Modern Methods for Solid Phase Synthesis of Peptides

Dr Donald Alfred Wellings - Spheritech Ltd, United Kingdom

Abstract

Two novel concepts for the design and manufacture of polymer supports for solid phase synthesis of peptides are described. The first concept involves the encapsulation of polymers within the hole of short pieces of capillary tubing often referred to as seed beads. This provides a rigid exo-skeleton for the support of soft polymer gel and other mechanically fragile polymer based matrices. The rigidity of the support provides a polymeric media that is particularly suited to continuous flow based peptide synthesis. The second concept complements this by providing an inexpensive approach to the preparation of spherical polymer particles by coating commercially available impervious hollow glass microspheres with polymer. The added advantage of this approach lies in the buoyancy of the resultant polymer particles, which facilitates handling on a large scale.

Introduction

Polymeric particles used in the solid phase synthesis of peptides are typically be made by a dispersion or emulsion polymerization process in which a solution of monomers is dispersed in an immiscible solvent (continuous phase) prior to initiation of the polymerization. The polymer particles formed are typically then filtered, washed and classified to isolate the required particle size distribution. These processes are disadvantageous in some respects which includes monomer loss to the continuous phase, generation of a range of particle sizes and the undesirable generation of fine particles during the polymerization. The subsequent laborious particle size classification, for example sieving and air classification adds to the expense of the current manufacturing processes. In addition to undesirable costs of manufacture and wastage during preparation certain disadvantages may arise with the physical properties of the known polymeric particles. Microporous polymeric particles are generally soft and difficult to handle in both stirred tanks and column based reactors. In addition, the soft particles may be compressed undesirably and cause fouling, for example during filtration often leading to compressive intrusion into the sinter or mesh being used at the bottom of the reactor. Rigid macroporous and macroreticular particles are more suited to high flow rates in packed column beds. However, due to the rigid nature the particles may be fragile and fragment under physical stress.

Two technologies are described in this paper which provides alternative approaches to the current polymer particle design applied to continuous flow based peptide synthesis and batch-wise operations in stirred tanks. In the first technology, described in patent WO2008/012064, we describe a polymer-impregnated bead wherein the bead has a hole in it and the polymer is disposed in the hole. This technology involves the encapsulation of polymers within the hole of short pieces of capillary tubing often referred to as seed beads. These polymer filled seed beads are readily packed into columns and operate efficiently under continuous flow conditions.

In the second, patented technology we have now found that the problems described above and other problems associated with traditional polymer particles may be ameliorated by providing a particulate support comprising a preformed hollow microsphere having a polymer coated on its surface. The hollow microsphere centre not only provides a uniformly sized particle but also provides a particulate support of a low density resulting in buoyancy in a liquid phase.

Materials and Methods

Concept 1

The glass seed beads used to encapsulate polymer matrices were purchased from Miyuki Beads Co Ltd., Japan.

The seed beads used here were 0.6mm length with a diameter of 0.5mm and hole diameter of 0.4mm. The beads were etched, surface coated to allow covalent binding of the polymer and filled with polydimethylacrylamide based polymer as described in patent WO2008/012064.

Concept 2

Hollow glass microspheres supplied by Potters Beads Europe were etched, surface functionalized to allow covalent binding of the polymer and coated with polydimethylacrylamide based polymer as described in patent application GB0916281.9.

Peptide Synthesis

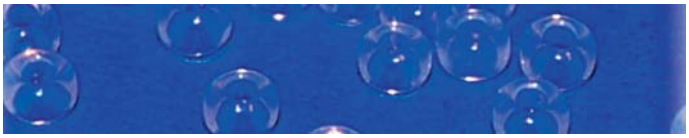
The immobilized polymers described in Concepts 1 and 2 were used to prepare Leu-enkephalin amide and ACP(65-74)amide. Standard Fmoc/TBTU based protocols described by Atherton and Wellings in Houben-Weyl, Methods of Organic Chemistry were used for peptide synthesis. Analytical HPLC was used to compare the crude products in relation to those prepared on traditional polydimethylacrylamide supplied by Polymer Laboratories, United Kingdom and polystyrene supplied by Bachem AG, Switzerland.

Results and Discussion

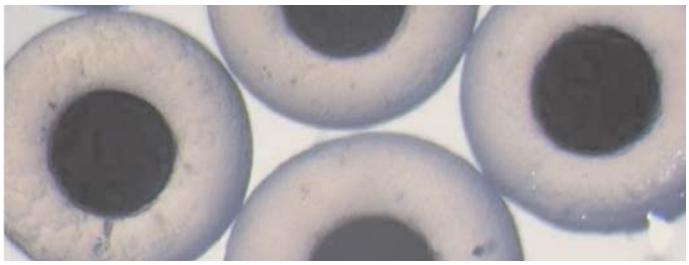
The concepts described in this paper are complementary yet inextricably linked in that the key starting constructs for each technology are commercially available at very large scale with an extremely low cost.

The new technology described for continuous flow based synthesis of peptides allows for the immobilization of extremely soft polymer gels that could not otherwise be used for peptide synthesis. Hydrophilic, highly solvated polymers with low levels of cross-linking will facilitate the synthesis of much longer peptides by solid phase chemistries moving us into the realms of protein synthesis. Small rigid pieces of capillary tubing can in theory be filled with any polymer matrix for a range of applications within and outside the peptide synthesis arena.

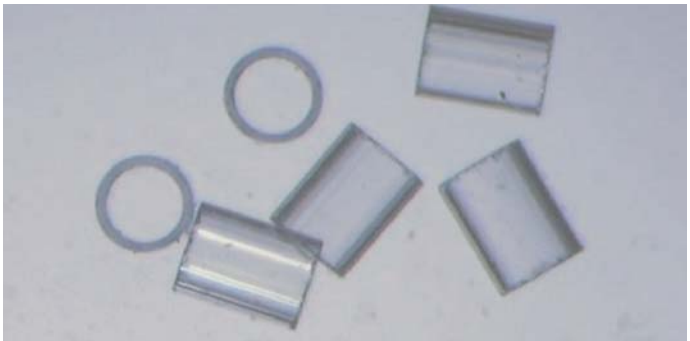
The original idea was stimulated by observation of a simple glass jewellery bead. These beads are called seed beads in the jewellery industry and are made by drawing out a capillary and cutting it into small pieces. The small pieces of capillary are then annealed at which point the ends of the tube become rounded and the resultant product is effectively a hollow glass sphere with holes at opposite ends as seen in the following picture.



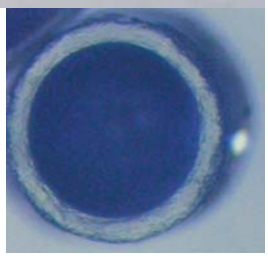
Before the polymer is encapsulated the glass is etched and functionalised to provide a covalent linkage of the polymer to the glass surface. The process for encapsulation of polymer is relatively simple because the capillary action draws monomer solutions into the void. Polymerisation is initiated by standard techniques known to those skilled in the art. Small amounts of polymer that form on the outside of the seed beads (seen below) are simply removed by abrasion.



The standard commercially available seed beads are approximately 1mm diameter with a 0.4mm diameter hole and although standard jewellery beads have proved to be extremely effective we have also custom manufactured smaller glass cylinders which are 0.6mm length, 0.5mm diameter with a hole diameter of 0.4mm as in the following picture.



The picture on the right shows polydimethylacrylamide encapsulated within these cylinders. It would not be impossible to miniaturise the technique for other applications.



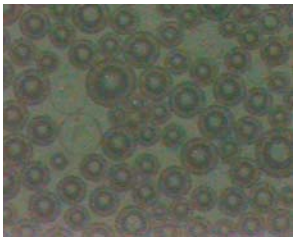
Standard glass seed beads are manufactured on an extremely large scale for the jewellery industry and in total approximately 30-40 tons are manufactured daily worldwide. As expected these beads come in a variety of colours and forms including striped beads so it is easy to envisage applications in combinatorial synthesis. Since these are made of glass they are also inexpensive and the cost is virtually negligible for applications in peptide synthesis and other solid phase applications.

The second technology described in this paper involves the application of an even less expensive key starting material. Known solid phase supports generally comprise polymer particles of a particular size and physical nature to suit the application. For ease of use these polymer particles are often spherical and have a defined particle size distribution. The spherical nature of the particles improves the flow and filtration characteristics of the polymer. Although the uses of solid supports have operational advantages there are disadvantages to the solid phase approach. For example, commercially available supports commonly used for solid phase synthesis of peptides are relatively expensive due to the complex of the manufacturing processes. Microporous polymers with a relatively low level of cross-linker are commonly used for solid phase peptide synthesis which allows the polymer particles to solvate and consequently swell in suitable solvents. Polymeric particles are typically made by a dispersion or emulsion polymerization process in which a solution of monomers is dispersed in an immiscible solvent (continuous phase) prior to initiation of the polymerization. The polymer particles formed are typically then filtered, washed and classified to isolate the required particle size distribution.

These processes are disadvantageous in some respects due to monomer loss to the continuous phase, generation of a range of particle sizes and the undesirable generation of fine particles during the polymerization. Laborious particle size classification, for example by sieving and air classification serves to increase the costs of manufacture.

In addition to the relatively high costs of manufacture and wastage during preparation certain disadvantages may arise with the physical properties of the known polymeric particles. Microporous polymeric particles are generally soft and when used on a large scale they often compress under filtration causing fouling and compressive intrusion into the sinter or mesh being used at the bottom of the reactor. We have now found that these and other problems associated with known polymer particles may be ameliorated by providing a particulate support comprising a preformed microsphere with a hollow centre and a polymer coated on its surface. The hollow centre provides a particulate support of a low density with controlled buoyancy in a liquid phase.

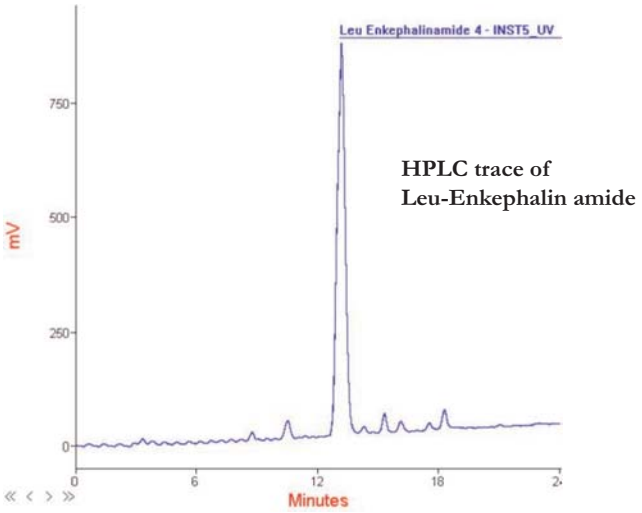
Hollow glass and polymeric microspheres are commercially available on a very large scale. These hollow microspheres are used industrially in many applications including, as examples, lightweight concrete blocks and as fillers in light-weight plastic fabrications. As in the first technology described here this key starting material is inexpensive and typically costs approximately £30 per cubic meter. The coating process is also straightforward and is described in patent application GB0916281.9.



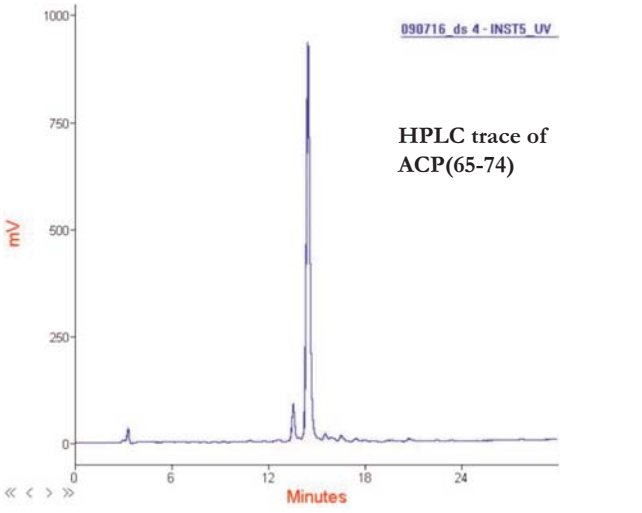
The coated hollow microspheres as seen in the picture on the left are buoyant enough to be used without a filter plate and the peptide synthesis described in this paper was actually performed in a separating funnel as seen below.

Discharging the reactor becomes a synch!

The use of these new technologies for peptide synthesis is demonstrated here by a proof of principle synthesis of Leu-enkephalin amide and in the traditional complex assembly of ACP(65-74). The following graphs show the analytical HPLC traces of the crude peptides prepared on polydimethylacrylamide based polymer encapsulated in glass seed beads.

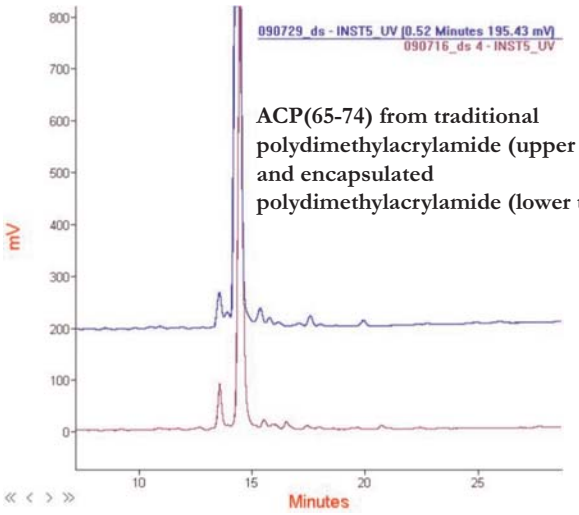


HPLC trace of Leu-Enkephalin amide

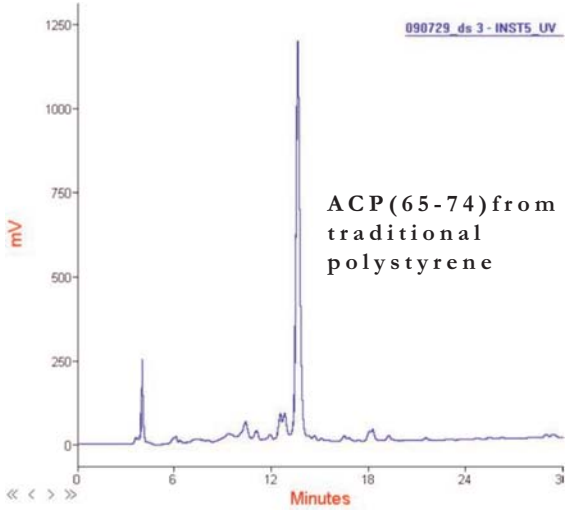


HPLC trace of ACP(65-74)

Comparison of ACP(65-74) prepared on the new seed bead technology, standard polydimethylacrylamide and traditional polystyrene based supports is shown in the following graphs.

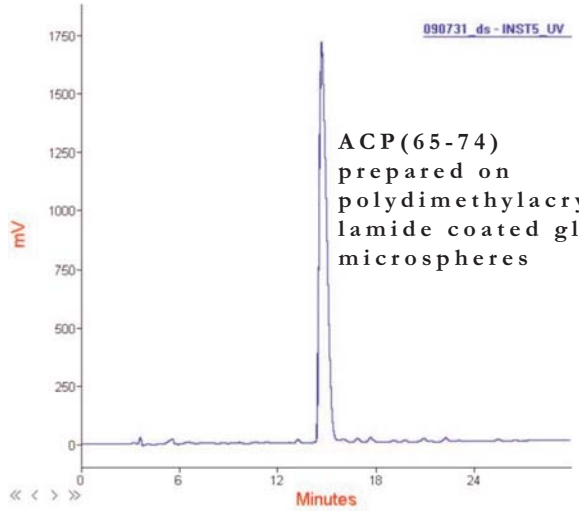


ACP(65-74) from traditional polydimethylacrylamide (upper trace) and encapsulated polydimethylacrylamide (lower trace)



ACP(65-74) from traditional polystyrene

The overlaid traces of ACP(65-74) prepared on standard polydimethylacrylamide based polymer and the same polydimethylacrylamide encapsulated in seed beads are similar, as expected. Whereas the same peptide prepared on traditional polystyrene is clearly inferior. The HPLC trace of the ACP(65-74) prepared on polydimethylacrylamide coated hollow glass microspheres is shown in the following graph. This polymer coating was prepared using a lower level of cross-linking (5 mole %) compared to standard polydimethylacrylamide (9 mole %). The quality of the resultant peptide synthesis is notably higher.



ACP(65-74) prepared on polydimethylacrylamide coated glass microspheres

Concluding Remarks

The two novel and complementary technologies presented here clearly demonstrate efficient and potentially inexpensive approaches to the presentation of polymeric matrices for the solid phase synthesis of peptides. In the first approach the polymer is encapsulated within the hole of a rigid glass exo-skeleton providing a simple inexpensive means of supporting mechanically fragile polymer matrices that could not be used under normal circumstances. The commercially available glass seed beads are extremely robust, can withstand very high mechanical pressures and there is virtually no resistance to liquid flow in a packed column.

The complimentary technology of coating preformed hollow microspheres with polymer matrices provides buoyant particles that simplify handling and filtration in stirred tank reactors. We have demonstrated in this paper that commercially available hollow glass microspheres can effectively be incorporated as the core of polymer particles. The manufacturing process to form polymeric microspheres is further simplified by coating 'preformed' hollow microspheres thereby avoiding the losses associated with traditional suspension and emulsion polymerization techniques.

This paper did not set out to create new polymers constructs for peptide synthesis but it has merely intended to demonstrate new ways of handling existing polymer matrices.

References

Atherton, E. and Wellings, D. A.. In 'Houben-Weyl Methods of Organic Chemistry. Volume E22A. Synthesis of Peptides and Peptidomimetics.' Edited by Murray Goodman, Arthur Felix, Luis Moroder, and Claudio Toniolo. 901 pp. ISBN 3 132 19604 5. Publisher: George Thieme Verlag, Stuttgart, Germany.

About the Author

Don Wellings founded SpheriTech in 2009 where he provides consultancy and laboratory based contract research and development to a range of industries.

Don has more than 30 years experience working in blue chip organisations holding senior scientific posts in ICI, Zeneca, Avecia and Polymer Laboratories. More recently he founded Chromatide where he worked as Chief Scientific Officer for 3 years and is credited as the sole inventor of all of the company's intellectual property.

Don is an internationally recognised authority on peptide synthesis, polymer particle design and chromatography. He is the author of numerous texts and book chapters and is the author of 'A Practical Handbook of Preparative HPLC'. With a track record of innovation he is the main inventor on 12 patents and the sole inventor on all patents filed by SpheriTech to-date.

Apart from the inventive nature exemplified by published intellectual property Don has been instrumental in the concept, design, building and launch of equipment for batch-wise and continuous flow based solid phase synthesis over the last two decades. More recently Don has intruduced new concepts in biotechnology based applications of continuous reactors which will be published in due course.

Spheritech Ltd
The Heath Business & Technical Park,
Runcorn, Cheshire, UK, WA7 4 QX

T: +44(0)1928 511331
M: +44(0)7540 803320
E: don.wellings@spheritech.com
W: www.spheritech.com