INTRODUCTION AND OBJECTIVES
1.1 Introduction and Objectives

Neurotensin (NT) is a 13 amino acid peptide originally isolated from calf hypothalamus by Carreway and Leeman.\textsuperscript{1} Like many other neuropeptides, it has a dual function of neurotransmitter or neuromodulator in the nervous system and of local hormone in the periphery. The biochemical and pharmacological properties of NT in the brain and in peripheral organs have been documented.\textsuperscript{2-7} NT is a neuromodulator of dopamine transmission and of anterior pituitary hormone secretion, and exerts potent hypothermic and analgesic effects in the brain. In the periphery, NT is a paracrine and endocrine modulator of the digestive tract and of the cardiovascular system of mammals and acts as a growth factor on a variety of normal or cancer cells.

In the last few years, several studies with synthetic analogues of neurotensin have been reported for investigating structure-activity correlations, resulting in some clear conclusions and also with divergent results. The first data\textsuperscript{8} indicated that the sequence could be considerably truncated at the N-terminus without an important decrease of biological activity. It was subsequently shown that the last C-terminal residue of the peptide could not be removed,\textsuperscript{9} amidated\textsuperscript{10} or N-methylamidated\textsuperscript{11} without a complete loss of biological activity. River et al.,\textsuperscript{11} after synthesizing a series of
neurotensin analogues in which each residue was systematically replaced by its D-isomer, found that the important amino acids for binding to mast cells were located in the C-terminal part of the molecule.\textsuperscript{12}

The above results indicated that the N-terminal region of the peptide was apparently not crucial for the expression of biological activities. In order to clarify the broad spectrum of results and determine the smallest chain length required for full activity, new well-defined truncated sequences of neurotensin were synthesised by solid phase technology. Other analogues were synthesised to determine and compare the presence of neurotensin in the central nervous system of diabetics and controlled rats.

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.\textsuperscript{13-16} 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. Applications of combinatorial peptide chemistry in drug industry fuelled the unprecedented challenges in peptide research. Synthetic and structural studies of the peptides help the development of new method 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 synthesise medium to large peptides with high purity. A number of approaches for the rapid and simultaneous synthesis of many peptides have been reported.

The fundamental premise of solid phase technique is that amino acids can be assembled into a peptide of any desired sequence while one end of the chain is anchored to an insoluble support. After the desired sequence of amino acids has been linked together on the support a reagent can be applied to cleave the chain from the support and to release the finished peptide into solution. 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. The virtue of using a solid support is that all the laborious purifications of intermediate stages for the
synthesis are substituted by simple washing and filtration of the polymer supported species.

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 mentioned characteristics can be obtained by the co-polymerisation of hydrophobic polystyrene with flexible hydrophilic cross-linking agents such as tetraethyleneglycoldiacrylate, 1, 6-hexanedioldiacrylate or 1,4-butanedioldimethacrylate.

The present work describes the preparation and application of a new resin, glyceroldimethacrylate cross-linked methyl methacrylate, for the synthesis of biologically active peptides, application of different linkers for the synthesis of neurotensin peptide analogues, synthesis of designed peptides and model peptides. After a brief review on recent development in solid supports and SPPS the thesis describes the following:
1. Preparation of GDMA-PMMA supports

The new flexible, chemically inert solid support of various cross-linking densities can be obtained by the aqueous suspension co-polymerization of glyceroldimethacrylate with methylmethacrylate. The highly flexible polar cross-linker can create more accessible reactive sites to various reagents that are used for SPPS. The presence of glycerol 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 GDMA-PMMA in a wide range of solvents that are commonly used in peptide synthesis.

3. Stability studies of GDMA-PMMA under various condition 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 GDMA-PMMA and various anchoring groups such as 4-hydroxymethylphenoxyacetic acid (HMPA)
and 4-(4-hydroxymethyl 3methoxyphenoxy) butyric acid (HMPB) 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 reaction and time dependent TFA cleavage of the target peptide from the resin are illustrated.

6. Synthetic capability of the supports is demonstrated by synthesising neurotensin peptide analogues.

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

8. Synthetic neurotensin peptide used as a tool for the bioassay of native neurotensin present in the central nervous system of controlled and diabetic rats.

1.2 Organization of the Thesis

The thesis is divided into 5 chapters. The contents of each chapter is as shown below.
Chapter 1 deals with the introduction of neurotensin peptide analogues, its importance and synthetic problems, SPPS and the objectives of the work.

Chapter 2 includes recent trends in solid phase peptide synthesis, problem associated with peptide synthesis and the various methods to circumvent it.

Chapter 3 contains the result and discussion of synthesis, functionalisation and characterisation of GDMA–PMMA support and synthesis of model peptides followed by experimental part.

Chapter 4 contains the synthesis of neurotensin analogues on hydrophilic polymer supports using Boc-chemistry and Fmoc-chemistry.

Chapter 5 includes the study of synthetic neurotensin peptide which is used as the standard for quantifying the native neurotensin present in the central nervous system of controlled and diabetic rats.

The references are included at the end of each chapter.
References


