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Core-shell functional microspheres by dispersion polymerization: 2. Synthesis and characterization

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Polystyrene microspheres ranging in diameter from 3 to 10 μm were prepared in alcoholic media by dispersion polymerization of styrene in the presence of a methacrylic acid/ethyl acrylate 1:1 statistical copolymer (Eudragit) as the stabilizer precursor. The effects of the initial Eudragit and free radical initiator concentrations on the microsphere dimensions, the microsphere size distribution and surface functionality were investigated. The particle dimensions were found to increase with increasing initiator concentration and decrease with increasing steric stabilizer precursor concentration. The percentage Eudragit in the microspheres was found to increase with the stabilizer precursor concentration and decrease with the initiator concentration. The resulting double-shell microspheres, with specific surface functionalities, are promising as tailor-made supports for biocatalysts.

(Keywords: dispersion polymerization; functional microspheres; polystyrene)

INTRODUCTION

Dispersion polymerization is a process which generates latex microspheres in the 0.5–20 μm diameter range[1–3]. These large microspheres are grown in a single step with no need for multiple stages as required by Vanderhoff's successive seeding method[3] or Ugelstad's two-stage swelling method[4]. This technique uses the polymerization of a monomer dissolved in an organic diluent in the presence of a polymeric stabilizer consisting of a graft copolymer or its precursor to produce an insoluble polymer dispersed in the continuous phase. The mechanism involves[2–6] the formation of a single-phase system constituted by an initiator, stabilizer or stabilizer precursor, monomer and solvent. In some cases[7], electrolytic costabilizers are also added to the reaction mixture. As radicals are formed, both grafting to the stabilizer precursor, leading to a graft copolymer which is the actual dispersion stabilizer, and polymerization of the monomer occur simultaneously. When sufficient polymer has been formed, nucleation takes place. The resulting microspheres are swelled by the monomers which bulk polymerize inside the microspheres. In favourable circumstances, polymer microspheres of uniform size are obtained. The particle surface nature[8] is determined by the choice of the steric stabilizer. Accordingly, dispersion polymerization represents a powerful tool in providing a great variety of core-shell microspheres with specific functionalities.

Owing to the versatility of this reaction, the macromolecular design can be directed to the preparation of microspheres with extensively hydrophilic and protein friendly surfaces with few non-specific interactions. A wide variety of applications[9,10] for these particles can be envisaged including support materials in liquid chromatography for aqueous biological separations and, once specific enzymes are anchored to the microspheres, as substrates in affinity chromatography and biocatalysts in industrial processes. For the last two applications, it should be recognized that if the steric stabilizer is soluble in the enzyme reaction medium, the formation a core-shell structure (*Figure 1*) constituted by a soft outer shell, made up of long, soluble arms able to fix the enzyme, anchored to a hard, insoluble core can be envisaged. Accordingly, the fixed enzyme still retains a high degree of freedom, thus giving significant accessibility of the enzyme catalytic sites to the substrates. Such biocatalysts may be advantageous for macromolecular substrates to minimize the diffusive control of the reaction.

In the first paper[11] of this series, we reported some preliminary results regarding the preparation of functional polymeric microspheres by dispersion polymerization of styrene using a methacrylic acid/ethyl acrylate 1:1 statistical copolymer (Eudragit) as the steric stabilizer. This commercial copolymer is of special relevance[12,13] in that it has been widely employed as a protein carrier. The endopectinylase (E.C.4.2.2.10) has been immobilized on a sample of these microspheres, and the pectin depolymerization reactivity of the resulting biocatalyst has been

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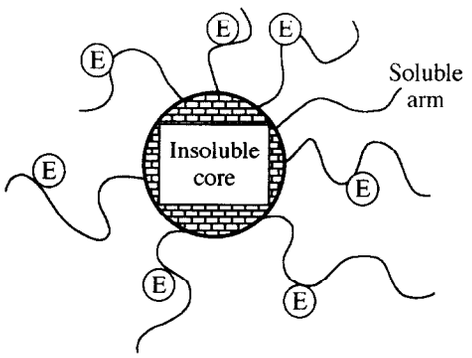

Figure 1 Schematic representation of the biocatalyst structure based on core-shell functional microspheres

described. A slight decrease in the enzyme activity was observed for this complex biocatalyst with respect to the free enzyme. However, the catalytic activity was still rather high, especially when considering the heterogeneous nature of the support and the macromolecular nature of the substrate.

Following our interest in this novel class of tailor-made biocatalysts, this paper reports the effects of the preparation conditions on the microsphere dimensions, the microsphere size distribution and surface functionality. A forthcoming paper will deal with enzyme immobilization and the reactivity of the resulting biocatalyst.

EXPERIMENTAL

Materials

Styrene, benzoyl peroxide (BPO), ethanol and 2-methoxyethanol were purchased from Aldrich and used without further purification. The methacrylic acid/ethyl acrylate 1:1 statistical copolymer (trade name Eudragit L 100-55) was supplied by Röhm Pharma as a powder sample and was characterized by a number average molar mass M_n of 135 000.

Dispersion polymerization procedure

In a typical polymerization, Eudragit (4.8 g) was dissolved under an argon atmosphere over 30 min in 220 ml of a 1:1 (v/v) mixture of ethanol and 2-methoxyethanol heated at 60°C. Under an argon atmosphere and with constant stirring, benzoyl peroxide (1.5 g) dissolved in styrene monomer (36.4 g) was added to the solution and the reaction was allowed to proceed for 24 h. The reaction mixture was then cooled and, after three cycles of centrifugation and redispersion with a 1:1 (v/v) mixture of ethanol and 2-methoxyethanol and with deionized water, the resulting microspheres were dried under vacuum. Reaction yields were between 75 and 85%.

Physicochemical characterization

The amount of Eudragit linked to the microsphere surface was determined by potentiometric titration using tetramethylammonium hydroxide solution in a 9:1 mixture of isopropyl alcohol and water according to the procedure described elsewhere[14]. Average molar masses were determined by size exclusion chromatography (s.e.c.) of chloroform solutions with a Waters 590

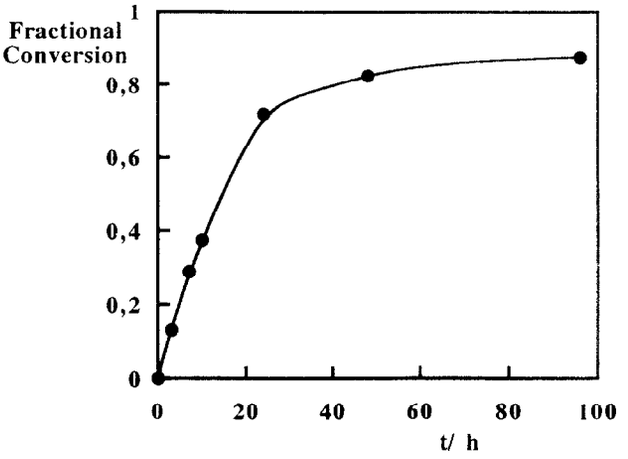

Figure 2 Fractional conversion as a function of time for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0 Eudragit and 1.5 g of BPO

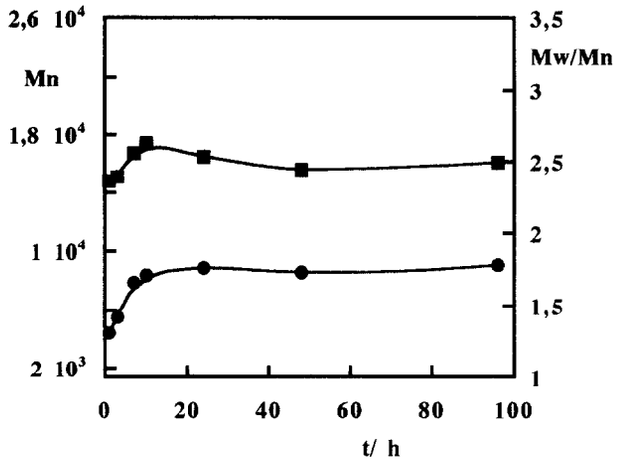

Figure 3 Number average molar mass (■) and first polydispersity index (●) versus time for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0% Eudragit and 1.5 g of BPO

chromatograph equipped with a Shodex KF-804 columm. An ultraviolet (u.v.) spectrophotometer set at 260 nm was used as a detector. Polystyrene standard samples were used for the universal calibration method[15]. The percentage conversion was determined from the residual monomer content by calibrated liquid chromatography using *o*-dichlorobenzene as the internal standard.

Particle dimensions and particle size distribution were measured using a JEOL JSM-35CF scanning electron microscope (operating at an accelerating voltage of 10 kV). The samples were sputter coated under vacuum with a thin layer (1–3 nm) of gold. The magnifications are given by the scales on each micrograph. The scanning electron microscopy (SEM) photographs were digitized using the Kodak photo-CD system and elaborated using the image processing program NIH Image (version 1.55, public domain). From 100 to 200 individual particle diameters were measured for each sample.

RESULTS AND DISCUSSION

All the dispersion polymerization reactions leading to

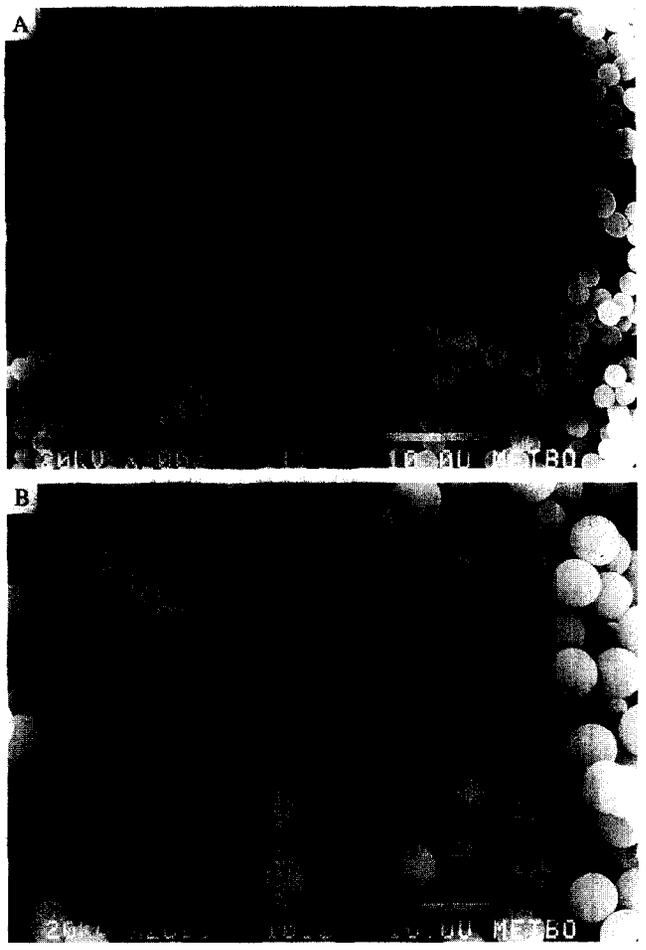

Figure 4 SEM micrographs of the polystyrene microspheres prepared from the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0 Eudragit and 1.5 g of BPO after 10 h (A) and 50 h (B)

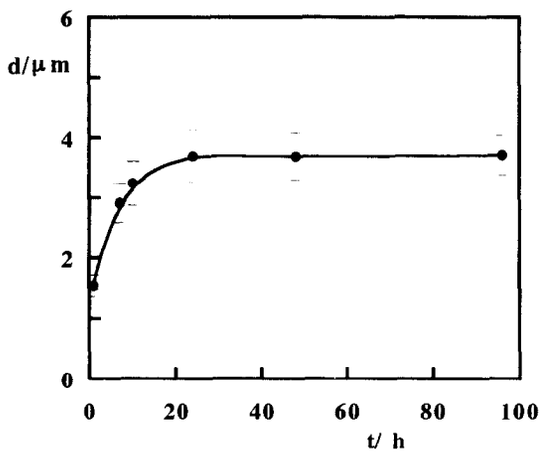

Figure 5 Polystyrene microsphere diameter (including the standard deviation limits) as a function of time for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0 Eudragit and 1.5 g of BPO

functional microspheres entailed polymerizing styrene in the presence of a methacrylic acid/ethyl acrylate 1:1 statistical copolymer (Eudragit) as the steric stabilizer precursor using benzoyl peroxide (BPO) as a free radical initiator in a 1:1 mixture of ethanol and 2-methoxyethanol as the solvent. Because the monomer was soluble in the organic solvent, the polymerization started in a clear, homogeneous system. After an induction period of about 15 min, a faint opalescence was observed. This became stronger after a while and the mixture turned into a white, stable dispersion. At the end of the reaction, the polymeric product was isolated, washed with water and ethanol, centrifuged and dried. The polymerization yield ranged from 70 to 85%.

As a typical example, *Figure 2* reports the fractional conversion versus time profile for the polymerization of styrene with 1.0% by weight of Eudragit and 1.5 g of BPO. The conversion curve is very similar to that typical of solution polymerization with little if any gel effect. *Figure 3* illustrates the changes in the number average molar mass (M_n) and the first polydispersity index (M_w/M_n) of the polystyrene, as determined by s.e.c., as a function of time. M_n initially increases with time and after about 10 h reaches the plateau value of 17 000. The trend in the first polydispersity index closely parallels that in M_n. Initially, M_w/M_n is 1.3 and increases slightly with time, reaching the plateau value of 1.8 after about 10 h. A relatively low number average molar mass (17 000) and a first polydispersity index lower than 2.0 indicate a negligible incidence of the gel effect as deriving from the presence of a parallel emulsion polymerization mechanism. This behaviour is in general agreement with the results[16] obtained in the polymerization of styrene using hydroxypropylcellulose as the steric stabilizer in the same solvent mixture and employing the same initiator.

Representative SEM micrographs of the polymer microspheres at different times are reported in *Figure 4*. *Figure 5* illustrates the particle growth curve as a function of time. The microsphere diameter increases with time steeply at first and then more gradually until the limiting value of 3.7 μm is reached. Irrespective of time, a unimodal distribution was observed with a standard deviation ranging from 0.17 to 0.40 μm. These data indicate that the polymerization reaction can be stopped after 24 h, at that point the product characteristics being stable. The Eudragit content was determined by potentiometric titration as 0.53% for the microspheres recovered after a 24 h reaction time.

To clarify the reaction characteristics as far as the dimensions, the size distribution and the surface functionality of the microspheres are concerned, we performed two sets of experiments on the dispersion polymerization reaction with different amounts of Eudragit and different quantities of BPO, respectively, keeping all the other reaction parameters constant.

In the first set of experiments, the polymerization reactions were carried out for 24 h at 60°C with 1.5 g of BPO and various weight percentages of Eudragit with respect to the total solution weight in the range 0.5–4.0%. For each composition, the reaction was repeated two or three times. The number average molar mass of the polystyrene sample tends to decrease with the Eudragit content, whereas the first polydispersity index is scarcely influenced (*Table 1*). The microsphere dimensions and functionality are substantially affected by the amount of steric stabilizer. *Figure 6* illustrates the change in microsphere diameter as a function of the percentage Eudragit in solution. Within the Eudragit compositional range investigated, the microspheres showed a unimodal

Table 1 Physicochemical characteristics of the polystyrene microspheres prepared for 24 h at 60°C with 1.5 g of BPO, employing various wt% of Eudragit in solution with respect to the total solution weight

% Eudragit in solution	d (μm)	dsdev[a] (μm)	M_n[b]	M_w/M_n[b]	% Eudragit on the microspheres
0.50	3.3	0.29	23 700	2.1	0.41
1.0	3.7	1.00	21 100	1.9	0.53
1.5	3.1	0.39	22 000	2.1	0.58
2.0	3.0	0.18	19 000	1.8	0.57
3.0	2.7	0.78	18 700	1.9	1.00
4.0	2.4	0.56	16 800	2.0	0.97

[a] Standard deviation
[b] By s.e.c., in chloroform at 25°C

Table 2 Physicochemical properties of the polystyrene microspheres prepared for 24 h at 60°C with 1.0% Eudragit in solution, employing different amounts of BPO

BPO/g	d (μm)	dsdev[a] (μm)	M_n[b]	M_w/M_n[b]	% Eudragit on the microspheres
0.5	3.1	0.70	28 500	2.4	1.11
1.5	3.7	1.0	21 100	1.9	0.53
3.0	7.7	0.60	10 300	1.5	0.50
4.5	8.8	3.5	8500	1.8	0.38
6.0	10.5	4.7	7900	1.3	0.27

[a] Standard deviation
[b] By s.e.c., in chloroform at 25°C

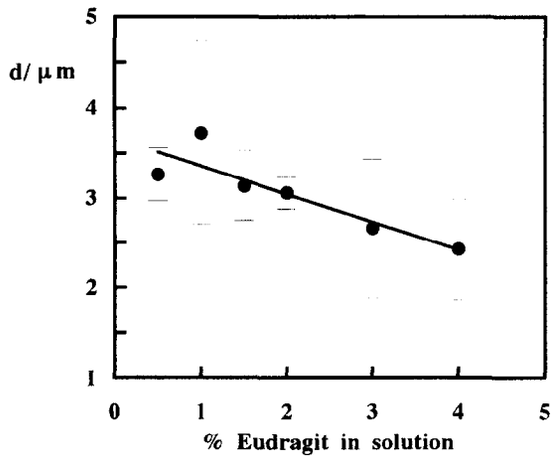

Figure 6 Polystyrene microsphere diameter (including the standard deviation limits) as a function of the Eudragit content in solution for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.5 g of BPO

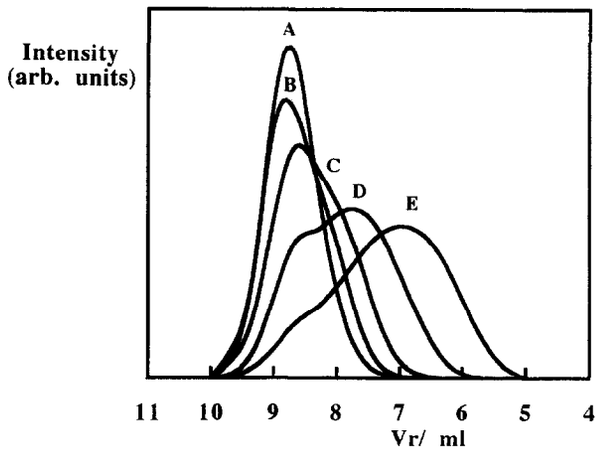

Figure 7 S.e.c. traces of the polystyrene samples from the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with different amounts of BPO: (A) 6.0 g; (B) 4.5 g; (C) 3.0 g; (D) 1.5 g; (E) 0.5 g

diameter distribution with mean diameter values in the 2.4–3.7 μm range and standard deviations ranging from 0.18 to 0.82 μm. Occasionally, bimodal diameter distributions were observed, possibly resulting from two successive nucleation processes. Therefore, the results of these preparations were excluded from the averaged values reported. The microsphere diameter decreases more or less linearly as the Eudragit content increases.

The Eudragit content of the microspheres, estimated as described above, increases from 0.41% corresponding to 0.5% Eudragit in solution, to 1.0%, corresponding to 4.0% Eudragit in solution, as reported in *Table 1*. These data indicate that as the amount of Eudragit in solution increases, so does the grafting rate to the stabilizer, producing more nuclei and hence smaller final microspheres, lower molar mass values for the polystyrene samples, and more graft copolymer, resulting in a greater Eudragit content.

In the second set of experiments, the polymerization reactions were carried out for 24 h at 60°C with 1.0% Eudragit in solution and different amounts of BPO in the range 0.5–6.0 g. Also in this set, the reaction was repeated two or three times for each composition. As a typical example, *Figure 7* reports the s.e.c. traces of the polystyrene samples prepared starting from different amounts of BPO. All the polystyrene samples show unimodal s.e.c. curves that are shifted towards lower values along the elution volume scale as the BPO content decreases. M_n and M_w/M_n values, collected in *Table 2*, decrease with increasing BPO content, the former going from 28 500 to 7900 and the latter from 2.4 to 1.3.

The microsphere diameter increases proportionally to the BPO content to the power 0.52, as illustrated in *Figure 8*. This exponent is slightly higher than reported in previous works concerning the polymerization of styrene using azobisisobutyronitrile as the initiator and poly(*N*-vinylpyrrolidone) as the steric stabilizer precursor, in which exponents in the range 0.38–0.40 are reported[6,17]. For BPO contents lower than or equal to 3.0 g, narrow and unimodal diameter distributions were obtained, whereas for higher BPO contents very broad, although still unimodal, distributions were detected. An increase in microsphere diameter with increasing free radical initiator content has been described for different polymerization systems[2,6,17]. This effect may result from the lower molecular mass of the formed polystyrene and hence of the graft copolymer, which is more soluble and less effective as a stabilizer. The Eudragit content of the microspheres decreases substantially with increasing BPO content, as illustrated in *Figure 9*.

CONCLUSIONS

Polystyrene microspheres ranging in diameter from 3 to 10 μm were prepared by dispersion polymerization of styrene in the presence of a methacrylic acid/ethyl acrylate 1:1 statistical copolymer (Eudragit) as the stabilizer precursor in alcoholic media. The effects of the Eudragit and initiator concentrations on the

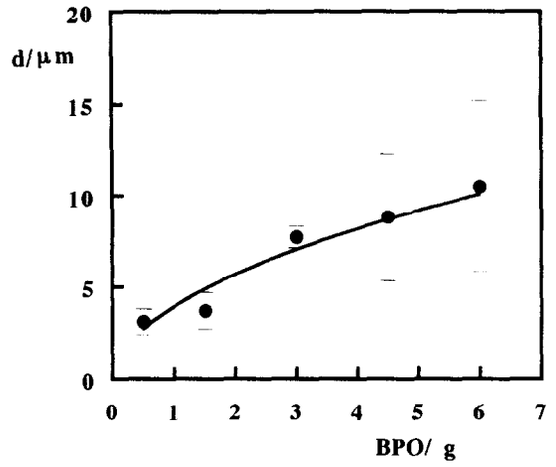

Figure 8 Polystyrene microsphere diameter (including the standard deviation limits) as a function of the amount of BPO for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0 Eudragit

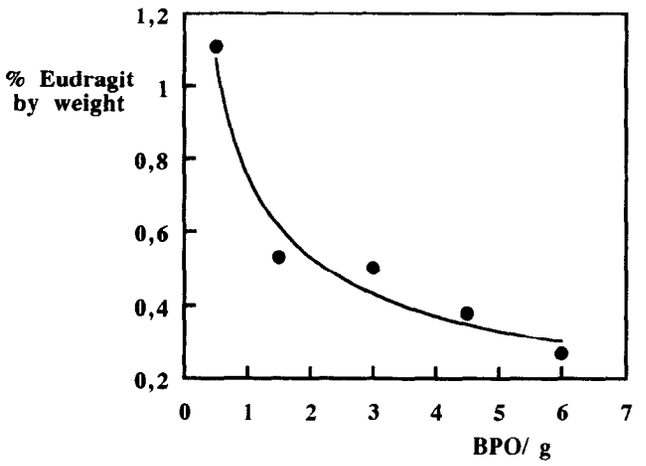

Figure 9 Eudragit content of the microspheres as a function of the amount of BPO for the polymerization of styrene in ethanol and 2-methoxyethanol (1:1 v/v mixture) with 1.0 Eudragit

microsphere dimensions, the microsphere size distribution and surface functionality were systematically investigated. Within the range of Eudragit contents employed, microspheres with narrow, although not monodisperse, size distributions were obtained. In contrast, the size distribution was found to be very sensitive to the initiator content leading to narrow size distributions for low initiator contents but very broad size distributions for high initiator contents. The particle dimensions were found to increase with increasing initiator concentration and decrease with increasing steric stabilizer precursor concentration. In contrast, the Eudragit content of the microspheres was found to increase with the stabilizer precursor concentration and decrease with the initiator concentration. This finding is of great practical relevance in that it allows the independent modelling of both the microsphere dimensions and the relevant surface functionality. Accordingly, the described approach to the preparation of monodisperse, core-shell functional microspheres as tailor-made biocatalytic supports is most promising because of its versatility and the possibility it offers to predetermine the microsphere dimensions and functional surface density.

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