Applications and Microwave Assisted Synthesis of Poly(ethylene glycol) modified Merrifield resins

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ABSTRACT

A microwave assisted methodology was developed to modify Merrifield resins (1-2% cross-linked containing 1.0-3.5 mmol Cl^-/g) with different nominal molecular weights PEG (200-1000). The synthesis was also carried out by conventional heating to assess the differences between the two procedures. The most efficient synthesis was achieved by using microwave and by using PEG with molecular weight 200 and MR 2% cross-linked containing 1.25 mmol Cl^-/g. The structural elucidation was carried out using Fourier transform infrared (FTIR) spectroscopy and elemental analyses. Upon pyrolsis-GC/MS analysis of the PEGylated MR, the PEG showed the tendency to undergo thermal degradation by the loss of a smaller PEG fragments. This observed degradation of PEG was less prominent during microwave assisted synthesis compared to conventional heating, in addition to faster reaction rates and higher yields. As expected, the PEGylated MR showed improved swelling properties in polar solvents. The chemical reactivity of the PEGylated Merrifield resin was confirmed by the esterification with pyruvic acid and by the substitution of hydroxyl group using thionyl chloride. In addition, the PEGylated MR was converted into **(1)** *polymer-supported acid/base or redox indicator* by the attachment of a blue organic dye – 2,6-dichloroindophenol (DCIP) through a nucleophilic substitution reaction and **(2)** *β-cyclodextrin trap*, a water insoluble inclusion-complex, by immobilization of β-cyclodextrin through cross-linking with 1,6-hexamethylene diisocyanate reagent.

RÉSUMÉ

Une méthode Assistée par Micro-ondes a été développée afin de modifier la résine de Merrifield (1-2% de réticulation contenant 1.0-3.5 mmol Cl^-/g) avec du PolyEthyleneGlycol (PEG) de différents poids moléculaires (200-1000 uma). La synthèse a aussi été faite par la méthode conventionnelle afin de comparer les deux procédures. La synthèse la plus efficace a été réalisée en utilisant la technique assistée par micro-ondes et le PEG de poids moléculaire de 200 uma et la résine Merrifield ayant 2% de réticulation et contenant 1.25 mmol Cl^-/g. L'élucidation structurale a été faite sur un spectromètre InfraRouge à Transformation de Fourier (IR-TF) et par analyse élémentaire. Suite à une pyrolyse et analyse par chromatographie en phase gazeuse couplée à un spectromètre de masse (CG-SM) de la Merrifield PEGylée, le PEG a démontré une tendance à subir une dégradation thermique par la perte d'un petit fragment. Cette dégradation observée du PEG fut moins importante lors de la synthèse assistée par micro-ondes que lors du chauffage conventionnel. Le réaction fut plus rapide par micro-ondes et le rendement plus élevé. Tel qu'attendu, la Merrifield PEGylée a démontré des propriétés améliorées de gonflement lorsqu'elle est dans des solvants polaires. La réactivité chimique de la résine de Merrifield PEGylée, fut confirmée par l'estérification avec l'acide pyruvique et par la substitution d'hydroxyle en utilisant le chlorure de thionyle. Également, la MR PEGylée a été convertie en (**1**) *réaction acide/base ou indicateur rédox supporté par un polymère*, par l'attachement d'une teinture organique bleue – le 2,6-dichloroindophenol (DCIP) par une réaction de substitution nucléophilique et en (**2**) *piege de β-cyclodextrine*, un complexe insoluble

dans l'eau par l'immobilisation de la β-cyclodextrine par réticulation avec le réactif diisocyanate 1,6 d'hexaméthylène.

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CHAPTER 1

INTRODUCTION

1.1. Background and Scope

In the last decade, the increased demand for pharmaceuticals and the need for variety of flavor compounds and nutraceuticals to be incorporated in new food products; have been the driving forces behind the development of new techniques for rapid synthesis. Preparation of these new compounds using traditional approaches usually require long incubation times often accompanied by product degradation. Such degradations reduce the overall yields and may complicate the separation of products from reagents, solvents and catalysts. Purification methods used for the isolation of the products such as liquid/liquid extractions and chromatography are often lengthy and expensive. Although, new synthetic methods such as enzyme based synthesis, is specific and side reactions are eliminated, the problem is the cost and limited availability of certain enzymes as well as difficulty of scale-up. For all these reasons, researchers have begun to explore new techniques that would allow faster and more efficient synthesis. Two such techniques are solid phase and microwave-assisted methodologies.

1.1.1. Solid Phase Synthesis (SPS)

Solid-phase synthesis is an approach in which reactions are performed on an insoluble matrix, referred to as a solid support, to overcome the labour intensive and time-consuming purification steps. R. Bruce Merrifield (1963) introduced this technique in the 1960's for the synthesis of peptides. In 1984, he was awarded the Nobel Prize in chemistry for this influential contribution. In SPS, large excess of soluble reagents can be used, driving the reaction to completion. Higher yield is often obtained as compared to the same reaction performed by the traditional solution phase method. Moreover, the ability to readily form and isolate the product through filtration allows for easy automation (Porco et al., 1996), further reducing the costs and time. In addition to solid phase synthesis, the solid supports are also used as scavenger resins, for purification of complex mixtures, and as resin bound reagent for immobilization of toxic intermediates thereby facilitating its removal from the reaction mixtures (McNamara et al., 2002).

1.1.2. Solid Supports

The choice of polymer support depends on the type of reaction to be performed. The first and still the most widely used support is the Merrifield resin (Figure 1.1); a polystyrene based resin with 1-2 % cross-linked divinylbenzene (DVB) (see Figure 1.2). Polystyrene based resins are hydrophobic with gel-like structures, and are easily functionalized due to the alkene side chains on the aromatic ring. They also exhibit good chemical and mechanical stability. These supports however, have limited use in

the organic reaction requiring polar solvents as the accessibility of their reactive sites is hindered.

One approach to overcome this limitation would be to modify the surface topology of insoluble polymers through covalent attachment of a short-chain soluble polymers such as poly[ethylene glycol] (PEG). Such hybrid polymers can incorporate the advantages of both types of polymers such as the physical stability of insoluble polymers and solvent-like character of liquid polymers that allow different substrates to approach the reactive sites more efficiently and hence increase the reaction rates.

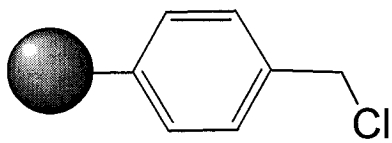


Figure 1.1 – Merrifield resin (O- represents polystyrene backbone)

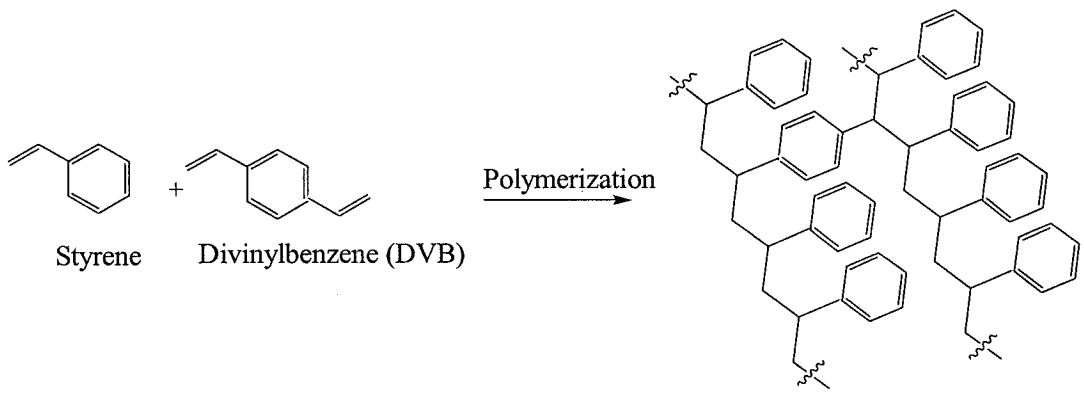


Figure 1.2 – Suspension polymerization of polystyrene resin

1.1.3. Microwave-Assisted Synthesis

Parallel to the developments in solid-phase chemistry, microwave-assisted process (MAP) (Paré et al., 1991, 1994; Paré 1994, 1995, 1996), is rapidly becoming recognized as an environment friendly technique due to the reduced amount of solvent and energy consumed in a variety of reactions (Lidström et al., 2001; Perreux and Loupy, 2001). MAP has been applied successfully to various liquid-phase and gas-phase extractions and is currently extensively used as a tool for many synthesis reactions, including the preparation of functionalized resins (Yang et al., 2001). Microwave heating is very different from the conventional heating which depends upon the thermal conductivity of the materials, it is an instantaneous heating of the molecules that will respond to dipole rotation or ionic conduction, the two mechanisms responsible for the microwave heating. Remarkable increase in reaction rates (up to 8 orders of magnitude), yield enhancement, as well as cleaner reactions with easier workup are the most important advantages of microwave heating (Giguere et al., 1986).

1.2. Research objectives

Recent advances in technology have made both the solid phase organic chemistry and microwave energy more efficient means of synthesizing new molecules, in medicinal and combinatorial chemistry. The development of new polymeric supports is expanding, and most functionalized resins are prepared by chemical modification of existing polymers (James, 1999). The traditional method for the modification reaction

is time consuming as the reagent must overcome the resistance of mass transfer and steric hindrance caused by the gel networks to access the reaction site of the polymer. Yang et al (2001) has first reported the use of microwave energy for preparing functionalized resins. The main objectives of this study were to develop:

(1) microwave-assisted methodologies to synthesize poly(ethylene glycol) modified Merrifield resins, and

(2) methodologies to functionalize PEGylated Merrifield resins for specific applications.

The specific objectives of the study were:

i) To study and optimize the effect of microwave parameters (time, power) on the yield and purity of products;

ii) To investigate the stability of the synthesized resins with respect to temperature and pH;

iii) To confirm the chemical reactivity of the modified resin by performing a) an esterification reaction and b) a nucleophilic substitution reaction;

iv) To develop redox or acid/base color indicators using PEGylated resins

v) To immobilize β-cyclodextrin through cross-linking of PEGylated resins with 1,6-hexamethylene diisocyanate to be used as a water insoluble inclusion-complex

CHAPTER 2

LITERATURE REVIEW

2.1. Solid-phase Synthesis

Limitations in efficiency of classical chemical synthesis resulting from tedious work-up and purification after each reaction step have been overcome by the use of insoluble, functionalized polymeric supports. Over the past 40 years solid-phase synthesis has been extensively studied and covers diverse chemistry extending from its early stages in solid-phase peptide synthesis (Gutte and Merrifield, 1969; Barton et al., 1973) to combinatorial chemistry for the discovery and optimization of lead compounds in various discovery programs (Gordon et al., 1994; Czaqnik and Ellman, 1996) and the now routine use of solid supported reagents and catalysts (McNamara et al., 2002). The solid-phase approach offers substantial advantages over the classical solution synthesis methods. The ease of purification which can be performed by simple filtration and washing, and use of excess reagents to drive reactions to completion were described in article by Labadie (1998). Most notably, reactions can be carried out with high yields and selectivity. Moreover, Porco et al. (1996) reported the amenability of solid-phase synthesis to automation.

Despite the successful development in solid phase synthesis it still exhibits several shortcomings, due to the nature of heterogeneous reaction conditions resulting from the

random processes of polymerization and cross-linking of the support as described by Hancock et al. (1973) and Sherrington (1998). Longer time is therefore required to develop chemistry, including the preparation of the resin due to unequal distribution and/or access to the reaction site. Also other authors (Gallop and Fitch, 1997) have observed the difficulty in characterizing compounds on resin beads.

2.1.1. Insoluble polymer: general considerations

The general requirements for a support are mechanical and chemical stability under the reaction conditions to be used. Supports also need to be functionalised, so that the intermediates can be covalently attached to the support via a suitable linker. Diverse functionalization reactions and different types of linkers are listed in the reviews by Gordon and Balasubramanian (1999) and Guillier et al. (2000). Moreover, because the diffusion of reagents into the support matrix is necessary (Bayer et al., 1970); the materials with sufficient permeability or swelling capacity need to be chosen.

Merrifield first demonstrated the use of a substituted cross-linked polystyrene (PS) resin in conjunction with dichloromethane (DCM) as solvent for solid phase peptide synthesis (1963). Until now the polystyrene based resin, also classified as the gel-type resin, is still the most commonly used. The use of other polymers, such as cellulose (Frank and Doering, 1988) and polyarcylamide resin (Kanda et al., 1991; Renil et al., 1998) has also been explored. The main advantages of PS are: some of the phenyl rings are easily functionalized to allow attachment of small molecules with a good loading

capacity of >0.5 mmol g^{-1} (Sucholeiki, 1999); it is usually cheaper than other resin types and it can withstand a wide range of reaction conditions. Some limitations however, have been observed in the synthesis of oligosaccharides (Frechet and Schuerch, 1971) and oligonucleotides (Köster, 1972) whose polarity is incompatible with the hydrophobic and non-polar nature of polystyrene. As describe by Bayer (1991), the steric factors and a lack of kinetic equivalents of the functional groups also play a certain role.

Although, cross-linked PS continue to be used successfully, the desire to improve upon their deficiencies has led to the development of new linkers and tether groups that can accommodate a wide range of reactions (Bergbreiter, 1999 and Sucholeiki, 1999). Of these, grafting of polyoxyethylene onto the PS resin, Tentagels[1] (Bayer, 1991) and ArgoGel[2] (Labadie et al., 1996) are the most notable alternatives. Other non PS based resins have also been developed, for example the cross-linked ethoxylate arcylate resin (CLEAR) support (Kempe and Baraby, 1996).

2.1.1.1. Factors affecting the reagent accessibility in the polymer

Since the bead swells when solvent is absorbed and the reactions take place within the solvated gel as well as on its surface areas (Seneci, 2000), therefore, the efficient swelling of support in the solvents chosen for synthesis is a crucial factor in solid-phase chemistry, especially for the successful use of polymer-supported reagents and

[1] TentaGel is a trade mark of Rapp Polymere GmbH, 72072 Tubingen Germany.
[2] ArgoGel is a trade mark of Argonaut Technologies, San Carlos, CA.

catalysts. Lightly (0.5-2.0%) cross-linked PS is usually used in solid-phase synthesis, as it gives a good ability to swell and a reasonable stability of the bead. Vaino and Janda (2000) explained in their review that, if a lightly cross-linked polymer does not swell when suspended in solvent, there will be little opportunity for reagents to interact, thus precluding reaction. According to Sherrington (1998) the DVB cross linked PS is very compact in the dry state, and the diffusion of even small molecules through this polymer network is very slow. The solvation, or swelling, of the resin in the solvent chosen for the reaction creates space, or 'solvent porosity', within the resin and allows small molecules easy access to the polymer network.

Pugh et al. (1992) stated that the extent of swelling of polystyrene resins in organic solvents is governed primarily by the amount of divinylbenzene cross linking of the polymer, and is also strongly affected by the addition of small molecules. It was recognized at the very beginning of solid-phase peptide synthesis that the amount of peptide on the resin changes swelling of polystyrene-DVB resins. Unsubstituted resin swells more in DCM than in DMF, whereas DMF is a better solvating medium for a resin bearing many amino acid residues (Sarin, 1980; Fields and Fields, 1991). In addition to the swelling ability, the other factor affecting the reagents accessibility in the polymer is the glass transition temperature (T_g), the temperature below which polymers have very little mobility. T_g is one of the defining properties of a polymer. Gutierrez and Ford (1986) showed in their studies that the rates of molecular diffusion decrease noticeably upon cooling through T_g, providing a guide to the useable temperature range of the resin. Also, interaction of the polymer with all solvents examined resulted in Tg

depression. For cross-linked PS, a highly amorphous polymer, T_g is lowered from 110 °C in the dry state to ambient temperature upon interaction with a good solvent (Ferry 1961, cited in Vaino and Janda 2000).

2.1.2. Soluble polymer – Poly(ethylene glycol) (PEG)

Soluble polymers, such as non-cross-linked polystyrene (Shemyakin and al., 1965) or poly(ethylene glycol) (Sauvagnat et al., 1998, 2000) have also been used as supports for organic synthesis. The aim of the synthesis on soluble support is to make the heterogeneous mixture more solution-like. The separation of the desired intermediate from the reagents is carried out by precipitation of the polymers in certain solvents or purification by membrane filtration or recrystallization. PEG has been the most notable polymer because it is available commercially in a wide range of molecular weights in monomethyl ether (MPEG) and free diol (PEG) forms. These polymers are soluble in water and most organic solvents, but can be precipitated with hexane, diethylehter, or tert-butyl methyl ether. PEG is also of interest to the food and pharmaceutical industry due to its non toxic nature, and is approved by the U.S. Food and Drug Administration for internal use in human (Moghaddam, 2001; Hunter et al., 1967). Gravert and Janda (1997) have reviewed the application of PEG and other soluble polymers for the synthesis of peptides, nucleotides, oligosaccharides (Douglas et al., 1991, 1995) and small molecules as an alternative to the solid-phase synthesis. This technology is however less attractive than the solid phase synthesis mostly because synthesis on soluble supports is difficult to automate (Bayer et al., 1985). Also, the polymers can be

difficult to recover, often contaminating the product and complicating reuse (Sherrington, 1998). Gerritz and co-workers (2000) have found that reactions on soluble polymers do not proceed significantly faster than those on insoluble, cross-linked polymers.

2.1.3. PEG-grafted Polystyrene resins

In order to overcome the problems in the synthesis using either the insoluble or soluble supports, attempt was made by Becker and co-workers (1982) to synthesize a hybrid polymer that incorporates the easy-handling characteristic of an insoluble support and the solvent like feature of the soluble polymer. PEG has a wide range of solvent compatibility and has been the choice for modifying the cross-linked PS resin. This new class of supports has been applied for the synthesis of various solubilized peptides reported by Hellermann et al. (1983). Moghaddam (2001) explained in his article that PEG attachment to solid surface provides a protective layer between a hydrophobic surface and molecules, reducing nonspecific interactions. Second, it tethers the reactive molecules into the microenvironment for chemical interactions. Most commonly, PEGs are attached to 1-2% cross-linked polystyrene. The first generation of commercially available PEG-grafted PS resin, TentaGel resin (Bayer and Rapp, 1988) had excellent chemical properties with respect to pressure resistance and stability. It also swells both in aqueous systems and inorganic solvents (Wilson et al., 1998) but have a lower loading capacity than the PS-based resins typically loading at < 0.5 mmol g^{-1}. Baytas and Linhardt (2004) reviewed the successful use of TentaGel in combinatorial

carbohydrate synthesis. In recent years, new graft-type PEG-PS with twice the loading capacity of TentaGel, called AgroGel (Labadie et al., 1996) has been introduced for solid phase organic synthesis. Similar studies on preparing poly(ethylene glycol)-polystyrene based resin were reported by Renil and Meldal (1996) and Burchardt and Meldal (1998). On the other hand, Itsuno et al. (1989) was the first to include the oligo(oxyethlyne) chain as cross-linking agent in the preparation of cross-linked PS beads.

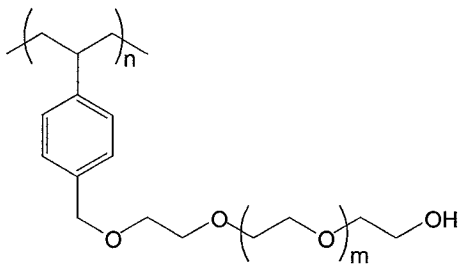


Figure 2.1 TentaGel – PEG-grafted PS resin

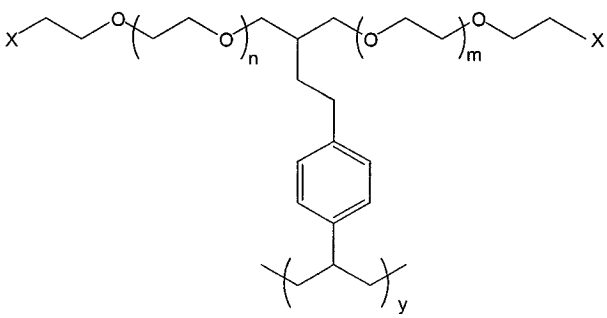


Figure 2.2 AgroGel - new graft-type PEG-PS with twice the loading capacity of TentaGel

Li and Yan (1998) compared the PS and TentaGel based resins and determined that TentaGel does not always provide faster reaction rates than PS resin. The choice always depends on the nature of the reaction and its requirement for polar and nonpolar medium.

2.2. Polymer supported reagents, catalysts and scavengers

An important practical problem in organic synthesis is the isolation of the pure products free from contamination due to reagents, solvents and catalysts and the purification step is often the most time consuming part of the synthesis. Following the successful introduction and extensive development of solid-phase peptide and organic syntheses, attention has focused in recent years on the development of polymer-supported reagents and catalysts for use in traditional solution-phase reactions. These reagents or catalysts are similar to their small molecule equivalents but they are insoluble materials that can be recycled after use (Nicewonger et al., 2002). Highly toxic chemicals can be rendered inert and harmless through attachment to a polymer support plus their release to the environment can be eliminated. The polymer supported reagent are non-volatile and odourless (Harris et al., 1998) and therefore are easy to handle. This combination of solution-phase reaction with polymer supported reagents allows the removal of excess reagents and by-products by simple filtration methods, without the need for chromatographic purification (McNamara et al., 2002), which is ideal for multi-steps reaction (Thompson, 2000; Ley et al., 2002). Scavenger resins act as reactive purification and/ or separation media, and are added after the reaction is completed to

quench or selectively react with excess reagent or by-product. A recent study by Hodge (2003) demonstrated the use of polymer supported reagents, catalysts and/or scavengers on reactions in flow systems, and their ability to enhance automation in solution-phase synthesis.

2.2.1. Polymer supported pH/ Redox indicators

Many colorimetric reagents showing optical interactions with specific analytes, are widely used as visual indicators for pH or for reduction–oxidation (redox) activity detection. These reagents are often immobilized by chemical or physical methods onto polymeric materials that are in the form of beads, powders or films, as reported by several authors as a successful approach to the construction of optical pH/redox sensors (Ensafi and Kazemzadeh, 1999; Newcombe et al., 1999; Ertekin et al., 2000; Goodlet and Narayanaswamy, 1994). The advantage of using polymer supported pH/redox indicators is the opportunity for recycling the spent reagent for repeated use. A successful indicator must give a fast response time and long term stability as mentioned by Jones and Porter (1988) and Cardwell et al. (1993).

2.2.2. Immobilization of cyclodextrin on an insoluble support

Cyclodextrins (CD) are cyclic oligosaccharides consisting of six or more D-glucopyranose units linked by alpha-(1,4) bonds (Figure 2.3) obtained by degradation of starch. As expected for carbohydrate molecules, CDs are very water soluble but they

have an apolar cavity in the center of the molecule, capable of forming inclusion complexes with organic substrates. Since they are non toxic (Rao et al., 2000) and edible they have been used for the encapsulation of flavours (Bhandari et al., 1998; Schatzman, 2002) and drugs (Perrakis et al., 1999). CDs have also been applied in chromatographic separation and purification methods as reviewed by Li and Purdy (1992).

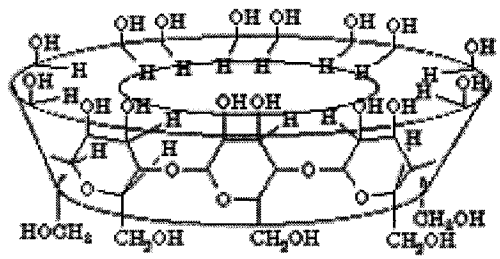


Figure 2.3 Cyclodextrin model

(http://www.usm.maine.edu/~newton/Chy251_253/Lectures/BiopolymersII/BiopolymersII.html)

The interest in recent research had been to introduce polymer having CD units as a part of the skeleton. Mizobuchi and co-workers (1980) in 1980 prepared a water insoluble cyclodextrin-polyurethane resins and later water soluble β-CD with epichlorohydrin was studied by Pöpping and Deratani (1992) and Renard and co-workers (1997). Such polymers posses high CD content and can be processed into various forms for practical applications, such as the removal of bitter components in juices (Shaw et al., 1984; Shaw and Buslig,1986), and the use as stationary phase in HPLC (Lee et al., 2002). An alternative way is to process CDs into insoluble solid forms through a linker arm attached to an insoluble polymer. These CD-polymers can be easily removed from the

reaction mixture and reused. David et al. (2001) integrated the polymerized form of β-cyclodextrin onto silica particles and had investigated its ability to form host-guest complexes with hydrophobically modified PEGs. In recent study, Bibby and Mercier (2003) reported the use of CD functionalized mesoporous silica for separation of water soluble aromatic molecules. Chiu and co-worked (2004) reported the immobilization of β-cyclodextrin in chitosan for the separation of cholesterol.

2.3. Microwave-assisted organic synthesis

Since 1970's microwave technology had been used in a wide variety of purposes, such as moisture analysis (Hesek and Williams, 1974) and regeneration of activated carbon (Katsuta, 1976), but the first interest in the application of microwave energy in organic synthesis was reported by Gedye followed by Giguere in 1986. Both demonstrated that the use of microwave energy in chemical reaction dramatically enhanced the rate of many organic reactions and formed cleaner products (Gedye et al., 1986; Giguere et al., 1986). Since then, microwave heating has been applied to series of reactions to reduce reaction times and to improve yields and selectivity, as listed in the review by Lidström et al (2001). Microwave-assisted process (MAP) was developed recently as a series of technologies that employ microwave energy for enhancing chemistry. Yaylayan et al. (1997) have reported the use of a two-stage microwave-assisted process in synthesizing and extracting selected Maillard reaction products.

The slow development of these techniques in organic synthesis in the beginning was principally attributed to the lack of control of microwave parameters due to the use of poorly designed domestic microwave ovens as reactors. Most early microwave-enhanced synthesis work was done in multimode systems (Loupy et al., 1998). These systems have large cavities and have been used successfully to process multiple sample formats, multiple-well plates, and larger scale reactions (greater than one litre). It presents however several drawbacks: due to the cavity design the distribution of the electric field is not homogeneous, creating hot and cold spots. Moreover, the power density in the cavity is low, making it difficult to heat small individual samples in a reproducible manner (Lew and al., 2002). These drawbacks led to the development of monomode reactors with good uniform energy distribution and the ability to couple microwave energy with small samples more efficiently.

2.3.1. The basics of microwave radiation

Microwaves are a form of electromagnetic energy that fall at the lower end of the electromagnetic spectrum between infrared radiation and radio waves, with wavelength ranges from 1cm to 1m which, correspond to frequencies between 0.3 and 300 GHz. In order to avoid interferences with radar and telecommunication, industrial and domestic microwave apparatus are regulated to frequency at 2.45 (± 0.050) GHz. Microwave heating is a very different process than the conventional method. It is an instantaneous heating of the molecules that will respond to dipole rotation or ionic conduction, the two fundamental mechanisms responsible for the microwave dielectric heating

(Lisdström et al., 2001). Dipole rotation is the results of dipole-dipole interactions between polar molecules and the electromagnetic field. As the molecules try to align themselves with the applied field, dielectric heating is induced as a result of molecular friction and collisions. Ionic conduction is the second pathway contributing to microwave heating. When there are free ions present in a substance being heated, the electric field generates ionic motion resulting in rapid heating. As the temperature of the substance increases, the transfer of energy becomes more efficient. The main benefits of using microwave heating lie in the effective heat transfer, homogenous heating throughout the sample and the selectivity to polar molecules. More importantly, both the reaction rates and the yields are enhanced (Strauss, 1999). Microwaves transfer energy faster than the molecules can relax, creating a nonequilibrium condition and high instantaneous temperatures that affect the kinetics of the system. The energy transmitted by microwave are very low (0.03 kcal/mol) compared to the typical energies of chemical bonds (80-120 kcal/mol) and will not account for any direct molecular activation (Larhed and Hallberg, 2001).

2.3.2. Microwave-assisted solid phase synthesis

Due to the relatively long reaction times usually associated with solid-phase synthesis, microwave-assisted solid-phase organic reactions have become the subject of several investigations during the past ten years. One of the first applications was reported by Yu and co-workers (1992) in solid-phase peptide synthesis. According to Stadler and Kappe (2001), the use of cross-linked polystyrene resin has been most established.

They have also shown that these resins are able to withstand microwave irradiation for prolonged periods of time, even at 200°C. Larhed and co-workers (1996) reported the use of TentaGel resins in microwave assisted solid phase organic synthesis, but some degradation of TentaGel resins has been observed during irradiation. Besides being used in solid-phase synthesis, microwave heating has also been employed in the preparation of functionalized resin (Yang et al., 2001). Only very recently, the use of microwave assisted organic reaction using polymer supported reagents has been reported in the literature (Lin and Sun, 2003).

2.4. Characterization of functionalized resins

The step by step monitoring of the preparation of the polymer support as well as the synthesis taking place on the solid phase is important yet problematic due to the insoluble nature of the support. According to Gallop and Fitch (1997) loading is the measure of how much ligand or reactive functional group is associated with the resin per unit weight, and is presented in millimoles per gram (mmol g^{-1}). Quantifying the loading is necessary so that the amount of reagent/ product, or reactive sites available in the support can be known. Standard analytical methods require the cleavage of intermediates from a support at various stages during the reaction; therefore it is not always practical or desirable. For example in the synthesis of supported reagents and catalysts that do not incorporate cleavable linkers. Combustion elemental analysis (Stranix et al., 1997), titrametric analysis, gravimetric analysis or colorimetric analysis (Kuisle et al., 1999; Kay et al., 2001; Cournoyer et al., 2002) are frequently reported as

methods for measuring loading, although inaccuracy and deviation in the result might be obtained due to low loading or contaminants (Fruchtel and Jung, 1996). Conventional NMR methods are of limited use due to the low mobility of the solid phase; line broadening occurs giving poorly resolved spectra. Also the signals from the polymer can swamp the area of interest in the spectrum (Fitch et al., 1994). Recently, the use of gel-phase high resolution magic angle spinning (HR-MAS) NMR has greatly improved this situation, allowing the investigation of noncovalent interactions between receptors and immobilized ligands (de Miguel et al., 1998).

Among the recent advances in analytical methods for on-bead analysis, single-beam FTIR or with the use of single bounce attenuated total reflection (ATR) has become the choice for many years for monitoring reactions in solid phase organic synthesis (SPOS). Giving the advantages of high sensitivity, ease of operation and rapid analysis (Larsen et al., 1993), infrared spectroscopy provide qualitative as well as quantitative detection of the changes of certain functional groups on the insoluble supports (Yan et al., 1995, 1996, 1998, 1999). Moreover, small sample requirement and nondestructive sampling method of single-bead FTIR/ ATR make it a more attractive technique particularly for the screening of polymer-supported combinatorial libraries (de Miguel and Shearer, 2000).

CHAPTER 3

MICROWAVE-ASSISTED SYNTHESIS OF PEGYLATED MERRIFIELD RESIN

3.1. Introduction

The use of cross-linked polystyrene based resins, such as Merrifield (MR) as solid support in combinatorial synthesis, is becoming increasingly important due to their stability, high compatibility and good swelling characteristic with a wide range of non-polar solvents. These resins, however, fail to perform when polar solvents are needed due to hindered accessibility to the reactive sites. Modification of solid surfaces of MR with polar and soluble polymers such as poly(ethylene glycol) (PEG) had been reported in literature. TentaGel and ArgoGel are the most widely used solid-phase synthesis support with PEG attached to 1-2% cross-linked polystyrene. The use of these hybrid polymers can be found in sample preparations, organic synthesis, sensor technology and as chromatographic support material. Synthesis of a functionalized resin using traditional method could be time consuming. Using the microwave-assisted process, we report here a convenient and fast PEGylation procedure starting with commercially available Merrifield resins. Merrifield resin containing the chloromethyl group could undergo a bimolecular nucleophilic substitution (SN_2) reaction with PEG bearing the hydroxyl group, commonly known as the Williamson ester synthesis. The overall

reaction is shown in Scheme 3.1. The resulting resin is functionalized with a modified attaching group containing the reactive site requires for solid phase synthesis.

3.2. Materials and Methods

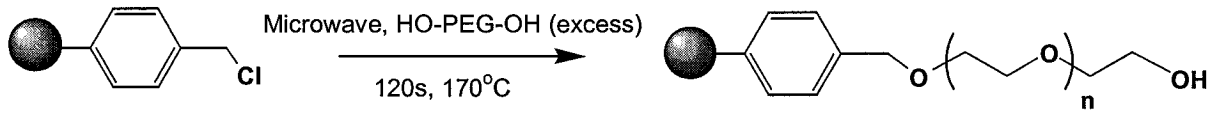
3.2.1. Reagents and Chemicals

Polyethylene glycol 200-1000 molecular weight; Merrifield's peptide resin, (1-2%) cross-linked, 200-400 mesh, (1.0-3.5mmolCl^-/g); sodium hydroxide (ACS reagent); silver nitrate ($AgNO_3$) volumetric standard 0.1025N solution in water; potassium chromate (K_2CrO_4), Fmoc-glycine 97%, 1,3-dicyclohexylcarbodimide 99%, ethanolamine and buffer solutions pH 3, 7 and 10 were purchased from Aldrich Chemical Company (Miwaukee, WI). Dichloromethane, methanol and tetrahydrofuran were purchased from Caledon Laboratories Limited (Georgetown, ON). Synthewave 402 was purchased from Prolabo (France)

3.2.2. Microwave-assisted synthesis of Poly(ethylene glycol) modified Merrifield resin

In a typical experiment, Merrifield's resin (1.22g ± 0.05) was suspended in excess poly(ethylene glycol) with a catalytic amount of solid NaOH. The mixture was then irradiated 3 x 40s for the total of 120 seconds ($T \cong 170^{\circ}C$) at 300W (unless otherwise specified) focused microwave power using Synthewave^{TM} 402 (Prolabo, France).

Excess PEG acts as solvent and at the same time prevents cross-linking of MR. The product was purified by washing with 30mL of water, 20mL of 10% HCl, 2 x 20mL of water and 4 x 20mL of methanol in succession and dried.



Scheme 3.1 Poly(ethylene glycol) attachment to Merrifield resin

3.2.3. Synthesis of polyethylene glycol modified Merrifield resin using conventional method

The conventional heating was carried out using a heated sand bath (Reacti-Therm, Pierce, Rockford, IL)

3.2.4 - Chloride determination by Mohr method

The efficiency of the reaction was determined by measuring the chloride ion released in the wash using the Mohr method. Mohr method uses CrO_4^{2-} as an indicator. AgCl is much less soluble than Ag_2CrO_4 so it will precipitate first. A precipitate of Ag_2CrO_4 forms in the presence of a slight excess of Ag^+ and signals the end point. The color changes from a yellow to a brownish-yellow. To determine the amount of chloride ions in the wash, keep the first wash, add 2 ml of indicator (0.1 M K_2CrO_4) and titrate. The mmole of Ag used is equivalent to the mmole Cl^- presented in the wash.

3.2.5. Estimation of the free hydroxyl groups on the PEGylated Merrifield resin by UV quantitation

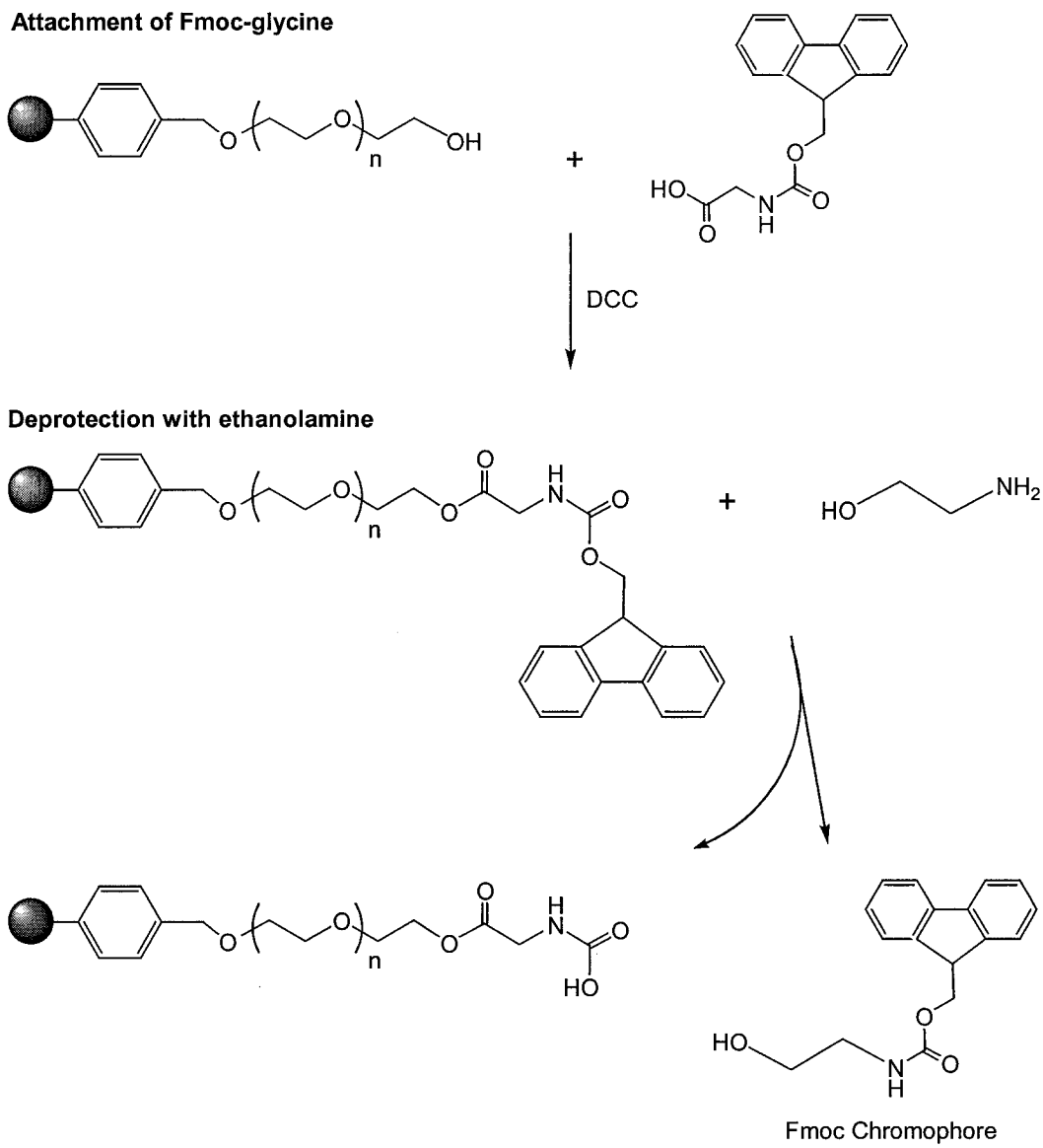
3.2.5.1. Reaction of PEGylated MR with Fmoc glycine (Scheme 3.2)

PEG-MR (1.50 ± 0.02g) and Fmoc-glycine (0.40 ± 0.02g; MW. 297.32) were suspended in 50 mL of dichloromethane. 1,3-Dicyclohexylcarbodimide (DCC; 0.30g ± 0.02g, MW. 206.33) was added to the solution. The mixture was stirred at room temperature (~20°C) for 48 hours. A white precipitate – dicyclohexylurea was formed. Additional Fmoc-glycine (2 mmol) and DCC (2 mmol) were then added. The stirring continued for approximately 8 more hours to complete the reaction. The precipitated DCU and the product were filtered and washed successively with water, DMSO (to remove DCU), water followed by methanol and was dried at room temperature.

3.2.5.2. UV quantitation of Fmoc chromophore

Deprotection of Fmoc: To the above obtained product (0.5 g) ethanolamine (0.5ml) was added. After 30min, the mixture was diluted with DCM (10.5ml). The supernatant containing the Fmoc chromophore was pipetted out and its absorbance was measured using an UV/Vis spectrometer (Perkin Elmer Canada Ltd., Montreal) at λ_{max} 258nm. The equivalent OH group site on the polymer was determined from the calibration curve. The calibration curve was constructed by measuring the absorbance of standards

with known Fmoc chromophore concentration (0.002-0.02M). The best-fit straight line was determined from linear least square method.



Scheme 3.2 Esterification of PEGylated MR with Fmoc-glycine, followed by Fmoc deprotection and UV quantitation of Fmoc chromophore.

3.2.6. The stability of PEGylated MR with respect to temperature and pH

The stability of the modified resin was determined by gravimetric analysis after storage. For pH controlled studies, PEG-MR (~ 0.200 g) was suspended in buffer solution (10 ml) of pH 3, 7 and 10 at room temperature for 24 hours. The resin was washed with water and methanol and allowed to dry. The weight difference, if any, was recorded. For temperature study, PEG-MR (~0.200 g) was suspended in de-ionized water. The mixture was heated in microwave at 270 and 300W for 3min. The resin was washed with water and methanol and allowed to dry. The weight difference, if any, was recorded.

3.2.7. Swelling Properties of the PEGylated MR

Swelling studies on the PEGylated Merrifield resin were carried out in a 10 mL graduated cylinder, the resin (~200 mg) was suspended in 10 mL of solvent and were allowed to stand at room temperature for 30 min. At the end of that time, the final volume of the swollen resin was recorded.

3.2.8. Identification Techniques

3.2.8.1 Pyrolysis-GC/MS analysis

A Hewlett-Packard GC/mass selective detector (5890 series II GC/5971B MSD, Palo Alto, CA) interfaced to a CDS pyroprobe 2000 unit (CDS Analytical Inc. Oxford, PA), through a quartz-lined and valved interface (CDS 1500), was used for the Py-GC/MS analysis. Samples (5mg) were introduced inside the quartz tube (0.3 mm thickness) plugged with quartz wool and were inserted inside the coil probe. The pyroprobe was set at 200 °C with a total heating time of 20 s. The pyroprobe interface temperature was set at 250 °C. The GC column flow rate was 0.8 mL/min. for a split ratio of 92:1 and a septum purge of 3 mL/min. Capillary direct MS interface temperature was 280 °C; ion source temperature was 180 °C. The ionization voltage was 70 eV, and the electron multiplier was 1682 V. The column was a fused silica DB-5 column (60 m x 0.25 mm i.d. x 0.25 mm film thickness; Supelco, Inc.). The column initial temperature (5 °C) was increased to 260 °C at a rate of 10 °C/min. and held at 260 °C for 15 minutes.

3.2.8.2. Elemental Analysis

Elemental analysis was performed by Guelph Chemical Laboratories Ltd. (Guelph ON, Canada). Data were reported as the average of duplicate measurements.

3.2.8.3 FTIR analysis

Infrared spectra were recorded with Merlin software on a Bio-Rad Excalibur Series FT-IR spectrometer (Bio-Rad, Cambridge, MA, USA) purged with dry air. The spectra of resin in dry state were acquired on a Golden Gate Single Reflection Diamond ATR. A total of 128 scans at 4 cm^{-1} resolution were co-added for the modification studies. Processing of the FTIR data was performed using GRAMS/32 AI version 6.01.

3.3. Results and Discussions

The actual amount of PEG grafted unto the MR was estimated by three methods, one based on the number of moles of chloride ion released (Table 3.1), the other based on the measured weight of the product (see Table 3.2), and the third based on the estimation of the free hydoxyl groups by UV quantitation (λ_{max} 258 nm) of Fmoc chromophore after reaction with Fmoc glycine.

3.3.1. Microwave assisted synthesis of PEGylated Merrifield resin vs. conventional heating

To determine the optimum conditions for the synthesis, the effect of the percent cross-link and the chloride load of MR, as well as the molecular weight of PEG, on the yield of the hybrid polymer was investigated. The data given in Table 3.1 show that the use of PEGs with higher molecular weights and MRs with higher number of reactive sites

resulted in lower %yields of the hybrid polymers. This can be explained by steric hindrance effect. Due to the bulkier structure of the higher molecular weight PEG, it becomes more difficult to approach the reactive sites on the MR. At 300W microwave power, the most efficient synthesis (76.5 % PEGylation sites) was achieved by the use of PEG (mol wt. 200) and Merrifield resin having 2% cross-link with an average of 1.25 mmoles Cl^-/g (see Table 3.1). This resin was used for further studies such as investigation of the effect of microwave power and conventional synthesis on the yield of the reaction. Table 3.2 summarizes the result of these experiments.

Table 3.1 Effect of PEG and MR on the % yield and % PEGylation sites during microwave-assisted PEGylation of Merrifield resin.

Experiment[a]	mmol of Cl^-	% Yield [b]	SD	% PEGylated sites[c]	SD[d]
PEG_{200}-MR2%(1.25)	1.17	91.9	0.9	76.5	5.5
PEG_{200}-MR2%(1.25)[e]	1.28	92.6	0.9	83.1	5.5
PEG_{200}-MR1%(1.75)	1.60	85.3	0.5	75.4	2.1
PEG_{200}-MR 2%(2.25)	1.61	80.3	0.2	59.5	0.6
PEG_{400}-MR1%(1.75)	1.57	78.1	2.2	73.1	5.3
PEG_{400}-MR1%(3.25)	1.54	54.5	0.6	41.2	0.7
PEG_{600}-MR2%(2.25)	1.54	57.9	0.3	56.0	0.5
PEG_{1000}-MR1%(1.75)	1.29	49.0	1.8	62.3	2.9
PEG_{1000}-MR1%(3.25)	1.43	34.0	0.5	35.8	0.6

[a] PEG_{MW}-MR % cross link (meq of cloride/g of resin); [b] % yield =actual wt x 100/ theoretical weight ; [c] % PEGylation is based on chloride ion released realtive to reported chloride ion content; [d] based on three replicate experiments; [e] performed under reduced microwave power from 300W to 210

Table 3.2 Comparison of the weight of grafted PEG in grams between conventional[a] and microwave-assisted synthesis

Experiment[b]	PEG $_{\text{theoretical}}$ [c]	PEG $_{\text{based on chloride ion}}$ [d]	PEG $_{\text{based on wt of product}}$ [e]	% PEGylation [f]	% Decomposition[g]
PEG_{200}-MR2%(120s)	0.307	0.235±	0.173±	56.3 (91.9)[i]	26.4
PEG_{200}-MR2%(120s)[h]	0.307	0.255±	0.189±	60.0 (92.4)	25.0
PEG_{200}-MR2%(10m)[a]	0.307	0.182±	0.145±	47.0 (90.5)	20.3
PEG_{200}-MR 2%(25m)[a]	0.306	0.192±	0.142±	46.4 (90.2)	26.0
PEG_{200}-MR2%(35m)[a]	0.306	0.213±	0.124±	40.5 (88.7)	41.7

[a] Reactions were performed in a React-Therm block heated at 170°C using 20 mL open vials. Each sample required 30 min to reach 170°C as measured by a fiber optic probe, the reported times in minutes, indicate heating times after reaching 170°C.;

[b] PEG_{MW}-MR % cross link (reaction time);

[c] based on the meq of chloride of the starting Merrifield resin (1.22 g, 1.25 meq/g);

[d] based on the meq of chloride ion released after the reaction;

[e] calculated from the weight difference between the starting resin and the product after correction for the loss of chloride;

[f] % PEGylation = PEG $_{\text{based on wt of product}}$ /PEG $_{\text{theoretical}}$ x 100;

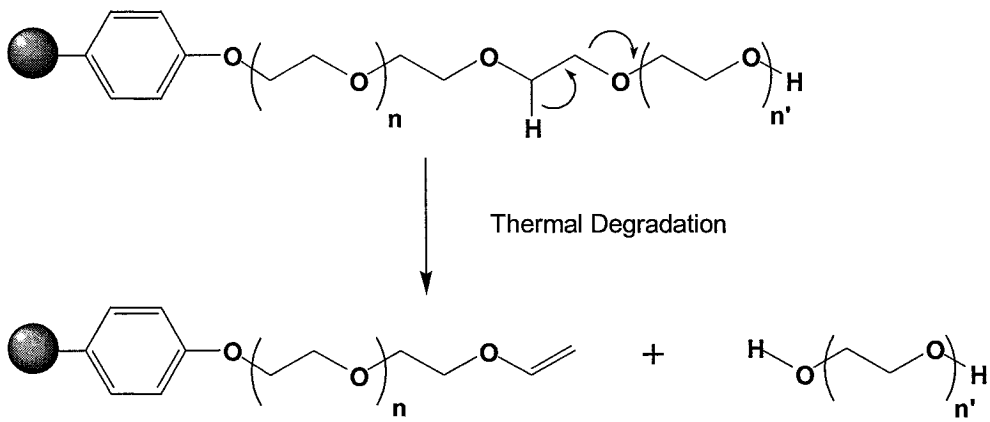
[g] % decomposition = (PEG $_{\text{based on chloride ion}}$ - PEG $_{\text{based on wt of product}}$)/PEG $_{\text{based on chloride ion}}$ x 100;

[h] performed under reduced microwave power from 300W to 210W ;

[i] % yield (as defined in Table 1).

The data in Table 3.2 indicate that not all predicted PEG (based on the chloride ion released) was incorporated into the MR (for example 0.173g instead of 0.235 g). This might be due to thermal cleavage of PEG after being grafted onto the MR backbone during synthesis (see Scheme 3.3). Pyrolysis-GC/MS analysis of PEGylated MR have indicated the propensity of PEG moiety to undergo carbon-oxygen bond cleavage to produce terminal ethenyloxy group instead of the intact ethanol group, by loss of smaller PEG fragments (Scheme 3.3). Lowering the microwave power to 210W

increased percent PEGylation (by 3.7%) by reducing the amount of side reactions (by 1.4%) that lead to thermal degradation of PEG.



Scheme 3.3 Thermal degradation of PEGylated Merrifield resin based on pyrolysis-GC/MS analysis.

The synthesis was also carried out by conventional heating at the same temperature, to assess the differences, if any, between the two procedures. The results listed in Table 3.2 indicated occurrence of similar thermal degradations, which was a function of heating time. Our results also show that the highest yield obtained by microwave (92.4%) was not achieved by conventional heating, even after 35 min of heating. In fact, the yield decreased with longer heating times due to decomposition of the grafted PEG as shown in Scheme 3.3. Although the amount of grafted PEG estimated based on the chloride ion released increased with increasing heating time, the actual amount of grafted PEG decreased over time, confirming the above conclusion. This was also corroborated by the determination of free hydroxyl groups remaining in the product, by UV quantitation (λ_{max} 258 nm) of Fmoc chromophore after esterification with Fmoc

glycine followed by the basic cleavage. The number of moles of hydroxyl groups estimated by this method was within 5% of number of moles of grafted PEG calculated based on the final weight of the product ($PEG_{\text{based on wt of product}}$) in Table 3.2. However, this method of determining the loading of PEGylation has certain drawbacks, such as the error associated with incomplete cleavage of the fmoc and the presence of contaminants that might absorb at the same wavelength would affect the accuracy of the result.

3.3.1.1. Determination of loading by elemental analysis

The extent of the loading reaction can be determined by elemental analysis. Results from the elemental analysis of MR and PEGylated MR are presented in Table 3.3. The amount of reacted Cl estimated from elemental analysis is consistent with the amount of Cl recovered from the reaction. And based on the weight % O gained (7.15 %) in the PEGylated MR, 0.894 mmol PEG/g were detected. However, this amount of PEG attached is more than the value determined from the weight gained of the product ($PEG_{\text{based on wt of product}}$). One possible explanation is that over storage period (6 months), the PEG moiety on the MR may absorb moisture, and results in higher O content.

Table 3.3 Chloride and Oxygen analysis of MR and PEGylated MR

	% Cl	%O	mmol Cl reacted/g resin	Loading (mmol PEG/g resin)
MR [a]	3.88	1.65 [b]		
PEG-MR	0.11	8.8		
Elemental Analysis			1.063 [c]	0.894 [e]
Experiment			1.064 [d]	0.662 [f]

[a] starting MR with 1.37 mmol Cl/g resin (value obtained from certificate of analysis from Aldrich Chemical Company)

[b] This % O presented in the MR is believed to be contaminant due to solvent or moisture and is subtracted from %O gained in the PEGylated MR;

[c] %Cl in MR - %Cl in PEG-MR

[d] mmol Cl recovered in the wash/ g of MR used

[e] net O weight gained per g resin/ molecular weight of O/ 5 mole O per mole PEG

[f] mmol PEG $_{\text{based on weight of product}}$/ weight of product (yield)

3.3.2. Effect of microwave power on the yield and purity of the product

As discussed in the previous part, the use of microwave energy allows faster and more efficient synthesis of PEGylated MR as compared to conventional heating. The short reaction time offered by microwave heating allowed the decrease of side reaction responsible for the thermal degradation of PEG, therefore improving the homogeneity of the product. Table 3.4 further indicates the possibility of modulating the microwave power to reduce degradative side reactions occurring during synthesis, such as using 210W microwave power instead of 240W. Finally, the synthesis of the hybrid polymer was reproducible on a larger scale (six-fold) under the same reaction conditions.

Table 3.4 Effect of microwave power on the yield and purity of the product

Experiment	Power (W)	% yield	%Pegylation	%Decomposition
PEG200-MR2% (160s)	240	93.1	61.3	25.4
PEG200-MR2% (160s)	210	93.4	63.0	19.3
PEG200-MR2% (160s)	180	92.1	53.0	16.3

3.3.3 Stability and swelling properties of the PEGylated Merrifield resin

The stability of the PEGylated Merrifield resin with respect to pH and temperature was studied. It was determined that there was no significant weight lost (<1 mg) over the exposure to buffer solutions of pH values ranging between 3 and 10. The resin was also found to be stable under microwave heating for 3 min at 300 W, with the final temperature reaching 92 °C.

The swelling properties of the PEGylated Merrifield resin in selected solvents are presented in Table 3.5. MR is a hydrophobic resin that swells well in non-polar solvents but does so poorly in polar solvents. As a result of the attachment of PEG, the resin showed improved swelling properties in polar solvent, suggesting that the reactivity of the support in polar solvents might be enhanced. For comparison, the studies were also carried out on 1% cross-linked MR resin. Our results indicate that the 1% cross-linked MR swells better in both non polar and polar solvents. This was expected due to the smaller extent of cross-linking that allow the support to be more flexible.

Table 3.5 Swelling properties of the PEGylated Merrifield resin in different solvents

Resins	Swelling (mL/g)			
	Methanol	Water	DCM	THF
MR2% (1.16)	1.78	--[a]	7.19	7.04
PEG200-MR2%(1.16)	2.16	1.94	5.6	5.44
MR1% (1.75)	2.55	--[a]	20.83	18.61
PEG_{200}-MR1% (1.75)	4.78	2.44	10.42	8.06
PEG_{400}-MR1% (1.75)	3.57	3.56	8.69	7.78
PEG_{1000}-MR1% (1.75)	3.06	3.56	8.57	7.47

[a] – density of the resin was lower than that of water

3.3.4 FTIR analysis of the PEGylated Merrifield resin

Structural elucidation of the PEGylated Merrifield resin was further confirmed by the FTIR analysis. The spectra of Merrifield resin (MR) and PEGylated Merrifield resin (PEG-MR) are shown in Figure 3.1. Disappearance of the C-Cl band at 1264 cm^{-1} and appearance of the broad absorptions at the O-H stretching (3200-3500cm^{-1}) and the C-O stretching regions (1030-1100 cm^{-1}) were observed in the spectrum of the PEG-MR. Moreover, the increase absorption at the C-H stretching region (2850-2910 cm^{-1}) confirmed the attachment of the PEG to the MR. Due to the condensed phase of PEGylated MR, hydrogen bonding of the hydroxyl groups is enhanced, which lowers and broadens the stretching frequencies of the participating O-H bonds.

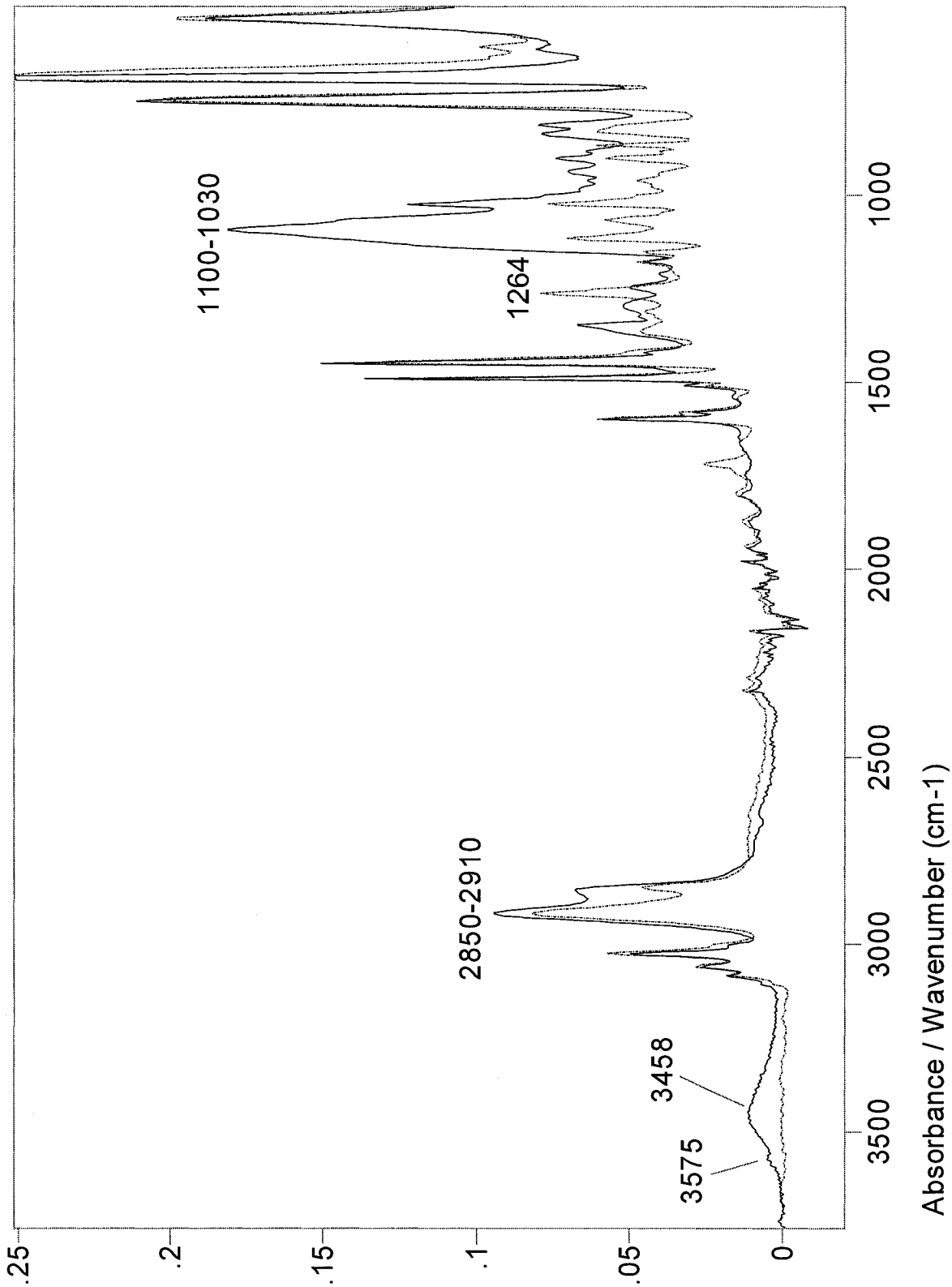


Figure 3.1 FTIR spectra of the Merrifield resin 2% cross-linked (1.0-1.5 mmol Cl/g) (------); Poly(ethylene glycol) (MW 200) modified Merrifield resin (———)

CHAPTER 4

CONFIRMATION OF THE CHEMICAL REACTIVITY OF THE PEGYLATED MERRIFIELD RESIN

4.1. Introduction

A microwave-assisted synthesize of the PEGylated Merrifield resin was reported in chapter 3. In view of the development and application of PEG-MR in the field of solid phase organic synthesis and in polymer supported reagents, we were particularly interested in investigating its chemical reactivity. In the present study, the chemical availability of the free hydroxyl end of the hybrid polymer was confirmed through esterification reaction using pyruvic acid (Scheme 4.1) and through substitution reaction using thionyl chloride (Scheme 4.2), thus converting the hydroxyl end into more reactive halide moiety.

4.2. Material and Methods

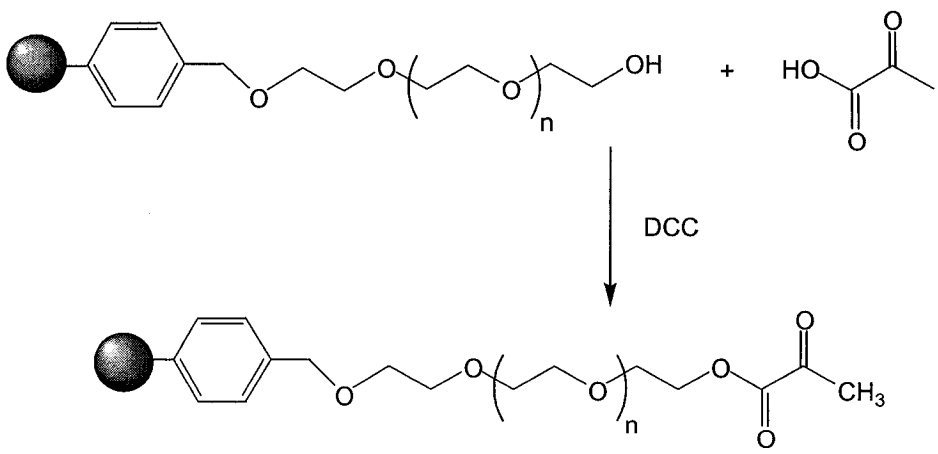
4.2.1. Reagents and Chemicals

PEGylated Merrifield resin (PEG_{200}MR2%(1.25)) was synthesized as described before. Thionyl chloride, pyruvic acid, and 1,3-dicyclohexylcarbodimide were purchased from Aldrich Chemical Company (Milwaukee, WI). Tetrahydrofuran, dichloromethane,

ethanol and methanol were purchased from Caledon Laboratories Limited (Georgetown, ON).

4.2.2. Attachment of pyruvic acid to PEG-MR

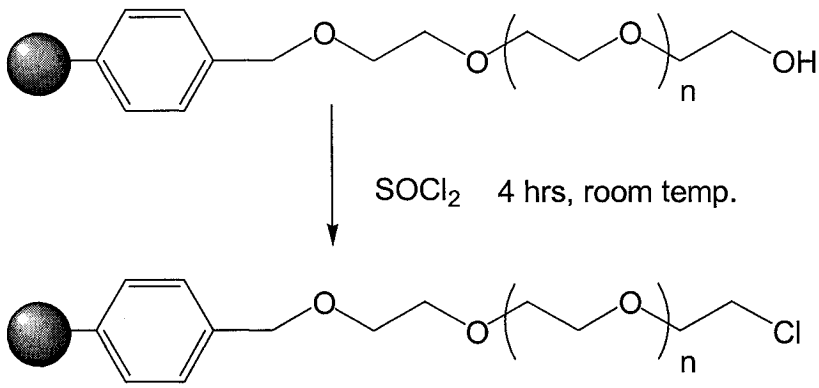
PEG-MR (1.30 ± 0.02g) and pyruvic acid (0.75μL) were suspended in dichloromethane (10mL). 1,3-Dicyclohexylcarbodimide (DCC) (0.207g) was added to the solution. The mixture was stirred at room temperature (~20°C) for 24 h. A white precipitate – dicyclohexylurea (DCU) was formed. Additional pyruvic acid (15μL) and DCC (0.04g, 0.25 mmol) were then added. The stirring continued for approximately 8 more hours to complete the reaction. The precipitated DCU and the product were filtered and washed with dimethyl sulfoxide (DMSO) to remove DCU, followed by methanol, water and methanol and was allowed to dry.



Scheme 4.1 Esterification of PEGylated Merrifield resin with pyruvic acid

4.2.3. Chlorination of the PEG-MR

PEG-MR (0.50 ± 0.02g) was suspended in 10 times excess amount of thionyl chloride ($SOCl_2$), stirred magnetically at room temperature for 4 hours. The product was filtered and washed with DCM and methanol.



Scheme 4.2 Substitution of the hydroxyl group of PEG using thinoyl chloride

4.2.4. FTIR analysis

Infrared spectra were recorded with Merlin software on a Bio-Rad Excalibur Series FT-IR spectrometer (Bio-Rad, Cambridge, MA, USA) purged with dry air. The spectra of resin in dry state were acquired on a Golden Gate Single Reflection Diamond ATR. A total of 128 scans at 4 cm^{-1} resolution were co-added for the modification studies. Processing of the FTIR data was performed using GRAMS/32 AI version 6.01.

4.2.5. Elemental analysis

Elemental analyses were performed by Guelph Chemical Laboratories Ltd. (Guelph ON, Canada). Data reported are the average of duplicate measurements.

4.3. Results and Discussion

Having confirmed that the PEG has been attached to the MR in previous chapter, the chemical reactivity of the free hydroxyl group on the PEGylated MR was investigated. The amount of pyruvic acid attached to the PEGylated MR and the amount of hydroxyl group converted to chloride ion was estimated by the measured weight gain, elemental analysis and by FTIR analysis.

4.3.1. Esterification of PEGylated MR with pyruvic acid

The availability and chemical reactivity of hydroxyl group on PEGylated Merrifield resin was verified by the esterification reaction with pyruvic acid. The % yield obtained was 96.6 % with a loading of 0.217mmole of pyruvic acid/g of resin. The FTIR spectra (Figure 4.1) show the formation of resin bound ester carbonyl group in the product at 1732 cm^{-1} and the keto group at 1750 cm^{-1}, confirmed the successful esterification of pyruvic acid with the PEGMR.

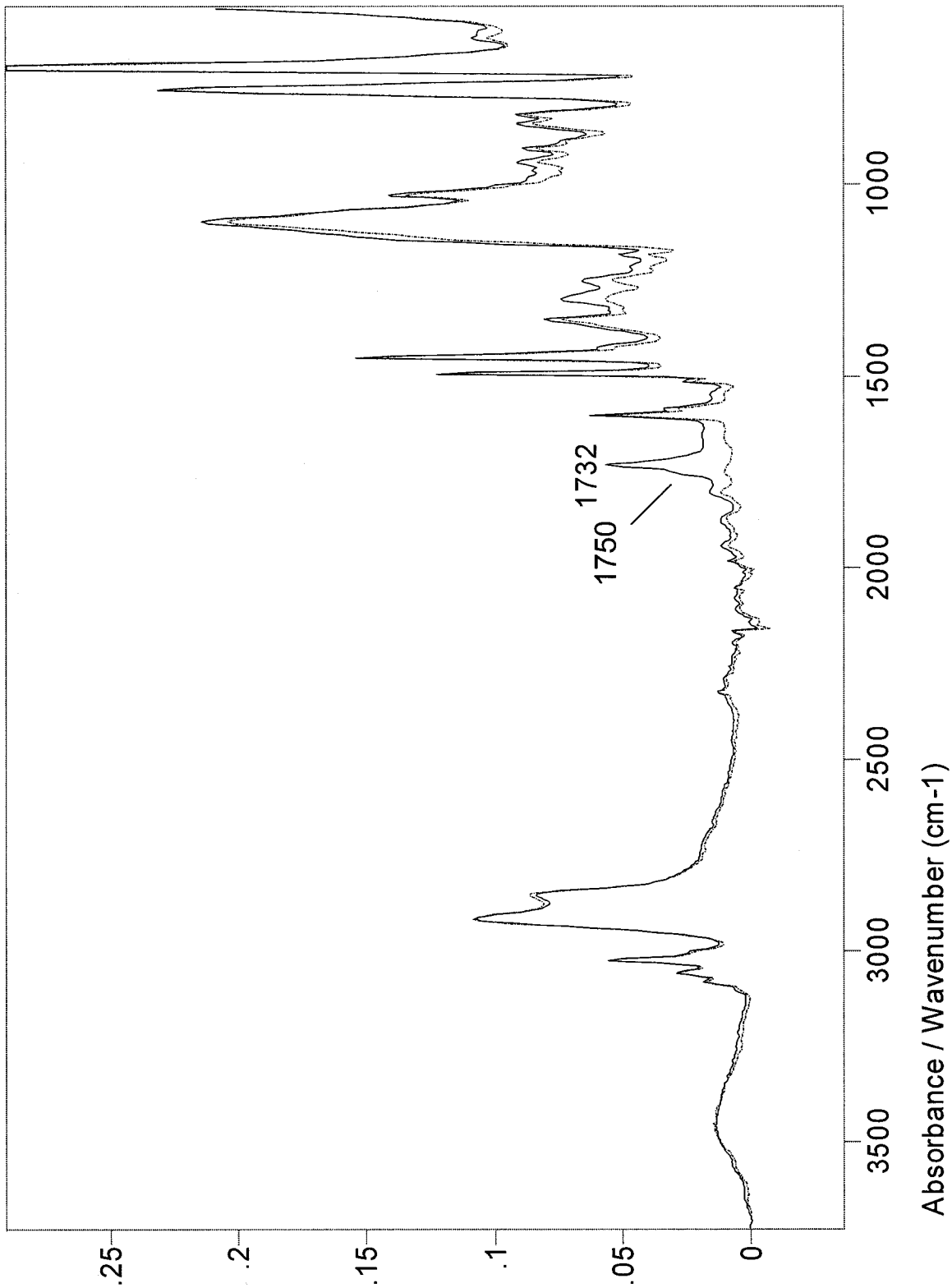


Figure 4.1 FTIR spectra of $PEG_{200}MR2\%$ (--------) and pyruvic acid-$PEG_{200}MR2\%$ (———).

4.3.2. Chlorination of PEGylated MR using thionyl chloride

Substitution of the hydroxyl group on the PEGylated MR was performed by reacting the resin with thionyl chloride. The chloride moiety is more reactive than the hydroxyl group and can react readily with other nucleophiles. There was no significant weight gained (<0.02g) in the product, and even weight lost was observed. The FTIR analysis of the product (see Figure 4.2) indicates the appearance of the typical CH_2-Cl wagging vibration band at 1264 cm^{-1} and the decrease in the intensity of the OH band at 3300-3500 cm^{-1}, hence validating the substitution of the hydroxyl group with chloride on the PEGylated MR. The results from elemental analysis (Table 4.1) also confirmed that there was 1.79 % chloride weight gained in the product, which correspond to a loading of 0.505 mmol Cl/ g resin.

Table 4.1 Elemental analysis of chlorinated PEGylated MR

	%O	%Cl	Yield $_{Calcd.}$	Yield $_{obs.}$	Loading (mmol Cl/ g resin)
Peg-MR	6.34	1.67			
Cl-Peg-MR	2.31 [a]	3.46 [b]	0.525[c]	0.521	0.505

[a] Total mmol O lost/g resin = %O $_{in\ MR}$ - %O $_{in\ Cl\text{-}PEG\text{-}MR}$/ molecular weight of O (2.519 mmol O/ g resin);

[b] mmol Cl gained/g resin = %Cl $_{in\ Cl\text{-}PE\text{-}MR}$ - %Cl $_{in\ MR}$/ molecular weight of Cl (0.505 mmol Cl/ g resin), this in theory should be same as mmol O lost due to leaving of OH group, as a result mmol O lost due to PEG degradation = total mmol O lost – mmol O lost from OH;

[c] Yield $_{calcd.}$ = PEG-MR + (g of Cl $_{gained}$ – g of O $_{lost\ from\ OH}$ – g O $_{lost\ due\ to\ PEG\ degradation}$)

On the other hand, weight lost observed in the final product can be explained by the degradation of PEG during the reaction. Thionyl chloride converts the alcohols to alkyl

chlorides plus producing hydrochloric acid (pH<2) as side product. The highly acidic medium may therefore lead to the cleavage of some PEG chains. Based on this hypothesis, the yield calculated (0.525g) from elemental analysis matched up closely with the yield observed (0.521g). This conclusion was corroborated by the FTIR analysis, where the band at 1100-1030 cm^{-1} from the ether bonds in PEG diminished in intensity.

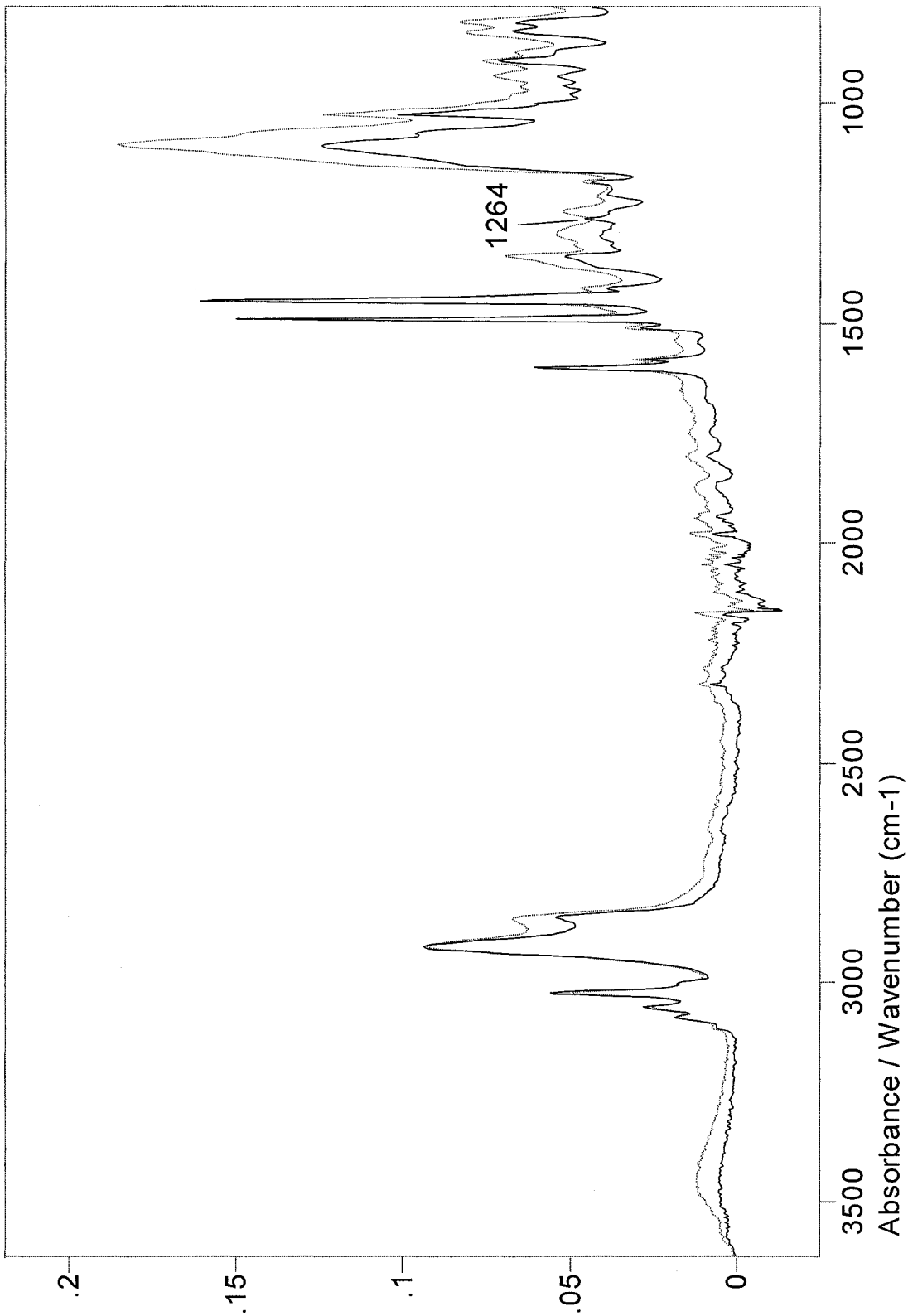


Figure 4.2 FTIR spectra of $PEG_{200}MR2\%$ (--------) and Cl-$PEG_{200}MR2\%$ (———).

CHAPTER 5

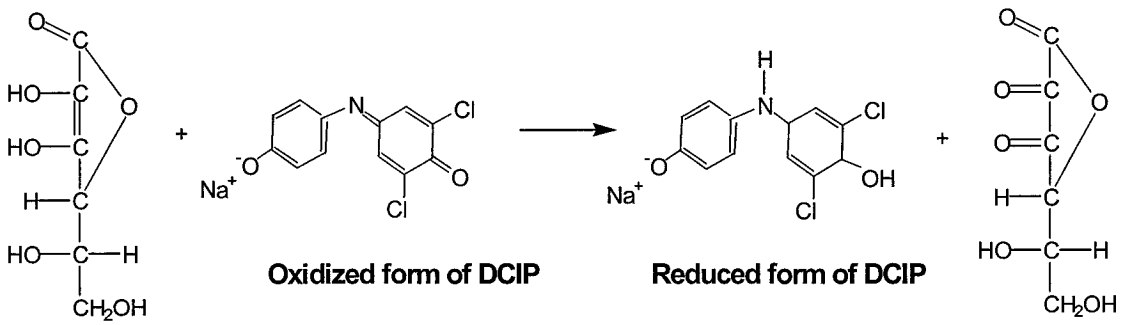
APPLICATION OF PEGYLATED MERRIFIELD RESIN I IMMOBILIZED 2,6-DICHLOROINDOPHENOL

5.1. Introduction

Due to the current interest in polymer supported reagents that offer convenient analytical and work-up procedures, the thionyl chloride activated hybrid resin was further used to immobilize 2,6-dichlorophenolindophenol (DCIP; Tellman's reagent) - a dye that has been used as a pH and redox indicators for various applications. It is blue in alkaline solution and red in acidic and can be reduced to a colorless form. One of the most important uses of DCIP is for the chemical analysis of ascorbic acid (vitamin C) using a titrametric method, in which the dye is reduced by ascorbic acid (Scheme 5.1) and the end point of the titration is indicated by the appearance of a faint color. Previously, Goodlet and Narayanaswamy (1994) reported the non-covalent immobilization of DCIP on amberlite XAD-4 resin and its successful application as an optical fibre sensor for the analysis of vitamin C in orange juice. It was also stated that the immobilized indicator must be kept in a buffer at pH 6 to avoid desorption from the resin which was observed at values above pH 7.

In this chapter we report the covalent attachment of DCIP on chlorinated PEGylated MR using a microwave assisted process. The use of insoluble polymer supported DCIP

indicator has the advantages of a typical polymeric reagent: ease of operation and reaction work up. Moreover, the immobilized DCIP can be regenerated and reused.



Scheme 5.1 Oxidation/ Reduction reaction of DCIP with ascorbic acid

5.2. Materials and Methods

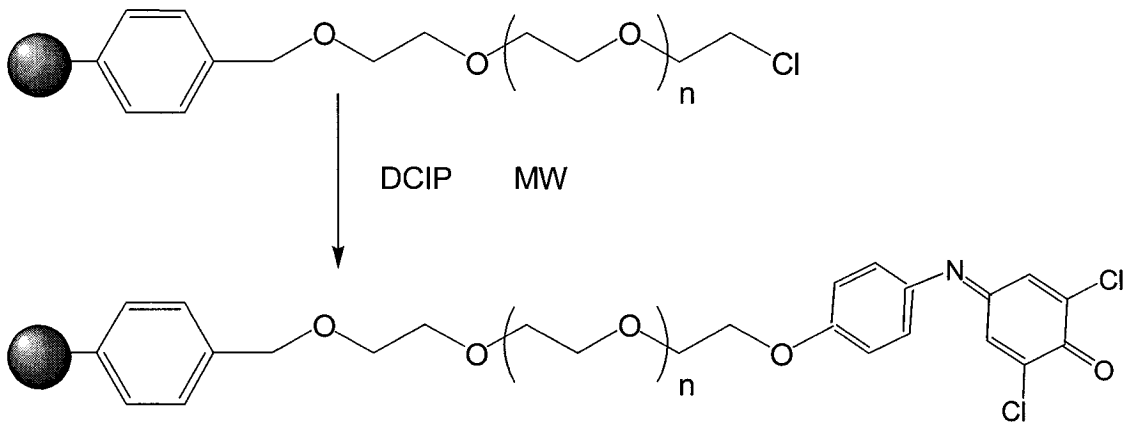
5.2.1. Reagents and Chemicals

PEGylated Merrifield resin ($PEG_{200}MR2\%(1.25)$) was synthesized as described before. Sodium 1,6-dichloroindophenol (DCIP) hydrate (ACS reagent) and L-ascorbic acid was purchased from Aldrich Chemical Company (Milwaukee, WI). Methanol and acetonitrile were purchased from Caledon Laboratories Limited (Georgetown, ON).

5.2.2. Attachment of DCIP to PEGylated Merrifield resin (Scheme 5.2)

PEGylated Merrifield resin (0.25 g ± 0.02 g) was suspended in acetonitrile (10 ml) and DCIP sodium salt (1 mg) was added. The mixture was irradiated at 300 W

(Synthewave 402, Prolabo, France) for 5min. The product was filtered and washed with methanol and water until no further discoloration was observed.



Scheme 5.2 Microwave assisted immobilization of DCIP to thionyl chloride activated PEGylated MR.

5.2.3. Detection of vitamin C using the PEGylated MR immobilized DCIP

The reactivity of the resin bound DCIP was tested. Resin bound DCIP (~0.10g) was packed in a column made from a pasteur pipette. The resin was swelled in DCM. Vitamin C (0.01mmole) solution was prepared in methanol and water (1:1) and was passed through the column. Any color changes of the resin was recorded.

5.2.4. FTIR analysis

Infrared spectra were recorded with Merlin software on a Bio-Rad Excalibur Series FT-IR spectrometer (Bio-Rad, Cambridge, MA, USA) purged with dry air. The spectra of

resin in dry state were acquired on a Golden Gate Single Reflection Diamond ATR. A total of 128 scans at 4 cm^{-1} resolution were co-added for the modification studies. Processing of the FTIR data was performed using GRAMS/32 AI version 6.01.

5.3. Results and Discussion

A fast and convenient method for the immobilization of DCIP on PEGylated MR was developed using the microwave assisted process. The resulting PEGylated MR with bound DCIP remained dark blue even after continuous washing with methanol and water. This indicated the successful attachment of DCIP to the resin, as the starting resin is off-white to light yellowish in color (Figure 5.1). It was observed that by attachment to the support, the odor of DCIP was eliminated. The chemical reactivity of the resin bound DCIP was tested using vitamin C. The resin changed from dark blue to light yellowish upon reaction with vitamin C as shown in Figure 5.1.

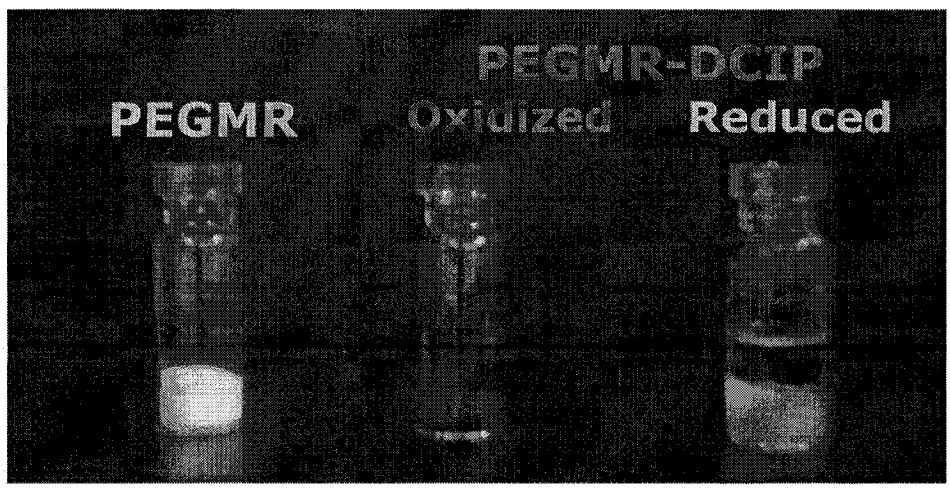


Figure 5.1 Reactivity confirmation of the resin bound DCIP demonstrated by the color changes

5.3.1. FTIR analysis of the resin bound DCIP

The FTIR spectrum of the immobilized DCIP was compared with that of thionyl chloride activated PEGylated MR and the spectra are shown in figure 5.2. Due to the overlapping bands, it was difficult to distinguish between the reactant and product without spectral subtraction. Figure 5.3 shows the subtraction (1:1 ratio) of the two spectra. The characteristic features of DCIP can be seen, such as benzene ring breathing at 685-760 cm^{-1}, 1030-1100 cm^{-1} C-Cl (aromatic), 1430-1470 cm^{-1} and 910 cm^{-1} correspond to the $-CH=CH_2$ and a weak carbon-nitrogen double bond stretches at 1590-1610 cm^{-1}.

5.3.2. Determination of the loading of resin bound DCIP

In theory, for each mole of DCIP attached to Cl-PEG-MR, there will be a net gain of 2 mole of O and 1 mole of Cl. Results from the elemental analysis (Table 5.1) indicated that, based on the % weight Cl gain in resin bound DCIP, 1.41×10^{-2} mmol DCIP/g resin was detected. However, higher molar equivalent of O was found. The possible reason for that may be the absorption of moisture.

Table 5.1 Chloride and Oxygen analysis of Cl- PEGMR and PEGMR bound DCIP

	%Cl	%O	Yield $_{calcd.}$	Yield $_{obs.}$	Loading (mmol DCIP/g resin)
Cl-PEG-MR	3.46	2.31			
DCIP-PEG-MR	3.51	2.51	0.381 [a]	0.383	1.41×10^{-2}

[a] Yield $_{Calcd.}$ = Cl-PEG-MR + DCIP $_{in\ g\ calculated\ from\ \%\ Cl\ gained}$

Finally, stability studies (three months of storage in the dry state at 4 °C) have indicated that polymer bound DCIP was not stable under storage conditions.

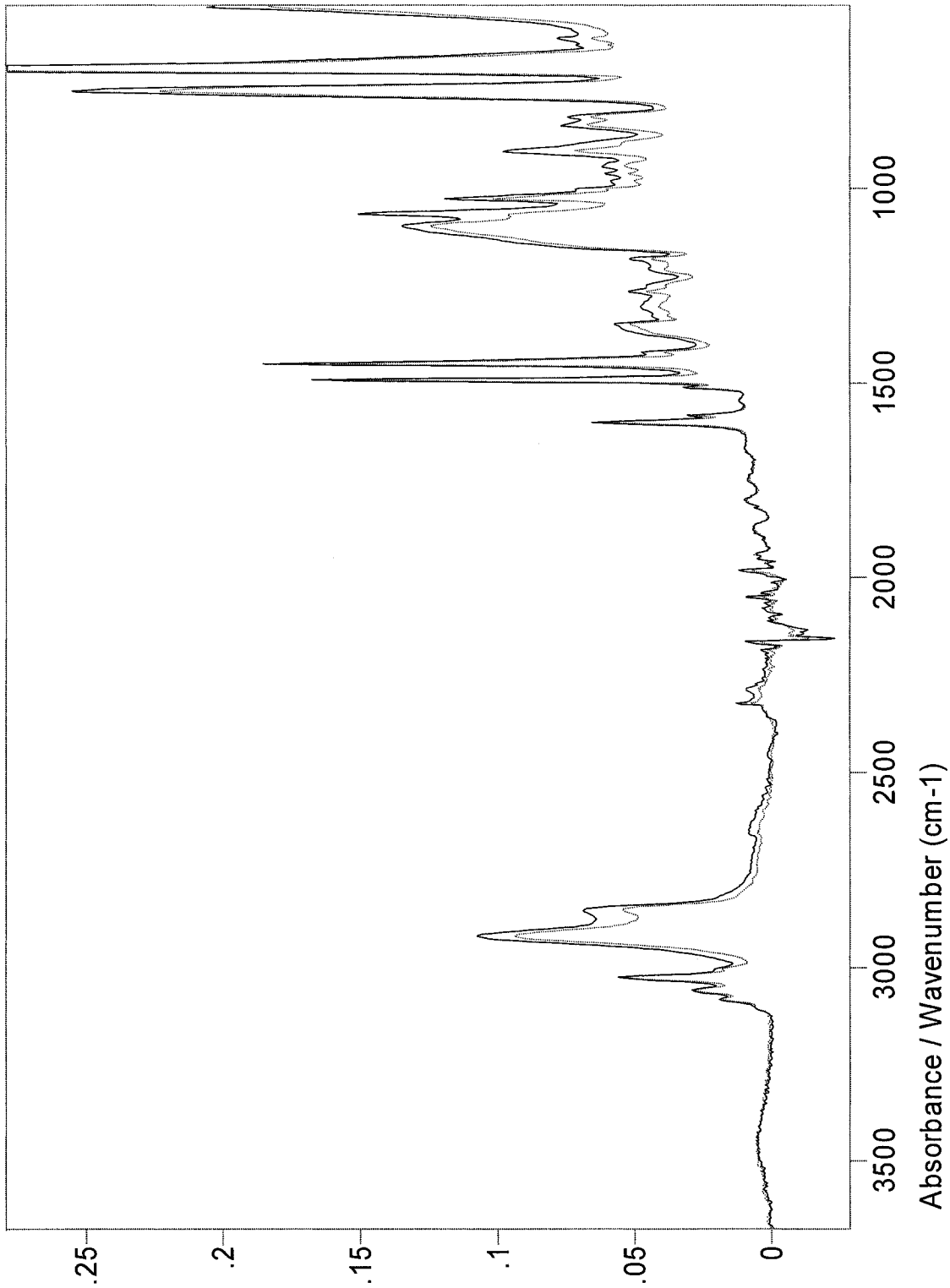


Figure 5.2 FTIR spectra chlorinated PEGylated MR (--------) and PEGylated MR with bound DCIP (———).

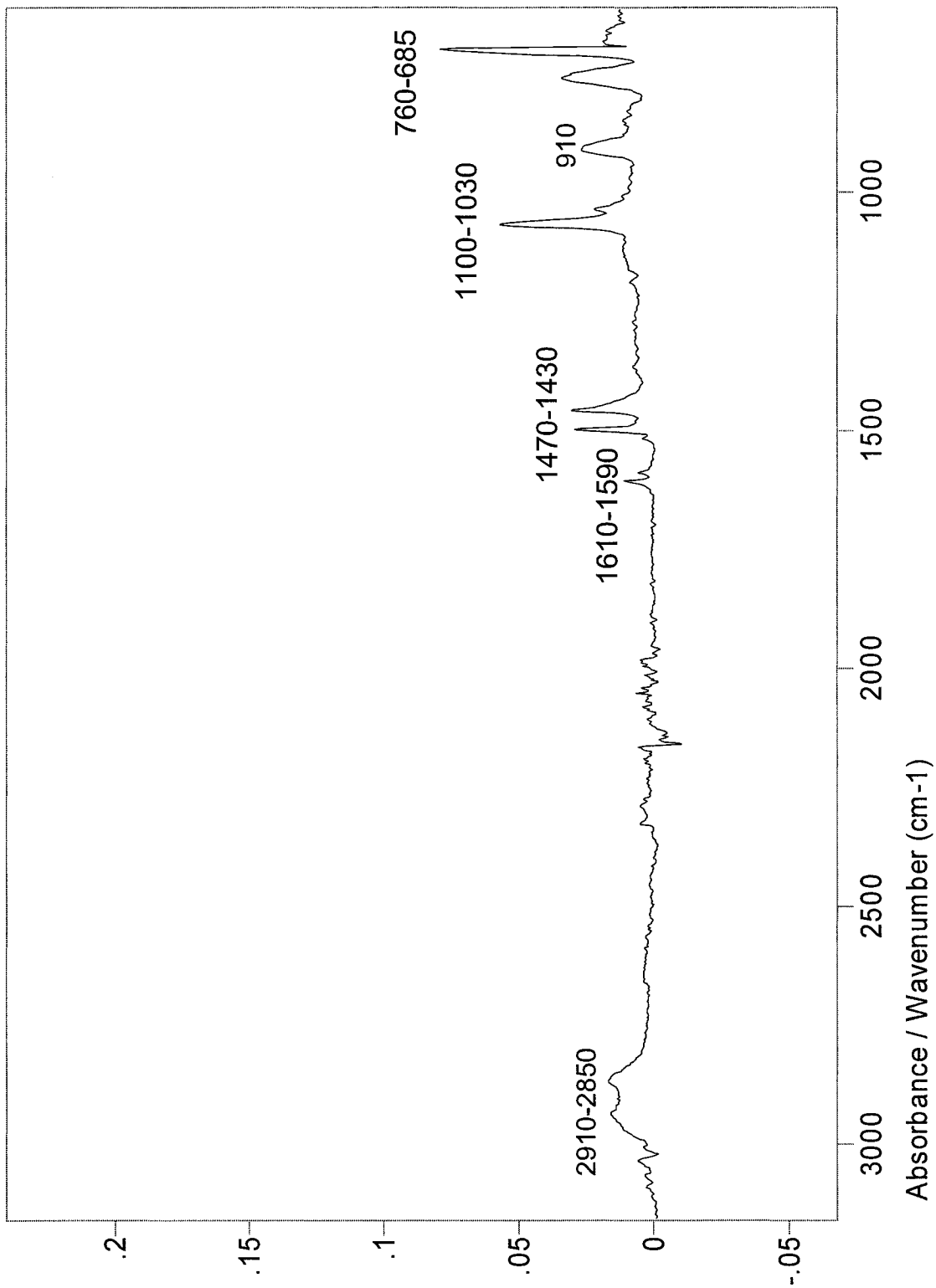


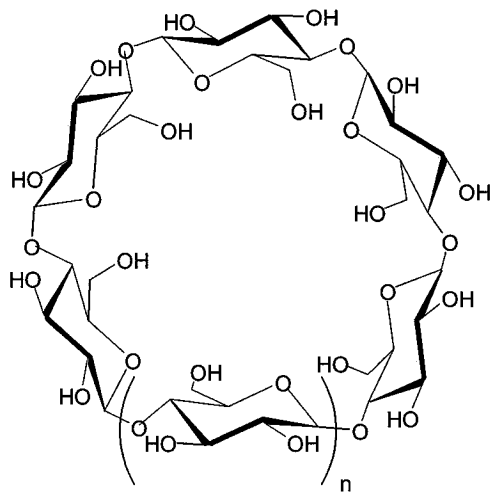
Figure 5.3 Spectrum of the subtraction of chlorinated PEGylated MR from PEGylated MR with bound DCIP.

CHAPTER 6

APPLICATION OF PEGYLATED MERRIFIELD RESIN II β-CYCLODEXTRIN TRAP

6.1. Introduction

Cyclodextrins are cyclic oligosaccharides composed of six to eight (1-4)-linked α-D-glucopyranosyl units. They are the most commonly used molecules which form host-guest type inclusion complexes with various organic molecules with suitable geometry and function. Applications for cyclodextrins are sought in various areas of chemistry, such as in chromatography, for encapsulation of drugs and flavours and for purification of organic compounds. The conical shape of these molecules results in well-defined apolar central cavities. In an aqueous solution, these cavities are occupied by water molecules which are unfavored by the polar-apolar interaction, and therefore can be readily substituted by appropriate "guest molecules" which are less polar than water. While CDs are highly water soluble, in order to facilitate their removal from the mixture and their regeneration, they must be processed into insoluble forms before they can be implemented into practical separation tools. One of the current interests is to incorporate the cyclodextrins molecules into some existing polymers. This chapter describes the use of PEGylated Merrifield resin as the insoluble polymer to immobilize β-cyclodextrin or polymerized β-cyclodextrin using 1,6-hexamethylene diisocyante (HMDI) as a linker.



n=1 α-CD
n=2 β-CD
n=3 γ-CD

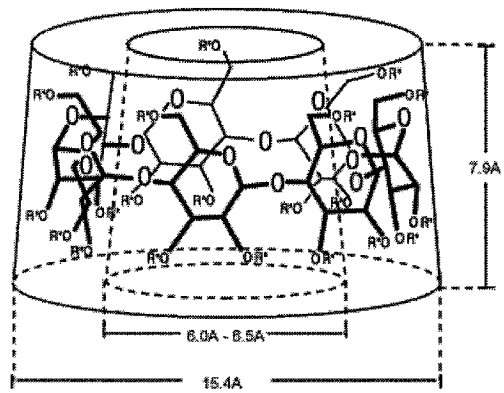


Figure 6.1 Cyclodextrin molecules

6.2. Materials and Methods

6.2.1. Reagents and Chemicals

PEGylated Merrifield resin [PEG_{200}MR2%(1.25)] was synthesized as described in chapter 2. β-Cyclodextrin (CD), epichlorohydrin (EP), 1,6-hexamethylene diisocyante (HMDI), tin (II) 2-ethylhexanoate and dimethylformide were purchased from Aldrich Chemical Company (Milwaukee, WI). Polymerized β-cyclodextrin was synthesized following the procedure of Renard et al. (1997).

6.2.2. Preparation of water soluble β-cyclodextrin-epichlorohydrin polymer

The procedure for the EP catalyzed polymerization of CD using a molar ratio of EP/β-CD of 5 was obtained from Renard et al. (1997). A mixture of β-CD (10 g) in 16 mL of NaOH solution was mechanically stirred (Eurostar, IKA work Inc.) overnight at room temperature. The mixture was heated at 30°C and EP was added (3.44 mL) rapidly. The temperature was kept at 30°C during polymerization. The stirring was kept constant during reaction. The reaction was stopped after 3hr by addition of acetone. After decantation, acetone was removed. The pH of the aqueous solution was decreased to 12 with 6N hydrochloric acid. The solution obtained was kept at 50°C overnight. After cooling, the solution was neutralized with 6N HCl and diafiltered (Slide-a-lyzer dialysis cassettes, molecular weight cut-off 3500-10000, Pierce, Rockford, IL). The solution obtained was evaporated and the solid triturated with acetone. Structures of polymerized CD with EP (polyCD-EP) are shown in Figure 6.2.

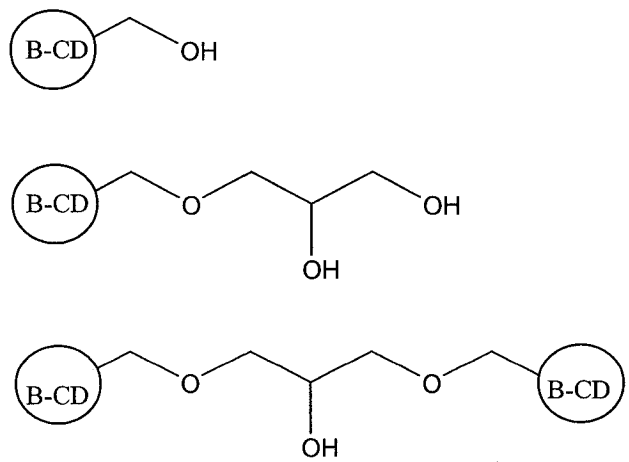
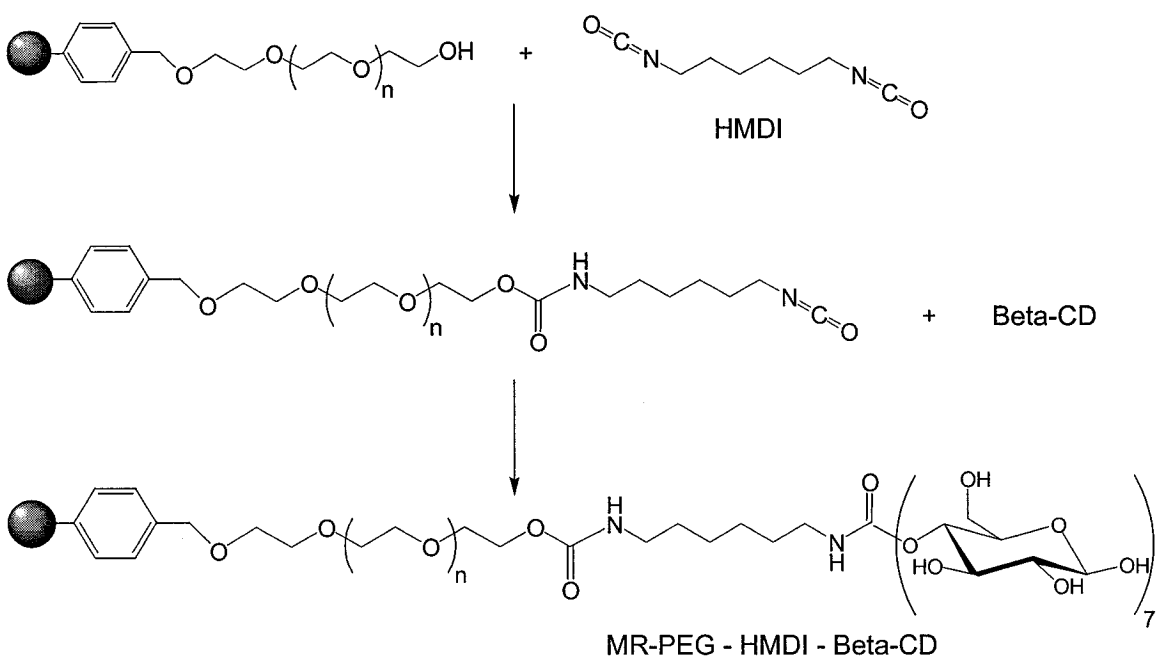


Figure 6.2 Suggested molecular structures of β-CD-EP polymer obtained from the polymerization reaction (For simplicity, only reactions on primary alcohols are shown.)

6.2.3. Immobilization of β-cyclodextrin or polymerized β-cyclodextrin on PEGylated Merrifield resin

In a typical experiment 1.00g ± 0.05g of PEGylated Merrifield resin was suspended in 10 mL of 10% (v/v) HMDI solution in toluene with a few drops of Tin (II) 2-ethlyhexanoate. The mixture was stirred at room temperature for 2 hours and the supernatant was removed by pipetting. The resin was washed several times with toluene and was dried under nitrogen. A 10 mL of 10% (w/v) β-cyclodextrin or polymerized β-cyclodextrin solution in DMF and a few drops of Tin (II) 2-ethlyhexanoate were added to the resin. The mixture was stirred overnight and the supernatant was removed by pipetting. The product was filtered and washed with DMF, water, ethanol and water again and was dried under nitrogen.



Scheme 6.1 Immobilization of β-Cyclodextrin on PEGylated Merrifield resin

6.2.4. Trapping of vanillin using immobilized β-cyclodextrin

Vanillin was used as a model compound for trapping experiments. Immobilized β-cyclodextrin or polymerized β-cyclodextrin (0.30 ± 0.02 g) was suspended in 3 mL of 5 ppm vanillin solution (in de-ionized water), stirred magnetically at low speed for 30-60 min. The resin was filtered and washed with de-ionized water.

6.2.5. Pyrolysis-GC/MS desorption of vanillin from the β-cyclodextrin trap

Py-GC/MS analysis was performed on a CDS Pyroprobe 2000 unit equipped with a CDS 1500 valved interface (CDS analytical, Oxford, USA), coupled to a Varian Gas chromatograph CP-3800 / ion trap mass spectrometer Saturan 2000 (Varian, Walnut Creek, USA). The separations were carried out on a DB5-MS column (5% diphenyl, 95% dimethyl-polysiloxane) with a dimension of 50m x 0.2mm i.d. x 0.33 mm film thickness (J&W Scientific, Folson, CA). The sample (~3mg) was packed in a quartz tube and was plugged with quartz wool on both ends. The total pyrolysis heating time was 20s and the desorption temperature was at 250°C. The volatile products generated by the sample were trapped in a sample pre-concentration trap (SPT; Tenax) for 4 min at room temperature and subsequently were desorbed by heating SPT at 250°C into the GC column with Helium as the carrier gas. The GC column flow rate was initially set at 70psi with a split ratio of (15:1) for the first 6 mins. Then, the GC column flow rate was set at 1.5 ml/min constant flow with a split ratio of 100:1 for the rest of the run. The column initial temperature was 50°C for 4 mins and was increased to 250°C at a rate of

20°C/min and kept at 250°C for 8 min. The Mass Spectrometer transfer line temperature was 250°C and the ion trap temperature was 175°C. The ionization voltage was 70eV and the electron multiplier was 1700V.

6.2.6. Elemental analysis

Elemental analysis was performed by Guelph Chemical Laboratories Ltd. (Guelph ON, Canada). Data reported are the average of duplicate measurements.

6.3. Results and Discussions

6.3.1 Immobilization of β-cyclodextrin and polymerized β-cyclodextrin

β-Cyclodextrin and polymerized β-cyclodextrin were both successfully immobilized on the PEGylated Merrifield resin using 1,6-hexamethylene diisocyanate (HMDI) as a linker. The isocyanate functional group is sufficiently reactive and has been used in many applications as useful linker for the attachment of small molecules to the solid support or as scavenger for nucleophiles. The absorption at 1716 cm^{-1} showed in the IR spectra (Figure 6.2) indicates the presence of O-C=O and confirmed the attachment of the linker to the resin. The increase absorption at O-H stretching regions (3330-3400 cm^{-1}) proved the attachment β-cyclodextrin moiety. However, the presence of the isocyante group (N=C=O) at 2270 cm^{-1} suggested that there are remaining reactive sites i.e. reaction did not go to completion.

We have investigated the polymerized CD with EP (polyCD-EP) in addition to CD, for the purpose of increasing the CD content per mole of the resin. As indicated from the elemental analysis (Table 6.1), higher loading of CD per g resin was obtained in polyCD-HMDI-PEGMR than in CD-HMDI-PEGMR. Moreover, the yield calculated from the elemental analysis agrees with the yield observed.

Table 6.1 Elemental analysis of PEGylated MR and Resin bound β-CD and poly β-CD

	%Cl	%O	% N	Yield $_{Calcd.}$ [a]	Yield $_{Obs}$	Loading (mmole β- CD/g resin)
PEG-MR	1.69	5.85				
CD-HMDI-PEG-MR		8.34	1.79	1.145	1.146	7.95×10^{-3}
polyCD-HMDI-PEG-MR		9.28	1.70	1.140	1.133	2.55×10^{-2} [b]

[a] Yield $_{Calculated}$ = PEG-MR + weight linker $_{based\ on\ \%N}$ + weight of CD/ CD-EP $_{based\ on\ \%O\ -\%N}$;

[b] Calculated with the assumption that the polyCD-EP contains 4 CD units and has an average MW of 5000

6.3.2. Trapping ability of the insoluble resin bound β-CD and poly β-CD-EP

Due to the well know property of β-cyclodextrin to form inclusion complexes with various compounds, the trapping ability of the resulting insoluble resin bound β-cyclodextrin was tested using vanillin as a model. As described under experimental conditions, thermal desorbtion of vanillin was performed using pyroysis-GC/MS. The data have indicated presence of vanillin in both samples. Current interest is to optimize the loading of β-cyclodextrin on the resin and to implement this insoluble resin bound β-cyclodextrin in trapping of environmental pollutants in rivers and lakes.

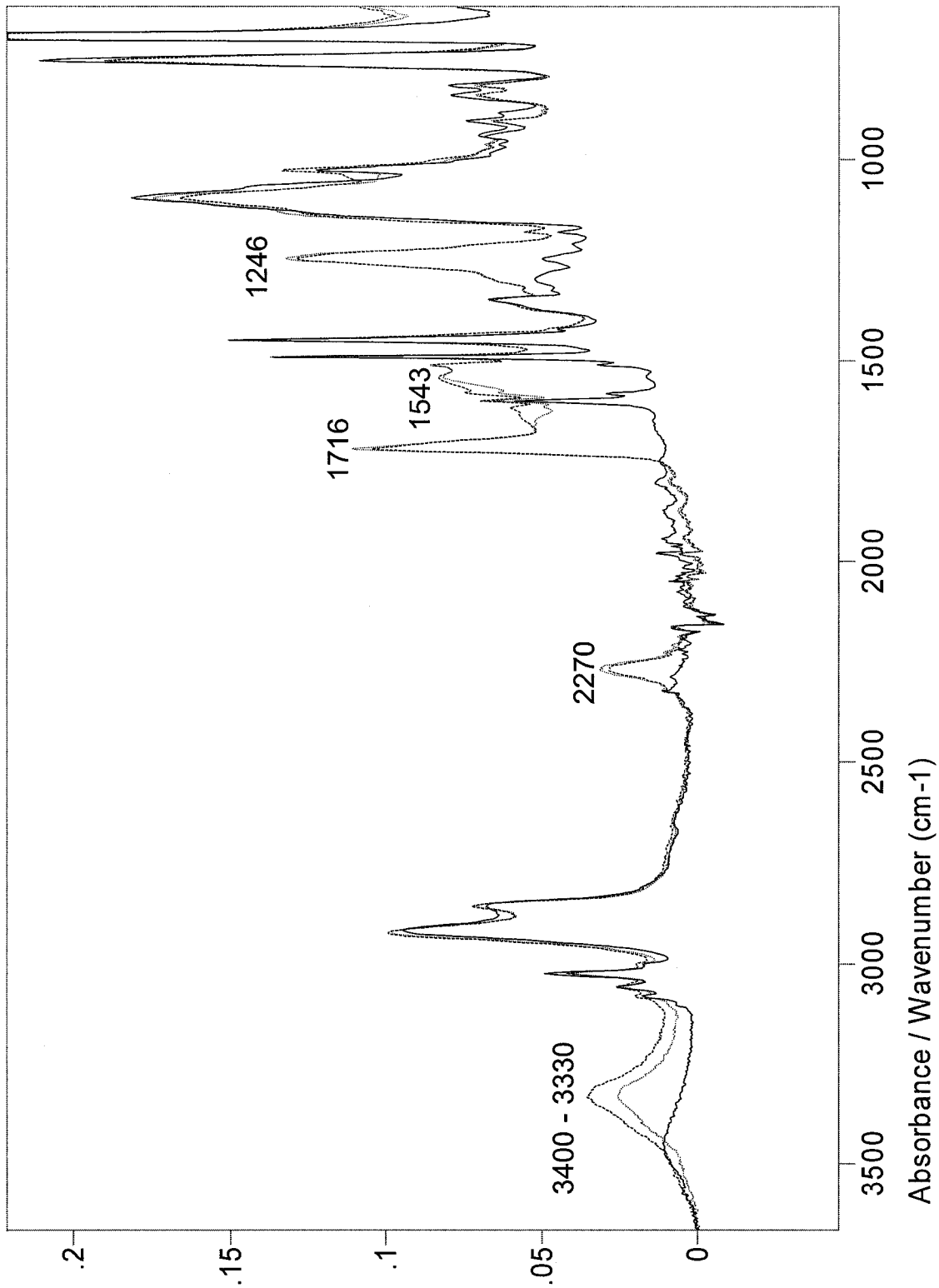


Figure 6.2 FTIR spectra of PEG200MR2% (———); β-cyclodextrin-PEG200MR2% (- - - - - -) and polymerized β-cyclodextrin-PEG200MR2% (·····)

CHAPTER 7

GENERAL CONCLUSIONS

Microwave-assisted process (MAP) has proven to be a major enabling technique for many chemical applications requiring rapid processing and efficient energy use, particularly in the pharmaceutical/combinatorial chemistry field. This work has clearly demonstrated that microwave-assisted synthesis of PEGylated Merrifield resin saves significant time and improves yields with lower product degradation as compared to traditional approach of synthesis. By modulating the microwave power, it was possible to reduce the degradative side reactions occurring during synthesis. The results have also shown that microwave energy allows for higher % PEGylation i.e. resin substitution, and increases the scaling-up ability. In consideration of bringing the synthesized PEGylated MR into practical uses, its stability with respect to temperature and pH was investigated. The resin was found to be stable over the pH 3-10 and with temperature reaching 92°C under microwave irradiation. The resin also shows improved swelling properties in polar solvents.

The chemical reactivity of the PEGylated MR was confirmed by the esterification of pyruvic acid with PEGylated MR and through substitution reaction using thionyl chloride, thus converting the hydroxyl end into more reactive halide moiety.

In addition, two applications of the PEGylated MR were studied in this work. First being the microwave assisted immobilization of the blue organic dye – 2,6-dichloroindophenol (DCIP) through a nucleophilic substitution reaction which resulted in a blue pigmented resin. The polymer-supported DCIP combines the advantages of a polymer-supported reagent with the properties of DCIP that is as acid/base or redox indicator. The reduced form of the reagent is easily separated by simple filtration and can be regenerated by oxidation for repeated use. The second application introduced the immobilization of β-cyclodextrin through cross-linking with 1,6-hexamethylene diisocyante reagent on the PEGylated MR to form a water insoluble inclusion-complex. The trapping ability of this immobilized β-cyclodextrin was not studied to great extend, but its ability to trap vanillin, as an example, was confirmed by the pyrolysis/GC/MS. Structural elucidation of the resins synthesized using FTIR analysis has proven to be a fast and convenient technique for characterizing functional groups changes occurred on solid support.

Further research is needed to fully characterize the synthesized functionalized resins and generate application data for their commercial exploitation.

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