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Synthesis of magnetic beads for solid phase synthesis and reaction scavenging

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Abstract

A new and efficient route for the large-scale preparation of magnetic, chloromethylated functionalized polystyrene-based resin beads has been developed. These new beads were found to compare favourably with standard Merrifield resin under a range of standard chemistries and displayed identical physical swelling properties. Iron leaching was not observed to be a problem even under TFA treatment although vigorous treatment with 12 M HCl did cause iron loss. These materials were used as a scavenger resin in an array synthesis of sulfonamides. These materials should find ready application in the area of scavenger resins and multiple parallel synthesis.

Magnetic particles that have been encapsulated within highly crosslinked polystyrene beads and then functionalized have found many uses in fields ranging from immunochemistry to molecular biology. For example, Dynabeads[1] have been used in the immunomagnetic purification of cells[2–5] and in affinity chromatography,[6] the method being based on the immobilization of a ligand on the surface of the magnetic bead and then the separation of the successful bound bead conjugate from the pool of non-binding ligands. Magnetic beads derivatized with a range of oligonucleotides have also been used in a number of DNA related applications including DNA sequencing[7] and purification.[8] The beads used in these applications typically range from agarose-based materials to polyurethane coated polystyrenes and are typically in the order of a few micrometres in diameter.

The continued interest in the development of new supports for solid phase synthesis[9] has recently led to the preparation of magnetic beads, which in principal could simplify bead handling issues and perhaps avoid the use of 96/384 well filtration plates. In this area, Szymonifka[10] described the synthesis of magnetically manipulatable solid supports for organic synthesis. This was achieved by the derivatization of standard 1% DVB-PS resin beads by nitration and subsequent reduction by ferrous sulfate and eventual conversion of the incorporated iron into magnetite. Recently, Sucholeiki[11] reported the synthesis of PS-based magnetic beads for SPS by the encapsulation of preformed, highly crosslinked

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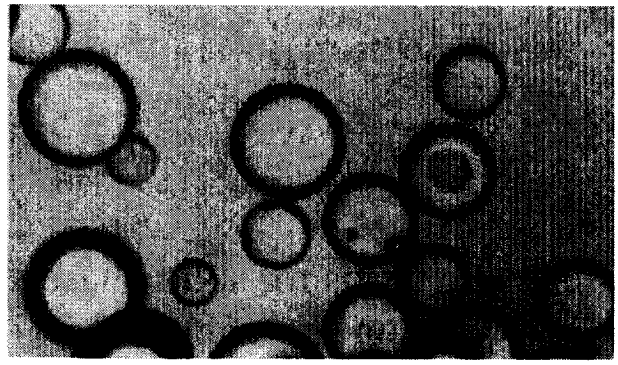

Figure 1. Optical microscope view of magnetic beads

magnetic polystyrene beads and their subsequent derivatization. Here we demonstrate a cost effective and potentially large-scale alternative by the direct incorporation of magnetite into the polystyrene beads by its inclusion into the polymerization mixture. Although this method has been applied to the synthesis of latexes by emulsion polymerization methods[12] its application to suspension polymerization reaction to produce beads for solid phase organic synthesis has not been reported.

The magnetic beads were synthesized by suspension polymerization.† The beads were then suspended in DCM and the buoyant fraction decanted, sonicated (to remove loosely bonded magnetite) and dried. The beads were then sieved and a fraction corresponding to beads with diameters of between 45 and 125 μm collected (13% by total mass of beads). The magnetic chloromethyl resin was then converted to amino methyl resin using potassium phthalimide followed by hydrazine treatment.[16]

Iron levels in the beads were determined spectroscopically. Thus, treatment of the beads with concentrated hydrochloric acid (12 M) gave rise to a yellow Fe^{3+} solution after a few seconds. Quantification was achieved using the Fe(III) ligand 7-iodo-8-hydroxy-quinoline-5-sulphonic acid (ferron) in sodium acetate buffer and detection at 360 nm.[14,15] Treatment of the resin in a similar way with 12 M NaOH, TFA (100%) and water gave rise to no detectable Fe(III) in solution. Beads from the polymerization mixture with magnetite introduced into the mixture at a level of 1 mol% with respect to the organic monomers were found to contain 4 mg of Fe(III)/g resin (0.7 μmol/g). The loss of magnetite from the beads was investigated by a range of mechanical shaking, stirring and sonication techniques which showed that the magnetite was stably incorporated into the resin beads. The level of magnetite incorporated resulted in beads, which were magnetic to such a degree that they could be stirred by a magnetic stirrer (without a stirrer bar) and captured by small bar-magnets.

Although the beads appeared en masse to be light grey, optical microscopy showed the beads to be optically transparent (Fig. 1). Electron micrographs and X-ray analysis indicated that the magnetite was incorporated throughout the beads (Fig. 2).

Swelling properties of the resin were measured by comparison against a batch of standard 2% DVB-PS aminomethyl resin (100–200 mesh) and showed that the beads behaved in an almost identical manner (Fig. 3). Again, no magnetite was released during these swelling studies.

These magnetic beads were used in a typical solid phase synthesis of the tripeptide Phe-Ser-Ala-NH_2 (0.25 mmol). This involved conversion of the chloromethyl resin to aminomethyl resin,[16] Rink linker[17]

† Styrene (14.7 ml), divinyl benzene (0.41 ml) and chloromethyl styrene (2.4 ml), polyvinyl alcohol (2.5 g, 87–89% hydrolyzed, Mr 85–150 KDa) and benzoyl peroxide (0.25 g) were suspended in water (1000 ml) at 80°C.[13] The magnetite was added once homogeneity was achieved. The beads were washed sequentially with hot solvents (water, dioxane:water (1:1), dioxane, DMF, EtOH, MeOH), DCM, ether and then dried in vacuo.

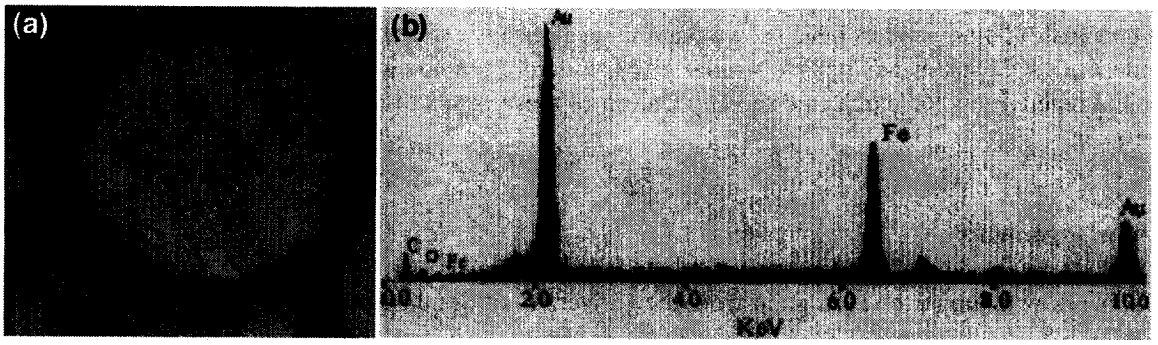

Figure 2. (a) Backscattered electron micrograph image of a magnetic bead. (b) X-Ray (K_α) analysis of surface of the main bead region shown in (a) indicating high Fe incorporation. Gold presence is due to the sample preparation process

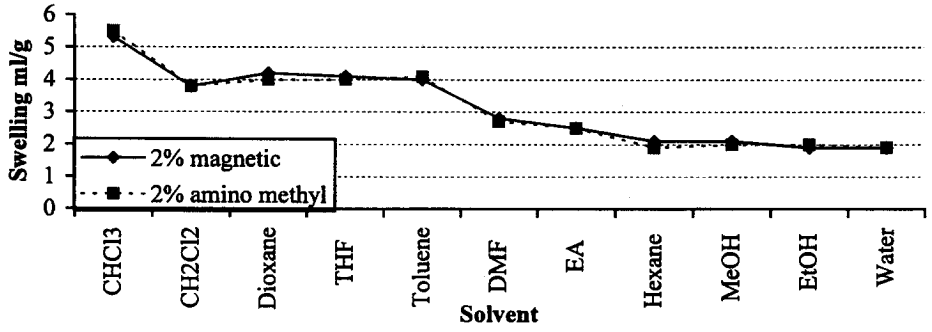

Figure 3. Swelling of magnetic beads in various solvents compared to standard 2% DVB-PS aminomethyl resin

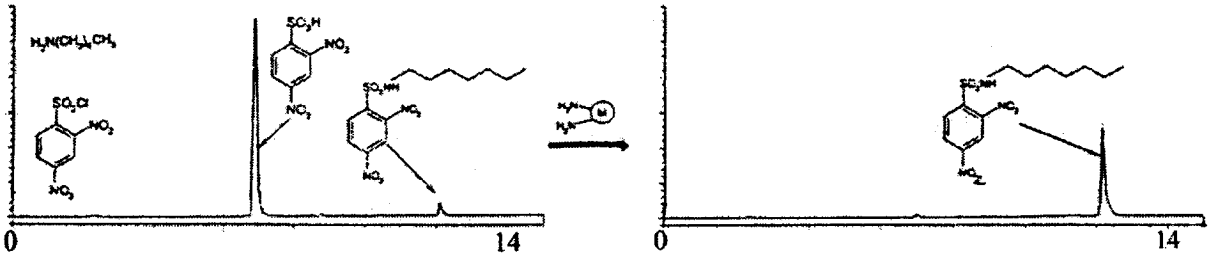

Figure 4. HPLC analysis of sulfonamide formation using magnetic scavenger beads

attachment and traditional Fmoc peptide synthesis.[18] Peptide synthesis was analysed by the standard quantitative ninhydrin assays,[19] and final peptide purity was determined by RP-HPLC and MS analysis of the final peptide.‡

To demonstrate the potential of these resins in a scavenger[20,21] role, a number of sulfonamides were prepared by reaction of amines (1 equiv.) with a number of sulfonyl chlorides (2 equiv.). Upon completion, the excess sulfonyl chloride and hydrolyzed sulfonic acid were removed by aminomethyl magnetic resin beads (aminomethyl) and the beads were removed by an array of small magnets. In each case, the final products were resin free and essentially pure (Fig. 4).

‡ Cleavage from resin by 95% TFA, 2.5% DCM, 2.5% water, RP-HPLC (analysis using HP 1100 Chemstation, 150×3 mm column), gradient 0–100% MeCN over 20 mins, retention time 7.3 mins and mass spectrometry analysis, expected peak mass 323.25 ($(M+H^+)^+$).

In conclusion, these studies have shown that magnetic beads can be produced by analogous suspension polymerization. These beads behave similarly to standard aminomethyl resin both in their physical and chemical reactivities. Research into the application of these beads to new chemistries is currently being undertaken.

Acknowledgements

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