CHAPTER 1

INTRODUCTION AND OBJECTIVES
Synthetic peptides and their derivatives are powerful tools in modern biological research. Recent advances in biotechnology made major impacts on understanding the life process of living organisms and health science. These advances made it possible to develop new synthetic vaccines that can compact with bacterial and viral infections, enzyme mechanism and kinetic study, understanding the action of hormones and neuro-peptides.\(^1\) Peptides and their derivatives with antibacterial, antiendotoxic, antibiotic-potentiating or antifungal properties are being developed for the use as a novel class of antimicrobial agents and as the basis for making transgenic disease-resistant plants and animals.\(^5\) Applications of combinatorial peptide chemistry in drug industry fuelled the unprecedented challenges in peptide research. Synthetic and structural studies of the peptides are essential for the development of new methods for the preparation of peptides and also for the action of proteins in living systems. Realising the full potential of synthetic peptides, new strategies for rapid synthesis and testing of large number of peptides must be developed. Numerous investigations are underway to develop new strategies to synthesize medium to large peptides with high purity. A number of approaches for the rapid and simultaneous synthesis of many peptides have been reported.\(^6\)\(^-\)\(^8\)

Synthesis of biologically active peptides for clinical purposes requires a strategy to enable a side-product free synthesis of every intermediate and allows analytical procedures for control of reactions and purity of sequential peptides. In the classical liquid-phase method of peptide synthesis, two approaches are possible (i) step-wise elongation of peptide chain starting at the C-terminal amino acid (ii) assembly of small number of protected fragments (segment condensation approach). In these methods, the coupling reaction is carried out in solution and the reaction can be monitored by the analysis and characterization of intermediate peptides. In stepwise synthesis, activated N\(_\alpha\)-blocked amino acids with correct choice of protecting groups and suitable coupling procedure are used. To keep the optical purity of the peptide, chemical reagents used in the stepwise process should minimise racemisation during the coupling and deprotection steps. In fragment-condensation method, the pre-formed side chain protected peptide segments obtained in stepwise manner can be linked together by various chemical approaches.\(^9\)
Classical solution phase method has been employed extensively for the synthesis of peptides, but the preparation of peptides containing more than a few amino acids is still a laborious process using this technique. This is because after each coupling steps the intermediate peptide has to be precipitated, purified and characterized before proceeding to the next coupling step. The main challenge in peptide synthesis is to establish an efficient synthetic route to homogenous products of defined structure. In 1963, R. B. Merrifield introduced a new chemical synthetic strategy called solid phase peptide synthesis (SPPS), in which the C-terminal Nα-protected amino acid is covalently linked to the resin, deprotection and stepwise assembly of activated Nα-protected amino acid residues are continued till the desired peptide sequence is formed. In this technique, the circumventing lengthy intermediate purification steps of classical peptide synthesis can be substituted by simple washing with suitable solvents. The synthesized peptide can be selectively cleaved from the resin by applying a suitable reagent. The chloromethylated 2% divinyl benzene cross-linked polystyrene was the polymeric support used in the synthesis. All the reactions involved in the synthesis can be carried out in quantitative or near quantitative yields, so that a homogeneous target peptide can be obtained. This synthetic protocol has continually been formulated towards greater efficiency in peptide assembly and cleavage from the solid support. The operational simplicity of the polymer supported reactions over conventional methods make an outburst in peptide chemistry and biology.

PS-DVB support has been used for the synthesis of large number of peptides and small proteins of various chain lengths. However, the side reactions still plague many syntheses, often resulting in low yields and poor homogeneity of the target peptide, especially when the number of amino acid residues were grown beyond twenty. The rate of incorporation of a particular amino acid residue has been found to be decreasing with increasing the chain length. The main reasons for the low yield and purity in SPPS are the physicochemical incompatibility of the PS-DVB matrix with the attached peptide and the development of unfavourable conformational characteristics of the growing peptide and protein sequences. The influence on mass transport of reagents, solvation of polymers as well as the peptide, reaction rates of acylation and deprotection have been mostly negative. This is because of the physicochemical incompatibility of polystyrene
network with the growing peptide chain. A number of modifications have been introduced to overcome the difficulties associated with polystyrene based resin. New type of polymers like poly-acrylamide, polyvinylpyrrolidone, polyethylene glycol-polyacrylamide (PEGA) and cross-linked ethoxylate acrylate resin (CLEAR) were developed to increase the polarity of the support and to make it more compatible with growing peptide chain. Though these supports are highly efficient in peptide synthesis, their mechanical stability is less compared to styrene based supports. It was generally considered that the cross-linked macromolecular network of the support would act as an inert and passive solid carrier. Extensive research with polystyrene-based resins has shown that the insoluble support does actually have a dynamic influence on the synthesis of peptides. An efficient polymeric support for peptide synthesis should facilitate the different types of organic reactions occurring in both polar and nonpolar medium. This is possible only for macromolecular matrix with optimum hydrophobic-hydrophilic balance. The mechanical stability of the polymer matrix is also an important factor in peptide synthesis. The success of solid phase synthesis depends on the solvation of the cross-linked polymer and the peptidyl resin in different solvents. A new class of polymer supports having the above mentioned characteristics can be obtained by the copolymerization of hydrophobic polystyrene with flexible hydrophilic cross-linking agents such as tetraethylene glycol diacrylate or 1,6-hexanediol diacrylate. A new polymer support having optimum hydrophilic-hydrophobic balance and mechanical stability was developed by the aqueous suspension polymerization of styrene with 1,4-butanediol dimethacrylate (BDODMA).

Strong acidic or basic cleavage of peptide from the resin may lead to undesired side reactions. Such risks can be avoided by employing mild conditions for the selective removal of the finished peptide from the support. This can be achieved by introducing suitable anchoring group between the resin and the peptide. New multi-detachable linker between the resin and peptide can help the selective cleavage of the peptide from the linker at different positions by using appropriate cleaving agents. The introduction of photolabile anchoring groups between the peptide and the resin leads to a mild cleavage or deprotection procedure for solid phase peptide synthesis. C-terminal modified peptides are required for the biological and pharmacological studies and their synthesis is
rather difficult. By introducing suitable photolabile anchoring linkage, C-terminal modified peptides can be synthesized by stepwise incorporation of respective protected amino acids. The finished peptide can be cleaved in the protected form from the support under mild conditions of photolysis without affecting the identity of molecules.

The present work describes the preparation and application of 1,4-butanediol dimethacrylate cross-linked polystyrene support for the synthesis of biologically active peptides, application of different linkers for the synthesis of peptide acids, C-terminal and N-terminal modified peptides and synthesis of different designed peptides. After a brief review on recent developments in solid supports and SPPS the thesis describes the following:

1. **Preparation of PS-BDODMA supports**
   
   The new flexible, chemically inert solid support of various cross-linking densities can be obtained by the aqueous suspension co-polymerization of styrene with 1,4-butanediol dimethacrylate. The highly flexible polar cross-link can create more accessible reactive sites to various reagents that are used for SPPS. The presence of styrene enhances the mechanical stability of the support and it also provides appropriate site for functionalisation. The presence of hydrophilic cross-linker renders the support more compatible with the growing peptide chain.

2. **The solvation and swelling studies of PS-BDODMA in a wide range of solvents that are commonly used in peptide synthesis.**

3. **Stability studies of PS-BDODMA under various conditions of peptide synthesis.**

4. **Functionalisation and characterization of the supports.**

   Different anchoring groups for peptide synthesis are introduced in the newly developed resin. Peptide synthesis using chloromethyl-PS-BDODMA, photolabile 4-chloromethyl 3-nitro and 4-aminomethyl 3-nitro PS-BDODMA, hydroxymethyl PS-BDODMA, aminomethyl PS-BDODMA and various acid, base and photolabile anchoring groups such as 4-hydroxymethylphenoxyacetic
acid (HMPA), 4-(4-hydroxymethyl 3-methoxy phenoxy) butyric acid (HMPB),
p-[(R,S)-α-1-(9H-fluoren-9-yl)methoxyformamido]-2, 4-dimethoxybenzyl] phenoxy-
acetic acid (Rink amide), 4-hydroxy methylbenzoic acid (HMBA),
4-bromomethyl 3-nitro benzoic acid and 4-aminomethyl 3-nitro benzoic acid attached PS-BDODMA resin are described.

5. Attachment of C-terminal amino acid under various conditions are optimized.
Time dependent acid and base removal of temporary Nα-amino protection and
time and temperature dependent coupling reactions and time dependent TFA cleavage of the target peptide from the resin are illustrated.

6. Synthetic capability of the support is demonstrated by synthesizing medium to
large peptides, protected peptide acids, amides and alkyl amides. A comparative study of newly developed PS-BDODMA resin is carried out with the commercially available Merrifield and Sheppard resins.

7. Purification, characterization and the optical purity of the peptides are achieved by
using various techniques like column chromatography, HPLC, CD, amino acid analysis and MALDI-TOF-MS.
References