Chapter 2

POLYMER-SUPPORTED SOLID PHASE REACTIONS: AN OVERVIEW

During 1980’s research in synthetic organic chemistry was dominated by asymmetric synthesis. The significant progress made by the synthetic organic chemist is the development of chiral auxiliaries for asymmetric carbonyl group transformation and reagent for enantioselective chemocatalysts. While intensive research in asymmetric synthesis continues and its importance remains, this decade has seen its popularity equaled and position as vogue subject in synthetic organic chemistry challenged by solid phase peptide synthesis.

Merrifield pioneered solid phase synthesis back in 1963 and his primary focus was on the solid phase peptide synthesis. The breadth of synthetic organic chemistry applied to solid phase peptide synthesis has been widened owing to the emergence of combinatorial chemistry, pharmaceutical and agrochemical research. Moreover the interest was focused in creating structurally diverse analogous on resins using multicomponent condensation reaction. The preparation of small molecules and drug like compounds on solid phase has undergone exhaustive study. Specific target molecules for drug discovery programme
such as hydroxamic acids,\textsuperscript{11} polyamines,\textsuperscript{12} biotinylated probes,\textsuperscript{13} peptidomimetics\textsuperscript{14} and benzodiazepines\textsuperscript{15} have been prepared.

Here, an attempt is made to discuss the new trends in polymer supported reactions, its morphology related aspects and an overview of solid phase peptide synthesis (SPPS).

2.1 Polymer support

A reactive functional group attached to a polymer has quite different reactivity due to the so called 'polymer effects'. The origin may be physical (viscous, diffusion effect, steric effect, site isolation, local concentration effect) or chemical (micro environment interaction, coordination interaction).\textsuperscript{16,17}

The reactivity of the polymeric reagent is influenced by nature of the solvents and reagents to which the polymer is likely to be subjected during the course of its functionalisation and subsequent application as a reagent. It depends also on the chemical behaviour of the polymer support which in turn depends upon the physical form, crosslink density, flexibility of the chain segments and the degree of substitution.\textsuperscript{18,19} Appropriate choice of the support as well as reaction condition can overcome the problem of low reaction rate and product yield compared to homogeneous reactions.\textsuperscript{20}
2.2 **Nature of the support**

A polymeric support not only binds the reactive species but also play an important role in determining the reactivity of the attached species. The major requirements of the polymer support are:

1. ease of preparation with controlled degree of crosslinks to give spherical porous beads,
2. compatibility with most organic solvents,
3. chemical and mechanical stability,
4. inertness of the backbone towards reactants and reagents,
5. low cost and commercial availability of the monomers, and
6. cost of functionalisation.

A wide variety of supports was developed giving due consideration to the above mentioned requirements. Several types of natural and synthetic polymers have been chemically functionalised for use as reactive supports.

2.3 **Linear and crosslinked supports**

Linear and crosslinked organic macromolecular species have found application as synthetic reagents, catalysts and substrate carriers. Each type possesses its own desired advantages and disadvantages depending on the final utilisation. The advantage associated with the use of linear polymers include the fact that the reaction can be carried out in a
homogeneous medium without much diffusional problems and with equal accessibility to all the functional groups of the polymer. This is important in a reaction which is known to be sluggish, for substrates having large size and which cannot penetrate the pores of crosslinked polymer matrix. But here separation of the polymer from the low molecular weight material is difficult and it can be achieved only by ultrafiltration. When the functional groups were attached to the crosslinked sites, they were not available to the reactants due to steric and other microenvironment factors.

Linear N-chlorinated nylon polymers with high chlorine capacity were used to oxidise primary and secondary alcohols to aldehydes and ketones. The halogenation of Nylon 66 requires 3 hours at 15°C and converted 94% of N-H bonds in the original polyamide into N-Cl bond. In addition, the polyamide which was originally insoluble became readily soluble after chlorination in chloroform. This may be due to the reduced intramolecular H-bonding. The possibility of the side reactions producing unwanted crosslinks and the difficulty in the recovery of support after the completion of the reaction are the two major problems when linear polymers are used as supports. The insolubility of a low molecular weight species in the precipitating medium prevents the complete removal of impurities from the precipitated polymer. Gel formation is also another problem associated with linear polymer.
In contrast, crosslinked polymers, being insoluble in all solvents, offer great ease of processing and they can be prepared in the form of spherical beads. Low molecular weight contaminants can be removed from the insoluble crosslinked polymer by simple filtration and washing with various solvents. In suitable solvents, beads with low degree of crosslinking, swells extensively exposing the inner reactive groups to the soluble reagents.\(^{26}\)

Macroporous or gel type resins are generally prepared by suspension polymerisation\(^{27}\) using a mixture of vinyl monomer and small amounts of crosslinking agents containing no additional solvents. Macroporous and macroreticular resins are also prepared by suspension polymerisation with higher amount of crosslinking agents and inclusion of an inert solvent. Small particles are obtained by increasing the water/monomer ratio or diluting the organic phase with a solvent for the polymer to be produced. Increasing the amount of crosslinking agent has opposite effect. Temperature also has a significant influence on the morphology of crosslinked polymer bead. However, the most important factors are the choice of dispersing agent and stirring rate.

Crosslinked polymers are also prepared by popcorn\(^{24,34}\) polymerisation, by gently warming a mixture of vinyl monomer and a small amount of crosslinking agent (0.1-0.5%) in the absence of initiators or solvents which results in a white glassy opaque granular material fully
insoluble and porous with low density. In such cases the growing polymer is propagated as a helix in the presence of high concentration of monomer. In styrene-DVB copolymers, the monomers act as good solvents for the growing polymer.

Particle with narrow distribution are easy to obtain in the submiron range by emulsion polymerisation with the help of an emulsifier. A simple process has been described involving the dispersion polymerisation of styrene in a non solvating medium containing the dissolved cellulose polymer. The particle thus obtained has a bead size around 10-20 microns.

The beads with 100-600 microns are preferred as a polymer support for functional group in organic synthesis or catalysts. The simplest way to get beads with a given particle size is by sieving process.

Gel type resins are highly crosslinked and have no permanent porosity. They are found to be less reactive than linear polymers as reaction will be limited by diffusion of the reagent within the resin pores. These resins are referred to as microporous and less sensitive to the choice of solvents.

2.4 Problems faced in polymer supported solid phase reactions

A proper choice of the polymer matrix is an important factor for successful utilisation of the polymeric reagents. The industrial applications of the polymer supported reagents are not frequent except for Merrifield synthesis. This is due to certain limitations of the polymeric system. Stability is one of the major limitations of polymer supported catalysts.
hydroformylation of olefins with supported rhodium catalyst has been extensively studied but not developed commercially. Another limitation especially of the stoichiometric reaction is the resin capacity which is controlled by the skeleton of the support itself.\textsuperscript{32} The third limitation is the non accessibility or non availability of active site to low molecular weight substrate. Most of them are not on the solid surface as it is in the case of classical heterogeneous catalysts. They are buried deep in the highly viscous crosslinked polymer matrix swollen with solvent system.

The support interacts with the surrounding medium. It may or may not swell depending on its thermodynamic affinity with the medium and its method of synthesis. It may selectively absorb one of the reactants or products as a result of preferential solvation. The macromolecules can be a linear species capable of forming a molecular solution in a suitable solvent. Alternatively crosslinked species, the so called resin, which though ideally being solvated by a suitable solvent remain macroscopically insoluble.\textsuperscript{33}

The ease of chemical modification of a resin and success of its application as a reagent or as a catalyst depend substantially on the physical properties of the resin.\textsuperscript{34} Functionalised polymer support must possess a structure which permits enough diffusion of the reagents into the reactive site. The kinetics of chemical reaction is controlled by the diffusion into the microparticles up to the point where there are enough functional groups on the pore surface.
While considering the reactivity of the polymer supported reagents, one important factor which is relevant is the diffusion and distribution of the substrate between the support and the bulk reaction medium. Entropy of mixing also contributes to the reactivity. Favourable interaction between the support and the substrate may enhance the rate of the reaction. But if the support and the substrate are mutually incompatible, the effective concentration of the substrate in the support volume will be diminished considerably which may lead to decreased reaction rate. The concept of diffusion controlled transport of the substrate has given importance to the consideration of the size of the substrate as well as the particle size of the support.

A soluble low molecular weight species when attached to a crosslinked polymer acquires the latter's property of complete insolubility in all common solvents. Moreover, if the polymer is highly porous, the bound molecules will remain fully accessible to the solvent and solute molecules. So, it does not loss much of its reactivity which they exhibit in a homogeneous medium. The polymer matrix contributes a special microenvironment for carrying out the reaction. Polymer matrix will generally impose a restriction to molecular diffusion which is controlled by pore parameters, chemical structure of the substituents on the polymer backbone, and the distance between the attached molecule and the polymer backbone.35
For polymer supported solid phase reactions, the reagent moieties are attached to linear as well as crosslinked polystyrene, polyacrylamide, vinylheterocyclic polymers, polyvinylpyridine and polyacrylic acid supports. These resins provide functionalities for the direct attachment of the reagent species to it.

One of the major disadvantages cited for polymeric reagents was slowness with which a functional group conversion takes place compared to the homogeneous reactions. Polymer aided heterogeneous reactions are comparatively very slow and requires vigorous conditions. This decrease in reactivity is due to the close proximity of the macromolecular matrix and the functional group. This is more pertinent in the case of crosslinked polymers where the active groups are either flanked by crosslinks or buried in the interior of the polymer. Such groups are not readily accessible to the reagent or substrate in the continuous phase. It has been observed that the reactivity of the immobilised functional groups can be considerably increased if the active site is separated from the polymer backbone. Such enhancement in property is observed when the functional group is separated from the polymer backbone by flexible arm. This makes the active function protrude away from the matrix into the solution phase where they are accessible to low molecular weight species and solvents.36,37

The hydrogenation of carbonyl compounds catalysed by poly(benzimidazole)Pd38 catalyst demonstrated the enhancement through
spacers. Better results are obtained when the catalytic function is removed from the benzene ring by a three carbon spacer. When the active site is close to the polymer backbone, a higher activation energy is required for effective molecular collision between the low molecular weight substrate and polymeric reagent resulting in the retardation of the reaction rate. Such limitations are encountered with polymeric t-butyl hydroperoxides also. To overcome these limitations, studies are carried out to determine the efficiency of polymeric t-butyl hydroperoxide reagents in which the reactive sites are well separated from the polymer backbone.

2.5 Diffusion of solvents into the polymer matrix

Penetration of the solvent into the polymer matrix brings the polymer to a state of complete solvation and this allows easy diffusion of low molecular weight substrates into the polymer matrix. The degree of penetration of the solvent will determine the effective pore size and molecular weight exclusion limit for the diffusion of substrate molecules. It is possible to cause a reaction to occur at a fraction of the available sites by the control of the swelling properties of the polymer. Reaction in swollen 1-2% crosslinked polymer bead resemble to those in homogeneous solution. Highly crosslinked beads are less reactive due to hindered diffusion of the substrate into the polymer. If the amount of crosslinks in the polymer is comparatively small, network chains are fairly long and the molecules of low molecular weight substrate can easily
penetrate into the solid matrix. When the network links are short, the polymer completely loses its ability to swell. Even though a crosslinked network of polymer cannot dissolve, the individual segments can solvate to give a swollen gel. The maximum swelling occurs, when the dilution accompanying the swelling equals the contraction force of rubber like elasticity of the polymer chain. That is, when the solubility parameters of the solvent and the polymer are substantially equal. It is believed that crosslinked matrix behaves like an osmotic membrane.

The swelling and diffusion characteristics of a polymer bead depends on its porous structure, which in turn depends on the conditions of polymerisation such as monomer to diluent ratio and temperature. The influence of diluent on the pore parameters of the crosslinked bead was studied by Coutinho et al. They used scanning electron microscopy for observing the variation of the matrix structure with the change of diluent type and amount, when styrene and DVB were copolymerised using toluene and heptane as diluent mixtures. The increase of thermodynamic affinity of the diluent mixture promoted the formation of the beads with a smooth surface. The copolymer synthesised with toluene/heptane in the ratio (45/55) had the characteristics of a smooth surface with small channels regularly distributed. The beads obtained by using large contents of the non solvent had a rough surface. In these cases, the channels were bigger and more irregularly
distributed. Evidently the polymer domains separated by voids were bigger when large content of heptane was employed in the copolymer synthesis.

The physical parameters of a solid support can be characterised in terms of total surface area, total pore volume and average pore diameter. The reactivity of a polymer bead can be changed by the variation in structural parameters like porosity and surface area. In the swollen state, the size and shape of the polymer networks continuously change with the solvating effect of a good solvent and hence the mobility of the polymer segments.

Gel type supports having relatively small pore diameter and a large effective surface area under certain circumstances give rise to high binding capabilities up to approximately 10 mmol/g. The macroporous or macroreticular supports have large pore diameters but relatively small surface area. Chemical modification occurs largely on the pore surface. Highly crosslinked and entangled polymer chains within the matrix are not readily available for functionalisation. A resin of high surface area can be prepared by using a good solvent as porogenic agent. The total pore volume and pore size distribution depends upon the type and relative volume of the diluent, degree of crosslinking and reaction conditions. The high porosity of the matrix has two desirable effects. It leads to good flow properties and does not hinder the penetration of the molecules of high molecular weight substrate.
The swelling characteristics of a crosslinked polymer largely depends upon the nature and amount of crosslinking agent. The crosslink ratio controls the behaviour of a resin in contact with a solvent and the swelling is found to be inversely proportional to the crosslink density of the polymer backbone.\textsuperscript{4c}

At low crosslink density the solvent swollen polymer may resemble a homogeneous solution. Here, the gel network consists largely of the solvent with only a small fraction of the total mass being the polymer backbone. But with increasing crosslink density, the tendency of the polymer backbone to expand in a good solvent is reduced and penetration of the reagent into the interior may become impaired. In the presence of solvents which are not able to swell the polymer network, the movement of the reagents within such a network become diffusion controlled. At higher concentration of the crosslinking agent, during suspension copolymerisation, in addition to crosslinking, considerable chain entanglements also occur. This reduces the extent of swelling in the presence of a good solvent. Polymers with large pores, macroporous and macroreticular resins also absorb reasonable amount of solvent simply by filling the available voids. Good solvent may penetrate and solvate highly entangled areas of the polymer. Solvent compatibility of the support can be adjusted by incorporating suitable monomer unit in the polymer chain by copolymerisation. In the case of highly swollen gel type resin, pools of solvent appear within the polymer
matrix through which molecules can diffuse quickly without the requirement of molecular motion of the polymer backbone.\textsuperscript{41}

In the case of crosslinked polymer networks, the distribution of the functional group on the polymer backbone is non homogeneous and there is some extent of non equivalence of the functional group. It might be expected that groups placed in the vicinity of the crosslink points are less accessible to reagents and solvents than groups situated away from the crosslinks. In the solvolysis of p-nitrophenyl acetate catalysed by Poly 2-acrylamido pyridine, the reaction rate was increased upto a certain crosslink ratio and then decreased. At very high crosslink density, due to decreased swelling of the gel, the accessibility of the pyridine group is less. In the bromination of polyacrylamide the molecular character and extent of crosslinking was found to influence the functionalisation and reactivity. According to Pillai, V.N.R \textit{et al.}, reactivities of brominated 5\% and 10\% NNMBA crosslinked polymer are higher than those of the linear ones\textsuperscript{42}. But with higher amount of crosslinking, the polymer network becomes rigid and the penetration of the solvent and substrate molecules into the active site becomes difficult. The initial delayed activity of linear polymeric reagent was due to the conversion of the linear chains into blocks during the course of the reaction. The crosslinked reagents appear as small particles and upon wetting they swell into small beads and the extent of swelling decreases with increase in crosslinking.\textsuperscript{43,44}
One of the major areas in polymer supported solid phase reaction that requires modernisation is in the design of polymer supports since synthetic peptides comprising several amino acids have emerged as a powerful tool in biological research. The study of the mechanism of hormone action, enzyme-substrate, antigen-antibody and protein-DNA interaction is possible.\textsuperscript{45-47} Chemical synthesis of peptides can confirm the naturally occurring structures. It can be made available in greater quantities for further investigations to delineate antigenic determinants, to allow preparation of artificial vaccines, and potent new drugs by judicious chemical substitutions that change functional groups and conformation of the parent peptide. Chemical synthesis is the practical way of providing useful quantities of material. In addition, it allows systematic variation of the structure necessary for developing peptides for therapeutic use. The main challenge in peptide synthesis is to establish synthetic routes to homogeneous products of defined covalent structure. The use of polymeric resins as a solid support for the synthesis of peptides have become popular ever since divinylbenzene crosslinked polystyrene (PS-DVB) was introduced by Merrifield in 1963.\textsuperscript{45} Cumulative advances have made it possible to synthesise large peptides by stepwise synthesis using different supports.\textsuperscript{45,46}

Chemical assembly of amino acids to form peptides is achieved either by the ‘solution phase’ or by the ‘solid phase’ method. The classical solution phase method for the synthesis of polypeptide began with Fischer
at the beginning of the 20th century when he synthesised the first peptide and coined the word 'peptide'. Chemical synthesis of peptide involves the blocking of the carboxyl group of one amino acid and the amino groups of the second amino acid. Then by activating the free carboxylic group of the second amino acid the peptide bond was formed. The protected peptide was then precipitated. C-terminal and N-terminal protections were selectively removed and the resulting dipeptide was purified before proceeding to the incorporation of the third amino acid. This method of peptide synthesis is laborious and time consuming because intermediate peptides have to be removed, purified and characterised before proceeding to the next coupling step.48

In 1963, Merrifield introduced the concept of solid phase peptide synthesis. The feasibility of this method was demonstrated by synthesising a tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine.5 In the same year Letsinger and Kornet reported the synthesis of a dipeptide L-leucyl-glycine on a 'Popcorn polymer support' using a different chemical approach. Since then several methodological improvements and refinements have been effected in the synthesis of peptide. The design of polymer support, its chemistry and application of the solid phase peptide synthesis have been well documented.49-57 Here, an updated overview of solid phase peptide synthesis emphasising new polymeric supports is given.
2.6 Principle and strategy of solid phase peptide synthesis

The basic idea of solid phase approach involves the covalent attachment (anchoring) of the C-terminal amino acid of the target peptide chain to an insoluble polymeric support in all stages of the synthesis. The target peptide sequence is formed in a stepwise manner in the C→N direction using activated N°-protected amino acids. After the incorporation of the N-terminal amino acid N-terminal Boc protection is removed and the target peptide is cleaved from the support and purified. The general steps involved in the Merrifield solid phase peptide synthesis using chloromethylated divinyl benzene crosslinked polystyrene support is outlined in Scheme 2.1.

In Merrifield's solid phase peptide synthesis, the C-terminal amino acid with a temporary N°-blocking function like t-butyloxy carbonyl (Boc) was attached to the chloromethyl resin by a benzyl ester linkage. The temporary amino protecting group Boc can be removed with 1M HCl in glacial acetic acid, 4N HCl in dioxane or with 30% TFA in DCM. The resulting amine salt was neutralised with a tertiary amine like triethyl amine.
Scheme 2.1. Merrifield solid phase peptide synthesis, using chloromethylated divinyl benzene-crosslinked polystyrene support.
The free amino group of resin bound amino acid was then coupled to the next Boc-amino acid using DCC as a coupling agent. Another important feature of this technique is that all the coupling and deprotection steps can be monitored using ninhydrin. All the above reactions are carried out under non aqueous conditions in organic solvents like DCM, DMF, and NMP. Finally, the completed peptide was deprotected and cleaved from the support with HF, or neat TFA in presence of scavengers.

The classical solution phase peptide synthesis produced peptides of high purity, but it still suffers from the following drawbacks:

- Peptide synthesis is slow, tedious and laborious. To obtain peptides with high purity, intermediate peptides has to be purified and characterised. This leads to longer synthesis time.
- Insolubility of the intermediate peptide in solvents that are used during the synthesis causes problems in purification and in the next coupling reaction it results in abrupt termination of chain elongation, and
- Large number of manipulations result in considerable reduction in overall yield of the peptide.

Solid phase peptide synthesis has a number of advantages over the classical solution phase peptide synthesis. They are:
The peptide can be synthesised while its C-terminus is covalently attached to an insoluble polymeric support. This permits easy separation of the by-products or constituent amino acids from the growing peptide.

All the reaction steps can be driven to completion by using an excess of reactants and reagents.

No loss occurs because the growing peptide is retained by the polymer in a single reaction vessel throughout the synthesis.

Due to the speed and simplicity of the repetitive steps, physical operations involved in the solid phase procedure are amenable to automation, and

The spent resin can be recycled.

Merrifield's solid phase method possesses several limitations and have been critically discussed by several authors. The major limitations of solid phase peptide synthesis are:

Non compatibility of the resin and the growing peptide chain,

Stability of peptide - resin linkage under the conditions of peptide synthesis,

Formation of error peptides due to deletion and truncated sequences,
Changes in the peptide conformation occur in macroscopic environment within the polymer matrix due to peptide-resin linkage.

Solid support plays an important role in determining the purity and homogeneity of the peptide. In order to achieve this, the reagents and solvents must be freely accessible to the growing resin bound peptide chain. Divinylbenzene crosslinked polystyrene (DVB-PS) support has been widely used in solid phase peptide synthesis with considerable success. However, low penetration of the reagents, difficulty in selecting a good solvent for both polymer and growing peptide, and low rates of acylation and deprotection are the major limitations of using the support.48

Swelling characteristic and mechanical stability of the support also play an important role in solid phase peptide synthesis.5 Swelling characteristic in non polar solvents was poor due to the hydrophobic macromolecular environment of the polymer.50 Since the growing peptide is hydrophilic, it gets aggregated in non polar solvents, which will affect the purity, and the homogeneity of the synthetic peptides.51,52

The solid support polystyrene crosslinked with divinyl benzene (1) introduced by Merrifield, has been widely used for the peptide synthesis using Boc chemistry.66,67
The ideal resin with optimum swelling and stability was 1% crosslinked polystyrene. 0.5% crosslinked resin was found to be too fragile while above 2% crosslinked polystyrene does not swell sufficiently in DCM. As the peptide grows within the resin beads, increased swelling of the resin was observed. This increased swelling is due to the change in the chemical potential of a swollen network. It has been attributed to lowering of the network free energy based on the additional solvation of the growing peptide chain.

Merrifield's technique has undergone a series of modifications and improvements because of the physico-chemical incompatibility of the growing peptide chain and the rigid hydrophobic macromolecular environment created by the PS-DVB network of the support. In order to
optimise the resin structure in SPPS, Sheppard introduced a polar polydimethyl acrylamide resin, which is structurally similar to peptide backbone.\textsuperscript{73,74} This helps easy solvation of the peptidyl resin and thus reduces the steric hindrance during deprotection and coupling reactions.\textsuperscript{75-80} Crosslinked and functionalised polydimethyl acrylamide gel can be detained within the pores of fabricated Keiseguhr which can be used as a matrix in continuous flow method. The support shows effective swelling in polar solvents but in non polar solvents it is very poor. The chemical stability of the resin is also less comparable to that of polystyrene supports.

In multi-pin synthesis technology (PIN) acrylic acid coated polyethylene rods are used as support. In simultaneous multiple peptide synthesis (tea bag method) polystyrene in polypropylene mesh packets are used as support.\textsuperscript{81} In multicolmum methods Macrosorb-SPR resin was used.\textsuperscript{82} The mixed PEG-PS resin, a highly promising class of solid support, is used successfully in polypeptide synthesis.\textsuperscript{83-85} A crosslinked polystyrene-polyethylene glycol graft copolymer with a 2-nitrobenzyl anchoring group has been used as a solid support for the stepwise synthesis of peptides.\textsuperscript{86} Swelling, which is a sign of good solvation of the resin, is good for polystyrene resins in non polar solvents like DCM, whereas polyacrylamide resins swell much better in DMF. Mixed PEG-PS polymers show excellent swelling in common solvents such as THF, acetonitrile and alcohols.\textsuperscript{83} The similar polarity of peptides and polyacrylamide support, both well
solubilised in DMF, makes the support suitable for SPPS. On the basis of solvent-resin interactions, increasing effort has been made to introduce polyacrylamide supports where initially polystyrene supports were used.

Bis-2-acrylamidoprop-1-ylpolyethyleneglycol crosslinked dimethyl acrylamide (PEGA) has been introduced as a hydrophilic, biocompatible and flexible solid flow stable support in peptide synthesis (2). The first flow stable synthesis resin was obtained by polymerisation of the soft polydimethyl acrylamide gel inside a solid matrix of supporting Keiselguhr. Small and Sherrington replaced the irregular Keiselguhr with more regular rigid 50% crosslinked polystyrene sponge containing a grafted polydimethyl acrylamide gel. This technique was developed for grafting polyethylene glycol on to 1% crosslinked polystyrene, which were monodispersed, spherical and flow stable. Polystyrene grafted to
films of polyethylene has been used for synthesis of peptides under non-polar conditions (3). Polyhydroxypropyl acrylate coated polypropylene and cotton has shown some promise as supports under polar conditions. A copolymer of bis-acrylamido polyethylene glycol, N, N'-dimethyl acrylamide and acryloyl sarcosin ethyl ester was successfully employed for the synthesis of peptides.

The inert polyethylene glycol crosslinked resins such as polyoxyethylene-polyoxypropylene (POEPOP) (4) and polyoxyethylene polystyrene (POEPS) (5) were efficiently used as flexible and biocompatible resins in SPPS. Crosslinked Ethoxylate Acrylate Resin (CLEAR) supports developed by Kempe and Barany were also used successfully in SPPS.
Different concepts for multiple peptide synthesis (MPS) or simultaneous multiple peptide synthesis (SMPS) were developed to respond to the rapidly growing demands for a large number of peptides with completely different sequences. The various methods can be distinguished by the differences in the polymeric supports employed, the number of peptides possible and the amount of products obtained. The multiple synthesis methods are mostly applied in hormone and inhibitor research.

For the identification of relevant side chains, every amino acid of a biologically active peptide can be systematically substituted, the chain length can be varied, and N-as well as C-termini can be modified.99
In the initial work of SPPS, the various N-terminal modifications such as acetylations, biotinylations, succinimidilations or couplings for preparing immunogens could be achieved by multiple methods or by consecutive synthesis. In multiple peptide synthesis it is possible to prepare simultaneously the same peptide bound to different anchors and cleaved to obtain peptide acid, peptide amide, alkylated amide, hydrazide or a fully protected fragment. The 'tea-bag' method proposed by Houghten belongs to the oldest strategies of multiple peptide synthesis.\textsuperscript{100} In 'tea bag' method, polystyrene in polypropylene mesh packets was used as supports. Geysen \textit{et al.} developed the concept of multi-pin synthesis technology (PIN) and several hundred peptides can be simultaneously prepared using this procedure.\textsuperscript{112} Acrylic acid coated polyethylene rods were used as supports in multi-pin synthesis technology. Valerio \textit{et al.} used 2-hydroxy ethyl methacrylate grafted polyethylene supports in multi-pin peptide synthesis.\textsuperscript{101,102} In multicolumn methods Macrosorb-SPR resin was used.\textsuperscript{63} Frank \textit{et al.} proposed an inexpensive procedure for the preparation of polymer bound peptides in which the first amino acid was coupled to a sheet of cellulose paper.\textsuperscript{103} Crosslinked enzyme crystals (CLECs) of thermolysin are also used for peptide synthesis.\textsuperscript{104}

For effective swelling of the resin and solvation of the peptide, the polymer should have optimum hydrophobic-hydrophilic balance.\textsuperscript{105,106} The structure-reactivity correlations in crosslinked polymeric systems helped
Pillai, V.N.R et al. to design new series of supports with optimum reactivity, mechanical stability and other essential requirements of a polymeric support. Compared to other supports, styrene based polymer supports showed high mechanical and chemical stability towards various reagents and solvents that were used for polypeptide synthesis. The problems associated with PS-DVB resin could be overcome to some extent by the judicious choice of the crosslinker that can provide optimum hydrophobic-hydrophilic balance to the resin. This increases the swelling of the polymer in various organic solvents and thus enhances the coupling and deprotection rate. These new resins are highly solvated in various solvents. This results in fast and quantitative reactions. Their polarity must also be compatible with those of reagents and solvents used.

The tetraethyleneglycol diacrylate crosslinked polystyrene (PS-TTEGDA) (6) was developed by introducing hydrophilic flexible TTEGDA crosslinker to polystyrene. The polymer was synthesised by aqueous suspension polymerisation of styrene with TTEGDA using benzoyl peroxide as radical initiator, toluene as the diluent and 1% polyvinyl alcohol (MW ~ 75,000) as suspension stabiliser. Polymer was obtained in spherical uniform beads of 200-400 mesh size.
Polymerisation in diluents like hexane and cyclohexane results in the formation of polymer in fine powdered form. The physico-chemical property of the polymer was found to be determined by the chemical nature of monomer and the mole percentage of the crosslinker. Reproducible results are obtained by adjusting the monomer to diluent ratio, amount of the stabiliser, geometry of the vessel and the stirring rate. Compared to various crosslinking density a 4% PS-TTEGDA resin showed optimum hydrophobic-hydrophilic balance, mechanical stability, excellent swelling properties and performance in the stepwise synthesis of medium to large peptides in high yield and purity.

A second polymer developed by introducing hexanediol diacrylate as crosslinker to polystyrene network was proved to be a very
good candidate for the solid phase polypeptide synthesis. This polymer was synthesised by the aqueous suspension polymerisation of the respective monomers using toluene as diluent and benzoyl peroxide as radical initiator.

A third support has been developed by the copolymerisation of styrene and butanediol dimethacrylate (BDODMA) for the solid phase synthesis of peptides (7). The resin support was synthesised with varying crosslinking densities (1%, 2%, 3%, 4%, 6%, 8% and 10%) by the free radical aqueous suspension polymerisation using toluene as diluent and benzoyl peroxide as initiator. The insoluble polymer support was obtained as spherical uniform beads. The beads were sieved and the main fractions obtained were of 100-200 mesh size. Reproducible results were obtained in the preparation of beads of 100-200 mesh size by adjusting the amount of stabiliser PVA, geometry of the vessel and stirrer, and stirring rate.
These supports were stable under all peptide synthetic conditions. The ester crosslinkage of the resin was stable enough to withstand strong acidic and alkaline conditions. The optimum hydrophobic-hydrophilic balance of the resin results in high swelling in different polar and non-polar solvents. So, the range of chemistry that could be conducted on the support makes it an efficient one for different organic reactions. The ease of preparation, functionalisation and workup procedure are the major advantages of these new polymers over conventional polymer supports. The enhanced coupling rate during peptide bond formation, high sensitivity in monitoring the coupling reactions, economical use of reagents, reactants and solvents, and the yield and purity of the peptides are the other advantages of these new resin supports. The physico-chemical
compatibility of the macromolecular support and the growing peptide chain helps to synthesise peptides of very high purity and homogeneity.

2.7 Recent techniques in solid phase peptide synthesis

2.7.1 The resin linkages

In solid phase method the covalent attachment of the growing peptide chain to the polymeric support has been found to be one of the critical problems of efficient peptide synthesis. A chemical linkage of peptide to the support by using a suitable 'handle' unit, allows the whole synthesis under precise conditions. Handles are bifunctional spacers incorporating the features of an easily cleavable protecting group on one end and the other end allows coupling to a previously functionalised support. Their linkages have to be easily formed, stable to repeated cycles of acylation and deprotection and yet easily cleaved at the end of the synthesis without damage to newly formed peptide bond. These handles also help to cleave the polypeptides as free acids, side chain protected acid fragments, amides, hydrazides and as carboxyl derivatives.

An orthogonal protection involves classes of groups that are removed by different chemical mechanisms. It can be removed in any order using appropriate reagents. For the synthesis of peptide acids a $p$-alkoxybenzyl alcohol resin (1) was developed by Wang. Here, the $p$-alkoxy substituent of the benzyl alcohol moiety enhances the acid sensitivity between the peptide and the resin. So, this anchoring bond can be cleaved with TFA. The support (1) was used for the peptide synthesis
using Fmoc amino acids in which N\textsuperscript{α}-protection was removed by an organic base\textsuperscript{109,110}.

![Diagram 1](image)

From the support (1) N\textsuperscript{α}-protection Boc was removed with 30\% TFA in DCM. It can also result in a minute loss of peptide from the resin. This problem was serious when large peptides were synthesised. So, a new support, containing oxymethylphenylacetamidomethyl handle (Pam-resin) (2) was used for the solid phase peptide synthesis\textsuperscript{111-113}. The electron withdrawing linker makes the ester bond hundred times more acid stable than the simple alkoxybenzyl alcohol resin.

![Diagram 2](image)

4-Hydroxylphenylthiomethyl resin (3) can be used for the solid phase peptide synthesis using Boc amino acids\textsuperscript{114,115}. The peptide can be cleaved from the support either by using H\textsubscript{2}O\textsubscript{2}/AcOH or by nucleophiles.
\( p\)-Nitrobenzophenone oxime-resin (4) was prepared from the corresponding ketoresin by the reaction with hydroxylamine hydrochloride and pyridine in refluxing ethanol. The finished peptide can be cleaved from functionalised support by using a range of nucleophiles like aqueous or alcoholic hydroxide, thiophenoxide in DMF to give peptide acid. Alcohols in presence of tertiary amine gives peptide ester, ammonia gives peptide amide and hydrazine gives peptide hydrazide.\(^{116-119}\)

Benzhydrylamine-4-oxycarbonyl methyl-resin (5) can be used for the solid phase synthesis of peptide. Cleavage of the resin bound peptide with HF gives peptide amide.
Supports like 4-hydroxymethylbenzamidomethyl resin\textsuperscript{51,107} (6) 4-hydroxy-methylphenoxyacetamidomethyl resin\textsuperscript{120} (7) or 3-methoxy-4-hydroxymethyl-phenoxyacetamidomethyl resin\textsuperscript{121} (8) are used in solid phase peptide synthesis. The peptide acids can be cleaved from the support by using TFA under various conditions.

Peptide amides can be synthesised by stepwise addition of Fmoc amino acid to a 4-(2',4'-dimethoxyphenylaminomethyl) phenoxyethyl resin (9).\textsuperscript{122,123} The peptide amide can be cleaved from the support using AcOH or dilute TFA.
Peptide amide can also be synthesised using a 4,4'-methoxybenzhydryl phenoxyacetamidomethyl resin (10). Cleavage of the peptide amide from the support was effected using TFA.\textsuperscript{124}

Acid labile 9-xanthenyl resin (11) prepared from 3-hydroxyxanthone can also be used for the synthesis of peptide amides by Fmoc method.\textsuperscript{125}

2.7.2 Multidetachable supports in solid phase peptide synthesis
Multidetachable anchoring groups in solid phase peptide synthesis gives maximum flexibility in the methods used for removing the peptide
from the resins. It provides greater chemoselectivity between the protecting groups of the α-amine side chain and the anchoring bond which connect the peptide with the solid support. The multidetachable resin supports can be cleaved at different positions with acids, nucleophiles or by photolysis. After the synthesis, the final peptide can be obtained as a free acid, as a protected peptide which is suitable for segment condensation or in a form which contains the removable spacer which can be again attached to suitable resin support for further elongation.

Based on the above concept Tam et al. have designed two resins 2-(4-hydroxymethyl) phenylacetoxypropionyl resin (12) and 4-[4-(hydroxymethyl) phenylacetoxyethyl]-3-nitrobenzamidomethyl resin (13) which can be cleaved selectively by acidolysis, at position A or by photolysis at position B.
Benzhydrylamine-4-oxycarbonylmethyl-resin (14) can also be used as a multidetachable support which can be cleaved with acid at position A and with a variety of nucleophiles at position B.

2.7.3 Fmoc protection in solid phase peptide synthesis

Merrifield's solid phase peptide synthesis requires the use of protecting groups of differential stability towards acidolytic cleavage. The temporary N\textsuperscript{α}-protecting Boc group is cleavable by mild acid treatment and anchor bond is cleavable by strong acid. These repeated acid treatments may result in partial loss of side chain protecting group.\textsuperscript{130} Nucleophilic side chain of Tyr, Trp, Met and His can undergo trifluoroacetylation during TFA treatment.\textsuperscript{131}

Reactions on solid support are influenced by the dissimilar solvation properties of the polymer and growing peptide chains. The difference in the solvation characteristics of polystyrene bound polymer support and peptide chain causes the decreased rate of peptide bond forming reaction, and deprotection. Fmoc polyamide solid phase synthesis was designed by
Sheppard et al. to overcome some of the problems experienced with Merrifield's solid phase peptide synthesis using Boc chemistry. These supports are well solvated in polar, aprotic solvents like DMF and NMP.\textsuperscript{131-134} In this strategy, Fmoc protection was used for amino blocking of the respective amino acid. Fmoc group can be easily removed by using non hydrolytic bases like piperidine. Fmoc protection was used with t-butyl side chain protection. p-Benzylxy benzyl ester resin anchorage offers a novel approach to solid phase peptide synthesis without repetitive acidolysis.\textsuperscript{135} The target peptide from this Fmoc polyamide solid phase peptide synthesis was exposed only once to acidolysis with TFA at the final stage.

Fmoc removal occurs rapidly in polar medium like DMF than non polar medium like DCM. Fmoc group was found to be completely stable to acids like TFA, HBr/HOAc or HBr/nitromethane.

A number of model peptides and protein sequences were prepared using these polar polyamide supports. Continuous flow Fmoc solid phase peptide synthesis has been demonstrated for resins encapsulated inside a low density, highly permeable inorganic matrix that can withstand continuous flow pressure. The inorganic support Kieselguhr was successfully used as a continuous flow matrix with polyamide resin for Fmoc solid phase peptide synthesis.\textsuperscript{136}
2.7.4 Attachment of C-terminal amino acid

The usual procedure for the synthesis of peptide acid is to attach the C-terminal amino acid to the polymeric support via an ester bond. In Merrifield method esterification was carried out by heating TEA salt of Boc-amino acid in EtOH with chloromethyl resin. This reaction was slow and complete esterification was usually not possible while there was a possibility to form quaternary ammonium group on the polymer. If esterification was not complete, then quarternisation can occur at every neutralisation step during the synthesis (Scheme 2.2). By converting the Boc amino acid to tetraethyl ammonium salt or cesium salt, esterification reaction can be driven to completion.

Scheme 2.2. Quarternisation of chloromethyl resin by trimethylamine.

Amino acids containing easily alkylatable functional groups like His, Cys and Met can cause difficulty during esterification reactions. If EtOH was used as solvent for esterification reaction, some ester interchange may occur with Asp and Glu. All these problems can be avoided by using hydroxy methyl resin or resins with suitable linkers containing the hydroxy group. Boc/Fmoc amino acids were attached to the resin in a variety of methods. Esterification with preformed symmetric anhydride of Boc or Fmoc amino acid
was the most popular method. 4-(Dimethylamino)pyridine (DMAP) was used as the catalyst for esterification reaction.\textsuperscript{137} This catalyst was able to promote hydrolysis of activated Fmoc amino acid as well as reaction with resin bound hydroxyl group. So, less than one equivalent (relative to resin functionality) of DMAP was sufficient. The resin and the solvents used for the first attachment should be perfectly dried. DMAP also significantly promote racemisation of activated urethane protected amino acids.\textsuperscript{138} It can also cleave Fmoc protecting group leading to the formation of dipeptide on the resin. These side reactions could be avoided by repeating the esterification with fresh reagents after 50 minutes, if the esterification reaction was not complete in 50 minutes.

2.8 Sequential attachment of amino acids and deprotection

The factors that depend on the efficiency of coupling reactions include the nature of the acylating agent and the activated species of protected amino acids. The solvation of the resin-bound growing peptide chain can also have a profound influence in the coupling steps. Subsequent amino acids must be added to the growing peptide-resin in a highly activated form to ensure rapid and quantitative coupling. The common activating species used in solid phase synthesis are the amino acid symmetrical anhydride and the amino acid active ester with HOBt. The symmetrical anhydride method using DCC (1) provides the derivatives of greatest reactivity. But their instability and the formation of insoluble DCU during acylation are its
drawbacks. Diisopropyl carbodiimide (DIPCdI, 1) and t-butyl ethyl carbodiimide (3) are the other carbodiimdes used in SPPS.\textsuperscript{139,140}

\[
\begin{align*}
\text{(1)} & \\
& \text{H}_3\text{C} \text{N}==\text{C}==\text{N} \text{CH}_3 \\
\text{(2)} & \\
& \text{H}_3\text{C} \text{N}==\text{C}==\text{N} \text{CH}_3 \\
\text{(3)} & \\
& \text{H}_3\text{C} \text{N}==\text{C}==\text{N} \text{CH}_3 \\
\end{align*}
\]

The symmetrical anhydride method suffers from a number of disadvantages. Some of them are:

i) they must be prepared just before use because of their instability,

ii) amino acids are wasted during the anhydride formation as two moles of amino acids are required for each mole of symmetrical anhydride produced,

iii) the most efficient solvent (DCM) for rapid symmetric anhydride formation is not the solvent of choice (DMF/NMP) for peptide synthesis, and
iv) the possibility of racemisation.

EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) (4) have also been used for the coupling of successive amino acids in SPPS.\textsuperscript{141}

![Chemical Structure of EEDQ](image)

The limitations of symmetrical anhydride method can be overcome by using the pentafluorophenyl (Pfp) (5), HOBt (6) and 3-hydroxy-2, 3-dihydro-4-oxo-benzotriazine (oDHbt) (7) esters of amino acids.\textsuperscript{142,143} These esters suppress the racemisation during coupling reaction.

![Chemical Structures of Pfp, HOBt, and oDHbt](image)
Benzotriazol-1-yl-oxy-tris(dimethyl amino) phosphonium hexafluoro phosphate (8) along with DIEA is one of the advancing acylating agents in peptide synthesis.

The synthesis of hydrophobic peptides are difficult because of internal aggregation of constituent amino acids via \(\beta\)-sheet formation or by association between protected chain with the synthetic support matrix. The use of 2-(1H-benzotriazol-1-yl) 1,1,3,3-tetramethyl uroniumhexafluorophosphate (HBTU) (9) or 2-(1H- benzotriazol-1-yl) 1,1,3,3-tetramethyl uroniumtetrafluoroborate (TBTU) (10) during the coupling reaction can avoid these problems.\(^{145-147}\) The coupling reaction proceeds rapidly at low level of racemisation. HOBt can act as a catalyst for this coupling reaction and the base like DIEA can activate the reaction.
1-Hydroxy-7-azabenzotriazol (HOAt) (11) and its uronium and phosphonium salt derivatives such as o-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexa-fluorophosphate (HATU) (12), o-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HAPyU) (13), 7-azobenzotriazol-1-yl oxytris-dimethyl amino-phosphonium hexafluorophosphate (AOP) (14), and 7-azobenzotriazol-1-yloxytris-pyrrolidino-phosphonium hexafluorophosphate (PyAOP) (15) in presence of DIEA can be used as acylating agents in acylation reactions.148
The reduction in product loss, minimisation of failure sequences, greater control over coupling steps, increased reaction efficiency, decreased racemisation and reduced coupling time are the advantages of using HOAt and its derivatives, especially in the preparation of peptides containing hindered amino acids.\textsuperscript{149}

2.9 Cleavage

Solid phase peptide synthesis was primarily designed for acidolysis of peptide-linker bond by TFA with suitable scavengers. Time required for cleavage of the peptide from the resin depends upon the type of linker that is used between the peptide and the resin. During cleavage all acid labile side chain protection is removed. Peptides can also be cleaved from the resin in fully protected form to produce a unique peptide carboxyl termination by using photolysis, fluoride ion, alkali or hydrogenation. Trifluoromethane sulphonic acid (TFMSA) and HF have both been used for the final cleavage and side chain deprotection.\textsuperscript{150} PAM linker can be readily removed by TFMSA.\textsuperscript{150} Trimethyl silyl trifluoromethane sulphonate (TMSOTf) in the
presence of thioanisol can be used as a cleaving agent.\textsuperscript{151} It can also remove several side chain protections.

Cleavage of peptide from benzyl ester type linkers by 2-dimethylamino ethanol or N,N-diethyl hydroxylamine yield side chain protected peptide acids.\textsuperscript{93} Protected peptide alkylamide may be generated by alkylamine cleavage and side chain protected peptide acids by catalytic transfer hydrogenation. Silyl containing linkers have been designed for cleavage of protected peptide by fluoride ion. Tetrabutyl ammonium fluoride was used to generate fluoride ion.
REFERENCES


18) Nair, V. A.; Suni, M.M.; Sreekumar K. Designed Monomers and Polymers. 2003, 6, 81.


61) Arunan, C.; Pillai, V.N.R.; Protein and Peptide Lett. 1999, 6, 391.


70) Flory, P.J. Macromolecule. 1974, 12, 199.


76) Kents Clark, L.J. Synthetic Peptides in Biology and Medicine, Alitalok.


131) Yaron, A; Schlossmann, S.F. Biochemistry. 1968, 7, 2673.


144) Kisfaludy, L.; Schon, I. Synthesis. 1983, 325


