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**Tailored ammonium-bearing and
ammonium-binding copolymers:
Pharmaceutical and industrial applications**

by

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A thesis

submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy



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Abstract

The post-polymerization functionalization, characterization and application of block copolymers, hydrogels and cross-linked polymeric micro-beads to particular pharmaceutical and environmental applications are the focus of this thesis. The synthesis of a new amphiphilic and cationic block copolymer, polystyrene-*b*-poly(N,N,N -trimethylammoniummethylene acrylamide chloride) (PS-*b*-PTMEACl), from polystyrene-*b*-poly(*tert*-butylacrylate) (PS-*b*-P(*t*BuA)) is presented. The morphology of the self-assembled copolymers in water is assessed, and finally, we evaluate the potential of this nanoscale system as a bile acid sequestrant. Bile salts represent the major elimination route for cholesterol from the body and bile salt sequestrants have an established reputation as highly effective agents in the facilitation of this elimination.

We also present the functionalization of a series of carbomers and their favorable bile salt binding properties. The synthetic methods were adapted from the post-polymerization functionalization of PS-*b*-P(*t*BuA).

The peculiar insolubility of ammonium tetraphenylborate in water was the fundamental property exploited in the research described in the final Chapter, where rather than ammonium-ion bearing polymers, ammonium-ion binding polymers are presented. Cross-linked PS resins were functionalized to contain pendent tetraphenylborate moieties and the ammonium-ion sequestering properties of this novel material were investigated. (Organo)ammonium ions from chemical and agro-industrial sources represent a major environmental hazard and our efforts contribute to the elimination of these toxins from effluent.

Résumé

Nous avons étudié la fonctionnalisation de macromolécules, ainsi que la caractérisation et certaines applications particulières de copolymères à blocs, hydrogels et micro-perles de polymères réticulés dans les domaines pharmaceutique et environnemental. Nous présentons la synthèse d'un nouveau copolymère bloc amphiphile et cationique, polystyrène-*b*-poly(N,N,N-triméthylammoniuméthylène chlorure d'acrylamide) (PS-*b*-PTMEACl) à partir de polystyrène-*b*-poly(*tert*-acrylate de méthyle) (PS-*b*-PtBuA). Nous avons déterminé la morphologie des copolymères auto-assemblés dans l'eau, et enfin nous avons évalué le potentiel séquestrant de ce système nanométrique sur les acides biliaires. Le cholestérol est éliminé principalement grâce aux acides biliaires, et les agents d'absorption de ces acides biliaires possèdent la réputation bien établie de rendre cette élimination considérablement plus importante.

Nous présentons aussi la fonctionnalisation d'une série de carbomères qui ont la propriété intéressante de fixer les acides biliaires. Nous avons adapté les méthodes de synthèse à partir de la fonctionnalisation consécutive à la polymérisation de PS-*b*-PtBuA.

Le tétraphénylborate d'ammonium est singulièrement insoluble dans l'eau, et nous avons exploité cette propriété fondamentale dans le dernier chapitre, où nous présentons des polymères qui se lient avec les ions ammonium, au lieu de polymères portant des ions ammonium. Nous avons fonctionnalisé des résines réticulées de PS afin qu'elles possèdent des groupements tétraphénylborates pendants, et nous avons investigué la propriété d'absorption des ions ammonium de ce nouveau composé.

Les ions organo-ammonium provenant des sources chimiques et agro-industrielles représentent un grand danger environnemental et nos efforts contribuent à l'élimination de ces toxines de l'effluent.

Preface

In accordance with the Thesis Specifications of the “Guidelines for Thesis Preparation” (Faculty of Graduate Studies and Research, McGill University), the following text is cited:

“Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.

If this option is chosen, **connecting texts that provide logical bridges between the different papers are mandatory**. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, a series of papers must be integrated.

The thesis must still conform to all other requirements of the “Guidelines for Thesis Preparation.” The thesis must include: A Table of Contents, and abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a review of the literature, a final conclusion and summary and a thorough bibliography or reference list.

Additional material must be provided where appropriate (*e.g.*, in appendices) and in sufficient detail to allow clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, **the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent**. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate’s interest to make perfectly clear the responsibilities of all the authors of the coauthored papers.”

This dissertation consists of eight chapters, with an introduction and general conclusions being the first and last, respectively. The second chapter details the post-polymerization functionalization of polystyrene-*b*-poly(*tert*-butylacrylate) (PS-*b*-(PtBuA)). The third chapter corresponds to a review article written by me and coauthored by both Prof. A. Eisenberg and Muriel Corbierre. The fourth chapter describes the self-assembled aggregate morphology of the functionalized polymers introduced in Chapter 2. The subject of Chapters 5 and 6 is bile acid sequestering block copolymer aggregates and hydrogels respectively. The final experimental chapter presents a new ammonium-scavenging polymeric system.

With the exception of Chapter 2, which has been published, and Chapter 7, which is the subject of a patent, manuscripts for publication are in the process of being prepared from this thesis. All will be coauthored by my research directors, Profs. G.R. Brown and A. Eisenberg. Drs. Lifeng Zhang and Yisong Yu graciously provided a few of the parent block copolymers discussed in Chapters 3 and 5. It was comforting to know that their library of samples was a reliable source of starting material should they ever be needed. Dr. F. Morin carried out all solid-state NMR spectroscopy. The question at the heart of Chapter 7 came from Prof. T.S. Cameron (Dalhousie University, Halifax), but the answer we propose is our own. Other than the aforementioned collaborations and assistance, and the valued input of my research directors, the contents of this thesis are the product of the author.

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*'The time has come' the Walrus said,
'To talk of many things:
Of shoes - and ships - and sealing wax - ,
Of cabbages - and kings'*

Lewis Carroll's *The Walrus and the Carpenter*¹

Chapter 1: Introduction

Our health and welfare are of paramount importance. Hence it is no surprise that a great deal of effort is invested in trying to repair the damage we cause ourselves in a society of excesses. To this end, and despite the ease with which chemistry, chemicals and chemists are condemned,² synthetic compounds of great utility are routinely discovered, characterized and put to use. This thesis focuses on a novel approach to human serum cholesterol reduction. Also presented is a new quantitative method of removing amines from aqueous effluent, in keeping with the positive role that synthetic polymers can play. Very broadly speaking, the study, characterization and application of a few polymers to better health and a cleaner environment were our goals.

Polymers: Some basic definitions

Schmierenchemie, or “greases chemistry,” as it was called 75 years ago, is a relative newcomer to the field of physical science. Hermann Staudinger was a founding-father of polymer science, having elevated it from the consideration of a resinous mass at the bottom of the flask for a reaction gone horribly wrong to an elegant and rich subject in its own right. By 1927 he began to convince skeptical colleagues not only that polymers actually exist, but that they are worthy of study.³ Whereas before 1927 it was thought that all compounds were molecules of low molecular weight that could be purified and characterized, we now know that polymers are large molecules composed of smaller structural units, or monomers. Biopolymers include DNA, RNA and all proteins.

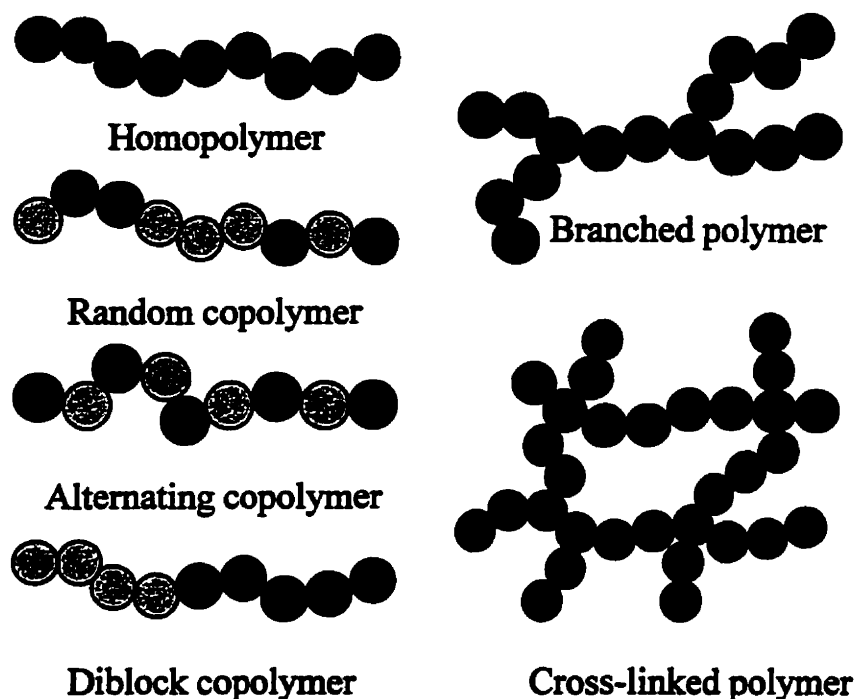


Figure 1: Schematic representation of random, alternating and block-copolymers.

In these cases, the smaller structural units are the nucleosides and the amino acids. This thesis focuses on synthetic polymers where vinylic monomers are polymerized and modified by a variety of chemical means to achieve the desired architecture. Used in this context, *architecture* refers not only to the tertiary structure (shape or morphology), but also to the way in which the monomer units are chemically attached. A polymer chain built completely from one monomer species is called a homopolymer; however, given a pool of monomer  and a pool of monomer , the resulting copolymer can be a random chain or a more ordered alternating polymer. The bulk of this thesis considers diblock copolymers that are prepared in such a way to have a block of one monomer followed by a block of the other (see Figure 1).

Each class of linear polymers has its own characteristics both in the bulk phase and in solution. Polymers composed of chiral sub-units will also have overall *tacticity* or handedness. An *atactic* polymer has no pattern to the handedness of its chiral centers, whereas *isotactic* and *syndiotactic* polymers are composed of monomers that have the same or alternating handedness, respectively. It should also be noted that not all polymers are linear: the final chapters of this thesis concern branched and cross-linked chains (see Figure 1).

Polydispersity: Molar mass and polymers

Small molecules and some biopolymers have a precisely defined molar mass; for example, any water molecule consists of exactly two hydrogen atoms and one oxygen atom. Our enzymes, DNA, and proteins can all be defined *exactly* by the number of residues present – indeed the activity of these molecules depends on a tightly controlled architecture. In general, synthetic high polymers cannot be so precisely constructed. Where the polymerization is a step-wise addition of monomer units to a growing chain, the rate of reaction for any given chain will depend on its micro-environment, and the total time of reaction for any given chain will depend on the lag time between initiator addition and actual initiation. Once polymerization is complete, there will therefore be a population of different macromolecules composed of varying numbers of the same sub-units. The number average molar mass ($\overline{M}_n$) is determined by experiments that count numbers of molecules. The weight average molecular mass ($\overline{M}_w$) is determined by experiments that measure their mass. Alternatively, the average degree of polymerization may be cited, but this gives no indication of the breadth of the polymer size distribution. Often, the polydispersity, or ratio of $\overline{M}_w / \overline{M}_n$ is given along with one of the average molar masses. In this manner, we have not only an average molar mass, but also an indication of the significant range of molar masses in the polymer sample.³

Classical free radical and condensation polymerizations can lead to high polydispersity values where $\overline{M}_w$ can be as much or more than twice $\overline{M}_n$. For very precise study of the self-assembly of amphiphilic copolymers in aqueous media, polymers synthesized via living ionic mechanisms are required, as these techniques yield

high polymers with narrow molecular weight distributions such that the polydispersities approach 1.0. The molecular weight profile is a Poisson distribution. When synthesizing polymers via anionic polymerization, initiation of all chains is nearly instantaneous. As this is a “living” process, even with all the available monomer having been consumed, the growing end of the polymer remains reactive in the absence of termination. If the reaction takes place above the de-polymerization ceiling temperature, de-polymerization/re-polymerization is also possible. The high reactivity of both the carbanionic initiator and the living end of the polymer, coupled with the essentially instantaneous initiation of all polymer chains in a limited monomer pool are the main contributors to the exceptionally narrow molecular weight profile typical for living polymerizations.

Homopolymers in solution

One particularly interesting property common to most linear and branched polymers is that a solvent can usually be found that will lead to a homogenous solution; even cross-linked polymers will swell in a suitable solvent. Polymer solutions are nevertheless much more complex than their small molecule analogues.³ Whereas the solubility of a small molecule in a given solvent generally increases with temperature, most polymers have an accessible lower critical solution temperature (LCST) above which the polymer will precipitate, as well as the intuitive upper critical solution temperature (UCST) below which the polymer will precipitate. These phenomena are explained as follows: starting with a polymer-solvent mixture at low temperature, there

is insufficient thermal energy to overcome the polymer-polymer interactions and thus solution does not occur. As the temperature is raised, the thermal energy begins to favour polymer-solvent interactions. Thermodynamically, this is expressed in Equation 1:

$$\text{Eq. 1} \quad \Delta G^M = \Delta H^M - T\Delta S^M$$

where ΔG^M , the free energy of polymer-solvent mixing, is a function of both the enthalpy (ΔH^M) and entropy (ΔS^M) of mixing. The entropy of mixing for non-polar polymers is usually positive, thus as T increases, the free energy of mixing decreases and solution occurs at the UCST.

The LCST is perhaps less easily understood. A simple explanation for the LCST in the particular case of polar systems is that hydrogen bonding between the polymer and the solvent aids the dissolution. Hydrogen bonds (and other polymer-solvent interactions) are thermally labile, however, so as the temperature is raised above a threshold, hydrogen bonding is decreased sufficiently that precipitation can occur. A more general explanation considers solvent order and free volume where a polymer-solvent mixture is superimposed on a hypothetical flexible lattice. As the temperature is raised above the LCST, the expanded liquid fraction of the lattice will no longer be able to accommodate the less expanded polymer, which is restricted by its connectivity. Clearly, both the UCST and the LCST are profoundly dependent on entropy.

The Flory-Huggins parameter

The enthalpy of transferring polymer A from a purely “A” environment to a solvent (B) environment, ΔH^M , is often defined in terms of the dimensionless Flory-Huggins parameter, χ_A , defined by

$$\text{Eq 2.} \quad \Delta H^M = kT\chi_A N_A \phi_B$$

where k is the Boltzmann constant, T is the temperature, N_A is the number of polymer molecules and ϕ_B is the volume fraction of the polymer in solution. Solvent quality increases as χ decreases from 0.5.

The Flory-Huggins parameter is also useful for parameterizing polymer-polymer blends. Except where there are favourable interactions between polymers (*e.g.*, hydrogen bonding), χ is usually positive, so the enthalpy and free energy of mixing are also positive. Most mixtures of homopolymers are therefore immiscible and macroscopic phase separation will take place in much the same way as an oil and water emulsion will de-mix.

When two homopolymers are covalently joined to form a diblock copolymer and χ (polymer-polymer) is unfavourable for the two blocks (as is often the case), macroscopic phase separation will be impossible under normal circumstances.*

Microphase separation of diblock copolymers has been a subject of much study: a series of morphologies has been observed as a function of block volume fraction using families

* Metaphorically one might imagine a cat and a dog tied together by the tail! (with thanks to Dr. M. Moffitt, Ph.D. McGill 1997, for pointing this out)

of copolymers. The bulk microphase separated morphology of polystyrene-*b*-polyisoprene with a short isoprene block, for example, is small spheres of polyisoprene arranged with body-centered cubic symmetry in a polystyrene matrix. As the volume fraction of polyisoprene increases, hexagonally packed cylinders and other morphologies are observed. When the volume fractions of each block are approximately equal, the dominant morphology is lamellar and as the polyisoprene fraction increases further, a morphology inversion is observed. When the polystyrene volume fraction is sufficiently low, small spheres of polystyrene arranged with body-centered cubic symmetry in a polyisoprene matrix are observed.⁴

Diblock copolymers in solution

Having established some of the parameters involved in the mixing of a homopolymer and a solvent, one homopolymer with another homopolymer and the result of the covalent attachment of two homopolymer blocks, the obvious extension of the argument is to consider what happens when a diblock copolymer is placed in a solvent. The interactions of each block with the solvent as well as the interactions between blocks must be considered. Furthermore, interactions within the block must also be accounted for. In the same way as small molecule surfactants self-assemble in a solvent that is “good” for either the polar head-group or the non-polar tail to form nanoscale aggregates, so do macromolecular diblock copolymers self-assemble (see Figure 2). The kinetics of copolymer self-assembly and morphological transition are slower than for small-

molecule analogues, but many morphologies are accessible including simple nanospheres, rods, vesicles and a host of more complicated architectures.⁵

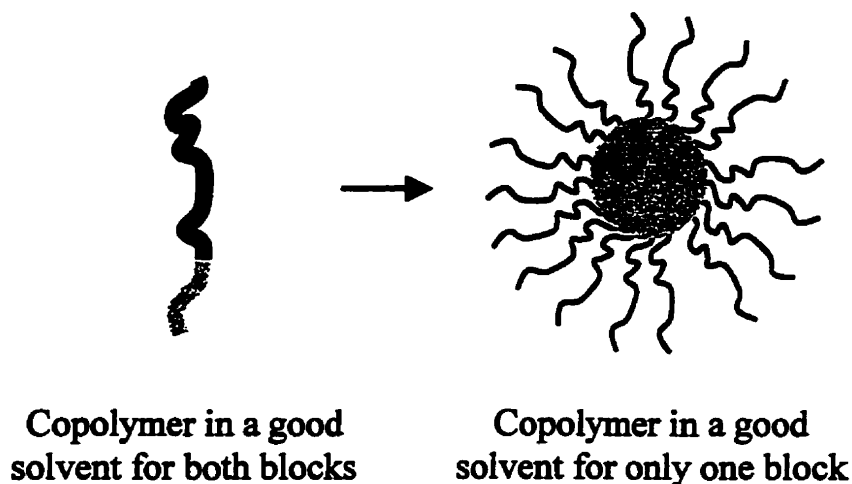


Figure 2: Block copolymer self-assembly in a block-selective solvent.

Hydrogels: structure, properties and applications

Some polymers dissolve completely in solvents with an increase in viscosity to form a homogeneous solution. According to a recent definition,⁶ polymers that absorb more than 20% of their weight in water *while maintaining* a distinct three-dimensional structure can be considered to be hydrogels. Aerogels absorb water into macro-pores without a great deal of overall swelling and xerogels are non-porous and absorb water by swelling.

Hydrogels are attractive for pharmaceutical applications, not only because they are water-compatible, but also because their mechanical properties can mimic organic tissue. They can also be employed for controlled drug release. When mechanical

robustness is required, hydrogels can be bonded to a strong substrate. Hydrogels have been manipulated to function in various oral dosage forms and suppositories as well as in direct blood and tissue contact applications including injectable nanoparticles and implants within the body.⁶

Resins: structure and properties

The term “resin” is a historical throwback as it was originally used to refer to amorphous solids such as shellac and rosin.⁷ Resin and bead are used together here to suggest cross-linked, small polymeric microspheres. Since all of the applications discussed in this thesis take place in an essentially aqueous environment, the beads under consideration are at least wettable, and swell within the limits of their cross-linking. It is therefore reasonable to think of them as restricted hydrogels.

Polymers: a summary

Polymers are therefore a unique class of chemical compounds with physical, chemical and engineering properties that render them extremely useful in a variety of applications. Thermosetting and thermoplastic polymers are often used structurally.⁸ Polymers are also used as friction-reducing additives and have been transformed into many synthetic fibres for the textile industry. Here, we consider instead the application of polymers to two aqueous systems when the goal is to sequester or “absorb” small molecules. Bile salts, which are integrally linked to serum cholesterol levels, are the

target molecules in an effort to control heart disease. The other target is (organo)ammonium waste in industrial water effluent.

Coronary Heart Disease

Causes, prevalence, outcome and cost

Coronary heart disease (CHD) is the leading killer in North America.⁹ In Canada alone, it is responsible for slightly more than one third of all deaths and is the third leading cause of death under the age of 75.¹⁰ The effects of heart disease are not, however, limited to mortality: chronic pain or discomfort, restricted activity, disability and unemployment as well as a profound impact on family life, sexuality, psychological health and reaction to treatment (side effects) are among the non-fatal yet important possible health implications. Recent surveys (1985-1990) found that more than one third of all Canadians between the ages of 18 and 74 had two or more of the major risk factors (smoking, hypertension, elevated cholesterol, physical inactivity or obesity).¹¹ Cardiovascular disease is the leading cause of hospitalization for both men and women, with the exception of childbirth, and the age onset is 45 and 55 years for men and women, respectively.¹⁰ The annual cost of CHD in Canada (1993) has been estimated at about 8 billion dollars (CAD).¹² Given the enormous price both in financial terms and in overall health, the search for CHD cures has therefore commanded an enormous combined research effort from the scientific community. The Victoria Declaration on Heart Health states:

Cardiovascular disease is largely preventable. We have the scientific knowledge to create a world in which most heart disease and stroke could be eliminated.¹³

It is widely accepted that one's risk of coronary heart disease increases with age, family susceptibility (genetic predisposition), smoking, physical inactivity, obesity, hypertension, diabetes and with dyslipidemias including high plasma cholesterol concentrations. Chemistry does not yet have a 'cure' for either the effects of age or family, but the other CHD risk factors can often be controlled. In most cases, lifestyle adjustments to smoking habits, eating habits and physical exercise can reduce the risk of CHD dramatically. In some cases, however, more intervention is required, particularly for those with elevated cholesterol levels.

Cholesterol's biological role

Cholesterol is vital to the human body. It is not only a stabilizer of the cellular fluid mosaic membranes, but also its four fused rings lay the framework for the steroid hormones, including progesterone, testosterone and estradiol (the sex hormones), cortisol (a glucose metabolism regulator) and aldosterone (which controls salt excretion). Cholesterol has a valuable role to play in regulating the fluidity of cellular membranes¹⁴ and is also bio-transformed into vitamin D. Finally, cholesterol is the precursor for the bile acids.¹⁵ Despite the fact that the severe lack of cholesterol in childhood results in extreme mental retardation, failure to thrive, and is associated with high infant-mortality,¹⁶ there nevertheless seems to be a threshold over which there can be too much

of a good thing.^{17,18} Elevated serum cholesterol levels have been discussed heatedly in medical and media circles as a CHD promoter.¹⁹ Cholesterol statistics for the Canadian population determined from the Heart Health Surveys (1985-90)¹¹ are as follows:

- 30% of men and 27 % of women were in the moderate risk group (5.2-6.1 mmol/L)
- 18% of men and 17 % of women were in the high risk group (≥ 6.2 mmol/L)
- the absolute number of men and women with high cholesterol levels increases dramatically with age.

Cholesterol transport

Cholesterol is a largely hydrophobic molecule and as such cannot be circulated directly in the blood. Instead, the hydroxyl group is esterified with a fatty acid (see Figure 3) and the whole molecule is transported in a mixed micelle with phospholipids. Thus, amphiphilic molecules (phosphatidyl choline) form a shell, and triglycerides with cholesterol esters are able to travel through the vasculature in micelle cores. There is a

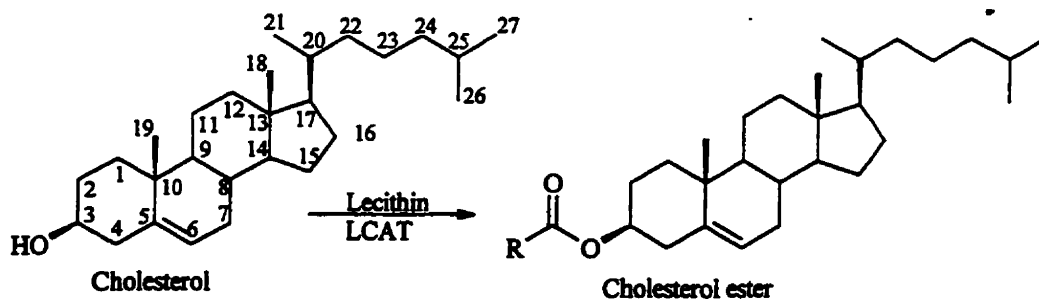


Figure 3: Cholesterol and the lecithin-cholesterol acyl transferase (LCAT) catalyzed cholesterol ester. (The standard sterol carbon numbering system is illustrated for cholesterol.)

family of these micelles called the lipoproteins due to the lipophilic fats, phospholipids and cholesterol, as well as the targeting proteins built into their shell. Lipoproteins are further categorized according to their density.

Thus, a system of very low density, low density and high density lipoproteins is developed. Very low density lipoproteins (VLDL) have a small amount of cholesterol in their cores, and are primarily involved in the transport of triglycerides. Once the fats have been deposited, then the density of the micelle increases, hence low density lipoproteins (LDL) travel in the blood stream. It is this form of solubilized cholesterol that is the most important for the body. Cholesterol transport and availability is vital for the production of bile acids in the liver and, to this end, physiological mechanisms in the body ensure a basal level of cholesterol. LDL receptors on the cell surfaces throughout the body bind LDL's. The cholesterol is ultimately brought to the endoplasmic reticulum through endocytosis.

Nascent high density lipoproteins (HDL) are cholesterol deficient; however, lecithin-cholesterol acyl transferase (LCAT) on the HDL surface catalyzes the esterification of cholesterol from lecithin (phosphatidyl choline), and mature, cholesterol-rich HDL has been postulated to carry cholesterol from the extrahepatic tissues to the liver as required (see Figure 4).^{14,15}

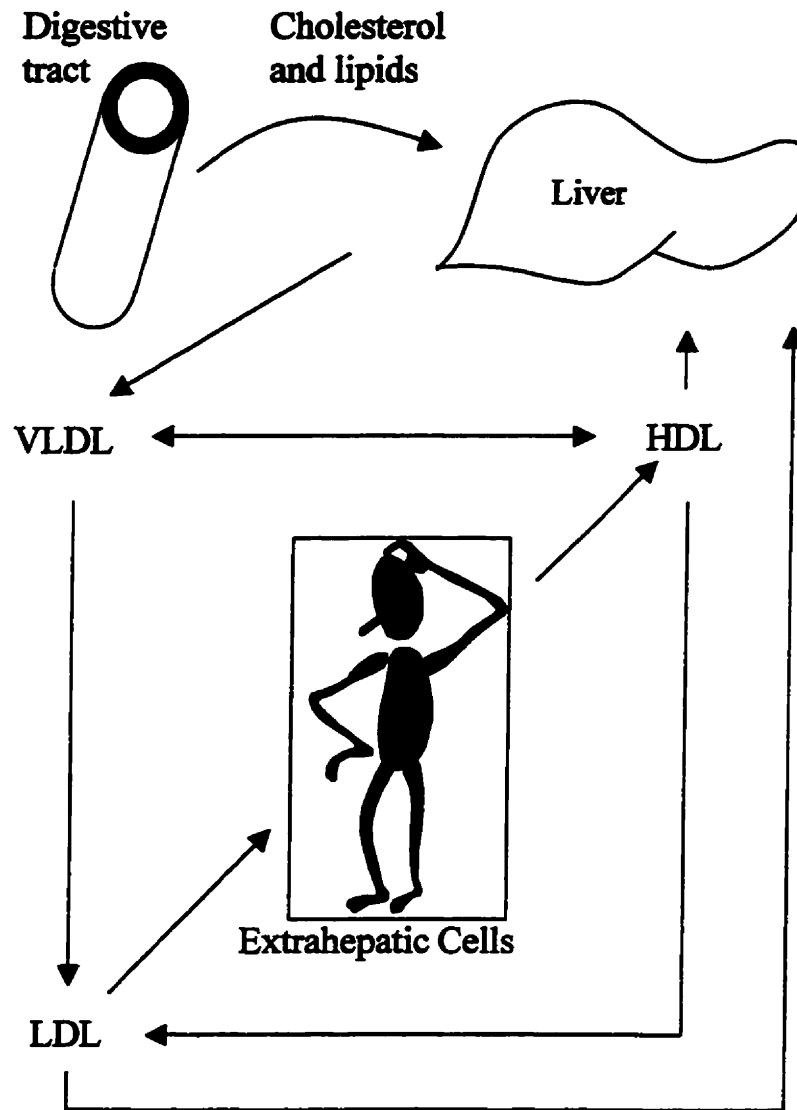


Figure 4: Cholesterol transport: Ingested lipids and cholesterol are transported across the intestinal lumen and are carried to the liver. Very low-density lipoprotein (VLDL) initially carries the hydrophobic compounds in the bloodstream and has two possible fates (conversion to HDL or LDL). All cells in the body including the liver take up LDL. HDL takes cholesterol from the extrahepatic cells (all non-liver cells in the body) to the liver.

Good and bad cholesterol

Given that high HDL levels indicate that the liver requires cholesterol and furthermore that cholesterol metabolism regulation is functioning properly, it is no surprise that elevated HDL has a negative correlation with coronary heart disease (CHD).²⁰ Broadly speaking, the VLDL and LDL are specific for the movement of cholesterol to the liver and on to the extrahepatic tissues for storage, and the HDL (*good cholesterol*) carries the cholesterol back to the liver for chemical modification.²⁰ There is substantial evidence that when the LDL (*bad cholesterol*) transport system is overloaded, or when it does not function properly, the excess is deposited on the arterial walls^{17,18} leading to atherosclerosis and CHD. This is the crux of the argument for moderating plasma cholesterol levels.

Total serum cholesterol concentration is not the most revealing indicator of CHD risk. In most cases, HDL and LDL levels are given in conjunction with the total, where, according to the National Institute of Health (USA),^{21,22} HDL > 35 mg/dL and LDL < 130 mg/dL are desirable serum concentrations, LDL from 130-159 mg/dL is borderline and LDL levels exceeding 160 mg/dL are high. For individuals with high-risk lipoprotein levels, cardiologists often follow the HDL/LDL ratio as a function of treatment.

Cholesterol sources

There are two pathways for the generation of plasma cholesterol. Cholesterol is scavenged from our diet, but the body is also equipped to synthesize all it needs from acetyl-coenzyme A (Acetyl Co-A). The metabolic pathway is controlled through feedback inhibition. The tightest regulatory control occurs in the reduction of 3-hydroxy-3-methylglutaryl coenzyme A (see Figure 5).²³ There is feedback inhibition at this step; if there is sufficient cholesterol stored in the cellular cytosol, then HMG-CoA reductase activity is limited and new cholesterol will not be synthesized. For diets high in cholesterol, *de novo* cholesterol synthesis ought to be low or insignificant. The most obvious way of controlling plasma cholesterol for “normal” individuals, therefore, is to control the amount of cholesterol in our diets. By limiting cholesterol intake, the endogenous cholesterol will eventually be cleared from the body and a desirable steady-state plasma cholesterol level will be achieved (this varies with age but seems to be about, or under 200 mg/dL (<5 mmol)).

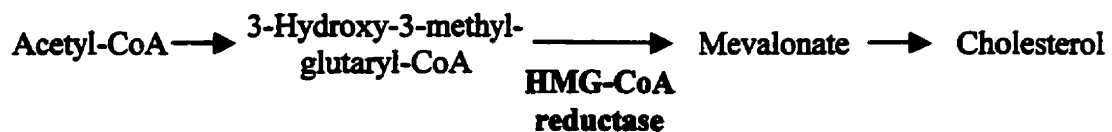


Figure 5: *de novo* cholesterol biosynthesis.

Hypercholesterolemia

For some people, however, low-cholesterol diets are not enough to prevent elevated LDL levels. The genetic disorder hypercholesterolemia is characterized by non-functioning LDL receptors at the level of the cells so that LDL particles circulate until they deposit in the arteries. Furthermore, since the feedback inhibition of cholesterol synthesis does not function correctly, the liver synthesizes cholesterol *de novo* and compounds the problem. For these people, and for others who suffer from chronic or extremely elevated levels of high cholesterol, there is an arsenal of available pharmaceutical agents to help them get their cholesterol levels down to an acceptable level. Here, there are two primary strategies. The first, and chemically more complicated, involves the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase²⁴ (see Figure 5). By minimizing the endogenous production of cholesterol, the body will rely on dietary or endogenous cholesterol, thereby lowering the plasma cholesterol. Until recently, the commercial drugs were all derived from a fungus, however there is now a fully synthetic, indole-based drug on the market. The mechanism of the inhibition does not seem to be very well understood for any of these drugs. Compactin,²⁵ Lovastatin,^{18,26,27} Simvastatin,²⁸ Pravastatin²⁹ and Fluvastatin (Lescol) are the major players.

Bile acid sequestrants

A less invasive technique is to use bile acid sequestrants (BAS). The BAS strategy depends on the fact that it is through bile salts that the majority of excess cholesterol is cleared from the body (see Figure 6).

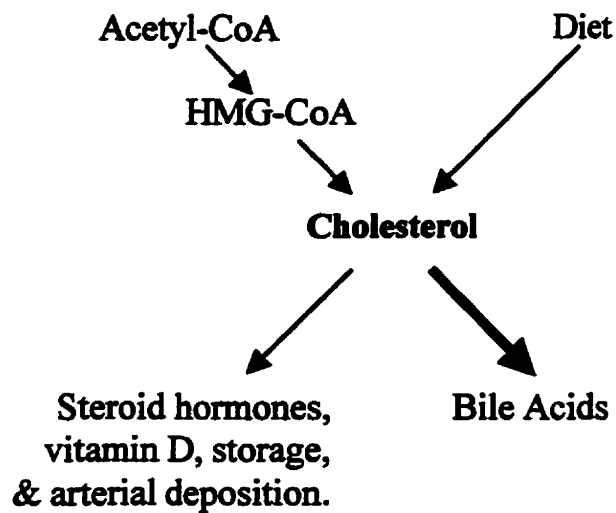


Figure 6: A schematic representation of the two sources of cholesterol and its fate. The main elimination route is via the bile acids.

Enterohepatic circulation

Bile acids are metabolized from cholesterol and are conjugated with glycine or taurine. The structure and energy-minimized conformation³⁰ of the parent bile acid are shown in Figure 7. Note that the terms bile salt and bile acid are often used interchangeably despite the possible confusion this causes. Bile acids are protonated and are insoluble in water. Bile salts, as found in bile, are ionic and water-soluble. Cholic acid derivatives represent 40% of all bile acids, chenodeoxycholic acid (dehydroxylated at C12) represents another 40% and finally, deoxycholic acid (dehydroxylated at C7)

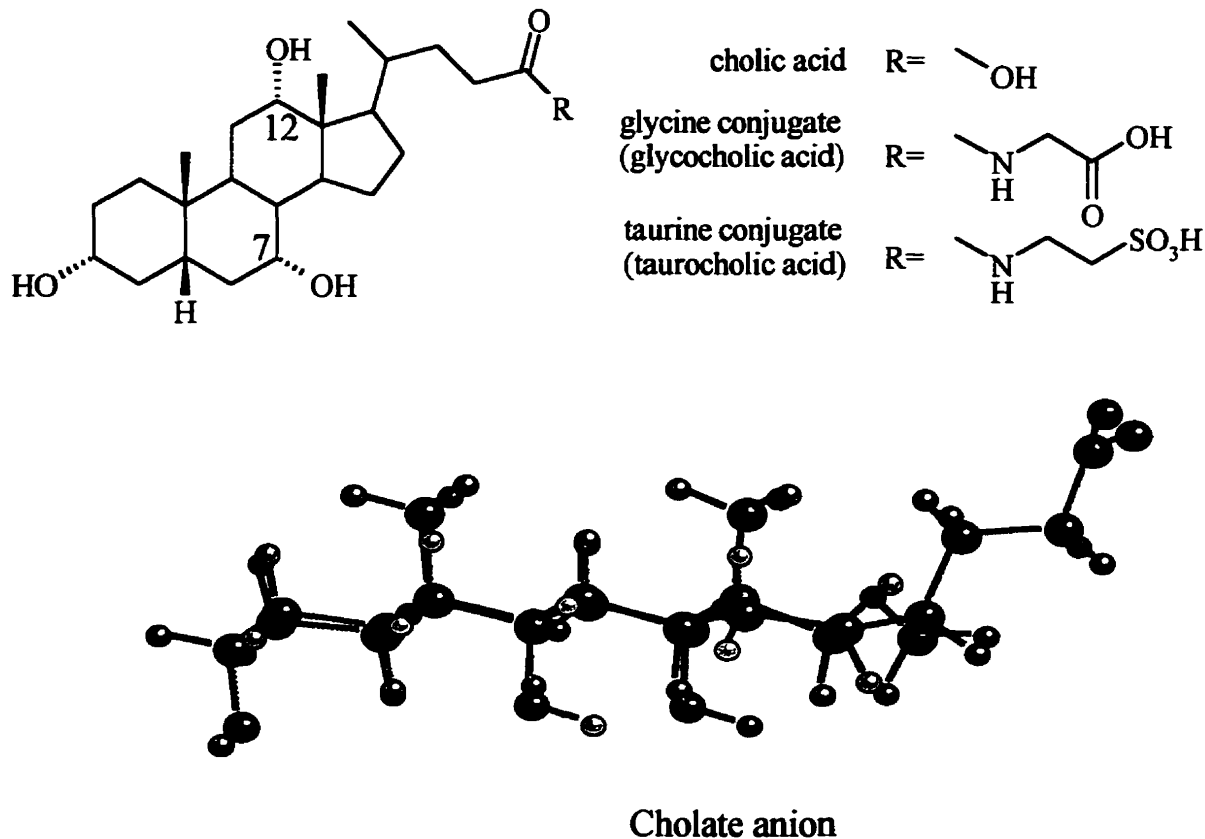


Figure 7: Bile salts (cholic, chenodeoxycholic (12-dehydroxylated cholic) and deoxycholic (7-dehydroxylated cholic acid) are conjugated with glycine or taurine to form surfactant molecules with a polar head-group, as well as a hydrophobic face and a hydrophilic face. The lower structure is an energy-minimized conformation of cholate (Nemesis)³⁰ viewed edge-on.

accounts for the remaining 20%. The conjugation (glycine:taurine) ratio is 3:1.

On ingestion of a meal, bile salts are passed into the gastrointestinal (GI) tract to emulsify fats and thereby facilitate their catabolism. Once the fats have been absorbed from the bile salt/fat micelles, the bile salts are re-absorbed primarily via active transport across the ilial brush border membrane and are passed through the hepatic portal vein to the liver and are ultimately passed back into the GI tract following re-conjugation as necessary³¹ (see Figure 8).

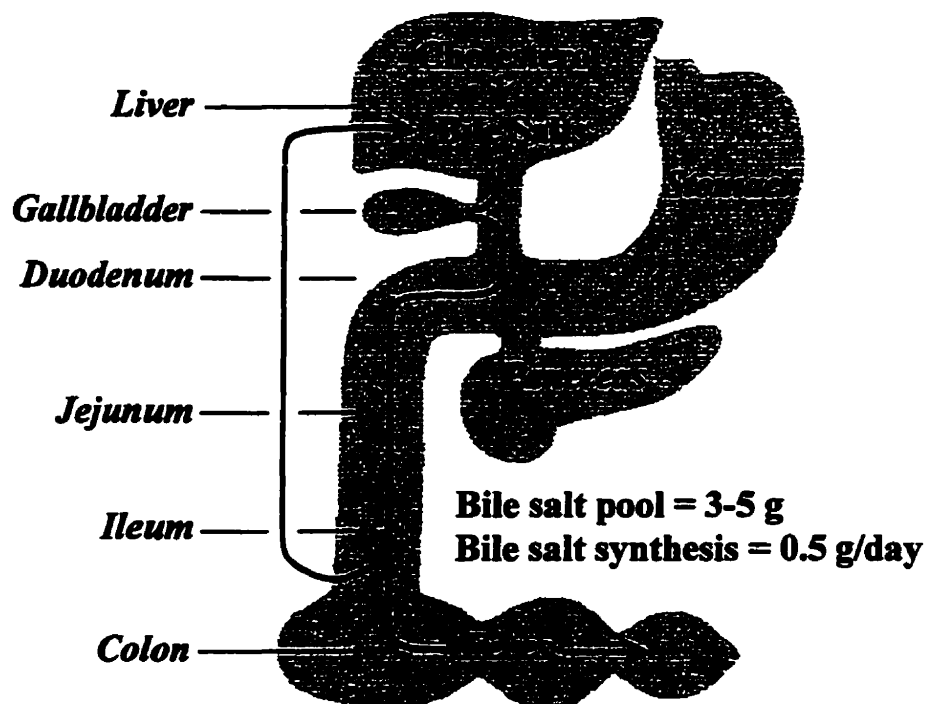


Figure 8: A schema of the gastrointestinal (GI) tract. The heavy line represents enterohepatic circulation of bile salts.

Re-absorption is very efficient (>95%), but some bile salts are excreted over each cycle. The body maintains a bile acid pool of up to 5 grams; thus, excreted bile salts are replaced by the synthesis of new bile salts from cholesterol. If the body can be “tricked” into excreting more bile salts than normal, then more new bile salts will be synthesized (see Figure 6).³² This is where BAS become useful. Whereas many drugs require controlled-delivery systems, sequestrants are controlled *extraction* agents and are actually

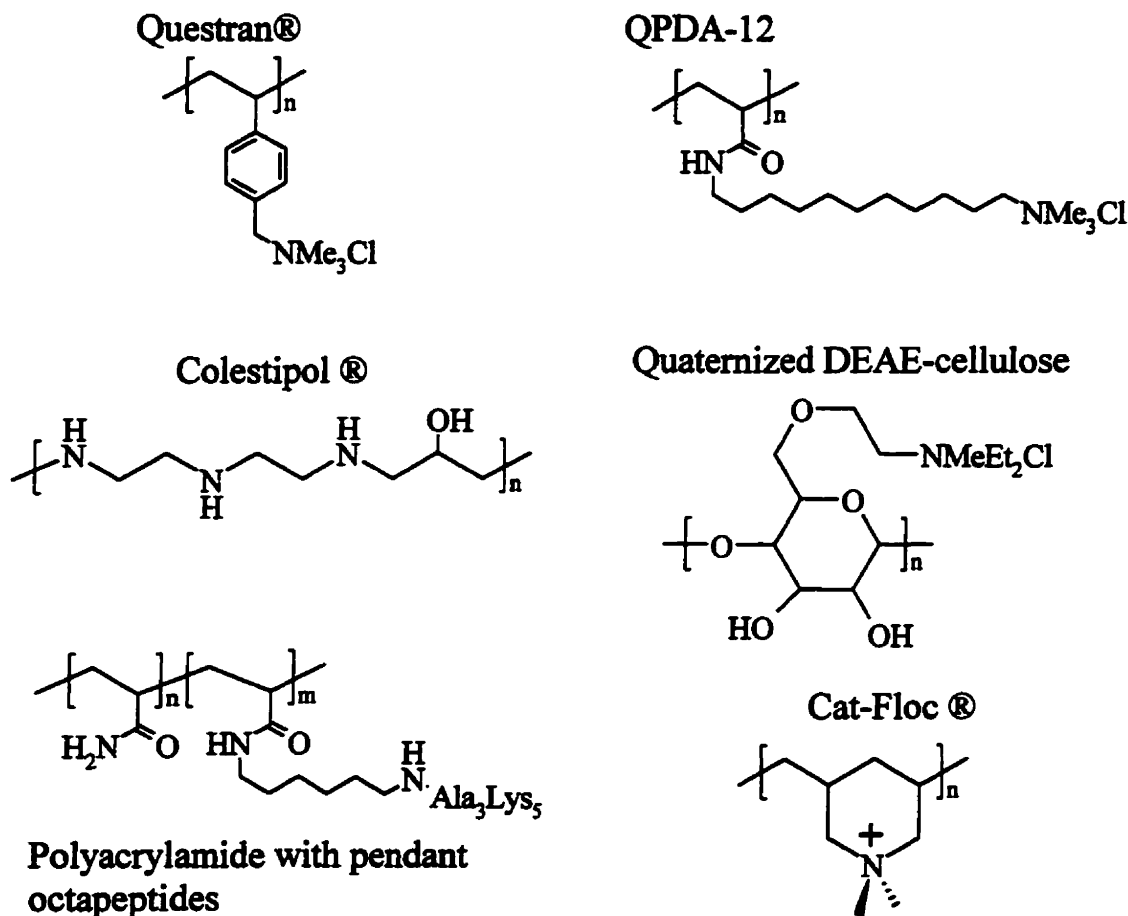


Figure 9: A variety of asystemic cholesterol-reducing agents.

asystemic. Most orally ingested drugs pass from the gastrointestinal tract into the blood stream where they are active, but sequestrants simply pass through the digestive tract. They are usually anion exchange resins³³ (e.g., cholestyramine, QPDA-12) which are polymeric networks characterized by quaternized pendant amines (see Figure 9). The positive charge of the quaternary ammonium ion pairs with the negatively charged bile salt and thus the very efficient active transport across the intestinal walls can be partially circumvented. Extensive work carried out under the direction of Drs. Brown and St-Pierre over the last few decades has significantly added to the body of knowledge concerning optimal bile acid sequestrants.³⁴

As shown in Figure 7, bile salts are surfactants with tri-faceted character. The fused rings are essentially coplanar and have a hydrophobic face dominated by methyl groups and a hydrophilic face dominated by hydroxyl groups. Furthermore, in either conjugated or unconjugated form, bile salts have a pendant carboxylate or sulfonate group carrying a negative charge. The recurring theme in most bile acid sequestrants (see Figure 9) is that they are amphiphilic, *i.e.*, they have both hydrophilic and hydrophobic character, and carry positive charge. In this way, the very strong short-range electrostatic attraction between bile salt and sequestrant is supplemented by hydrophobic and hydrophilic interactions.

Under normal circumstances, bile salts form mixed micelles with lipids in the gut but sequestrants provide an alternative and less reversible substrate. The optimal system developed by Drs. Brown, St-Pierre *et al.* is a polyacrylamide conjugated with N,N,N-trimethylammoniumdodecane, a sequestrant that has binding characteristics superior to the generally prescribed cholestyramine (Questran®). The quaternary ammonium group

therefore provides the positive charge, the hydrophobic character is provided by the dodecyl spacer and the hydrophilic requirement is met not only by the charge-bearing group, but also by the chemically stable amide group. Earlier work in our laboratory has established that the electrostatic component of the attraction dominates, but for an effective sequestrant, all three potential binding mechanisms must be exploited.³⁵ It is from these observations and conclusions that the work presented in this thesis was conceived. The goal is to encourage bile salt excretion thereby necessitating *de novo* bile salt synthesis from its precursor, cholesterol.

Commercial bile acid sequestrant resins

There are two commercially available BAS resins: colestipol and cholestyramine sold under the trade names Colestid (Upjohn) and Questran A (Bristol-Meyers).³⁶ Many more resins have been developed such as QPDA-12,³⁷ SK&F 97426-A³⁸ and very recently, GelTech has presented a new hydrogel that structurally resembles that of QPDA-12 and shows promise in phase III clinical trials.³⁹ The therapeutic dose for the commercial resins can be as much as 36 grams per day. A significant fraction of patients complain of nausea, constipation, indigestion, and if BAS therapy is prolonged, there may be an increase in bleeding due to vitamin K depletion. Patient compliance is very low with these treatments due, in part, to the unpleasant delivery of the medication. Polymeric resins are gritty with the appearance of sand, and 12 gram doses before each meal would certainly reduce the pleasure of eating. Nevertheless, when faced with CHD from high cholesterol levels, this seems a small price to pay.

Cholestyramine

The most venerable of the BAS is cholestyramine,^{29,40} which was introduced in the 1950's. It has remained the traditional standard, and new BAS's tend to be measured against cholestyramine. Many resins have been developed now with binding properties that are superior to those of cholestyramine, which has relatively poor affinity for the relatively hydrophilic trihydroxy bile salts. *In vivo* assays show only 1-3% of the binding capacity for bile salts demonstrated in the *in vitro* tests. This dramatic difference in efficiency from the non-physiological trials is almost certainly due to the active-transport-facilitated diffusion of the bile salts from the resin in the ileum, *i.e.*, rather weakly bound bile salts are released from the BAS. Further decrease probably results from competitive binding with other anionic species (*e.g.*, vitamins, etc.). Cholestyramine shows a preference for the dihydroxy bile salts and for the taurine-conjugated bile salts. Glycocholic acid, which makes up the majority of the bile salts in humans, is not so irreversibly bound in the resin.⁴¹

QPDA-12

QPDA-12 is a cross-linked resin that is very similar to cholestyramine in terms of the carbon backbone.³⁴ However, the quarternized nitrogen pendant groups are attached through an amide linkage with an aliphatic spacer, whereas the pendant group for cholestyramine is *para* benzyl-trimethylammonium chloride. Following extensive *in vitro* experiments, the *in vivo* assays for these new resins tend to include a battery of plasma fraction concentration tests, enzyme activity tests and any changes in liver or body weight are monitored. *In vivo* and *in vitro* tests, comparing the efficacy of QPDA-12 with cholestyramine, show conclusively that QPDA-12 is more effective at least for

rabbits, though QPDA-12 is yet to be tested on humans. Unfortunately, due to the active transport of bile salts across the lower intestinal lumen, *in vitro* assays must be interpreted with caution. Not only the capacity of the sequestrant, but also the irreversibility of the binding must be optimized. Animal trials always precede human trials and for hypercholesterolemia, rabbits or hamsters seem to be the species of choice. Hamsters have been shown to have a physiological bile salt blend and cholesterol physiology that is similar to that of humans. They have LDL analogues and LDL analogue receptors.⁴² QPDA-12 appears to cause a lowering of the total plasma cholesterol levels without adversely affecting plasma glucose and other important fractions.

SK&F 97426-A

SK&F 97426-A (Smith Kline Beecham)^{38,43} has also undergone both *in vitro* and *in vivo* pre-clinical testing but has been dropped as a BAS lead by Smith Kline Beecham following a recent paper describing the synthesis of ¹⁴C radio-labeled resin. Where QPDA-12 has an acid stable amide linkage and cholestyramine has a virtually totally stable benzylic linkage, SK&F 97426-A is a 1% cross-linked 11-trimethylammonio-undecylmethacrylate chloride-co-ethylene glycol dimethacrylate polymer. The ester linkage of the pendant group may be unstable in the GI tract which might explain the results of the isotope labeling experiment and subsequent silence.

Other polymeric sequestrants

Many other bile acid sequestrants have been explored. Dimethylaminocellulose (DEAE-cellulose) has been reported as a possible alternative to the commercially available resins, but is five times less active than cholestyramine on a weight basis.⁴⁴ Oligopeptide-containing resins^{45,46,47} have been extensively studied by G.R. Brown *et al.* Two water-soluble polymers, 3,3-ionene chloride and CAT-FLOC,⁴⁸ have also received some attention.

Neomyacin and niacin

Neomyacin⁴⁹ is an antibiotic typically used in the prevention of hepatic coma. However, a concomitant decrease in neomyacin-treated patients' serum cholesterol levels was noted. This drug appears to have an effect on the formation of bile acid micelles in the intestine and facilitates the excretion of neutral sterols. The side effects include possible kidney damage, as well as itching and abnormal intestinal function. Niacin (or nicotinic acid) has also been studied⁵⁰ and although one study reports that there was no significant decrease in fatal cardiovascular events, the total serum cholesterol was reduced by 10%. The combined decrease in fatal and non-fatal myocardial infarction was 15%.⁵¹

Fibrates⁵²

Clofibrate, gemfibrozil, fenofibrate, benzofibrate and ciprofibrate are a series of aryloxyisobutyric acids that appear to reduce total serum cholesterol levels.⁵³ Clofibrate was the first in this class to be explored as a cholesterol reduction agent under the tradename Atromid-S, but the other fibrates, particularly gemfibrozil, have since proven more effective. Fibrates are best suited to treating those with hypertriglyceridemia and are not as effective at reducing serum cholesterol levels as bile acid sequestrants or HMG-CoA reductase inhibitors. Nevertheless, most patients suffering from elevated triglycerides and cholesterol levels will see improvement in both if gemfibrozil is administered. The most common side effect with this treatment is intestinal distress (abdominal pain, diarrhea and nausea: 2-5% of patients), and other side effects include skin rashes, alopecia[†], blurred vision, weight gain, impotence, leukopenia[‡] and anemia.

Other cholesterol reduction agents

Dietary fibre,⁵⁴ a high-protein diet⁵⁵ and even walnuts,⁵⁶ have also been shown to reduce serum cholesterol. Saponins and the following inorganic compounds have also been reported in this context: aluminum hydroxide and CaCO₃,⁵⁷ bismuth subsalicylate and montmorillonite⁵⁸ and also ferric chloride⁵⁹ which was tested on cholesterol-fed chickens in the early 1950's.

[†] baldness

[‡] an abnormally low white blood cell count

The French provide several clues to possible CHD reduction techniques. Despite the fact that the average total cholesterol level in France exceeds the North American average by 20 mg/dL, the coronary death rate for French men is only 80 per 100 000 as compared with 180 per 100 000 Americans. Hypotheses abound trying to explain this paradox. Some believe that since the French consume most of their daily food before early afternoon, that there is a metabolic effect. Calcium from cheese, anti-oxidants in red wine, relaxed life-style, abundance and consumption of fresh fruits and vegetables have also been postulated as explanations for this puzzle. Reduced risk of CHD notwithstanding, heart disease and cholesterol levels do correlate in France and elevated cholesterol levels remain a factor in cardiovascular health.⁶⁰

Block copolymer micelles and hydrogels

It has been recently proposed⁶¹ that a colloidal suspension of block copolymer micelles with suitably engineered pendant groups might prove to be more efficient in the binding of bile salts. Several aspects of this idea are appealing, one being that the colloidal domain maximizes surface area and thereby potentially lowers dosage and side effects. Furthermore, it has been conclusively shown that hydrophobic interaction between bile salts and resins enhances sequestration. Originally, the hope had been that a polystyryl micelle core might provide such an environment, though it now seems much more likely that such a core would be glassy and impenetrable in an aqueous environment. Nevertheless, the idea of a comparatively large hydrophobic domain near the bile salt binding sites remains interesting. The self-assembly of amphiphilic diblock

copolymer actually presents a wealth of accessible architectures of varying complexity, therefore the characterization of the self-assembly properties of our system allowed us to select likely candidate copolymers giving us some measure of control over the colloidal surface area, availability of binding sites, and, in principle at least, control over the hydrophobicity of the hydrophilic block.

A corollary advantage of bile salt binding micelles is born out of the intuitive observation by Brown *et al.*⁶² that additional cross-linking of ion-exchange resins *reduces* their *in vitro* effective capacity to sequester bile salts. The established functionalization technique involved the condensation of polymethyl(methacrylate) with an α,ω -diamine, but Asgari improved on this route using a mono-protected diamine for the ester-amide transformation followed by deprotection and N-methylation, thereby eliminating additional cross-linking during functionalization and increasing the activity of the resin. Clearly, any amine consumed in cross-linking cannot be available to bind bile salts. Furthermore, additional cross-linking renders the beads less susceptible to diffusion and thereby renders some binding sites inaccessible. Cross-linking also reduces the flexibility of the polymer resulting in a resin that is less able to adopt a tertiary conformation amenable to the shape of the bile salt molecules. A potential advantage offered by micelles is that there is no cross-linking in the corona and, concomitantly, little to hinder the diffusion of bile salts into the reach of the positively-charged ammonium groups. It appears to be widely accepted that the majority of the disagreeable side effects associated with bile acid sequestrants can be traced to the enormous dosages required for treatments (up to 30 g/day). If the effective capacity of the sequestrant can be raised, then the dosage can be reduced, thereby reducing, or perhaps eliminating, side effects.

Quaternary nitrogen has been the most widely studied 'hook' for bile salts in the GI tract due to the known binding of bile salts to lysine residues in physiological conditions. Before other 'hooks' are developed, it is perhaps advisable to optimize a system that mimics nature. Our initial approach was to synthesize PS-*b*-P*t*BuA by now well-established anionic polymerization techniques, and to then displace the butoxide leaving group with N,N-dimethylethylenediamine.⁶³ Quarternization of the dimethyl amine was carried out with methyl iodide and then the iodide was exchanged for chloride.

Although the approach appears to work *in vitro*, we are obliged to admit that the synthesis is labour-intensive and that the final polymer concentration in solution is severely limited. We propose in addition to capitalize on lightly cross-linked and branched polymeric particles that do not imply the complications of self-assembly yet remain highly water-swallowable. In either case, the first and possibly main advantage over the commercially available bile acid sequestrant resins is the improved delivery system. One might envision gelcaps or a pleasantly flavoured liquid as potential media for the ingestion of the BAS.

Amine waste

Amine waste in effluent is a problem of sufficient proportion that the Monsanto Corporation recently offered one million dollars for a "cost-effective and commercially practical technology for recovering ammonia."⁶⁴ Monsanto's chemical operations typically generate amine-poisoned effluent water at the rate of 300 gallons per minute, and the presence of other organic waste prevents the easy conversion of the ammonia into

nitrogen fertilizer. The centrate ammonia recovery process, developed by the American Department of Energy's Pacific Northwest National Laboratory, Richland, Wash.; Battelle Columbus (Ohio) Operations and ThermoEnergy employs zinc-loaded ion exchange columns to remove up to 90% of the ammonia in waste water. The columns are regenerated with sulphuric acid and the ammonia is recovered as ammonium sulfate, which can then be sold as fertilizer.⁶⁵

Based on an on-going study of hydrogen bonding to π -systems at Dalhousie University,⁶⁶ it was noted that the tetraphenylborate anion is an aggressive ammonium cation scavenger and that the ion pair is essentially insoluble in water. Our goal was to take this observation and marry it to a cross-linked polymer system in order to investigate its utility as an (organo)ammonium scavenging material.⁶⁷

Summary and scope of this thesis

The diversity of subjects covered in this thesis is aptly reflected in the Walrus's discussion with the Carpenter from Lewis Carroll's *Through the Looking Glass*. Shoes and ships and sealing wax are unrelated at first blush, but actually have macromolecules in common as do self-assembling diblock copolymers, branched and cross-linked copolymers, and treatments for heart disease and amine-rich wastewater.

In this thesis, we describe the characterization and present the synthesis of a series of macromolecules designed to interact favorably with bile salts and with amines. Within this Introduction, the endeavor has been to provide a general overview of the problems to be addressed as well as the necessary background material for an understanding of the role of cholesterol in coronary heart disease. Chapter 2 is a recently published review of

the solution morphologies that are accessible from highly asymmetric amphiphilic block copolymers. Chapter 3 presents a description of the synthesis of polystyrene-*b*-poly(N,N,N-trimethylammoniummethylene acrylamide) and provides the preparative details for Chapter 4 which deals specifically with the solution morphology of the polystyrene-*b*-polyacrylamide system. Bile salt binding is the focus of Chapters 5 and 6 where self-assembling aggregates and branched polymers are considered in greater detail. The penultimate chapter presents the preliminary characterization of the recently patented polymer-tethered tetraphenyl borate ammonium binding system.

The elements of this thesis are therefore:

1. the synthesis and characterization of a new amphiphilic diblock copolymer;
2. the study of the nano-scale, self-assembling system's accessible solution morphologies as a function of diblock architecture;
3. aggregate stability on the addition of electrolytes including bile salts;
4. the functionalization of lightly-branched poly(acrylic acid) and its binding characteristics; and
5. the synthesis and characterization and (organo)ammonium-binding of tethered tetraphenylborate.

The final chapter summarizes the conclusions to be drawn from the studies of these potentially applicable polymers. Although ostensibly unrelated, an excess of either cholesterol in the body or (organo)ammonium ions in the environment can have serious consequences. Bile salt sequestration in the human gut demonstrably reduces serum cholesterol levels and amine-rich wastewater is a harmful, carcinogenic pollutant. It is

therefore our hope that the reader will be left with the impression of having learned more about polymers, coronary heart disease and water detoxification rather than only about “cabbages and kings.”

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Chapter 2

Morphology I: Asymmetric Amphiphilic Block Copolymers in Solution*

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Chapter 2 - Asymmetric Amphiphilic Block Copolymers in Solution: A Morphological Wonderland

Abstract

Asymmetric amphiphilic diblock copolymers self-assemble in selective solvents. Since 1995, when we first reported the systematic preparation of a sequence of various ‘crew-cut’ aggregate morphologies from this class of copolymer in solution¹, we have identified a vast array of structures and have begun a detailed investigation of the thermodynamic and kinetic parameters that induce morphogenesis. Not only spheres, rods, bilayer and bicontinuous architectures, as well as inverted structures are observed, but also a selection of mixed, combined and much more complex aggregates has been documented. All of these aggregates have a phase-separated insoluble core and a ‘crew-cut’ soluble corona. Thus all parameters that permit selective modification of the component of either phase or of the interface provide a window for morphological control. By carefully adjusting the polymer chain environment, it has been possible to develop a systematic understanding of morphogenic parameters which include, among

others, polymer composition, common solvent, initial concentration, temperature, type and concentration of added ions, method of preparation, and added homopolymer. To date, more than 30 publications have appeared in the literature from our group alone on this subject. One of the problems inherent with such a complicated system is the taxonomy or classification: which morphologies correspond to equilibrium positions and which are intermediate or trapped? An attempt at a logical presentation of the observed aggregates is given, preceded by a qualitative discussion of the thermodynamic framework for this system. Where possible, the transitions between morphologies are explained in the context of the thermodynamic parameters. Finally, parallels are drawn between the copolymer aggregates and biological architectures.

Introduction

Self-assembly of highly asymmetric amphiphilic block copolymers in selective solvents has been of paramount interest in our laboratories since 1994² but morphologies of amphiphilic species, such as small molecule surfactants, have attracted a great deal of interest for many decades.³ The underlying principle is that two fundamentally different sub-units of the amphiphile are driven to phase-separate like oil and water, with the limiting condition that they are covalently attached to each other. Molecules, and in our case, *macromolecules*, are therefore thermodynamically driven to self-assemble in order to minimize the contact between the immiscible components (the solvent and the insoluble block). Block copolymer self-assembly in solution has been a topic of active research for more than 30 years.⁴ A convenient term for the most commonly observed morphology is the star-micelle. 'Star' refers to the fundamental core-corona structure

where the core is small and the corona large; micelle reflects the dynamic equilibrium conditions under which these aggregates frequently form. Star micelles can be further divided into two types: regular and reverse. Regular micelles are formed in polar solvents such as water and alcohols; thus the corona has hydrophilic character. Micelles formed in apolar solvents such as toluene have a hydrophobic corona and are referred to as 'reverse'. Recently, we have devoted most of our attention to highly asymmetric block copolymers which form aggregates characterized by a large core and a thin coronal shell of the soluble block: the 'crew cut' systems.^{1,2,5-31}

Small molecule amphiphiles

Self-assembly of molecules is a rich field for scientific investigation and a great deal of groundwork has already been completed in the study of small molecule surfactants. Biologically, one of the most important surfactants is the phospholipid that makes up all cell walls. Without highly controlled self-assembly, our living cells could not exist. A typical small molecule surfactant such as sodium dodecyl sulfate (SDS) aggregates at concentrations in excess of 8 mM, the critical micelle concentration (cmc). The free energy of micellization in aqueous media has three components: a favourable hydrophobic component due to the association of the hydrocarbon tails, a surface term that takes into account the opposing requirements to both fully shield the hydrocarbon tails from the polar environment and the electrostatic repulsion inherent for the charged head groups and also a packing term that determines the morphology.³ Aggregation of surfactants has been described as a start-stop mechanism in that self-assembly occurs until all surfactant molecules are directly involved in the equilibrium morphology

whereupon only dynamic chain exchange occurs. Addition of further surfactant molecules induces further self-assembly of aggregates similar to the pre-existing ones.

Many morphologies have been observed for these small-molecule amphiphilic systems including simple micelles, rods, planar bilayers, inverted micelles, bicontinuous structures and vesicles. With increasing surfactant concentration, the morphology spectrum runs from micelles (L_1), through a cubic array of micelles (I_1), hexagonally packed rods (H_1), cubic bicontinuous spheres (V_1), to lamellae (L_3) where phase inversion takes place and the inverted morphologies develop: cubic (V_2), reversed hexagonal (H_2), cubic (I_2) and reversed micelle (L_2).^{32,33} Furthermore, for systems such as didodecylammonium bromide in oil, micro-emulsions characterized by interconnected tubes are observed.³ The addition of water causes tube dilation until the increased curvature induces droplet formation. Further water addition causes the droplets to grow until the system is no longer able to maintain curvature and phase separation occurs. Thus, the self-assembly of small molecule surfactants is characterized by relatively high cmc's, three contributions to the free energy of micellization, and dynamic morphology changes.

Diblock copolymers in the bulk

Diblock copolymers in the bulk also exhibit a spectrum of morphologies analogous to the series observed for small molecule surfactants. Much of the experimental work in this regard has been carried out on polystyrene-*b*-poly(isoprene) (PS-*b*-PI) and polystyrene-*b*-polybutadiene (PS-*b*-PBD). With these systems it has been possible to study phase separation in the strong segregation limit where $\chi N \gg 10$ (χ is the

Flory-Huggins segment-segment interaction parameter, and N is the degree of polymerization). As an example, with increasing volume fraction of PI, the morphological transitions go from body-centered cubic (BCC) spheres of PI in a PS matrix through hexagonally packed cylinders of PI in PS, gyroid (G), metastable hexagonally perforated layers (HPL)³⁴ and finally lamellae (LAM) form when the volume fractions of the two blocks approach equality. As the PI volume fraction increases further, phase inversion occurs and HPL, G, HEX and finally BCC morphologies are observed where PI forms the continuous phase. The full spectrum of morphologies is not observed for all polymers, as not only the volume fraction of each block, but also the total degree of polymerization plays a role.³⁵

Diblock copolymers at the air-water interface

Various morphologies are also observed in two dimensions for block copolymers at the air-water interface. Systems investigated include polystyrene-*b*-poly(4-vinyl pyridine.alkyl iodide) (PS-*b*-P4VP.RI)^{36,37} and the structures identified with changing relative block composition progressed from circles ('starfish') to ribbons and lamellae. Inverted circles (two dimensional nanofoams) were also observed for polystyrene-*b*-poly(sodium acrylate) (PS(90)-*b*-PANA(32)). The diblock copolymer is dissolved in an organic solvent which is then spread on a water surface in a Langmuir trough. Upon evaporation of the solvent, aggregation occurs. The 2D 'films' have been studied by X-ray and neutron scattering (SAXS, SANS),³⁸ as well as atomic force microscopy (AFM) and transmission electron microscopy (TEM).³⁹

Diblock copolymers in solution

Self-assembly of amphiphilic block copolymers in selective low molecular weight solvents has been studied now for many years. The principle of aggregation of the amphiphilic block copolymers is similar to that for small molecule amphiphiles in solution; however, the critical micelle concentration (cmc) is usually much lower in the case of block copolymers.⁶ The dynamics of the chain exchange between the micelles are also different for block copolymers and small molecule surfactants. Indeed, at equilibrium, the micelles exchange molecules with one another, and this exchange is fast in the case of small molecules. However, the molecular exchange in block copolymer systems is generally much slower, due to a number of factors including the high molecular weight, the low cmc, possible entanglement and low mobility of the chains in the core.¹⁴ For example, when the glass transition temperature (T_g) of the core-forming chains is very high (T_g of PS *ca.* 100 °C) and no plasticizer is present, the micelles are considered to be 'frozen' since the mobility of the core-forming chains is extremely low.

Depending on the relative block lengths, either star or crew-cut micelles are formed. A small core surrounded by a thick corona characterizes star micelles, whereas crew-cut micelles consist of a large core and a thin corona. The term crew-cut was originally proposed by Halperin, Tirrell and Lodge.⁴⁰ Two features enhance the difference between crew-cut and star micelles: the process of the formation of the micelles and the morphologies of the aggregates. Star micelles can often be prepared by direct dissolution of the block copolymer in a solvent selective for the long block. Thus the insoluble short blocks associate and form the core, and the soluble long blocks form the corona of the micelles. This process is usually impossible to apply to the formation

of crew-cut micelles since the insoluble blocks represent a very high fraction of the block copolymer. Instead of the direct dissolution process, the diblock copolymers are first dissolved in a solvent good for both blocks, and then a precipitant for the long block is added, resulting in the aggregation of these long blocks. The preparation of crew-cut micelles therefore involves a more delicate process and the resulting aggregates present a more complicated system than star-like micelles, since they involve solvent mixtures of variable composition. Star micelles are essentially spherical aggregates. Aggregates in the star regime other than small solid spheres have only rarely been observed and have not been studied as extensively.⁴¹⁻⁴⁸ Very recently, the groups of Winnik and Manners have developed a novel organometallic block copolymer of ferrocenyldimethylsilane and dimethyl siloxane (PFS-*b*-PDMS).⁴⁹ This copolymer self-assembles in hot hexane to form worm-like micelles with an iron-rich core.

Starting in 1995,¹ a wide range of different crew-cut morphologies has been observed among aggregates prepared from asymmetric amphiphilic block copolymer systems. The aggregation behavior shadows small molecule surfactants, where a long hydrophobic tail and a small polar head group generate a wide variety of morphologies in solution. For asymmetric amphiphilic diblock copolymers, the observed morphologies include, but are by no means limited to spheres, rods, bicontinuous structures, vesicles, lamellae, large compound micelles, large compound vesicles, tubules, 'eggshells', baroclinic tubules, porous spheres and multilamellar vesicles. Overall, we have identified on the order of thirty morphologies,^{1,5-31} but their taxonomy presents problems. Some of the morphologies are thermodynamically induced, in which case equilibrium trends are observed from simple spheres to inverted micelles; others are kinetically controlled and

their classification is more difficult. Where the mode of aggregation is primarily under thermodynamic control, we observe a spectrum of morphologies (as is also seen both in the bulk and in small molecule surfactant systems) from spheres through rods, bicontinuous rods, bilayers (lamellae and vesicles) to inverse rods and spheres as well as mixtures. Polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA), polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO) and polystyrene-*b*-poly(4-vinyl pyridine) (PS-*b*-P4VP) are the diblock copolymers that have received the most attention in our studies.

Others working in the field

More recently, other groups have also studied crew-cut morphologies of copolymers other than simple spheres, including the group of G. Liu who investigate, among others, polyisoprene-*b*-poly(2-cinnamoyl ethyl methacrylate) (PI-*b*-PCEMA) in THF with the addition of hexanes, a selective precipitant for PCEMA⁵⁰ They also prepare spherical micelles from polystyrene-*b*-poly(2-cinnamoyl ethyl methacrylate) (PS-*b*-PCEMA) in THF/cyclohexane or chloroform/cyclohexane mixtures.⁵¹ PCEMA-*b*-PAA has also been shown to give various morphologies, including nanofibres⁵² and hollow nanospheres, among others.⁵³ One particularly interesting characteristic of PCEMA is that it can be cross-linked, thereby adding stability to the aggregates. The group of Nose has also published examples of crew-cut aggregates.⁵⁴

Statement of purpose

In this review, the intent is to present and interpret the conditions leading to the formation of aggregates of the various morphologies of highly asymmetric amphiphilic diblock copolymers. The thermodynamic framework as well as parallels to small

molecule systems are considered. A detailed review of the various morphogenic parameters including block length, additives, temperature, solvent and block composition will be presented as a foundation for a survey of the solution morphologies we have observed. Parallels will be drawn between these aggregates and biological sub-cellular architectures.

Thermodynamic and experimental parameters

Three components of the free energy of aggregation

The morphology of self-assembled block copolymer aggregates is governed primarily by three components of the free energy of aggregation: i) core-chain stretching, ii) interfacial energy and iii) inter-coronal chain interactions.^{21,40} The effects of these parameters can be more easily understood using the following two examples: in the first, only the hydrophilic block length is varied; in the other, a polymer of constant composition is used in a binary solvent mixture of DMF and water, but we increase the water concentration.

Using a block copolymer with a constant polystyrene block length and starting with a relatively long PAA block, spherical micelles are observed initially. As we decrease the PAA length, the repulsive interactions between coronal block chains decrease, thus more chains can aggregate in a given micelle, leading to larger spheres. Some of the polystyrene blocks, however, must reach from the centre of the core to the core-corona interface and, in general, the larger the PS core, the greater the average degree of core-chain stretching beyond the unperturbed chain-length. When the

aggregates become very large, the entropic penalty of core-block stretching renders simple spheres unfavourable as a low energy morphology, and rods are formed with a decreased cross-sectional diameter. Further reduction of the PAA block leads to an increase in the rod diameter as the aggregation number increases due to reduced coronal block repulsion. Ultimately, in the same manner as for the simple sphere-rod transitions, the core-block stretching term will dictate a further morphological transition to the bilayer structures.

On the other hand, starting with a diblock copolymer of a particular composition in pure common solvent, as we increase the water concentration, we get self-assembly of spheres at some critical concentration (cwc). As the water content increases, the total interfacial energy increases, so the drive is to minimize the total interface surface area. In the limit, a single styrene sphere would minimize the interfacial energy, but the finite styrene block length precludes this option. As soon as the free energies associated with chain stretching and coronal terms exceed the interfacial energy term, a new rod-like morphology with a smaller radius becomes favourable. When the PAA block length exceeds some critical length, however, then the spherical micelles formed are thermodynamically 'stuck' and do not undergo the transition. In this case, the net surface area decrease does not compensate for the electrostatic repulsion implied by the long acrylic acid block. Where the PAA block is short enough, the rod to bilayer morphology change can be induced by more water addition.

If any of these free energy parameters is stressed, for example, by further water addition or reduction of the hydrophilic block length, morphological transition to inverted structures is possible. The PS stretching term and corona-chain repulsion term are

balanced by the interfacial term to give a comparatively smooth series of transitions in the total free energy of aggregation²⁸ (see Figure 1).

Preparation of the aggregates

Small molecule surfactants can usually be dissolved directly in a solvent that is good for either the hydrophobic or the hydrophilic component, and due to rapid molecule exchange, equilibrium morphologies are rapidly established (*c.f.* start-stop mechanism). This approach does not work easily for aqueous systems involving amphiphilic PS-*b*-PAA because of the extreme insolubility of polystyrene in water. As mentioned earlier, in an effort to achieve equilibrium conditions, dilute polymer solutions (1% w/w) in dimethyl formamide (DMF) are prepared and then de-ionized water is slowly added, thereby approaching the critical water concentration (cwc) where self-assembly begins. As the water concentration increases from the cwc, it is possible to trap the then-present morphologies since the kinetics of chain exchange are slow. The cwc is usually around 5% H₂O. At about 10% H₂O, the three dimensional architectures are considered to be 'frozen' and when the water content reaches 25% (w/w), the sample is dialyzed against distilled water to remove DMF.

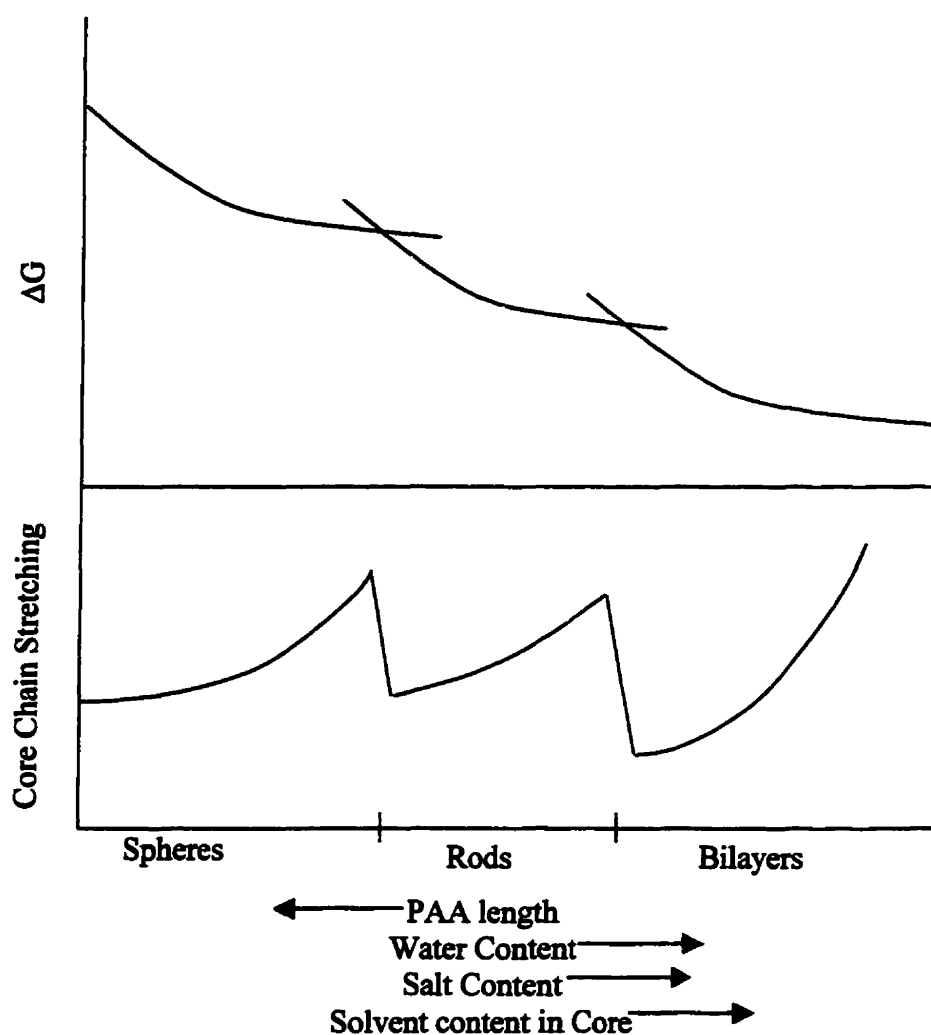


Figure 1: Schematic plot of relative free energies and degree of core-chain stretching to indicate morphological boundaries as a function of morphogenic parameters.

Environmental parameters

Having detailed the contributing factors to the free energy of aggregation, it follows that any parameter that perturbs the core stretching, coronal repulsion or

interfacial energy terms provides a window to morphological control. While the stability of the aggregates, once formed, is very high, the self-assembly of crew-cut PS-*b*-PAA is, in fact, extremely sensitive to the preparative conditions. We have therefore been able to probe the effects of parameter modification that directly affect one or more of the three contributions to the free energy of aggregation: precipitant addition, initial polymer concentration, additives (salt, acid, base and homopolymer), block composition, temperature and common solvent.

Water addition

Two methods of aggregate preparation are exploited for the DMF-water system. In the vast majority of our samples, aggregation is induced by the water addition method (WA) as described above. The direct dissolution method (DD) in DMF/Water mixtures has also been exploited where, for example, the PS-*b*-PAA is dissolved in a solvent mixture containing between 5.5 and 9.5 wt % water. Table 1 demonstrates the effect of WA from 0 to 7.5% as compared with direct dissolution into a binary solvent mixture at 7.5% water on the aggregate morphology.²⁶

Table 1. Major morphologies vs. water content as a function of preparative method and initial polymer concentration for PS(190)-*b*-PAA(20).²⁶

Pol. wt %	7.5 %Water content in DMF (w/w)	
	<i>DD</i>	<i>WA</i>
1.0	Long rods	Spheres
1.5	Long & Interconnected Rods	Spheres
2.5	Bilayers	Spheres
3.5	Bilayers	Rods, Vesicles & Spheres

These methods of sample preparation provide further insight to the thermodynamic and kinetic parameters of morphogenesis. At low water concentrations, the kinetics of exchange are relatively rapid and therefore it is the thermodynamically most stable morphology that will dominate. At higher water concentrations, the speed at which aggregation occurs or morphologies change controls the prevalent morphology to a much larger extent. The WA method certainly optimizes chain exchange, since during the onset of aggregation the non-solvent concentration is low, whereas the DD method is limited by the kinetics of chain exchange at a specific precipitant content. In both cases, the thermodynamics of aggregation appreciably control morphology at low water contents; however, due to the initially high water concentration for DD, rods are possible, for example, in a system that forms simple spheres using the WA method. On the whole, the aggregates formed via DD exhibit more complicated morphologies, whereas the morphologies observed via WA reflect the faster kinetics of chain exchange and the morphologies formed at lower water concentrations which freeze when the rate of water addition exceeds the dynamics of morphological transition. A possible mechanism for these method-dependent differences depends on the initial state of the polymer. For WA, the polymer is in a molecularly disperse solution of DMF, and aggregation is induced at a low water concentration. At higher water concentration, chain exchange is very slow and thus, despite the same final water concentration (e.g. 7.5% in Table 1), the exhibited morphology is often the simple sphere. For DD, polymer is added as a solid to a binary solvent mixture and swelling followed by agglomerate fission can lead to more complex morphologies that may better reflect thermodynamic equilibrium. In the limit of very

slow water addition or very long storage times for direct dissolution, the observed morphologies are observed to converge.

Initial polymer concentration

Table 1 not only highlights the effect of water concentration and the preparation method, but also indicates the strong dependence of morphology on the initial polymer concentration. As with most of the trends which we observe by progressively modifying the aggregation conditions, the morphological progression is from spheres to rods to vesicles and on to inverted and more complicated architectures with various intermediates. Thus, at low initial polymer concentration, simple spheres dominate; however, at higher polymer concentrations, more complicated morphologies become possible.²⁶

Additives

Water is not the only additive that can have a profound impact on morphology. Any reagent that affects the coil dimensions, inter- and intra- coronal block interactions, or solvent quality, can have an effect on aggregation morphology. The results from experiments involving acid, base, and various salts as well as homopolymer are described below. For the ionic additives, the absolute concentration of additive, though important, is not as interesting as the molar ratio, R , which relates the number of ions added to the number of coronal repeat units (in our case AA, 4VP etc.) present. The morphogenic effect of added ions has been investigated over a molar ratio range up to 4.0 (see Table

2).^{8,12} The morphogenic effects of salt have also been studied at millimolar ratios (mR) for PS(310)-*b*-P4VP(58). More extraordinary is that *micromolar* ratios (μ R) of acid and base induce morphological transitions for this system.²⁴

Table 2. Summary of the effect of salt or acid addition on the dominant morphology for PS(410)-*b*-PAA(25).^{8,12}

[Acid or salt] (mM)	R	Dominant Morphology
NaCl		
<2.1	<0.40	Spheres
3.2-5.3	0.60-1.0	Rods
5.3-16	1.0-3.0	Vesicles
21	4.0	LCV's
CaCl ₂		
<0.12	<0.023	Spheres
0.14-0.20	0.026-0.038	Vesicles
0.24-0.48	0.045-0.09	LCVs
HCl		
0.19	0.035	Spheres
0.21	0.040	Rods
0.24	0.045	Vesicles
0.27	0.050	LCVs
NaOH*		
0	0	Vesicles
0.028	0.005	Spheres

R = molar ratio (salt: acrylic acid groups)

* PS(410)-*b*-PAA(13)

Salt

The addition of salts induces electrostatic shielding along the partially ionized corona-forming block (for the polyelectrolytes). As the charges along the chain become shielded from one another, the coil dimension decreases thereby reducing steric and electrostatic repulsion among the chains. It is no great surprise then that for a given polymer (e.g. PS(410)-*b*-PAA(25)) the progressive addition of NaCl to a final R value of 4.0, while the system is dynamic, can be used to induce the formation of rods, vesicles or large compound vesicles from a suspension of spheres (see Table 2).^{8,12} By adding a divalent cation, (CaCl₂), bridging becomes possible and morphological transitions occur at even lower concentrations and R values (Table 2). PS(310)-*b*-P4VP(58) follows the same trend from spheres to bilayers on addition of simple electrolytes.²⁴

Acid

The addition of a protic acid is also a potent morphogenic device since protonation of any anionic acrylate pendant groups will be assured. Thus, protonation provides a valid mechanism for morphological transitions (see Table 2). At a molar ratio (R) less than 0.035 HCl per acrylic acid unit for PS(410)-*b*-PAA(25 or 13), only spheres are observed. By raising the molar ratio progressively to 0.050, rods, vesicles and LCVs are obtained.^{8,12} Amphoteric PS(310)-*b*-P4VP(58) undergoes a sphere-rod transition on the μ R addition of HCl. Further addition induces a rod-sphere transition due to increased charge density and continued addition reverses the trend direction again due to electrostatic shielding.²⁴

Base

The effect of the addition of a strong base is simply to enhance the charge density of the coronal block for PS-*b*-PAA due to deprotonation of the pendant acrylic acid. As charge density increases, coronal repulsion also increases, thus aggregation number and core diameter decrease and the morphological trend is reversed (Table 2).^{8,12} For the PS(310)-*b*-P4VP(58) system, the situation is more complicated. The micromolar ratio addition of NaOH causes a transition from spheres to rods, but the progressive addition increases charge density due to the abstraction of the α backbone hydrogen so the anticipated sequence of rods to spheres is observed. Still more NaOH leads to shielding and the trend is again reversed in the direction of spheres to bilayers.²⁴

Homopolymer

The addition of homopolymer to a molecularly disperse solution of PS-*b*-PAA can be used to induce minor variations in diameter within the spherical morphology regime or regime transitions from rods or vesicles to spheres (see Table 3).^{7,25} The effect of added homopolystyrene is to decrease the cwc due to the higher overall content of hydrophobic polymer, but the onset of rod formation is delayed since the additional homopolymer can volume-fill the core, thereby relieving the core-chain stretch. Experimentally, the block copolymer was dissolved in DMF along with homo-PS and then water was added in the standard way. It was necessary to use homo-PS samples that were shorter than the PS block of the copolymer in order to avoid premature precipitation of the homopolymer. As long as the copolymer and homopolymer self-assemble before the homo-PS precipitates, then the DMF swollen nascent cores solubilize the homo-PS chains and therefore, within the spherical regime, the core diameters increase. The

addition of homo-PS to samples that form aggregates other than solid spheres may induce spherical morphologies since it is primarily the resistance (and ultimately, inability) of the PS block to stretch and completely fill a sphere that leads to non-sphericity in the ‘native’ state (see Table 3).

Table 3. Effect of homo-PS addition on aggregation.⁷

PS- <i>b</i> -PAA (units)	Homo-PS Units	Weight ratio PS-PAA:PS	Morphology	Diameter or wall thickness (nm)
500- <i>b</i> -58	-	100:0	Sphere	30
500- <i>b</i> -58	180	90:10	Sphere	31
500- <i>b</i> -58	180	80:20	Sphere	36
500- <i>b</i> -58	180	70:30	Sphere	37
180- <i>b</i> -14	-	100:0	Rod	23
180- <i>b</i> -14	38	95:5	Sphere	27
200- <i>b</i> -8	-	100:0	Vesicle	18
200- <i>b</i> -8	38	95:5	Sphere	33

Block length (scaling relations)

As discussed within the context of the section on the three components of the force-balance, the block length of each block affects the morphology. Scaling relations have been determined that relate core radius (R_{core}), as well as the related surface area per chain (A_c) and the degree of PS stretching (S_c) within the core for crew-cut primary spheres.⁷ For example $R_{\text{core}} \propto N_{\text{PS}}^{0.4} N_{\text{PAA}}^{-0.15}$ (equation 1) shows that the radius increases with PS length due to the increased volume of PS in the core. With increasing PAA length, however, increasing coronal chain repulsion decreases the aggregation number,

therefore the aggregate size decreases. By varying just the PAA content of the polymer, many of the equilibrium-type morphologies can be induced (see Table 4).⁷

Table 4. Diblock composition and morphology.⁷

PS- <i>b</i> -PAA (units)	PAA (mol %)	Morphology
170- <i>b</i> -33	16.3	Sphere
180- <i>b</i> -28	13.5	Sphere
180- <i>b</i> -14	7.2	Rod
410- <i>b</i> -20	4.7	Vesicle
410- <i>b</i> -16	3.8	Lamella
200- <i>b</i> -4	2.0	LCM

Temperature

The single solvent preparation of aggregates from highly asymmetric diblock copolymers is very desirable not only from the perspective of potential industrial applications, but also as a means of simplifying the system in order to control it better. A non-toxic single solvent would be even more desirable if the applications were to have pharmacological leanings. For PS-*b*-PAA, where the PS block is very much longer than the PAA block, direct preparation in water, even at 200 °C and elevated pressure, has proven impossible. However, in less polar low alkanols (methanol, ethanol, *sec*-propanol and *n*-butanol), elevated temperatures are sufficient to permit at least chain transfer from the bulk to nano and mesoscopic aggregates including spheres, vesicles, tubules and interconnected vesicles. Given the monodisperse spheres of diameter ~25nm observed for PS(386)-*b*-PAA(79) formed on cooling in *n*-butanol (0.07 wt %, 170 °C), we infer that while at 170 °C, the complete dissolution of the polymer may have been achieved in

this solvent, or at least that the kinetics were fast enough for morphogenesis.²³

Essentially, this method mimics the effect of water addition to a copolymer solution in a common solvent since solubility decreases both as the water content increases for binary solvent mixtures and as the temperature decreases for our polymers in single solvent systems.

Common Solvent

The role of solvent is crucial to all aspects of the free energy of aggregation.

Polymer-solvent interactions determine the coil dimension for each block of a diblock copolymer, which, in turn, has a profound impact on the aggregate dimensions and modes of assembly.

Table 5. Solvent and polymer parameters.⁵⁵

Material	Solubility parameter δ	Dielectric constant ϵ
DMF	24.8	38.2
Dioxane	20.5	2.2
THF	18.6	7.5
Water	47.9	80.1
Styrene	19.0	
Acrylic acid	24.6	

Dissolution of polymers is optimal where the δ values for polymer and solvent are similar (see Table 5).⁵⁵ As these values diverge, the polymer chain dimensions decrease. High dielectric constant (ϵ) values improve the solubility of the corona block. The choice of common solvent therefore permits a qualitative exploration of the principal terms of

the free energy of aggregation since the common solvent directly affects both the core and corona chain dimensions, and the dielectric constant of the non-solvent affects the coronal chain dimension as well as the interfacial energy.

Solvents common for both blocks in the PS-*b*-PAA system other than DMF and low alkanols have also been investigated. The core dimension of a nascent aggregate during the early stages of water addition is highly dependent on the polymer-solvent interaction parameters. Where the common solvent is better for the core forming block, during the addition of water the partition of organic solvent will dictate that the core be more swollen than is the case for DMF. Due to the swelling, more complex morphologies are possible. Some examples of the available morphologies from PS-*b*-PAA copolymers using DMF, dioxane or THF as a common solvent are given in Table 6. Using just one polymer that exhibits different morphologies in different solvents, any of the intermediate morphologies is made accessible through solvent mixtures.¹⁸

Table 6. Morphology of PS-*b*-PAA from different common solvents.¹⁸

Polymer	PAA content (Mol %)	Common Solvent		
		DMF	Dioxane	THF
PS(168)- <i>b</i> -PAA(59)	26.0	Sphere	Sphere	Sphere
PS(490)- <i>b</i> -PAA(87)	15.1	Sphere	Rod	Sphere
PS(500)- <i>b</i> -PAA(58)	10.4	Sphere	Vesicle	Vesicle
PS(420)- <i>b</i> -PAA(26)	5.8	Sphere	Vesicle	LCM

Other block copolymers

Most of the variable parameters discussed above have been presented in the context of PS-*b*-PAA; however, other asymmetric diblock copolymer systems have also been investigated in our laboratories albeit less extensively (see Table 7). The hydrophilic block characteristics change significantly for the different samples; nevertheless, the underlying principles for the self-assembly of asymmetric amphiphilic diblock copolymers are universal. Due to narrow morphological boundaries in their respective phase diagrams, as seen for the polymer bulk morphologies described earlier, it may be that some morphologies are not observable for practical reasons. Some morphologies may not be stable at all for some systems under the conditions of the experiment.

The PS-based systems have a glassy styrene phase at room temperature in water. The other core-forming blocks, poly(caprolactone) (PCL) and poly(butadiene) (PBD), have T_g 's of about $-65\text{ }^{\circ}\text{C}$,⁵⁶ and -90 to $-8\text{ }^{\circ}\text{C}$,¹⁴ respectively. Polyelectrolytic coronae can be formed for PAA and derivatives of poly(4-vinyl pyridine) (P4VP). Uncharged coronae arise from PEO systems. The PS-PMMA-PAA triblock system²⁰ permitted a preliminary investigation of the effect of a second hydrophobic block on the cwc and subsequent morphologies. Despite the incomplete investigation to date for some of the systems described in Table 7, it is clear that the morphology development for all of these highly asymmetric amphiphilic diblock copolymers follows the same rules. By varying the parameters discussed in some detail above, each system has been induced to form a range of morphologies and each adds to our understanding of micro-phase separation and aggregation for this particularly rich class of macromolecule.

Table 7. Morphologies observed to date for our various crew-cut systems.^{1,5-31}

System	Observed morphologies
PS- <i>b</i> -PAA	S, R, V, L, LCM, LCV, T, other
PS- <i>b</i> -PEO	S, R, V, L, LCM, LCV, other
PS- <i>b</i> -PVP.MeI	S, R, V, L, LCM
PS-PMMA-PAA	S, R, V
PBD- <i>b</i> -PAA	S*, R, V, other
PCL- <i>b</i> -PEO	Large aggregates d~0.5 μ m.

Key: S, sphere; R, rod; V, vesicle; L, lamellae; LCM, large compound micelle; LCV, large compound vesicle; T, tubule; other = more complex structures

* near sphere

Phase diagram and kinetics studies

Summarizing multidimensional phase behavior is a complicated undertaking; however, we have begun the construction of a ternary phase diagram as a function of water, dioxane, and the polymer PS(310)-*b*-PAA(52).²⁸ Given the detailed information on the necessary conditions for the formation of the various equilibrium and near-equilibrium morphologies presented below, it becomes feasible to design systems for desired properties based on phase diagrams.

A further consideration is the speed at which aggregates can be formed. Early studies started with two populations of solid spheres formed from two different PS-*b*-PAA samples and followed the time scale over which they formed a single population when mixed.^{13,57} More recently, attention has been devoted to the formation of complex

inverted structures (HHH and TWV, discussed below) from vesicles^{17,22,27} and bilayers from rods.²⁹

Equilibrium and Near-Equilibrium Morphologies

As a consequence of the kinetics and thermodynamics studies, most aggregates can now be sorted into equilibrium, near-equilibrium and metastable morphologies. Equilibrium studies have positively identified spheres, rods, and vesicles as well as wide boundary regions between each morphology where, for example, spheres and rods coexist at equilibrium.²⁸ Furthermore, in a similar fashion to small molecule surfactants and also diblock copolymers in the bulk, a phase inversion occurs in the spectrum of morphologies. In a limited sense to be discussed further, the inverted equilibrium morphologies include hexagonally packed hollow hoops (HHHs) and large compound micelles (LCMs). As observed under the electron microscope, these aggregates represent morphologies at or near equilibrium that were quenched and isolated. Equilibrium and near equilibrium morphologies (Figures 2 to 5) are described below. A discussion of non-equilibrium morphologies (Figure 6) follows. Some morphologies presented are paradigms from the polymer literature, and some are novel for polymers (*e.g.* tubules – see below). Still others are novel in their own right, and to our knowledge are without precedent in the self-assembly literature (*e.g.*, pincushions – see below).

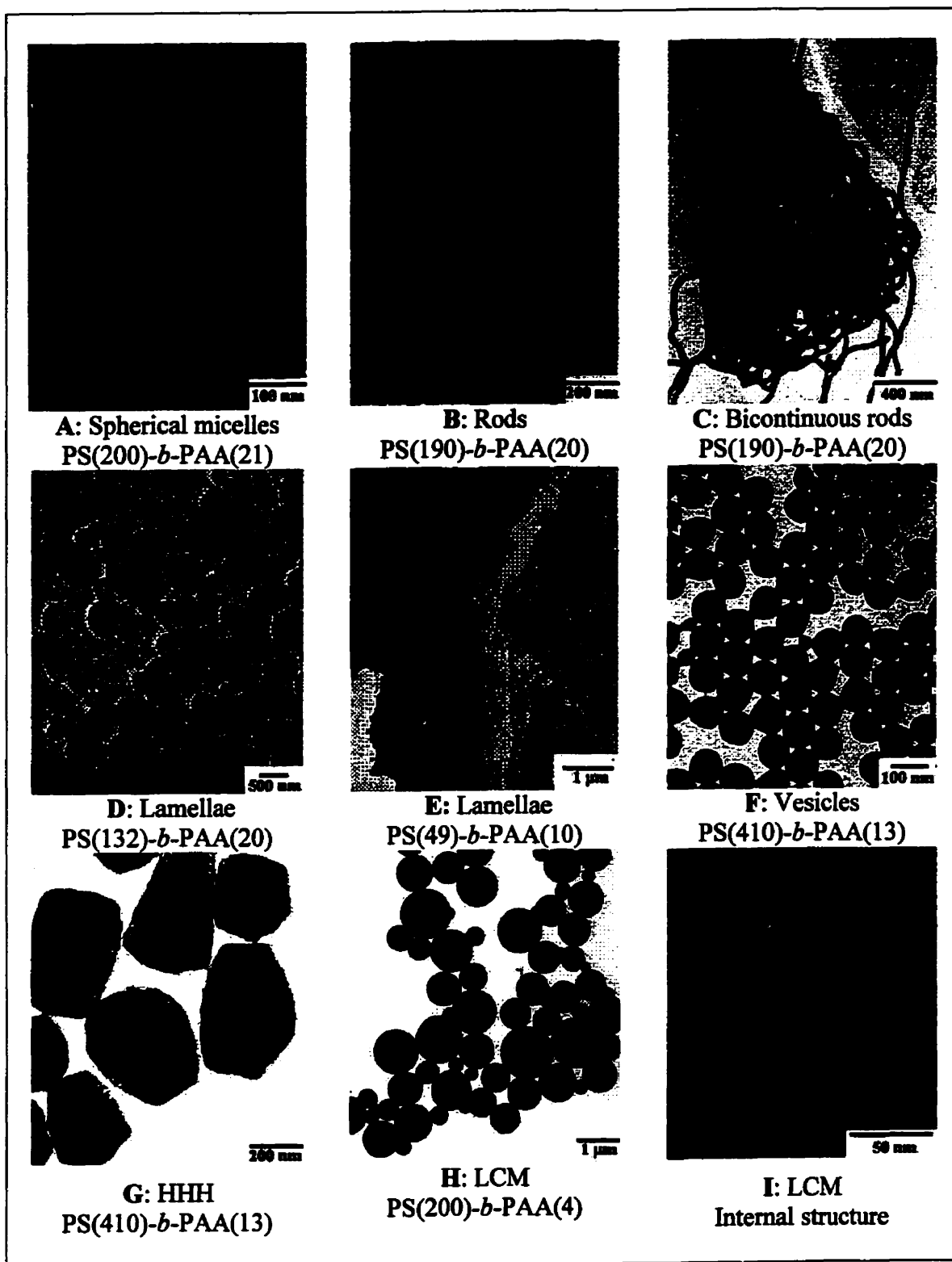


Figure 2: Transmission electron micrographs (TEM) of “pure” morphologies: the degree of polymerization for each block is given in parentheses.

Equilibrium

Simple spheres, shown schematically in Figure 3, of PS-*b*-PAA block copolymer formed in an aqueous environment, consist of a PS core surrounded by PAA coronal chains.^{1,2} Usually, PS-*b*-PAA block copolymers with a relatively high PAA content (> 10 mol%) form simple spheres. The polydispersity of such micelles is low ($2\sigma_r/d < 5\%$)[†], and the size of the spheres is affected by a number of factors, such as the block lengths, addition of salts (HCl, NaCl, CaCl₂) or homopolystyrene to the solution. An obvious restriction for simple spheres is that the total hydrophobic block length in its planar zigzag configuration must not exceed the radius of the core as some chains must reach the center of the aggregate. The entropic penalty of chain stretching dictates, however, that well before the chains become fully extended, a morphological transition is induced. The simple sphere is the parent morphology for all crew cut aggregates, since under our classical preparative conditions, it is usually the first aggregate to form. We take this morphology as the most basic example of the effect of the force balance equation. The subsequent morphologies are explained as a function of their deviation from this standard where the core-chain stretching, core-corona interfacial energy and the coronal chain repulsive forces are optimized with respect to the lowest accessible free energy of aggregation available (*c.f.* Figure 1). Figure 2A demonstrates the typically low polydispersity of spherical crew-cut micelles formed, for example, from PS(200)-*b*-PAA(21).

[†] σ_r : radial standard deviation; d: diameter of the sphere

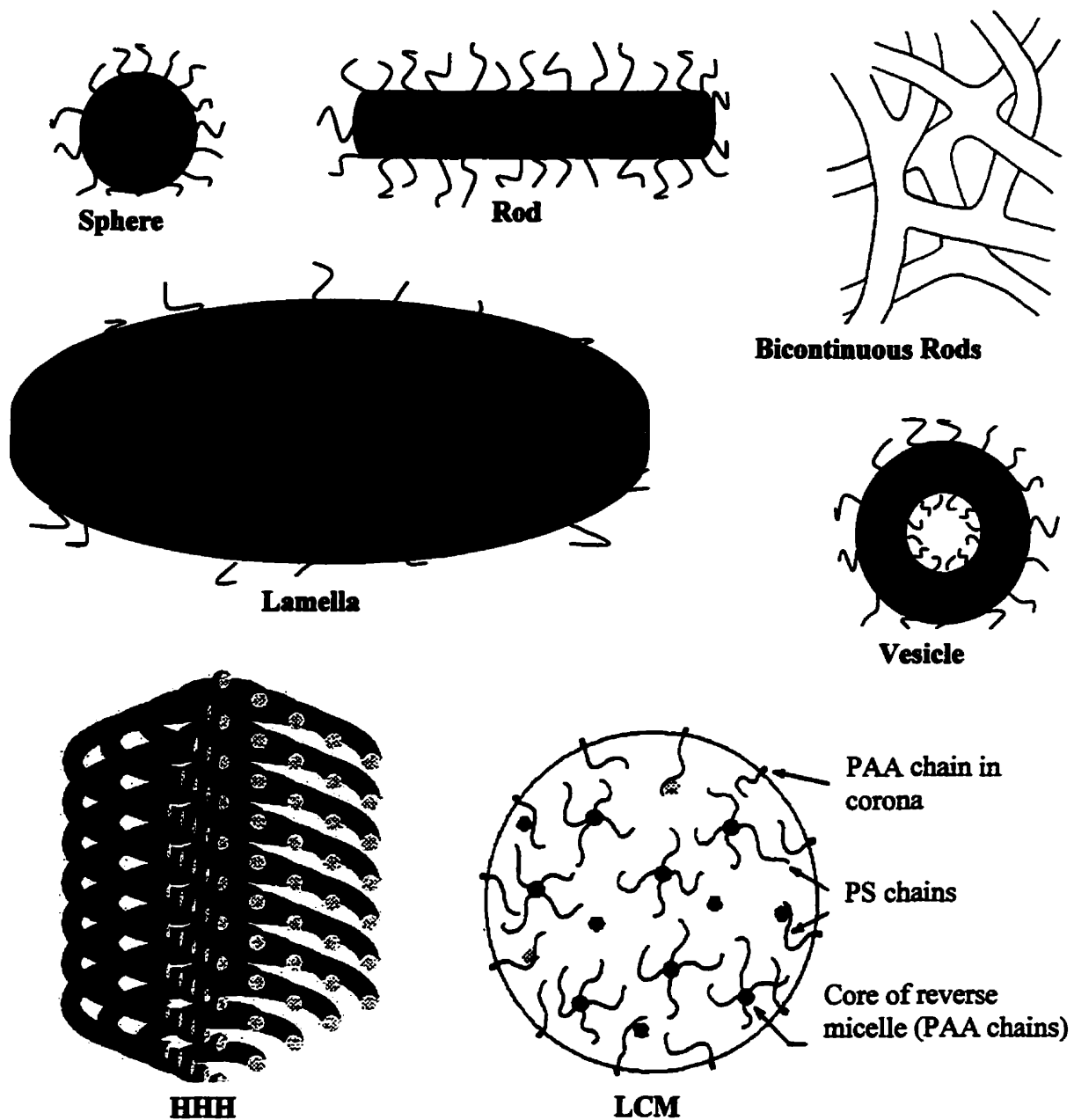


Figure 3: Schematic drawings of equilibrium morphologies. Where convenient, coronal chains are indicated; core-forming chains are symbolically represented for the sphere. The surface for HHH represents the interface between hydrophilic and hydrophobic domains. Thus, for PS-*b*-PAA, these hoops contain water-swollen PAA and the whole structure is embedded in a PS matrix.

Rods^{1,7,21} as sketched in Figure 3, need little description. They are characterized by diameters of the same order of magnitude as those of primary micelles (*ca.* 30 nm)

but can be of widely variable length that can exceed 10 μm (Figure 2B). It should be noted that the energetic contributions to the free energy of aggregation still include core-chain stretching which, at the transition, is decreased slightly relative to the simple sphere, but generally is comparable since the diameters are comparable, and also corona-chain repulsion, which experiences a net decrease due to a decrease in the chain length or due to the addition of simple electrolytes. While the radii of spheres and rods are comparable, the greater length of the rods means that the total aggregate volume increases, thereby reducing the net interfacial energy per chain between the core and the solvent. A further contribution is the end-capping energy arising from the high curvature of the hemispherical caps at rod ends. Under a wide range of conditions, this term is not especially significant although hooped rods ('doughnuts') have been observed. For example, in the case of some of the more complex aggregates presented later, the end-capping energy penalty is sufficiently large that hoops are formed despite the extra curvature energy.

The **bicontinuous rod** morphology (Figure 2C) consists of networks of interconnected branched rods in three dimensions. The diameters of the rods are monodisperse, and the origin of the branch points is the same as for the bicontinuous morphology in the bulk. Disordered bicontinuous rods are schematically represented in Figure 3. We have observed not only disordered bicontinuous morphologies,²⁵ but also highly ordered examples.³¹ It is not especially surprising that we find a range of morphologies for asymmetric diblock copolymers in solution. Detailed equilibrium

studies have confirmed the status of the simpler solution morphologies; however, due to the fact that this morphology is not seen for all samples and, furthermore, where it is seen, it is only over a very narrow range of parameters, we are unable to categorically assign equilibrium status. It is likely that the highly ordered bicontinuous samples better represent equilibrium.

Bilayers (Figures 2D-F) represent the next step in the arrangement of diblock copolymers as block or environmental parameters change. As shown schematically in Figure 3, **lamellae** (Figures 2D, 2E) and **vesicles** (Figure 2F) are characterized by flat or curved bilayer ‘walls’ of polymer. It should be noted that under some preparative conditions, lamellae appear in vesicular suspensions, but under other conditions, pure lamellae can be prepared.²⁹ Figure 2D demonstrates isolated lamellae, whereas Figure 2E shows the superposition of much larger lamellae, in which the different shades of grey represent different numbers of lamellar layers. Vesicles are a particularly versatile morphology as they can be prepared from many polymers. Small, nearly monodisperse vesicles are often formed, but larger, more polydisperse aggregates can also be obtained.

It is highly possible that the **inverse bicontinuous rod** morphology exists under certain equilibrium conditions. To date we have had only indications of its existence in our studies of the transition from vesicles to the HHH morphology; however, we have not been able to identify it positively. This morphology is the phase-inverted counterpart of the bicontinuous rod described above.¹⁷

The preparation of **hexagonally packed hollow hoop** structures (HHH) (Figure 2G) involves first the formation of small vesicles of PS-*b*-PAA by addition of water; subsequent addition of NaCl ions to the solution of vesicles leads to the assembly and

rearrangement of the vesicles into HHH aggregates.¹⁷ The HHH structure of the aggregates is depicted in a computer visualization (Figure 3), and consists of an arrangement of multiple ‘hollow’ concentric rings in a hexagonal pattern. The external surface of the aggregates, as well as the internal surface of the hollow hoops consists of short PAA chains. The hoops are embedded in a PS matrix.

The addition of NaCl to the single vesicles allows for the decrease of the repulsion between them since NaCl increases the electrostatic screening among the PAA chains, thus reducing the electrostatic repulsion. The vesicles, therefore, come into contact and form large compound vesicles (LCVs). Minimization of the surface free energy and that of chain stretching lead to the formation of HHH aggregates. The end-capping energy of a rod is replaced by a mesoscopic curvature energy on this size scale (*i.e.*, several hundreds of nanometers), imposing the hoop morphology. Indeed, there are two ways of packing rods in a cylindrical space: one consists of a hexagonal packing of straight rods parallel to the direction of the principal axis, in which case there is an end-capping energy penalty, resulting from the formation of hemispherical caps at the end of the rods. The other way avoids the end-capping energy penalty by forming hoops, but involves a curvature energy penalty. In the present case, for particle sizes less than 1 μm , the end-capping energy is more important than the curvature energy, and hollow hoops are formed in a hexagonal pattern. This morphology may represent equilibrium as long as the particle is isolated, in analogy to the thermodynamic aspects of the vapor pressure of a small drop. Near a large surface, a small drop is unstable due to its higher vapor pressure, however, an isolated small drop can be considered to be at equilibrium.

Secondary copolymer aggregates present a further solution to the problem of how to arrange a large, insoluble block where the solubilizing block is very short. **Large compound micelles (LCMs)**, illustrated in Figure 3, bear a strong resemblance to bulk reverse micelles. For LCMs, islands of PAA chains are found in a continuous phase of core-forming polymer (PS) all of which is surrounded by the solvent-soluble PAA corona.¹

It should be noted that LCMs are highly polydisperse (Figure 2H), and that they tend to settle due to gravity because of their large size. Figure 2I represents the internal morphology of an LCM obtained by microtoming a sample embedded in epoxy resin. The 60 nm sections were stained with CsOH(aq) and rinsed with deionized water to remove the excess CsOH. Staining increases the contrast between the PAA and PS regions. The elongated shape of the spheres is most likely due to the strong shear force when microtoming.⁷

Aggregates of spheres

HHH is an excellent example of the end result of a morphological rearrangement in that vesicles are induced to form inverse rods on addition of salt. Another type of rearrangement involves coronal and core-chain interactions for simple spheres which, on addition of acid, yields predominantly one-dimensional ‘**pearl necklaces**’ (Figure 4A) in transition from spheres to rods.⁸ Drying conditions can yield **2D sheets of micelles** (Figure 4B includes sheet folding) which might conceivably be precursors to lamellae;⁵⁸ when drying is performed at elevated temperatures (60-80 °C), crack patterns develop which give very large three-dimensional **birefringent needles** (Figure 4C), which re-suspend in water to give simple spheres if the PAA block is sufficiently long.¹

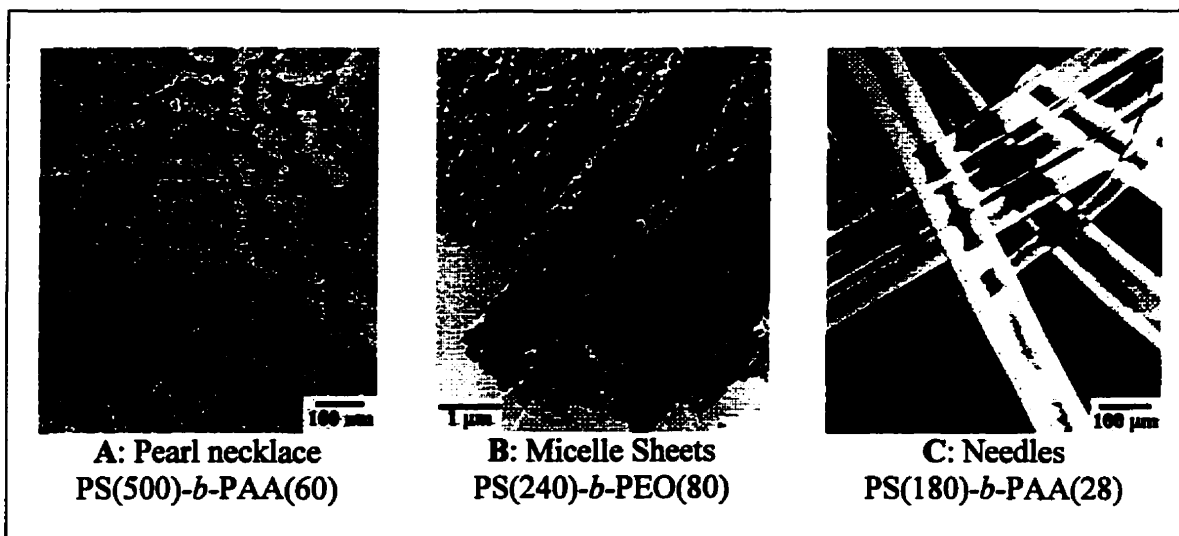


Figure 4: Primary spheres induced to rearrange into necklaces (1-dimensional), sheets (2-dimensional); and even mesoscopic, birefringent needles (3-dimensional).

Near equilibrium

We have identified several other discrete morphologies that structurally resemble pure equilibrium morphologies. We are unable to unequivocally classify them; however, it seems clear that the aggregates are at least near-equilibrium.

Large rod-shaped compound micelles (LRCMs) have been observed (Figure 5A); we believe their internal structure to be identical to that of large compound micelles. Their lengths can reach several micrometers, and their diameters, several hundreds of nanometers.¹¹

Branched short rods (Figure 5B) may be the result of a situation in which the branching energy is comparable to the end-capping energy. Short branched rods may represent an intermediate structure between rods and bicontinuous rods.¹¹

The hexagonally perforated bilayer morphology obtained in the case of small molecule amphiphiles, where the pores are hexagonally arranged throughout a bilayer of

molecules, has not yet been observed for asymmetric diblock copolymers in solution.

However, we commonly isolated **disordered perforated bilayers** (Figure 5C).²⁶



Figure 5: Near-equilibrium morphologies of asymmetric block copolymers in solution.

The **eggshell** morphology (Figure 5D) consists of a bent lamella, and therefore could possibly be an intermediate structure between a lamella and a vesicle.¹⁹

Tubules (Figure 5E) were rarely isolated with PS-*b*-PAA block copolymers except in low polarity solvents,^{18,23} but were observed very often with non-ionizable PS-*b*-PEO block copolymers.¹⁹ The wall thickness of the tubules is typically *ca.* 20 nm and their outside diameter is *ca.* 100 nm. The length of the tubules can reach many micrometers. The difference between rods and tubules is that the latter are hollow. Tubules therefore belong to the family of bilayers; however, it is not yet clear whether they are true equilibrium morphologies.

Branched tubules (Figure 5F) most probably occur when the free energy of the branching points is energetically comparable to the linear mid-sections. This morphology arises when there is no appreciable penalty associated with branching. The diameters of branched tubules are similar to the diameters of the single tubules.¹⁹

The ‘**bagel**’ structure (Figure 5G) is an example of many three-dimensional bilayer oddities.¹⁹ It can be described as a tubule in which the two ends are connected. This structure was occasionally observed among other bilayer structures for PS-*b*-PEO block copolymers, including structures with several holes that can be considered hollow ‘pretzels’.

Baroclinic tubes (Figure 5H) can be considered to be strings of attached vesicles, and are possibly an intermediary state between tubules and vesicles. This morphology was isolated for several polymers under radically different conditions.^{19,23}

Bicontinuous bilayers (called ‘plumber’s nightmare’ in small molecule systems) are three-dimensional arrangements of bicontinuous tubes.^{11,19} As in Figure 5I, they are

observed along with vesicles, which may be the building units for such complicated structures.

Structural parallels

There are many parallels to be drawn between the 'solid' and bilayer structures. The theme of morphological transition seems universal among small molecule amphiphiles, copolymers in the bulk, copolymers at the air-water interface and copolymers in solution. Similarly there is an obvious parallel to be drawn between the sequence of spheres, pearl necklaces, rods, 'doughnuts' and bicontinuous rods, and the sequence of vesicles, baroclinic tubules, tubules, hollow doughnuts (or 'bagels') and bicontinuous bilayers within the family of copolymers in solution. The formal similarity of the block copolymer bicontinuous architectures to small molecule amphiphilic bicontinuous cubic phases³ is also noteworthy.

Non-equilibrium

The equilibrium and near equilibrium morphologies previously discussed and demonstrated both schematically and in TEM images represent only a fraction of the aggregates we observe. We also observe kinetically trapped morphologies, metastable morphologies and also various combinations of otherwise stable morphologies. The classification of some of the observed structures into one of these groups is by no means trivial. Furthermore, the definition of each distinct morphology is, in some cases, quite arbitrary, and these morphologies remain open to interpretation and further investigation.

Large compound vesicles, LCVs,^{8,12} some of which resemble compartmented spheres or aggregated soap bubbles (Figures 6A, 6B) are perhaps among the best examples of a metastable morphology where clear classification is difficult. The inset in Figure 6B is a schematic representation of the internal bilayer structure. Despite the fact that we can choose a polymer and conditions to create a suspension solely of LCVs, the polydispersity remains quite high and the substructure is highly irregular. Essentially spherical LCVs as well as LCVs with clearly protruding vesicular structures have been observed. It is tempting to conclude that this morphology is in transition. PS(410)-*b*-PAA(13) at 1% initial concentration (w/w) in DMF forms spacious LCVs on the addition of water and very dilute HCl (225 μ M). By increasing the HCl concentration to 253 μ M, the LCVs formed are much more dense with smaller cavities. As the cavities get smaller, the aggregate begins to resemble more and more the inverse bicontinuous rod or even HHH morphology.

Porous spheres (Figure 6C) are morphologically between LCMs and LCVs though where exactly they fit in the continuum remains to be seen. The continuous phase is hydrophobic and there are large cavities within each aggregate which suggests a 'Swiss cheese' analogy. These aggregates form not only from PS-*b*-PEO,¹¹ but also from PS-*b*-PAA with additional homo-PS.²⁵

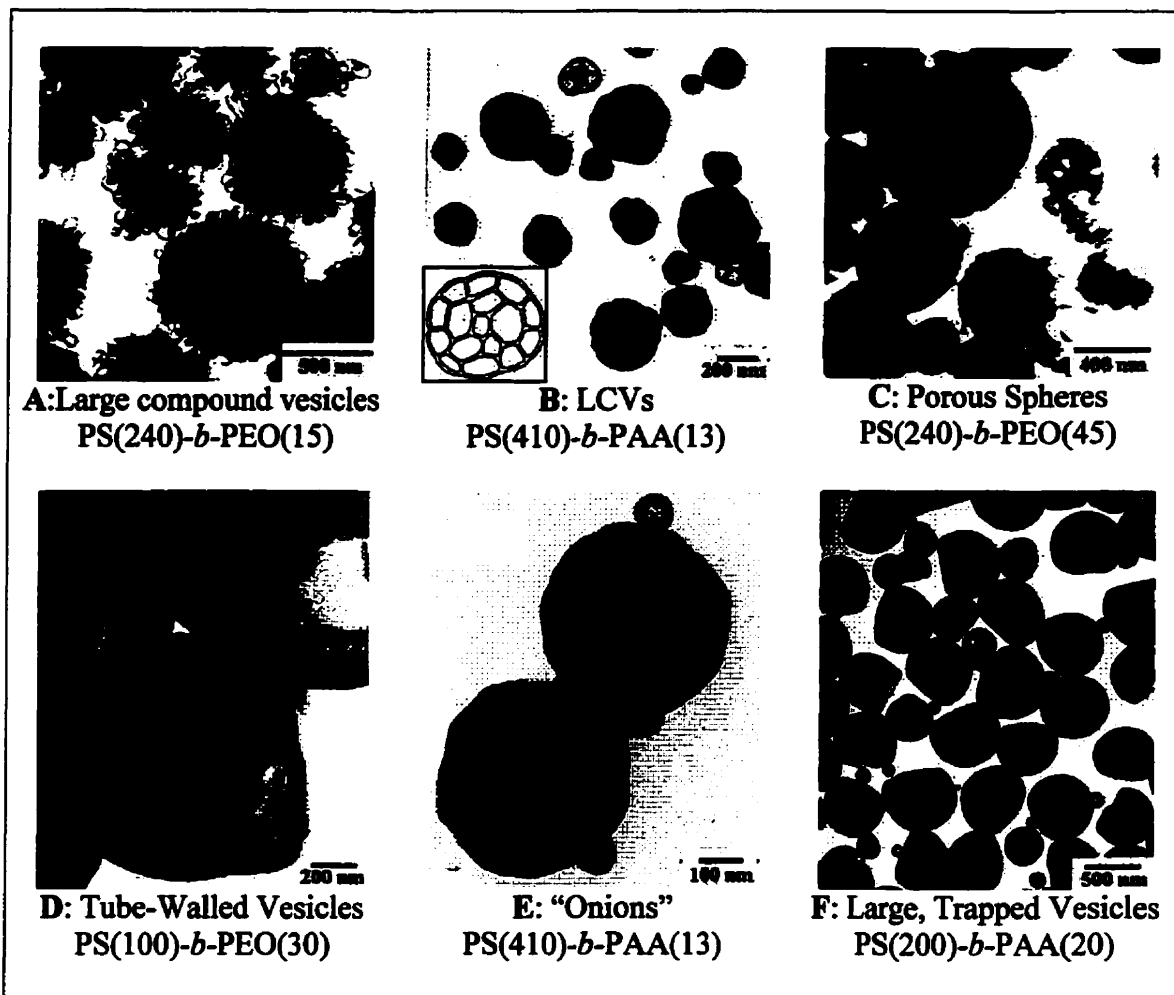


Figure 6: Non-equilibrium trapped morphologies.

Tube-Walled Vesicles (TWVs), seen in Figure 6D, are an interesting hybrid of bilayers and inverse rods.^{22,27} Although vesicular, the walls are also constructed of hollow tubes. We have many examples of aggregates that follow this motif and believe these aggregates are formed in transition from vesicles to HHH, where for steric reasons the vesicles do not fuse but rather the wall thickens and hollow tubes develop.

'Onions'⁵⁹ and **entrapped large vesicles**¹⁹ (Figures 6E, 6F) are variations on a theme. Multi-lamellar vesicles have been observed in at least two of our polymer systems and are likely possible in the rest. TEM shadowing experiments confirm the sphericity of the 'onions' that for PS(410)-b-PAA(13) are formed from a suspension of

vesicles in DMF with 5% water on the addition of salt. Onions have also been observed from PS(260)-*b*-P(4VP,DecI) prepared from a dilute solution in THF by simply adding water drop-wise to a final concentration of 50% (w/w) followed by dialysis.

Interestingly, there is a precedent for diblock copolymer ‘onions’ where symmetric diblock copolymers in the bulk have been shown to form multilamellar spheres when a polymer solution is sprayed, giving small drops that are subsequently dried.⁶⁰

An important feature of the large and trapped (‘pregnant’) vesicles is their size. While nearly monodisperse small vesicles (Figure 2F) are often possible for asymmetrical diblock copolymers, under other conditions, we also observe very much larger vesicles that are usually quite polydisperse. Each large vesicle appears to have an indentation; however, this is an artifact of sample preparation since each TEM grid is subjected to and released from high vacuum in shadowing and there is plenty of opportunity for an imbalance of internal and external pressure.

Some samples under certain preparative conditions give **mixed morphologies**. Careful phase diagram work³⁰ demonstrates appreciably wide equilibrium co-existence regions between the pure sphere and pure rod as well as between the pure rod and pure bilayer regimes. Figures 7A and 7B are representative examples of such mixed morphologies.

Combined morphologies should be distinguished from the transitional morphologies such as TWVs in that for true combination, simple morphologies are fused. The structural elements of LCMs and rods combine to give a variety of ‘pincushions’ (Figures 7C and 7D) where the ‘pins’ can be long or short.¹¹ Rods and lamellae fuse to

give ‘many-armed starfish’ (Figure 7E) and vesicles and rods are also occasionally joined (Figure 7F).

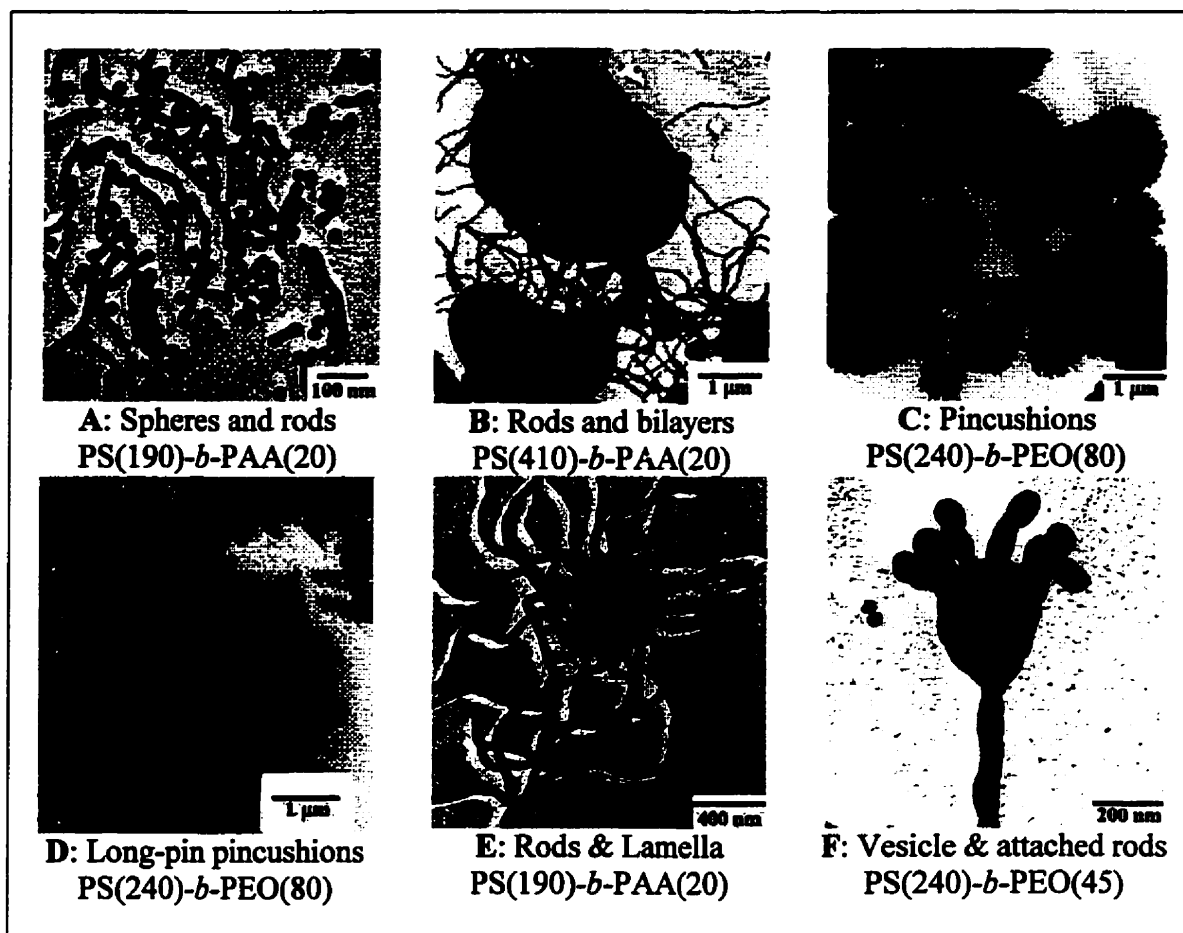


Figure 7: Mixed and combined morphologies.

Structurally Biomimetic Morphologies

It is interesting to note that many of these architectures and their combinations superficially resemble much more complicated biological and cellular systems. Consider the following pairs: lamellae and cell walls, tubules and cellular microtubules, rods and biological filaments, nucleoli and short branched rods or small pincushions, the smooth endoplasmic reticulum and the bicontinuous rod and finally microvilli vs. the pincushion.

As with lipid-based biosystems, we observe budding transitions at the fusion points for combined morphologies of copolymer aggregates.¹⁹ A further point of similarity is the extreme versatility of both biolipids and asymmetric amphiphilic diblock copolymers. In summary, although the biomimetic analogy cannot be taken very far, what remains true is that nature chooses low-energy biological structures and we observe low-energy architectures that, with small changes in environment, are subject to morphogenesis.

Conclusions

A morphological wonderland is made possible by asymmetric copolymers that mimic, on a macromolecular level, highly asymmetric small molecule amphiphiles. The critical components of the free energy of aggregation are the core-chain stretching, the coronal chain repulsion and the interfacial energy. Any parameter that can be induced to modify these terms has a morphogenic effect, and due to the slow diffusion kinetics of macromolecules, we have enormous control over their ultimate aggregated morphology. Furthermore, slow diffusion/displacement kinetics lend themselves to detailed study of morphological transitions better than the kinetics of small molecules, which are fast. The range of equilibrium morphologies is broad and follows, in a sense, the same sort of sequence as observed for small molecule systems as well as diblock copolymers in the bulk. The many near-equilibrium, non-equilibrium and combined morphologies make unequivocal classification difficult and, furthermore, the definitions of the non-equilibrium morphologies are sometimes arbitrary.

What is a taxonomical nightmare, however, is an engineer's dream as the various morphologies formed by asymmetric diblock copolymers in solution have many potential

applications. We are actively pursuing the use of our systems as drug-delivery vehicles,⁶¹ but other useful roles include nanoconductors⁴⁹ and insulators, nano-filters, timed and chemically induced release capsules⁵³ as well as nano-reactors^{62,63} for this rich morphological field.

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Chapter 3

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Chapter 3: Polymerization and post-polymerization functionalization of block copolymers

Abstract

The synthesis of polystyrene-*b*-poly(*N,N,N*-trimethylammoniummethylene acrylamide chloride) (PS-*b*-PTMEACl) was accomplished employing both polystyrene-*b*-poly(*tert*-butyl acrylate) (PS-*b*-PtBuA) and its hydrolyzed derivative, polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) as starting materials and coupling them with *N,N*-dimethylethylenediamine (DMED). The various intermediates we examined include aluminum amides, acid chlorides, and imides derived from carbodiimides. We present below our investigation of several synthetic routes and conclude that the carbodiimide coupling of PS-*b*-PAA with DMED followed by quaternization and counter-ion exchange was the most effective method of achieving our target. A brief discussion of the merits of each procedure in the context of block copolymers is given and IR spectroscopic evidence for the post-polymerization synthesis of the poly(acrylamide) block is provided.

Introduction

By definition, bile salt sequestrants are asystemic, *i.e.*, they are ingested, pass through the gastrointestinal (GI) tract and are ultimately eliminated. The currently used macroscopic resins suffer from poor patient compliance due to intestinal discomfort because of the bead size, bead activity, and concomitant high dosage requirements. Small molecules are incapable of sequestering bile salts against the active transport

enterohepatic recirculation mechanisms and furthermore may cross the intestinal wall thereby becoming *systemic*. Self-assembling nano-scale architectures are attractive as potential bile salt sequestrants because they are large enough to remain in the GI tract, but small enough that dosage and side effects may be minimized. Our criteria in choosing the diblock system for study were as follows:

1. the copolymer should be amphiphilic for self-assembly to take place leading to water-compatible 'hairy' nanospheres. Based on previous work in our laboratories, establishing that poly(N-alkylammonium)acrylamide is an effective sequestering agent, it was therefore reasonable to choose a hydrophilic block having this structure;
2. once formed and in water, aggregate-solution chain exchange had to be essentially non-existent to avoid potential polymer uptake across the intestinal wall;
3. accessibility to self-assembled architectures other than simple spheres was important; and
4. any possibility of cross-linking in the synthetic method had to be avoided as the protocol for self-assembly requires that the copolymer be initially dissolved for aggregate formation.

Polystyrene was chosen for the hydrophobic block, in part because of the simplicity of its synthesis but also because its extreme hydrophobicity precludes appreciable chain exchange in water. The hydrophilic block was largely predetermined by earlier successes using lightly cross-linked poly(N,N,N-trimethylammoniumdodecyl acrylamide chloride) (QPDA-12). However, the dodecyl spacer between the acrylamide and the ammonium groups was determined to be too hydrophobic to be practicable in self-assembly; hence a two-carbon spacer was ultimately chosen.

The synthesis of the block copolymer polystyrene-*b*-poly(N,N,N-trimethylammoniummethylene acrylamide chloride) (PS-*b*-PTMEACl) with tightly defined block lengths directly from monomers is complicated due to its multiple functional sites. We therefore opted to synthesize and then functionalize a precursor copolymer.

Choosing a particular polymerization technique depends very much on the polymer properties of interest. For the study of the self-assembly of amphiphilic block copolymers, carefully controlled block-lengths and architectures are of paramount importance and, where systematic study is desired, families of copolymers must be synthesized. Living polymerization techniques lend themselves to clearly defined polymer populations with low polydispersities. Anionic polymerization techniques in our laboratories have yielded a significant opus of publications concerning crew-cut aggregates of polystyrene-*b*-poly(acrylic acid) as well as a variety of other block copolymers all based on the precept that when a diblock copolymer is placed in a solvent that is good for only one of the blocks, the polymer chains will self-assemble to minimize the contact between the solvent and the insoluble block.^{1,2}

In aqueous media, amphiphilic asymmetric diblock copolymers having a soluble block that is very much shorter than the insoluble block form a series of about thirty morphologies and morphological intermediates depending on composition and preparation.³ When the soluble block of the block copolymer is not very much shorter than the insoluble block, the prevalent morphology in dilute solution is the simple small (primary) sphere.

Most living polymerization techniques are extremely sensitive to monomer functionality. In the case of anionic polymerization as performed routinely in our laboratories, the reactive carbanion is both a nucleophile and a base. Clearly, these polymerizations must be performed in the complete absence of any acidic protons or any other quenching agent and the monomer must be susceptible to nucleophilic attack only at the polymer backbone sites.

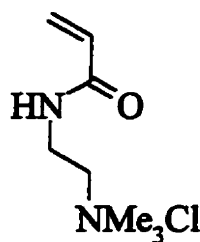


Figure 1: Hypothetical monomer required for “one-pot” polymerization of the acrylamide block.

Since the synthetic goal we describe here was PS-*b*-PTMEACl, the corresponding acrylamide monomer would have vinyl, secondary amide and quaternary ammonium groups (see Figure 1); therefore, one-pot anionic polymerization was deemed not feasible.⁴ Nevertheless, since the direct block copolymerization of the component monomers would have simplified the system, to satisfy curiosity, micro-scale preliminary investigations into the synthesis of the N,N-dimethylamino monomer were carried out and infrared spectra (see Appendix 2)^{*} indicated the successful synthesis of the acrylamide. However, purification in the absence of significant polymerization was not possible.

^{*} Useful IR band assignments are given in Appendix 1

Instead, we began by preparing comparatively simple polystyrene-*b*-poly(*tert*-butyl acrylate) (PS-*b*-PtBuA) samples following established protocols as described in exhaustive detail in the literature⁵⁻⁷ and summarized below in the Experimental section. The synthetic challenge was therefore to convert pendant *tert*-butyl ester moieties from PS-*b*-PtBuA to pendant ammonium groups with an ethylene spacer and an amide linkage to the hydrocarbon backbone (see Figure 2). The direct conversion from ester to amide by reaction with an amine as well as with two different aluminum amides was attempted with various degrees of success. The hydrolysis product of PS-*b*-PtBuA, polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA), was also converted to the acrylamide derivative via the acid chloride generated using either thionyl chloride or oxalyl chloride. Finally, the carbodiimide coupling reaction of the acid with a monodentate diamine was also accomplished. The pendant amine in all cases was quaternized with methyl iodide and the iodide was replaced with chloride by ion exchange.

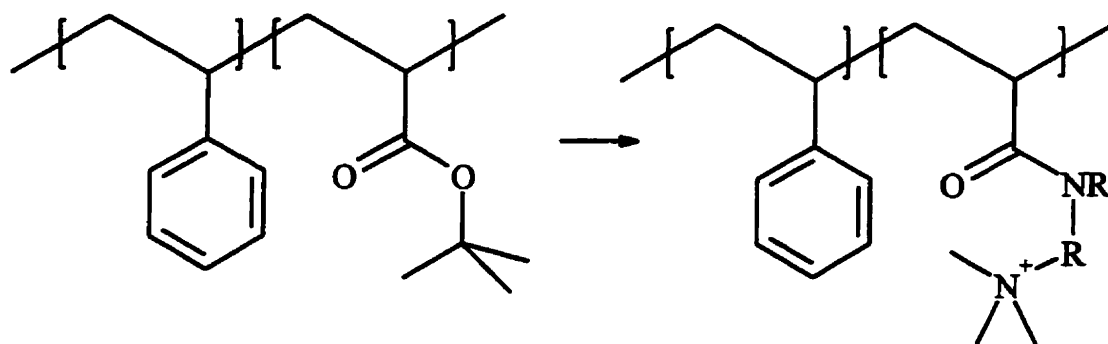


Figure 2: The synthetic challenge where R = hydrocarbon spacer, R' = H, Me.

Summary of synthetic strategy

The intent, as outlined above, was to generate an amphiphilic diblock copolymer system that self-assembles in water and sequesters bile salts. The strategy, therefore was:

1. to synthesize families of diblock copolymers of various block sizes, composed of styrene and *tert*-butyl acrylate that were predisposed to functional group conversion;
2. to compare the direct amidation of the pendant ester with the conversion of its derivative acid to determine the preferred synthetic route; and
3. to optimize the functionalization of the material and to obtain useful quantities of the polymer.

It might be argued that if the only goal were to obtain a material that sequesters bile salts, then the complete functionalization of the parent copolymer should not be necessary. For pragmatic reasons, it would indeed be convenient to accept non-quantitative functionalization. However, there are several factors that favour total replacement of the ester (or acid) groups: (i) residual ester groups render the ostensibly hydrophilic block less water-soluble and thereby complicate the process of self-assembly, (ii) residual carboxylate groups will interact with the pendant ammonium moieties to form ionic pseudo cross-links, and finally, (iii) every residual ester or carboxylate group is a wasted bile salt binding site. We therefore limited the system interactions (block-block, polymer-polymer, block-solvent, etc.) by limiting the repeat-unit species incorporated in the block copolymer to simple incarnations.

Experimental

Instrumentation

Gel permeation chromatography (GPC) was performed on a Waters® instrument (two columns in series) interfaced to a PC running Millenium® software. A calibration curve was constructed using polystyrene standards. The flow-rate was 0.6 mL/minute and the eluent was tetrahydrofuran (THF).

FTIR spectra were collected on a Perkin Elmer® 16PC instrument interfaced to a PC running either IRDM® or Spectrum® software. Samples were typically prepared as KBr pellets; though in some cases, films were cast on KBr windows (Wilmad). Except in rare cases, 16 scans were sufficient to get clean spectra. A summary of the relevant peaks is given in Table 1 just before the spectra at the end of the chapter.

Parent copolymer synthesis

The parent copolymer to the post-polymerization functionalization product PS-*b*-PTMEACl was either PS-*b*-P*t*BuA or PS-*b*-PAA. The PAA-containing polymer was derived by hydrolysis of the acrylate by well-established means. While it is true that the hydrolysis of PS-*b*-P*t*BuA is a post-polymerization modification of the polymer, we draw a distinction between the preparation of the parent P*t*BuA or PAA blocks (described immediately below) and their more challenging PTMEACl sibling in the following section.

PS-*b*-P*t*BuA

The parent block copolymer samples were synthesized following established protocols *via* anionic polymerization under an ultrapure nitrogen atmosphere (Matheson) in flame-dried glassware. Solvent and monomer transfers were accomplished *via* cannula and septa. Freshly distilled THF was collected from sodium/benzophenone under nitrogen. In a typical polymerization, 500 μ L of α -methylstyrene (α MS) was added to 200 mL of THF containing LiCl to provide *gegen* ions in 5-10 fold excess as compared with the number of polymer chains to be initiated. The reaction was titrated dropwise with a suspension of *sec*-butyl lithium (1.3 M in THF, Aldrich) until a dark red colour persisted indicating stable α MS anionic monomers and/or nascent oligomers. The reaction was then initiated with *sec*-butyl lithium in 10% excess and the temperature was reduced to approximately 240 K in a dry ice/acetone bath. Care was taken to avoid overcooling at this stage due to the relatively high ceiling temperature for the polymerization of α MS. Since both conjugate addition and addition-elimination reaction mechanisms are possible with the α,β unsaturated *t*-butyl acrylate, an orderly polymerization of the *t*BuA block is much more likely with α MS initiating anion as compared with the sterically less hindered and therefore more aggressive styryl anion. The incomplete consumption of the α MS monomer before the addition of the styrene monomer facilitates the next step.

Cryo-distilled styrene monomer that had been stirred over CaH_2 and then further dried with fluorenyl lithium immediately prior to distillation was then added to the reaction and the deep red color temporarily turned orange, the colour associated with the styryl anion, until all the styrene monomer was consumed. The styrene block was then

auto-encapped with traces of remaining α MS and the deep red α MS colour associated with the living α MS anion was regenerated.

Although exact calculations of the quantity of *sec*-butyl lithium required for any desired degree of polymerization are trivial, the delivery of precisely any given volume of an imperfectly uniform suspension of the salt in hexanes is not possible. An aliquot of PS was therefore removed for gel permeation chromatography (GPC) analysis and based on the confirmed degree of polymerization, the quantity of *tert*-butylacrylate (*t*BuA) to be added for the desired architecture was determined. The acrylate monomer was pre-purified over CaH_2 and was sometimes further dried with triethylaluminum immediately before cryo-distillation.[†] The reaction temperature was reduced to 195 K in a dry ice/acetone bath[‡] and on addition of *t*BuA, the deep red colour of the α MS anion was replaced by the nearly colorless acrylate anion. An aliquot ranging in volume from several drops to a large fraction of this reaction mixture was extracted and “killed” in degassed, cold methanol to avoid coupling. Since the residual reaction mixture still contained living polymer chains, further addition of *t*BuA was possible thereby generating a family of polymers based on a fixed PS block length with a series of different *t*BuA block lengths (see Figure 3).

[†] In our hands, triethylaluminum often induced polymerization and so the benefit of its use was usually outweighed by the loss in distillable monomer during the purification process.

[‡] Below the ceiling temperature for α MS and sufficiently cool to favour Michael addition.

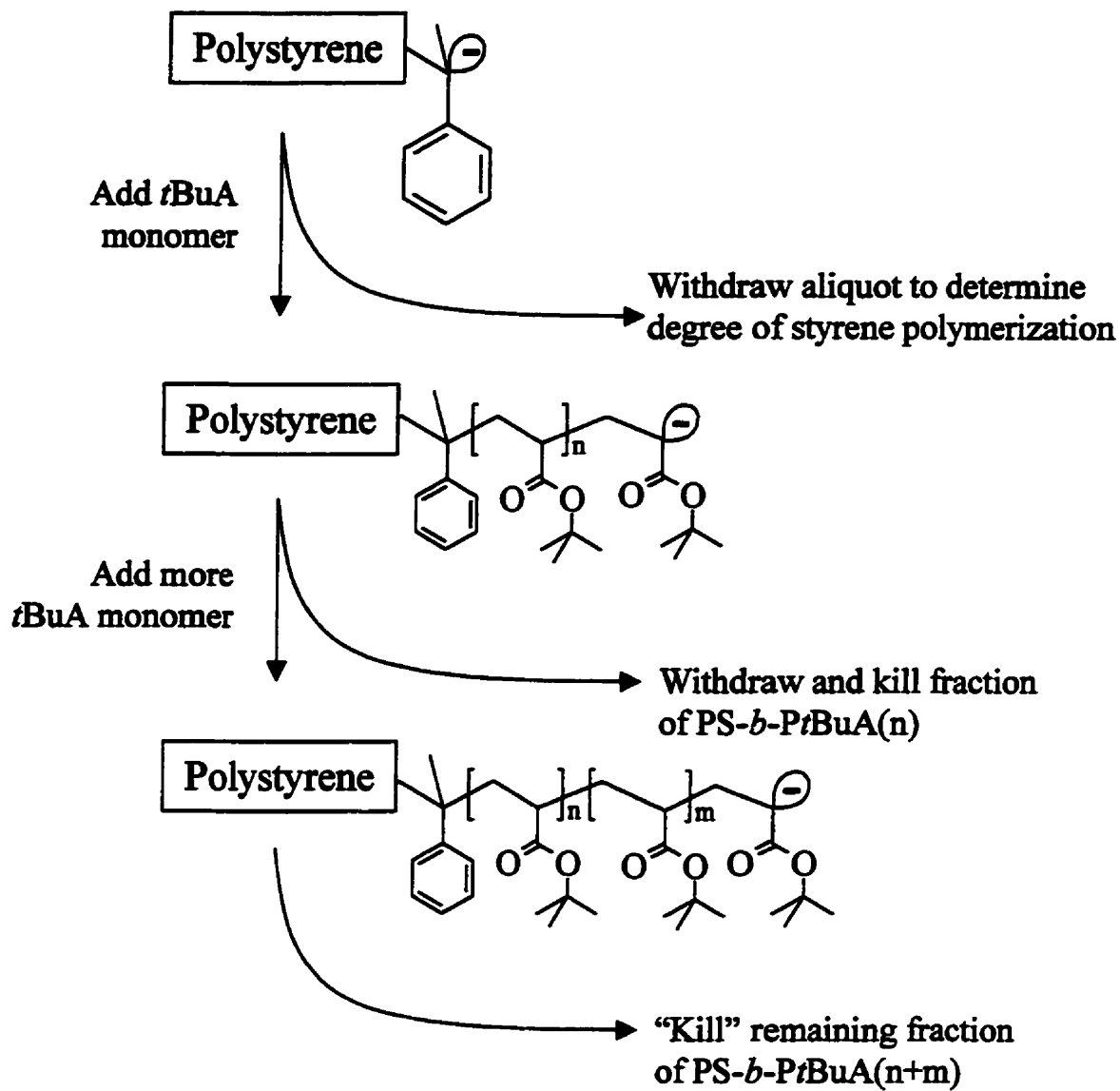


Figure 3: Family of polymers generated via living anionic polymerization.

PS-*b*-PAA

Polystyrene-*b*-poly(acrylic acid) was prepared by acid-catalyzed hydrolysis of PS-*b*-PtBuA. The acrylate-containing polymer was dissolved in toluene and 2-5% toluene-sulphonic acid (TsOH) was added relative to the number of ester groups to be converted (*i.e.*, mole percent). The reaction was left at reflux for several hours whereupon the toluene was removed by rotary evaporation and the residue was re-dissolved in THF. The polymer was precipitated in water and infrared spectroscopy of cast films or KBr pellets confirmed the *quasi*-complete hydrolysis where the characteristic carboxylic acid bands were present (O-H at 3000-2500 cm⁻¹ and carbonyl at 1725-1700 cm⁻¹).⁸ Furthermore, there was little or no evidence for residual ester carbonyls (~1730 cm⁻¹) or *t*-butyl groups (a doublet at 1395-1385 cm⁻¹ and 1365 cm⁻¹) (see Appendix 3).

Post-polymerization functionalization

There are many possible routes for the conversion of carbonyl-containing functional groups to amides. Our challenge was to determine which of the alternatives was best suited to our system. Amidation of these block copolymers was accomplished with varying degrees of success using several different synthetic pathways (see Figure 4). The pendant ester groups were reacted either directly with the corresponding amine or with an aluminum amide. Alternatively, the pendant ester groups were hydrolyzed to carboxylic acids, which were either converted to the acid chloride and reacted with an amine, or were coupled using a carbodiimide and an amine. Pellets (KBr) were prepared

containing analyte and the solid state IR amide I ($1680\text{--}1630\text{ cm}^{-1}$) and amide II ($1570\text{--}1515\text{ cm}^{-1}$) diagnostic bands were employed to follow the reactions.⁹

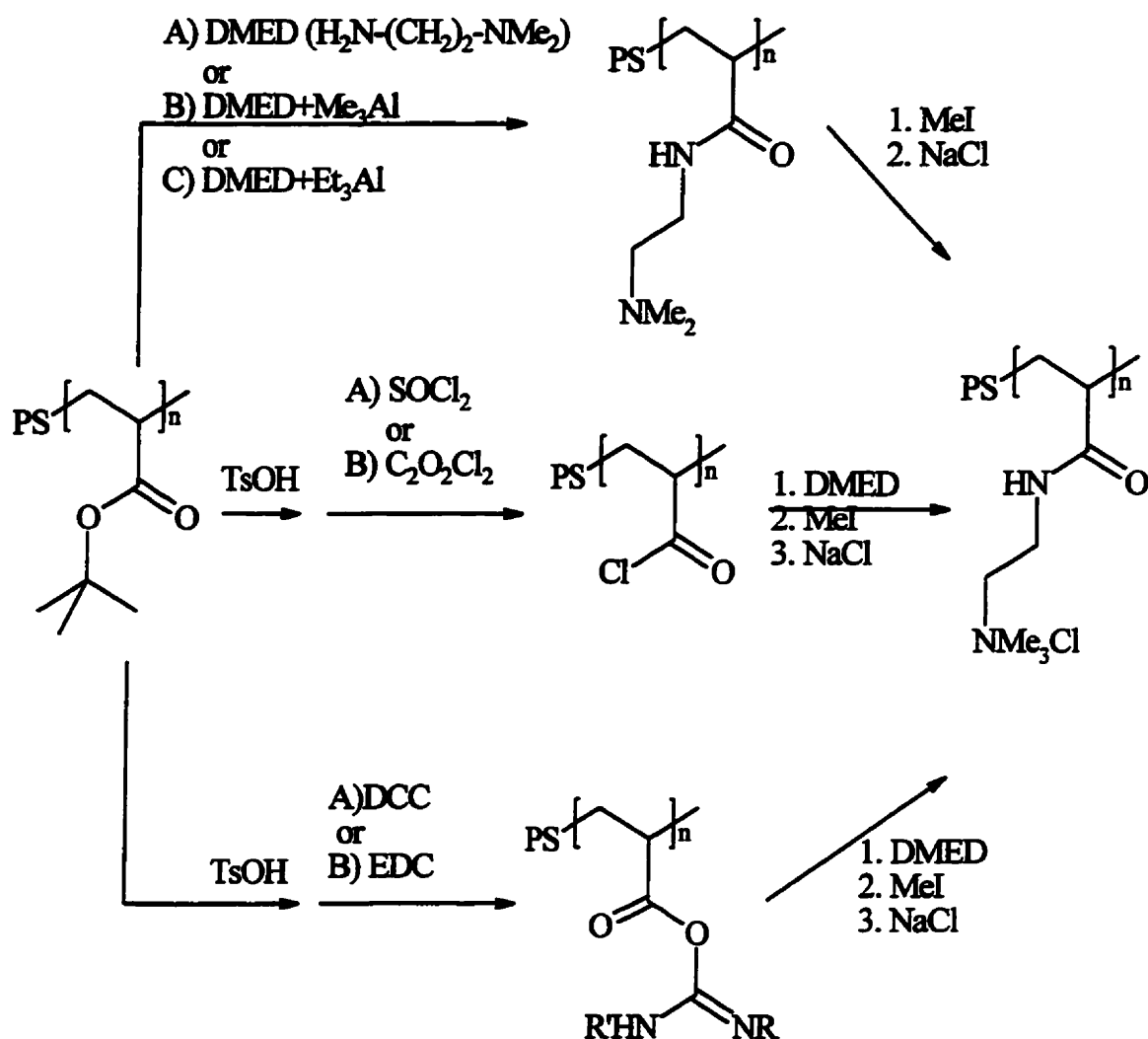


Figure 4: A functionalization summary for PS-*b*-PtBuA. The R and R' reflect the different substituents for the 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and dicyclohexylcarbodiimide (DCC) adducts.

Direct ester to amide transamidation

The direct functional group conversion from ester to amide was attempted by simply refluxing a solution of ester-containing polymer in an excess of mono-dentate diamine (neat or in dimethylformamide, DMF). In a typical reaction, 1 mmol of *t*-butyl acrylate units from PS(175)-*b*-P*t*BuA(65)[§] were refluxed with 4 mmol N,N-dimethylethylenediamine (DMED, Aldrich, re-distilled) in dioxane. Reaction times were on the order of one week at elevated temperature approaching, but not exceeding reflux (374 K). The polymer was then precipitated and washed with methanol/water (milliQ). Sample FTIR spectra may be found in Appendices 4 and 5 (the discussion of these and all subsequent spectra will be found below)

Ester-amide (aluminum intermediate)

The aluminum amide mediated functional group conversion of an ester to an amide was attempted starting with either triethyl- or trimethylaluminum (AlEt₃ or AlMe₃).¹⁰ In a typical AlEt₃ reaction, 2 mmol of AlEt₃ were stirred with 4 mmol N,N-dimethylethylenediamine (DMED) in 5 mL freshly distilled CH₂Cl₂ for at least 15 minutes. Then 270 mg PS(25)-*b*-P*t*BuA(280) containing 2 mmol of ester groups was added in 5 mL CH₂Cl₂ and the reaction was left at reflux overnight. The reaction was quenched with acidic water yielding a gel-like white precipitate.

[§] the numbers represent the degree of polymerization for their respective block.

Spectroscopy indicated incomplete conversion (see Appendix 6). Therefore, to increase reactivity, AlMe_3 was employed under similar conditions. $\text{PS}(25)\text{-}b\text{-PrBuA}(280)$ containing 1 mmol of ester groups was added to the reaction product of 3 mmol Me_3Al and 4 mmol DMED in 5 mL freshly distilled CH_2Cl_2 . The reaction was allowed to proceed at reflux for 72 hours and was then quenched with acidic water. The aqueous phase was then rendered basic and the organic phase was collected, the solvent was removed by evaporation and the residue was re-dissolved in dioxane. The dioxane solution was dialyzed against distilled water and then lyophilized, yielding a faintly yellow powder. Sample spectra may be found in Appendices 7, 8 and 9.

To confirm the potential utility of this reaction pathway, the small-molecule analogue reaction was also carried out on ethyl acetate. Me_3Al was added to DMED in dioxane (freshly distilled from P_2O_5) and was stirred for 25 minutes at room temperature. Ethyl acetate was then added and the reaction mixture was left overnight at 298 K. Dilute $\text{HCl}_{(\text{aq})}$ was then added to quench the reaction and the product was extracted into CH_2Cl_2 once the aqueous phase had been rendered basic with NaOH . The organic phase was dried over MgSO_4 , filtered, evaporated and IR spectra consistent with successful amidation were obtained (see Appendix 7).

Ester-acid-amide (SOCl_2)

To increase susceptibility of the acrylate block to nucleophilic addition/elimination, the ester was hydrolyzed and from the resulting carboxylic acid, the acid chloride intermediate was synthesized. A sample of $\text{PS}(385)\text{-}b\text{-PAA}(80)$ was

dissolved in freshly distilled THF. To this solution, SOCl_2 was added in slight excess and the reaction solution was allowed to stir at 316 K for 14 hours whereupon the reaction was cooled to 273 K and excess DMED was then added. The reaction was allowed to proceed for 4 hours and was quenched with 25 mL water (milliQ). An FTIR spectrum of the product may be found in Appendix 10.

Ester-acid-amide ($\text{ClC}_2\text{O}_2\text{Cl}$)

Water was added drop wise to a solution of PS(80)-*b*-PAA(330) (100 mg) in 5 g DMF in order to induce micellization at about 10% water content (w/w). The micelles were then dialyzed against an aqueous solution of NaOH at pH 12 and the sample was lyophilized. The resulting PS-*b*-PANa was re-suspended in 100 mL THF to which 5.5 mmol of oxalyl chloride was added. The reaction was stirred overnight under an argon atmosphere whereupon a large excess of DMED was added. The reaction mixture was stirred for 24 hours, was then dialyzed against water, lyophilized and was then quaternized. The FTIR spectra may be found in Appendix 11.

Ester-acid-amide (DCC)

Dicyclohexylcarbodiimide was added to a solution of PS-*b*-PAA in THF in 50% excess. After 3–4 hours the solution became cloudy and the reaction was allowed to continue overnight. Excess DMED was then added and the reaction solution was stirred for 1 day. The polymer was precipitated, collected and re-dissolved in DMF whereupon MeI was added and left for up to 12 hours. Excess MeI was removed by rotary

evaporation and then aggregation was induced by addition of deionized water (milliQ). The suspension was dialyzed against distilled water for 4 days. One reaction was followed spectroscopically and the resulting spectra may be found in Appendix 12.

Ester-acid-amide (EDC)

To a solution of 94 mg PS(80)-*b*-PAA(330) in 5mL CH₂Cl₂, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (395 mg) was added and was stirred for 5-10 minutes. Excess DMED (500 μL, or 4.5 fold excess over all acid groups) was then added and was stirred for 24 hours whereupon the polymer was precipitated into MeOH/H₂O and dialyzed against milliQ H₂O. The functionalized copolymer was then re-precipitated in saturated NaCl_{aq} and was dialyzed against milliQ water to remove impurities. The polymer was lyophilized to yield 140 mg product and was quaternized in MeOH with MeI in 20-fold excess. The iodide counter-ion was exchanged for chloride by precipitation in NaCl_(aq), followed by dialysis against 0.1 M NaCl_(aq) and then against milliQ H₂O. Sample FTIR spectra may be found in Appendix 13.

Quaternization

Over the course of these syntheses, two different quaternization conditions were employed. Either the system was swamped by a huge excess of MeI or less MeI was used in methanol with a weak base (20 mL MeOH, 1 g KHCO₃, 1 mL MeI).

Discussion

Given the relative simplicity of transforming an ester to an amide in the absence of other chemically sensitive groups, we would have had reason to be extremely disappointed had it not worked. However, the development or modification of the amphiphilic nature of the diblock copolymers as conversion occurs presents special problems.^{17,18} Additionally, the requirements for successful nano-scale self-assembly also provided unanticipated challenges. Consequently, we explored various synthetic routes to develop a better understanding of the limitations imposed by our system.

Inherent challenges to diblock copolymer functionalization

Cross-linking

Previous work has demonstrated conclusively that the transamidation of poly(methyl methacrylate) (PMMA) resins using diamines usually implies further crosslinking.¹¹ In a study by Asgari,¹² rather than refluxing PMMA resins with α,ω diamines where each end of an aliphatic chain has a primary nucleophilic nitrogen atom, one end was first protected with *t*-butylcarbonyl (BOC) and the other was allowed to displace methanol from PMMA to form the poly(acrylamide). Finally, the now-pendant amine was deprotected and trimethylated to yield the desired pendant trimethylammonium group. While this method worked rather well for PMMA, had it been employed here for the diblock functionalization, the work-up would probably have been prohibitively complicated due to polymer-solvent interactions as described above, compounded with the additional synthetic steps required for deprotection. A possible solution, where the α,ω diamine is mono-protected, then dimethylated and deprotected

prior to coupling was not explored. Instead, we chose a commercially available monodentate diamine: N,N-dimethylethylenediamine, where one amine is primary (a good nucleophile) and the other is tertiary (quite a poor nucleophile, but a reasonably good base). The presence of the tertiary amine not only precludes cross-linking, but also simplifies the quaternization step as only one more methyl group is required. It is possible that this general procedure of preparing a mono-dentate diamine where the protecting groups need not be removed might also be useful in the synthesis of bile acid sequestering resins.

Solubility

Although in principle, the amidation of an ester is a relatively simple operation,^{13,14} in practice, the problems with the post-polymerization functionalization of amphiphilic diblock copolymers are multi-fold. A reaction medium that will solubilize the starting material, the reaction intermediate(s) and the product is required since instantaneous and complete functionalization can never be achieved (*i.e.*, the reacting polymer must be in intimate contact with the reagent(s) at all times regardless of the degree of functionalization, otherwise the reaction either never starts or decelerates as soon as it is underway). Furthermore, the reaction medium choice must take into account the static, and in our case, generally insoluble block. In the event of aggregation (self-assembly) during the reaction, it is important that the non-reactive block serve as neither shield nor barrier to the reacting species. The polymer handbook¹⁵ lists many solvents for polystyrene, poly(acrylic acid) and poly(*tert*-butylacrylate). However, polyacrylamide is insoluble in nearly all of them: morpholine and water are the only solvents listed for this polymer. The amidation of PMMA with mono-BOC protected

1,6-hexanediamine works well in DMF at high temperatures (~150 °C) over several days suggesting that the alkylated polyacrylamide at least swells in DMF. The solvents of choice were therefore either the diamine itself or hot DMF, though others were tested.

Macrosurfactants

For purification of these amphiphilic block copolymers, solvent extraction work-up techniques have proven largely ineffective since the macromolecules in this case are also macro-surfactants. Even when extracted or 'salted out' with saturated aqueous NaCl, the amphiphilic nature of the polymers in question precluded any hope of clean phase separation. Instead, the laborious cyclical process of dissolution, micellization, dialysis, lyophilization and redissolution was employed to remove reaction byproducts and impurities. Though it was tempting to carry out the reactions on micellized copolymers,¹⁶ the threat of cross-linking and the inaccessibility of the coronal units near the core-corona interface rendered this option less attractive.

Microscale and scaled-up reactions

A final consideration was the reaction scale. For morphological study, essentially quantitative conversion was required, though the absolute quantity of copolymer necessary for the investigation was less than 10 mg. For practical bile anion sequestering studies, however, 50 mg of the sample was sufficient to yield indicative results, though more would have been preferable. The scale-up of any successful microscale reaction could not sacrifice degree of conversion without losing the advantages of nanoscale self-

assembly. Without complete conversion of the corona-forming block, the whole process of nano-aggregation was compromised.

Functional group conversion

For PMMA cross-linked resins, it has been possible to convert the pendant esters to amides directly with diamines by heating for protracted reaction times.^{17,18,19} Under these conditions, near-quantitative conversion was generally achieved. Since the initial purpose of the present study was to determine the self-assembly properties of this novel diblock copolymer, and since it has been conclusively demonstrated that self-assembly is extremely sensitive to any number of variables, cross-linking, degradation and incomplete functionalization reactions had to be avoided. Extended reaction times for the direct amidation of *tert*-butyl ester-containing copolymer at elevated temperature were not sufficient to ensure reproducible high conversion as determined by FT-IR, and furthermore, the rigorous conditions involved concomitant cross-linking side-reactions possibly mediated by N-methyl migration.

At reduced temperature, even over very long reaction times, there was no appreciable conversion. The spectra shown in Appendix 4 indicate good amidation as a function of time as the amide carbonyl peaks become dominant ($< 1700\text{ cm}^{-1}$); however, the final product, showing no residual ester ($\sim 1730\text{ cm}^{-1}$), was insoluble in all common solvents and only swelled in water suggesting that cross-linking was extensive. Two clean and functionalized samples were obtained, albeit in small quantities (Appendix 5). A minor ester carbonyl peak ($\sim 1730\text{ cm}^{-1}$) is observable. However, the amide peaks dominate.

Despite many attempts, the scale-up reaction was less successful. Therefore, the ester-amide conversion by simply refluxing the ester in the presence of excess amine (Figure 5) was neither reproducible nor ideal due to the extreme conditions required for the transformation - a disappointment due to the relative simplicity of the reaction work-up. Nevertheless, based on the success of the microscale reaction, we were able to initiate an exploration of the aggregation properties of these copolymer samples.

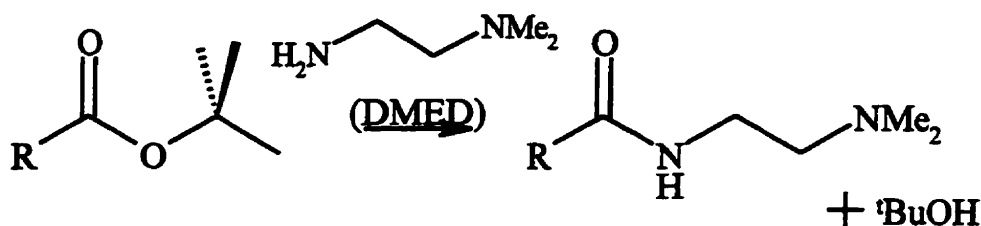


Figure 5: Scheme for the direct transamidation of PS-*b*-P*t*BuA (*R* = polymer backbone). For simplicity, only one pendant ester is shown; the degree of polymerization of the ester-containing block will determine the number of ester moieties to be converted.

Dialkylaluminum amides

Not only reactivity and solubility, but also steric hindrance plays a significant role in the successful functionalization of the coronal block. In the case of the aluminum amide mediated reaction (see Figure 6), the intermediate is a four-center cycle (see Figure 7). Crowding from the tertiary carbons of the *t*-butyl acrylate groups as well as the polymer coil itself slowed the kinetics of reaction significantly as compared with the small-molecule analogue reaction, such that even switching from the diethyl to the dimethyl aluminum amide had profound effects on the kinetics and yield.¹⁰ The reaction

involving Et_3Al -mediated transamidation was so slow as to be essentially unsuccessful. Nevertheless, a hint of amidation was observed in the carbonyl region of the IR spectra (see Appendix 6) so the use of aluminum amides was pursued further.

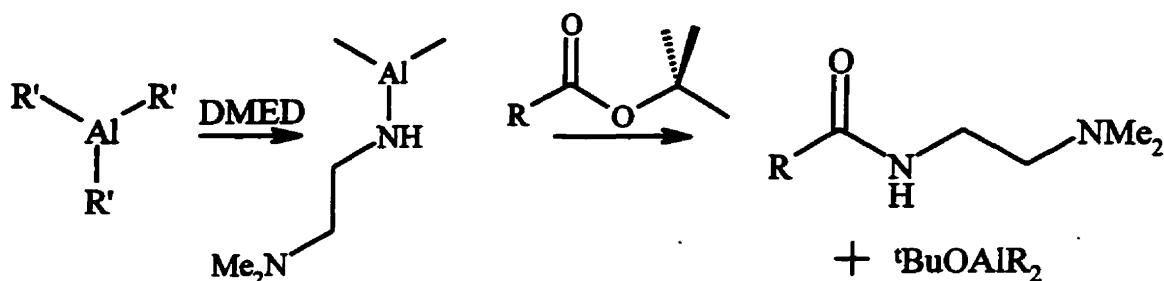


Figure 6: Transamidation scheme via dialkylaluminum amide (R = polymer backbone and $\text{R}' = \text{Me}$ or Et).

As a side note, it is interesting to consider that the tacticity of the polymer chain was not proactively controlled. However, due to the bulky *t*-butyl ester groups and their sequential addition during anionic polymerization, it is reasonable to expect syndiotactically-weighted random tacticity. Given the space requirements for the four-centre intermediate (see Figure 7), reactions with an isotactic polymer would most probably have been unsuccessful. The ester-free amide product that was obtained using unhindered, small-molecule ethylacetate as the starting material demonstrates that the reaction is highly dependent on spatial requirements (see Appendix 7).

Appendices 8 and 9 show IR spectra for the Me_3Al -mediated functionalization of $\text{PS}(175)\text{-}b\text{-PrBuA}(35)$, $\text{PS}(175)\text{-}b\text{-PrBuA}(65)$, as well as the functionalization and quaternization of $\text{PS}(25)\text{-}b\text{-PrBuA}(280)$. Residual ester carbonyl groups are indicated by

the peaks around 1730 cm^{-1} in Appendix 8, but the dominance of the amide peaks Appendix 9 suggests high conversion. However, the work-up of these reaction mixtures was not trivial.

A perennial *bête noire* of block copolymer synthesis and functionalization, especially where nano-scale self-assembly is concerned, is purity due to the sensitivity of the polyelectrolytic block to its microenvironment. Infrared spectroscopy indicated few residual ester groups;^{**} however, the separation of copolymer from the aluminum oxide required an aqueous acid/organic extraction, but the pendant amide has a proton acceptor, so both the product *and* the byproduct were soluble in the aqueous phase. Despite attempts at centrifugation, dialysis and a variety of extraction techniques, reliably clean, pure copolymer in workable quantities was ultimately elusive due to steric constraints in the synthesis and solubility issues in the work-up.

^{**} Although the spectra are presented in transmission mode and therefore Beer's law can only be directly applied qualitatively, it remains true that the ester peak is a sensitive 'canary' as it is typically both strong and sharp.

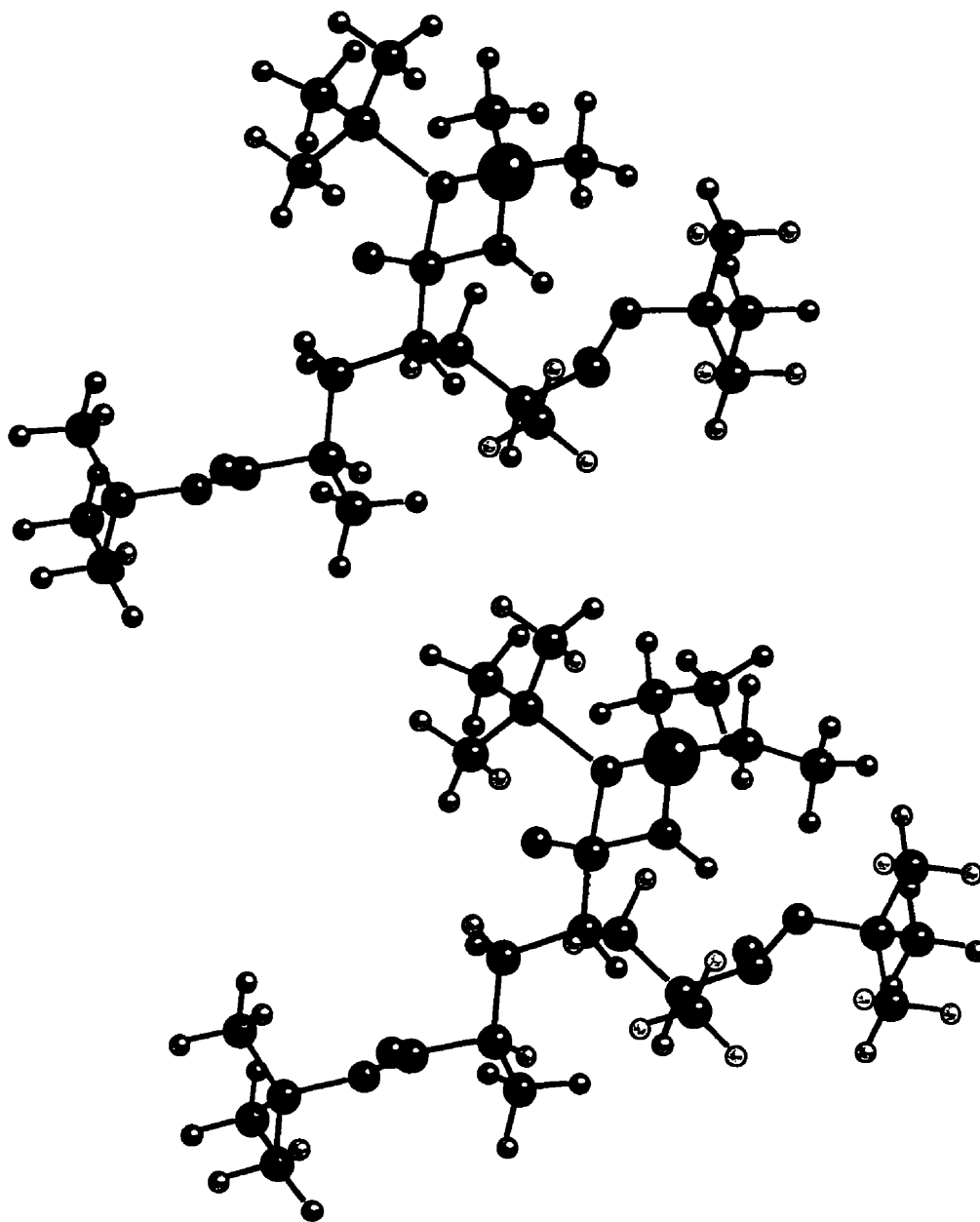


Figure 7: Energy minimized conformation (Nemesis) of 'syndiotactic' *t*-butyl acrylate trimer showing the aluminum amide four-centered intermediate at the middle carbonyl. Carbons (green), hydrogens (grey), nitrogen (blue) and aluminum (blue-grey) are shown. The upper structure is a representation of the dimethylaluminum amide-polymer intermediate and the lower structure illustrates the diethyl aluminum amide-polymer intermediate.

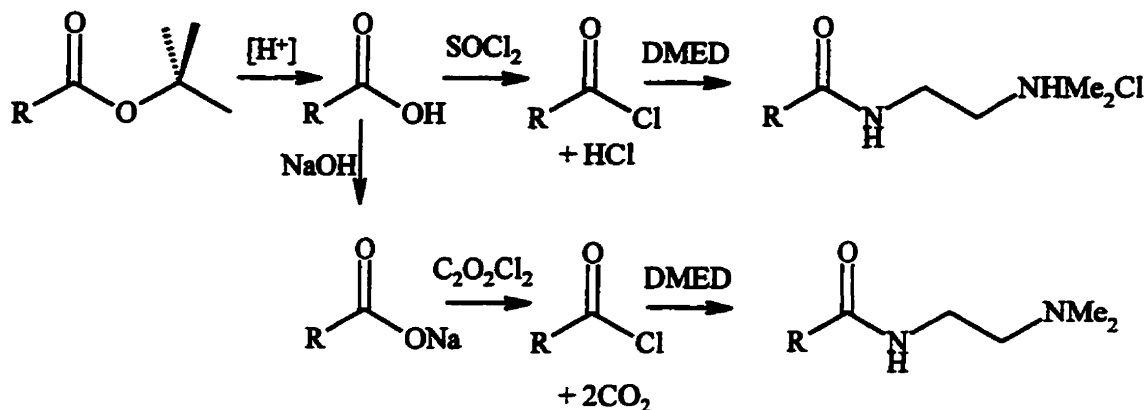


Figure 8: Acid-chloride mediated amidation using either thionyl or oxalyl chloride-generated acid chlorides (R = polymer backbone). Dimethylethylenediamine was added in large excess as a proton scavenger.

Acid chlorides via thionyl chloride or oxalyl chloride

Due to the relatively non-reactive ester moiety, it was necessary to consider alternative routes including a step to activate the carbonyl to nucleophilic attack. The ester was therefore hydrolyzed to the corresponding carboxylic acid and was then predisposed to nucleophilic addition-elimination by the formation of the acid chloride. Early trials with thionyl chloride successfully functionalized the material to the acid chloride, but subsequent reaction required a large excess of amine due to the generation of hydrochloric acid at both the chlorination and amidation steps (see Figure 8). An IR spectrum of the most successful amidation via this route may be found in Appendix 10 where the amide carbonyl peaks are clearly present.

An alternative route to the desired product proved to be quite successful via the carboxylate using oxalyl chloride (see Figure 8) as the chlorinating agent. Since the

byproduct of this reaction was CO_2 , which bubbled out of the reaction mixture, there was no deactivation of the nucleophile. The complete initial ionization of the pendant carboxylate moieties in this case rendered the reaction complicated and reproducibility was an issue due to the insolubility of the reaction intermediate. Furthermore, the complete amidation of all pendant groups was not accomplished as suggested by the spectra in Appendix 11. The hydrolysis of the initial ester followed by the deprotonation of the carboxylic acid groups is indicated by the shift in the FTIR carbonyl band from $\sim 1730\text{ cm}^{-1}$ to $\sim 1600\text{ cm}^{-1}$. The amide bands ($< 1700\text{ cm}^{-1}$) appear following the formation of the acid chloride and the addition of DMED. A methyl ester peak appears in the spectra after the quaternization step due to the methylation of un-coupled carboxylates.

Carbodiimides

Proposed in 1955, carbodiimides represent an attractive route to the desired amide in that they activate carboxylic acids to nucleophilic attack.²⁰ For small-molecule

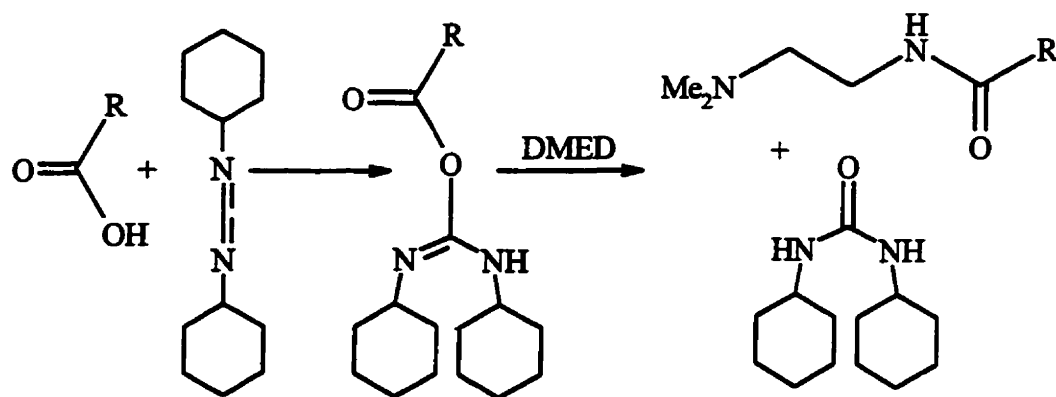


Figure 9: The dicyclohexylcarbodiimide (DCC) coupling reaction of PS-*b*-PAA with N,N-dimethylethylenediamine (DMED) showing the insoluble dicyclohexylurea. R represents the rest of the polymer.

chemistry, the work-up usually requires only the separation of the desired amide from the urea byproduct. The reaction scheme shown in Figure 9 presents the classic dicyclohexylcarbodiimide (DCC) mediated coupling of carboxylic acid with an amine.

There are, however, several *caveats*. Of some concern is a competing side rearrangement where the newly generated NH from the diimide acts as a nucleophile (see Figure 10). By this rearrangement, urea is covalently incorporated into the polymer.

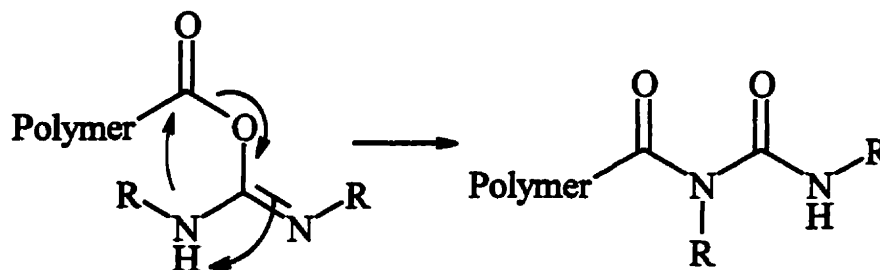


Figure 10. N-acyl rearrangement mechanism in carbodiimide coupling

In small-molecule reactions this reaction byproduct would be removed by any number of separation techniques. However, in a polymeric system, individual repeat units cannot be selectively eliminated. Ensuring the nucleophilic amine is present in significant excess can minimize the rearrangements. Diimides also react directly with amines. However, the coupling reaction to the carbonyl is typically much faster.²¹ Anhydrides can also

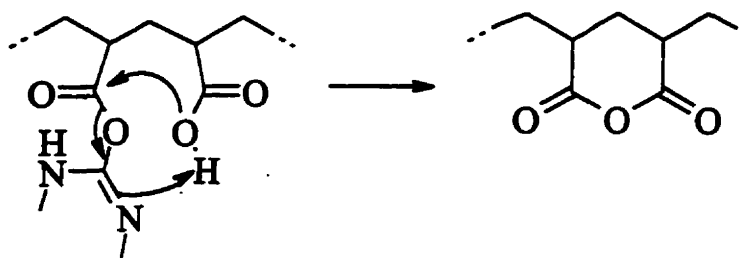


Figure 11: Anhydride formation mechanism in carbodiimide coupling

form, particularly in cases where the reacting carboxylic acid groups are held in proximity to each other (see Figure 11), but should they persist, they are evidenced by a pair of bands in the IR spectrum around 1800 cm^{-1} .^{8,9}

DCC

In the small-molecule literature, there are many examples of DCC coupling reactions. One of the strengths of this pathway is that the urea byproduct is insoluble in virtually all common solvents except alcohols²¹ and thus filtration followed by a purifying column is usually sufficient to render the product clean. Here again, the solubility of the copolymer proved to be an issue. Neither filtration nor columns are practical, and although an obvious alternative, centrifugation followed by dialysis against water, is effective, it is often either not completely quantitative in removing the *N,N'*-dicyclohexyl urea or the samples suffered some *N*-acyl rearrangement. Small quantities of some spectroscopically clean samples were nevertheless obtained. The spectra in Appendix 12 follow the coupling reaction as a function of time. In the uppermost spectrum typical PS and PAA peaks may be found. Addition of DCC is paralleled by the appearance of a strong band at $\sim 2100\text{ cm}^{-1}$, which recedes with time as the amide I and II peaks grow. The final quaternized product spectrum provided no evidence of residual urea.

EDC

The overwhelming advantage of using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) as the coupling reagent (see Figure 12) is that the

urea-hydrochloride byproduct is water soluble. Given that extractions are exceptionally difficult to perform on amphiphilic diblock copolymers, the purification process is much more dependent on dialysis and centrifugation. The disadvantage of this reagent is that it appears less reactive than its dicyclohexyl cousin and the reaction conditions employed for the block copolymers may not have minimized N-acyl rearrangements. The spectra in Appendix 13 clearly indicate amide H-bonding (3250 cm^{-1}) and amide carbonyl bands. However, the sharp peaks at 1630 cm^{-1} may reflect some tethered urea. Despite this potential complication, the reaction provided sufficient material not only for self-assembly, but also for bile salt binding assays.

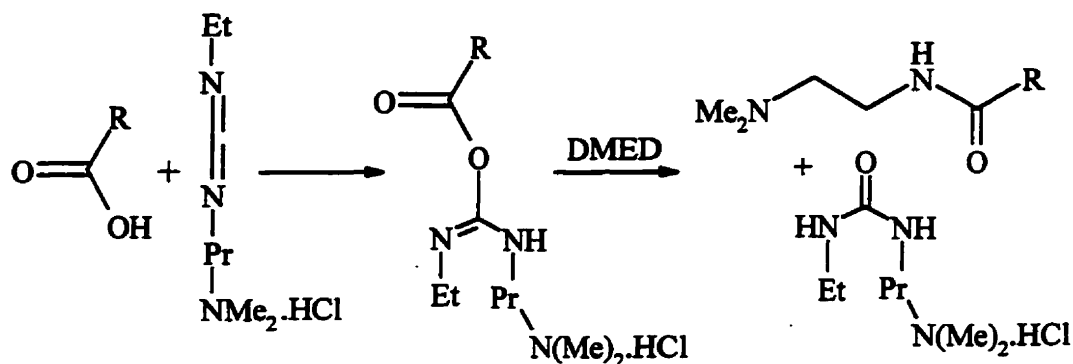


Figure 12: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) mediated amidation indicating the water-soluble urea. R represents the rest of the polymer.

Summary

The post-polymerization conversion of PS-*b*-P*t*BuA to PS-*b*-PTMEACl is a formidable task that was ultimately best addressed by water-soluble carbodiimide coupling reagents. A high degree of functionalization from any of the synthetic routes above is certainly possible, but despite model reactions that behave well not only for

small-molecule analogues, but also on linear homopolymers, the complete, repeatable and large-scale amidation of our starting PS-*b*-P*t*BuA or -PAA block copolymers in substantial quantity and high purity remains challenging. Forming self-assembled block copolymer aggregates was a precondition to both the examination of morphology as well as potential bile acid sequestrant utility. Therefore, in several cases, while the coupling was successful, rendering the product sufficiently clean for further study proved impossible.

The direct transamidation of the ester by reaction with the corresponding amine requires conditions and reaction times that result in side reactions. While these can be minimized to a certain degree by using ultra-pure reagents, inert atmospheres, moderate reaction temperatures and UV shielding, this route is not a particularly good one if high conversion, clean, non-cross-linked polymer is desired.

Milder conditions implied by the dialkylaluminum amide transamidation reaction are extremely attractive. The reaction times are, in principle, shorter and the risk of side reactions is low. The challenges presented by this route are: (i) steric, the intermediate is very bulky and thus the kinetics of the reaction are dramatically slowed for the polymer systems as compared with the small-molecule analogues;^{††} and (ii) work-up, in that the aluminum oxide byproduct is typically washed out of the water-insoluble product in acidic water; however, the functionalized block has a tertiary amine that quaternizes in acidic water thereby also solubilizing the product.

^{††} Indeed, this was perhaps the most revealing example of the differences between small- and macro-molecular chemistry. The dimethylaluminum amide transamidation of ethyl acetate required just one day to accomplish from beginning to isolated product on my first (and only) try. Similar reactions on the diblocks took several weeks.

Hydrolysis of PS-*b*-P*t*BuA to PS-*b*-PAA is an example of highly successful post-polymerization functionalization of a diblock copolymer. Initially, the polymer is completely soluble in toluene and as the acid-catalyzed hydrolysis occurs, the reaction medium becomes milky. Since the hydrolysis initially requires only the migration of a proton to the ester, refluxing for several hours is sufficient to achieve essentially quantitative conversion of P*t*BuA to PAA. The direct transamidation from carboxylic acid to amide is not possible since any nucleophilic amine will deprotonate the acid before it adds to the carbonyl. Thionyl chloride and oxalyl chloride each provide good routes to acid chlorides, which are in turn highly reactive to amines. This multi-step functionalization is a victim of its torturous route and the highly reactive acid chloride intermediate. For ease of handling, the oxalyl chloride reaction perhaps merits closer attention than the thionyl chloride reaction, but both ultimately remain complicated.

The most successful (and repeatable) reactions were the carbodiimide couplings. Nevertheless completely separating the polymer from the dicyclohexylurea byproduct was a formidable obstacle given the solubility issues described above. It is quite likely that in even the purest of samples that there remained some contamination. Samples prepared via EDC coupling are much cleaner.

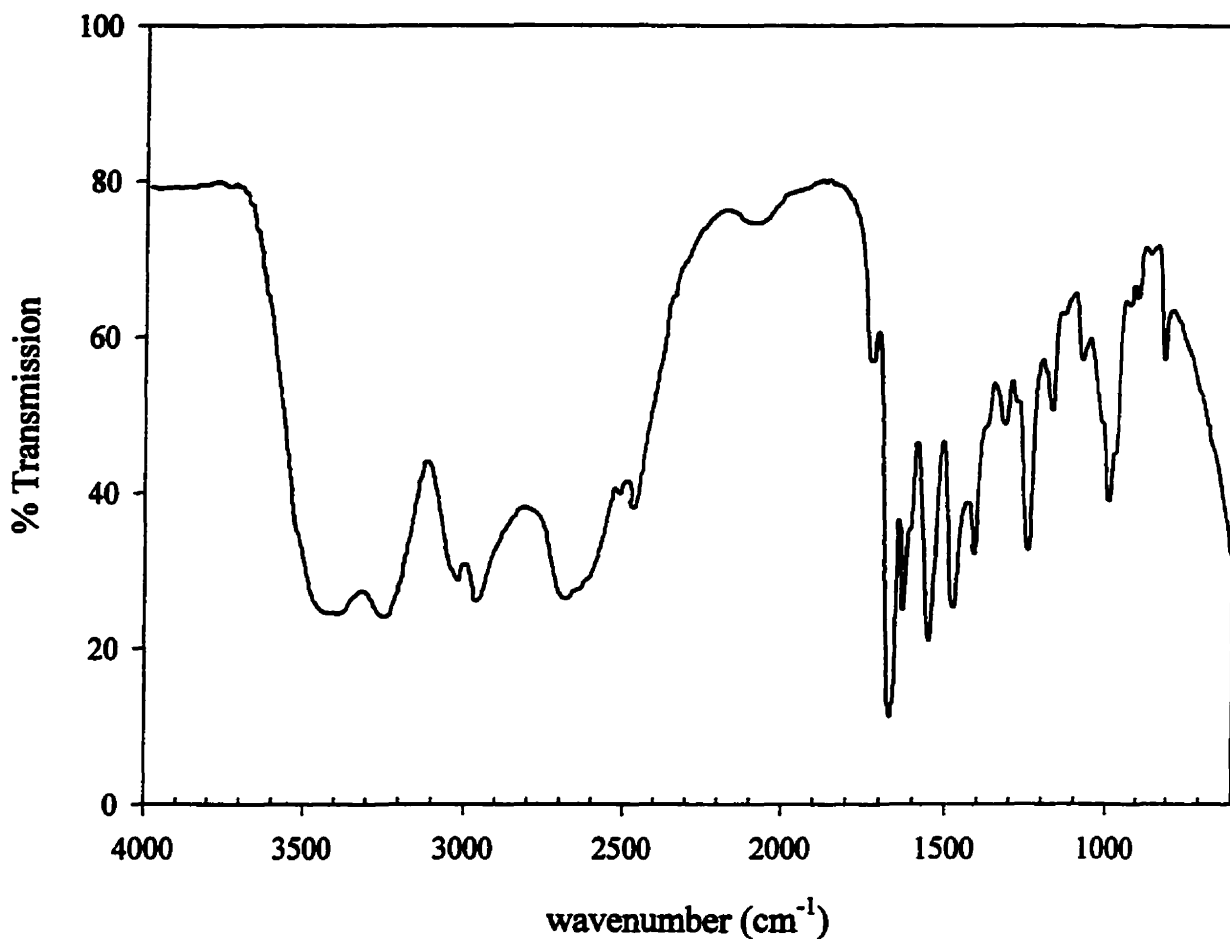
Given recent advances in living free radical polymerizations^{22,23} (nitroxide-mediated polymerization and ATRP), it seems quite clear that should there be any need to develop significant quantities of uniform, architecturally controllable diblock copolymers of the type described in this chapter, thus future work would be well advised to reconsider pseudo *ab initio* synthesis.

The challenges associated with this system notwithstanding, it remains true that, in small quantities, we were able to isolate reasonably clean, nearly quantitatively converted diblock copolymers that permitted us to carry out a survey of the self-assembly properties of this novel amphiphilic diblock as described in Chapter 4. We also investigated these polymer aggregates as potential matrices for bile acid sequestering in the human gut as described in Chapter 5.

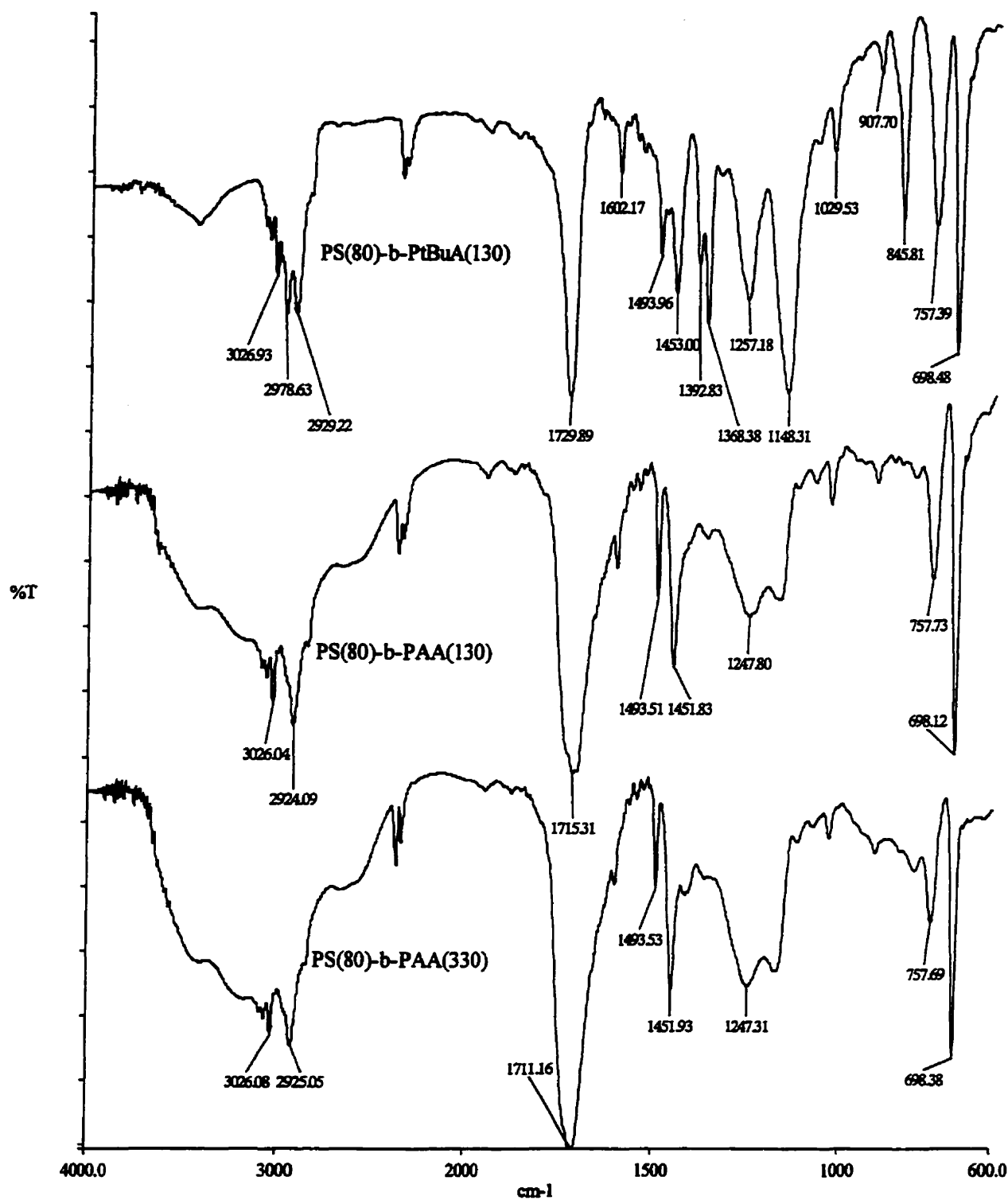
Appendices

Wavenumber (cm ⁻¹)	Assignment
<i>PS-b-PtBuA</i>	
3025	sp ² C-H stretch
2980	sp ³ C-H stretch
2930	sp ³ C-H stretch
1900, 1800	aromatic overtones
1730	ester carbonyl stretch
1395, 1370	<i>tert</i> -butyl deformation
1260, 1150	C-O stretch
845	olefinic C-H (unreacted monomer?)
760, 700	monosubstituted phenyl
<i>PS-b-PAA</i>	
3200 (v. br.)	OH hydrogen bonding
3025	sp ² C-H stretch
2930	sp ³ C-H stretch
1900, 1800	aromatic overtones
1715	carboxylic acid carbonyl stretch
1600, 1500, 1450	phenyl group
1250	C-O stretch
760, 700	monosubstituted phenyl
<i>PS-b-PTMEACI</i>	
3200	secondary amide NH bonding
3025	sp ² C-H stretch
2930	sp ³ C-H stretch
~2500	R ₃ N-H
1900, 1800	aromatic overtones
1680-1630	amide I
1570-1515	amide II
1600, 1500, 1450	phenyl group
	C-N
760, 700	monosubstituted phenyl

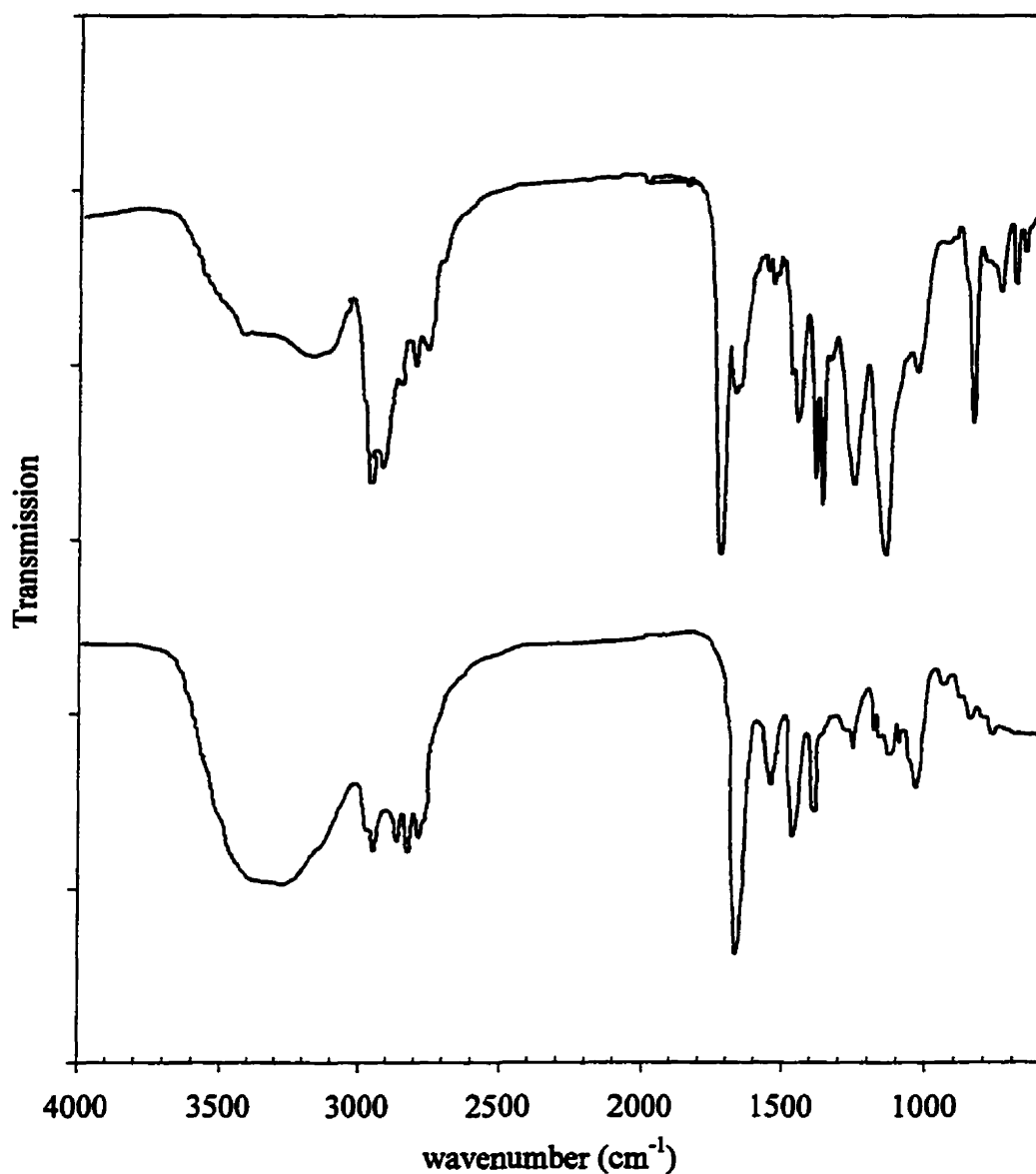
Appendix 1: Table of IR band assignments



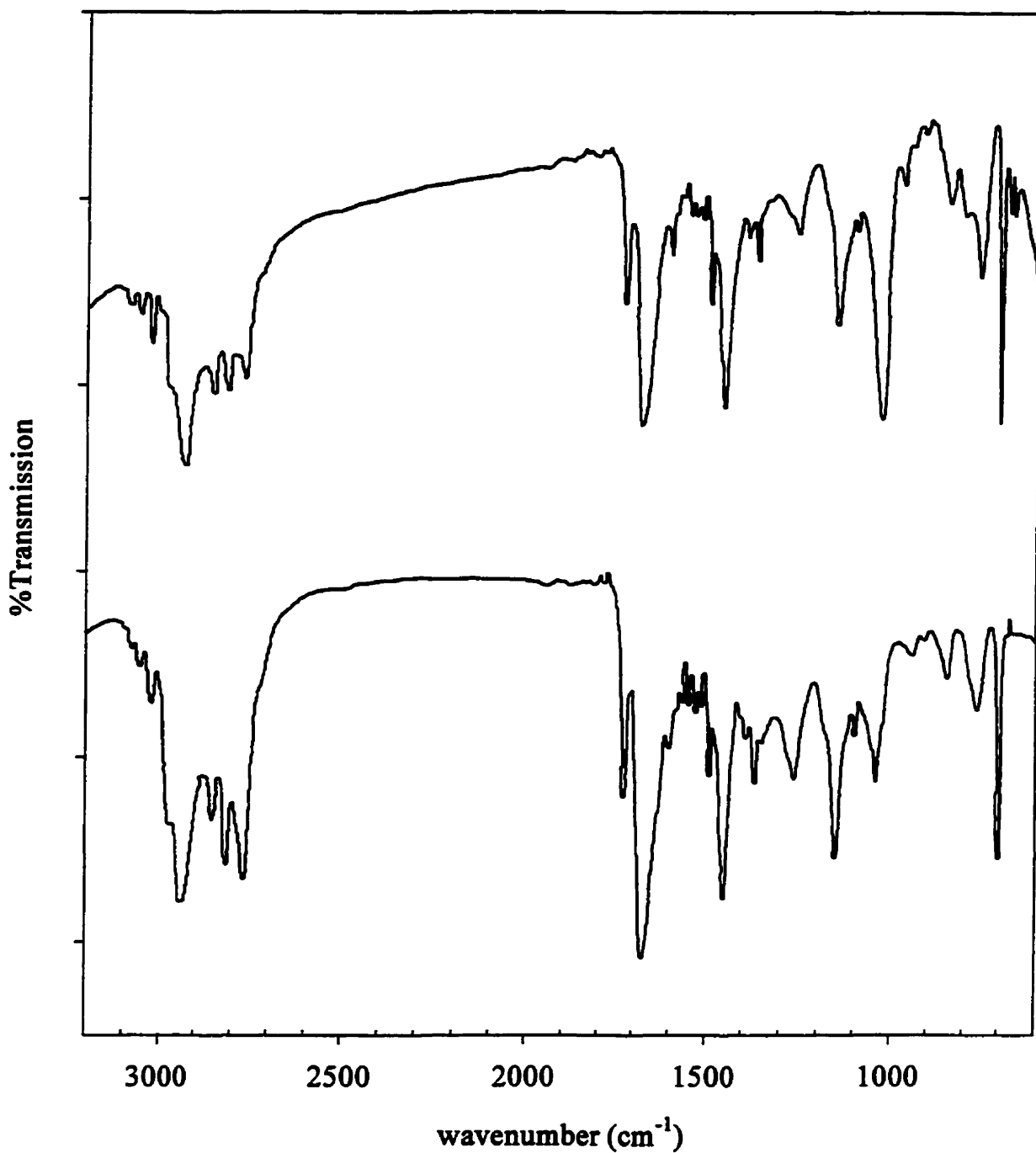
Appendix 2: FTIR spectrum for N,N-dimethylaminoethyleneacrylamide synthesis. The amide I and II bands are strong, but there is a carboxylic acid shoulder due to the hydrolysis of the acryloyl chloride starting material. The complicated H-bonding region ($\sim 3500\text{ cm}^{-1}$) and the ammonium bands ($\sim 2600\text{ cm}^{-1}$) bear out the difficulty in purification.



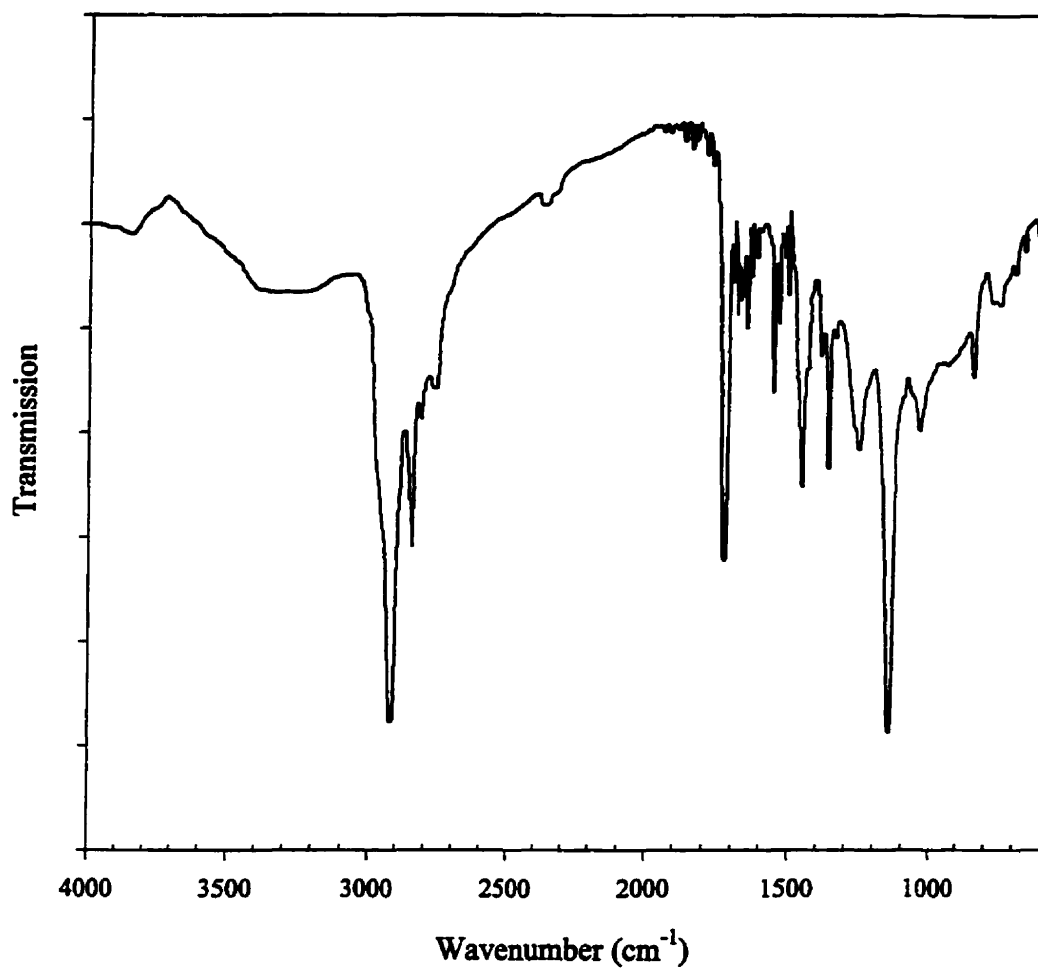
Appendix 3: FTIR spectra of one of the parent PS-*b*-PtBuA samples and a pair of derivative hydrolyzed polymers based on the same polystyrene block. Note the carbonyl band displacement (1730 cm^{-1} – 1710 cm^{-1}) and the *t*-butyl bands' disappearance. ($\sim 1390, 1370\text{ cm}^{-1}$).



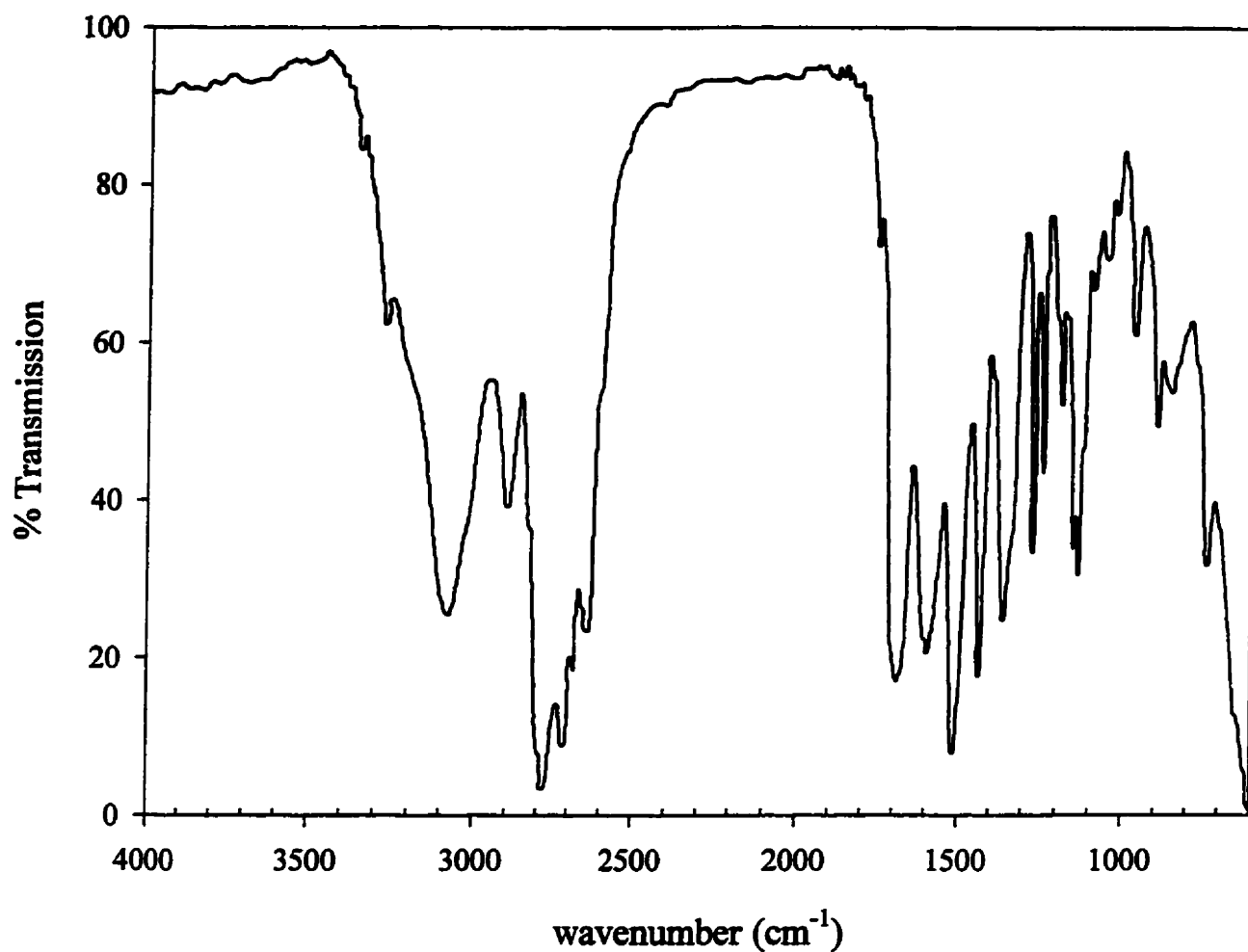
Appendix 4: FTIR spectra indicating PS-*b*-PtBuA transamidation with DMED at 4 hours (upper) and several days (lower). Note the growth of the amide I peak ($\sim 1650\text{ cm}^{-1}$), the complete effacement of the ester carbonyl peak ($\sim 1730\text{ cm}^{-1}$) and the weak amide II peak ($\sim 1600\text{ cm}^{-1}$).



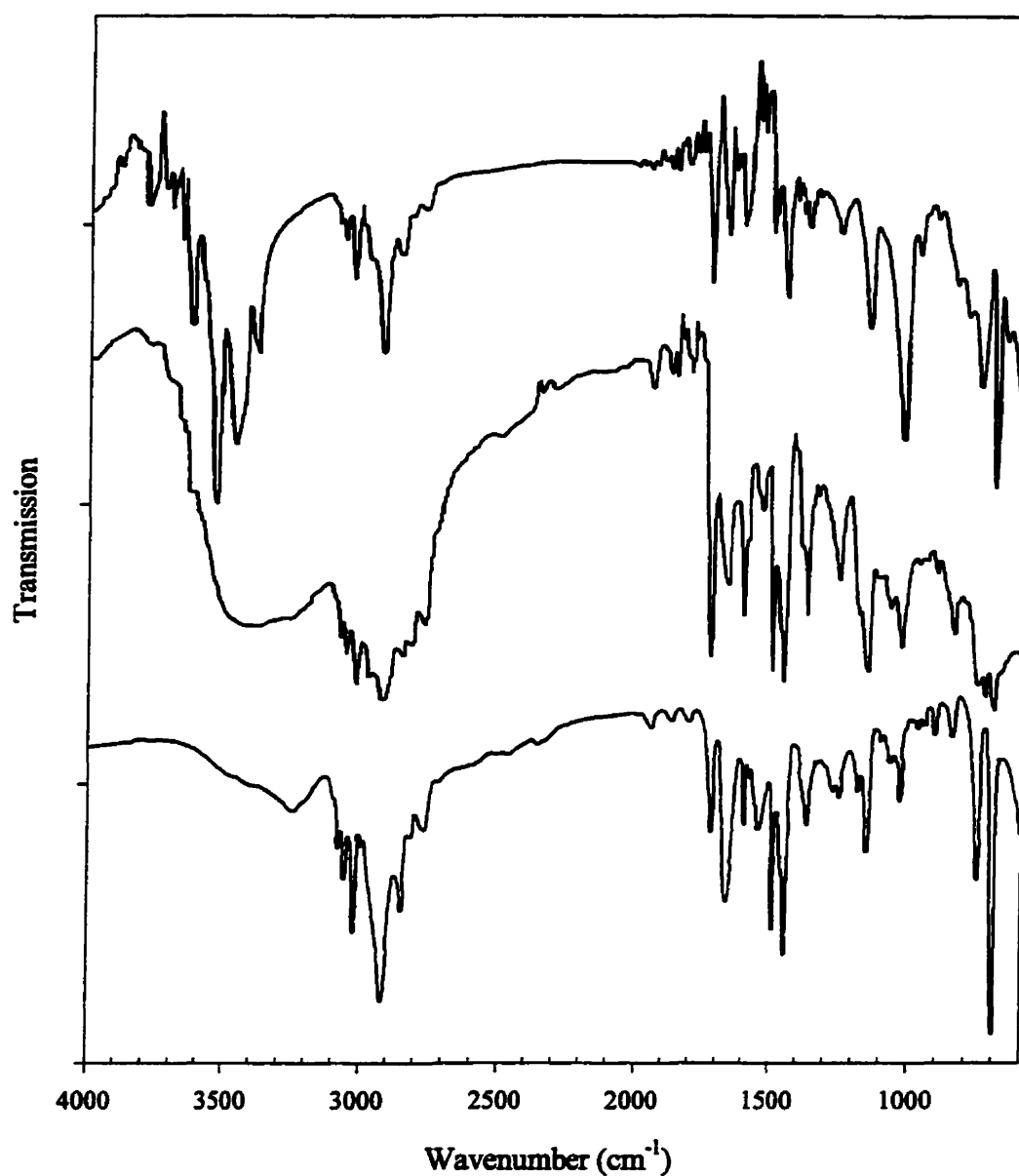
Appendix 5: FTIR spectra showing incomplete, but nevertheless substantial transamidation of two copolymers with DMED alone based on a PS(175) block (upper: PS(175)-*b*-PTMEACI(35), lower: PS(175)-*b*-PTMEACI(65)).



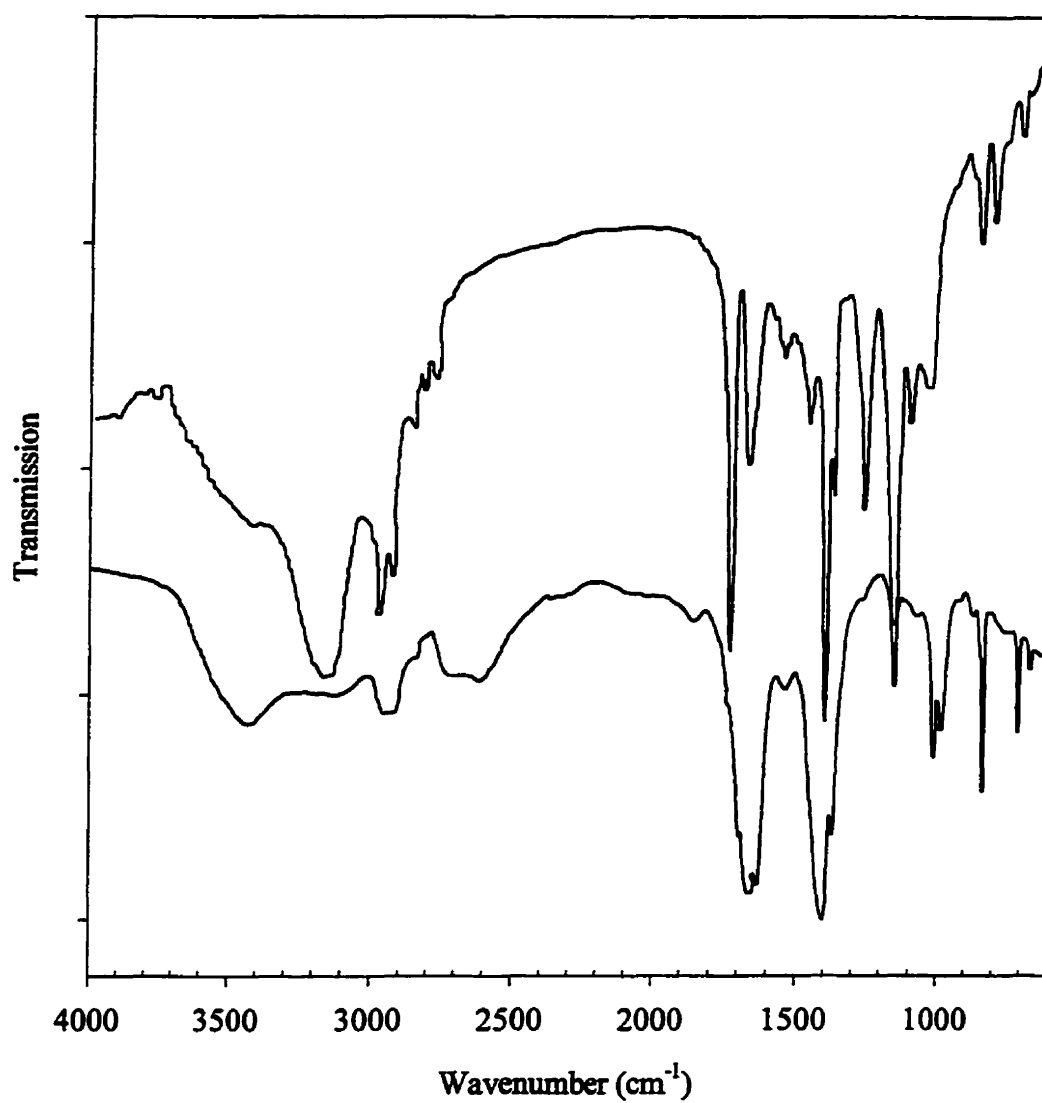
Appendix 6: FTIR spectra for the diethylaluminum amide functionalization of PS(25)-*b*-P*t*BuA(280). Note the dominant ester peak ($\sim 1735\text{ cm}^{-1}$) that nearly masks the hint of amide I ($\sim 1650\text{ cm}^{-1}$).



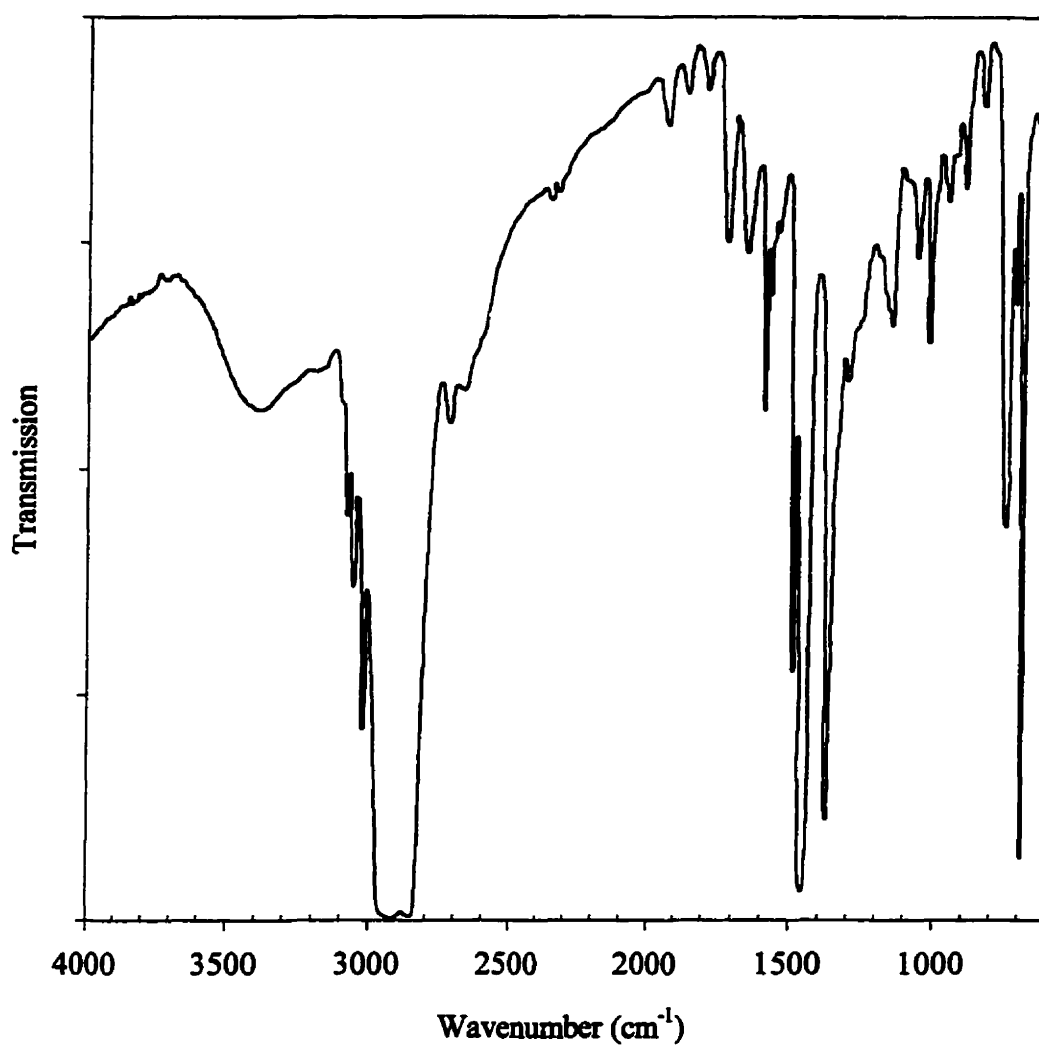
Appendix 7: FTIR product spectrum from the synthesis of N,N-dimethylaminoethyleneacetamide via the dimethylaluminumamide transamidation of a model small-molecule ester (ethyl acetate) showing virtually no residual ester carbonyl ($\sim 1735\text{ cm}^{-1}$) and strong amide I and II bands (~ 1650 and $\sim 1600\text{ cm}^{-1}$).



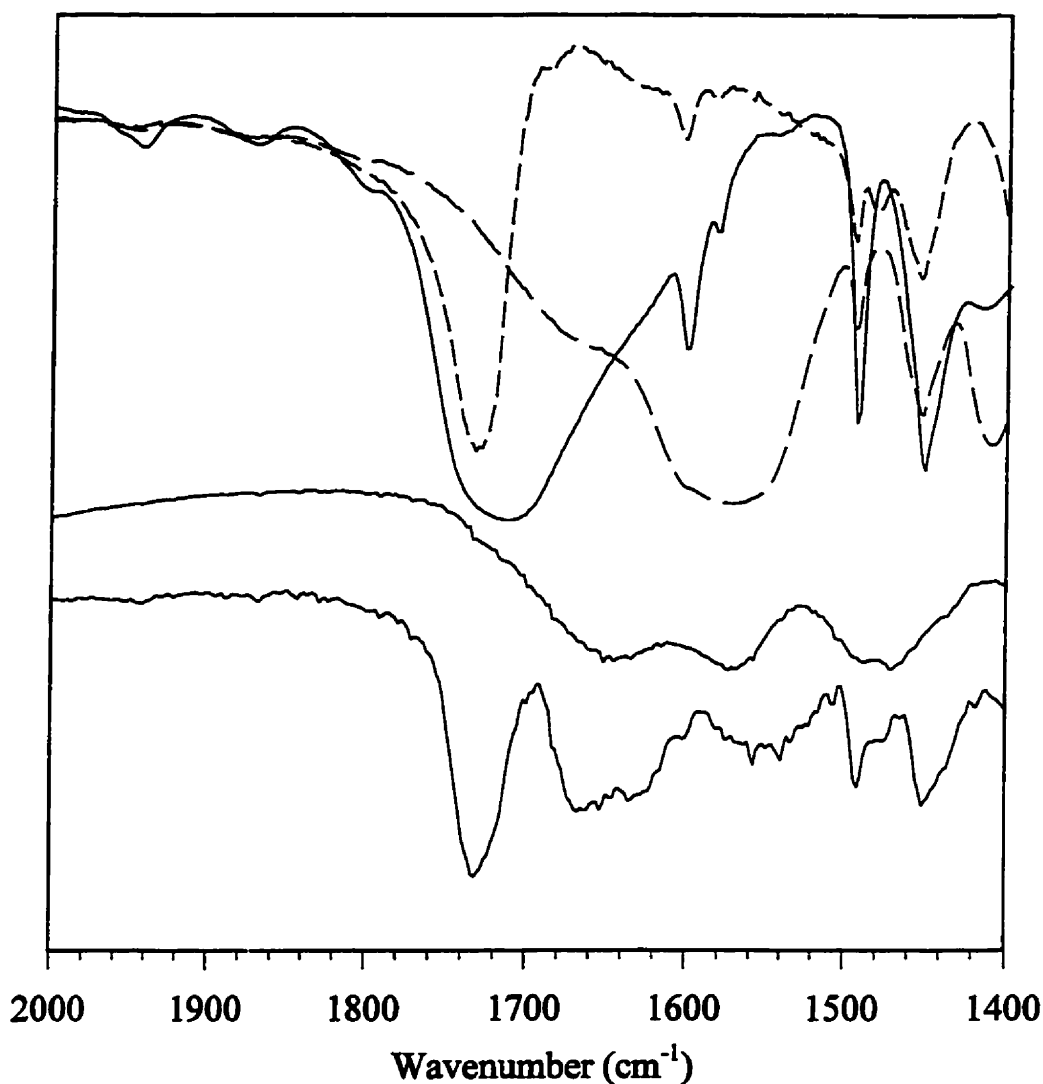
Appendix 8: FTIR spectra for the pendant amide copolymers from the PS(175)-*Pr*BuA family synthesized via the dimethylaluminum amide. Note the very weak residual ester peak ($\sim 1735\text{ cm}^{-1}$). Upper: direct transamidation of PS(175)-*b*-*Pr*BuA(35). Middle and Lower: transamidation of PS(175)-*b*-*Pr*BuA(35) and PS(175)-*b*-*Pr*BuA(65) via Me₂Al-amide.



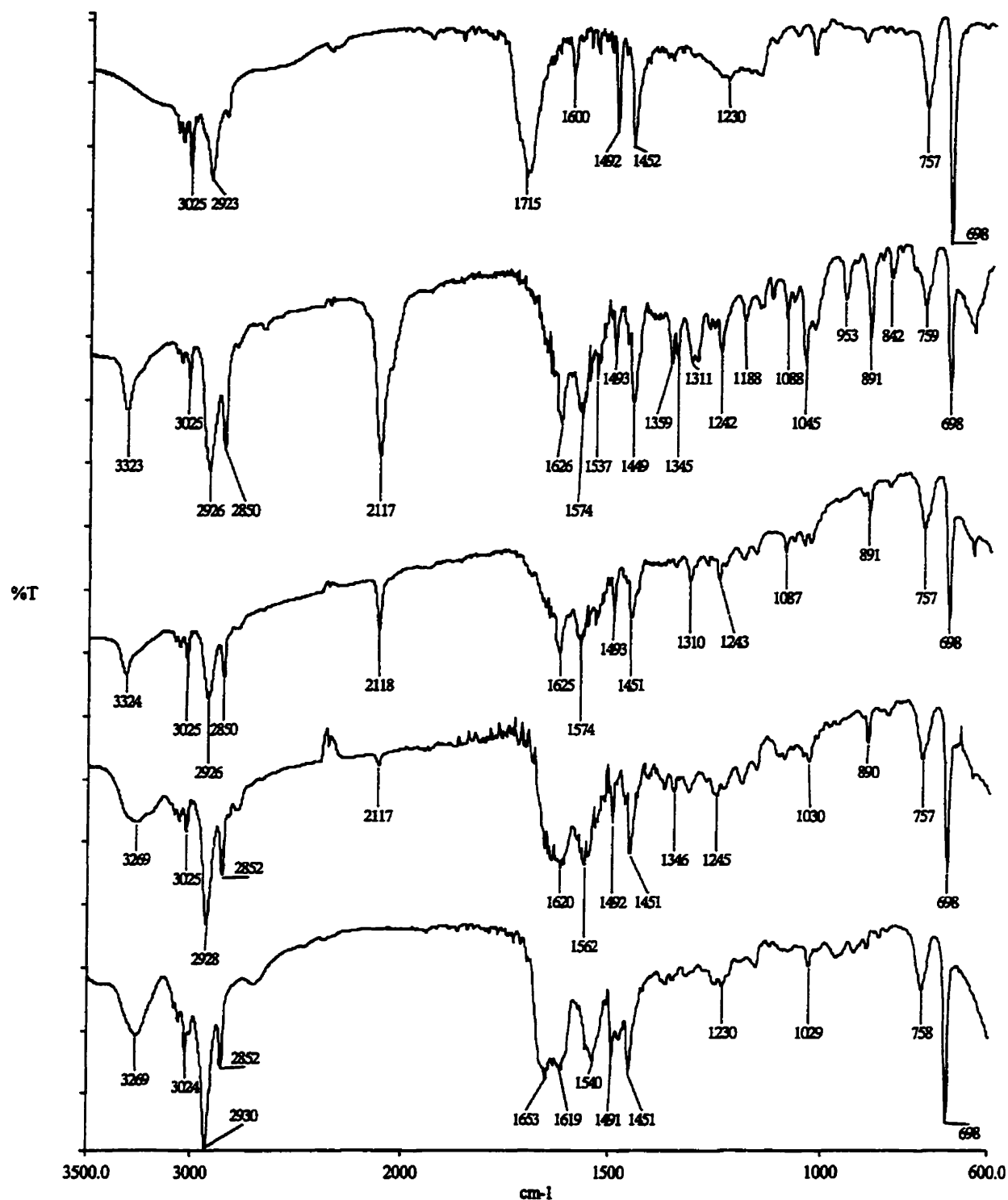
Appendix 9: FTIR spectra for the Me₂Al-amide transamidation of PS(25)-*b*-PtBuA(280) (upper) and quaternization (lower).



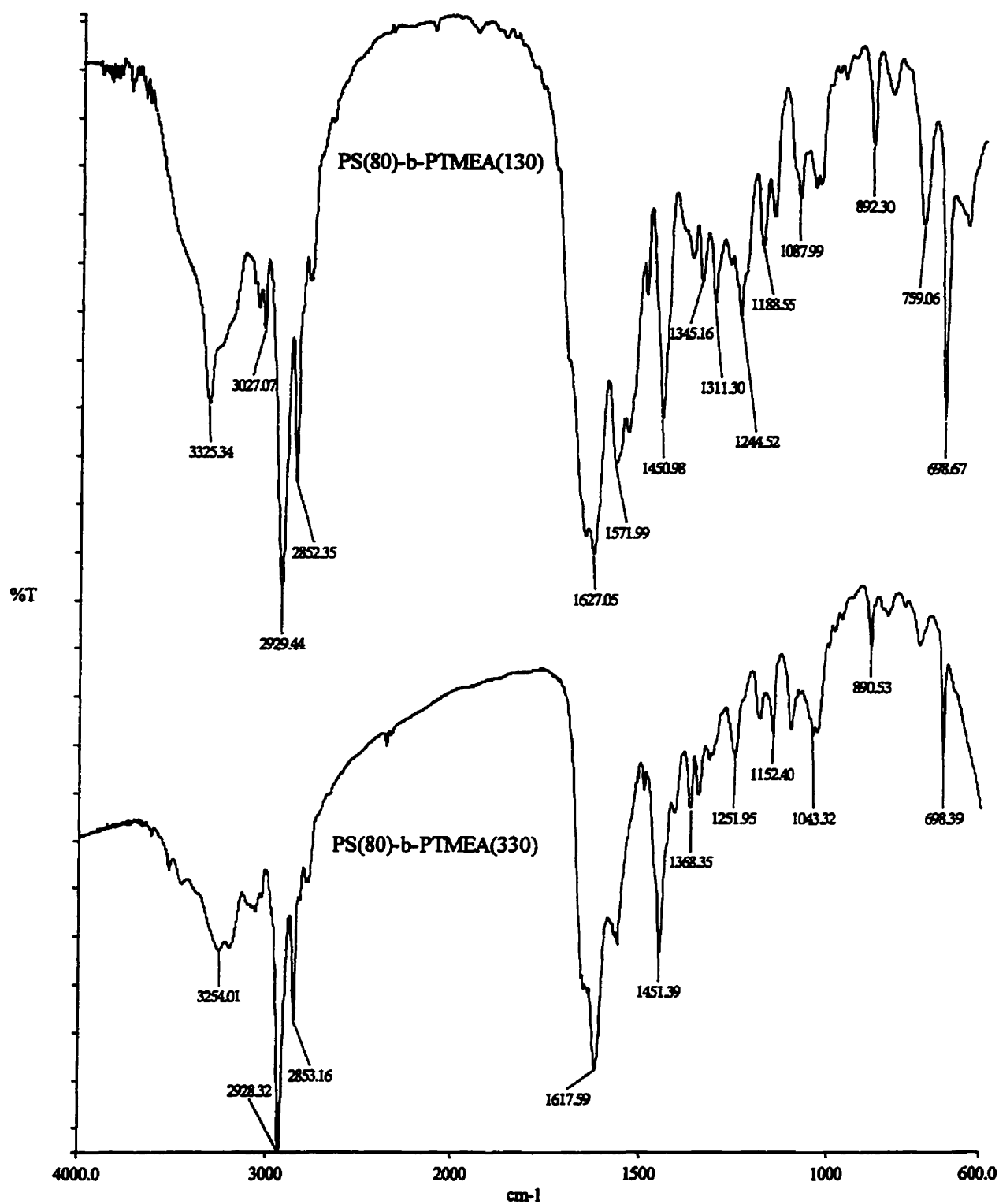
Appendix 10: FTIR product spectrum where the carboxylic acid groups of PS(385)-*b*-PAA(80) were converted to acid chlorides with SOCl₂, which were in turn reacted with DMED. Note the weak ester and amide carbonyl peaks in this spectrum (~1700 cm⁻¹).



Appendix 11: FTIR spectra for the oxalyl chloride mediated functionalization of PS-*b*-PAA showing in the upper triplet (from left to right) the ester (1730 cm⁻¹), acid (1710 cm⁻¹) and the sodium acrylate (1600 cm⁻¹). The lower pair shows the amidation product followed by the quaternization product. Note the reappearance of the ester peak due to incomplete amidation; unreacted acrylate units as well as N,N-dimethylamino pendant groups are methylated with methyl iodide hence the methyl ester.



Appendix 12: FTIR spectra for the DCC coupling of PS(95)-*b*-PAA(65) at $t = 0, 4$, and 30 hours, isolated product and quaternized product (from top to bottom). Note the lack of IR evidence for both impurities and N-acyl rearrangements as well as the strong amide I and II bands ($\sim 1650, 1600 \text{ cm}^{-1}$).



Appendix 13: FTIR spectra of the carbodiimide (EDC) coupling products. Traces of urea or N-acyl rearrangement are present ($\sim 3325 \text{ cm}^{-1}$ and shoulders on the amide peaks).

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Chapter 4

Morphology II: Multiple Morphologies of Polystyrene-*b*-Poly(N,N,N-trimethylammoniummethylene acrylamide chloride)

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Chapter 4: Multiple Morphologies of PS-*b*-PTMEACl

Abstract

This chapter presents studies of the new amphiphilic diblock copolymer polystyrene-*b*-poly(N,N,N-trimethylammoniumethyleneacrylamide chloride) (PS-*b*-PTMEACl) (see Figure 1), a derivative of PS-*b*-poly(*tert*-butylacrylate) (PS-*b*-PtBuA). The trimethylammonium-bearing copolymer differs significantly over the polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA)-type systems, reported previously, not only in that the charge on the corona-forming block is cationic, but also in that the full ionization of the hydrophilic block is expected. Whereas PS-*b*-PAA is extremely sensitive to pH, self-assembled aggregates of PS-*b*-PTMEACl present a uniformly charged polyelectrolytic brush. While ionizable systems such as PS-*b*-PAA are tunable by addition of acid or base, by comparison, PS-*b*-PTMEACl is expected to be comparatively immune to pH. The aggregation and self-assembly properties of this PS-*b*-PTMEACl system can, in principle, be adjusted by modifying the hydrophobicity of the hydrophilic block. The hydrophobic spacer between the charge and the amide-link to the backbone of the corona-forming block (in this case, ethylene) can be exchanged for more, or indeed less, hydrophobic moieties. For PS-*b*-PTMEACl, simple spheres (diameter ~ 20 nm), large compound micelles (diameter >100 nm) and larger, more complex architectures are presented below and the morphologies are discussed and compared with the systems presented in Chapter 2. The colloidal stability with respect to sodium chloride and as a function of particle concentration is also considered.

Introduction

The self-assembly of amphiphilic block copolymers in a solvent that is specific for only one of the blocks has been studied extensively¹, as discussed in Chapter 2. Of particular interest is the range of morphologies that is developed. In an aqueous environment, micro-phase separation of amphiphilic diblock copolymers gives aggregates with a core formed from the hydrophobic block, which is shielded from the solvent by the hydrophilic blocks. Among the simplest morphologies is the ‘star’ which is observed for strongly segregated diblock copolymers with short core-forming and long corona-forming blocks.² Studies of amphiphilic diblock copolymers in the crew-cut regime, where the core-forming block is much longer than the corona-forming block, have identified a vast array of aggregate morphologies, including spheres, rods, vesicles, large compound micelles (LCM), large compound vesicles (LCV) and hexagonally packed hollow hoops (HHH).³

Block length, common solvent, nature of the precipitant, concentration, and salt content as well as the method and rate of self-assembly induction are among the parameters that can be modified to induce morphogenic changes. Thermodynamic arguments for these complex modes of aggregation are beginning to be developed, as is our understanding of these morphologies and the driving forces to morphogenesis.⁴ *Grosso modo* it has been proposed that in the crew-cut regime, three contributions to the free energy of aggregation control the ultimate morphology: (i) the “stretching” of the hydrophobic segments in the core beyond their random coil dimensions, (ii) the interfacial energy between the core and the corona, and (iii) the repulsion among the corona-forming blocks, particularly near the core-interface. The minimum energy conformation of the hydrophobic segments is the unperturbed random coil, the interface tends to a minimum area and the coronal chains also seek to become a self-avoiding random coil. For

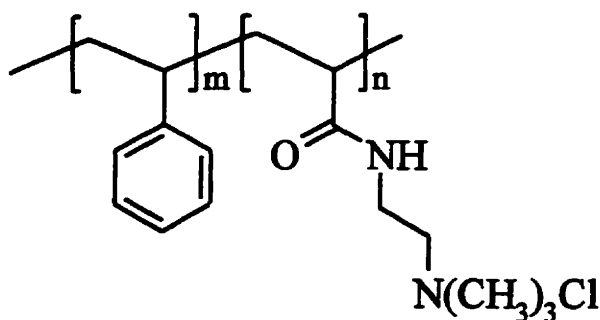
example, the minimum interfacial area for an amphiphilic diblock copolymer in an aqueous environment would require that all hydrophobic blocks be aggregated into one large sphere surrounded by a corona of water-soluble blocks. In order to have such a sphere, however, at least some hydrophobic chains would have to stretch into the center of the sphere. This entails at best a large entropic penalty. Furthermore, for short coronal chains, a high proportion of the repeat units have little freedom due to crowding near the interface. This combination leads to high-energy species.

Extensive studies in our laboratory, described in some detail in Chapter 2, have considered crew-cut aggregates of amphiphilic diblock copolymers where the core-forming block is polystyrene (PS), and the corona-forming block is poly(acrylic acid) (PAA).⁵ Although various methods have been investigated,⁶ we typically induce equilibrium aggregation of minimum energy by dissolving our polymers in a common solvent for both blocks to create a macromolecularly disperse solution whereupon we slowly add a precipitant for one of the blocks. The common solvent for the PS-*b*-PAA system has usually been N,N-dimethylformamide (DMF), the precipitant was de-ionized water and the initial copolymer concentration was on the order of 1% (w/w). It was demonstrated that as long as the acrylic acid content exceeds 10% mole fraction, nearly all aggregates formed are primary small spheres.⁷ As the acrylic acid content is decreased to 6-7 mol%, rods are observed and at 3-4 mol% acrylic acid, vesicles are formed. Furthermore, the addition of HCl changes the available morphologies radically. In the case of PS(410)-*b*-PAA(13), for example, the addition of HCl to a final concentration of 250 μ M induces the formation of large compound vesicles (LCV's) despite an initial copolymer concentration of only 1% (w/w).⁵ The addition of a protic acid quenches the polyelectrolytic character of the coronal blocks within the limits of the proton availability and the pK_a's

involved, thereby reducing the hydrophilic character of the corona-forming block. It also introduces the possibility of inter- and intra-chain hydrogen bonding between carboxylic acid groups. The addition of HCl to a suspension of previously formed micelles induces gelation, which upon sonication yields a ‘pearl necklace’ morphology.⁸

This Chapter presents preliminary studies of the new amphiphilic diblock copolymer polystyrene-*b*-poly(N,N,N-trimethylammoniummethyleacrylamide chloride) (PS-*b*-PTMEACl) (see Figure 1), a derivative of PS-*b*-poly(*tert*-butylacrylate) (PS-*b*-PtBuA). Due to the pendant trimethylammonium chloride groups, complete ionization, hence, the hydrophilic nature of the corona-forming block is assured. However, with the ethylene spacer separating the amide and the backbone from the charge-bearing group, the block retains a certain hydrophobic character. The hydrophilicity of the cationic corona-forming block is well suited to being fine-tuned via changes in the spacer size (and pH for the dimethyl analogue).

In this study, the mole fraction of acrylamide units ranged from 17 to 81%, *i.e.*, this group of samples touched only marginally what was expected to be the crew-cut regime. Nevertheless, both primary micelles and large compound micelles (LCMs) were observed in



nearly all cases, with the exception of PS(175)-*b*-PTMEACI(35) for which *only* LCMs were observed. Vesicles and non-equilibrium morphologies were also observed. The various block copolymer solution morphologies prepared under equilibrium and non-equilibrium conditions offer insight not only into the mechanism of aggregation, but also demonstrate the effect of hydrophobicity and hydrogen bonding in the corona.

Experimental

Polymer samples

Anionic polymerization

The PS-*b*-PTMEACI diblock copolymers summarized in Table 1 were prepared in several steps. First, PS-*b*-PtBuA was synthesized by sequential anionic polymerization in tetrahydrofuran (THF). *Sec*-butyl lithium was used as the initiator and the polystyrene block was end-capped with α -methyl styrene units. A sample of end-capped polystyrene was withdrawn for reference and the reaction temperature was reduced before the addition of the *tert*-butyl acrylate monomer. The details of this anionic polymerization method can be found elsewhere.^{9,10} The polymers generated were characterized by gel permeation chromatography (GPC) to obtain $\overline{M}_n$, $\overline{M}_w$, and the polydispersity index. For all samples of homopolystyrene and the diblock-copolymers, a narrow peak typical for anionic polymerization was observed. The degree of polymerization of the acrylate blocks was determined via NMR and/or FTIR.

Hydrolysis

The pendant ester groups were hydrolyzed to the corresponding carboxylic acid by refluxing at 110 °C in toluene in the presence of a catalytic amount of *p*-toluene sulfonic acid

overnight. FTIR showed no evidence of residual ester groups at either the carbonyl or the *t*-butyl bands and we therefore consider hydrolysis to be complete.

Post-polymerization functionalization

As described in Chapter 3, a variety of coupling reactions were employed in the course of this study in order to achieve quantitative conversion from the acid to the amide corresponding to the addition of *N,N*-dimethylethylenediamine (DMED). The qualitative criteria employed in judging a reaction pathway were: synthetic simplicity, reaction rate, completeness of reaction and ease with which pure product was obtained. The simplest and most successful reactions employed carbodiimides as coupling reagents. The resulting functionalized polyacrylamide was quaternized with methyl iodide in methanol in the presence of sodium bicarbonate. The iodide was exchanged for chloride, as described in Chapter 3, and the PS-*b*-PTMEACl (see Figure 1) was then dialyzed against de-ionized (milli-Q) water to render the copolymer clean. Any uncoupled carboxylic acid was esterified on addition of iodomethane, but FTIR spectroscopy showed little or no residual esterified carboxylic acid. These methods of functionalization as well as functionalization via acid chloride generated with either oxalyl chloride or thionyl chloride and direct conversion of the ester to the amide with an amine or via an aluminum amide intermediate were discussed more fully in Chapter 3.

Micelle suspension preparation

Salt-free

Following purification via dialysis, the polymer was lyophilized and the resulting powder was dissolved in DMF ($\leq 1\%$ w/w). De-ionized water was then added at a rate of about one drop every 10 seconds until the water content reached 6-10%. The resulting turbid or blue-tinged suspension was then dialyzed against distilled water for several days with frequent water changes to remove DMF.

With salt

In previous studies,¹¹ salt was added before micellization. In this study, however, we added sodium chloride to a suspension of micelles *after* dialysis in order to confirm the solution stability of the colloidal system in the presence of simple electrolytes.. As a control, a stock suspension of micelles was diluted with de-ionized water and also with an aqueous solution of sodium chloride to a final NaCl concentration of 5 mM. The resulting samples were then analyzed via transmission electron microscopy (TEM).

Transmission Electron Microscopy

The TEM instrument used was a Phillips EM410 operating at an acceleration voltage of 80 kV. A drop of aggregate-containing suspension was deposited on a copper grid coated first with a thin film of formvar and then with carbon. The specimen was left to permit evaporation of water at atmospheric pressure for several minutes. The sample concentration was chosen to maximize grid coverage without excess impingement, overlapping and possible flocculation.

Residual water was “wicked” away with a piece of absorbent paper (Kimwipe) and the grids were shadowed with palladium/platinum at an angle of about 30° using either a Baltzers or an Edwards Auto 306 evaporator.*

Image analysis

TEM negatives were scanned directly with a UMAX Astra 1200S scanner and a Relisys negative adapter operating at 150 dpi, 200% enlargement as grayscale images. The images were then imported into a SigmaScan Pro 5.0 (SPSS inc.) worksheet and calibrated automated area measurements were carried out to measure 100 or more aggregates per sample. An estimation of the diameters based on the reasonable assumption that the area of each aggregate was circular was calculated using simple algebra and the resulting data were manipulated into histograms using SigmaPlot 4.0/5.2 (SPSS inc.). The histogram data were then peak-fitted (PeakFit 4.0, SPSS inc.) with Gaussian bell curves to determine average diameters and modality.

Results and Discussion

For all polymer compositions, the aggregates observed were nearly all spherical to a first approximation. A typical grid had an abundance of discrete, rough-surfaced aggregates. Over all samples, diameters ranged from about 20 to 450 nm. Essentially there appears to be a bimodal distribution of particles for most samples. Despite the fact that LCMs may appear as the most distinct feature of the images, a carpet of primary aggregates often surrounds them. Occasional baroque structures are also observed and are presumed to be a result of kinetic

* The Baltzers' shadowing angle is much more precise and the quality of the carbon and metal films is exceptional. However, the operation of this evaporator is a time-consuming black art.

trapping due to the extremely localized high concentration of water as the drops are added to DMF near the critical water concentration (cwc).

PS- <i>b</i> -PTMEACl	ϕ ammonium units	SSS d (nm)	LCM d (nm)	PS length (nm)*	Chain length (nm)*
PS(80)- <i>b</i> -PTMEACl(330)	0.81	26	93	20	103
PS(95)- <i>b</i> -PTMEACl(65)	0.40	33	75	24	39
PS(175)- <i>b</i> -PTMEACl(65)	0.28	60	118	43	59
PS(175)- <i>b</i> -PTMEACl(35)	0.16	none	250, 328	43	51
PS(385)- <i>b</i> -PTMEACl(80)	0.17	45	73	86	116

Table 1: Summary of polymers synthesized. (ϕ = mol %, SSS= small simple sphere, d = diameter measured from TEM micrographs, *theoretical planar zigzag (fully extended) conformation).

Morphologies

Primary characteristics

A defining characteristic of a micelle is the critical micelle concentration (cmc) above which aggregation occurs and an equilibrium exists between aggregated and free molecules. It should be noted that styrene has a relatively high glass transition temperature ($T_g \sim 100^\circ \text{C}$) and furthermore is not wetted in water. Therefore, as water is added and the styrene is segregated from the increasingly aqueous environment, the architecture is essentially “frozen”. Since the cmc for such a polymer in water is so low as to be essentially meaningless, it is therefore a simplification to refer to these aggregates as micelles. Nevertheless, since these aggregates resemble morphologies seen in true micelles where the aggregates and unimers are in dynamic

equilibrium, block copolymer aggregates are conventionally referred to as micelles and therefore these terms will be used interchangeably.

Primary spheres of PS-*b*-PTMEACl in aqueous media are expected to have a styrene core and ammonium-containing corona. The electron density contrast between core and corona-forming blocks was not sufficient to distinguish the two via TEM. The fully extended polystyrene block dimension provides ample bulk to fill all the primary spheres observed. The predominance of primary micelles can be indirectly observed on sample preparation. As one might expect from light scattering, due to the colloidal size and low concentration of the particles, they create a blue-tinged turbid suspension. A suspension of larger particles is more opaque. For the largest particles, settling was observed, though re-suspension involved only gently shaking the sample vial.

Considering that for PS-*b*-PAA, when the mole fraction of acid unit exceeds 10%, only primary spheres are observed under standard conditions and that in all cases for PS-*b*-PTMEACl, the mole fraction of charge-containing groups equals or exceeds 16%, we might expect to see primary spheres. Indeed, primary spheres were observed for all samples except PS(175)-*b*-PTMEACl(35) (see Figure 2). The size of this smallest aggregated morphology is regulated to a large extent by the length of the core-forming block and the aggregation number. Very large primary spheres with high aggregation numbers require the core-forming blocks to stretch well beyond their minimum energy conformation until other aggregation modes (such as the LCM formation) become energetically preferred. For the PS(175)-*b*-PTMEACl(65) sample (see Figure 2), the average diameter of primary micelles was near 60 nm. If the actual structure is not core-swollen by polystyrene homopolymer and if the overall architecture is star-like, as is expected, this corresponds to nearly fully extended polystyrene blocks (86 nm). It is perhaps less

surprising therefore that the PS(175)-*b*-PTMEACl(35) sample gives a population of what appear to be LCMs (bi-modal with average diameters of 250 nm and 328 nm) where primary micelles are not possible due to the short coronal chains that favor higher aggregation numbers. The large ‘transitional’ primary spheres of PS(175)-*b*-PTMEACl(65) are composed of chains that when fully extended are only 59 nm long (average). Indeed, these spheres may be nascent LCMs. Thus, we infer that 65 coronal units per chain is near the solubilizing limit for primary spheres in this polymer family. By comparison, the PS(175)-*b*-PAA(35) derivative of from this family of samples (for which the coronal block is very hydrophilic), has been independently studied¹⁰ and forms only small spheres in pure water with a diameter of 25 nm having a standard deviation of 1.4 nm.

LCM formation mechanism

Large compound micelles (LCMs), such as those formed from PS(175)-*b*-PTMEACl(35) can form by at least two pathways: (i) on addition of non-solvent for one of the blocks, the non-soluble blocks aggregate to create a continuous phase with islands of corona-forming blocks nested throughout and surrounded by a coronal shell at the core-solvent interface. (ii) Alternatively, simple spheres can form, but corona-chain interpenetration occurs and thus the continuous phase is coronal blocks with pockets of insoluble blocks (Figure 3). These two alternatives correspond to the extremes of morphology observed in the bulk for diblock copolymers. A ‘spectrum’ of spheres to lamellae followed by continuous-phase inversion and the inverse morphological progression to spheres observed for PS-*b*-PI as a function of volume fraction¹² parallels the ultimate architecture of LCMs formed in solution. The especially rough texture of the LCMs arising from PS(385)-*b*-PTMEACl(80) (see Figure 4), suggests that in this

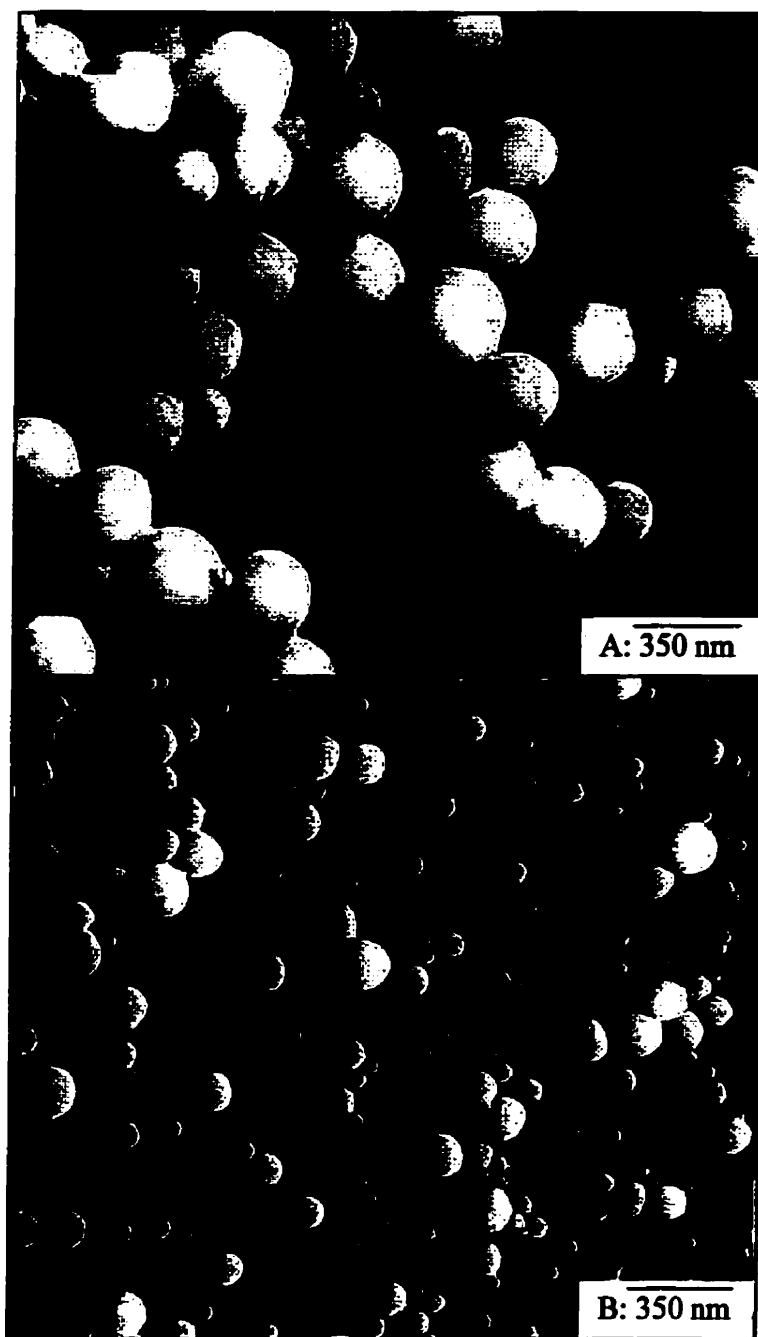


Figure 2: Transmission electron micrographs (TEM) demonstrating the morphologies within a family of PS-*b*-PTMEACl. A: LCM's from PS(175)-*b*-PTMEACl(35); B: Mixed morphologies from PS(175)-*b*-PTMEACl(65).

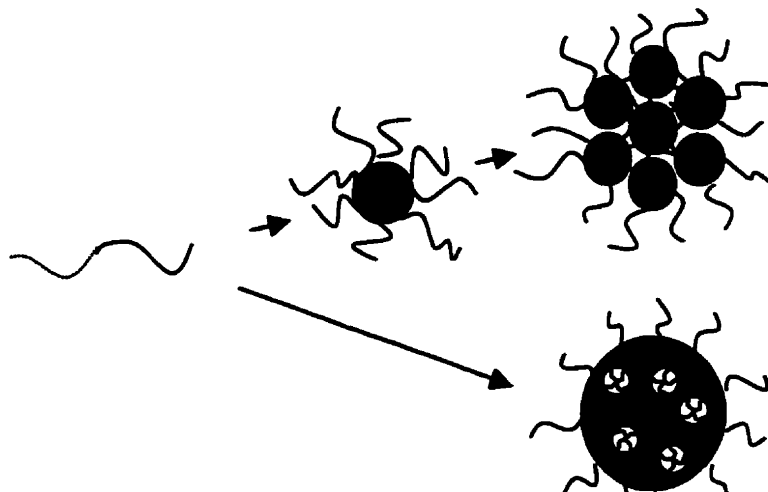


Figure 3: Schematic representation of chain entanglement and direct LCM formation pathways.

case, chain entanglement is the primary mode of formation in effect. This mode of aggregation is somewhat counter-intuitive given the charge-bearing coronae. However, the hydrophobic pendant spacer and possible hydrogen bonding between amide groups may compete with electrostatic repulsion.

The PS(175)-*b*-PTMEACI(35) sample formed the least visually complicated LCMs by TEM, but this observation needs some explanation as PS(385)-*b*-PTMEACI(80) has the same mole fraction of acrylamide units but forms primary spheres. In this latter case, the styrene block is more than twice as large as the styrene in the PS(175) family and therefore the free energy of stretching of the core blocks is perhaps less important. If there was significant ‘killing’ between the PS block polymerization and the acrylate block addition, residual homo PS may contaminate this sample. Hence, for PS(175)-*b*-PTMEACI(35), the force balance between stretching of the core blocks, extension of the corona blocks and the interface energy as well as the possibility of residual homo core-forming polymer dictates that primary micelles are energetically too expensive to permit their formation..

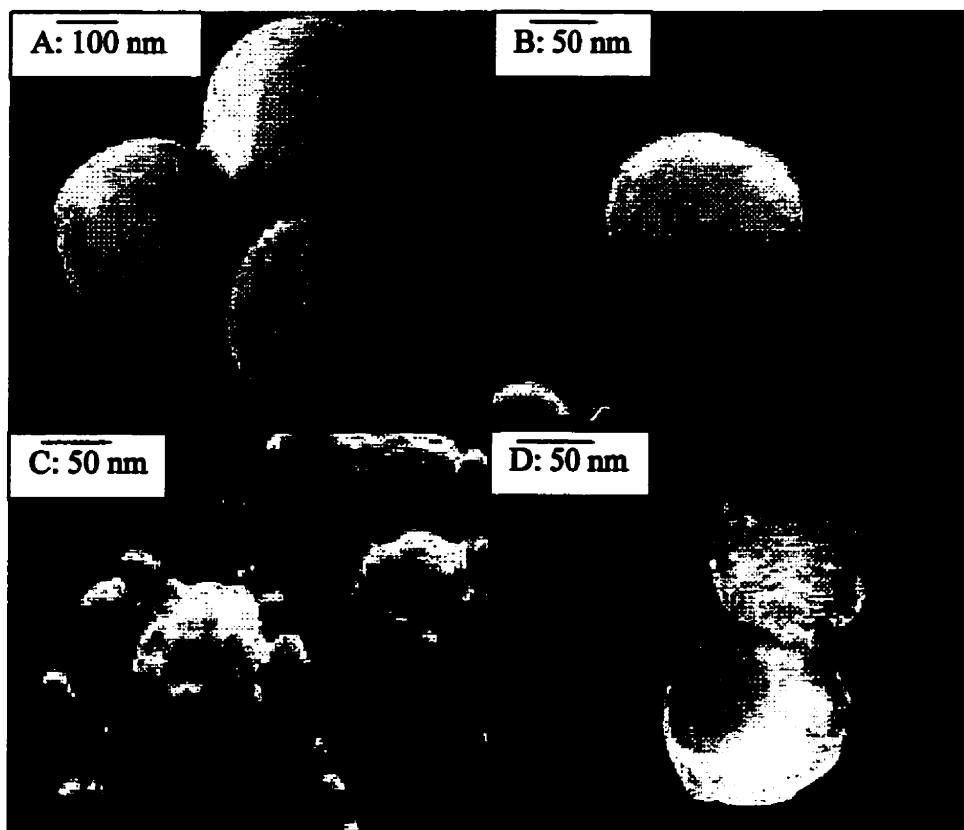


Figure 4: TEM micrographs of various large compound micelles (LCMs):
 A = PS(175)-*b*-PTMEACI(35); B = PS(175)-*b*-PTMEACI(65);
 C = PS(385)-*b*-PTMEACI(80); D = PS(80)-*b*-PTMEACI(330).

Bilayer structures

The formation of non-equilibrium structures including vesicles and lamellae was also observed (see Figure 5). While primary spheres and LCMs dominated, other structures were occasionally observed suggesting that kinetic trapping also occurs. It is probable that in the process of formation, some aggregates do not have sufficient time to achieve a structure with an energy minimum. Since the cores become essentially glassy, even at low water concentration, macromolecular dynamics and chain transfer processes are dramatically slowed down. As the

water concentration approaches the cwc, the annealing time between water drop addition would have to be substantially increased from roughly ten seconds per drop in order to avoid these kinetic oddities.

Figure 5A shows a hollow sphere with internal surface features. The emptiness of this structure can be inferred from the electron-transparency of the subject. Were it not for this polymer void, the surface texture and occlusions would be much more difficult to observe. Figure 5B focuses on a dimpled aggregate that may be a collapsed, thick-walled vesicle. Figure 5C demonstrates a mesoscopic bilayered architecture that hides a significant population of much smaller primary spheres and vesicles. The center of Figure 5C is enlarged and presented in Figure 5D where the corpuscular features are more obvious. Though these aggregates were rare, they do indicate that PS-*b*-PTMEACl can offer the same sort of morphological variation exhibited by the PS-*b*-PAA and PS-*b*-PEO crew cut systems that have been studied so intensively (see Chapter 2).

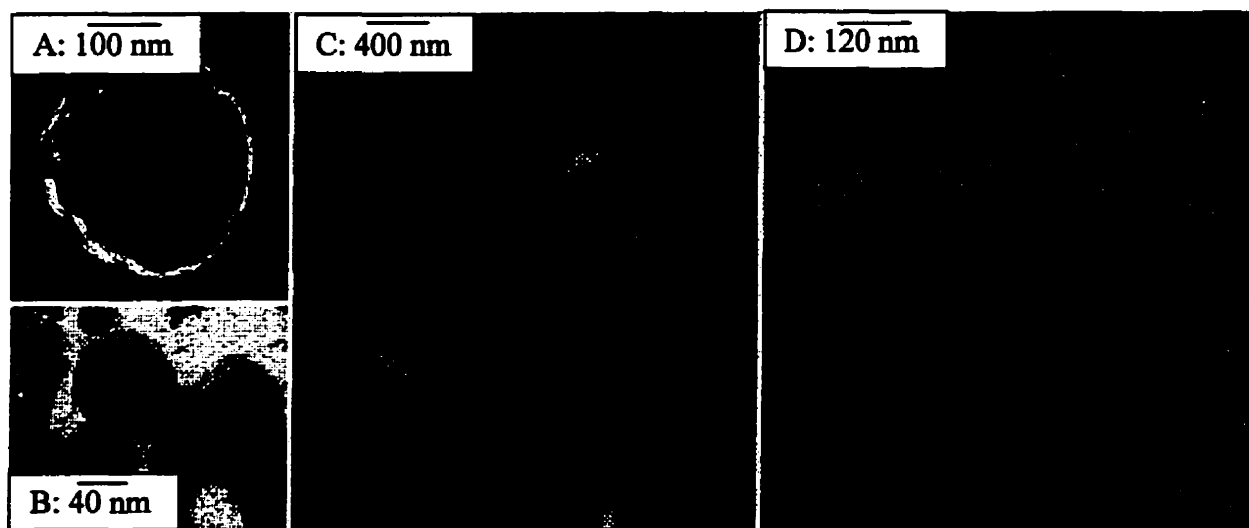


Figure 5: TEM micrographs of *baroque* structures. A: PS(80)-*b*-PTMEACl(331) shell structure of diameter (300nm); B: PS(385)-*b*-PTMEACl(80) dimpled architecture (55nm); C: PS(175)-*b*-PTMEACl(65) interconnected vesicles with simple spheres; D: PS(175)-*b*-PTMEACl(65) micrograph enlargement from (C) showing small spheres, interconnected spheres and vesicles.

Population histograms

When the populations of aggregates (see Figure 6) are put in the context of their parent chain maximum dimensions, the morphologies become clearer. As long as the sphere diameter remains less than twice the maximum chain dimension, then simple “micelles” are a likely possibility, especially given that these polymer samples are not monodisperse and therefore necessarily contain some chains that exceed the average degree of polymerization. These chains facilitate larger aggregates. In practice, it is easy to distinguish simple spheres from higher order morphologies, especially when both are present since the simple spheres are so much smaller than the structurally more complex LCMs.

The population histograms presented in Figure 6 offer two interpretations: a profile with a single peak and tailing, or a bimodal distribution. Given the visual impact of the micrographs and the low polydispersity characteristic of the anionically synthesized parent polymers that ought to propagate through to the aggregates, it seems most reasonable to assign multiple peaks to the histogram profiles, with the maxima at small diameters referring to the simple spheres, and the larger populations to the LCM aggregates.

The histograms in Figure 6 are presented in order of decreasing corona-forming repeat unit mole fraction ϕ . The uppermost histogram in Figure 6 corresponds to PS(80)-*b*-PTMEACl(330) where ϕ is 0.81. For this sample, the long corona-forming blocks limit the aggregation number by electrostatic repulsion and crowding. The simple sphere diameter for this polymer is therefore relatively small (mode = 30 nm). However a substantial fraction of the aggregates are present as LCMs with an average diameter of 93 nm. The following sample, PS(95)-*b*-PTMEACl(65) has a similar size of core-forming block, but ϕ is 0.40. With fewer corona-forming units, the decreased crowding in the corona favors an increase in the aggregation

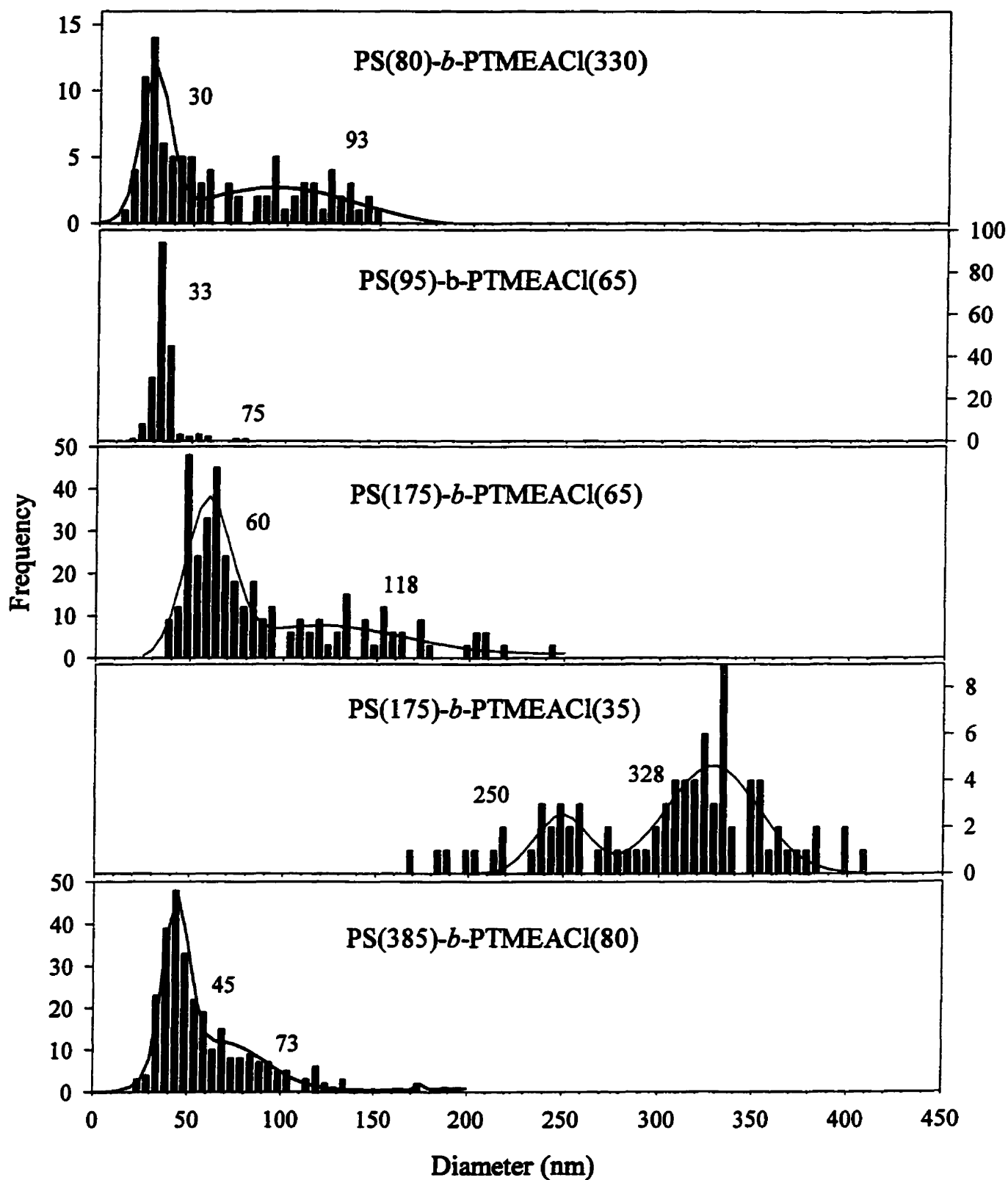


Figure 6: Population histograms for PS-*b*-PTMEACl. Peak maxima are indicated.

number, as reflected in the slightly larger simple spheres (33 nm). Very few LCM's are observed. When ϕ is 0.28 (PS(175)-*b*-PTMEACl(65)), the modal small sphere diameter is 60 nm, *i.e.*, substantially increased. These large simple spheres can be considered transitional, since the related PS(175)-*b*-PTMEACl(35) sample, where ϕ is 0.16 yields no small spheres and forms only LCMs. Finally, when the hydrophobic styrene block is sufficiently long to space fill the core of a simple sphere as dictated by the aggregation number determining corona-forming block, small spheres are once again formed as demonstrated for PS(385)-*b*-PTMEACl(80), where ϕ is 0.17 (see Figure 5B).

Comparison of the size distributions of PS(175)-*b*-PTMEACl(35) and PS(175)-*b*-PTMEACl(65) shows that by changing only the hydrophilic block length, drastic morphogenesis can be induced. For diblock systems within the same morphological regime (for example, simple spheres as presented in Chapter 2), the aggregate diameter is primarily dependent on the core-forming block: PS(80)-*b*-PTMEACl(331) and PS(385)-*b*-PTMEACl(80) follow this trend where short styrene blocks form small primary aggregates (average diameter = 30 nm) and long styrene blocks form large primary aggregates (45 nm). In order for simple spheres to form, there must be sufficient ammonium-bearing units to solubilize the styrene, to limit the aggregation number and the PS blocks must not be obliged to stretch beyond the limits imposed by the free-energy penalty to space-fill the core. For PS(175)-*b*-PTMEACl(65), where there are insufficient ammonium-bearing groups, transitional and LCM spheres self-assemble (60 nm and 120 nm) leading to the higher-order aggregates formed by PS(175)-*b*-PTMEACl(35) (250 nm and 328 nm).

Trends in the LCM diameters for all five samples are less easily explained. From the outset, it seems counter-intuitive that a polymer sample of low polydispersity that is induced to

self-assemble would form a mixture of aggregates. However, in Chapter 2, we presented examples of mixed morphologies that have been shown to arise from wide co-existence regions in the phase diagrams between pure morphologies. Self-assembly for PS-*b*-PTMEACl is more complex than for PS-*b*-PAA due to the range of possible interactions within and among the corona-forming blocks. The compositional heterogeneity of the coronae suggests that the most probable explanation for the various LCMs presented here is that at some stage in the self-assembly process, they were thermodynamically stable, and on continued addition of non-solvent for PS, they remain kinetically trapped. It is also possible that in the anionic synthesis of the parent diblock copolymers in the PS(175) family, significant killing on the addition of the acrylate left the sample contaminated by homo-polystyrene. Work in our laboratory has confirmed that the addition of homo core-forming polymer leads to core swelling for PS-*b*-PAA.¹³

The baroque structures may be best understood by drawing parallels to the PS-*b*-PAA system. We have demonstrated that PS-*b*-PAA is extremely sensitive to counter-ion valence and concentration.¹¹ Adding CaCl₂, for example, during the formation of aggregates from PS(410)-*b*-PAA(13) at extremely low concentration (as little as 0.03 Ca²⁺ ions per repeat unit) induces vesicle formation from a polymer that forms simple spheres in the absence of added ions. Due to the added complexity of the amidation for PS-*b*-PMEACl, it is possible that despite repeated dialysis and the lack of IR evidence for substantial incomplete functionalization, these samples were very slightly contaminated by reaction byproducts or ionic species beyond our detection limit. Due to the hydrophobic spacer between the backbone and the ammonium group, we expect the polymer to be even more sensitive than PS-*b*-PAA to nanoscopic changes in

environment, thus while any given sample ought to be self-consistent, there remains the possibility of occasional eccentricities.

Effect of simple salt on corona

Preliminary investigation of micelle stability demonstrates that the aggregates are robust. Addition of sodium chloride before and during micellization to a block polyelectrolyte such as PS-*b*-PAA induces morphogenesis. Previous studies show that a polymer that self-assembles to form simple spheres with de-ionized water as the precipitant (e.g., PS(410)-*b*-PAA(25)), can be induced to form short rods, long rods, vesicles and large compound vesicles (LCV's) by adding salt to the precipitant (water).⁸ Similar morphogenic effects are to be expected for PS-*b*-PTMEACl. However in the present work, where an indication of colloidal stability was required, sodium chloride was added *after* micellization and dialysis.

We expect the polystyrene core to be glassy, solvent-free and as a consequence we expect the micelles to be stable. It should be noted that block copolymer micelles appear to be intrinsically stable. Even when the glass transition temperature (T_g) of the core-forming block is very low, as can be the case for polybutadiene-*b*-poly(acrylic acid) (PBD-*b*-PAA), LCMs, rods, and primary structures are formed.¹⁴ In this context it is not surprising that the addition of small amounts of salt to "frozen" aggregates (primary and transitional spheres) of PS-*b*-PTMEACl perturbs only the micellar dimension without disrupting the integrity of the structure (see Figures 7 and 8). Indeed, the size of the simple spheres appears to be only slightly affected by salt addition and the main impact is on the sizes of the larger aggregates. Due to the polyelectrolytic coronae, addition of counter-ions shields the charges on the chain from each other and thus the interface to chain-end distance decreases, thereby decreasing the free volume within the corona.

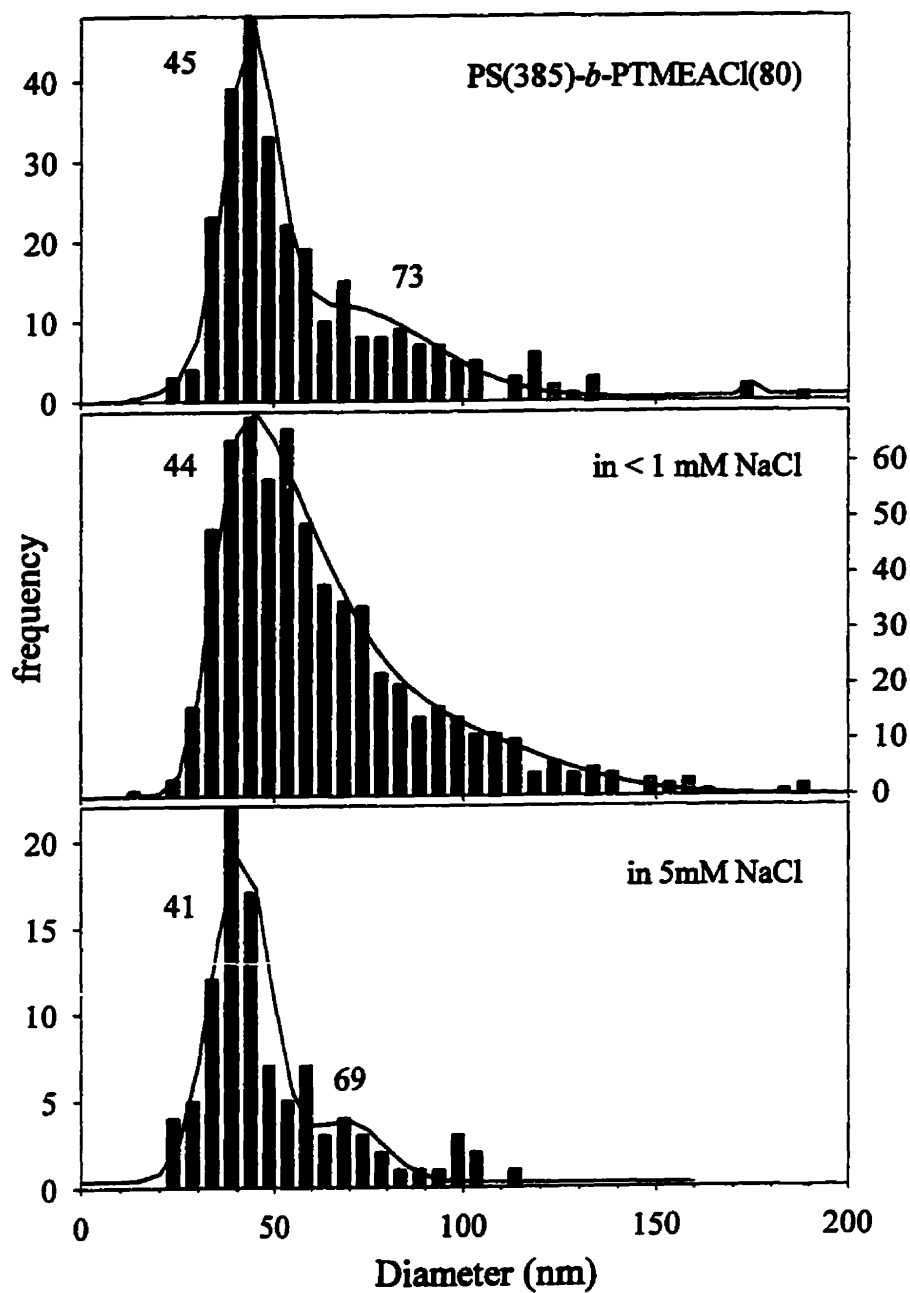


Figure 7: Population histograms showing the NaCl effect on aggregate diameter.

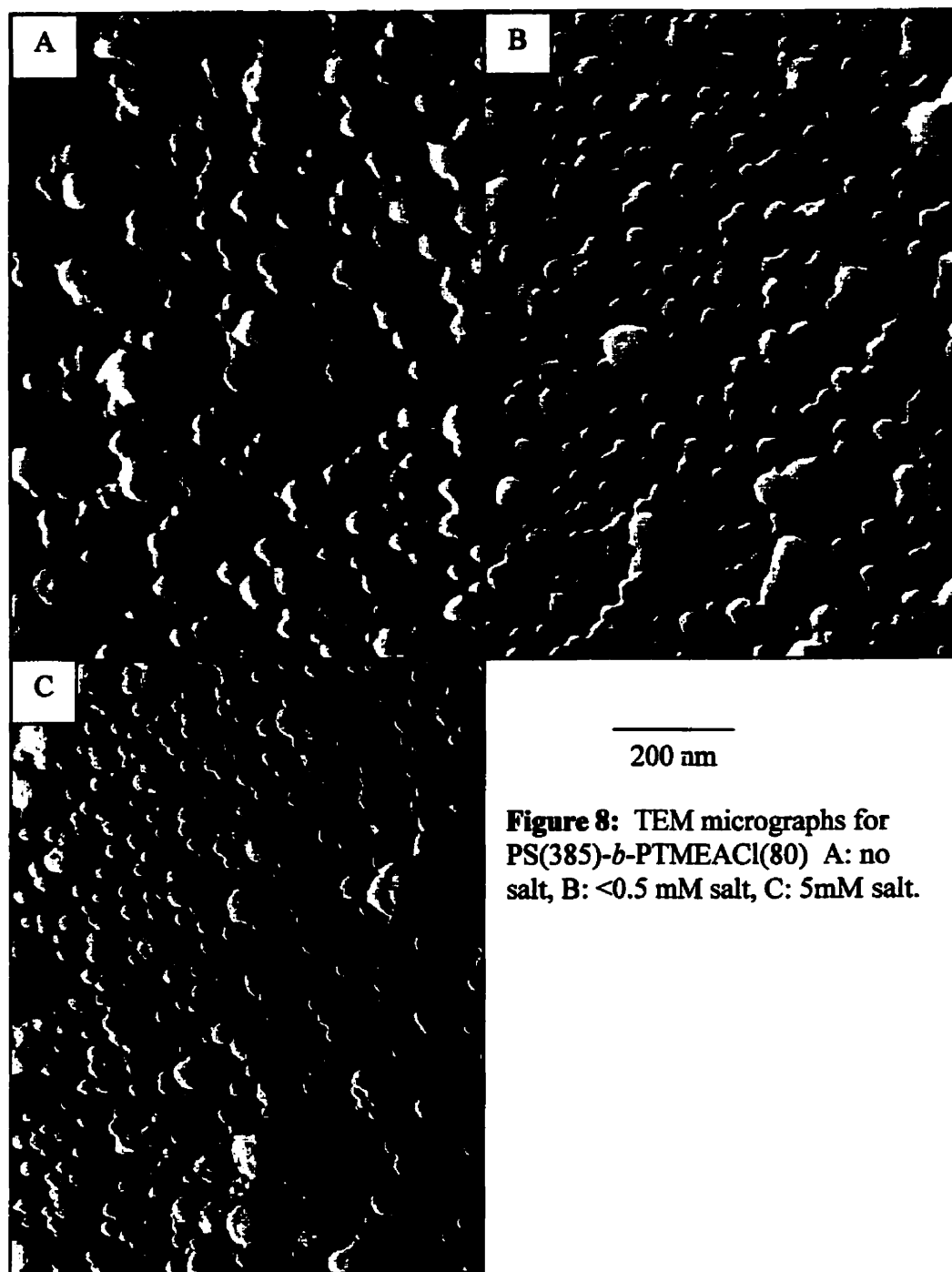


Figure 8: TEM micrographs for PS(385)-*b*-PTMEACl(80) A: no salt, B: <0.5 mM salt, C: 5mM salt.

LCMs and primary micelles with polyelectrolytic coronae collapse slightly in the presence of salt, but once fully formed and in pure water, close proximity does not appear sufficient to induce coronal chain entanglement and morphogenesis (see Figure 8). An early TEM assay, where the aggregate concentration was too high to give isolated spheres on the grid led to the micrograph in Figure 9. The intimate contact of these micelles is an artifact of the TEM grid preparation. However it remains clear that the aggregate concentration was higher than for the other samples yet each sphere retains coherence.



Figure 9: TEM micrograph of PS(385)-*b*-PTMEACl(80) with high polymer and salt content induced flocculation.

Summary

We have presented a novel amphiphilic diblock copolymer that self-assembles into nano and meso structures composed of a polystyrene hydrophobic phase and a cationic trimethylammonium chloride containing hydrophilic phase. The most prevalent observed morphologies are primary spheres and LCMs. However, more complex, apparently kinetically trapped structures are occasionally observed and indicate a rich field of morphological investigation. Among the interesting facets of this polymer is the hydrophilic corona-forming block with hydrophobic characteristics, which makes PS-*b*-PTMEACl more sensitive than PS-*b*-PAA to precipitation and renders the corona block less 'able' to solubilize the styrene blocks. The ammonium-containing block is also interesting because of the secondary amide link between the ammonium group and the backbone and the implied potential for hydrogen bonding. Hydrophobic interactions and hydrogen bonding make chain entanglement a reasonable mechanism for LCM formation. Once the plasticizing common solvent is completely removed from the system via dialysis, the micelle architecture is stable, though the coronae can be induced to collapse slightly in the presence of sodium chloride. Even at high polymer concentration, the aggregates seem to maintain coherence.

Future work will include a more detailed investigation of the solution morphology, hydrophobic, electrostatic and hydrogen-bonding interactions as well as post-polymerization functionalization and potential industrial applications.

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Chapter 5

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Chapter 5: Bile salt binding I: Block copolymers

Abstract

Elevated human serum cholesterol levels are recognized as a significant risk factor for coronary heart disease, and bile salts represent the primary metabolic elimination route for cholesterol. Composed of cross-linked beads, conventional bile salt sequestrants are plagued by low efficiency, high dosage, disagreeable side effects, and concomitant poor patient compliance. We now present new self-assembled block copolymer aggregates with stable, glassy, polystyryl cores and N,N,N-trimethylammonium chloride-bearing coronae that were prepared and tested as a potential alternative to the commercial resins. This system focuses on the electrostatic attraction between anionic bile salts and cationic sequestrants but possess minimal hydrophobic character. Electron micrographs were employed to visualize the polyelectrolyte-biosurfactant interaction and chromatographic separation analytical methods were used to quantify the sequestration. The results indicate that although they require laborious preparation, self-assembled aggregates show promise as bile salt sequestrants.

Introduction

Bile salts and sequestration

Despite the recent popularity of the hydroxymethylglutaryl-coenzymeA (HMG-CoA) inhibitors for the treatment of hypercholesterolemia, bile acid sequestrants remain therapeutically attractive due to the simplicity of their action, as described in Chapter 1. There is no doubt that bile acid sequestrants, when correctly administered, are successful cholesterol reduction agents. Furthermore, there are strong indications that their use as a complement to HMG-CoA reductase inhibitors is very effective. However, there is also no doubt that patient compliance is low with this treatment, partly due to the high dosages and associated unpleasant, albeit non-critical, side effects.

The aggregation behavior of amphiphilic diblock copolymers is herein shown to be a potentially exploitable phenomenon with respect to the sorption of bile salts. Pharmaceutically available bile salt sequestrant resins such as Cholestyramine[®] have a proven track record of reducing serum cholesterol levels.¹ Nevertheless, their *in vivo* performance is weak not only because of poor patient compliance, but also because the bile salt sorption by the resin is in direct competition with the active transport bile salt uptake mechanisms in the lower small intestine. Furthermore, since the sequestration is an anion-exchange, and not covalent process, it is therefore both dynamic and reversible. Nano-scale amphiphilic di-block copolymer architectures have been proposed as alternative materials to improve on existing technology in the battle against cholesterol-induced coronary heart disease.²

The amphiphilic nature of bile salts leads to aggregation that occurs in a step-wise fashion at concentrations of the order of 10 mM.³ The ionic head groups are hydrophilic, the methylated face of the surfactant is extremely hydrophobic, and the variably hydroxylated face is somewhat hydrophilic. It is widely accepted that for ion-exchange sequestrants, the driving forces to bile salt sorption are electrostatic attraction coupled with hydrophobic interactions.⁴ While it is attractive to say that the electrostatics dominate, the electrostatic force between two fixed charges, one in the resin and the other on the sorbate, no more than follows Coulomb's law

$$F = \frac{Q_1 Q_2}{4\pi\epsilon_0 \epsilon r^2} \text{ (Equation 1)}^5$$

where Q is the charge, ϵ_0 is the permittivity of a vacuum, and ϵ is the relative dielectric constant. Since the attractive force falls off with the second power of the separation, r , it is primarily important when the sorbate and site are proximate. Clearly, it is an oversimplification to suggest that electrostatic attraction alone is responsible for the success of bile salt sequestrants. Substantial indications including optical evidence of ordering within the beads⁶ and comparative work on cholestyramine and QPDA-12⁴ suggest that the whole process begins with the partition of hydrophobic bile salt anions into the hydrophobic polymeric matrix. Once there, these bio-surfactants are anchored by their charge-bearing "tails" and participate cooperatively in sequestering further bile salts.

Bile salt binding to trimethylammonium-bearing resins has been found to increase with decreasing cross-link density and with decreasing particle size.⁷ These observations support the hypothesis that bile salt diffusion into the beads in conjunction with bile salt self-association are key variables to be optimized if sequestrants are to be improved.

Block copolymer aggregates are particularly well suited to addressing the issue of binding site accessibility. In the case of the simple sphere, all ammonium groups are located within a few nanometers of the outer surface, and furthermore, due to the nanoscopic colloidal size range, the total surface area/volume ratio is extremely high. The expression for this ratio is $4\pi r^2 / \frac{4}{3}\pi r^3$, or $3/r$, where r = radius. When $r \sim 30$ nm, the surface area is ~ 100 km² per m³ of material. For resin beads, where $r \sim 10$ μ m, the surface area is ~ 0.3 km² per m³ of material. Even in larger aggregates such as the highly ordered large compound micelle (LCM) (see Chapters 2 and 4) or disordered agglomeration architectures, the particles remain largely sub-micron and the hydrophilic, charge-bearing blocks remain accessible. The absence of cross-linking within the ammonium-bearing domain enhances the potential availability of binding sites. The entire assembly may be considered to have three domains: (i) a glassy polystyrene core, that is essentially a spectator, (ii) a densely packed hydrophilic region in the corona at the corona-core interface that may have a limited binding role to play, and (iii) a highly solvent-swollen ammonium-group bearing domain. LCM's and other large multiple-core aggregates have a fourth domain, (iv) where the polymer is highly solvent-swollen, but due to isolation or distance from the aggregate-suspension medium interface, sorbate diffusion is limited (see Figure 1).

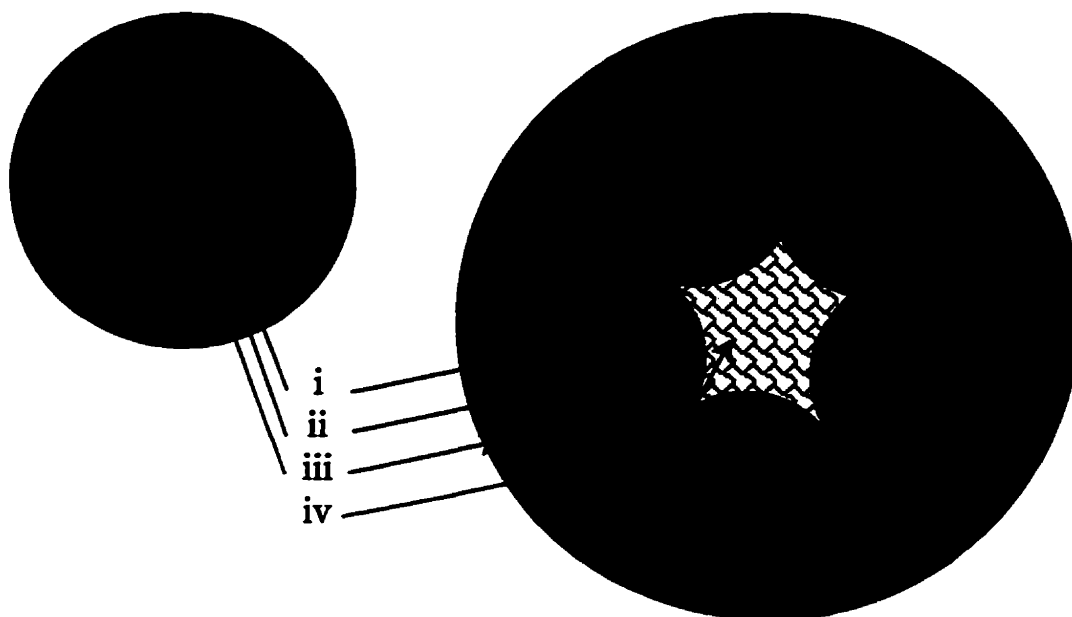


Figure 1: Bile salt sorption domains for small spherical aggregates and large compound aggregates: (i) glassy polystyrene core, (ii) restricted mobility region at core-corona interface, (iii) highly solvent-swollen domain, and (iv) solvent swollen, but less accessible region.

Nano-scale aggregates as ion exchangers

Ion Exchange

Before dealing directly with the application of block copolymer aggregates to ion exchange, the topic warrants introduction as it has a rich history and several terms that need precise definition. Ion exchangers are referred to in the Bible when Moses “cried upon the Lord; and the Lord shewed him a tree, which when he had cast into the waters, the waters were made sweet”.⁸ Rotted cellulose is a good exchanger for magnesium ions. Aristotle (384-322 BC) also knew that salt and impure water could be rendered potable by percolating it through layers of sand and rock. In the modern age, the phenomenon of ion exchange was recognized as such by Thomson, Spence and Way in the mid-19th century.^{9,10} Following the development of Arrhenius’ theory of electrolytic dissociation

(1884) it became possible by the early 20th century to describe general properties of ion exchange. Ion exchangers are insoluble polyelectrolytes having free ions that can reversibly interchange with ions in surrounding solutions. A cation exchanger has fixed anions covalently bonded to the polymer and its initial cation is exchanged in an equilibrium process with any cation(s) in solution. Anion exchangers have fixed cations in the polymeric matrix and exchange their counter-anions with anions in solution. Inorganic ion-exchangers as such were first synthesized by Gans in 1905 (permutites)¹¹ and synthetic organic ion exchange resins were discovered by Adams and Holmes in 1935.¹²

Ion exchange is a clearly defined process and should be distinguished from adsorption and absorption. Absorption is the phenomenon of uniform penetration of the sorbing species into the sorbant following the rule of *similia similibus solvuntur* (like dissolves like) with no change in concentration. One substance therefore takes up another and distributes it evenly throughout the matrix. Adsorption occurs at the interface of a solid and the solute-bearing medium and is due to physical attraction associated with the surface of the sorbant (e g., van der Waals forces). Chemisorption is also possible, as in the case of catalysts. Ion exchange is characterized by the retention of charge-bearing solute species by association with ionic groups in the matrix. Phenomenologically, absorption, adsorption and ion exchange are similar; all are sorption processes, but they differ substantially in the manner by which they retain sorbing species.¹³

Binding and applicability of nano-scale technology

Bile salt sequestrants are therefore ion exchangers and, depending on their structure, can be adsorptive or absorptive. The primary desired characteristics for effective bile salt sequestrants are specificity, high capacity and high affinity. High capacity is a function of the degree and availability of functionalization. In this study, the *quasi* quantitative amide conversion of the block copolymers under study was necessary for the self-assembly process, since the nano-aggregates were formed by the slow addition of a block selective solvent to a solution of the polymer. Non-quantitative functionalization would render this process impossible. Intra-chain interactions between unreacted carboxylates and ammonium groups lead to suspension instability. Unreacted butylacrylate groups in the corona would render the nominally hydrophilic block less water-soluble. Attempts to generate nano-scale aggregates from incompletely functionalized copolymers were unsuccessful. Since nearly all of the binding sites are near the surface of the aggregates in question and since bile salt diffusion into the solvent-swollen corona is unhindered by covalent cross-linking, potential binding capacity for these aggregates is expected to be a function of the hydrophilic trimethylammonium-containing block length.

High affinity is a function not only of the pendant sorption group but also of the counter-ion being displaced by the bile salt.^{2,14} In the present case, chloride was the counter-ion being displaced though other counter-ions have been explored for other systems. Selectivity or specificity is the preference of the binding species for a particular sorbate and is determined by the binding functional group itself. The trimethylammonium-containing binding group chosen for this study focuses on the

electrostatic component of bile salt sorption. Earlier studies have identified a hydrophobic spacer to have a pivotal role in optimizing bile salt sorption in cross-linked resins. However, due to the extreme sensitivity of self-assembly, the ammonium-containing block was engineered to be as hydrophilic as possible. A simple two-carbon spacer was therefore incorporated into the pendant binding groups. The system presented below addresses therefore the competition between bile salts and chloride ions with solvent and tethered trimethylammonium groups.

Scope of this chapter

With the synthesis and morphological characterization of PS-*b*-PTMEACl accomplished, the obvious extension of the work was to establish potential utility of the material. Explicitly, the goal was to determine to what extent self-assembled block copolymer aggregates containing pendant trimethylammonium groups with short hydrophobic spacers linking the positive charge to the backbone participate in ion-exchange sequestering of the glycocholate anion. In the first instance, attempts were made to qualitatively indicate binding by TEM. Quantitative determination of the interaction was obtained using HPLC analysis of equilibrium bile salt concentration. Due to limited material, the investigation was somewhat restricted. However, attempts were made to determine the effects on bile anion sorption of aggregate morphology, coronal chain length, coupling reagent, and concentration both of bile salt and polymer.

Experimental

Block copolymers:

Synthesis summary for PS-*b*-PTMEACl

Self-assembled aggregates of polystyrene-*b*-poly(N,N,N-trimethylammonium ethyleneacrylamide)chloride (PS-*b*-PTMEACl) (see Figure 2) are shown below to be bile salt sequestrants. These polymers were derived from anionically synthesized polystyrene-*b*-poly(*tert*-butyl acrylate) parent macromolecules that were hydrolyzed and then functionalized in neat N,N-dimethylethylenediamine (DMED) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) or dicyclohexylcarbodiimide (DCC) as the coupling agent (see Chapter 3). The functionalized copolymers were quaternized with methyl iodide and the counter-ions were exchanged for chloride.

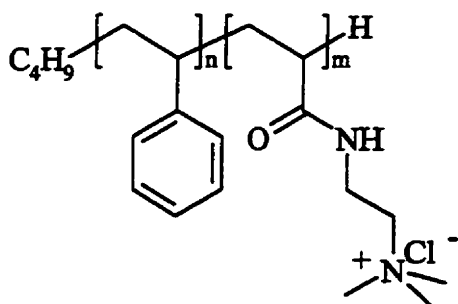


Figure 2: Polystyrene-*b*-poly(N,N,N-trimethylammonium methylene acrylamide) chloride (PS-*b*-PTMEACl).

Self-assembly summary for PS-*b*-PTMEACl

When amphiphilic copolymers such as PS-*b*-PTMEACl are placed in a solvent that interacts favorably with only one of the blocks, the polymer chains self-assemble to minimize the contact between the solvent and the insoluble block (see Chapter 2). The survey of morphologies observed from the micro-scale functionalization of a series of parent polystyrene-*b*-poly(*tert*-butylacrylate) (PS-*b*-PtBuA) copolymers as presented in Chapter 4 indicates two samples that are particularly well suited to investigative bile salt binding assays. When the corona-forming hydrophilic block is comparatively long, the dominant solution morphology for the self-assembled aggregates is the simple small sphere. For samples with shorter hydrophilic blocks, more complex and kinetically trapped solution morphologies were often observed. Following the preliminary TEM studies on PS(385)-*b*-PTMEACl(80) (see Figure 3 below), the two samples chosen for this study were PS(80)-*b*-PTMEACl(130) and PS(80)-*b*-PTMEACl(330) which provide microscopic non-uniform aggregates and nanoscale spheres respectively.

Transmission electron microscopy (TEM)

Procedures for these studies were identical to those described in Chapters 2 and 4.

Bile salt sorption studies

Since this laboratory has a long history of bile salt sorption studies,^{7,15,16,17,18,19} efforts were made to replicate earlier experimental conditions. Equilibrium bile acid concentrations were determined with reverse-phase high performance liquid chromatography (HPLC). The flow rate of the pump (LKB Bromma 2248(10)) was typically set to 1.0 mL/min, and the eluant was a Millipore-filtered binary mixture of

methanol and de-ionized water (milliQ) at a volume ratio of 80:20. Glacial acetic acid was added to a final concentration of 0.1 M in order to protonate the bile salt. Under these conditions, the back-pressure did not exceed 15 MPa. During longer periods of inactivity, the system was flushed with filtered methanol to prolong the useful life of the column. The back pressure with methanol alone at a flow-rate 1.0 mL/min was about 10 MPa. The analyte was detected with a Waters 410 differential refractometer interfaced to a Pentium computer running Waters Millenium[®] software in a Windows[®] environment.

Before any injections were initiated, the refractometer reference and sample cells were purged and the column was allowed to equilibrate with the acidic binary eluant for at least one hour. The baseline was also monitored to ensure that the refractometer had equilibrated with respect to temperature and that the base signal was therefore stable.

Standard solutions of bile salts were prepared in 2.5 mM or 5.0 mM tris(hydroxymethyl)amino ethane (Tris hereafter) buffer adjusted to pH 7.2 with 0.1 M HCl. Aliquots were simply filtered and loaded onto the injection loop (50.0 μ L) and a linear relationship between peak area and bile salt concentration was observed. Attempts at micro-filtration of the copolymer-containing suspensions resulted in immediate filter blockage or no polymer retention. Therefore, measuring the equilibrium bile salt concentration (C_{eq}) following incubation with copolymer aggregates required centrifugation. Aliquots of bile salt and copolymer-containing suspension (1 mL) were placed in Epindorf[®] tubes and were subjected to 14k r.p.m. for 2 minutes (Fischer Scientific Micro14) which cleared the polymer from the suspension. Unbound bile salt molecules participating in true micellization were not appreciably affected by this procedure.

Results and Discussion

Indicative TEM study

The first indication that block copolymer assemblies could function as bile salt sequestrants was strikingly visual. We think we have some understanding as to the *how* and *why* of bile salt and sequesterant interactions, but it was profoundly satisfying to see the effect of bile salt addition to a suspension of nano-spheres prepared from the PS(385)-*b*-PTMEACl(80) sample (see Figure 3). To a stock suspension of aggregates, NaCl and solutions of sodium glycocholate (NaGC) in Tris buffer were added. The suspensions to which NaCl was added were unchanged to the unaided eye, in keeping with the observations reported in Chap. 4. However, the addition of NaGC ultimately led to macrophase separation over the course of several hours.

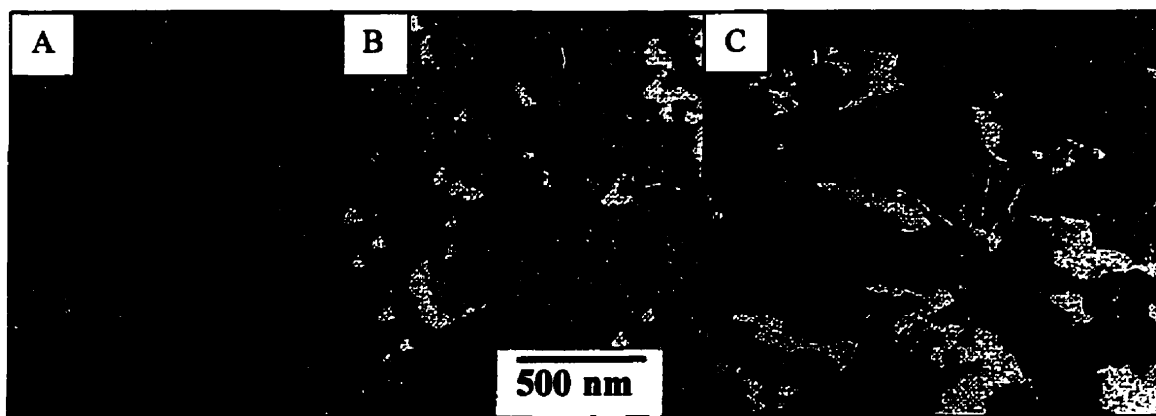


Figure 3: TEM micrographs of PS(385)-*b*-PTMEACl(80) samples prepared in the presence of A: 5 mM NaCl; B: 0.6 mM NaGC; and C: 1.2 mM NaGC. The simple electrolyte slightly reduces the diameter of the aggregates as compared with the salt-free sample (see Chapter 4). However, the addition of bile salt surfactant molecules, even in very low concentrations, causes agglomeration.

One would expect a polyelectrolyte chain to collapse somewhat on the addition of salt due to electrostatic shielding of the charges on the polymer.²⁰ However, the macrophase separation of the samples treated with NaGC is clearly much greater than observed on addition of NaCl and appears to be the result of surfactant-mediated agglomeration. It seems reasonable to suggest that ion exchange, electrostatic shielding and the mechanisms of bile salt self-assembly cooperatively lead to the macrophase separation.

Low polymer concentration sorption study: HPLC

Due to synthetic complications which resulted in limited sample quantity, we were obliged to continue the study of bile salt binding by copolymer aggregates with other samples. The first polymerization and functionalization (DCC-mediated) that provided sufficient material for quantitative trials was PS(80)-*b*-PTMEACl(330) (see Figure 4).

A stock suspension of nanoscale self-assembled PS(80)-*b*-PTMEACl(330) was prepared as described in Chapter 4 at a concentration roughly measured to be 0.5 g/mL. To 1.00 mL aliquots of this suspension, 1.00 mL aliquots of NaGC solution were added to final NaGC concentrations of 1.50 mM and 3.00 mM. The resulting mixture was agitated for at least one day using a mechanical flatbed shaker at room temperature. The equilibrium NaGC concentration under the assay conditions was 7% less than the initial NaGC concentration.

A recurring complication with the copolymer aggregate-glycocholate binding assays was the limited quantity of polymeric material. For these trials, less than 5 mL of a clean suspension were isolated. The actual polymer concentration was determined by evaporation of a 1 mL aliquot in a pre-weighed vial. Furthermore, assay duplication was not possible. Therefore, the error associated with this procedure was high, and the quantitative data should be interpreted with caution. Nevertheless, since the polymer for these assays was delivered from a stock suspension, the data shown in Figure 4 are self-consistent. Assays described below support the general trend observed here, though a more accurate determination of the actual polymer mass employed suggests that the extent of binding for these aggregates is probably considerably lower than indicated by these preliminary trials.

The total polymer concentration for these assays following dilution due to the addition of NaGC solution was measured to be 0.25 mg/mL, as described above. Assuming that this value approximated the true polymer concentration, on the order of 40 bile salt molecules were bound per chain nominally holding 330 trimethylammonium pendant groups. The buffer concentration was chosen to follow the example of previous studies on resins carried out under the supervision of G.R. Brown. The role of the buffer is to ensure that the bile salt remains unprotonated and therefore carries a negative charge but the addition of buffer also affects the ionic strength of the solution and thereby introduces competition for the binding sites.

Further studies with this sample were hampered by limited material.

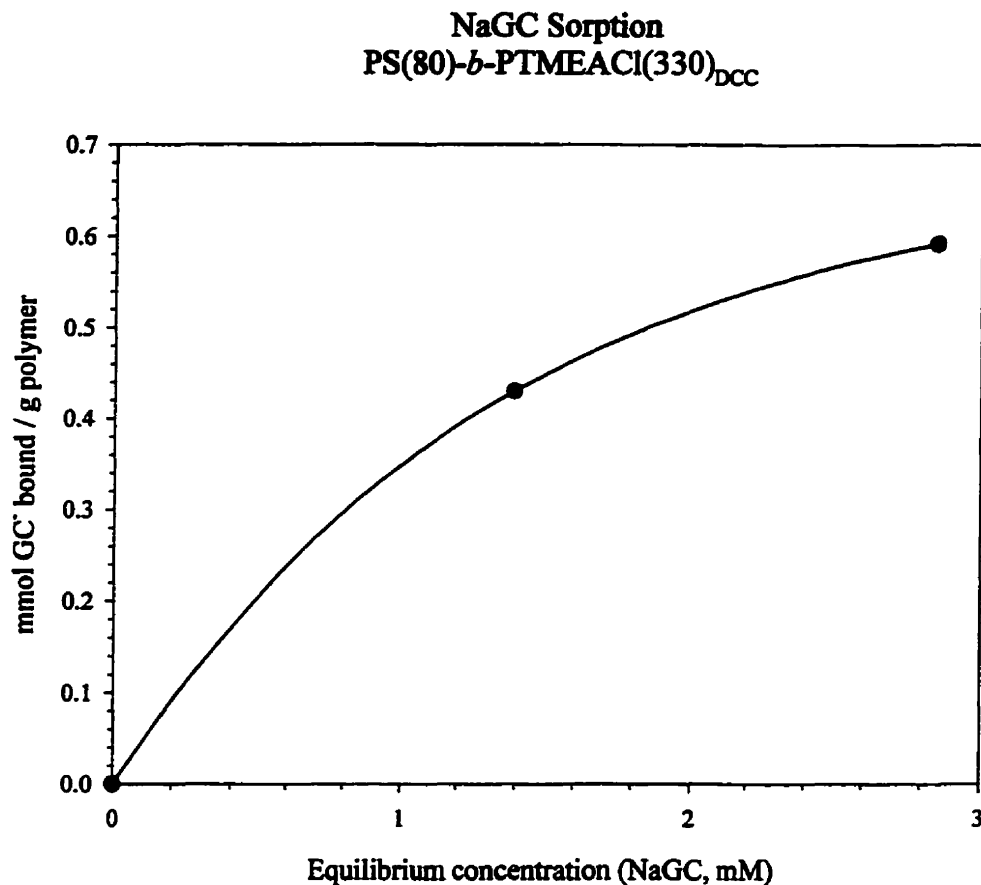


Figure 4: This binding isotherm is derived from data indicating that a 1.00 mL micellar suspension (0.5 mg/mL) sequesters up to 7.2% of the available NaGC when added to a 1.00 mL NaGC solution. The absence of a plateau suggests non-saturation; however, further data are required for a complete assessment of the glycoholate-binding properties of this polymer. The high binding values should be regarded with caution due in part to the sub-milligram quantities of polymer employed and the associated error.

High polymer concentration sorption study: HPLC

When working with low concentrations of polymer, a persistent worry is that results can be badly flawed due to errors in mass determination. Although the data above were self consistent since the copolymer aggregates were introduced from a stock suspension, there could be no measure of their accuracy. To ensure that the binding phenomenon observed in Figure 4 was repeatable and furthermore that the binding was due to the polymer (Figure 2) and not due to spurious effects, such as impurities remaining from the functionalization reactions, further material was prepared. A water-soluble carbodiimide (EDC, see above and Chapter 3) was employed to convert two related samples of PS-*b*-PAA to PS-*b*-PTMEACl (see TEM micrographs, Figure 5).

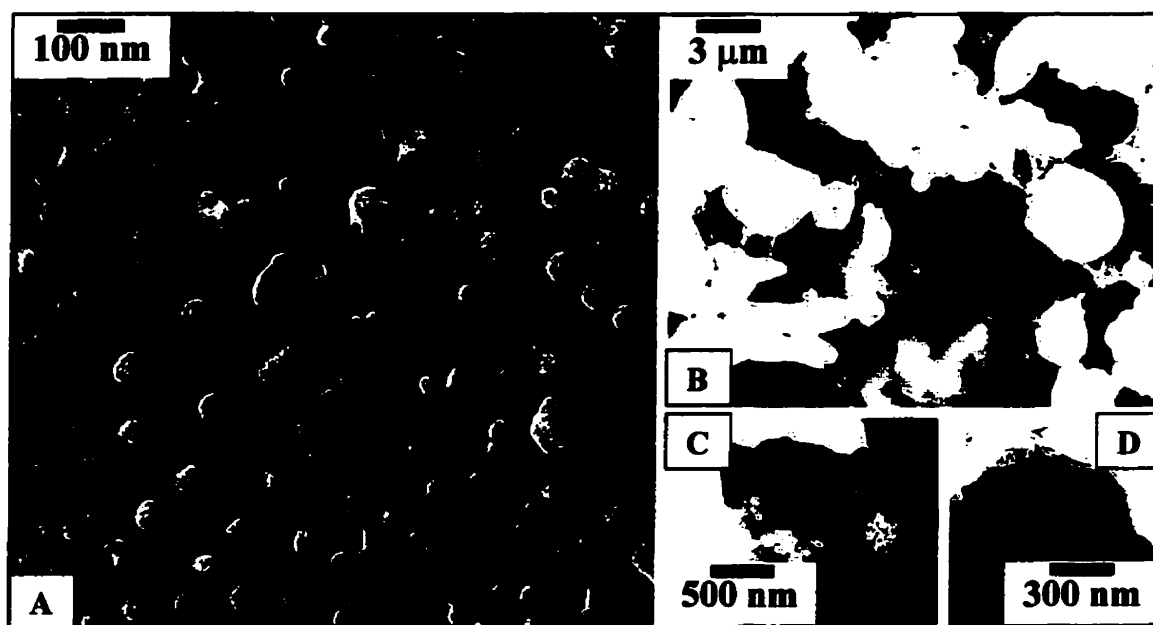


Figure 5: TEM micrographs of PS-*b*-PTMEACl self-assembled architectures (EDC mediated functionalization). A: PS(80)-*b*-PTMEACl(330) showing primarily simple small spheres though nascent rods and lamellae (one perforated) are observable; B, C and D: PS(80)-*b*-PTMEACl(130) larger aggregates and agglomerates clearly dominate, but small spheres and bilayer structures are also present.

Once these aggregates were isolated in pure water, the samples were lyophilized and aliquots of NaGC solutions were tested with sufficient polymer to ensure a significant decrease in C_{eq} (NaGC). The dry polymer (~10 mg) was added directly to the NaCG/Tris solutions (2.0 mL) to avoid potential errors resulting from dilution and HPLC injections were carried out in duplicate. Under these conditions, PS(80)-*b*-PTMEACl(330)_{EDC} reduces the NaGC concentration by up to one third of the initial concentration. Figure 6 presents the resulting GC⁻ binding isotherm .

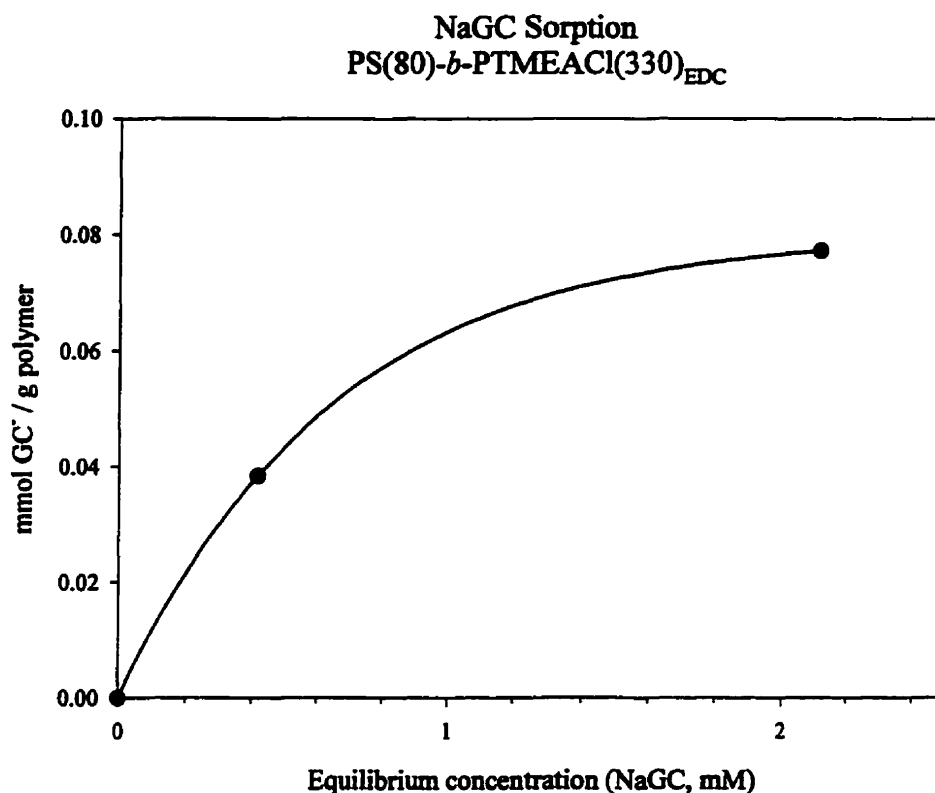


Figure 6: A GC⁻ sorption isotherm for PS(80)-*b*-PTMEACl(330)_{EDC}. The EDC-mediated functionalized polymer (10 mg) in a micellar suspension removes up to 32% of the initial glycocholate from 2 mL of a bile salt solution (5.0 mM Tris, pH 7.2).

Following a summary of the copolymer samples given below in Table 1, the quantitative binding results for all polymers tested using HPLC analysis of the C_{eq} (NaGC) are given in Table 2. The first four lines of Table 2 give data for the polymers functionalized using EDC and the last two concern the DCC-mediated polymers. The data show that when nearly equal masses of polymers are used, the sample with a higher mole fraction of TMEACl units is twice as effective per chain at removing NaGC from solution. The difference in aggregate morphology between Sample 1 and Sample 2 does not seem to have a strong influence on bile salt anion sorption. Very low polymer concentrations seem to result in the best ratio of bound GC (mmol) to polymer mass (g), but direct comparison of the efficacy of the DCC and EDC derived polymers is complicated by the limited amount of polymer available for the study. Sample 3 assays employed ~0.5 mg of polymer, whereas Sample 1 and 2 assays tested 3-10 mg copolymer. A secondary difference-causing effect may involve the assay buffer concentrations. For compatibility with ongoing investigations into the nature of bile salt sorption,²¹ 5.0 mM Tris was used for the EDC-derived polymers whereas dilution gave 2.5 mM Tris for the DCC-derived samples. Bile salts are sequestered most effectively from pure water, less well from Tris buffer, and phosphate buffer surrenders bile salts under protest. As the buffer concentration increases, the ionic strength of the solution increases and the sorption decreases although for these experiments this effect is expected to play only a minor role.¹⁹

Table 1: A summary of PS-*b*-PTMEACl samples.

Sample	PS	PTMEACl	ϕ	Coupling agent
1	80	130	0.62	EDC
2	80	330	0.80	EDC
3	80	330	0.80	DCC

ϕ = Ammonium-bearing fraction of the total

Table 2: A summary of PS-*b*-PTMEACl binding experiments.

Sample	Polymer mass (mg)	-NMe ₃ Cl (μ mol)	Initial [NaGC] (mM)	C _{eq} (mM)	GC bound per gram polymer (mmol/g)	GC bound per chain
1	9.9	13.1	0.62	0.43	0.039	1.3
2	3.0	14.0	0.62	0.48	0.096*	6.7*
2	10.6	49.5	0.62	0.42	0.038	2.3
2	10.6	49.5	2.53	2.12	0.077	5.6
3	0.5	2.3	1.5	1.39	0.43*	31*
3	0.5	2.3	3.0	2.85	0.59*	43*

* values to be interpreted with caution due to the mass of polymer in the assay

Conclusions

The N,N,N-trimethylammoniumethylene-functionalized PS-*b*-poly(acrylamide) samples presented in Chapters 3 and 4 are herein shown to be bile salt sequestrants. The ion exchange sorption of bio-surfactants into the highly charged hydrophilic coronae of the self-assembled nano-aggregates derived from this family of polymers has been established. Furthermore, indications of a significantly high capacity are suggested in the isotherms.

Utility of technology

Synthetic and practical challenges notwithstanding, these materials do participate in ion-exchange reduction of free glycocholate when placed in a glycocholate solution. Self-assembled aggregates of the parent copolymer can be isolated by lyophilization and on addition of water, re-suspended immediately to give a turbid 'solution'. The maximum relative reduction in bile acid concentration reported here was 33% (10 mg PS(80)-*b*-PTMEACl(330)_{EDC}, 2 mL, 2.5 mM [NaGC]_i) and the maximum ratio of GC⁻ bound per gram of copolymer that can be considered with confidence was 0.08 mmol/g (10.6 mg PS(80)-*b*-PTMEACl(330)_{EDC}, 2 mL, 2.5 mM [NaGC]_i). When the fraction of available ammonium groups is very low compared with the absolute number of glycocholate molecules, up to 40 binding sites per chain consisting of 330 potential sites may be occupied (assuming that each bound molecule completely occupies one site). Given the region of restricted chain mobility due to crowding near the core-corona interface, the true number of available sites will be somewhat less than the degree of polymerization of the sorption block. When a greater quantity of PS(80)-*b*-PTMEACl(330) copolymer was tested, 5.6 glycocholate anions were bound per polymer chain.

Block copolymer aggregates do have a major handicap, however. The synthesis of an appropriate amphiphilic system, its purification and the optimization of the conditions required for self-assembly are non-trivial problems. From the perspective of a chemical engineer, this material is not well suited to large-scale application as a sequestrant in pharmaceutical industry, though nitroxide-mediated²² or atom transfer

radical polymerization (ATRP)²³ of appropriate monomers may well make the aggregates more readily available and therefore more attractive.

In summary, despite the absence of a long hydrocarbon spacer used in previous sequestrants and the expected concomitant dramatic increase in binding constant, these materials show some promise as bile salt sequestrants. The primarily electrostatic attraction between glycocholate and polyelectrolyte as well as the partition of glycocholate between the highly polar aqueous medium and the hydrophilic, but less polar matrix favors surfactant sorption. Electron microscopy visually confirms the effect of the addition of bio-surfactants to nanoscale aggregates formed from amphiphilic diblock copolymers. It is in the study of these interactions that these aggregates may prove most fruitful.

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Chapter 6

Bile salt binding II:

Branched polymers

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Chapter 6: Bile salt binding II: Branched polymers

Abstract

Hydrogels present an attractive alternative to nanoscale block copolymer aggregates and microscale resin beads as potential asystemic serum cholesterol reduction materials. Not only would the oral delivery of these materials be more pleasant than the sand-like bile salt anion sequestrant beads, but the hydrogel preparation is much simpler than the copolymer aggregate analogues (see Chapters 2-5). Finally, light cross-linking, typical of hydrogels, does not overly restrict diffusion of the target bile salt anions into the matrix. In this Chapter, following an introduction to hydrogels and their application to hypercholesterolemia, the synthesis, characterization and glycocholate binding properties of poly(N,N,N-trimethylammoniummethyle acrylamide chloride)_{gel}, (PTMEACI)_{gel}, are presented.

Introduction

Bile salt sequestrants

In developing bile salt sequestrants for the treatment of hypercholesterolemia, the goals include high capacity, high specificity and strong affinity. Low dosage is expected to lead to lower serum cholesterol levels with a concomitant reduced risk of coronary heart disease, fewer drug-related side effects and higher patient compliance. Fundamentally, bile salt sequestrants are usually ion-exchange polymers and bile salts are

bio-surfactants. The literature provides a wealth of information on polymer-surfactant interactions^{1,2,3,4} and the specific interactions between methylated ammonium groups, hydrocarbon spacers and bile salt anions is a topic of active research.^{5,6,7} It seems clear that sequestration is a function of both electrostatic and hydrophobic effects.

Bile salt sequestrant characteristics

The accessibility of bile salt binding sites is an additional consideration worthy of further study. An increase in the degree of cross-linking in functionalized poly(acrylamide) resins clearly reduces bile salt uptake.⁷ Charge density, segment mobility, ionic strength and network topology as well as cross-linking have roles to play in surfactant-polyelectrolyte interactions. Increased cross-linking in hydrogels suppresses segmental motion leading to a large osmotic pressure, which is balanced by the elasticity of the gel. For example, a water-swollen gel containing pendant sulfonic acid groups will contract on the addition of a surfactant such as cetyl pyridinium chloride – a process that is aided by incremental cross-linking. Cooperativity effects, on the other hand, are reduced since a constricted gel has less free-volume for surfactant-surfactant interactions.⁸ Elegant studies on poly(*N-isopropylacrylamide*) gel disks (diameter 0.7 – 10 mm) and surfactants show conclusively that the majority of surfactant uptake occurs near the outer edge of the disk thereby adding a confirming voice to the self-evident belief that diffusion is limited in cross-linked polymers, at least on a micrometer scale.³

Chapter 5 provides evidence that self-assembled block copolymer colloidal particles can be effective bile salt sequestrants. The volume fraction of polymer with

accessible binding sites is high since nearly all sites are within a few nanometers of the aggregate surface and since there is no covalent cross-linking in the corona, diffusion to those sites is limited only by 'crowding'. On the other hand, the preparation of high concentrations of block copolymer aggregates remains a non-trivial problem. Therefore, the absolute number of binding sites in real colloidal suspensions under the conditions reported in Chapter 5 is very low when compared with the cross-linked resins that have always been the mainstay of the bile salt sequestrants.^{6,9,10,11}

Clearly one possible method of attaining high binding site concentration without sacrificing availability or accessibility is to incorporate the binding sites onto linear or gel-forming polymers.^{12,13,14,15} As a compromise position between the colloidal domain presented in Chapter 5 and the functionalized poly(acrylamide) resins that have been the subject of study by G.R. Brown *et al.* for several decades, we now present the synthesis, characterization and preliminary bile salt binding characteristics of hydrogels formed from N,N,N-trimethylammoniummethylene-functionalized poly(acrylamide), (PTMEACl_{gel}). Among the novel aspects of this hydrogel is its simplicity. While the bile salt anion binding hydrogels in the literature are composed of a variety of functional groups including secondary and tertiary amines, amides and hydrophobic groups, notably pendant cholesteryl moieties,¹⁴ we are aware of none that have the same fundamental structure as PTMEACl_{gel}: an aliphatic backbone with a pendant amide lending chemical stability and the possibility of hydrogen bonding, a hydrophobic spacer group and a quaternary amine to anchor bile salt anions. Furthermore, with a short ethylene spacer, this material is an appropriate vehicle against which to compare any future materials with more hydrophobic character and more complex polymer-bile anion interactions.

Hydrogels

Hydrogels are lightly cross-linked hydrophilic polymers that swell, but cannot dissolve in water to give a macromolecularly disperse solution. They have come to enjoy wide use as absorbents and in a variety of household applications.¹⁶ They are also used as drug delivery vehicles, in contact lenses, artificial organs and limbs, and various pharmaceutical applications.¹⁷

It is convenient to imagine that hydrogels are composed of uniformly cross-linked or branched polymers yielding a homogenous network bearing charge. More realistic is to accept that real hydrogels have many defects: heterodispersity of the chain lengths, as well as loops, loose ends, entanglements and interpenetrating networks.

Some understanding of the driving forces to hydrogel swelling is necessary to appreciate diffusion and the effect of added electrolytes. A recent theoretical treatment of the swelling and elastic properties of hydrogels attempts to model the extraordinary capacity of hydrogels to absorb water.^{18,19} When immersed in an electrolyte-containing solution, a polyelectrolyte gel swells due to osmotic pressure. The total osmotic pressure, Π , equals zero once the process reaches equilibrium since the total free energy, ∂G , is at a minimum with respect to the swelling volume, ∂V

$$\Pi = -(\partial G / \partial V)_T = 0 \quad (\text{Equation 1})$$

The total osmotic pressure is the sum of four contributions

$$\Pi = \Pi_{bare} + \Pi_{rep} + \Delta \Pi_{Donnan} + \Pi_{mix} \quad (\text{Equation 2})$$

The structural contributions are from the uncharged backbone, Π_{bare} , and the repulsion of charges interacting along the chains, Π_{rep} . The Donnan effect relates to the exclusion of external salt by ions covalently associated with the hydrogel. In the absence of constraints, ion-exchange is a statistical redistribution of counter-ions between binding sites within the polymer matrix and the solution. Donnan theory treats systems of electrolytes where at least one of the ionic species is covalently restricted. In the present case, the ammonium ions are fixed within the polymeric gel, which implies an uneven distribution of ionic species leading to $\Delta\Pi_{\text{Donnan}}$. The restricted migration of free ions gives rise to the Donnan potential.²⁰ The mixing term, Π_{mix} , refers to the polymer-solvent interaction and is described by the Flory-Huggins equation:

$$\frac{\Pi_{\text{mix}}V_w}{RT} = -(\ln(1 - \phi) + \phi + \chi\phi^2) \quad (\text{Equation 3})$$

where V_w is the molar volume of water, ϕ is the polyelectrolyte volume fraction, R is the gas constant, and χ is the polymer-solvent interaction parameter.

Objectives

Hydrogels are hydrophilic, cross-linked or branched polymers that swell within the limit of their structure in water until the osmotic pressure across the gel/solvent boundary equals zero. Polyelectrolyte gels swell as a function of polymer-polymer interactions, charge repulsion, polymer-solvent interactions and the Donnan effect. On the addition of simple electrolytes, electrostatic shielding in the matrix leads to decreased electrostatic repulsion, and hydrogels de-swell. On the addition of charged bio-surfactants such as bile salts, to hydrogels such as PTMEACl_{gel} it is anticipated that ion exchange (chloride by bile salt anion) and hydrophobic interactions (bile salt anion – bile

salt anion) will lead to de-swelling. Enhanced bile salt anion uptake and enough affinity to combat the highly effective enterohepatic recirculation of bile salts *in vivo* are the obvious extensions of this phenomenon. Patient compliance with the commercial bile salt sequestrant resins currently available is low because of low *in vivo* bile salt uptake, high dosage, gastrointestinal discomfort,^{*} and the unpleasant nature of the gritty, sand-like resins themselves. Hydrogels present highly accessible binding sites, a flexible matrix to accommodate the diffusion, sorption of bile salts and subsequent gel-collapse, and a smoother texture. Clearly, the hope is that selectivity and binding activity can be enhanced sufficiently over the currently available resins that the dosages required and concomitant side effects can be minimized.

Our strategy, therefore, was to capitalize on the pre-existing structure and ready availability of the carbomers[†] and to adapt them to the chemistry now described in Chapter 3. For comparison with the systems presented in Chapter 5, the ethylene hydrophobic spacer between the amide linkage to the backbone and the quaternary nitrogen moiety was retained.

Experimental

Carbopol

All hydrogel samples discussed below were derived from a series of BF Goodrich thickening agents: Carbopol 934, 940 and 941, all of which are cross-linked and branched poly(acrylic acid)^{21,22,23} (see Figure 1). These materials were manufactured by reflux polymerization in the presence of an undisclosed catalyst and inert solvent.

^{*} nausea, constipation, flatulence, etc.

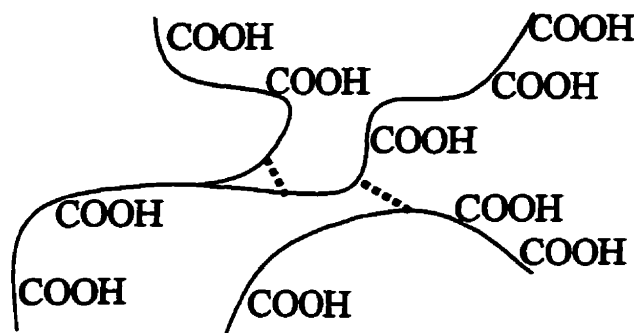


Figure 1: A schematic representation of the Carbopol family of polymers showing carboxylic acids groups, branching and light cross-linking.

These carbomers range in molecular weight from about 500 000 to 5 000 000 g/mol where 934 is the smallest, and 941, the largest. The main differences among these samples are in molecular weight and cross-link density. Since these polymers are proprietary, BF Goodrich is understandably reluctant to divulge complete structural information. However, the cross-linking agent has been reported to be a polyalkenyl polyether. There has also been some indication that its actual structure is a vinyl group with pendant sucrose (see Figure 2).

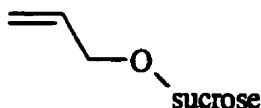


Figure 2: Unconfirmed, but reported Carbopol cross linking agent.

The carbomers are provided as a white, mobile powder that swells dramatically when added to water. The viscosity of the Carbopol polymers in solution (0.5% aqueous,

[†] *carbomer* is the generic term for this family of polymers

CPs) is reported as follows: (934) 30 500 -39 000, (940) 40 000 – 60 000, and (941) 4 000 – 11 000. Carbopol 940 yields a clear gel in water whereas the others are turbid. Carbopols are used extensively in the cosmetics industry where there are at least 1084 product formulations including creams, soaps, sunscreen, deodorants, eye shadow, and lipstick. Safety studies indicate that the polymers pose essentially no risk if ingested, spread on the skin, placed on the cornea or even injected intravenously at low to moderate levels. Carbopol polymers are used in pharmaceutical applications as thickening, emulsifying, dispersing and suspending agents. They are also used in controlled release tablets, bulking agents in laxatives and as a gel base for jellies, ointments and pastes. These polymers have even found use in industrial specialties including wallpaper removers, waxes, waterproof coatings, inks and lattices.

Each of three Carbopol samples were functionalized with N,N-dimethylethylenediamine (DMED) using carbodiimides as coupling agents. They were then quaternized with iodomethane (MeI) and the counter-ion (iodide) was exchanged for chloride.

Synthesis

Six reaction flasks were prepared, each containing 500 mg of Carbopol 934 (C-934), Carbopol 940 (C-940) or Carbopol 941 (C-941) (~7 mmol carboxylic acid groups) and the polymer was allowed to swell in ~50 mL tetrahydrofuran (THF) for 4 hours. Excess 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) or dicyclohexylcarbodiimide (DCC) was added (7.8 mmol) to the flasks so that each Carbopol was functionalized in parallel by both EDC and DCC to give F-934_{DCC}, F-934_{EDC}, F-940_{DCC}, F-940_{EDC}, F-941_{DCC}, and F-941_{EDC}. The reaction mixture was

stirred for 5 minutes and then freshly distilled DMED was added in excess (7.9 mmol). The reactions were stirred at room temperature for two days. The gels were washed with ethanol, neutralized with 5% $\text{Ca}(\text{HCO}_3)_2(\text{aq})$, and the water was then removed by rotary evaporation and lyophilization. The samples were re-suspended in methanol and a 20-fold excess of MeI was added to quaternize the product. The reactions were stirred for 1 week and a further excess of MeI (5 mL) was added and the reaction was allowed to continue for several days. Unreacted MeI was removed by rotary evaporation, and the quaternized gels were washed with a saturated aqueous solution of NaCl and were then purified by dialysis in Spectrapor® dialysis tubing (MW 50 000 cut-off) against de-ionized water (Millipor®) for at least one week.

Characterization Methods

IR

Infrared spectra were obtained using a Perkin-Elmer 16PC FTIR running Spectrum® software. Of particular interest was the carbonyl stretching region, that in transmission mode qualitatively indicated the degree of amidation. A quantitative estimate of the functionality was obtained by determining the extinction coefficient ratio for the ester carbonyl and amide I stretching bands in the IR using model compounds. Solutions of ethyl acetate (see Figure 3), a small-molecule analogue of the polymeric pendant methyl ester, and N-acetythylenediamine (see Figure 3), an analogue of the polymeric pendant amide were prepared in chloroform (4×10^{-2} M to 2×10^{-1} M). The Beer-Lambert relationship, $A = \epsilon cl$, where A is the absorbance, ϵ is the molar absorption (or extinction) coefficient, c is the molarity of the solution, and l is the solution path

length in centimeters, permits the calculation of concentration from absorbance values if ϵ is known. The ratio of $\epsilon_{\text{ester}}/\epsilon_{\text{amideI}}$ determined from the model compounds above was $1.01 \cong 1.0$ (see Appendix). The carbonyl region of the FQ_{EDC} FTIR absorbance spectra were deconvoluted using PeakFit 4.0 (Jandel Scientific) and the relative areas of the ester and amide I carbonyl bands (normalized by the extinction coefficient ratio) provided a measure of the functionality of each sample.

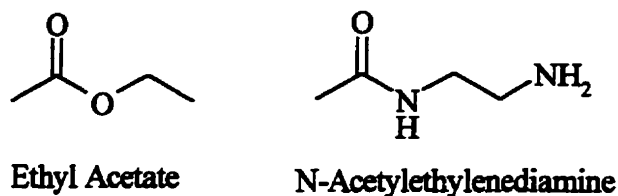


Figure 3: Small molecule model compounds for the determination of $\epsilon_{\text{ester}}/\epsilon_{\text{amideI}}$.

NMR

¹³C CP/MAS solid-state NMR spectra were collected for the dry EDC products on a ChemMagnetics M100 instrument at the McGill Chemistry NMR Center. The contact time was 2 ms and the delay was 1 s.

Static Light Scattering (SLS)

Attempts were made to characterize the starting material by light scattering measurements to confirm the vague solution characterization provided for C-934, -940 and -940 in BF Goodrich literature²² which suggests that the polymer chains swell in water to give discrete particles 2-7 μm in diameter. Although static light scattering (SLS) may have been useful in confirming the molecular weight of these samples, reliable dn/dc measurements (Wyatt refractometer) proved elusive.

Dynamic Light Scattering (DLS)

To measure hydrodynamic radii of polyelectrolytes, a dilute, uniform suspension of polymer molecules must be prepared in a salt solution to normalize against electrostatic repulsion within and among the polymer chains. In 0.1 M NaCl, the FQ carbomers do not swell sufficiently to render DLS particle sizing measurements fruitful. However, a Brookhaven BI9000 dynamic light scattering (DLS) instrument was employed to measure the hydrodynamic radius of the parent Carbopol polymer chains (and thereby the diameter). As the photomultiplier tube (PMT) is mounted on a swing arm for the BI9000, multiple angle experiments were comparatively simple to run. The incident light source was a laser at a wavelength of 532 nm. Data were collected over a range of angles from 60° to 120°.

Solution characterization of polyelectrolytes is usually carried out in salt solutions in order to avoid micro-variations in chain conformation due to the possibility of ionic impurities. Schliessel and Pincus treat the effect of simple, non-surfactant counter-ions on a polyelectrolyte in depth with clarity in recent work.²⁴ BF Goodrich literature suggested diameters on the order of 10 μm , which stretches slightly the useful upper detection limit of the technique.²⁵ Nevertheless, particle sizing of C-934, -940 and -941 at 0.02%, 0.02% and 0.01% (w/w) in 0.100 M NaCl using CONTIN-autocorrelation fitting²⁶ suggested that the unfunctionalized carbomer macromolecules swell to an average diameter of about 5 μm (934), 11 μm (940), and 8 μm (941) (see Figures 4, 5, and 6).

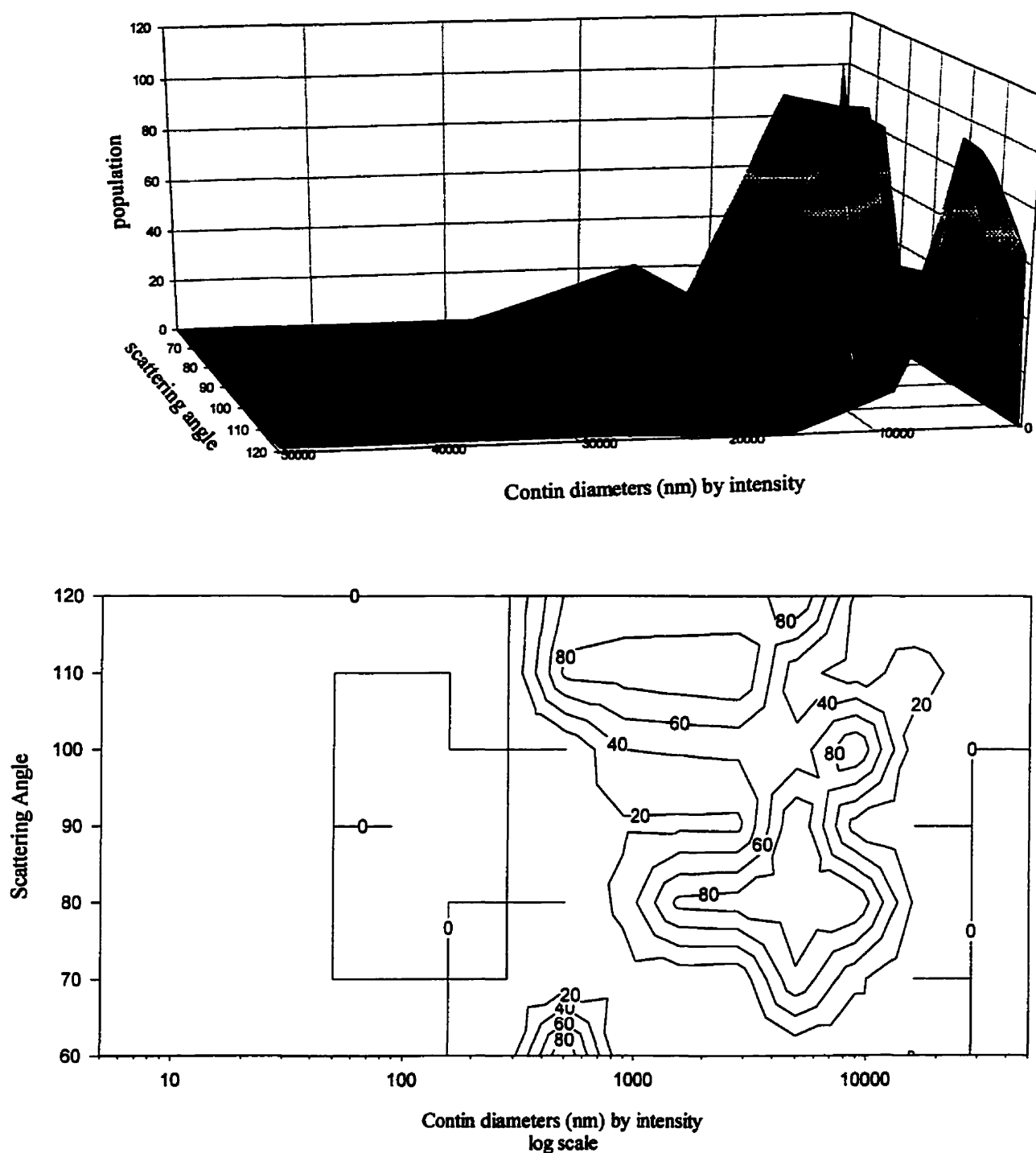


Figure 4: DLS results for C-934 (0.02% w/w in 0.100 M NaCl) indicating particles about 5-6 μm in diameter. The angular independence of this result suggests spherical architecture. The lower plot is a two-dimensional contour representation of the three-dimensional histogram above (the diameter is plotted in log scale).

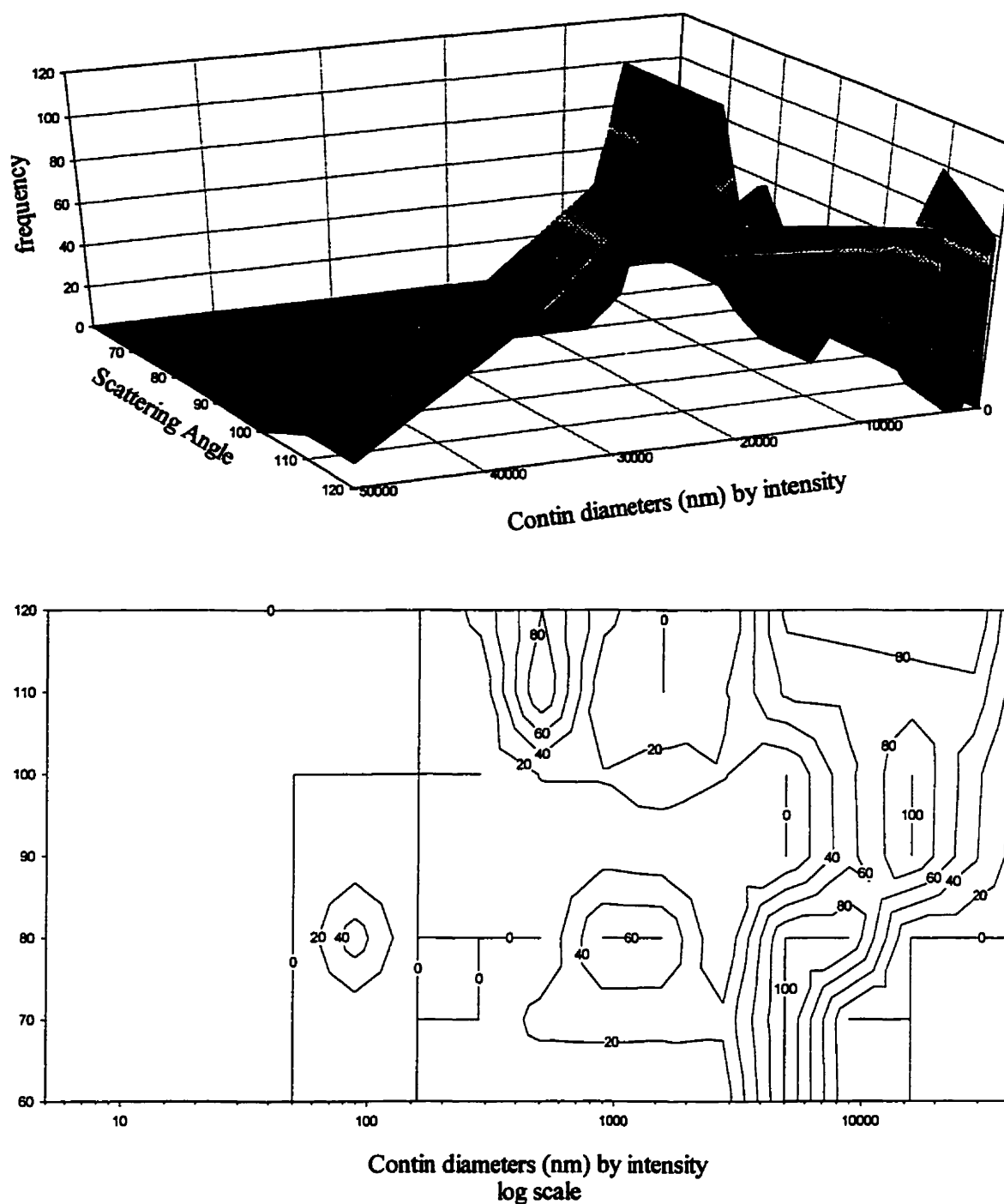


Figure 5: DLS results for C-940 (0.02% w/w in 0.100 M NaCl) indicating roughly spherical particles about 11 μm in diameter. The lower plot is a two-dimensional (log) contour representation of the three-dimensional histogram above.

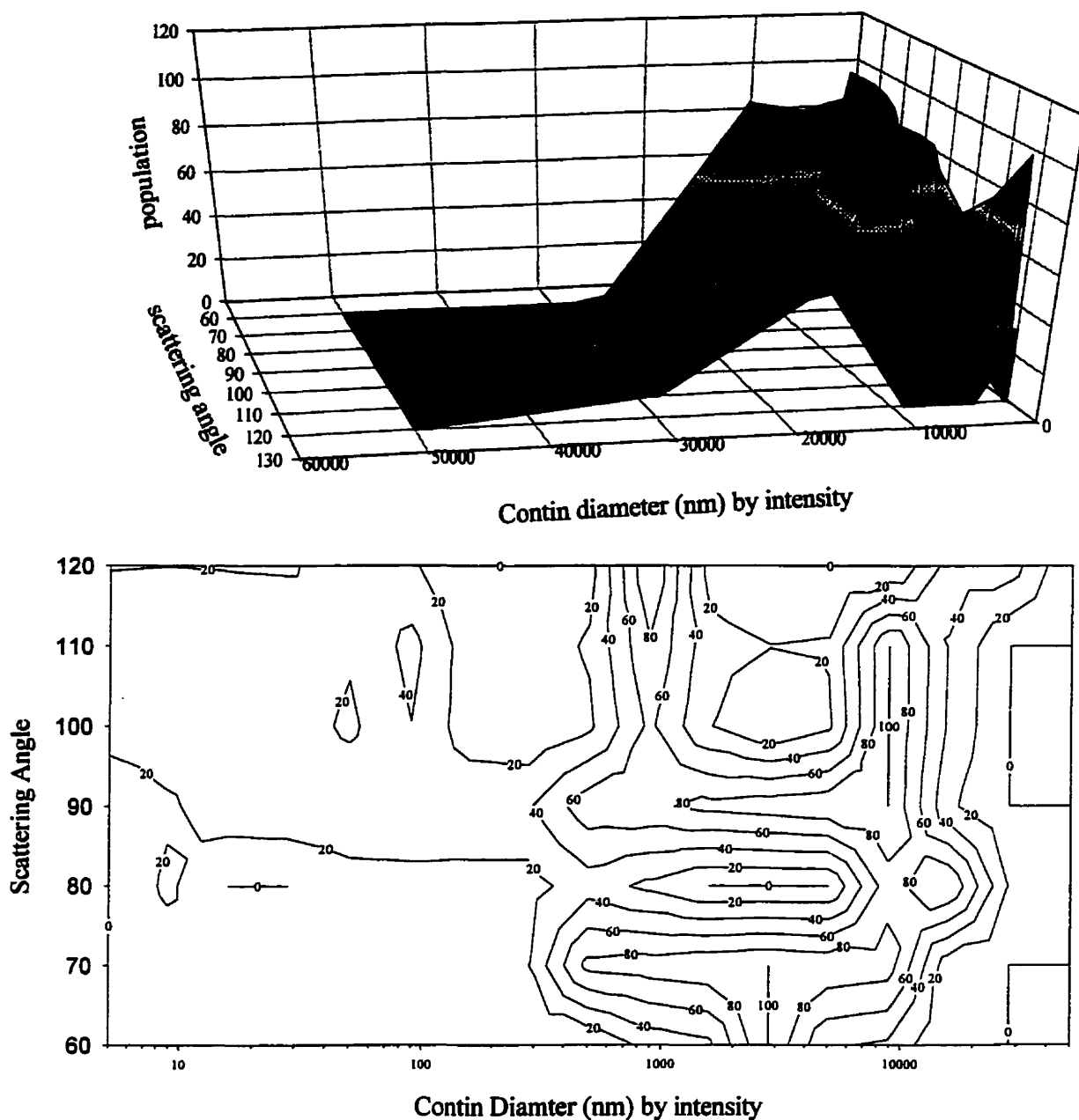


Figure 6: DLS results for C-941 (0.01% w/w in 0.100 M NaCl) indicating roughly spherical particles about 8 μm in diameter. The lower plot is a two-dimensional (log) contour representation of the three-dimensional histogram above.

HPLC

As in Chapter 5, reverse-phase HPLC was employed to measure the FQ polymers' bile salt sorption by changes in solution concentration. The eluent was a binary mixture of methanol and water (80:20 v/v) containing 0.1 M acetic acid to ensure protonation of bile salts. The pump flow-rate (LKB Bromma 2248(10)) was 1.0 mL/min. The refractive index detector (Waters 410) was interfaced to a computer, and the analyte peak areas were calibrated with sodium glycocholate (NaGC) standards in 5 mM tris(hydroxymethyl)amino ethane (Tris hereafter) buffer adjusted to pH 7.2 with 0.1 M HCl. Further instrumental details may be found in Chapter 5.

To identify the most sorbent polymer, all samples were tested as follows: ~10 mg of polymer powder were weighed into vials to which 2 mL of buffered bile salt solution (5 mM Tris, pH 7.1) were added at five concentrations ranging from 0.1 mM to 2 mM. The vials were agitated for at least one day, they were centrifuged as described in Chapter 5 and the supernatant was injected into the HPLC apparatus. FQ-934_{EDC} was identified as a promising candidate, and three more vials were prepared using 20 mg polymer samples of this material.

Results and Discussion

Preliminary binding evaluation for the FQ hydrogels

Binding data can be presented in several forms. For ease of comparison, isotherms where the quantity of bound sorbate per gram of sorbent (or equivalent) is plotted against the equilibrium sorbate concentration are preferred and are presented below. However, even in their simplest form, the raw HPLC data give a clear indication as to which hydrogel samples merit attention. For a bile salt anion sequesterant to have potential pharmacological and physical utility, its addition to a bile salt solution must result in a decreased equilibrium bile salt concentration. The data plotted below in Figure 4 suggest that two FQ_{EDC} samples (-934 and -940) show promise in that under the experimental conditions reported above, after hydrogel addition, the equilibrium free NaGC concentration was about 25% less than the initial [NaGC]. FQ-940_{DCC} and FQ-941_{EDC} yielded similar, intermediate results (~10% reduction) and the two remaining FQ_{DCC} samples (934 and 941) were poor sequesterants. The FQ_{DCC} and FQ-941_{EDC} samples were not pursued further as IR evidence indicated residual urea following the functionalization despite laborious work-up procedures. The DCC coupling byproduct, dicyclohexylurea, (DCU) is insoluble in all common solvents except alcohols in which it is sparingly soluble. Both DCU and the EDC-derived urea (EDU) were retained within their hydrogel matrices rendering purification difficult, though the hydrophilicity of EDU made its removal less troublesome. Residual DCU was likely responsible for the spurious binding results from FQ-934_{DCC} and -941_{DCC} for which negative binding is

suggested in the data (see Figure 7) since the alcoholic HPLC eluent was a fair solvent for this compound. The FQ_{DCC} and FQ-941_{EDC} samples may indeed be effective sequestering agents, but our best efforts were not sufficient to render these materials clean.

Although this plot of the initial bile salt concentration against the final equilibrium NaGC concentration following incubation with the FQ polymers gives a clear indication as to which polymers measurably sequester bile salts, the functionality of each sample was required before plotting the isotherms.

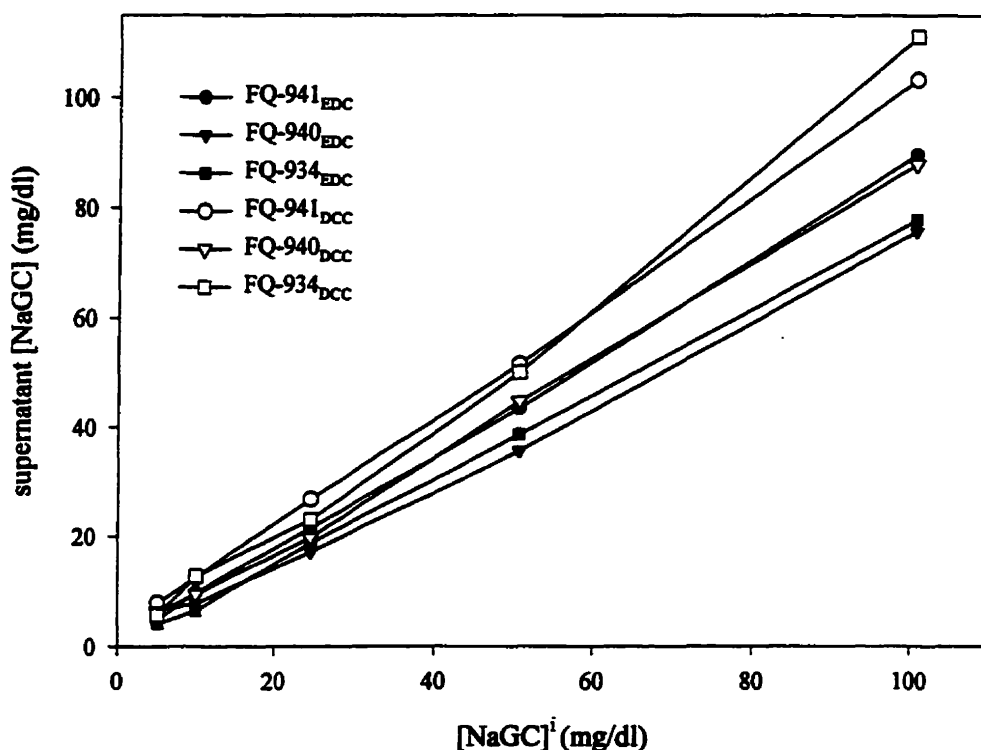


Figure 7: Raw bile salt sorption data for all FQ samples (10 mg polymer, 2 mL buffered bile salt solution, >1 day agitation prior to measurement). The FQ_{EDC} samples appear to substantially outperform their FQ_{DCC} counterparts.

Functionality determination (FTIR)

The FTIR spectra below (see Figure 8) clearly indicate ester and amide carbonyls in each sample. Of particular note are the strong amide I and II bands for FQ-940_{EDC} and FQ-934_{EDC} (1664 and 1572 cm⁻¹). Furthermore, the spectrum for FQ-941_{EDC} provides strong evidence for residual urea byproduct from the functionalization, which may explain the poor performance of this material (and the FQ_{DCC} materials) when compared with FQ-934_{EDC} and -940_{EDC}. The results of plotting the carbonyl region in absorbance mode followed by peak deconvolution and integration are given below in Table 1.

Despite the cross-linked starting materials, EDC reaction conditions successfully coupled the majority of all available carboxylic acid groups with DMED. The apparent degree of functionality for FQ-941_{EDC} suggested that this sample ought to perform exceptionally well; however, these data should be interpreted with caution as the deconvolution of the overlapping amide I and urea peaks for this spectrum was imperfect due to the differing peak shapes.

Table 1: FQ_{EDC} functionality as determined by FTIR.

Sample (FQ _{EDC})	Amide peak area (~1665 cm ⁻¹ , %)	Ester peak area (~1735 cm ⁻¹ , %)	Fit (r ²)	Acrylamide functionality (mmol TMEACl / g FQ _{EDC})*
934	58	42	0.994	3.9
940	53	47	0.992	3.8
941	67	33	0.994	4.3

* Since the molar absorptivity ratio for these bands is 1.0, the relative peak areas correspond to the mole fraction of repeat units bearing either ester or amide pendant groups.

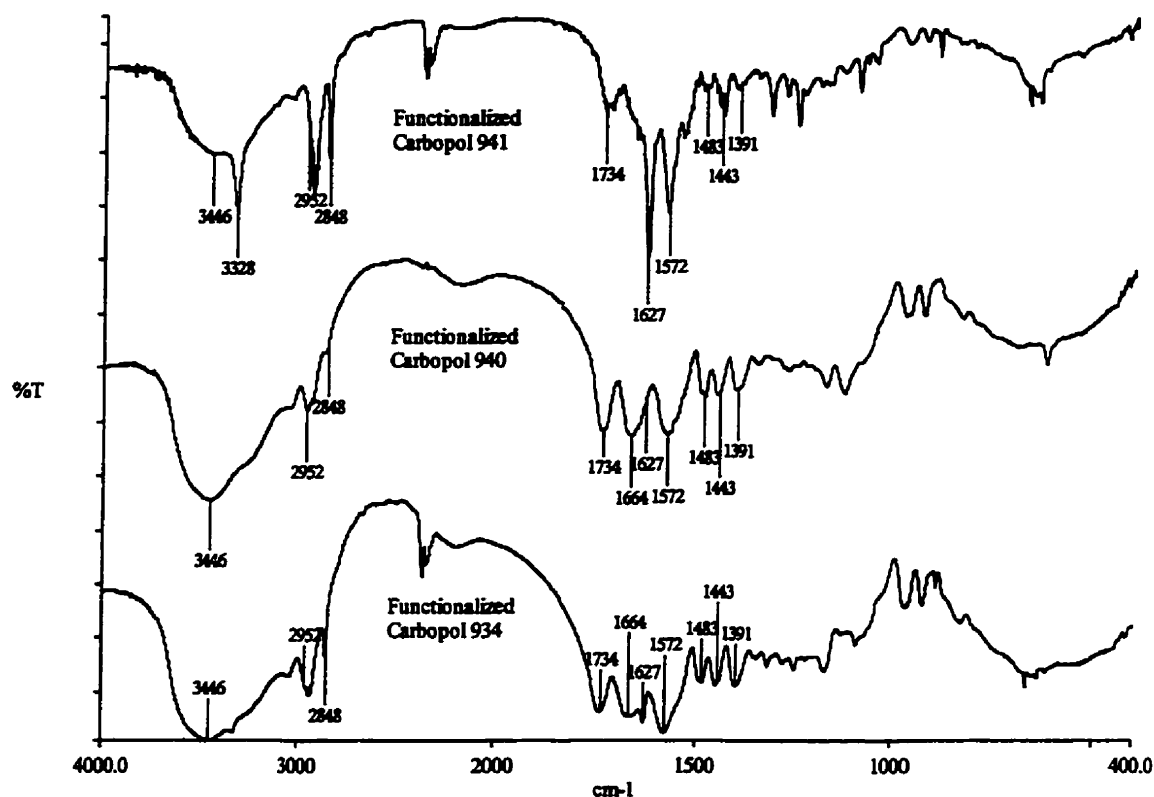


Figure 8: FTIR spectra of the EDC-mediated DMED-coupled Carbopols. Of particular note are the strong amide I and II bands for FQ-940_{EDC} and FQ-934_{EDC} (1664 and 1572 cm^{-1}) as well as the residual urea or N-acyl peaks for FQ-941_{EDC} (3328, 2848 and 1627 cm^{-1}). All spectra show ester carbonyl peaks (1734 cm^{-1}) due to the methylation of unreacted carboxylic acid groups under basic conditions.

NMR results

Solid state ^{13}C NMR spectra (see Figure 9) qualitatively support the functionality determined above. The dominant broad peaks in the spectra are amide carbonyls (~ 177 ppm), and backbone carbons (~ 40 ppm). The sharp peak at about 55 ppm corresponds to the N-methyl carbon signal and the ethylene spacer appears at about 65 ppm. Residual ester carbonyl signals are observed as discrete peaks or shoulders at about 181 ppm (see Figure 9).

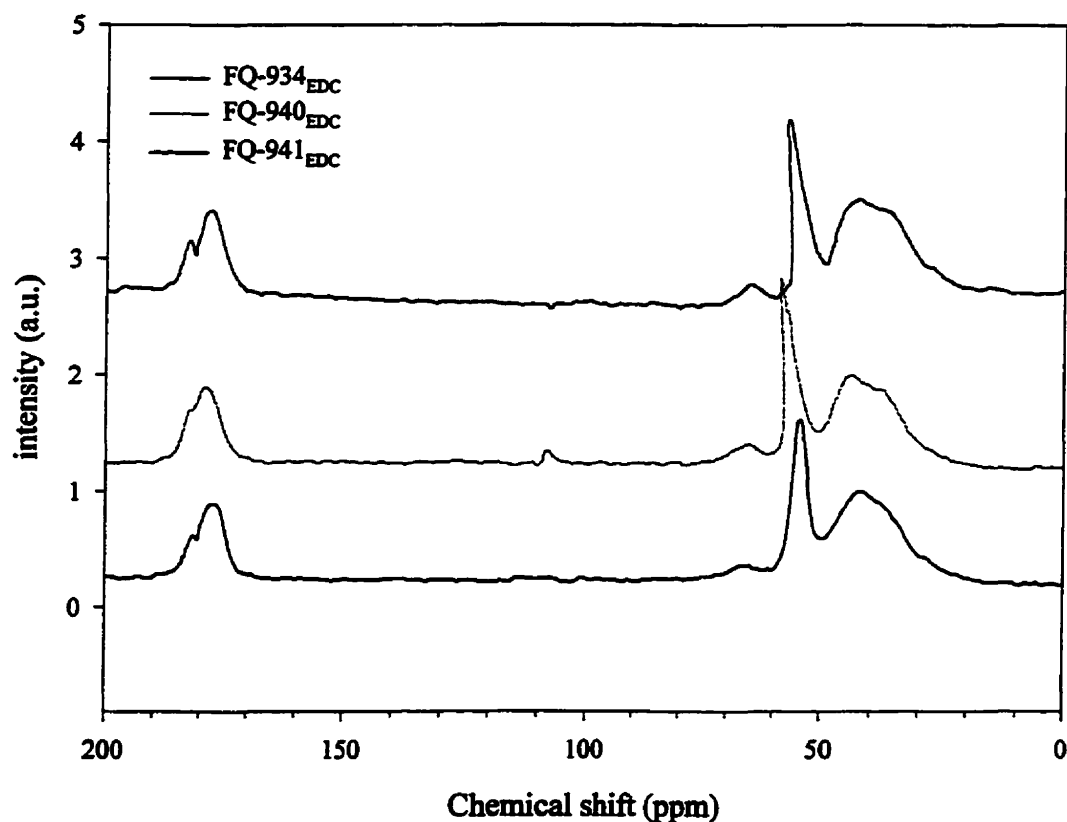


Figure 9: ^{13}C Solid-state CP/MAS NMR spectra of FQ-934_{EDC}, FQ-940_{EDC} and FQ-941_{EDC}. Qualitatively, the strength of the ester 'shoulders' parallels the functionality determined by FTIR.

Glycocholate binding isotherms

Under the experimental conditions leading to the isotherms below, sorbent addition caused a substantial decrease in the NaGC concentration for FQ-934_{EDC} and FQ-940_{EDC} and a significant, but lesser, decrease for the remaining sample, FQ-941_{EDC} (see Figure 10). As long as the hydrogel-induced [NaGC] decrease did not fall off dramatically, the binding sites were not saturated.

The binding isotherms (see Figure 11) indicate not only high capacity for the FQ_{EDC} hydrogels, due to the steep slope and lack of a sorption plateau, but the sigmoidal shape also suggests a cooperative binding process where early binding amplifies further interactions. Limited quantities of FQ-940_{EDC} precluded further assays with this very promising material; however, addition of 20 mg samples of FQ-934_{EDC}, instead of 10, reduced the NaGC equilibrium concentration below the HPLC detection limit at low initial concentrations, suggesting quantitative removal of bile salt anion.

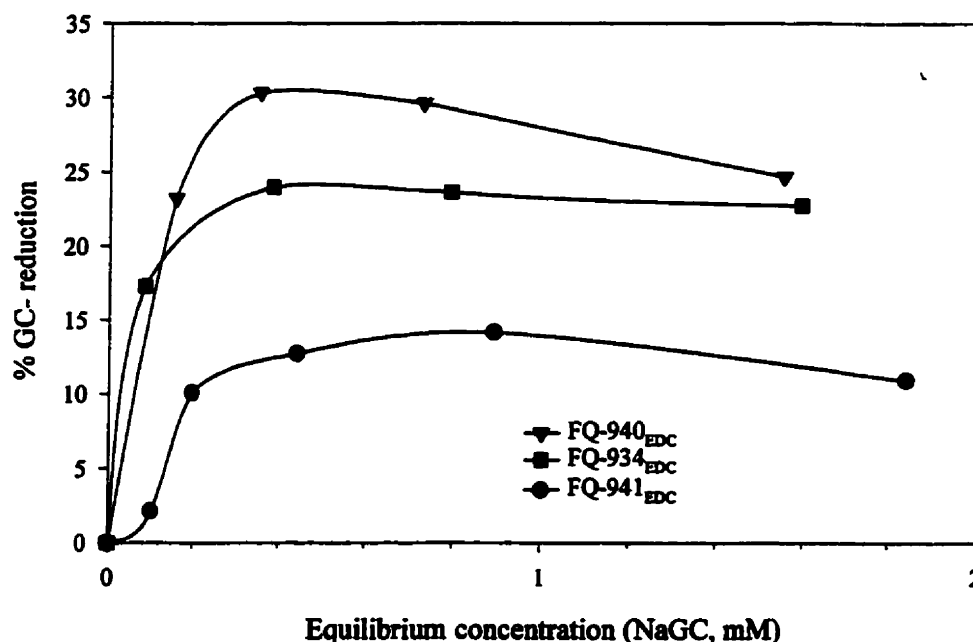


Figure 10: Supernatant [GC⁻] reduction (%) on FQ_{EDC} hydrogel addition. (The lines are spline curves to guide the eye.)

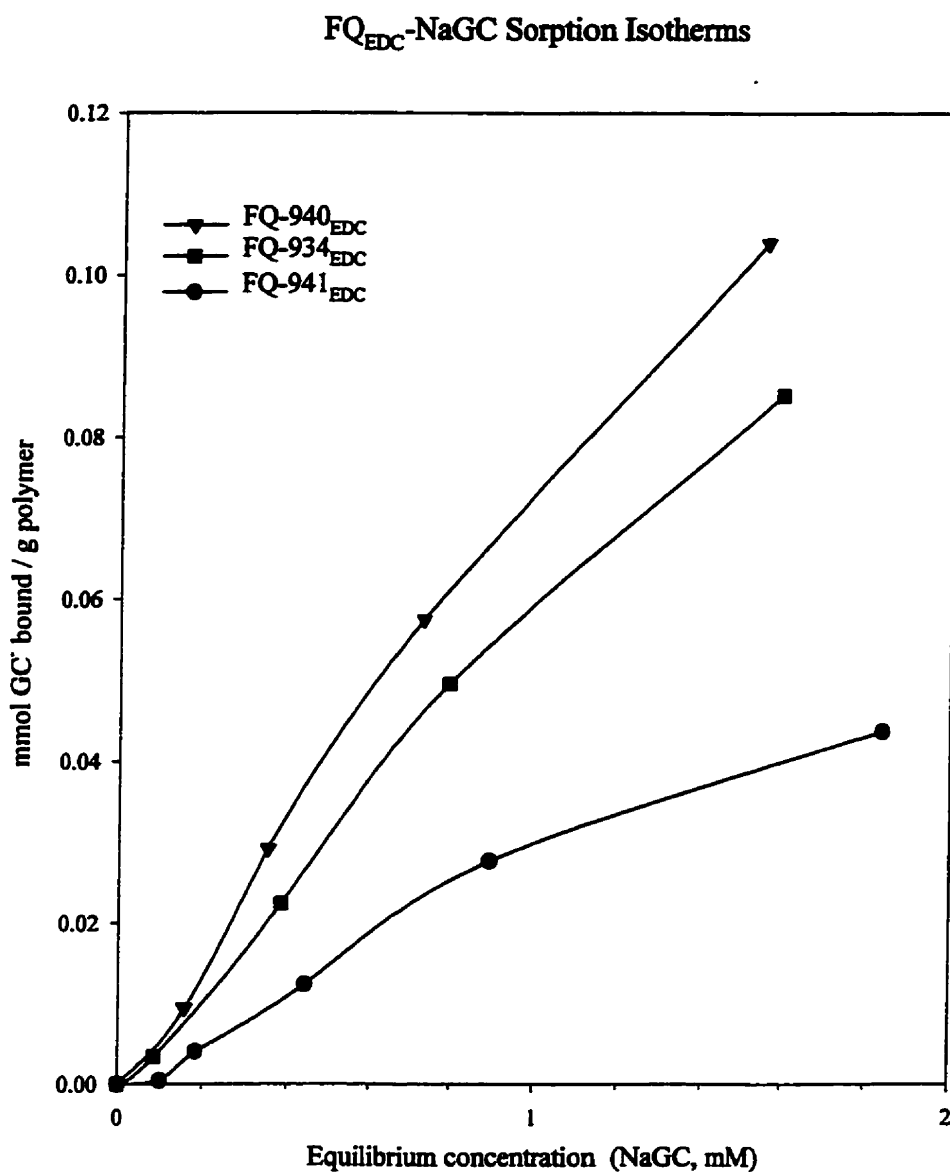


Figure 11: Glycocholate sorption isotherms for the FQ_{EDC} hydrogels. Polymer samples (10 mg) were incubated in 2.00 mL NaGC solutions and the equilibrium bile salt anion concentration was determined by HPLC. The functionalities of FQ-934_{EDC}, FQ-940_{EDC}, and FQ-941_{EDC}, were 3.9, 3.8 and nominally 4.3 mmol TMEACI/g respectively. (The lines are spline curves and are intended only to guide the eye.)

Polymer dimensions and sorption

It is tempting to try to explain the bile salt sorption behavior of the FQ_{EDC} polymers based solely on the hydrodynamic radii of their parent carbomers reported above. However, the hydrodynamic radius is a function not only of the molecular weight of the polymer, but also of the cross-link and branch point densities. Branches and cross-links restrict swelling and therefore the intuitive correlation between molecular weight and hydrodynamic radius need not hold.⁷

Due to the long incubation of bile salt solutions and hydrogels with agitation (several days), we expect that the kinetics of diffusion were not a factor in explaining the different sorption performance of these materials. Instead, it emerges that despite repeated washing and protracted dialysis, the least active of the FQ_{EDC} polymers (-941) may have been contaminated with residual urea. Alternatively, this sample may have been subject to N-acyl rearrangements, as discussed in Chapter 3 (see Figure 8). Given this observation, two possible explanations for residual urea leap to mind: (i) The cross-link and/or branch point density is sufficiently high that EDC can diffuse into the matrix but that once the urea has been displaced, it is not easily removed thereby reducing the number of available binding sites; and (ii) A corollary to the supposition of high cross-link density is reduced availability of binding sites so that despite a high degree of synthetic conversion, the cationic binding sites could not ‘see’ the sorbate anions.

The two hydrogels that performed best, -934 and -940, were synthesized from the carbomers that yielded the smallest and the largest hydrodynamic radii, respectively. It follows that for FQ-934, derived from the small hydrodynamic diameter (5 μm)

carbomer ensuring that all or most potential functionalization sites are available, the byproduct will diffuse out of the structure and the sorbate will have access to the pendant quaternized amines. The molecular weight of this sample probably approaches the lower limit of the range suggested by BF Goodrich.²²

Given the 15 μm hydrodynamic radius of C-940, the clarity of the gel in water and its nearly limitless swelling capability, the cross-link and/or branch point density must be quite low. The high viscosity of C-940 aqueous suspensions, on the other hand, suggests a high polymer. It seems reasonable to conclude that C-940 and C-934 swell sufficiently to allow for EDC-mediated DMED functionalization and work-up. Furthermore, once functionalized, N,N,N-trimethylammonium pendant binding sites are available to participate in ion-exchange NaGC sorption.

Proposed mechanism of binding

Earlier work has shown conclusively that electrostatic and hydrophobic interactions each have a significant role to play in the ion-exchange sorption of bile salts.⁶ With the relatively short ethylene hydrophobic spacer between the pendant ammonium groups and the polymer backbone, the cooperative hydrophobic interactions with the polymer are expected to be minimal. One must therefore assume that for this system, electrostatic attraction between the carboxylate groups on the bile salts and the methylated ammonium groups on the polymer is of fundamental importance. The cross-linking agent, which may resemble sucrose but is at least a polyether, may have a role to play in hydrophobic polymer-surfactant (bile salt) interactions.

Conclusions

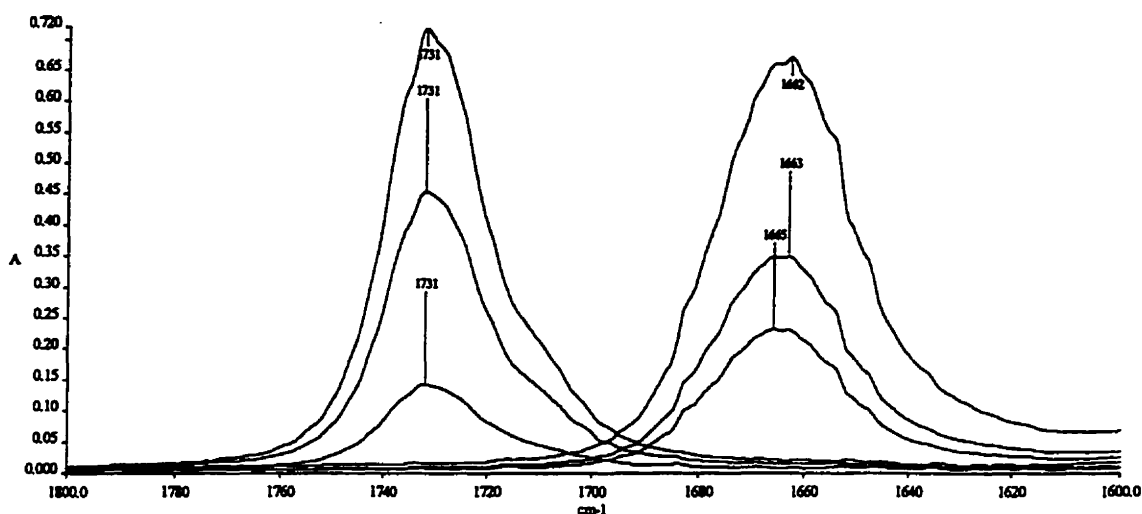
Chapter 6 presents the comparison of two methods of Carbopol functionalization. The problems inherent with the purification of hydrogels are underscored with the FQ_{DCC} materials; however using EDC, a hydrophilic carbodiimide, as the acid-amine coupling agent improved the synthesis and work up, thereby providing bile salt sequestrant polymers that may well be worth pursuing. Primarily employing electrostatic attraction, these sequestrants appear capable of near-quantitative ion-exchange bile salt sorption at low bile salt concentrations. The maximum equilibrium concentration reduction achieved here was about 40% and the binding isotherms suggest positive cooperativity.

To be practical, a bile salt sequestrant must have a high capacity and affinity for bile salts, preferably be selective, and it must also be comparatively simple to synthesize and prepare. As presented in Chapters 3 and 5, block copolymer aggregates offer an incredible surface area relative to total mass (or volume), they are promising as bile salt sequestrant candidates and represent an interesting platform from which to probe polyelectrolyte-surfactant interactions in a curved brush. They remain, however, complicated to prepare. Using industrial-scale polymers such as the carbomers for starting material, on the other hand, is attractive. Furthermore, the preparation of a hydrogel for ingestion involves no more than the suspension of a highly swellable powder in water.

Bearing in mind that earlier work has shown that using a longer hydrocarbon spacer between the acrylamide and the ammonium groups dramatically improves the performance of highly cross-linked resins, synthesizing α -permethylated diamines with varying hydrocarbon spacers and then coupling them to Carbopol samples may well

provide an interesting avenue for future work. It is nevertheless worth stressing that although the primary thrust of this work has been to synthesize polymers to test their applicability to the problem of coronary heart disease, the potential for pure investigation is also significant. As with the block copolymer aggregates, FQ-type polymers offer an interesting material for the study of polyelectrolyte-surfactant interactions but have the additional advantage of being a potential template for investigating the role of hydrophobic interactions.

Appendix



Above are solution FTIR spectra (CHCl_3) for ethylacetate ($\text{EtAc} \sim 1730 \text{ cm}^{-1}$) and N-acetythylenediamine ($\text{NAED} \sim 1660 \text{ cm}^{-1}$) focusing on the carbonyl stretching bands. The concentrations for EtAc were 0.0356 M, 0.111 M and 0.183 M. The concentrations for NAED were 0.0650 M, 0.100 M, and 0.197 M. With a solution cell path length of 0.01 cm, the extinction coefficient for the ester ϵ_{ester} was $10500 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ and for the amide, $\epsilon_{\text{amide I}}$, was $10400 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The ratio of $\epsilon_{\text{ester}}/\epsilon_{\text{amide I}}$ was therefore 1.01.

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Chapter 7

Ammonium Binding Resins: Polymer Tethered Tetraphenylborate

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Chapter 7: Tethered Tetraphenylborate

Abstract

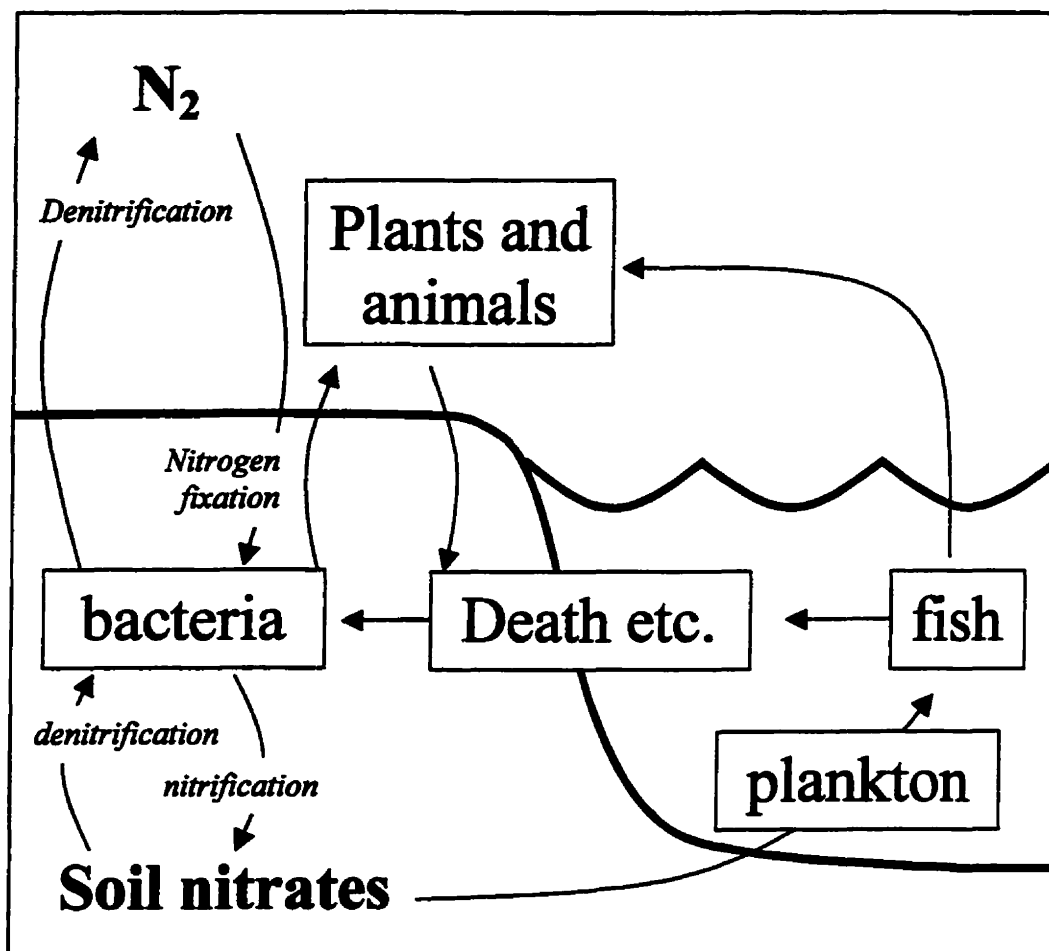
A novel approach to the problem of removing (organo)ammonium ions from industrial effluent and agricultural waste is herein proposed. Capitalizing on the insolubility of (organo)ammonium tetraphenylborate, we have designed, synthesized and tested a polymer-tethered system that eliminates (organo)ammonium species from aqueous solution. Furthermore, the binding can be controllably reversed thereby regenerating the material, by deprotonating the ammonium ion in strong base, without significantly reducing the binding properties of the matrix. Whereas the work presented in previous chapters employed tethered ammonium groups to sequester anionic bio-surfactants, this final experimental chapter presents a polymeric system bearing anionic tetraphenylborate moieties that sequester (organo)ammonium cations.

Introduction: Why is ammonium bad?**The Nitrogen cycle**

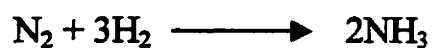
Hydrogen, oxygen, carbon and nitrogen together represent more than 99% of human body mass, a rule that is generally applicable across the biosphere. Most introductory organic chemistry textbooks underscore the importance of C, H, O, and N, (along with phosphorus and sulfur), as they describe the properties and reactions of these elements. The two elements that restrict plant growth the most are nitrogen and phosphorus due to limited availability.¹ Obviously, the biosphere will remain coherent only as long the elements of life are balanced. Nitrogen gas, as N_2 , is chemically inert. This stability accounts for the observation that about 80% of our atmosphere is composed of gaseous nitrogen. The nitrogen cycle (see Figure 1) can be summarized as an equilibrium, facilitated by bacteria, between soil nitrates, ammonia and atmospheric nitrogen where ammonia has a critical side cycle through the plant and animal kingdom. Once nitrogen is fixated as ammonia in the soil, other organisms can chemically manipulate the element to synthesize bio-molecules and biopolymers.

Environmental effects: algal blooms and fish kills

The environment is a delicate balance of chemical reactions. Under normal conditions, soil nitrates and atmospheric N_2 together provide a limited, but sufficient source of ammonia for organic use. Nitrifying bacteria are usually limited by the availability of ammonia and are in competition with other living organisms for the accessible supply. When these bacteria are given far more ammonia than is usually in the



Nitrogen fixation



Nitrification

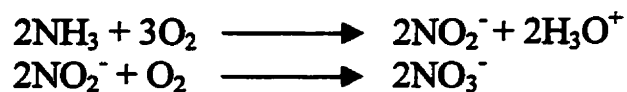


Figure 1: A simplified version of the nitrogen cycle (adapted from ref. 1, p. 486). The arrows represent the movement of nitrogen.

environment (for example in effluent water from some industries, or from sewage ponds, etc.), then they prosper or *bloom*. One might expect that a boon at the algal level of the food chain should enrich all its dependants, but the nitrification of ammonia consumes oxygen, which in turn leads to the de-oxygenation of the affected ponds and the death of many aquatic organisms.

Ironically, the farming community that depends so heavily on a healthy nitrogen cycle must shoulder a lot of the responsibility for elevated ammonia levels in the environment.² Advances in farming methods have lead to highly specialized operations. Though there are fewer hog farms now than there have been in the past, the total number of pigs owned at each site has increased dramatically. The nitrogen-rich animal waste is collected in lagoons that are periodically emptied when the waste is pumped off and sprayed over the fields as fertilizer. As long as the soil can absorb the waste and the water table is not contaminated, then there is little reason for concern; however, according to a recently published review, 72% of the variability of air-born ammonia (in Samson County, North Carolina) is a direct result of swine farming.² Furthermore, when natural disasters strike, the waste lagoon dykes can rupture, thereby contaminating the environment in a concentrated manner.

How do we already control ammonium?

Given the highly toxic effects of elevated ammonia levels, there is clearly a need for an efficient 'sponge' to mop-up after industrial chemistry and farming wastes. The centrate ammonium recovery process employs a proprietary ion-exchange system loaded with zinc that scavenges ammonium, which is collected by regenerating the polymer with

sulfuric acid. Not only does this make the product reusable, but also, as an added benefit, ammonium sulfate can be sold as fertilizer.

Centrate,³ the supernatant from centrifuged, anaerobically digested sewage is passed through ion-exchange columns at a rate of about 10 g ammonia per liter of resin. A sulfuric acid solution of zinc is employed to concentrate the ammonia to about 15 000 ppm which is, in turn, further concentrated to about 60 000 ppm. On cooling, zinc ammonium sulfate crystals are obtained and are aggressively heated to push the ammonia into a sulfuric acid scrubber yielding the desired ammonium sulfate and waste water containing at least 90% less dissolved ammonia.

Zeolites,⁴ high temperature and pressure oxidation,⁵ CO₂ extraction,⁶ and microbial degradation⁷ are examples of other patented processes that have ammonium cleansing properties. The zeolite method requires many reaction chambers and addresses only NH₄⁺ ions. High pressure and temperature reactions are expensive and require large on-site apparatus. Microbial degradation effectively reduces nitrogen-containing compounds to ammonia, but the efficient recovery of pure water is not trivial.⁸

Dalhousie University tetraphenylborate work

Though a variety of procedures exist already for the purification of wastewater, there remains room for an inexpensive, simple, re-usable alternative. The Dalhousie University X-ray crystallography center has had an on-going interest in ammonium-containing structures for several decades and in the course of their study of hydrogen bonding to π electrons, it was observed that the tetraphenylborate salt (TPB) of ammonium is essentially insoluble in water. Recrystallization of NH₄TPB (ATPB) in 1:1

water: acetone yielded robust prisms that were subjected to X-ray diffraction and FTIR experiments. Low-temperature results (120 K) clearly indicate tetra-radial boron centers with two pairs of orthogonal phenyl groups where the ammonium cation is trapped in a tetraphenyl cage between two boron hubs as shown in Figure 2.⁹ The study has grown to include more complex nitrogen-containing species that can accept a proton and thus

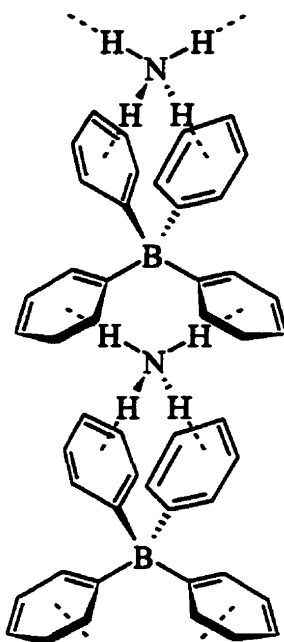


Figure 2: A schematic representation of ammonium tetraphenyl borate (ATPB) showing hydrogen bonding from the H(N)'s to the plane of the phenyl rings.

become cationic.¹⁰ When NaTPB_(aq) is added in slight excess to a solution of ammonium chloride (200 ppm NH₄⁺), ammonium tetraphenylborate (ATPB) precipitates and the supernatant is ammonium-ion free within the limits of colorimetric detection by Nessler's solution. More quantitatively, mass spectrometric analysis was sensitive enough to detect ammonium in distilled water, yet showed no trace (<2 ppm) of ammonium in the supernatant.¹¹ Very recently, others have also begun investigating the properties of NaTPB.¹²

Given that the goal, in part, was to create a method of removing ammonium from wastewater, NaTPB clearly represents an interesting possibility. A need was nevertheless recognized to adapt this technology to a re-usable and environmentally friendly system that is stable, easy to use, and has a high capacity. NaTPB alone is reasonably stable and is very efficient, but it would be complicated to apply directly to industrial situations without some method of filtration or precipitate recovery and regeneration.

Scope of this chapter

Using polystyrene as our basic starting material, we examined pathways by which this polymer support can be functionalized with TPB. To this end, we have synthesized poly(4-NaTPB)styrene (PTPB) and have initiated preliminary feasibility studies for its application to ammonium binding. Small-molecule work has demonstrated that TPB will precipitate with protonated (organo)ammonium and diammonium species as well as heterocyclic ammonium and even tetramethyl ammonium ions. It would appear that TPB is an effective scavenger for most, if not all, (organo)ammonium species including caffeine, nicotine and other alkaloids such as most street drugs. This chapter focuses on

the synthesis, characterization and ammonium binding properties of PTPB. It is our hope that PTPB will prove to be an efficient, inexpensive, recoverable, reusable, easy and environmentally friendly method of removing nitrogen-based contaminants from water.

Synthesis

The synthetic goal presented here was to produce cross-linked polymeric beads with tethered TPB moieties. To optimize the synthetic route, we brominated, lithiated and then triphenylboronated commercial cross-linked PS resin beads (see Figure 3) as described in detail below.

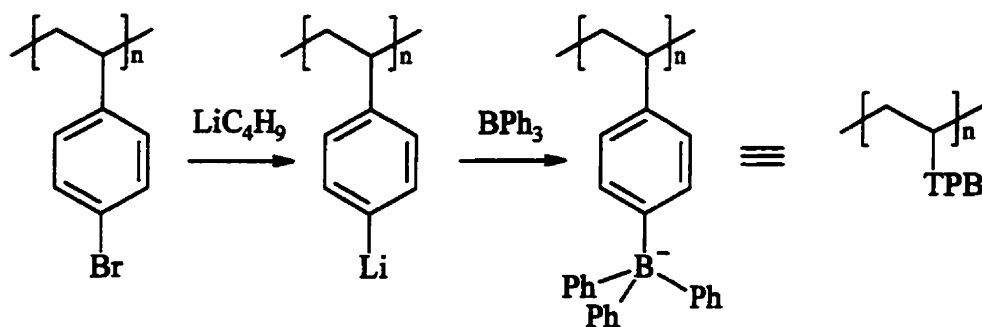


Figure 3: Reaction scheme indicating lithiation and triphenyl-boronation. The lithium counter ion is ultimately replaced by sodium.

There are several possible routes for the synthesis of NaTPB. One method calls for the tetraphenylation of trifluoroboron¹³ using the phenyl Grignard reagent (PhMgBr) followed by precipitation and recrystallization with NaCl or Na_2CO_3 . Rather than starting with BF_3 , we chose to couple BPh_3 , which is commercially available (Aldrich), and poly(4-bromo)styrene (P4BS) (see Figure 3).

To test the feasibility of this strategy, we began by reactions of non-cross linked P4BS that had been polymerized from the 4-bromostyryl monomer, thereby ensuring

essentially complete bromo-functionality. Our ultimate goal, however, was to generate cross-linked beads.

Bromination of PS beads

In order to produce brominated PS beads, we considered two routes: (i) bromination of pre-synthesized cross-linked PS beads, or (ii) polymerization of styrene, (4-bromo)styrene and divinyl benzene along with a pore-forming agent. In the interests of time, we opted to brominate 200-400 mesh PS beads containing 2% divinyl benzene (DVB) (Polysciences) with iron(III) bromide.*

The beads were washed in hot THF (Soxhlet apparatus) for 24 hours and were then dried *in vacuo* at 50 °C. The PS beads (5 g) were suspended in CH₂Cl₂ with 500 mg iron filings, and the reaction temperature was reduced to 0 °C. Bromine (3 mL) was added in 1 mL aliquots and after one hour, the reaction temperature was raised to reflux for 3 days.

The product was washed in hot dichloromethane for 12 hours and then extracted with hot THF for 3 days (Soxhlet). The sample was dried to constant weight giving 9.6 g. All FTIR spectra (see Figures 4 and 5) and solid-state NMR spectra (see Figures 6-9) are found and discussed in the Characterization section below. The weight-fraction of bromine was determined by elemental analysis (QTI Technologies) to be 52.7%, which corresponds well to the weight gain (48%). Given the high bromine content, it seems reasonable to conclude that surface bromination was accompanied by bromination within the beads. Ordinarily, alkyl-substituted phenyl rings are predisposed to *ortho*- or

* The formation of iron bromide is exothermic, so appropriate precautions are required.

para- electrophilic aromatic substitution, though the *para* position is usually preferred for steric reasons. Once mono-brominated, the carbons *ortho* to the bromine are the most susceptible to further bromination. It is therefore not surprising that the FTIR spectrum for the brominated beads indicate not only 1,4 disubstitution, but also 1,2,4 trisubstitution in the aromatic out-of-plane distortion region, whereas the commercially polymerized P4BS yields an FTIR spectrum with only 1,4-disubstituted phenyl bands (compare Figures 4 and 5). The slight difference between the theoretical and actual final mass may be attributed non-homogeneity and an over-brominated sample. Since the bromination is a necessary precursor to the nucleophilic sites, maximum bromination was desirable.

The Grignard intermediate

Synthesizing phenyl Grignard is a fairly standard introductory organic chemistry experiment, but it does not adapt well to P4BrS since this is a heterogeneous reaction where Mg insertion depends on favorable contact between the aryl bromide in solution and the metal surface. For the cross-linked P4BrS the coil dimensions and steric constraints conspired to make this reaction *extremely* slow, despite trying either freshly ground Mg turnings or Mg powder. Since the resulting Grignard is highly reactive, long reaction times should be avoided. Generally, the kinetics of Grignard killing are extremely fast. Although we saw some evidence of the proton-quenched Grignard intermediate, the yield was not sufficient to render this route useful.

The aryllithium intermediate

A better alternative to the synthesis of the phenyl Grignard proved to involve the synthesis of the phenyl lithium intermediate. Bromine-lithium exchange is a much more

controllable reaction to run and for small molecules occurs at temperatures as low as – 120 °C. For small molecules, this chemistry is well established,¹⁴ but to our knowledge, it has not been employed to derivatize polymers; rather it is used in the synthetic route to monomer precursors. Bromine and lithium exchange occurs due to the comparative stability of the sp^2 carbanion, yielding the equivalent of the phenylmagnesium bromide (phenyl Grignard). Since it partially dissolves in THF, butyl lithium is a much more potent reagent than Mg metal.

Lithiation and TPB functionalization of linear homo-P4BrS

In a typical test reaction of linear homo-P(4BrS), 240 mg (1.3 mmol aryl bromide) of vacuum-oven dried polymer was dissolved in 20 mL of dry THF freshly distilled from the sodium/benzophenone complex, all in an ultra-pure nitrogen atmosphere. The reaction temperature was reduced to -78 °C using a dry ice/acetone bath and 1.1 mL of a 1.3 M solution of *sec*-butyl lithium (1.4 mmol or ~10% excess) was added drop-wise over 1-2 minutes.

The reaction was left to stir at low temperature for several hours whereupon an aliquot was removed and quenched with acidified methanol/water, which initially gave a suspension of translucent organic droplets that gelled and solidified on stirring. Redissolution of the gel in THF was not possible, thereby suggesting a cross-linked intermediate terpolymer of cross-linked styrene, debrominated styrene (via the lithiated product) and unreacted 4-bromostyrene. A new peak in the phenyl fingerprint FTIR spectrum at 700 cm^{-1} indicated the formation of the proton-trapped lithiation intermediate (styrene) and diminished peak at 718 cm^{-1} suggested unreacted bromide (see Figure 4).

Having established the successful, albeit incomplete, lithiation of homo-P4BS, conditions for the successful triphenyl-boronation of the linear polymer were as follows: 6.0 mL triphenyl boron solution (0.25 M in THF or 1.5 mmol - Aldrich) was added to the lithiated polystyrene reaction mixture at $-78\text{ }^{\circ}\text{C}$ and the reaction was allowed to warm to ambient conditions overnight. A sandy-coloured precipitate with a faintly cloudy supernatant was observed 12 hours after the triphenyl boron addition.

The reaction was quenched in rapidly stirred distilled water giving rise to small solid particles (295 mg dry mass) (PTPB-1) for which IR spectra indicated successful functionalization (*i.e.*, mono-substituted phenyl groups). Both the polymeric starting material and regular homopolystyrene are very soluble in THF, yet the particles were only THF swellable and did not redissolve in any solvent suggesting that the product PTPB-1 was a cross-linked resin.

The aqueous fraction was blue-tinged (turbid) but otherwise transparent. On evaporation of the water, a film and some solid residue remained suggesting triphenyl boron side reactions and perhaps residual non-crosslinked polymer.

Lithiation and TPB functionalization of PS-co-PDVB-co-PBrS

Several triphenyl boronations of PS beads (PS-co-PDVB-co-PBrS) were carried out using the brominated PS beads prepared as described above. Based on the experience gained by reaction of the noncross-linked P4BrS, the beads were functionalized as follows: PS-co-PVDB-co-PBrS (3.0 g containing 20 mmol Ar-Br groups determined by elemental analysis) was stirred in 10 mL THF that was freshly distilled from Na/benzophenone and the reaction temperature was reduced to $-78\text{ }^{\circ}\text{C}$. The lithiating

reagent, *sec*BuLi (17 mL, Aldrich, nominally 1.3 M) was added and the lithiation was allowed to proceed for 3.5 hours whereupon 21 mmol Ph_3B were added in THF and the reaction was allowed to warm to ambient conditions. Two days later, the reaction was quenched by addition of 4 M NaCl. The reaction mixture was filtered and then washed with THF, ethanol, water and was finally dried to give 2.8 g of product (PTPB-2).

The highest degree of functionality was achieved under the following conditions: PS-co-PVDB-co-PBrS was dried *in vacuo* at 40 °C overnight and 1.02 g (6.7 mmol Ar-Br) were suspended in 50 mL THF that was freshly distilled from Na/benzophenone under N_2 . The reaction temperature was reduced to -78 °C whereupon *sec*BuLi (5.4 mL, 1.3 M in THF or 7.0 mmol, Aldrich) was added and the reaction was stirred for 75 minutes. Triphenylboron (BPh_3 , 3.5 g) was dissolved in 50.0 mL of THF, as above, and the solution was added to the lithiated PS beads. The mixture was left to stir and warm up to ambient temperature overnight until 20 hours after initiation, when the reaction was 'killed' by addition of 2 mL milliQ water. The beads were stirred in 0.1 M NaCl for 24 hours and were filtered. Soxhlet extraction (48 hours, THF) was employed to wash the beads, which were then dried *in vacuo* and weighed to give 0.78 g, PTPB-3.

Characterization

FT infrared spectroscopy

A Perkin-Elmer 16-PC FTIR using Spectrum® software was used to collect FTIR spectra. With one exception, the samples were prepared as pellets prepared with spectrograde KBr (Aldrich). The P4BrS precursor to PTPB-1 was cast as a film from

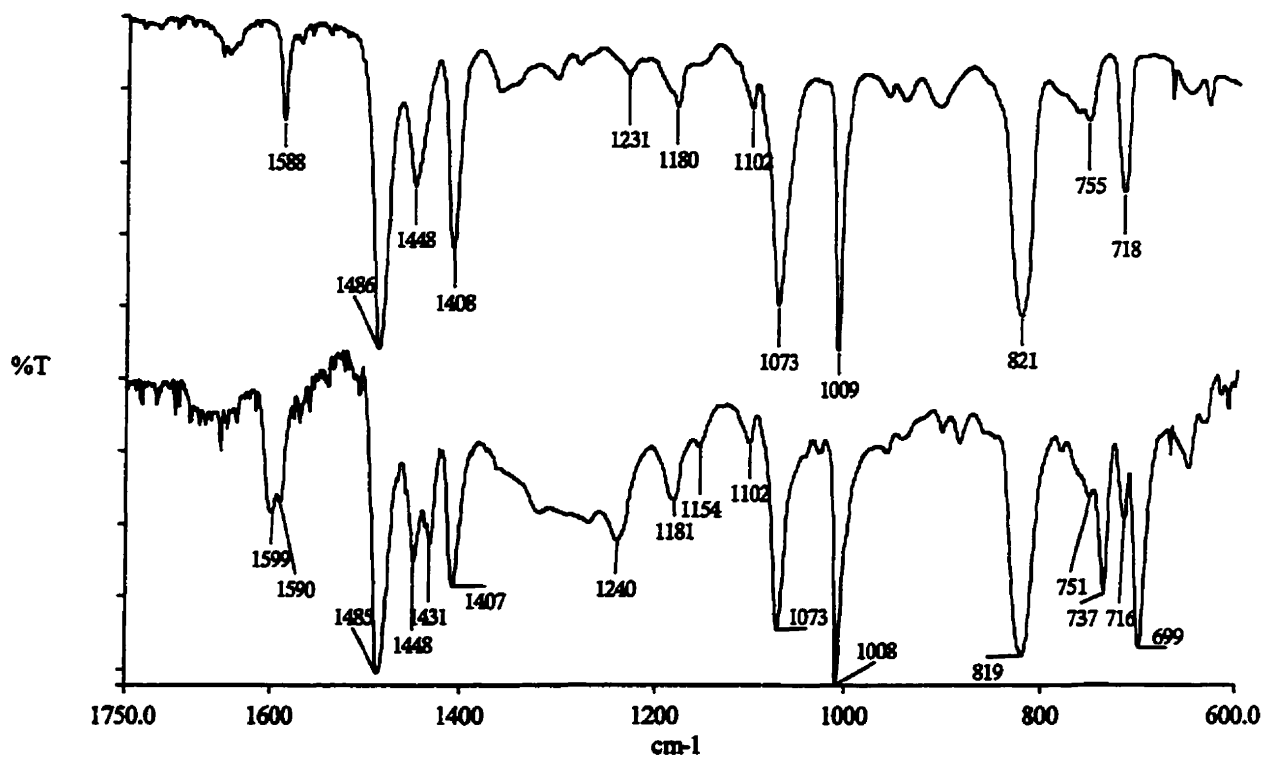
THF on a KBr window (Wilmad). In all cases, the nominal resolution was 2 cm^{-1} and the number of scans was either 32 or 64.

To follow this series of reactions by FTIR is comparatively simple, since the degree of substitution of a phenyl ring can be determined from the Ar-H out-of-plane bending region (see Table 1). Under many circumstances, the so-called fingerprint region ($<1600\text{ cm}^{-1}$) is too complex for complete assignment. However, given the comparative simplicity of the starting material and the reaction scheme, most bands in these spectra can be identified with reasonable certainty. Where PS was the starting material, mono-substituted phenyl rings were expected, giving two characteristic bands at $770\text{--}730\text{ cm}^{-1}$ and $720\text{--}680\text{ cm}^{-1}$. Para-disubstituted phenyl rings have a strong band at about 820 cm^{-1} .

FTIR spectra for the test reactions on the linear starting material (P4BrS) and its product, PTPB-1, are given in Figure 4. In the upper spectrum, we see evidence of monosubstituted (non-brominated styrene) as well as the dominating 1,4 disubstituted bromostyryl rings (see Table 1). The other peaks are assigned as C-C sp^2 stretches and C-H distortions. Following lithiation and triphenyl-boronation, the C-C sp^2 stretching region and the C-H in-plane distortion regions become more complex reflecting the new phenyl groups, and the boron (mono)-substituted phenyl groups appear in the out-of-plane distortion region (note especially 737 cm^{-1}). New peaks at 1430 cm^{-1} and around 1240 cm^{-1} support the conclusion that the polymer has tetraphenylborate pendant groups.

Table 1: Diagnostic IR bands^{15,16}

Moiety	IR Bands (cm ⁻¹)
Aromatic Bands	
C-H stretch	3100-3000
C=C stretch	1650-1430
C-H in-plane distortion	1275-1000
C-H out-of-plane distortion	900-690
monosubstituted	770-730 and 710-690
1,4 disubstituted	860-800
1,2,4 trisubstituted	885-870 and 825-805
Boron-Carbon Bands	
C-B-C antisymmetric stretch	1280-1250
B-Ph ring vibration	~1430
Bromine-Phenyl Bands	
Ar-Br ring vibration	1070-1020
C-Br	750-500

**Figure 4: FTIR spectra of P(4-bromo)styrene (upper) and PTPB-1 (lower).**

The spectra in Figure 5 are (from upper to lower) PS beads (Polysciences), brominated beads, PTPB-2 and PTPB-3. As one might expect, the PS beads give a comparatively featureless IR spectrum with C=C, Ph-H in-plane and out-of-plane deformations that are characteristic of mono-substitution (*i.e.*, pendant, but otherwise unsubstituted phenyl groups). A small peak at 840 cm^{-1} reflects the 2% 1,4-disubstituted divinyl benzene moieties and the band at 905 cm^{-1} may be due to unreacted vinyl groups. The spectrum of the brominated product shows evidence of 1,4 and 1,2,4 substitution, and the mono-substitution bands are rendered comparatively insignificant (*e.g.*, at 755 cm^{-1}). Following lithiation, the para-position is preferentially occupied by triphenyl boron. Other lithiated sites are sterically restricted by the extremely bulky boron moiety and therefore may be quenched during the work-up with H^+ . As with PTPB-1, new peaks at 1436 cm^{-1} , $\sim 1240\text{ cm}^{-1}$ and 737 cm^{-1} reflect the TPB moieties. Note that the TPB peaks are more pronounced relative to the neighboring peaks for PTPB-3 when compared with PTPB-2, suggesting a higher degree of functionalization.

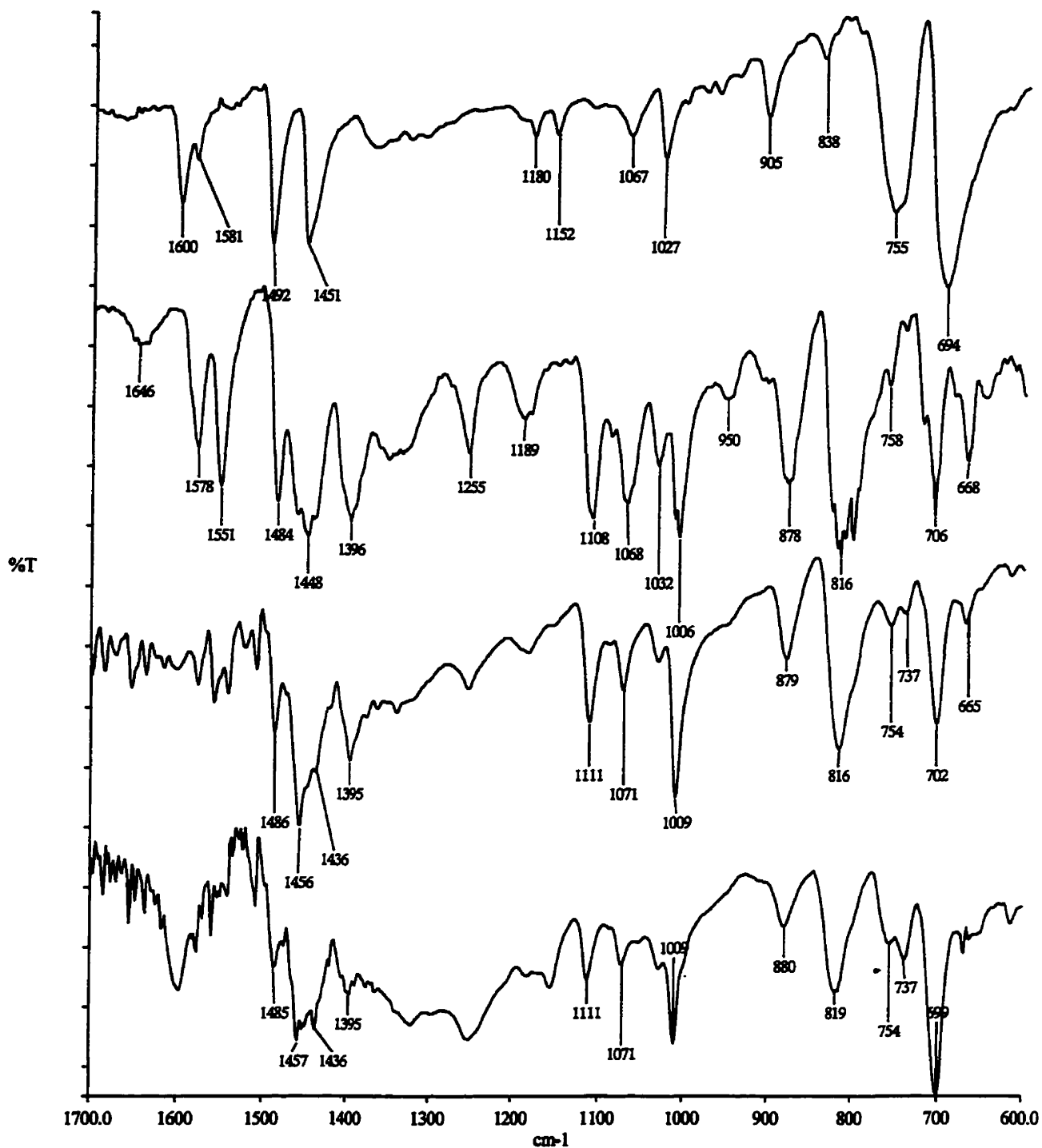


Figure 5: FTIR spectra (upper to lower) of PS beads (2% divinylbenzene, Polysciences), brominated PS beads, PTPB-2 and PTPB-3.

Nuclear magnetic resonance spectroscopy

Solid-state ^{13}C NMR spectra (CP-MAS[†] with TOSS[‡] pulses) for the brominated PTPB-2 and PTPB-3 beads were obtained on a 300 MHz spectrometer (75 MHz for ^{13}C ; see Figures 6-8) with a contact time of two or three milliseconds. The delay time between pulses was two seconds. Dipolar dephasing (DD, sometimes called gated decoupling) experiments were also employed to suppress nearly all protonated carbon signals leaving just quaternary, brominated phenyl and methyl carbon signals (see Figures 7 and 8).¹⁷ Methyl carbon signals survive this experiment since C-H coupling for methyl groups is weak due to rotational mobility. Boron-11 NMR spectra were collected for PTPB-2 and PTPB-3 (see Figure 9).

The successful bromination of the PS beads is supported by the residual peaks in the DD spectra (Figure 6) in the aromatic region where the carbon *ipso* (*i.e.*, the substituted, or attached to) to the backbone appears at 145 ppm and the brominated aromatic carbon appears at 124 ppm ($\text{C}_{\text{Ar-Br}} \sim 120$ ppm).¹⁸ Residual THF peaks (~ 20 and 70 ppm remain due to the mobility of the small molecules). The spectra in Figures 6-8 are normalized with respect to the backbone carbons near 40 ppm (upper spectrum in each Figure). On this basis, the spectra for PTPB-2 (Figure 7) suggest some additional phenyl groups when compared with the starting material (~ 130 ppm in the CP-MAS/TOSS without DD). The DD experiment indicates residual $\text{C}_{\text{Ar-Br}}$ at 126 ppm. The carbon *ipso* to boron may appear as a very weak signal near 165 ppm (163.4 ppm for

[†] Cross Polarization – Magic Angle Spinning

[‡] TTotal Suppression of Sidebands

BPh_4^- , $^1J(^{11}\text{B}, ^{13}\text{C}) \sim 50 \text{ Hz}$),¹⁹ but positive assignment is not possible in these spectra due to noise.

The aromatic carbons *ipso* to boron in PTPB-3 (Figure 8), on the other hand, do appear in both the CP-MAS/TOSS and CP-MAS/TOSS/DD spectra, though the DD spectrum still indicates residual brominated carbons ($\sim 129 \text{ ppm}$). It should be noted that the intensities of the C-Br signals in the DD spectra for PTPB-2 and PTPB-3 are very much weaker than for the brominated beads (Figure 6).

The chemical shifts for the ^{11}B NMR spectra (see Figure 9) were assigned relative to NaBH_4 , placing the boron signal for PTPB-2 and PTPB-3 around 40 ppm . ^{11}B has spin $3/2$ and the quadrupolar relaxation mechanism usually dominates. The Pake peak that one might expect for quadrupolar nuclei is reduced to a dominant sharp peak due to the symmetry of the tetrahedral center. The second peak may be attributed to unsymmetrical arrangements due to crowding and neighboring group effects. These boron spectra thus confirm the presence of pendant TPB moieties.

Samples of PTPB-2 and PTPB-3 with equal mass (80 mg each) were analyzed; thus, the peak heights are indicative of the relative degree of boronation. It appears that PTPB-2 is less functionalized than PTPB-3, which is supported by the binding data to follow.

Brominated PolySciences Beads

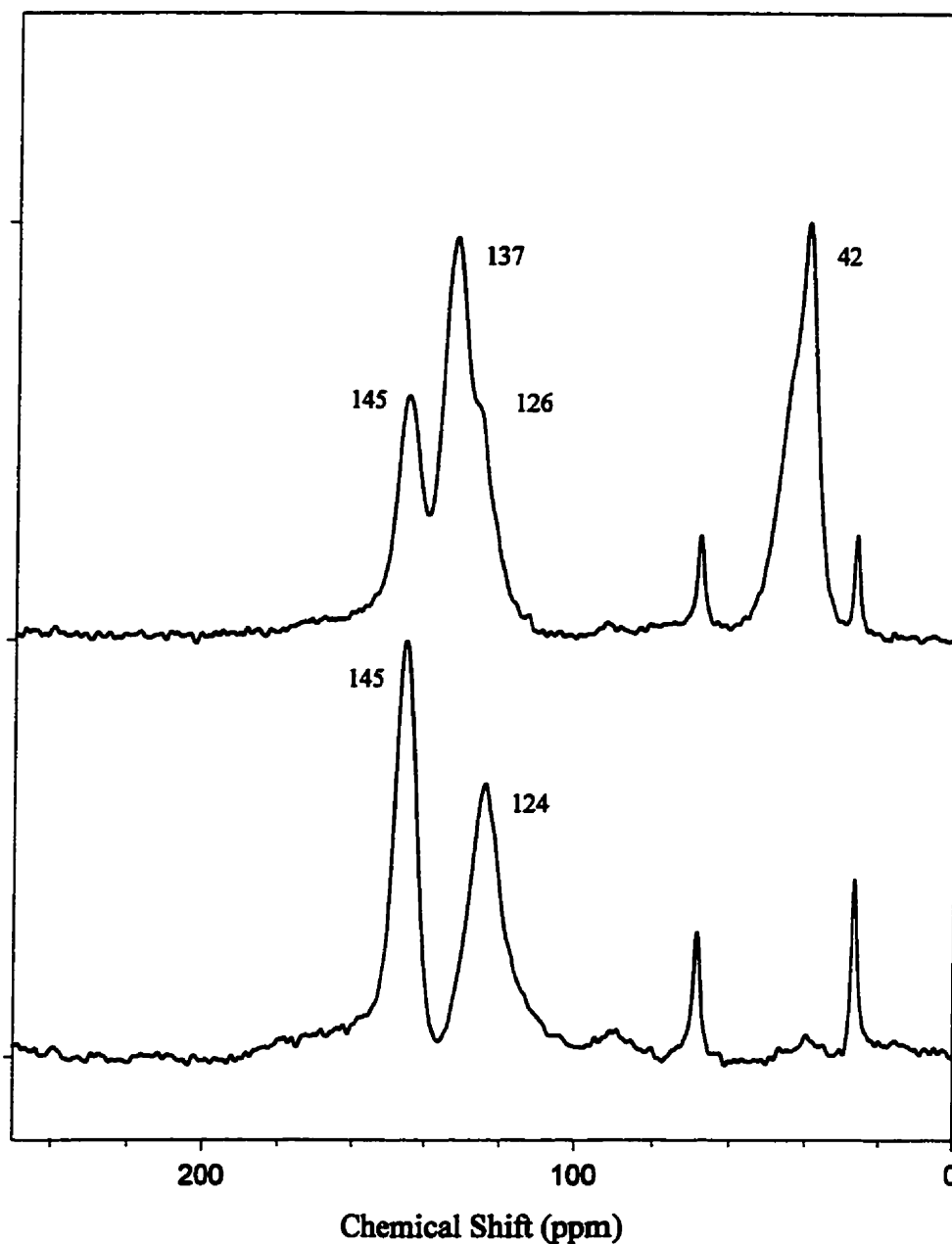


Figure 6: CP-MAS/TOSS (upper) and CP-MAS/TOSS/DD (lower) ^{13}C NMR spectra of brominated PS beads. In the upper spectrum signals appear for all carbons, whereas the lower one shows only the C_{Ar} -backbone and $\text{C}_{\text{Ar}}\text{-Br}$ signals.

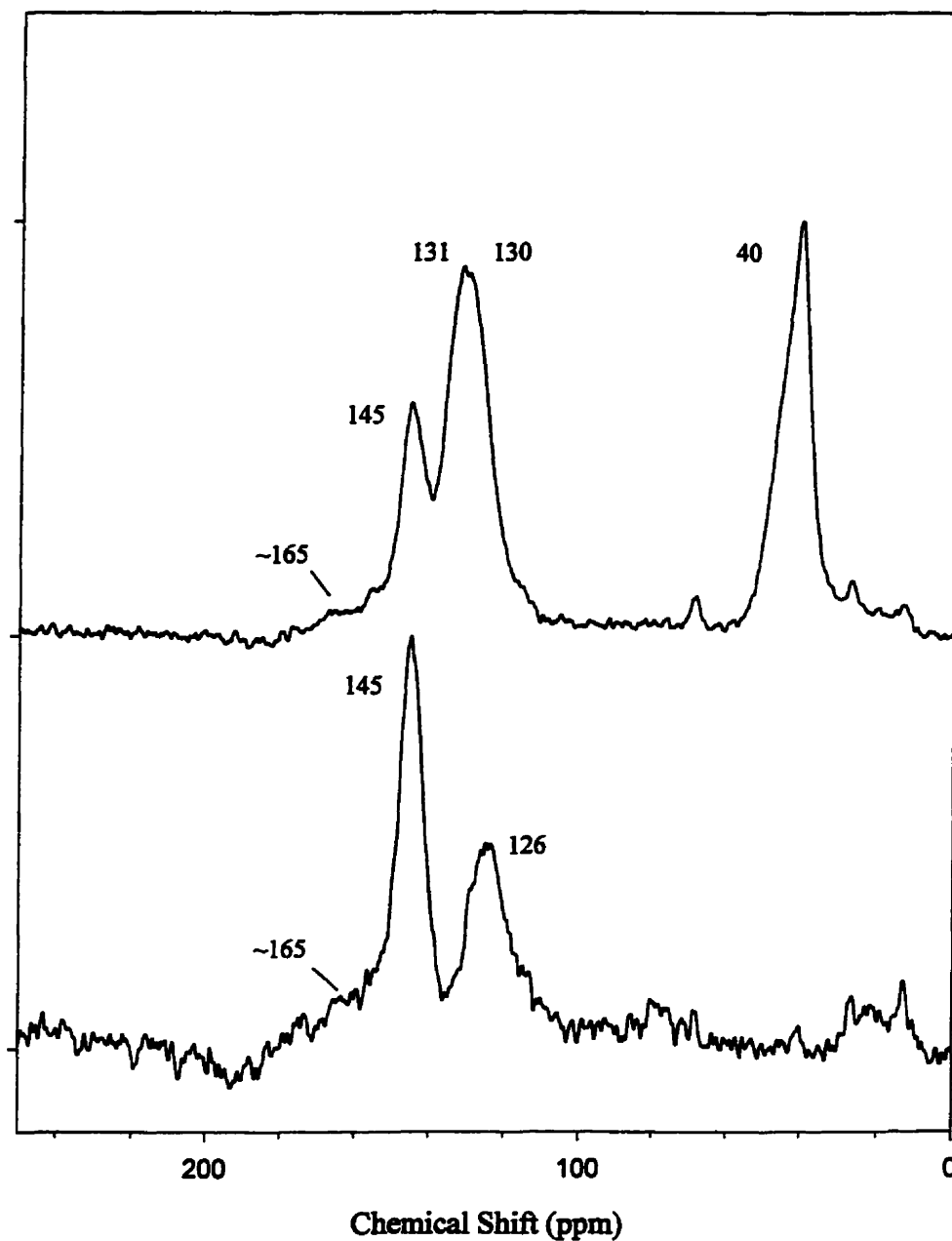
PTPB-2 Solid-State C^{13} NMR

Figure 7: CP-MAS/TOSS (upper) and CP-MAS/TOSS/DD (lower) ^{13}C NMR spectra of partially triphenylboronated PS beads (PTPB-2). In the upper spectrum signals appear for all carbons appear, whereas the lower one shows only the C_{Ar} -backbone and C_{Ar} -Br signals. There may be some evidence of the C_{Ar} -B signal (~165 ppm).

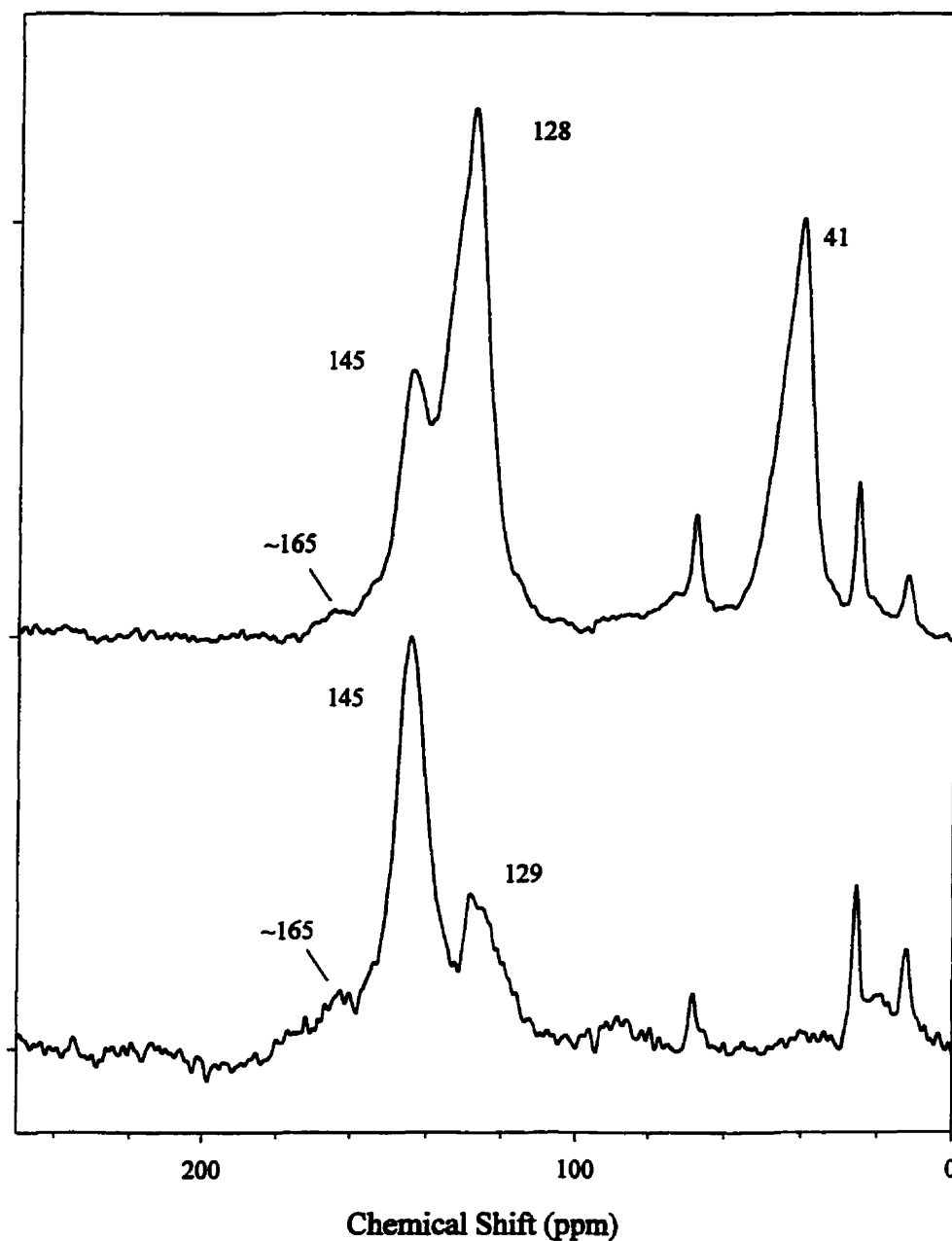
PTPB-3 Solid-State C^{13} NMR

Figure 8: CP-MAS/TOSS (upper) and CP-MAS/TOSS/DD (lower) ^{13}C NMR spectra of partially triphenylboronated PS beads (PTPB-3). In the upper spectrum signals appear for all carbons, whereas the lower one shows the C_{Ar} -backbone (145 ppm), C_{Ar-Br} (129 ppm) and C_{Ar-B} (~165 ppm) signals.

¹¹B Solid-State NMR
PTPB-2 and PTPB-3 (normalized)

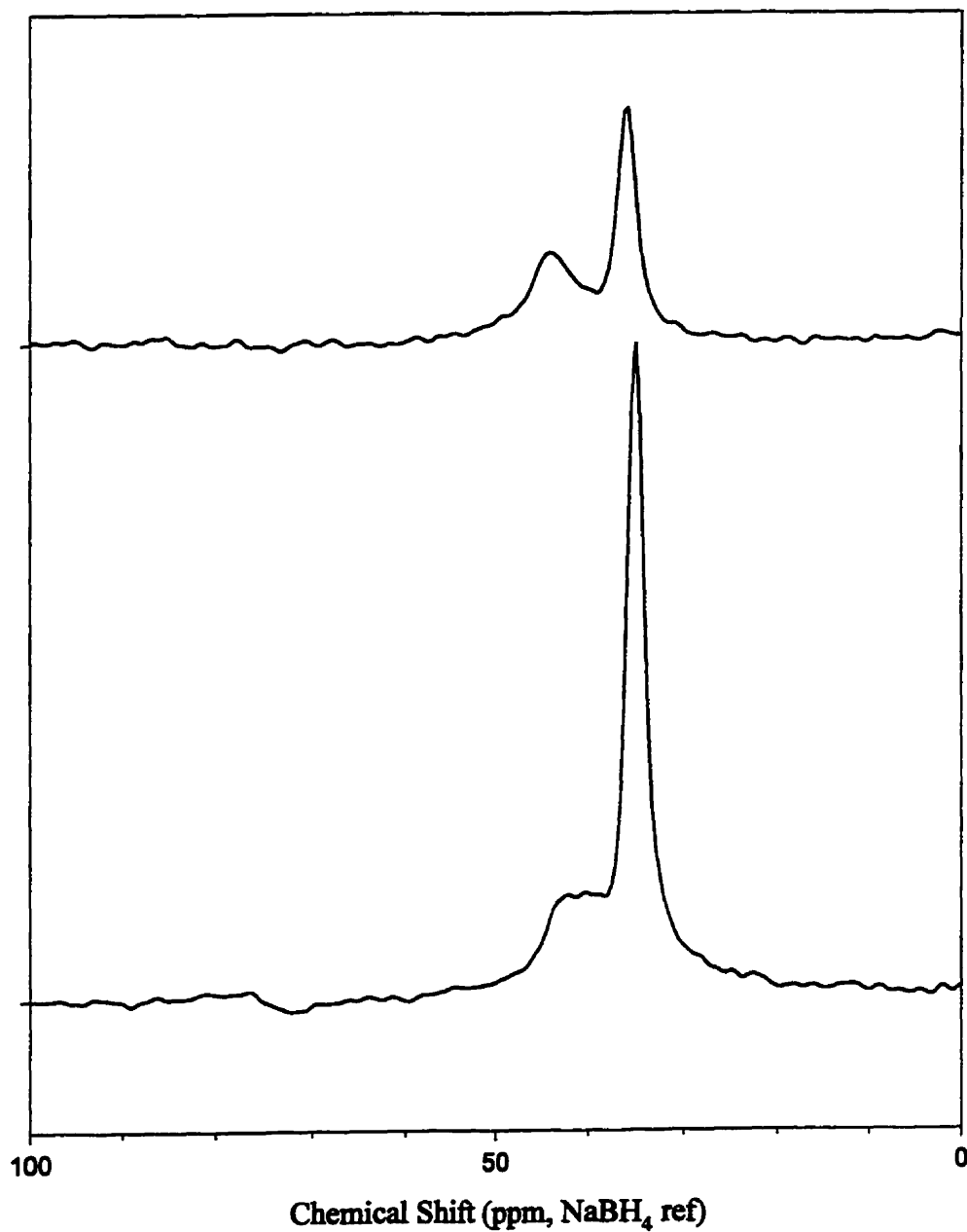


Figure 9: Solid-state ¹¹B NMR spectra of PTPB-2 (upper) and PTPB-3 (lower). (The chemical shift is given relative to NaBH₄).

Binding

Nessler's reagent and UV-Vis colorimetric assays

Nessler's reagent[§] is used to detect low concentrations of ammonia in water.²⁰ It is an alkaline solution of mercury (II) potassium iodide (K_2HgI_4), prepared by dissolving 5 g HgI and 3.5 g KI in 5 mL water. This was added to a cold solution of 35 mL water containing 8 g NaOH and was made up to 50 mL with water.²¹ The solution was clear and light yellow in color. In the presence of ammonia, the color changed to dark orange as a function of the ammonium concentration and the change in absorbance at 412 nm was linear with concentration. We used deionized water (milliQ) to prepare the solutions. Since this solution was to be used for analytical purposes, it was critical that the water be as ammonia-free as possible.

All UV-Vis data were obtained using an HP 8452A spectrometer interfaced to a PC and set to monitor absorbance at 412 nm. A quartz flow cell with a path length of 0.100 cm was used. Before each series of measurements, a milliQ water blank was run and both the cell and the standards were calibrated by testing a 1.000 mL aliquot of each standard with 50.0 μ L Nessler's solution. Initially, each concentration was injected four times and measured five times; however, the data showed that there was no significant variance between injections. Subsequent assays were injected once and were each measured several times. The data were plotted as absorbance versus $[NH_4^+]$ and the

[§] sometimes called Meyer's reagent

linear regression provided a relation between absorbance and concentration. The quantitative function of the UV software was employed to generate calibration curves. To accommodate absorbance values at extremely low ammonium concentrations (<5 ppm), a second-order fit was determined to follow the data most closely with the error on any given standard run being less than 2%. A first order fit led to larger errors, particularly at low concentrations. Ammonium concentrations were determined in ppm for simplicity; however, the binding isotherms were reported in mmol; the conversion factor is 5.544×10^{-3} mM / ppm.

PS beads: Control experiment

To establish that the unfunctionalized starting material had no binding activity, four samples of PS beads (2% DVB, 200-400 mesh, Polysciences Inc.) weighing 5.07, 10.2, 50.3 and 100.3 mg each were shaken with 1.000 mL milliQ water for 24 hours at which point 4 mL ammonium chloride solution (20 ppm) was added. The initial ammonium ion concentration was therefore 16 ppm. The suspension was agitated for 72 hours and then the sample vials were centrifuged for approximately 15 minutes. 1.000 mL aliquots were withdrawn and tested with 50.0 μ l Nessler's solution. Absorbance values at 412 nm were converted to concentration from the calibration data and were plotted as a function of resin mass. The data plotted in Figure 10 indicate that unfunctionalized PS beads have no sequestering effect on ammonium ion concentration.

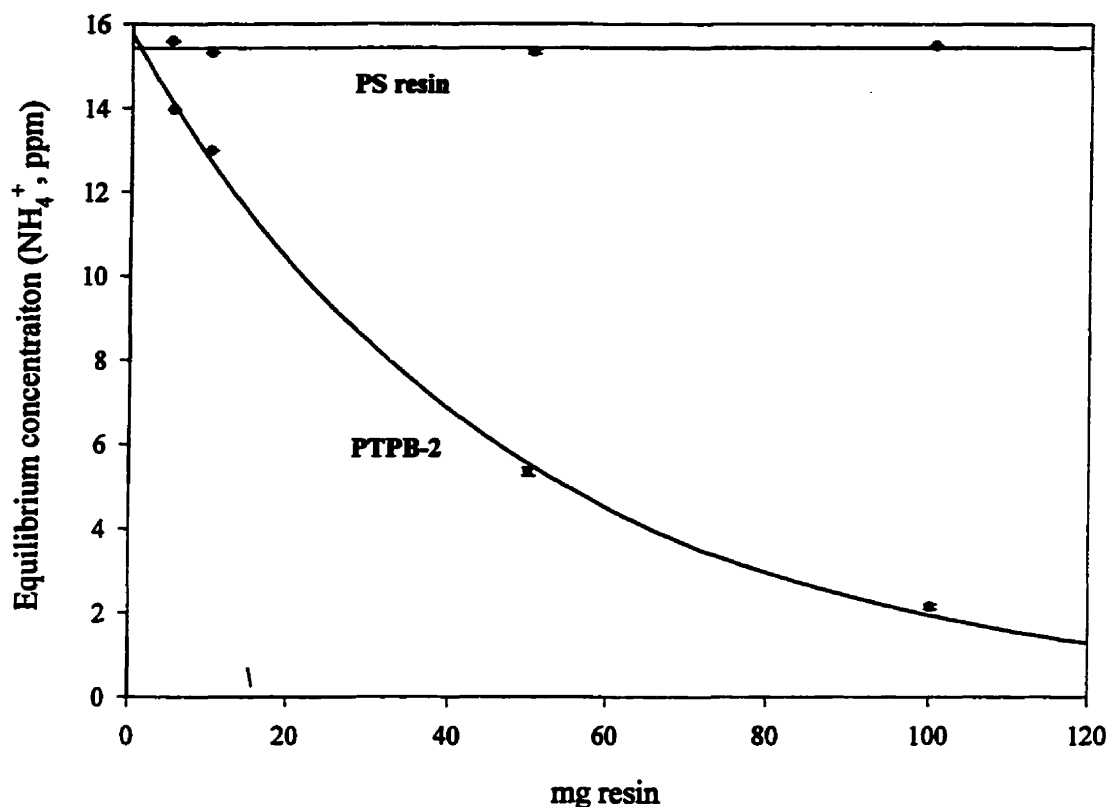


Figure 10: Ammonium sorption as a function of resin mass for PS and PTPB-2 in 5mL, 16 ppm (NH₄⁺) solution.

PTPB-1

To test the functionalized homopolymer for ammonium-binding properties, 2 mL of an aqueous solution of ammonium chloride (115 ppm) was added to 30 mg of the functionalized homopolymer and was stirred overnight in an airtight vial. Stock ammonium chloride solution (115 ppm) was diluted to 77 and 38 ppm. One drop of Nessler's solution was delivered to 0.300 mL of each standard as well as to 0.300 mL of distilled water. The colors observed ranged from orange (115 ppm) to colorless (0 ppm). The supernatant from the polymer assay was again tested in duplicate and the color generated was visually determined to be very slightly darker than that of the 38 ppm

standard. The concentration of ammonium ions was therefore reduced by the polymer-bound tetraphenyl borate to approximately one third of its initial value. This corresponds to a functional binding capacity of about $0.28 \text{ mmol NH}_4^+ / \text{g polymer}$ (see Table 3), or about 10% of the maximum possible capacity assuming triphenyl-boronation of every styryl monomer and that each pendant group binds one ammonium ion.

PTPB-2

Since nearly three grams of PTBP-2 were synthesized, this sample was the subject of a series of ammonium sorption, desorption and resorption experiments. Initially, conditions identical to the control were employed for comparison. Four PTBP-2 samples weighing roughly 5, 10, 50 and 100 mg each were shaken with 1.000 mL milliQ water for 24 hours at which point 4 mL ammonium chloride solution (20 ppm) were added giving an initial ammonium ion concentration of 16 ppm. The suspension was agitated for 72 hours. The sample vials were centrifuged for approximately 15 minutes and 1.000 mL aliquots were withdrawn and tested with 50.0 μL Nessler's solution. Absorbance values at 412 nm were converted to concentration terms from the calibration data and were plotted as a function of resin mass (see Figure 10). A parallel experiment tested 10 mg samples of PTPB-2 with 2 mL aliquots of ammonium chloride solutions at 1, 5, 10 and 20 ppm (NH_4^+). To obtain more data points at lower equilibrium ammonium ion concentrations, larger PTPB-2 samples (50 mg) were also tested with 5 mL aliquots of ammonium chloride solutions at various initial concentrations. An ammonium ion binding isotherm similar to the isotherms in Chapters 5 and 6 was constructed from all PTPB-2 data (see Figure 11). The data were fitted with a hyperbolic curve.

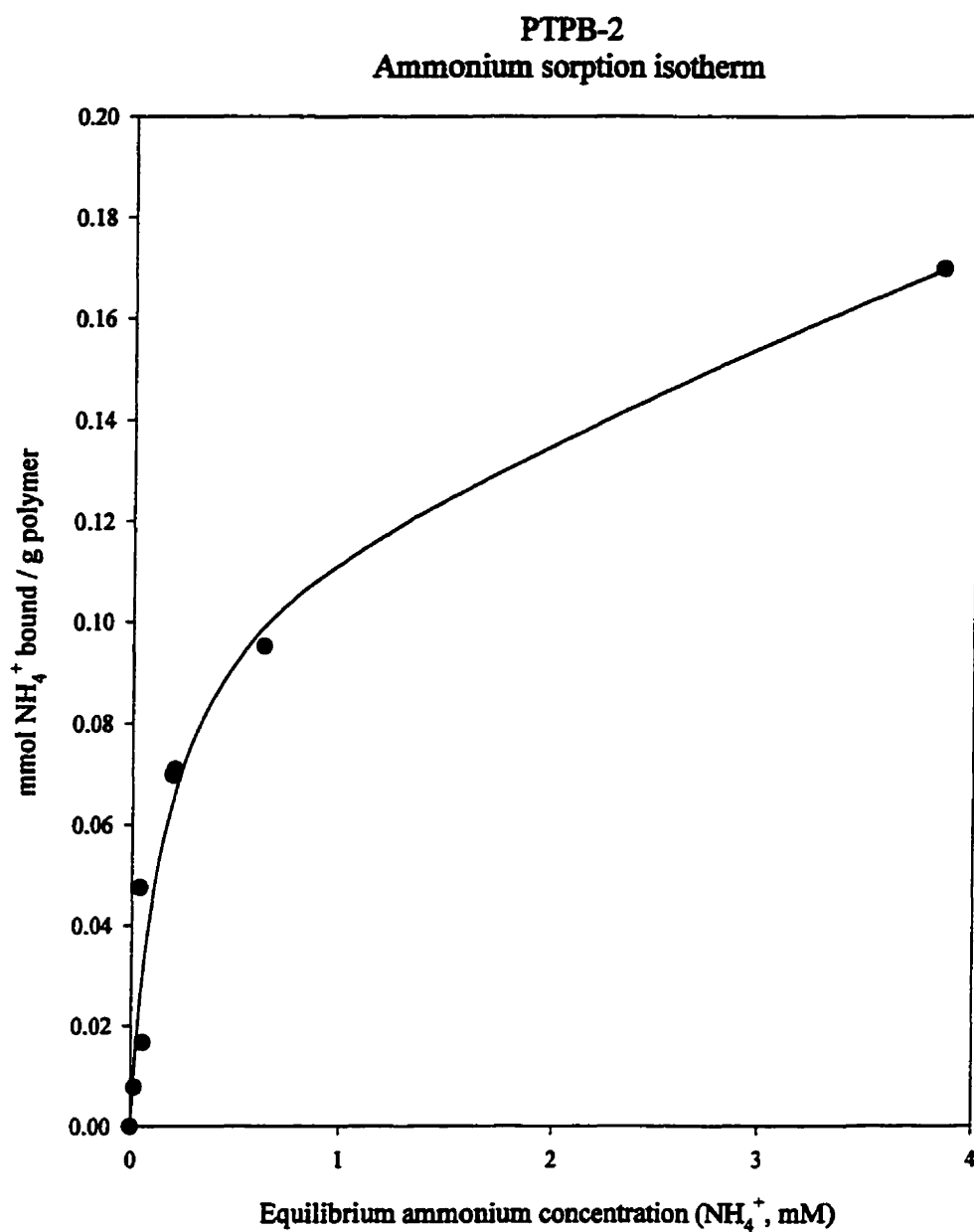


Figure 11: Ammonium sorption for PTPB-2. (The data were fitted with an exponential curve.)

PTPB-3

As indicated by the NMR spectra, PTPB-3 was more heavily functionalized than PTPB-2. In order to mimic the study for PTPB-2, 50 mg samples of PTPB-3 were prepared, to which 5.0 mL of solutions containing ammonium ions were added at the following concentrations (in ppm): 1, 5, 10, 20, 61 and 100. In the first instance, only 1-20 ppm solutions were employed; however, since the sorption was essentially quantitative after only 3 hours, additional samples with 61 and 100 ppm solutions were prepared and tested following 48 hours of agitation (see Figures 12 and 13).

The isotherm shown in Figure 12 shows that PTPB-3 effectively binds ammonium ions. The steep slope at low concentrations is indicative of strong binding, *i.e.*, a high binding constant, consistent with the data shown in Figure 13. The plateau at approximately 0.4 mmol/g of dry polymer suggests that all of the available sites are binding ammonium ions. (Although an absolute value for functionality is not available, estimates based on molar masses, indicate that the maximum that can be expected is 2.6 mmol/g ignoring the presence of cross-linking.)

Quantitative binding appears to take place up to an initial ammonium concentration of about 60 ppm; however binding is significant, even up to 100 ppm (see Figure 13). Following the addition of PTPB-3 to an NH_4Cl solution ($[\text{NH}_4^+]_i = 100$ ppm) the final supernatant concentration was about 30 ppm (70% of the initial ammonium ions were removed from solution). Without dilution, measuring 60 ppm ammonium ion concentrations is near the upper limit of the Nessler reagent under these conditions. At higher concentrations, a solid orange precipitate was observed.

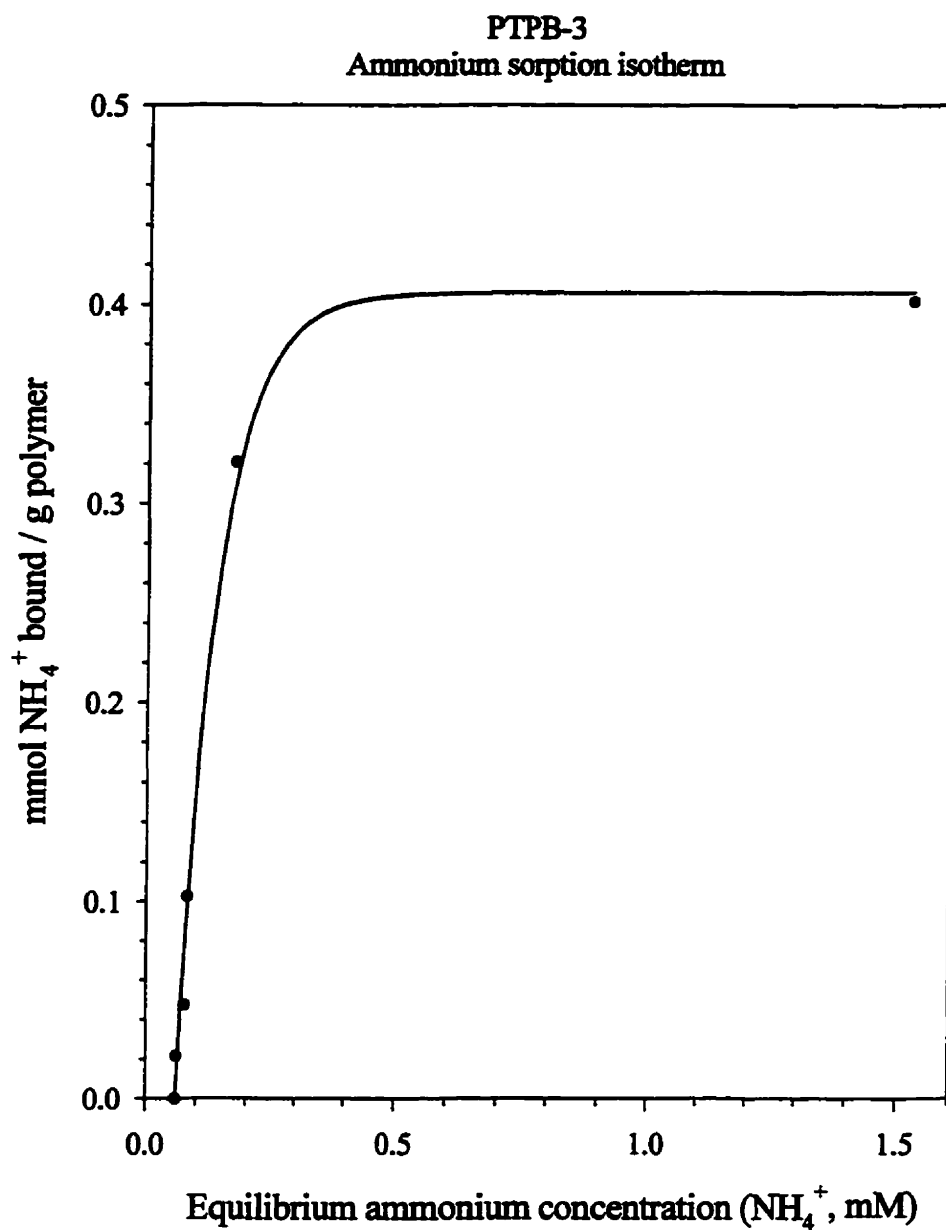


Figure 12: PTPB-3 ammonium ion sorption isotherm. The data were fitted with a hyperbolic curve. (Sorption conditions were 50 mg of polymer and 5 mL of ammonium chloride solution.)

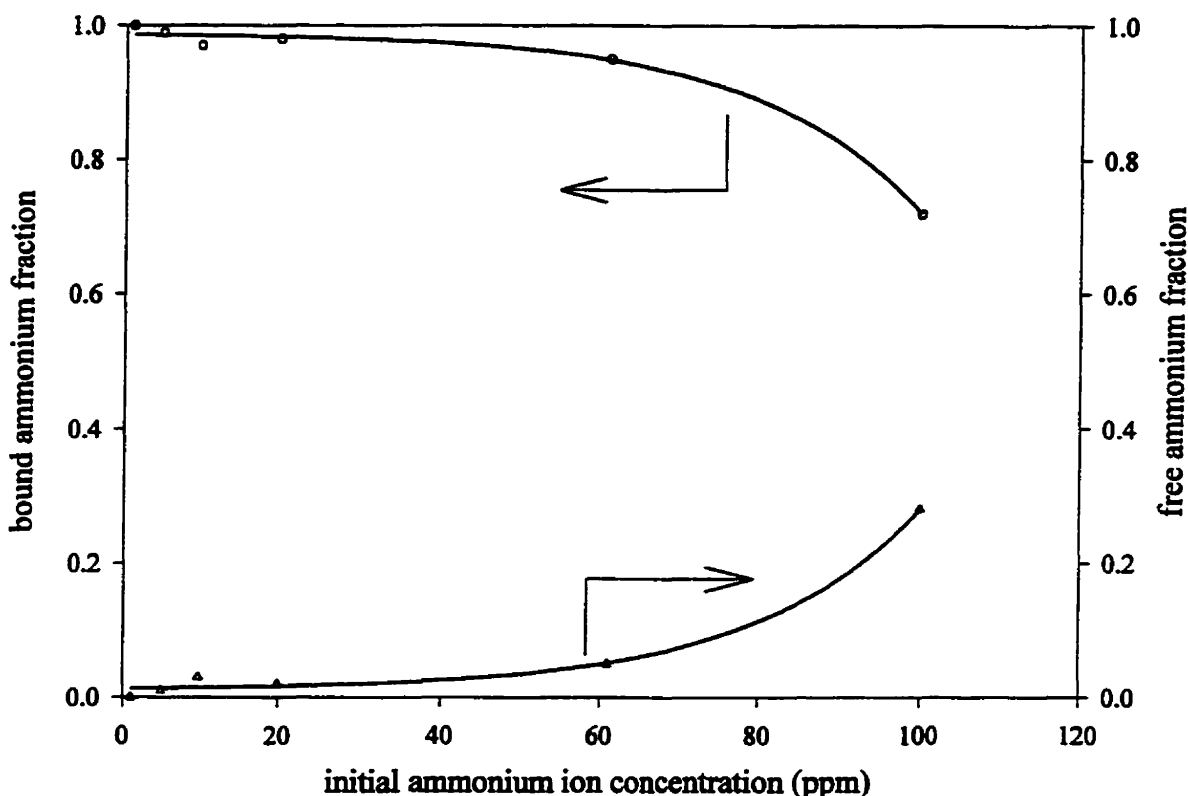


Figure 13: Bound or unbound ammonium fraction vs. $[\text{NH}_4^+]^i$ for PTPB-3. Under these experimental conditions (50 mg PTPB-3, 5 mL NH_4Cl solutions) even where sorption is not quantitative, the free ammonium fraction remains very low.

Sorption discussion

The utility of PTPB materials for the extraction of (organo)ammonium ions from aqueous solution depends on several factors including: (i) strong binding that can be 'switched off,' and (ii) high capacity. The strength of the binding is a function of the matrix and most importantly the interaction between the binding moiety and the sorbate. The solubility, of NH_4TPB in water is so low as to be essentially immeasurable; therefore, it is comforting that the binding isotherms for the PTPB systems indicate equally strong binding leading to extreme insolubility. Clearly, PTPB is an excellent ammonium ion scavenger, since once the phenyl cage has entrapped an ammonium group

(see Figure 2), it essentially precipitates the ammonium ions out of solution, even when attached to the polymeric backbone.

The capacity of the material appears to be determined by the degree of functionality. Indications are that the fraction of PTPB-1 repeat units bearing TPB is substantial due to at least two factors. Not only was the original starting material uniformly and completely mono-brominated, but also the earliest indication of cross-linking during the functionalization process came subsequent to the addition of triphenyl boron. Therefore, all potential sites were in intimate and free contact with the reagents. The scale of this trial (300 mg) rendered more extensive binding assays impossible. However, the results gave clear evidence for the formation of tetraphenyl borate functional groups and were sufficiently encouraging to warrant the functionalization of PS beads.

The data from the PTPB-2 sorption trials (see Figures 10 and 11) indicate the effectiveness of the binding sites despite qualitatively lower functionality than for PTPB-1 and -3 (*c.f.* FTIR, Figure 5). The minimum bound fraction of ammonium ions from 5.00 mL of NH_4Cl solutions was 30% following addition of 50 mg PTPB-2. At very low ammonium concentrations, the sorption efficiency is much higher ($\sim 75\%$ at $[\text{NH}_4]^i = 4$ ppm). For PTPB-2, the ammonium binding constant appears to be much lower than for free TPB anions, which may be explained by considering the PTPB-2 degree of functionality and the NH_4TPB crystal structure. For the small molecules, NH_4^+ and TPB^- can form highly regular H-bonded columns where each ammonium ion is held in a cage of four electron-rich phenyl groups. Since the FTIR spectra suggest that the functionality of PTPB-2 is lower than for PTPB-3, it is possible that π -H bonding is insufficient to

produce the quantitative ammonium extraction observed for free TPB anions due to the restricted availability of cage-forming phenyl groups. The binding isotherm plateau is at about 0.18 mmol per gram PTPB-2. If PTPB-2 were completely functionalized, it would provide 2.7 mmol binding sites per gram, ignoring the presence of cross linking, thus a conservative estimate** of the functionality of this polymer is 7%.

Table 2: Summary of polymer samples and ammonium binding values.

Sample	Total mass (g)	Binding (mmol NH ₄ ⁺ / g polymer)	TPB-bearing groups (%)
PTPB-1 ^a	0.3	0.28	10
PTPB-2 ^b	2.8	0.18	7
PTPB-3 ^b	0.8	0.40	15
starting materials: ^a linear PS, ^b 200-400 mesh PS beads (Polysciences)			

The PTPB-3 functionalization reaction was designed to increase the degree of triphenylboron incorporation into the polymer matrix and this material outperformed both PTPB-1 and PTPB-2. Even beyond the quantitative binding regime, significant ammonium reduction was observed (0.4 mmol ammonium / g PTPB-3). The fraction of TPB-bearing repeat units for this polymer was at least 15%. Although not conclusive, quantitative binding for a more heavily functionalized polymer supports the hypothesis that increased availability of phenyl groups makes phenyl cages for the ammonium ions possible. It is also probable that the elevated degree of functionalization favors binding within the polymer matrix.

** assumes that one binding site binds one ammonium ion and that all binding sites are occupied.

Reusability

The sorption studies on PTPB-1, -2, and -3 clearly indicate not only that the functionalization was successful, but also that the extraordinary insolubility of ammonium tetraphenylborate in water does indeed carry over to polymer systems as PTPB-3 is an excellent ammonium ion scavenger. Bearing in mind the ill-effects of excess (organo)ammonium ions in the biosphere, this alone makes the PTPB materials interesting. However, a further necessary characteristic of an environmentally friendly polymer system is its reusability. In order for PTPB to be of any real industrial importance, one must be able to strip any collected ammonium ions from the polymer and regenerate the system for continued use. Preliminary wet and dry desorption as well as resorption experiments with PTPB-2 and a weak base (KHCO_3) were followed by quantitative desorption and resorption experiments with PTPB-2 and a strong base (NaOH).

Dry desorption

Resin samples from earlier PTPB-2 sorption experiments were pooled, filtered and air-dried. The release of ammonium ion from 111 mg of this resin was followed spectroscopically as the dry material was added to 11.0 mL of 0.1 M aqueous KHCO_3 and a 1 mL aliquot was withdrawn and tested with Nessler's reagent immediately. Further aliquots were withdrawn over a 30-hour period (see Figure 14). An initial sharp rise in the supernatant ammonium ion concentration on the addition of base to ammonium-exposed beads stabilized over the course of a few hours and there was no further measurable increase in free amine. There were three factors that complicated these data: (i) the resin was initially dry and therefore the very steep rise in onset ammonium ion

concentration may well have been an artifact caused by dry ammonium-rich PTPB-2.

The kinetics of release depend primarily on the deprotonation of NH_4^+ and the kinetics of wetting and swelling are almost certainly much slower. (ii) The ammonium and carbonate-containing supernatant reacted with Nessler's solution yielding the anticipated yellow-orange color, but the resulting solution was not photostable. On irradiation in the UV-Vis spectrometer, the absorbance values increased dramatically with successive scans. When left on the bench, these solutions turned cloudy over the course of an hour or two. (iii) In order to have sufficient material to ensure measurable ammonium ion release, the resin was pooled from all previous sorption experiments. Though the total ammonium ion released from this pool is certainly calculable, there is no way of accurately determining the total amount of ammonium ion available to be released and so the degree of regeneration can only be measured by the material's subsequent ability to re-sequester ammonium ions.

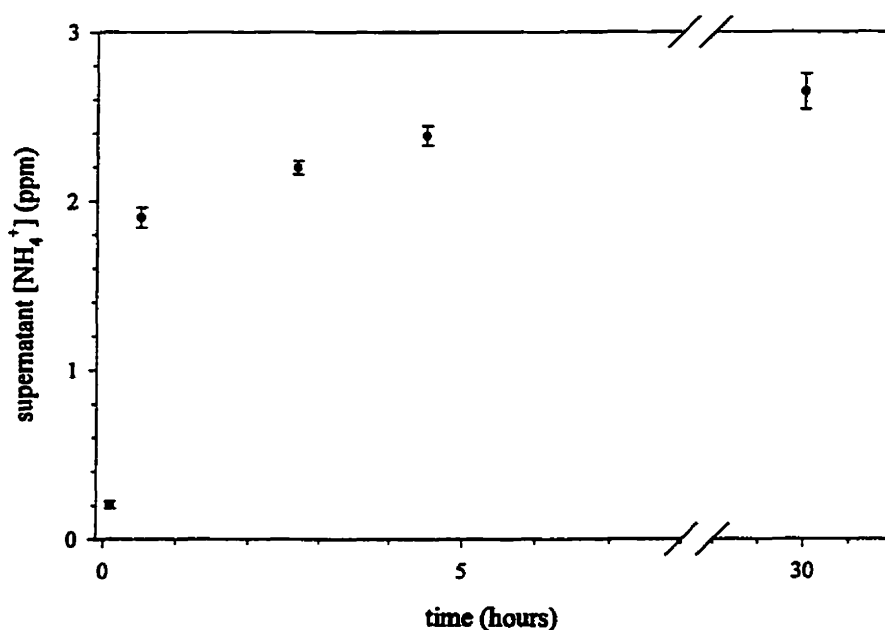


Figure 14: Bicarbonate mediated (0.1 M KHCO_3) ammonium ion desorption for PTPB-2.

Wet desorption

A small sample of PTPB-2 from a mixture of ammonium-containing solutions was collected via centrifugation and was decanted. It was immediately re-suspended in KHCO_3 (0.10 M, 10 mL) and 1 mL aliquots were tested at 5, 15 and 50 minutes (see Figure 15). The time scale for desorption from wet beads seems to be about 10 minutes under these conditions, but the total ammonium ion released was only 1 μg . This protocol was designed to show desorption from completely swollen PTPB-2, therefore the sample mass was not determined since the beads were wet and the % release can be

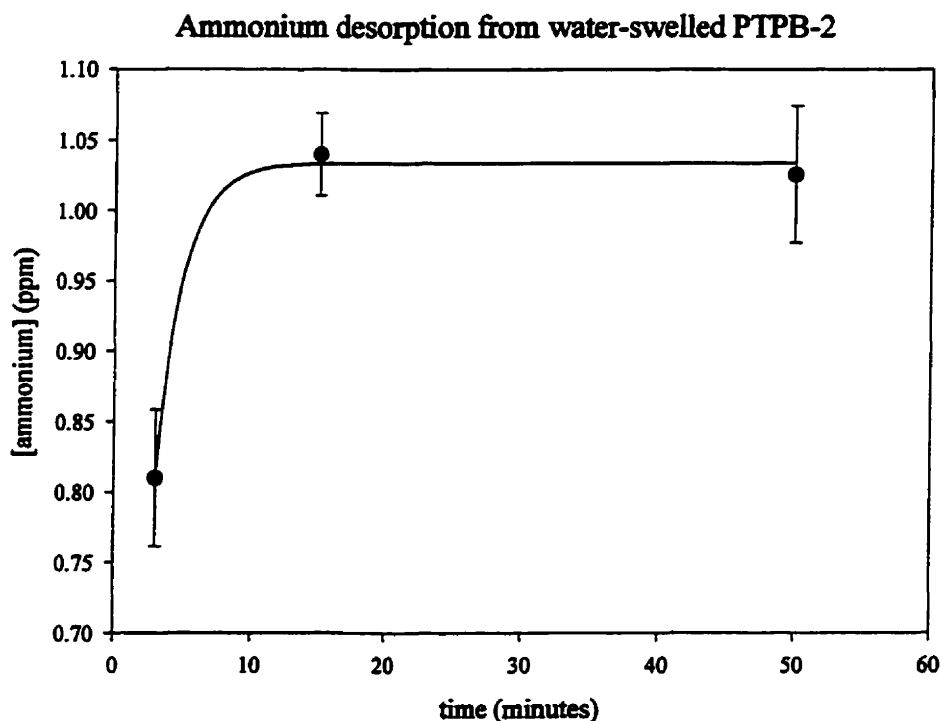


Figure 15: PTPB-2 was collected from an ammonium chloride solution and was immediately subjected to mild base (KHCO_3).

estimated only by the material's capacity to take up further ammonium ions. Nevertheless such limited ammonium release suggested that the ammonium ions were not appreciably liberated.

Resorption

The resin from the dry desorption study was filtered and three binding assays were prepared: (i) two samples of previously exposed PTPB-2 (10 mg and 60 mg); (ii) two samples of previously exposed PTPB-2 that had been washed in KHCO_3 (10 mg and 60 mg); and (iii) two samples of unexposed PTPB-2 (10 mg and 60 mg). Each sample was pre-swelled with 1.00 mL milliQ water for 48 hours whereupon 4 mL of ammonium chloride solution ($[\text{ammonium ion}] = 20 \text{ ppm}$) was added to each vial. Following 4 hours agitation, the vials were centrifuged and 1 mL aliquots were tested with 50.0 μL of Nessler's solution (see Figure 16). Unsurprisingly, previously unexposed beads bind ammonium ions quite effectively, previously exposed beads hardly bind at all and nominally stripped beads appear to take up at least some ammonium ions. Clearly, desorption via potassium bicarbonate did not fully restore previous activity.

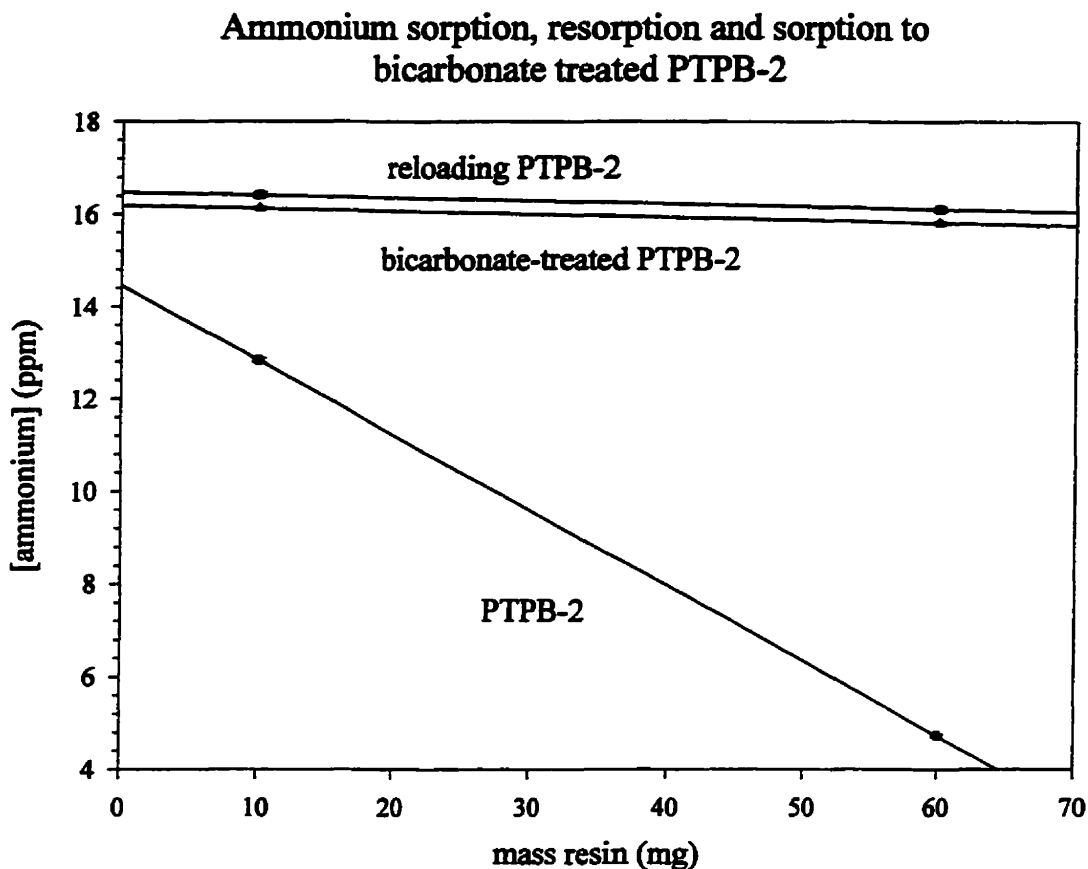


Figure 16: Comparative graph plotting the final ammonium concentration against the PTPB-2 mass. (The lines are intended only to guide the eye.)

Desorption: NaOH vs. NaHCO₃

Two 100 mg samples of PTPB-2 were suspended in ammonium containing solution (8 mL, 10 ppm). The final ammonium concentration in the supernatant was 1.8 ppm; therefore each 100 mg sample bound 3.6 μmol ammonium ion.^{††} Each vial was centrifuged and the supernatant was decanted. The residual resin in one vial was then suspended in 0.10 M NaHCO₃ (5.0 mL) and 0.10 M NaOH was added to the other

^{††} The conversion factor is $5.54 \times 10^{-5} \text{ mol L}^{-1} \text{ ppm}^{-1}$.

sample (5 mL). After 75 minutes of agitation, 1 mL aliquots were filtered (0.45 μm filters) and assayed with Nessler's solution. As observed for KHCO_3 , the UV-Vis absorbance from the NaHCO_3 exposed aliquots increased dramatically with successive scans. The values for ammonium concentration increased from 3.2 ppm to more than 10 ppm over the course of 10 scans despite a delay of 5-10 minutes between the addition of Nessler's solution and collection of the UV data. The sample in the cell was visibly turbid and sonication was required to render the cell clean. Clearly, the resultant assays for bicarbonate-mediated regeneration are unreliable.

The sample that had been exposed to sodium hydroxide appeared stable on addition of Nessler's solution and UV irradiation so further assays with bicarbonate were abandoned. The remaining supernatant of the hydroxide trial was further agitated with the resin and after a total of 5 hours, it was tested. As this assay consumed all remaining supernatant, 5.0 mL more of 0.10 M NaOH was added and the sample was shaken for 48 hours and then the concentration of ammonium ions in the supernatant was measured as before (see Figure 17). The first hydroxide wash released nearly 40% of the resin-bound ammonium ions, and would have released more had all the supernatant not been consumed by the assays. The second wash released a further 30% of the initially-bound ammonium ions. Therefore, at least 70% activity was anticipated for the reactivated PTPB-2

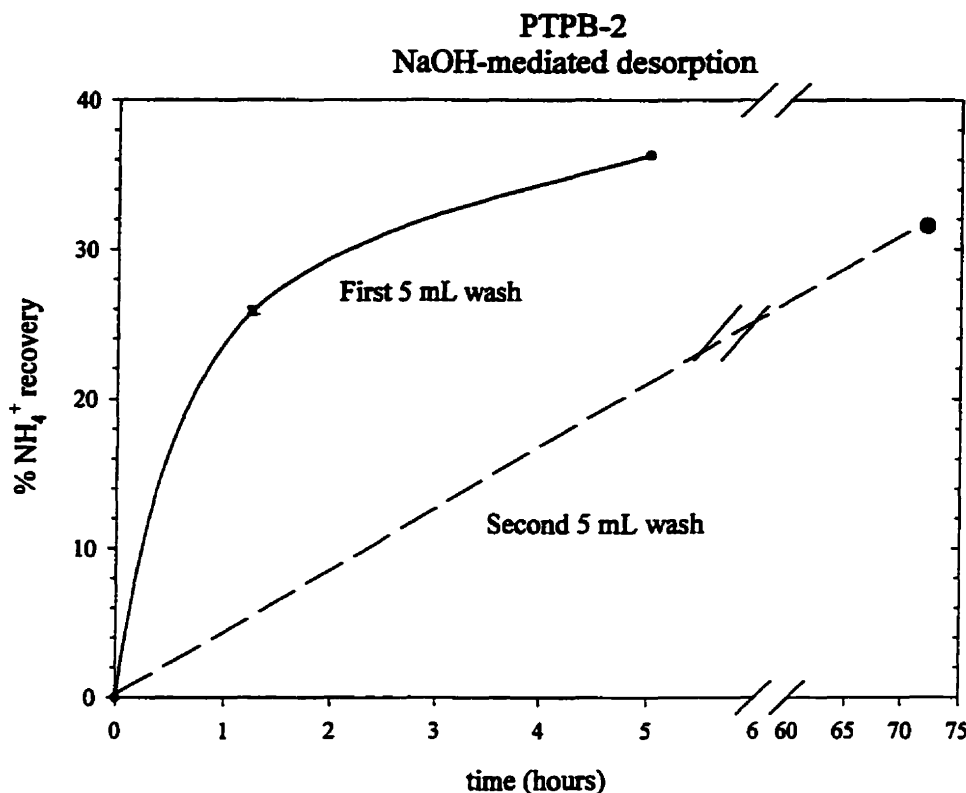


Figure 17: 100 mg PTPB-2 preloaded in 8 mL, NH_4Cl ($[\text{NH}_4^+] = 10$ ppm). Desorption conditions were ~100 mg PTPB-2 washed twice with 5 mL 0.1 M NaOH aliquots. (The lines are intended to guide the eye and to underscore each desorption experiment.)

Although the bicarbonate assay with Nessler's solution was not photostable leading to a cloudy solution and ultimately, precipitation, the hydroxide-containing assay was unsurprisingly robust (Nessler's solution is 4 M in NaOH). The final ammonium concentration that resulted for the first washing was 4.5 ppm and the final ammonium concentration for the second washing was 3.94 ppm (see Figure 17). Since the concentration reduction for the pre-loading in 8 mL was from 10 ppm to 1.8 ppm, the total sorption was 8.2 mg/L in a volume of 8 mL, or 66 μg . The desorption for the first washing was 23 μg and for the second, 20 μg : *i.e.*, 43 μg representing about 67% of the total.

Resorption: hydroxide-washed beads

A re-sorption experiment with the hydroxide-washed beads was employed to determine if the base wash was destructive to the polymer. The resin recovered after being treated with hydroxide was washed with milliQ water, filtered and divided among three vials (9 mg, 25 mg and 37 mg). Ammonium ion containing solution (5.0 mL, $[\text{NH}_4^+] = 10 \text{ ppm}$) was added to each vial and the sample was agitated for six hours. The supernatant ammonium concentration and binding values were determined as before (see Table 3).

Since the hydroxide wash indicates the successful removal of ammonium ions from PTPB-2, further ammonium sorption should be possible with the regenerated material. The hydroxide-exposed resin, when shaken with an ammonium chloride solution does indeed lower the supernatant ammonium concentration. The ammonium binding values for PTPB-2 before and after the hydroxide wash are given in Table 3. Once corrected to reflect the incomplete liberation of ammonium ions during the hydroxide wash (~70%), these data show that despite having been exposed to 0.1 M NaOH, PTPB-2 retains essentially all of its initial activity. Furthermore, the incomplete activity regeneration of PTPB-2 may also have been a reflection of the protracted exposure to strongly basic hydroxide anions (~80 hours), which may have been partially destructive with respect to the binding sites. While these experiments demonstrate the uncomplicated reusability of the PTPB resins, the parameters were by no means optimized due to limited material. Nevertheless, the PTPB samples show significant promise as (organo)ammonium scavengers.

Table 3: Bound ammonium ion fraction as a function of PTPB-2 resin mass comparing initial sorption data with re-sorption data following a hydroxide PTPB regeneration.

mass PTPB-2 (mg)	$C_{eq} \text{ NH}_4^+$ (mM)	bound NH_4^+ : washed PTPB-2 (mmol/ g)	Activity (%) ^a	Activity (corrected, %) ^b
8.6	0.46	0.052	58	82
25	0.38	0.034	42	60
37	0.20	0.023	32	46

^awhen compared with the activity of 'virgin' PTPB-2; ^bthe activity normalized to reflect the incomplete ammonium ion liberation (70%)

Summary

(Organo)ammonium species represent a very real threat in a variety of possible scenarios from the comparatively trivial, yet potentially devastating NH_4^+ ion, to more complex molecules including the alkaloids. While there are technologies available to scavenge ammonium ions from water, they tend to suffer from high cost and are not applicable to many situations. A need remains for an inexpensive, environmentally friendly, reusable system to clean wastewater, and polymer-tethered tetraphenylborate represents a potential alternative.

In this chapter, we have presented a method for the successful synthesis of polymer-supported TPB and have demonstrated its potential utility. We began by exploring the synthetic method and binding characteristics of the system on a linear homopolymer (PTPB-1) and then worked exclusively with 200-400 mesh PS beads. Unfunctionalized PS beads were shown to have no ammonium-binding capability. When the functionalization was comparatively low (PTPB-2), the ammonium-scavenging

characteristics were not optimal and the binding appeared to be somewhat reversible in aqueous environments. The ^{11}B NMR spectrum of this sample suggests that there are two environments for boron, and therefore the low activity may well be due to the limited availability of TPB pendants. At very low concentrations, the sorption is on the order of 75%; but at slightly elevated initial ammonium ion concentrations, only 30% of the ammonium was sequestered. The binding isotherm plateau occurred at about 0.18 mmol NH_4^+ / g PTPB-2. De-sorption and re-sorption experiments on PTPB-2 confirm that the ammonium can be stripped from the polymer and that the most of the previous activity is regained.

Spectroscopic analysis including ^{13}C and ^{11}B NMR, as well as FTIR spectroscopy forecast the dramatically increased activity of PTPB-3. For this polymer, ammonium sorption appears to be quantitative within the limits of the functionality (~ 0.4 mmol NH_4^+ /g resin).

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Chapter 8

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Chapter 8 - Conclusions, contributions to original knowledge, and suggestions for Future Work

Conclusions and contributions to original knowledge

At the heart of this work is the synthesis of a novel amphiphilic diblock copolymer (Chapter 3), the study of its self-assembly behavior in the context of other amphiphilic block copolymer aggregates (Chapters 4 and 2, respectively) and an investigation into the utility of the resulting aggregates as bile salt sorption agents (Chapter 5). Using the synthetic method developed in the post-polymerization functionalization of the block copolymer, a hydrogel analogue of the copolymer system was also prepared and studied as a potential bile salt sequestrant (Chapter 6). The immediate goal was to demonstrate that these polymeric materials were potential cholesterol reduction agents by determining their *in vitro* bile salt sorption.

In vitro sorption of ammonium ions using a post-polymerization functionalized polymeric matrix was the topic of the final chapter. Tetraphenylborate (TPB) moieties were tethered to polystyrene (PS) micro-beads and the resulting material (PTPB) was successfully employed to reduce the equilibrium concentration of ammonium ions in aqueous solution (Chapter 7). While the bile salt binding agents were ultimately designed for *in vivo* serum cholesterol reduction, PTPB was tailored to eliminate (organo)ammonium species from industrial effluent.

Synthesis and post-polymerization functionalization of vinylic block copolymer

The target copolymer for the initial study was poly(styrene)-*b*-poly(N,N,N-trimethylammoniummethylene acrylamide chloride) (PS(*n*)-*b*-PTMEACl(*m*)). The average degree of polymerization, *n* or *m*, of each block is given in parentheses where appropriate. The now well-established PS-*b*-poly(*tert*-butylacrylate) (PS-*b*-PtBuA) copolymer was chosen as the starting material and the goal was to chemically exchange the pendant ester groups for amide moieties including trimethylammonium functionality. Several synthetic methods for this inter-conversion were attempted. While each enjoyed some measure of success, solubility issues, work-up complications and cross-linking plagued the assays. Ultimately, the novel polymer PS-*b*-PTMEACl was best synthesized by first hydrolyzing the PS-*b*-PtBuA esters to carboxylic acid groups and by then coupling these reactive centers with N,N-dimethylethylenediamine (DMED) using 1-[3-[dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The polymeric product containing N,N-dimethylamino pendant groups was quaternized with methyl iodide and the resulting iodo counter ions were exchanged for chloride. The material, once prepared, successfully met our criteria in that:

1. it was amphiphilic and self-assembled in water leading to 'hairy' nanospheres with suitably available cationic groups in the coronae for bile salt-polymer interactions; and
2. with PS being the insoluble block, and given this polymer's extreme insolubility in water, aggregate-solution chain exchange was minimized to levels below detection thereby avoiding possible uptake *in vivo* across the intestinal wall; and

3. as with essentially all amphiphilic block copolymers comprised of fairly short corona-forming as compared with their core-forming blocks, these materials gave us access to several different solution nano-morphologies; and finally
4. the synthetic method avoided possible cross-linking to polymer chains which would not only complicate the process of self-assembly, but would also render some binding sites inaccessible to bile salt molecules.

Self assembly of amphiphilic block copolymers

Asymmetric amphiphilic diblock copolymers in the strong segregation limit self-assemble in aqueous media to give a plethora of morphologies ranging from the simple small sphere (~ 10 nm) to much larger and more complicated architectures (> 1 μm). Using PS-*b*-PTMEACl, we have demonstrated that by rendering the corona-forming block less hydrophilic, morphologies other than simple spheres can be obtained even when the core:corona degree of polymerization is > 1 . Mixtures including simple spheres were observed for nearly all samples, but one sample (PS(175)-*b*-PTMEACl(35)) gave only large compound micelles (LCMs). LCMs from some samples appeared to be agglomerates of small spheres, whereas others seem to have formed on addition of the non-solvent. The latter aggregates are true LCM's, the others are a result of the complicated corona-corona interactions facilitated by the multi-functional pendant groups. Small vesicles and large bilayered structures were also observed. Once the aggregates' genesis was 'frozen' in pure water, the colloids were stable to both elevated sodium chloride and increased polymer concentration. A slight decrease in particle diameter attributed to electrostatic shielding was observed on addition of the simple electrolyte (from 44 to 41 nm for PS(385)-*b*-PTMEACl(80)). The effect of elevated polymer concentration was observed on preparation of transmission electron microscopy

samples. A drop of colloid-containing suspension was delivered to a copper grid and the solvent (water) was left to evaporate under ambient conditions. Though the colloidal aggregates impinged, they did not coalesce.

Bile salt sorption with block copolymer aggregates

Drs. Brown, St-Pierre, *et al.*, have established that the three necessary conditions for successful bile salt binding matrices are: quaternized ammonium groups, a hydrophobic spacer between the ammonium pendants and the polymeric backbone and minimal cross-linking. Self-assembled nano-scale aggregates of PS-*b*-PTMEACl fulfill these requirements and we have now shown both qualitatively with TEM images and quantitatively with HPLC sorption studies that this material reduces the concentration of glycocholate (GC) when introduced to a NaGC solution. The greatest measured reduction was 33% (mol) for 10 mg PS(80)-*b*-PTMEACl(330)_{EDC}, 2 mL 2.5 mM NaGC, 5.0 mM tris buffer.

To ensure maximum hydrophilicity of the corona-forming block and concomitant colloidal stability, the hydrophobic spacer between the ammonium moieties and the backbone was restricted to an ethylene group. Earlier studies have shown that bile salt binding is much improved as the spacer is increased to a dodecyl unit, but this present study demonstrates that despite the reduced possibility of hydrophobic interactions between the binding agent and the bio-surfactants, electrostatic attraction remains a key element.

Bile salt sorption with hydrogels

Three carbomer samples (lightly branched and/or cross-linked poly(acrylic acid) (PAA) were coupled with DMED in parallel with EDC and DCC. The EDC-coupled functionalized and quaternized samples (FQ_{EDC}) appeared to outperform the FQ_{DCC} materials as bile salt sorption agents. The activity of these gels is superior to their copolymer aggregate analogue. At low initial NaGC concentration, the equilibrium sorbate concentration was below the detection limit following addition of FQ-934. Under similar conditions as employed for the binding experiments using block copolymers (summarized above) both FQ_{EDC}-934 and -940 reduced the equilibrium NaGC concentration by about 25%. Doubling the hydrogel mass doubled the bile salt sorption. The remaining FQ_{EDC} sample did not perform as well (10% NaGC reduction), but FTIR revealed residual urea thereby lending support to the reasonable hypotheses that the reaction byproduct or, more likely, N-acyl rearrangement hinders bile salt sorption. The binding isotherms indicate that the experimental conditions did not saturate the materials and that their capacity may be significant.

Hydrogels marry the advantages of the colloidal and cross-linked resin systems admirably and avoid many of the disadvantages. Block copolymers are non-trivial to synthesize and preparation of the nano-scale aggregates is both labor and time intensive. Nevertheless, once prepared, the surface area to volume ratio is enormous, the availability of binding sites is high and once dry, re-suspension of the colloids in water is trivial providing a mobile, turbid suspension not noticeably more viscous than water. Resin beads, the mainstay of hypercholesterolaemia treatment are effective at reducing serum cholesterol levels but suffer from poor patient compliance due to texture (sand),

low *in vivo* bile salt sorption (requiring high dosage) and side effects (intestinal discomfort). Beads are chemically quite simple to prepare and are very easy to handle. The manifold advantages of the carbomer-based hydrogels as cholesterol reduction agents include that

1. they swell in water sufficiently that availability of binding sites should not be an issue;
2. they are easy to handle, can be dried to a powder and re-swell on water addition within minutes;
3. they are comparatively uncomplicated to synthesize, requiring only that additional cross-linking be avoided on functionalization; and
4. once optimized, their *in vivo* sorption may permit lower dosage than for the beads thereby reducing side effects.

Furthermore, the texture of hydrogels resembles gelatin and would certainly be more pleasant than “sand” to ingest.

(Organo)ammonium sorption with polymer-tethered tetraphenylborate

Patient compliance and high dosage are central to the search for a bead-replacement in treating hypercholesterolaemia. Treating industrial effluent to remove (organo)ammonium ions, on the other hand, is most easily accomplished with a macroscopic system that can be filtered from the waste water.

Building on earlier work by Drs. Cameron, Knop *et al.*, where they investigate N-H... π hydrogen bonding, we have herein developed and presented a method for the functionalization of 200–400 mesh PS beads with tetraphenylborate (TPB) to give PTPB. We have also shown that the resulting material quantitatively removes ammonium ions from aqueous solution within the limit of its effective functionality. Finally, we have

confirmed that the process is reversible on addition of strong base and that PTPB, once stripped of ammonium ions essentially regains its previous activity. While the reduction of NH_4^+ ions was investigated here, it is anticipated that virtually all (organo)ammonium species are potential targets, since the list of water-insoluble organo(ammonium) tetraphenylborates is extensive.

Suggestions for future work

All three binding systems presented in this thesis would benefit from further optimization. Among the goals for each material was to have a “clean” product with high functionality in order to investigate and evaluate their potential utility pharmaceutically or environmentally. This goal having been reached, it remains to maximize the activity of the products prior to real-world application.

Bile salt sequestering matrices

Block copolymers

As asystemic cholesterol reduction agents, the block copolymer aggregates presented here are not ideal, though the system may yet be worth pursuing. To ensure immeasurably low chain exchange (*i.e.*, free polymer chains in solution) we employed an extremely hydrophobic core-forming block that yielded glassy, impenetrable domains at the center of the self-assembled architectures. To capitalize on hydrophobic bile salt-polymer interactions, it would be interesting to choose a core-forming block such as poly(ϵ -caprolactone) (PCAP) or poly(butadiene) (PBD). In the first case, hydrophobic interactions might be amplified by H-bonding to the backbone esters and the glass transition temperature (T_g) for PCAP is -65°C . For PBD, polymerization can be tuned

to give a T_g from -90 to -8 °C and, in principle, the residual degrees of unsaturation can be polymerized to give a cross-linked core. Conceivably, complex morphologies could thereby be covalently “frozen” and the benefits of uniform but highly variable colloidal particles could be evaluated.

The synthesis of the hydrophilic block also merits some attention. Laborious efforts to functionalize a copolymer synthesized via anionic polymerization demonstrate that if high conversion and clean product are required, then the synthetic steps must be rapid, high yield and work-up must be both simple and efficient. With the advent of more forgiving living free radical polymerization methods, the block-polymerization of *N,N*-dimethylamino(alkyl)acrylamide or of *N,N,N'*-trimethylamino(alkyl)acrylamide may give positive results. This system, were it to work, would be ideally suited to the study of the effect of the alkyl spacer and the availability of the amide proton on solution/suspension properties, aggregate morphology and bile salt binding. While it seems unlikely that colloidal stability could be maintained with the optimal dodecyl spacer, a likely candidate would be in the intermediate pentyl-, hexyl- or heptyl- range.

Hydrogels

Ammonium-bearing hydrogels are attractive as asystemic cholesterol reduction materials due, in part, to the ease of their synthesis. The Carbopol® precursors used herein are not, however, the only option. Indeed star polymers, dendrimers, and other branched/cross-linked materials may well prove to be better suited to the task. To ensure maximum functionality, the polymerization of a suitable acrylamide is an obvious first requirement and thereafter, both spacer length and cross-link/branch density will require

optimization. As these homopolymers are not plagued by the complications of self-assembly and colloidal stability, they are well suited to accommodating the dodecyl alkyl spacers that have been identified as important to bile salt sequestration. There is an exciting and real possibility that optimized hydrogels will provide high *in vivo* bile salt binding with the concomitant reduction in both required dosage and side effects. Furthermore, the delivery of hydrogels to the patient cannot help but be more pleasant than the currently available resins.

Ammonium ion sequestering beads

Quantitative elimination of ammonium ions from a stock solution at up to 100 ppm in the target species is a powerful argument for the utility of PTPB. There are several potential routes to the improvement of the system. The maximum possible functionality approaches 3 mmol borate moieties per gram of material and the maximum degree of functionality obtained here was 0.35 mmol ammonium bound per gram of material. The synthetic precursor to the triphenyl boron addition is poly(styryl lithium), which is highly reactive and can be trapped by any stray electrophile, including H^+ . To increase the conversion of this critical step, a large excess of Ph_3B should be added.

Since the non-quantitative conversion may be attributable to inaccessibility of the styryl lithium groups within the matrix of the bead, the system may be improved by using a bead with reduced cross-linking (the PS beads used in these experiments had 2% divinylbenzene (DVB) units) and induced pores. As for bile salt binding to ammonium-bearing polymer, in order for the functionalization of the styryl lithium centers and the ion exchange of ammonium for sodium in PTPB to occur, the sites must be physically available within the timeframe of the experiment, in other words, the kinetics of diffusion

into the bead must not be a significant factor. Since the most available sites are on the surface of the beads, the surface : volume ratio of smaller beads would be advantageous in this case, though some filterable threshold must be respected.

The TPB-support itself suggests a further avenue of optimization. Increased hydrophilicity of the polymer backbone might increase bead swelling and thereby render more potential sites accessible. Poly(styrene oxide) (PSO), for example, would still provide phenyl groups to be coupled with triphenylboron, but the ether groups increase the polarity of the backbone. Copolymers including some optimal combination of PSO, PS and other ethers such as poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) are attractive possibilities. Inorganic polymer networks such as silica and alumina are also worth consideration.

The last word

The work presented in this thesis has a distinctly application-based *raison d'être*. The goals from the outset were unashamedly utilitarian in the short term. Nevertheless, having laid the groundwork for these systems, there is no shortage of further “pure” physical experiments that may be employed to better understand the relevant interactions. In so many ways, the complicated chemistry of nitrogen is central to life and the study of hydrogen bonding (ammonium-PTPB), electrostatic and hydrophobic interactions (bile salt – (ammonium-bearing) polyelectrolyte), are worthy undertakings. It is in the distillation of the eclectic, but related “shoes and ships and sealing wax” that chemistry is most rewarding.