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New manufacturing process to produce highly uniform resins with excellent performances in Solid Phase Peptide Synthesis

KEYWORDS: Jetting manufacturing, SPPS, polystyrene, swelling, loading, peptides.

ABSTRACT

Peptides are an important class of Active Pharmaceutical Ingredients. Thanks to the implementation of the solid phase synthesis methodology pioneered by the Nobel Laureate R. Bruce Merrifield in the 20th century, it is now possible to synthesize peptides with up to forty amino acids in a multi-kilogram scale.

Herein, we describe a new manufacturing process to produce highly uniform resins that can be successfully applied to solid phase peptide synthesis (SPPS). The resins were employed for the synthesis of between 5 and 28mer peptides using either automatic heat-assisted microwave systems or manual mode. Excellent results are reported when PuroSynth™ CTC/S, PuroSynth™ Wang/S, PuroSynth™ Rink Amide/S and PuroSynth™ MBHA/S are used for SPPS.

INTRODUCTION

Fifty years ago, it was unthinkable to consider that chemically synthesized peptides could become part of the drug arsenal. The synthetic methodology available in that time did not allow for the preparation of these compounds at the multi-Kg scale, with the purity required by the corresponding drug agencies.

This paradigm started to change when, in the 1960s, R. Bruce Merrifield described the solid phase peptide synthesis (SPPS) methodology. Merrifield realized that anchoring the growing peptide chain to an insoluble polymeric support enhances the synthetic process (1).

Today, it is possible to produce multiple Kgs of a 10-40 amino acid peptide for the drug market; as has been the case for many years for so-called small molecules (< 500-700 Da). As a result, almost 100 peptides are in the market and, more importantly, more than 300 peptides are being evaluated in clinical trials. This assures that in the near future the number of peptide-based drugs on the market will vastly increase, with the resulting benefit of addressing all medical targets (1). However, although the SPPS process involves protected amino acids, coupling reagents, solvents and other reagents, the important entity is the resin (1), which is key for the success of a peptide synthesis. Herein we report on a new manufacturing process, which can increase the performance of resins for SPPS. We evaluate the 2-chlorotritylchloride

(CTC) and Wang resins, which can be used directly for the synthesis of C-carboxylic acid terminal peptides, and 4-methylbenzhydrylamine (MBHA), and aminomethyl (AM), ready for the incorporation of a broad range of handles.

RESULTS AND DISCUSSION

Manufacturing of PuroSynth™ resins by 'can jetting'

The most common method to manufacture spherical polymer beads is by means of suspension polymerization, whereby an organic monomer phase is dispersed as droplets in a vessel equipped with an agitator and an aqueous phase (2). The control this offers over final polymer particle size and distribution is limited because it is controlled by relatively nonspecific means (i.e. stirrer shape and speed of agitation). Suspension polymerization is useful for all applications where particle size distribution is not a key factor as a catalysis, ion exchanger, or for unselective adsorption. However, when it comes to applications where diffusion is a limiting step to obtain good purity and yield, such as chromatography and solid phase peptide synthesis, the particle size distribution plays a key role.

Commercial resins on the market for solid phase peptide synthesis are manufactured by suspension polymerization and then sieved to obtain the desired particle size range, with obvious decreased yields, increased costs and risk of polymer breakage. This presents both operating and production challenges including lower yields of final polymer and time required to sieve. It also poses application challenges due to low uniformity, including high back pressures in columns, and challenges associated with mass transfer and kinetics (3).

The patented technology - 'jetting' - developed by Purolite to produce highly uniform polymer particles, has been applied successfully to all chromatographic resins for both industrial applications (i.e. 350 micron for sugar fractionation) and for analytical and preparative scale (i.e. 75 micron for purification by RP of APIs).

Here we report on the use of 'can jetting' technology, and the manufacture of PuroSynth™; a range of products for solid phase peptide synthesis. 'Can jetting' is designed for the manufacture of a D50 ranging from 25µm to 250µm, with a uniformity coefficient lower than 1.3, and C.V less than 15%.

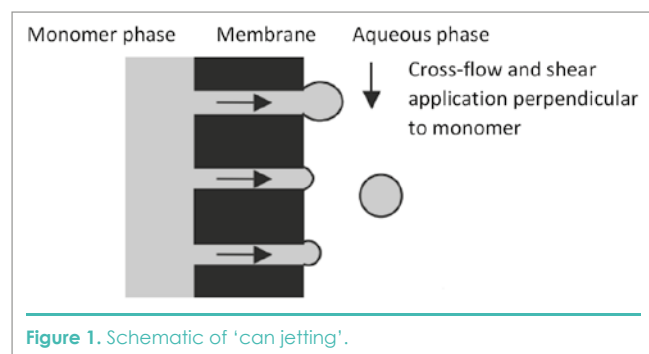
A typical suspension polymerization will normally achieve a uniformity coefficient of 1.6-1.7. Uniformity coefficient along with coefficient of variation indicate how uniform the emulsion and subsequent polymer will be. Uniformity coefficient (U.C) is where monodispersity is defined as:

$$U.C = \frac{D_{60}}{D_{10}} \quad (1)$$

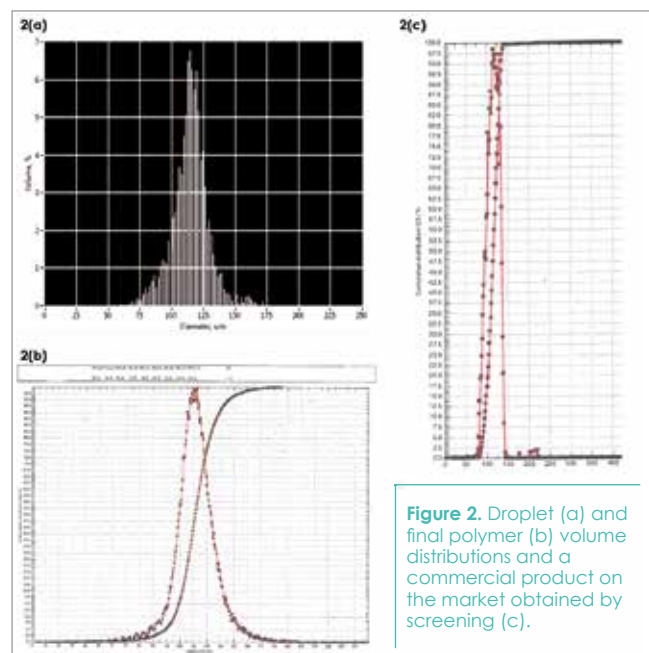
A uniformity coefficient of 1 represents monodispersity. Coefficient of variation (C.V) is where monodispersity is defined as:

$$C.V = \frac{\sigma}{D_{50}} \cdot 100\% \quad (2)$$

Where σ is standard deviation, a coefficient of variation closer to 0 % represents monodispersity. Coefficient of variation (C.V) is a measure of dispersion and is also referred to as the relative standard error. Figure 1 shows a schematic of can jetting - polymerizable monomer phase, pumped through a metallic membrane that contains uniformly drilled holes (5). D10, D50 and D60 are diameters that correspond to 10%, 50% and 60% of the particle size distribution (PSD) respectively, and the Uniformity Coefficient (U.C) is calculated as D_{60}/D_{10} .



Additionally, shear force in the form of oscillation is provided at a point of egression of the monomer phase into the aqueous phase. The direction of shear is perpendicular (like that of the flow of continuous phase) to the direction of egression of the monomer phase. In simple terms, shear force along with various membrane attributes such as pore size, offers control over D_{50} and droplet distribution (6).



'Can jetting' technology was applied to the production of polystyrene beads crosslinked with 1% divinylbenzene (DVB), which are used for the PuroSynth™ product range. We have achieved a U.C and C.V (see equation 1 and 2) of <1.20 and <13% respectively, and spherical beads as shown further by the distributions in Figure 2.

The challenge of this process is the capability of producing 'monosize' emulsions, and maintaining that uniformity in the final polymerized product. This stability is maintained using a combination of surfactants and stabilisers.

Figure 2(c) shows the particle size distribution of a commercial product on the market obtained by sieving. It is clear to see the screening performed at both fine and coarse ends to achieve a particle size distribution of 75-150 micron, as required for these applications in peptide synthesis.

APPLICATION OF PUROSYNTH™ RESINS TO SOLID PHASE PEPTIDE SYNTHESIS

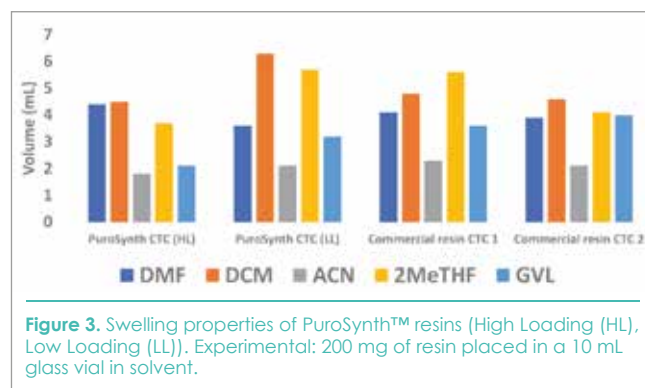
Following the manufacture of highly uniform copolymers based on styrene/1% DVB, the polymers were functionalized to achieve MBHA, 2-CTC, Wang, AM and CM and then tested in peptide synthesis.

Swelling in organic solvents

Swelling is one of the key requirements to achieve a good performance with a SPPS resin, since the reaction kinetic in SPPS is diffusion dependent. Resins that swell better will enhance the reaction yield, and decrease the reaction time by means of facilitating the diffusion. On the contrary, reduction in swelling capacity triggers the undesired peptide aggregation pathways and consequently, increases the coupling difficulties.

Five different solvents were considered for this test: [N,N-dimethylformamide (DMF), dichloromethane (DCM), acetonitrile (ACN), 2-methyltetrahydrofuran (2-MeTHF), and γ -valerolactone (GVL)]. DCM and DMF are two of the most widely used solvents for SPPS, while GVL and 2-MeTHF were reported for green SPPS, and also ACN - which although is not optimum for SPPS as it does not facilitate the swelling of the resin, can still be used in particular cases.

In the example shown in Figure 3, PuroSynth™ resins functionalized with 2CTC with high loading (HL, 1.0 mmol/g) and low loading (LL, 0.5 mmol/g) show excellent swelling properties in all solvents, (except in ACN as expected) and comparable or even better in the case of low loading, with other commercial products in the market.



Peptide synthesis using PuroSynth™ resins

Different peptides, from 5 to 28 mer, were synthesized on PuroSynth™ resins prepared by 'can jetting' and also compared with a commercial resin in the market (Figure 4).

The synthesized peptides were evaluated with respect to the purity by HPLC, and the percentage recovered from the resin after the global final cleavage and precipitation using ether. A green ether (CPME) was also successfully used as a precipitation agent, after the global deprotection step in all 5 peptides (7).

Results in Figure 4 show that all peptides were recovered with yields that were always very high, sometimes reaching recoveries of >93%. In the case of ACP-COOH the comparison with a commercial resin (see the orange bar in Figure 4) further demonstrated the benefits of jetting, as better recovery was achieved (59% vs 50%).

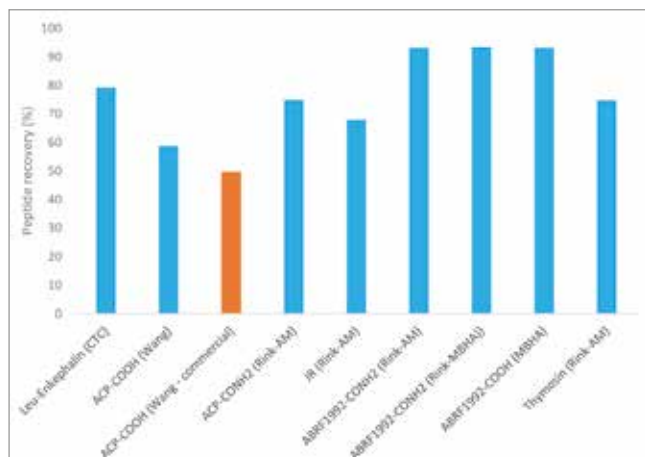


Figure 4. Peptides synthesized, resin used, and recovery. The following peptide sequences were selected: (i) the pentapeptide Leu-Enkephalin; (ii) the decamer acyl carrier protein (ACP); (iii) the Jung-Redemann decapeptide (JR); (iv) the 16-mer ABRF 1992; and (v) the 28-mer thymosin. Syntheses were carried out following standard methods previously reported by our laboratories (7).

Peptides obtained after cleavage from PuroSynth™ show very clean products, with a purity of 90.9% and a recovery of 58.8% for the 10 mer peptide ACP using PuroSynth™ functionalized with Wang linker (Figure 5a), and a purity of 89% and a recovery of 93.5% for the longer 16 mer peptide ABRF 1992 using PuroSynth™ functionalized with Rink-MBHA (Figure 5b).

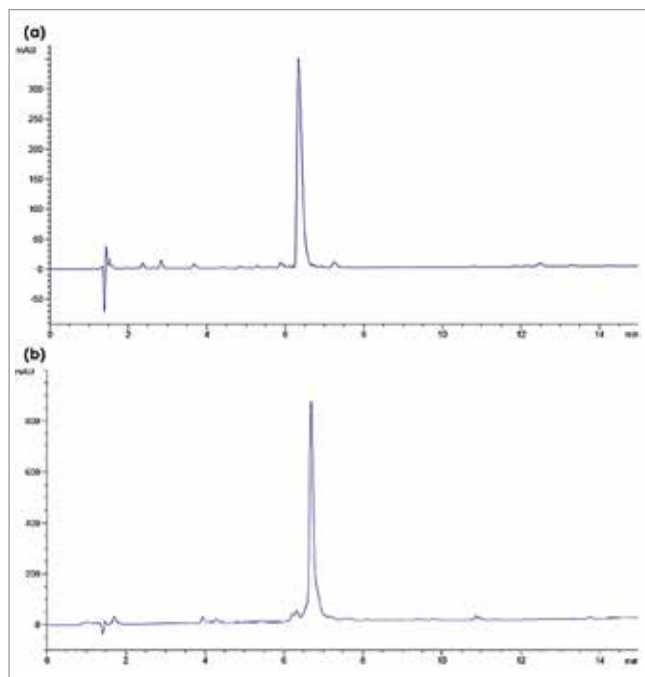


Figure 5. HPLC chromatograms demonstrate the peptide purity after cleavage using PuroSynth™: (a) ACP on Wang resin, (b) ABRF 1992 on Rink-MBHA resin.

CONCLUSION

The basis of the SPPS methodology is the quality of the solid support - the resin. Herein, we have introduced a new technology that allows the manufacture of highly uniform resins to be used in SPPS, with the advantages of high swelling, high peptide recovery after cleavage and the high purity of final peptides. This new technology has a significant impact from a scientific point of view. It can be applied in many fields where the good dispersion of particles has a number of advantages (for example in chromatography), and from a commercial perspective, can decrease the need to screen and thus decrease production costs.

ACKNOWLEDGEMENT

The work in the academic laboratories has been partially supported by the following National Research Foundation (NRF) (CSUR Grant No: 105892 and Blue Sky's Research Programme # 110960) and the University of KwaZulu-Natal (South Africa); and the Spanish Ministry of Economy, Industry and Competitiveness (MINECO) (CTQ2015-67870-P) and the Generalitat de Catalunya (2017 SGR 1439) (Spain).

REFERENCES

1. Y. E. Jad, A. El-Faham, B. G. de la Torre, F. Albericio: *Solid-Phase Peptide Synthesis, the State of the Art. Challenges and Opportunities*, Ch. 18, in *Peptide-Based Drug Discovery: Challenges and New Therapeutics*. Edited by V. Srivastava, Ed. Royal Society of Chemistry, London, UK. (2017), 10.1039/9781788011532-00518.
2. O. Okay, *Prog Polym Sci*, 25, 711-779 (2000), 10.1016/S0079-6700(00)00015-0.
3. D. Horák, *Acta Polym*, 47, 20-28 (1996), 10.1002/actp.1996.010470103.
4. S. R. Kosvintsev, Pat. 20140264984, 2013, Method of producing uniform polymer beads of various sizes. US Pat. 9028730 B2, 2015
5. J. M. M. Simons, *J Memb Sci*, 20-28 (2011), 10.6100/IR719470.
6. S. M. Joscelyne, G. Trägårdh, *J Membr Sci*, 169, 107-117 (2000), 10.1016/S0376-7388(99)00334-8.
7. O. Al Musaimi, Y. E. Jad, A. Kumar, J. M. Collins, A. Basso, B. G. de la Torre, F. Albericio, *Curr Opin Green Sustain Chem*, 11, 99-103 (2018), 10.1016/j.cogsc.2018.06.017. ■

ABOUT THE AUTHORS

In 2012, **Alessandra Basso** joined PuroLite to set-up the Life Sciences division; since then more than 600 new products have been launched. In close connection with R&D and the global sales network of PuroLite, Alessandra supports customers in developing applications, and expanding the scope of the business to the food, pharmaceutical and chemical industries. Alessandra is an author of more than 50 scientific papers in international journals.



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