CHAPTER IV

TETRAETHYLENEGLYCOL DIACRYLATE (TTEGDA)-CROSSLINKED POLYSTYRENE: A NEW POLYMERIC SUPPORT FOR PEPTIDE SYNTHESIS

The chemical nature and topographical structure of the polymer matrix are the two important factors which determine the physicochemical properties that render a polymer support favourable for peptide synthesis. The main aim of the current study is the search for monomers which give a polymer which interacts with a broad spectrum of solvents and the development of reproducible procedures for obtaining beaded resins. The topography of the polymer matrix is determined by the chemical nature of monomers and the mole percentage of crosslinking agent. The crosslinking provides the desired mechanical integrity for the resin.

Results and Discussion

IV.1. Polymer Synthesis

For the preparation of the solid support,
solution polymerization was employed initially. Styrene and tetraethyleneglycol diacrylate (TTEGDA) were freed from inhibitors by washing with sodium hydroxide solution or distillation under vacuum. On heating a mixture of monomers in methanol/chloroform at 80°C in the presence of benzoyl peroxide gelation occurs at the initial formation of an infinite network when the molecular weight of the macromolecule becomes effectively infinity. At this stage the mixture loses fluidity although some linear polymer chains and considerable amount of both monomers remain. The mixture was heated for some more time with addition of excess benzene. The product resin was obtained in an easily filterable form and washed free of monomers and linear polymer to obtain the amorphous insoluble polymer support.

Suspension polymerization has been proved to be the most useful technique for synthesising crosslinked polymeric supports, principally because of the extremely convenient physical form of the beaded product which lends itself to further conversions. Polystyrene with 2,3,4,5,8,10 and 20 mole percent of TTEGDA were prepared by aqueous suspension of monomers and benzene as diluent at 80°C using benzoyl peroxide as initiator (Scheme IV.1).
Scheme IV.1 Preparation of TTEGDA-Crosslinked Polystyrene Support
Table IV.1. Preparation of Tetraethyleneglycol Diacrylate-Crosslinked Polystyrene by Suspension Polymerization

<table>
<thead>
<tr>
<th>Mole % cross-linking agent in the feed</th>
<th>Weight of Styrene (g)</th>
<th>Weight of TTEGDA (g)</th>
<th>Yield of Polymer (g)</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25.5</td>
<td>1.5</td>
<td>20.5</td>
<td>75.90</td>
</tr>
<tr>
<td>3</td>
<td>20.17</td>
<td>1.89</td>
<td>17.0</td>
<td>77.07</td>
</tr>
<tr>
<td>4</td>
<td>33.32</td>
<td>4.02</td>
<td>31.70</td>
<td>85.63</td>
</tr>
<tr>
<td>5</td>
<td>32.99</td>
<td>4.04</td>
<td>30.40</td>
<td>79.95</td>
</tr>
<tr>
<td>8</td>
<td>9.58</td>
<td>2.33</td>
<td>9.8</td>
<td>82.27</td>
</tr>
<tr>
<td>10</td>
<td>9.37</td>
<td>3.02</td>
<td>10.0</td>
<td>80.60</td>
</tr>
<tr>
<td>20</td>
<td>8.32</td>
<td>5.99</td>
<td>11.5</td>
<td>80.34</td>
</tr>
</tbody>
</table>

High molecular weight polyvinylalcohol (PVA) or poly(vinylpyrrolidone) was used as suspension stabilizer. Beads of convenient shape and size could be obtained under these conditions. The polymer beads were obtained in high yield (Table IV.1.) and particle size ranging from 100-500 mesh. They show good mechanical properties and were obtained in easily filterable form. Reproducible results could be obtained in the case of particle
size distribution of the polymer beads by adjusting the amount of stabilizer (PVA), geometry of vessel, stirring rate and shape of the stirrer. The polymer beads were refluxed with trifluoroacetic acid at 80°C to remove any linear polymeric impurities and monomers.

Scanning electron micrographs (SEM) of the typical copolymer beads are shown in Fig.IV.1. The micrographs show that all particles are perfectly spherical and almost uniform in their size and that the surface is even and smooth. The shapes of the particles were inferred from the SEM patterns. The even and smooth surface of the particles also demonstrate that the monomer diluent is a good solvent for the polymer and the beads are microporous in nature. Guyot et al extensively used the SEM technique for studying the morphological features and the mechanism of formation of the beads129-131.

IV.2. Functionalization of Tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Polystyrene Resin

Functionalization of polymers involves copolymerization of substituted monomers directly
Fig. IV.1 Scanning Electron Micrograph of TTEGDA-Crosslinked Polystyrene Beads
or functionalizing the preformed polymer by polymer-analogous reactions\textsuperscript{132}. Introduction of functional groups into styrene polymer by copolymerization of suitably substituted styrene monomers gives polymers of more uniform functionalization. In addition they are not contaminated by small proportion of other functional groups remaining from incomplete prior chemical transformation. However, in the case of functionalization of the preformed polymer the functional groups will be more accessible to reactants for further chemical modification.\textsuperscript{133}

IV.3. Preparation of Chloromethyl Resin (2)

There are two standard methods for chloromethylation of crosslinked polystyrene resins\textsuperscript{134-136}. A mixture of formaldehyde and gaseous hydrochloric acid in presence of Lewis acid catalyst ($\text{H}_2\text{SO}_4$, ZnCl\textsubscript{2}) has been reported to effect the chloromethylation reaction. The second method utilizes chloromethylether or dichloromethyl ether in the presence of a catalyst such as AlCl\textsubscript{3}, ZnCl\textsubscript{2} and SnCl\textsubscript{4}. The advantage of this method is that chloromethylether is a good swelling agent for the copolymer. The main disadvantage is
the introduction of additional crosslinks in the polymer matrix. It is therefore important to establish the optimum conditions for the chloromethylation reaction taking into account the various physicochemical characteristics of the polymer matrix. The ability of the macromolecular matrix to allow penetration of the reagent into it and the byproducts to escape out of the reaction sites is one of the factors necessary for achieving optimum conditions\(^\text{137}\). When a sufficiently good swelling agent is used, the crosslinked network becomes extremely distended and gives a gel having low mechanical resistance making the reagent easily reach practically all the available sites. Svetlov\(^\text{138}\) studied the chloromethylation of poly(styrene-Co-DVB) with different amounts of DVB (2, 6, 12 and 20\%) in the presence of ZnCl\(_2\). The rate of reaction increases with increasing porosity, but the reaction takes place in a homogeneous phase of swollen polymer in spite of the heterogeneity of the system. By using the method of Kressman\(^\text{139}\) which involves the interruption of chloromethylation it has also been demonstrated that the diffusion is gel-type and not film type\(^\text{140}\). The rate of chloromethylation reaction is first order and it depends only on the reagent in the gel phase\(^\text{141}\).
For comparatively smaller degrees of conversion, IR spectral studies show that more than 90% of chloromethylation takes place at the para position and less than 10% in the ortho position\textsuperscript{142,143}. According to Greig and Sherrington only the aromatic sites which do not belong to the crosslinked unit can be chloromethylated\textsuperscript{144}. Manatt et al showed that the crosslinked and chloromethylated styrene-DVB copolymer, swollen by CDCl\textsubscript{3} can give \textsuperscript{13}C-NMR spectra amenable to interpretation; 99% of the chloromethylation occurs at the para position\textsuperscript{145}.

Tetraethyleneglycol diacrylate (TTEGDA)-crosslinked polystyrene was functionalized by electrophilic substitution of the aromatic ring. Chloromethylation of the styrene ring was carried out using chloromethylmethylether in the presence of Lewis acid and CH\textsubscript{2}Cl\textsubscript{2} as the solvent\textsuperscript{137,146}. Chloromethylmethylether can be conveniently prepared in good purity by passing dry HCl gas through methanol-formaldehyde mixture\textsuperscript{110}.

A mixture of chloroform and methylenechloride was employed as the cosolvent. Eventhough anhydrous AlCl\textsubscript{3} can be used as an effective Friedel-Crafts
Preparation of chloromethylated TTEGDA-Crosslinked Polystyrene

catalyst it incorporates into the polymer as a complex and cannot be washed away completely with common solvents. The product resin is darkly coloured and is of high chlorine capacity. Anhydrous SnCl$_4$ is found to be a better catalyst in chloromethylation reaction at low temperature. In the present case anhydrous ZnCl$_2$/THF was used as the catalyst for the controlled chloromethylation reaction$^{147}$ (Scheme V.2). The reaction can be easily controlled and chloromethyl polystyrene resin (2) of desired chlorine capacity can be prepared by varying the amount of reagent, catalyst, temperature and duration of reaction. The results are given in Table IV.2.
Table IV.2. Chloromethylation of Tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Poly-styrene using ZnCl₂/THF Catalyst (0.5 g resin 0.1 mL 1.0 M ZnCl₂ in THF, 3 mL ClCH₂OCH₃, 2.9 mL CH₂Cl₂).

<table>
<thead>
<tr>
<th>Duration of reaction (hours)</th>
<th>Temperature °C</th>
<th>CH₂Cl Substitution (m mol/g resin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.04</td>
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</tr>
<tr>
<td>15</td>
<td></td>
<td>4.20</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.54</td>
</tr>
</tbody>
</table>

The degree of chloromethylation in the resin was determined from the chlorine content obtained by the Volhard's method. A number of resins with widely ranging capacity were prepared and no crosslinking was observed during chloromethylation.
IV.4. Preparation of 4-Chloromethyl-3-nitro-tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Polystyrene Resin (3)

The chloromethyl resin (2) was nitrated using fuming nitric acid at 10°C to obtain 4-chloromethyl-3-nitro resin (3) which can be used in the preparation of protected peptides by photolytic cleavage under mild conditions. The use of crosslinked polystyrene incorporating a photolytically cleavable 2-nitrobenzyl anchoring linkage between the polymer support and growing peptide chain was first reported by Rich et al.148-150. The photolytic cleavage method under mild neutral conditions at room temperature offers the

\[
\text{Fuming HNO}_3 \quad -10^\circ C
\]

Scheme IV.3. Preparation of 4-Chloromethyl-3-nitro-tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Polystyrene Resin
possibility of obtaining fully protected peptide segments that can be directly used for conformational studies\textsuperscript{151-155}. In order to establish the utility of the new resin in solid phase peptide synthesis of protected peptides, 2-nitrobenzyl anchoring group was introduced into the resin (Scheme IV.3).

IV.5. Introduction of Aminomethyl groups in Tetraethyleneglycol diacrylate (TPEGDA)-Crosslinked Polystyrene Resin (5)

Chloromethylated 4\% TTEDGA-crosslinked polystyrene resin (2) was converted to the aminomethyl resin (5) by the Gabriel phthalimide method\textsuperscript{156} and hexamine method\textsuperscript{157}. In the phthalimide method, chloromethyl resin (2) was first converted to phthalimidomethylpolystyrene (4) by treatment with potassium phthalimide followed by hydrazinolysis. This method has the advantage of the exclusive formation of the primary amino polymer. But in the ammonolysis, secondary and tertiary amino derivatives can also be formed. TTEGDA-crosslinked polystyrene resin was found to be stable under the above functionalization conditions as revealed by IR peaks at 1720 (ester) and 1150 (ether) cm\textsuperscript{-1}. Amino
Scheme IV.4. Preparation of Aminomethyl Tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Polystyrene

group capacity of the product resin was estimated by picric acid titration method.\(^{41}\)

Phthalimide method cannot be used for polystyrene derivatives containing additional functional groups such as carbonyl or nitro group.\(^{157}\) Hence one pot-conversion of chloromethyl resin to aminomethyl resin by hexamine method was used (Scheme IV.4). In this method the chloromethyl resin
was treated with a 2-fold molar excess of hexamethylenetetramine in DMF at 80°C for 10h. The resulting resin on hydrolysis with ethanolic HCl followed by neutralization with 10% triethylamine (TEA)-CH$_2$Cl$_2$ afforded aminomethyl TTEGDA-crosslinked polystyrene resin. There was no detectable amount of chlorine in the product resin and the resin gave intense blue colour to ninhydrin test.$^{43}$

IV.6. Preparation of $\alpha$-Bromopropionyl (6) and $\alpha$-Aminopropionyl(7) Tetraethyleneglycol diacrylate (TTEGDA)-Crosslinked Polystyrene Resin

For the preparation of protected peptides and peptide amides by photolytic cleavage, $\alpha$-bromopropionyl and $\alpha$-aminopropionyl anchoring groups were introduced into TTEGDA-crosslinked polystyrene resin by the polymer-analogous reaction sequence depicted in Scheme IV.5. $\alpha$-Methylphenacyl ester linkage has been reported in the case of polymer-supported synthesis of protected peptide fragments on DVB-crosslinked polystyrene resin.$^{158-162}$ This strategy was found successful in the case of the liquid phase method of peptide synthesis on polyethylene glycol supports$^{163}$ and in multidetachable resin supports$^{164,165}$. 
Scheme IV.5. Preparation of $\alpha$-bromopropionyl and $\alpha$-aminopropionyl tetraethyleneglycol diacrylate-crosslinked polystyrene

$\alpha$-Bromopropionyl resin(6) was prepared by the Friedel-Crafts acylation of TTEGDA-crosslinked
Table IV.3. Preparation of α-Bromopropionyl Resin of Different Capacities

<table>
<thead>
<tr>
<th>Amount of resin (g)</th>
<th>Amount of α-bromopropionyl chloride (m mol)</th>
<th>AlCl₃ anhy. time (hours)</th>
<th>Capacity of resin (m mol/g)</th>
<th>Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>78.7</td>
<td>157.4</td>
<td>2.64</td>
<td>13.6</td>
</tr>
<tr>
<td>10</td>
<td>24.0</td>
<td>48.0</td>
<td>1.50</td>
<td>12.5</td>
</tr>
<tr>
<td>10</td>
<td>12.0</td>
<td>12.0</td>
<td>0.90</td>
<td>11.45</td>
</tr>
</tbody>
</table>

polystyrene resin with α-bromopropionyl chloride in presence of anhydrous AlCl₃. The IR spectrum of the resin showed a strong carbonyl absorption at 1685 cm⁻¹. The capacity of the resin can be varied from 0.9 m mol Br/g to 2.64 m mol Br/g by adjusting the amount of the reagent and duration of reaction (Table IV.3).

The use of α-methylphenacylamido linkage has been reported recently for the solid phase synthesis of peptide amides 166. α-Bromopropionyl TTEGDA-crosslinked polystyrene resin was converted to α-aminopropionyl resin(7) by treatment with
hexamine in DMF at 10°C for 10h followed by hydrolysis with ethanolic HCl and neutralization with 10% triethylamine in CH₂Cl₂. The product resin gave blue colour to ninhydrin test and there was no detectable amount of bromine. Amino group capacity was estimated by the picric acid titration method⁴¹. The IR spectrum of the resin showed absorption bands at 3400-3500 cm⁻¹ (NH₂) and 1685 cm⁻¹ (C=O).

IV.7. Preparation of 4-Bromomethyl-3-nitrobenzamidomethyl(8) and 4-aminomethyl-3-nitrobenzamidomethyl (9) tetraethyleneglycol diacrylate (TTEGDA)-Polystyrene Resin

The 2-nitrobenzyl ester linkage finds widespread applications as protecting and anchoring group in the polymer-supported methods of peptide synthesis¹⁶⁷-¹⁷². In solid phase peptide synthesis the introduction of anchoring group between the solid support and the growing peptide chain is a convenient strategy for the mild non-destructive cleavage of peptides. The anchoring linkage should be stable under the conditions of the various reactions which are repeated and at the same time it should be cleavable finally by mild and selective
reaction which does not affect the finished peptide. The use of different types of anchoring groups with varying stability between the polymer support and the first amino acid facilitates the attachment of the first residue and the final cleavage of the peptide in the free carboxyl form or as the peptide amide\textsuperscript{173-175}. The principle of photolytic deprotection of functional groups has been made use of to provide mildly and selectively cleavable anchoring linkages between the first amino acid and the polymer support. Photochemical cleavage from the polymer support permits the preparation of N\textsuperscript{\alpha}-amino and side chain protected peptides which are useful in segment condensation\textsuperscript{176-178}.

For the introduction of the anchoring group 4-bromomethyl-3-nitrobenzoic acid was prepared from p-toluic acid by two step reaction\textsuperscript{169}. Aminomethyl TTEGDA-crosslinked polystyrene resin was prepared from chloromethyl resin by Gabriel's phthalimide method and was coupled with 4-bromomethyl-3-nitrobenzoic acid in the presence of dicyclohexylcarbodiimide (DCC) to give the photolabile 4-bromomethyl-3-nitro benzamidomethyl TTEGDA-crosslinked polystyrene support (Scheme IV.6). The IR spectrum of this resin showed
Scheme IV.6. Preparation of 4-bromomethyl-3-nitro benzamidomethyl(9) and 4-aminomethyl-3-nitro benzamidomethyl(9) TEGDA-cross-linked polystyrene support

characteristic bands at 1350 and 1540 cm\(^{-1}\)(NO\(_2\)) and at 1650 and 3400 cm\(^{-1}\)(NH-CO). The resin was found to have a bromine content of 0.48 m equiv/g.
reaction only single coupling in the presence of pyridine was needed for complete reaction as compared to the double coupling and acetylation to block the remaining amino group in the case of DVB-crosslinked polystyrene resin. This suggests increased facilitation of the reaction in the case of TTEGDA-crosslinked polystyrene resin. 4-Bromomethyl-3-nitrobenzyamidomethyl TTEGDA-crosslinked polystyrene resin (8) was converted to 4-aminomethyl-3-nitro benzamidomethyl resin (9) by hexamine method as described earlier. The product resin gave a blue colour with ninhydrin reagent and the aminogroup capacity was determined by the picric acid titration method. The resin swells very well in dichloromethane, DMF and other solvents used in solid phase synthesis.

IV.8. Preparation of 4-(Hydroxymethyl) Phenoxy-methyl Tetraethyleneglycol Diacrylate (TTEGDA)-Crosslinked Polystyrene Resin (10)

The chemical linkage of the growing peptide chain to the resin support is crucial in solid phase synthesis. It has to be easily formed, stable to repeated cycles of acylation and deprotection reaction, and yet easily cleaved at the end of the
synthesis without damage to newly formed peptide bond. Reagent (A), 4-hydroxybenzyl alcohol, is the standard peptide-resin linkage agent in the Fmoc-solid phase technique. The p-alkoxy substituent labilizes the derived benzylester towards acids, giving reactivity comparable to t-butyl derivatives. Thus peptides linked to the resin through ester of (10) may be cleaved by mild acid treatment, usually trifluoroacetic acid under the same conditions as t-butyl side chain protecting groups are removed. In order to demonstrate the applicability of TTEGDA-crosslinked

![Scheme IV.7. Preparation of 4-(Hydroxymethyl) Phenoxyethyl TTEGDA-Crosslinked Polystyrene Resin](image)

\[
\begin{align*}
\text{(2)} & \quad \overset{\text{(A)}}{\text{HO-}}\overset{\text{CH}_2\text{OH}}{\text{CH}_2\text{Cl}} \quad \overset{\text{CH}_3\text{ONa, 50°C}}{\text{CH}_3\text{ONa}} \quad \overset{}{\text{CH}_2\text{O-}}\overset{\text{CH}_2\text{OH}}{\text{CH}_2\text{O-}}
\end{align*}
\]
polystyrene resin in Fmoc-peptide synthesis, 4-hydroxybenzyl alcohol anchoring group was attached to chloromethyl resin (2). Chloromethyl resin was swelled in DMA and a 2-fold excess 4-hydroxybenzyl alcohol was allowed to react in the presence of sodium methoxide (Scheme IV.7). IR spectrum shows peaks at 3420 cm⁻¹ (OH) and at 1220 cm⁻¹ (ether). There was no residual chlorine in the product resin as evidenced by the Volhard's method.

IV.9. Characterization of the Tetraethyleneglycol diacrylate-Crosslinked Polystyrene Resins

The adequate characterization of the polymeric support and the functional group conversion carried out on support is a major problem associated with polymer supported chemistry. Crosslinked macromolecular supports are highly insoluble and methods such as UV and nuclear magnetic resonance present major problems for providing detailed structural information. The two most powerful techniques of analysis which are readily applicable are elemental microanalysis and infrared absorption spectroscopy.

IR spectroscopy is the most widely used
technique not only in following polymer supported reaction but also for structural identification\textsuperscript{181}. Functional group attached to polymer support do not differ appreciably from those of small molecules and the technique of taking the spectra of polymeric solids (film, mull or KBr pellet) is well-developed. Infrared spectroscopy has been particularly useful in following polymeric transformations. The characteristic absorption due to a particular functional group often disappears completely on chemical transformation, with a simultaneous appearance of the characteristic absorption of the new group. IR spectroscopy has been used as a qualitative tool to show the presence of certain functional group and extent of transformation in polymer-supported reactions. Quantitative correlation has been reported in the case of hydrogen bonding in polymers\textsuperscript{182}. Crowley and Rapoport used IR to calculate the capacities of chloromethyl polystyrene resin\textsuperscript{181}. Recently, diffusion-reflected Fourier transform IR spectroscopy\textsuperscript{135} has been used for a more detailed analysis of peaks of the chloromethylated polystyrene. In solid phase peptide synthesis IR spectroscopy has been used for the study of conformational analysis of crosslinked polystyrene resin-bound oligoleucines in the swollen state\textsuperscript{183}. 
TTEGDA-crosslinked polystyrene was characterised by IR spectroscopy. IR spectra were recorded in solid KBr pellets since all the polymers were insoluble. TTEGDA-crosslinked polystyrene resin (1) shows an intense peak at 1720 cm\(^{-1}\) of ester carbonyl and a band at 1150 cm\(^{-1}\) of the ether linkages of the crosslinking agent besides the usual peaks of polystyrene (Fig.IV.2). In chloromethyl TTEGDA-crosslinked polystyrene resin (2) there is a sharp band at 1250 cm\(^{-1}\) corresponding to H-C-Cl.
Fig. IV.3. IR Spectrum (KBr) of Chloromethyl TTEGDA-Crosslinked Polystyrene Resin (2)

Bending vibration (Fig. IV.3). 4-Chloromethyl-3-nitro resin (3) shows characteristic absorption of nitro group at 1520 and 1350 cm\(^{-1}\) and 1250 cm\(^{-1}\) due to chloromethyl group (Fig. IV.4). Aminomethyl TTEGDA-crosslinked polystyrene (5) shows a peak at 3400 cm\(^{-1}\) of N-H stretching. This resin on coupling with 4-bromomethyl-3-nitrobenzoic acid gave 4-bromomethyl 3-nitrobenzamidomethyl TTEGDA-crosslinked polystyrene resin (8) with characteristic IR absorptions at 1650 cm\(^{-1}\) (amide),
Fig. IV.4. IR Spectrum (KBr) of 4-Chloromethyl-3-nitro Resin (3).

Fig. IV.5. IR Spectrum (KBr) of 4-Bromomethyl-3-nitrobenzamidomethyl Resin (8)
Fig. IV.6. IR Spectrum (KBr) of \( \alpha \)-Bromopropionyl TTEGDA-Crosslinked Polystyrene Resin (6)

Fig. IV.7. IR Spectrum (KBr) of 4-(Hydroxymethyl) phenoxyethyl Resin (10)
at 1340 and 1540 cm\(^{-1}\) (nitro) (Fig. IV.5).

\(\alpha\)-Bromopropionyl TTEGDA-crosslinked polystyrene resin (6) shows a peak at 1685 cm\(^{-1}\) arising from the carbonyl stretching (Fig. IV.6). This on conversion to \(\alpha\)-aminopropionyl TTEGDA-crosslinked polystyrene resin (7) showed absorption band at 3400-3500 cm\(^{-1}\) characteristic of the NH\(_2\) group. 4-(hydroxymethyl) phenoxy-methyl resin (10) shows a peak at 3420 cm\(^{-1}\) of the OH and 1220 cm\(^{-1}\) of the aryl alkyl ether linkages. The band at 1250 cm\(^{-1}\) due to CH\(_2\)Cl group completely disappeared confirming complete conversion (Fig. IV.7).

\(^1\)H, \(^13\)C and \(^19\)F-NMR spectroscopy have all been used in monitoring solid phase reaction and in the characterization of the supports\(^{184-186}\). \(^19\)F-NMR spectroscopy of trifluoroacetylated solid phase peptide products has been found to be a very sensitive method for the detection of error peptides\(^{185}\). Solid state deuterium NMR studies on polystyrene resins containing protected glycine revealed that particle aggregation of the glycine oligomers occur after the pendant chain reaches a critical length\(^{68}\) (n>5). These studies showed that the polystyrene matrix is concomitantly immobilized presumably due to additional effective crosslinks.
caused by the aggregation. High resolution $^{13}$C-NMR spectroscopy has been employed to study the additional crosslinking in chloromethyl DVB-crosslinked polystyrene resin. Gel phase $^{13}$C-NMR has also been employed to monitor solid phase peptide synthesis on most commonly used polystyrene based resins. This technique proved to be useful for characterising polystyrene-based starting supports as well as to determine the degree of functionality and purity. DVB-crosslinked phenyl acrylates have been characterized by solid state $^{13}$C-CP-MAS NMR method.

TTEGDA-crosslinked polystyrene resin was characterized by $^{13}$C-CP-MAS (solid state) NMR spectroscopy. The $^{13}$C-CP-MAS solid state NMR measurements were conducted on a Bruker 300 MSL CP-MAS instrument operating at 75.47 MHz. The spectra were run with fine powder of polymer beads at room temperature and KelF rotor was employed for MAS. The samples were rotated with a spectral width of 25000 Hz, the CP time was 22 ms and number of scans was in the range of 200-300. Each sample was rotated with two different spin rates and by comparing the resultant spectra, the spinning side bands were eliminated. Solid state $^{13}$C-CP-MAS NMR
Fig. IV.8. $^{13}$C-CP-MAS (Solid State) NMR spectra of TTEGDA-Crosslinked Polystyrene Resin (1)

spectrum of TTEGDA-crosslinked polystyrene resin (1) shows an intense peak at 127.89 ppm which corresponds to aromatic polystyrene rings and a small peak at 145.65 ppm arising from C-3 carbon of the polystyrene ring. The backbone methylene carbon of the polymer appears as single peak at 40.34 ppm.
The methylene carbon of the ether linkage of the crosslinking agent TTEGDA appears as a small peak at 70.65 ppm (Fig.IV.8). Chloromethyl resin (2) was also characterized by $^{13}$C-CP-MAS NMR method. Chloromethyl resin (2) gave additional peak at 46.11
Fig. IV.10. 75.47 MHz $^{13}$C-(solid state) CP-MAS-NMR Spectrum of 4-(hydroxymethyl) phenoxy-methyl tetraethyleneglycol diacrylate-Crosslinked Polystyrene. $\delta$(ppm); assigned carbon: 158.16 C-18, 144.16 C-9, 134.17 C-15/12, 129.18 C-11/16, 114.7 C-17, 69.70 C-13, 63.92 C-14, 39.89 C-7/8.

ppm due to the C-7 methylene carbon atom of chloromethyl group and a small peak appears in the
region 135.56 ppm corresponding to C-6 carbon of the polystyrene ring (Fig. IV.9). 4-(Hydroxymethyl) phenoxy methyl resin (10) was also characterised by C-13 solid state CP-MAS-NMR method (Fig. IV.10).

Eventhough not much investigations have been carried out using $^{13}$C-CP-MAS solid state NMR technique on crosslinked polymeric supports there is much scope for the method in the characterization and monitoring of solid phase reactions.

Elemental analysis of a polymer support gives an idea about the functional group capacity of polymer supports and their conversions. Volhard's method was used in the estimation of chlorine in the chloromethyl TTEGDA-crosslinked polystyrene resin (2) or bromine in α-bromopropionyl resin (6) and 4-bromomethyl-3-nitrobenzamidomethyl resin (8). Similarly estimation of nitrogen in the nitro resin (3) and amino resin (5) gave information on the amount of functional group transformation. Chemical methods include direct conversion of the functional group which are freely accessible in solution and to quantify the reaction product. Ninhydrin test was used to find out the presence of amino group in TTEGDA-crosslinked polystyrene resins and to follow
the solid phase peptide synthesis. Picric acid titration method was used to estimate the amino group capacity in amino resins. Amino acid analysis of the resin-bound peptide was used to quantify the amount of peptide in the resin. Hydroxyl and amino groups were estimated by the usual acetylation method.

IV.10. SWELLING, SOLVATION AND STABILITY OF TETRAETHYLENEGLYCOL DIACRYLATE-CROSSLINKED POLYSTYRENE RESINS

For the success of solid phase peptide synthesis the accessibility of the growing resin bound peptide chain to reagent and solvents is very important. For maximum accessibility of the reactive functional group in the resin, the polymer matrix should swell extensively in the solvating medium. This measure of swelling property of a polymer support is a criterion for its efficiency in solid phase synthesis. There are two main classes of supports, the gel type and macroporous resin. The gel type resins are generally lightly crosslinked (1-5%) and appear translucent. They have no permanent porosity but swell in various organic solvents. The space between the crosslinks occupied
by the solvent are considered as small pores in these resins. Macroporous resins are obtained when the polymerization is carried out with higher (>5%) amounts of crosslinking agent in the presence of porogens. In the present study microporous TTEGDA-PS resins were prepared by suspension polymerization and swelling studies were carried out. 4% TTEGDA-PS resin showed effective swelling in polar as well as non-polar solvents when compared to the DVB-polystyrene resin. To investigate the effect of the hydrophilic crosslinking agent (TTEGDA), the swelling characteristics of the resin in several solvents of varying polarity were measured. The swollen volumes of the resin in different solvents are given in Table IV.4.

Chloromethylated TTEGDA-crosslinked polystyrene also shows the same extent of swelling as the original TTEGDA-crosslinked polystyrene resin confirming that there was no additional crosslinking during chloromethylation. The swollen volumes of the chloromethyl resin in different solvents are 5.8 mL (DMF); 8.8 mL (CH₂Cl₂); 9.1 mL (NMP); 7.2 mL (benzene); 8.7 mL (dioxane) and 8.7 mL (THF). Swelling measurements were carried out by placing the beads in a graduated cylinder with excess
Table IV.4. Comparison of Swelling Characteristics of Crosslinked Polystyrene Gels

<table>
<thead>
<tr>
<th>Solvent</th>
<th>1% DVB-PS Resin mL</th>
<th>4% TTEGDA-PS Resin mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>5.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Toluene</td>
<td>4.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Pyridine</td>
<td>4.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Dioxane</td>
<td>3.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>4.3</td>
<td>7.3</td>
</tr>
<tr>
<td>DMF</td>
<td>2.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.8</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Determined by gain in weight as solvent/g of dry beads reduced to volume of solvent/g dry beads.

solvent and noting the initial and final volumes of the beads. These results show that the TTEGDA-crosslinked polystyrene resin has enhanced swelling behaviour in polar solvents. This helps maximum diffusion of soluble reactants and reagents into the polymer matrix and facilitates the reaction.
Another important criterion for a solid support to be suitable for solid phase peptide synthesis is that the resin should be mechanically stable under all conditions of repeated synthetic operations and the parts of the support other than the functional group should be chemically inert during the various synthetic reactions. The 4% TTEGDA-crosslinked polystyrene resin was found to be stable even after vigorous conditions of functionalization. This new polymer support has comparable physical and mechanical properties as that of divinylbenzene(DVB)-crosslinked polystyrene support permitting identical manipulation such as shaking and filtration when used as a support for solid phase peptide synthesis. In order to test the stability of the resin under various conditions of peptide synthesis the resin was subjected to the commonly encountered conditions in peptide synthesis. The resin should have enough stability to withstand the repeated acid treatment required for the Boc removal and base treatment of Fmoc removal. The stability of the resin was tested under the same acidic and basic conditions of Boc removal and Fmoc removal. There was no degradation of the polymer under these conditions and the IR spectrum of the resin after deprotection showed no peak due
to carboxylic acid group showing that no hydrolysis of the ester crosslinks has occurred. These observations indicate that the crosslinks are stable enough for repeated synthetic steps. Similar observations have also been made in the case of resins derived from glycidyl methacrylate crosslinked with ethyleneglycol dimethacrylate\textsuperscript{191}. 
PEPTIDE SYNTHESIS USING TETRAETHYLENE GLYCOL DIACRYLATE (TTEGDA)-CROSSSLINKED POLYSTYRENE RESIN