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A morphological study of molecularly imprinted polymers using the scanning electron microscope

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Abstract

Molecular imprinting is an emerging technique for producing polymers with applications in affinity-based separation, in biomimetic sensors, in catalysis, etc. This variety of uses relies upon the production of polymers with different affinities, specificities, sensitivities and loading capacities. Research into the development of molecular imprinted polymers (MIPs) with new or improved morphologies – which involves modification of the polymerisation process – is therefore underway. This paper reports a comparative study of non-covalent MIPs synthesised by "bulk" polymerisation using digoxin as template. These were synthesised under different conditions, i.e., changing the functional monomers employed (methacrylic acid or 2-vinylpyridine), the porogens (acetonitrile or dichloromethane) used, and by altering the volume of the latter. The polymerisation process was allowed to proceed either under UV light or in a thermostat-controlled waterbath. The surface morphology (was determined by scanning electron microscopy) and the ability of the different polymers to selectively rebind the template was then evaluated.
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Keywords: Molecularly imprinted polymers; Digoxin; Scanning electron microscope; Synthesis comparison

1. Introduction

Molecular imprinting technology is a new and revolutionary way of producing recognition sites for specific analytes in synthetic polymers [1–3]. The shape, size and positions of the functional groups in the recognition sites generated are complementary to those of the original analyte. Thus, molecularly imprinted polymers (MIPs) rebind their original analytes in preference to related molecules. Some of these polymers have affinity constants comparable to those of natural receptors such as antibodies [4]. MIPs have therefore been called "plastic antibodies" by some authors [5].

In recent years, molecular imprinting has been used in many areas of chemistry, biochemistry, biotechnology and medicine [6–8]. Given the versatility, high specificity and recognition that can be achieved, the future of MIPs would seem bright. These polymers have been used in the synthesis of receptors for many analytes, such as drugs, herbicides, proteins and toxins, etc., and as adsorbents in chromatographic and electrophoretic separation techniques. Owing to their affinity for their ligands, MIPs have also been used as sensors in industrial, diagnostic and environmental analyses.

The different applications of MIPs rely on the specific properties and configurations of the polymers on which their structures are based. In response to the demand for these molecules, new production methodologies are under development including "bulk" [1], suspension [9], two-step swelling [10], precipitation and emulsion and core-shell polymerisation [11–14]. Currently, however, there are few studies on how different synthesis conditions affect MIP structure. In this work, several MIPs were prepared for digoxin using the bulk polymerisation method, but under different synthesis conditions, i.e., different monomers and porogens, as well as different polymerisation and extraction processes were used. This produced polymers with different structural conformations and characteristics (hardness, porosity, stiffness, loading capacity, strength, etc.). The scanning electron microscope (SEM) was used to examine these MIPS morphologically; the excellent resolution provided by the SEM makes it one of the best tools for this purpose.

The analyte binding capacity, binding specificity and chemical and thermal capacities of these MIPs were found to depend directly on the characteristics of their surface morphology.

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2. Experimental section

2.1. Apparatus and materials

The intensity of fluorescence was measured using a Perkin-Elmer LS 50B spectrofluorimeter equipped with a 100 μL Hellma flow cell (Jamaica, NY, USA) with optical path 3 mm. This was used in conjunction with a Hewlett-Packard computer. All pH readings were made with a Metrohm 654 pH meter. A Gilson Minipulse 2 pump, Omnifit injection valve (six way) and PTFE tubes 0.5 mm i.d.) were employed to build the manifold. A Digiterm 3000542 thermostat-controlled waterbath was used to provide constant polymerisation temperatures.The morphology of the polymer particles was characterised by SEM using a Jeol model JSM-6400. MicrowavePAAR (Anton Paar, France).

2.2. Reagents

The ethylene glycol dimethacrylate (EDMA), methacrylic acid (MAA) and 2-vinylpyridine (2-VP) used were from Merck (Darmstadt, Germany). 2-2′-Azobisisobutyronitrile (AIBN) was from Fluka. Digoxin labelled with FTC (10 μmol L^{-1}) was purchased from Helena BioScience (Sunderland, UK). Digoxin (95%) was purchased from Sigma–Aldrich (Madrid, Spain). Phosphate buffer solution (PBS, pH 7.5, in NaCl 0.1 M, KH_2PO_4 1.4 mM, NaH_2PO_4 8 mM, KCl 2.7 mM and $MgCl_2$ 21.3 mM) was purchased from Merck (Darmstadt, Germany), and HPLC-grade solvents (acetonitrile, methanol and dichloromethane) from Sigma–Aldrich (Madrid, Spain).

2.3. Preparation of polymers by "bulk" polymerisation

Several polymers were prepared under different synthesis conditions using digoxin as template (Table 1). A mixture of a functional monomer (MAA or 2-VP), EDMA and AIBN was dissolved in the porogen (dichloromethane or acetonitrile) in a 25 mL glass tube along with the template molecule. This mixture was purged with nitrogen for 7–8 min. The glass tube was then placed in a thermostat-controlled waterbath at 60 °C for 10 h (polymers D6, D6.1), 16 h (A, A1), 20 h (B1, D2, D5, D7, D7.1, D9) or 24 h (D3, D10), or under a UV source (365 nm) at 10 °C for 24 h (B, D) (see Table 1). Once the polymers were produced, the glass tube was broken to obtain the polymer block. This was crushed and ground in a mechanical mortar to obtain particles of different size for analysis. Finally, the template was removed from the imprinted polymers to create the binding sites. This was done in two ways: soxhlet extraction with a mixture methanol:acetonitrile (50:50 v/v) for 10 h (polymer A), 17 h (D6.1), 20 h (D, D2, D7, D7.1, D9) or 25 h (D3, D5, D10); or microwave extraction (MW) for 10 min with acetonitrile (A1), dichloromethane (B) or a mixture of methanol:acetonitrile (D6) (see Table 1). Non-imprinted polymers were prepared in the same way but without adding the template molecule.

2.4. Fluoroligand binding assays

To verify that the polymers were specific for digoxin, the binding of this molecule was confirmed using FTC-digoxin as a labelled ligand. For this experiment, MIPs with a functional monomer–template molar ratio (T/M) of 10^{-3}/1 (polymers B, D, D2) and 10^{-3}/11 (D3, D6) were used. A 0.02 g of polymer particles were mixed with 100μL of a solution of FITC-digoxin 10^{-5} μmol L^{-1}) and 900 μL of ACN and PBS (D, D2, D3, D6, B) or dichloromethane (D, D2, D3, B). This mixture was incubated overnight at room temperature. The supernatant was collected and its fluorescence measured.

3. Results and discussion

3.1. Comparative study of MIP and non-imprinted structures

Fig. 1 is a scanning electron micrograph showing the morphology or structure of the digoxin-imprinted MIP and its

Table 1
Polymer compositions and methods of preparation

Polymer	Monomer (M) (mmol)	T/M	EDMA (mmol)	M/EDMA	AIBN (mmol)	Porogen (mL)	Reaction conditions	Method and time of extraction
A	MAA (2)	10^{-3}/1	6	1:3	2×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 16 h	Soxhlet (MeOH/ACN), 10 h
A1	MAA (2)	10^{-3}/1	6	1:3	2×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 16 h	MW (MeOH/ACN), 10 min
B	MAA (2)	10^{-3}/1	10	1:5	6×10^{-2}	CH_2Cl_2 (10 mL)	UV (365 nm), 10 °C, 24 h	MW (MeOH/CH_2Cl_2), 10 min
B1	MAA (22)	10^{-3}/11	10	11:5	6×10^{-2}	CH_2Cl_2 (8 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/CH_2Cl_2), 20 h
D	MAA (2)	10^{-3}/1	10	1:5	6×10^{-2}	ACN (10 mL)	UV (365 nm), 10 °C, 24 h	Soxhlet (MeOH/ACN), 20 h
D2	MAA (2)	10^{-3}/1	10	1:5	6×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/ACN), 20 h
D3	2-VP (22)	10^{-3}/11	1	22:1	6×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 24 h	Soxhlet (MeOH/ACN), 25 h
D5	MAA (4)	10^{-3}/2	6	2:3	18×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/ACN), 25 h
D6	MAA (22)	10^{-3}/11	10	11:5	6×10^{-2}	ACN (5 mL)	Waterbath, 60 °C, 10 h	MW (MeOH/ACN), 10 min
D6.1	MAA (22)	10^{-3}/11	10	11:5	6×10^{-2}	ACN (5 mL)	Waterbath, 60 °C, 10 h	Soxhlet (MeOH/ACN), 17 h
D7	MAA (22)	10^{-3}/11	10	11:5	6×10^{-2}	ACN (8 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/ACN), 20 h
D7.1	MAA (22)	10^{-3}/11	10	11:5	6×10^{-2}	ACN (15 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/ACN), 20 h
D9	2-VP (22)	10^{-3}/11	6	11:3	18×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 20 h	Soxhlet (MeOH/ACN), 20 h
D10	MAA (22)	10^{-3}/11	1	22:1	6×10^{-2}	ACN (10 mL)	Waterbath, 60 °C, 24 h	Soxhlet (MeOH/ACN), 25 h

Digoxin: template molecule (2×10^{-3} mmol); EDMA (ethylene glycol dimethacrylate): cross-linker; MAA (methacrylic acid)/2.VP (2-vinylpyridine): functional monomers; AIBN (α,α′-azoisobutyronitrile): initiator.

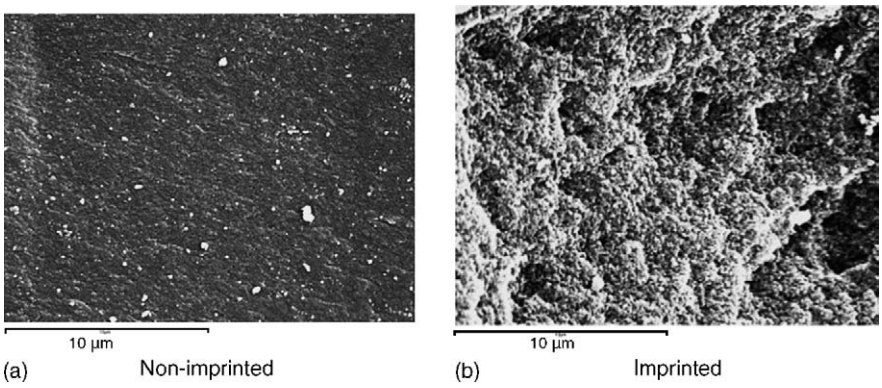

Fig. 1. SEM micrographs of polymer B: (a) non-imprinted and (b) imprinted with digoxin as the molecule template.

non-imprinted counterpart. Polymer B (Table 1) and its corresponding non-imprinted control were synthesised by exactly the same method, but by excluding the template in the latter (molar ratio of MAA:EDMA 1:5; porogen = dichloromethane; UV polymerisation; MW extraction in dichloromethane). Both polymers were ground to a particle size of 100–200 μm.

The images show appreciable differences in the morphology of the polymers. The non-imprinted polymer had a more uniform, smooth shape than the imprinted polymer which had an irregular, rough morphology (rather like microparticles with small cavities). The regular structure of the non-imprinted polymer was due to the fact that no specific binding sites had been created for the analyte. The cavities in the MIP were probably caused by the structure of the target molecule (digoxin).

3.2. Structural variations arising under different synthesis conditions

Polymers D3 and D10 were both synthesised using different monomers (2-VP and MAA) while maintaining the rest of the reactants and polymerisation variables constant. Both polymers were powdery; therefore, no grinding was required. The template was removed by soxhlet extraction (Table 1). Fig. 2 shows the morphology of the polymers obtained.

The polymers synthesised with 2-VP were harder than those made with MAA, and felt and looked very like amber with their transparent, yellowish colour. Their structure was very compact and the smooth surface had some 'parallel' grooves. Polymerisation with MAA led to a morphology rather like a conglomerate of beads. These polymers were more porous, with small cavities between larger ones. The D3 polymer had no surface cavities. This might mean that no binding occurred between the template and the monomer. However, in the D10 polymer synthesised with MAA, holes and cavities complementary to the template molecules were observed.

An important factor in the formation of polymers is the nature of the porogen and the volume used. The porogen plays an important role in the final polymer morphology, influencing its surface morphology and the pore diameter. This helps in its recognition of the specific analyte. Fig. 3 shows polymers B1 and D7, synthesised under the same conditions but with a different porogen (dichloromethane and ACN, respectively). The results obtained agree with those in the literature; in general, polymers synthesised with ACN showed more microporous shapes than polymers synthesised with others solvents such as dichloromethane or chloroform. Polymers D7 and D7.1 were synthesised with the same porogen (ACN) but with different volume (8 and 15 mL, respectively; see Table 1). These polymers were ground to a size of 200–355 μm. Fig. 3 shows that the larger volume produced better-defined spherical polymer microparticles, similar to those obtained in precipitation polymerisation.

The polymerisation method also influenced the specific recognition characteristics as well as the internal and external morphology of the polymer. Different conditions greatly

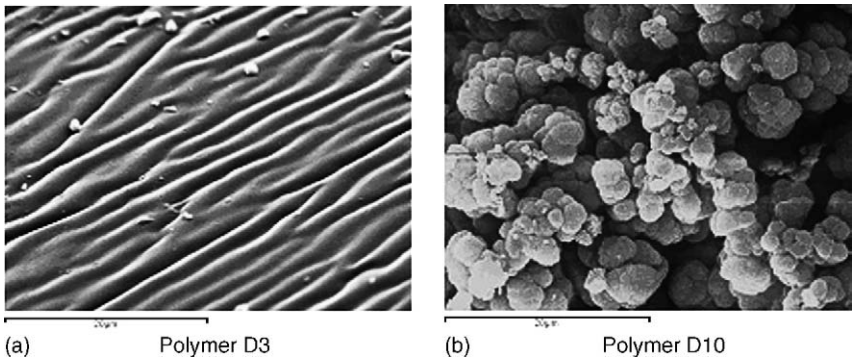

Fig. 2. SEM picture of digoxin-imprinted polymers made with different functional monomers. (a) D3 synthesised with 2-VP, (b) D10 with MAA. The porogen was ACN; polymerisation occurred in a thermostat-controlled waterbath (60 °C for 24 h).

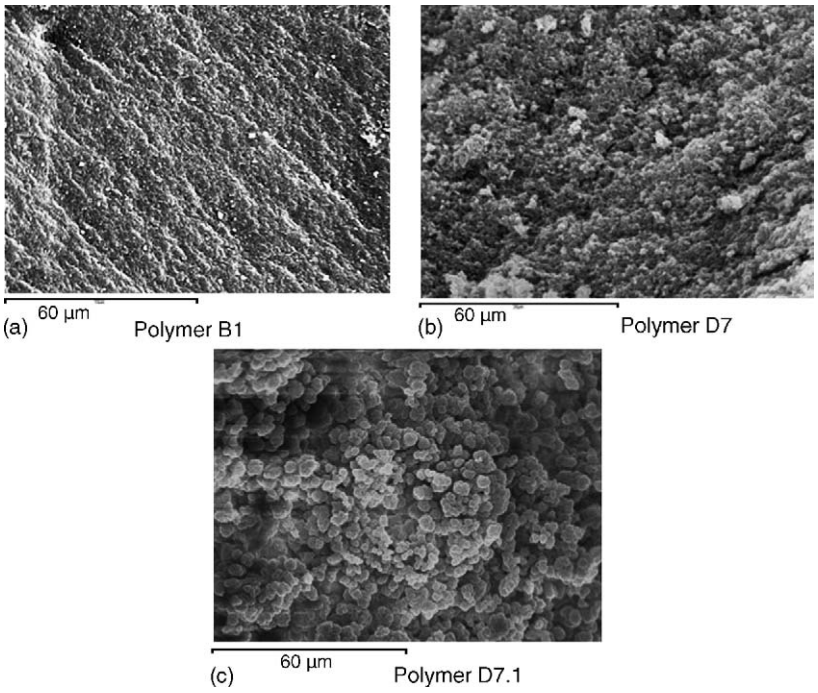

Fig. 3. SEM micrographs of imprinted polymers synthesised with MAA and different porogens: (a) Polymer synthesised with 8 mL of dichloromethane; (b) and (c) with 8 and 15 mL of ACN, respectively. Molar ratio of MAA:EDMA 11:5. Polymerisation occurred in a waterbath (60 °C for 20 h); the MW extraction (MeOH/ACN) fraction was 50% (v/v).

modified the polymer structure (Fig. 4). All polymers were synthesised with either MAA and ACN as the porogen. Polymers D and D2 were made under UV light and in a thermostat-controlled waterbath, respectively. Extraction was performed by the soxhlet method (MeOH/ACN: 50:50 v/v) over 20 h. The molar ratio of MAA:EDMA was 1:5. Both polymers were ground to a particle size of 355–600 μm. Scanning electronic micrography showed the textures of these polymers to be different. For both polymers, agglomerates of microparticles of different sized were obtained. Polymer D, obtained with UV initiation, showed a microporous structure. However, polymer D2 (obtained in the waterbath) showed particles with a beaded appearance. These particles were larger, more spherical and the entire structure was more compact. The slow polymerisation process gave more time for homogeneous, spherical particles to be formed.

Structural variations depending on the template extraction process were also analysed. Polymers D6 and D6.1 were synthesised under identical conditions (see Table 1), but the template was extracted by either the MW or soxhlet method (Fig. 5). Both polymers were ground to a particle size of 355–600 μm. The SEM micrographs obtained showed no appreciable differences, except that the structure was more hollow after soxhlet extraction. This was probably due to the fact that soxhlet extraction is a longer and more aggressive process; the polymer remains in contact with hot solvents for a long period of time, during which its structure is attacked.

3.3. Fluoroligand binding assay

Five polymers from the all synthesised were chosen for binding studies. This selection was made in order to choose the most representative ones using different synthesis conditions (functional monomer, porogen, synthesis reaction). Fluorescence spectroscopy was used to determine the binding affinity of the

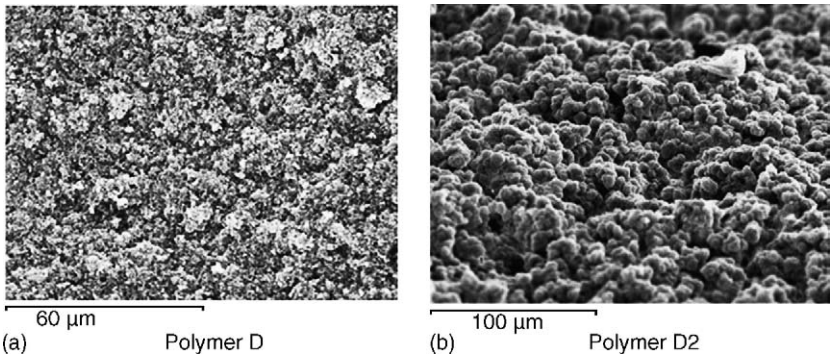

Fig. 4. SEM of MIP particles imprinted with digoxin under (a) UV light (365 nm) at 10 °C for 24 h and (b) in a thermostat-controlled waterbath (60 °C for 20 h).

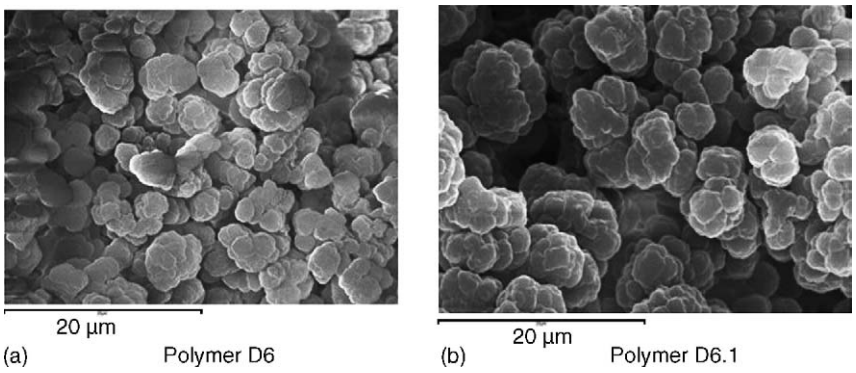

Fig. 5. The analyte-template was extracted with a mixture MeOH/ACN (50% v/v). (a) MW extraction for 10 min. (b) Soxhlet extraction for 17 h.

Table 2
Uptake of digoxin-FTC from a solution of ACN, PBS 10 mM and dichloromethane

Polymer	Percentage uptake in ACN			Percentage uptake in PBS			Percentage uptake in dichloromethane		
	Control	Imprinted	Specific	Control	Imprinted	Specific	Control	Imprinted	Specific
D	19	61	42	0	0	0	15	23	8
D2	37	89	52	37	77	40	13	47	34
D3	62	27	0	42	7	0	20	3	0
D6	41	61	20	75	85	10	–	–	–
B	10	63	53	0	67	67	13	58	45

digoxin MIP. Different uptake solvents were tested: an aqueous solution (PBS) and organic solvents (ACN or dichloromethane). Table 2 shows the results obtained expressed as the amount of digoxin bound to each polymer and in terms of specific uptake (the percentage of rebinding to the imprinted polymer minus the percentage of non-specific rebinding to the non-imprinted polymer).

The uptake behaviour in the different media depended not only on the uptake medium used, but also on the polymer structure. Polymer B showed the greatest specific binding in all uptake media especially in the aqueous solution (Table 2). It also showed a very low binding affinity for the control polymer. However, polymer D3 and D6, synthesised with larger amounts of MAA, showed higher levels of non-specific binding (control polymer). This suggests that an excess of free functional monomer might lead to strong interactions between the analyte and the control polymer. As expected, the specific binding recorded for polymer D3 was null in all the media tested. These results agree with those of the morphological studies above which show D3 to have a compact structure (i.e., with no specific cavities induced by the template).

4. Conclusions

MIPs show a very high degree of selectivity for their target substances. To date, most of the literature related to MIPs have been concerned with methods of synthesis; few have tried to explain the morphology and structure of these polymers. The morphological studies of the present work connect the structural characteristics of MIPs synthesised under different conditions (monomers, cross-linkers, polymerisation, etc.) with the results of ligand-binding assays.

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References

[1] K. Mosbach, O. Ramström, Biotechnology 14 (1996) 163.
[2] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812.
[3] K. Shea, J. Trends Polym. Sci. 2 (1994) 166.
[4] G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, Nature 361 (1993) 645.
[5] D. Barceló, Trends Anal. Chem. 18 (3) (1999) 137.
[6] K. Ensing, T. De Boer, Trends Anal. Chem. 18 (3) (1999) 138–145.
[7] Y. Tan, Z. Zhu, P. Wnag, L. Nie, S. Yao, Talanta 55 (2001) 337–340.
[8] M. Petcu, P.N. Schaare, C. Cook, J. Anal. Chim. Acta 504 (2004) 73–79.
[9] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769.
[10] K. Hosoya, K. Yoshizako, N. Tanaka, K. Kimata, T. Anaki, J. Haginaka, Chem. Lett. (1994) 1437.
[11] N. Pérez-Moral, A.G. Mayes, MRS Symp. Proc. 723 (2002) 61.
[12] N. Pérez, M.J. Whitcombe, E.N. Vulfson, J. Appl. Polym. Sci. 77 (2000) 1851.
[13] N. Pérez, M.J. Whitcombe, E.N. Vulfson, Macromolecules 34 (2001) 830.
[14] L. Ye, I. Surugiu, K. Haupt, Anal. Chem. 74 (2002) 959.