AmphiSpheres™

AMPHIPATHIC RESIN FOR SOLID PHASE PEPTIDE SYNTHESIS

For over 25 years the StratoSpheres™ range of polymer supports have been synonymous with quality and economy for solid and solution phase synthesis.

We are very pleased to introduce the latest addition to this StratoSpheres product family: AmphiSpheres.

As the name suggests, this type of material contains both hydrophobic (polystyrene, PS) and hydrophilic (polyethyleneglycol, PEG) components. This subtly changes the swell characteristics of the material allowing a broader range of solvents to be used. At the same time, the active functionality is located at the end of a PEG chain which helps promote reactivity.

Two versions are available with differing PEG content:

- *AmphiSpheres 20 has 20% w/w PEG content and a loading of 0.7 mmol/g.*
- *AmphiSpheres 40 has 40% w/w PEG content and a loading of 0.4 mmol/g.*

Key Benefits

- **High quality and exceptional performance.** The presence of a polyethyleneglycol chain imparts improved swell characteristics across a wide range of solvents providing better synthesis results.
- **Large scale production.** The method of manufacture, attaching a polyethyleneglycol chain to a predefined polystyrene particle, enables the scale to be increased.
- **Highly reproducible.** The underlying polystyrene particle used to prepare the AmphiSpheres products is an optimized copolymer. This provides exceptional reproducibility batch-after-batch.
- **Economical.** Unlike many grafted supports, the loading of AmphiSpheres resin is not compromized, thereby maximizing yield and so further minimizing production costs.

NOTICE: This document contains references to Varian. Please note that Varian, Inc. is now part of Agilent Technologies. For more information, go to **www.agilent.com/chem.**

Agilent Technologies

Amphipathic resin for solid phase synthesis

Since the early 1960's when Merrifield first introduced the concept of solid phase synthesis, polystyrene has been the resin of choice. The simplicity of filtration means that complex molecules such as peptides can be synthesized quite simply if they are assembled whilst attached to an insoluble polymer support. However, Merrifield recognized immediately that the interior of the bead needs to contain reactive sites and that these sites must be readily accessible. Polystyrene is a hydrophobic polymer and requires the use of relatively non-polar solvents, such as dichloromethane, to swell effectively and therefore gain access to these internal sites.

Peptides are polar polyamide molecules and have entirely different solvation properties, hence for the last twenty years or so, scientists have been looking for ways to alter the swell properties of polystyrene in order to improve its performance.

One of the most widely adopted approaches has been to combine polyethylene glycol within the polystyrene matrix in order to provide a material which will swell equally well in polar solvents.

Most polymers produced by this approach cannot be manufactured on large scale and are therefore expensive and of limited use to peptide manufacturers. These composite resins have found favor in smaller facilities producing large numbers of peptides for research.

AmphiSpheres resins are the first composite synthesis supports which can be manufactured on multi-kilogram scale. This provides numerous benefits:

- Highly effective in solid phase synthesis applications
- Economical manufacture
- Highly reproducible
- Exceptionally high quality

The table below shows the swell characteristics of AmphiSpheres resins.

Resin	Swell (mL/gram)				
	DMF	DCM	THF	MeOH	Water
AmphiSpheres 20 NH_2	7.3	9.6	7.5	2.6	2.0
AmphiSpheres 40 NH_2	6.1	8.4	6.8	3.5	2.2
Alternative PEG-PS Resin	5.0	6.0	4.5	4.0	3.1

High loading Synthesis Supports

AmphiSpheres resins are optimized for peptide synthesis. They retain the characteristic spherical shape of polymer supports for solid phase synthesis.

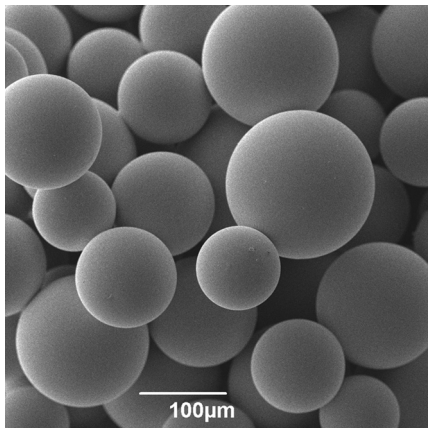


Figure 1. AmphiSpheres particles are spherical and easy to handle. They are also free draining during use.

Two versions of AmphiSpheres are available:

AmphiSpheres 20 contains 20% w/w polyethylene glycol and therefore retains a high loading per gram and has handling characteristics close to that of "glassy" polystyrene. This means that the yield of product is not compromized to the same degree as with larger PEG chains.

AmphiSpheres 40 contains 40% w/w polyethylene glycol and uses a longer PEG chain than AmphiSpheres 20. The amount of PEG is noticeable in that the material is more difficult to shrink down without becoming sticky. However the increased length of PEG chain can give significantly improved results in the synthesis of "difficult" peptide sequences.

Attachment of the appropriate linker or handle enables the material to be used for the synthesis of peptide acids and peptide amides:

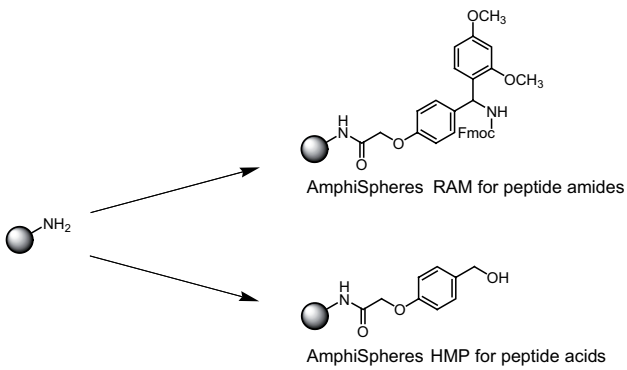
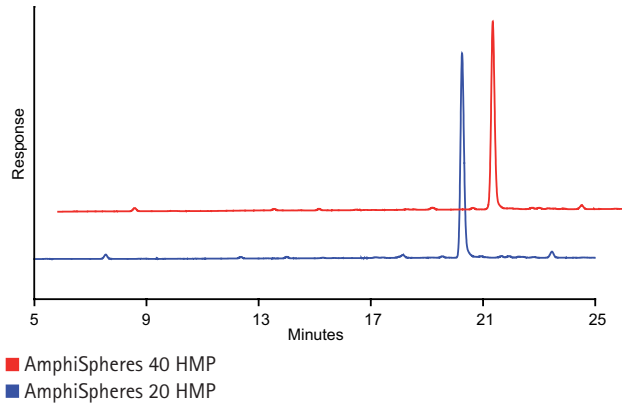


Figure 2. Linker attachment to AmphiSpheres resins.

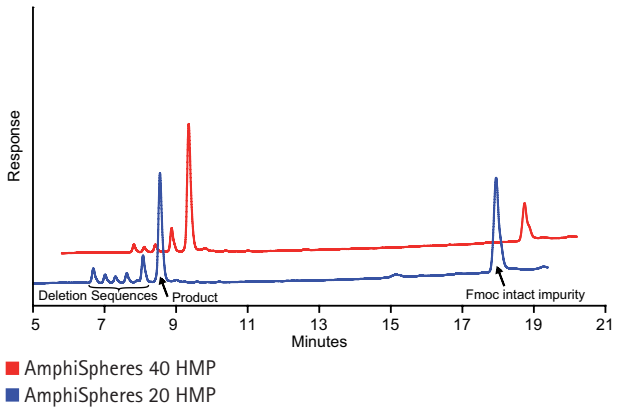
Synthesis of ACP 65-74 Decapeptide

ACP 65-74 is a commonly used "test" sequence, H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH. The hydrophobic residues, and particularly the most hindered residues (Ile & Val), can lead to poor coupling or deprotection efficiencies. Some coupling reagents can cause dehydration of the side chain of glutamine, resulting in the formation of pyroglutamic acid and hence chain termination, therefore trityl side chain protection was used. Without any optimization of synthesis conditions, crude peptide purities of >90% were found by HPLC (MS 1064 $[M+H]^+$).



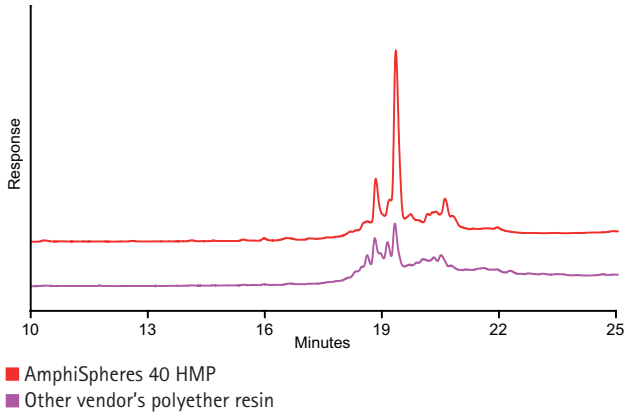
Synthesis of H-Ala$_{10}$-Val-NH$_2$

Decaalanine-valine (amide), H-Ala$_{10}$-Val-NH$_2$, is a well known problematical sequence. The polyalanine chain readily forms secondary structures (beta sheets) and its insoluble nature ensures that deprotection and coupling reactions are slow and frequently incomplete. HPLC analysis clearly shows des-Ala deletion sequences (6.5-8.5 mins) and also fragments with the Fmoc group still intact (18 mins), however the AmphiSpheres resins outperform a commercial PEG-PS resin.



Synthesis of HIV-1 gp4 Ectodomain Sequence

The 28 residue HIV-1 gp41 ectodomain sequence H-NEQELLELDKWASLWNWFNITNWLWYIK-NH2 is a more challenging sequence. Multiple tryptophan, leucine, asparagine and glutamic acid residues as well as other amino acids requiring side chain protection clearly make this a difficult prospect and would normally require optimization of the synthetic strategy. As can been seen, the synthesis using AmphiSpheres resin outperformed the synthesis using a commercially available polyether resin (HPLC/MS confirms the major peak as 3653 $[M+H]^+$).



Peptide Synthesis

All peptides were prepared using an ABI 461A peptide synthesizer on a 0.1mmol scale using 10 fold excess Fmoc-AA-OH (with standard side chain protection as required) and coupling reagent (HBTU) for coupling reactions. Single 9 minutes couplings were used throughout. Deprotection reactions (20% piperidine in DMF) were monitored by conductivity meter. Following peptide assembly and cleavage of the final Fmoc group on the synthesizer the peptide was cleaved from the resin by treating with TFA / TIPS / water 95:2.5:2.5 (v/v) for 2-3h. Cleavage reagents were removed by rotary evaporation followed by trituration of the resultant solid with cold ether. The peptide was then lyophilized from water or water / acetic acid mix.

HPLC Analysis

Analyses were carried out using Varian's Polaris 5 mm C18-A 150 x 4.6 mm column using either: 10 – 30% MeCN (0.1% TFA), 0 – 30 mins, or 5 – 95% MeCN (0.1% TFA), 0 – 30 mins. Flow Rate: 1 mL/min; UV detector: 220 nm; Injection Volume: 20 µL.

StratoSpheres Products

The StratoSpheres product line encompasses a wide variety of polymer supported reagents, catalysts, scavenger resins and synthesis supports. These products are specifically designed to provide enabling tools for organic chemists, particularly in the field of high throughput chemistry and drug design and development. The StratoSpheres range is synonymous with quality, at an affordable price.

Partners in Purification

Varian offers a full range of state-of-the-art instrumentation and consumables for HPLC analysis and purification. With fully integrated HPLC systems and prep-to-process packing stations to reversed phase and ion exchange columns and sorbents, Varian can provide you with a single source solution for the analysis and purification of your compound. Visit our website or contact your local Varian sales representative for further information.

AmphiSpheres Ordering Information

Product Description	5 g	25 g	100 g	1 kg
AmphiSpheres 20 RAM 0.7 mmol/g 75-150 µm	PL3867-1762	PL3867-3762	PL3867-4762	PL3867-6762
AmphiSpheres 20 HMP 0.7 mmol/g 75-150 µm	PL3863-1762	PL3863-3762	PL3863-4762	PL3863-6762
AmphiSpheres 40 RAM 0.4 mmol/g 75-150 µm	PL3867-1764	PL3867-3764	PL3867-4764	PL3867-6764
AmphiSpheres 40 HMP 0.4 mmol/g 75-150 µm	PL3863-1764	PL3863-3764	PL3863-4764	PL3863-6764

Patent pending.

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