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RESEARCH PAPER

Stereoselective Release Behaviors of Imprinted Bead Matrices

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ABSTRACT

In this work, the stereoselective release behaviors of "low"-swelling molecularly imprinted polymer (MIP) bead matrices in pressed-coat tablet type were studied. Either R-propranolol selective MIP or S-propranolol selective MIP was combined with excipients and racemic propranolol and fabricated into the matrix. Subsequently, the release of different propranolol enantiomers from the matrices was examined. Also, the microscopic structure of the hydrated "low"-swelling MIP matrix was determined using a cryogenic scanning electron microscope in order to compare with that of the hydrated "high"-swelling MIP matrix. In vitro release profiles of the "low"-swelling matrices showed a difference in the release of enantiomers, in that the non-template isomer was released faster than the template isomer. However, in the last phase of dissolution this difference reduced and later reversed, resulting at last in the type of specificity being similar to that obtained previously with "high"-swelling MIP matrices.

In summary, MIP beads can be fashioned into matrices and incorporated into different formulations to regulate the resultant stereoselectivity. From the behaviors of stereoselective release observed in MIP matrices, we can conclude that the enantioselective-controlled delivery mechanism of MIPs via formulations depends on the relative affinity of the enantiomer for the template sites, as well as the nature of the polymer, such as hydrophobicity and swellability.

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Key Words: *Enantiomer; Matrix; Molecularly imprinted polymer; Propranolol; Stereoselective release*

INTRODUCTION

The use of polymeric-based systems that allow the controlled release of drugs is a well-established strategy for modifying the delivery and absorption of therapeutically-active molecules (1–4). A recent study has developed the strategy further by examining the potential of producing molecularly imprinted polymer (MIP) bead-based matrices with a view to developing molecular-specific polymer release systems. Such systems offer the possibility of generating differential release between enantiomers from a racemic mixture. It might thus be feasible to engineer systems in which the release of the more therapeutically-active enantiomer is promoted, while the release of the less or non-active enantiomer is retarded.

In an earlier study (5), we reported the applicability of MIP bead-based matrices for enantioselective-controlled release of racemic propranolol. The preparation of such polymers depends upon generating an accurate molecular template of the enantiomer of interest. Such templates are required to retain their chiral recognition both during processing and then until the drug is released from the matrix. It is possible that the selectivity of sites within the polymer might be modified if, for example, the polymer matrix is compressed into a tablet. Drug release might then be a combined process involving desorption from highly selective sites and from sites that are less selective due to distortion caused by the compression process. However, our original study utilized a ''high''-swelling MIP matrix (5). If the MIP matrix is fabricated from a swelling polymer then as that polymer hydrates and structural spacing alters, the selectivity of release might gradually be lost. To investigate this, a ''low''-swelling MIP matrix was designed and the stereoselective release behaviors of this type of matrix were determined. In the present study, ''low''-swelling MIP matrices were prepared by compressing hydrogenated vegetable oil (HVO)-coated granules of the MIP beads together with other excipients. Subsequently, the in vitro release of the separate enantiomers of racemic propranolol was examined from the prepared matrices. Furthermore, cryogenic scanning electron microscopy (Cryo-SEM) of the MIP matrices was carried out to determine the microscopic structures of the matrices.

EXPERIMENTAL

Materials

The bead polymers employed were imprinted with either R-propranolol or S-propranolol of the same batches as in our earlier work (5). Polyvinyl pyrrolidone (PVP) K30 was purchased from BASF (Ludwigschaften, Germany) and HVO was supplied by Mendell (New York, USA). Eudragit®-E100 was obtained from Rohm Pharma (Weiderstadt, Germany). Racemic propranolol HCl was purchased from Aldrich Chemical Company (Wisconsin, USA). All other chemicals and solvents were of analytical–reagent grade and used as received.

Characterization of Polymers

The bead polymers were synthesized using a suspension polymerization method outlined by Mayes and Mosbach (6) using perfluoro cyclohexane as disperse phase and 2,2′-azobisisobutyronitrile as initiator, methacrylic acid as functional monomer and ethyleneglycol dimethacrylate (EDMA) as cross-linker. The polymers employed here were characterized in terms of swelling, size, surface area, and porosity. The approximate range in particle size was determined by measuring 300 particles per sample using an optical light microscope (Olympus, Tokyo, Japan). The swelling of polymers was determined from the ratio between the volume of swollen polymer and the volume of dry polymer in phosphate buffered saline (PBS) pH 7.4, as described previously (5), and the results are the averages of triplicate determinations. In this study, the porosity measurement of bead polymers was also performed. The determinations of pore volumes and specific surface areas were carried out by nitrogen adsorption/desorption techniques using an Autosorb-1 series surface area and pore analyzer (Quanta-

chrome Cooperation, Florida, USA) which enables pores between 20 and 3000 Å to be measured. The samples were degassed at 150°C and a 40-point pressure table was used. The surface area was determined from a Brunauer, Emmett, and Teller (BET) plot, whilst the average pore diameter and the cumulative pore volume were obtained using a Barrett, Johner, and Halenda (BJH) model of the adsorption isotherm.

Tablet Formulation

In this experiment, the tablet formulation was designed to be a slow-swelling matrix and was prepared as a pressed-coat tablet, containing either an R- or S-propranolol-imprinted polymer layer and racemic propranolol as a component of the core of the tablet. Further details of the tabletting procedure are as follows. First, the core drug tablets were prepared using a Manesty F3 single punch tabletting machine (Liverpool, UK) fitted with 1/8-inch diameter flat-faced punches from 10 mg of racemic propranolol under a compression force of 2 kN. To prepare the coated granules, 250 mg polymer was mixed with 16 mg HVO and 24 mg PVP as 20% (w/v) PVP solution in ethanol. The resulting mixture was then granulated by forcing the powder mass through an 18-mesh sieve and dried overnight. Finally, the pressed-coat tablets were prepared by placing 130 mg of the coated granules in a 3/8-inch diameter die and precompressing. The core tablet was carefully positioned in the center of the compact and 160 mg of the remaining coated granule was added. The granules were compressed around the core using flat-faced punches at a compression force of 4 kN, yielding 300 mg tablets (with about 4 kg tablet hardness). The tablets were then dip-coated with 20% Eudragit-E100 in acetone:isopropanol (1:1 v/v), using the same method as reported previously (5). The film-layer thickness was about 0.1 mm for all tablets.

In Vitro Release Testing

Dissolution tests were performed using the paddle method described in United States Pharmacopeia (USP) XXIII, with 1 L phosphate buffer pH 7.4 as dissolution medium, at 37 ± 0.5°C, with paddle rotation speed 50 rpm. A 2-mL sample was collected at appropriate time points and immediately assayed after withdrawal of samples from the dissolution medium. An equal volume of fresh dissolution medium was replaced to maintain the original volume. Then, the amounts of each propranolol enantiomer released were assayed using a chiral high performance liquid chromatography (HPLC) analytical method outlined in the next section. Each test was carried out in sets of six.

Analysis Methods

The analysis of propranolol enantiomer content in samples was performed directly by a chiral-HPLC method. The system comprised a Waters 600 HPLC (Bedford, MA) with a Waters 717 plus autosampler (100 µL injection loop), equipped with a Waters 996 photodiode array detector set at 290 nm. Chromatograms were recorded using Millennium PDA software. The column was a 150 mm × 3.1 mm i.d., particle size 5 µm chiral-AGP protein column (ChromTech, Hägersten, Sweden). The mobile phase was 0.5% (v/v) isopropyl alcohol in 50 mM ammonium acetate buffer (pH 4.2) with a flow rate of 1.0 mL/min. Typical retention times were 8.2 min for R-propranolol and 11.5 min for S-propranolol. The cumulative percentages of each enantiomer released were plotted against time. In addition, the ratios of the cumulative percentages of the propranolol enantiomers released were plotted as a function of time. The paired t-test was employed to compare the amounts of each enantiomer released at every time point. The significance level was set at $p < .05$. The release rate constant (k) was calculated using the time exponent: $M_t/M_\infty = kt^n$, where M_t/M_∞ is the fractional release of the enantiomers, t is the release time, and n is the release exponent indicating the type of drug release mechanism.

Cryogenic-Scanning Electron Microscopy

Matrices were hydrated in PBS pH 7.4 at room temperature. After 7 days, the samples were carefully removed and sectioned through an undisturbed portion of the tablet. The specimen was positioned on a sample holder, rapidly frozen in liquid nitrogen, etched under vacuum, and gold-coated. Images were obtained at 25 keV on a JSM 5800LV scanning electron microscope (Jeol, California, USA).

RESULTS

Characteristics of Bead Polymers and Tablet Preparation

Table 1 summarizes the characteristics of R- and S-propranolol selective MIPs in respect to swelling, size, surface area, pore volume, and pore diameter. Visually, both polymers contracted reversibly during drying and expanded on re-immersion in PBS pH 7.4. R-Propranolol selective MIP exhibited slightly higher swellability than S-propranolol selective MIP. The estimated size range of primary particles of the polymers was between 1 and 5 μm, indicating the relatively narrow size distribution of the polymers. The total pore volumes of S-propranolol MIP and R-propranolol MIP were 0.004 and 0.013 cm^3/g, respectively. The pore diameters of S-propranolol MIP and R-propranolol MIP were 120 and 100 Å, respectively. It is noted that the surface areas and pore volumes of the R- and S-propranolol MIPs are different. Further, the nitrogen adsorption–desorption isotherms of the MIPs are open hysteresis loops. The desorption plots indicate a lower average pore diameter with a narrow size distribution, in comparison to the pore sizes obtained from the adsorption plots. This suggests the materials have ink-bottle or narrow-neck pores, findings that concur with those from a previous work (7).

The MIPs employed in the current study were applied as pressed-coat matrices to a core containing the tabletted drug. Thus the MIP surrounded the whole of the tablet surface as a drug-free polymeric shell, having been applied by a double-compression technique. When the shell becomes completely hydrated, the medium penetrates into the core and starts to dissolve the drug. The release of the drug starts only after this lag time when the drug can diffuse outward. The drug always passes through the whole of the extended bulk phase of the hydrated polymer before leaving the matrix, and this process might enhance any possible interaction of the enantiomer with the MIP.

In a previous study (5), "high"-swelling matrices composed of either R-propranolol-imprinted polymer or S-propranolol-imprinted polymer containing the racemate of propranolol were prepared. Other excipients, including PVP and HVO, were employed to formulate the final matrix. The preparation method involved mixing, using low shear force, HVO with dry granules, the latter being composed of polymer and drug. The HVO is an excipient, generally added in this manner to produce lipophilic-based controlled-release formulations. However, it can also be exploited as a coating material such that there is a more intimate contact with the matrix components. Here, for example, granules of MIP were coated with HVO in an effort to counteract the swelling of EDMA-based polymers in aqueous environments. Thus, in the preparation of such matrices, a damp mass of the MIP and other additives was blended with HVO to form a film layer after granulation. The quantity of HVO used was the same as that employed to produce what was intended to be the "high"-swelling matrices. In addition, the original specificity of the MIPs in the "low"-swelling matrices was completely analogous to that of the "high"-swelling matrices, because the same batches of MIPs were used in the preparation of both tablet formulations.

The swelling behavior of the two different matrix formulations of the same MIP containing S-propranolol-imprinted polymer was examined in PBS pH 7.4. It was found that, after 3 days of solvent exposure, the "low"-swelling matrix showed no change in tablet diameter. In contrast, after the same period of time the diameter of the tablet which included the "high"-swelling matrix had increased by 4 mm (Fig. 1). After 7 days of

Table 1

Polymeric Characteristics

Polymer Type	Swelling (mL/mL)	Particle Size (μm)[a]	Surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Diameter (Å)
S-Propranolol-MIP	1.58	1–5	1.2	0.004	120
R-Propranolol-MIP	1.67	1–5	0.5	0.013	100

[a]The estimated size of primary particles.

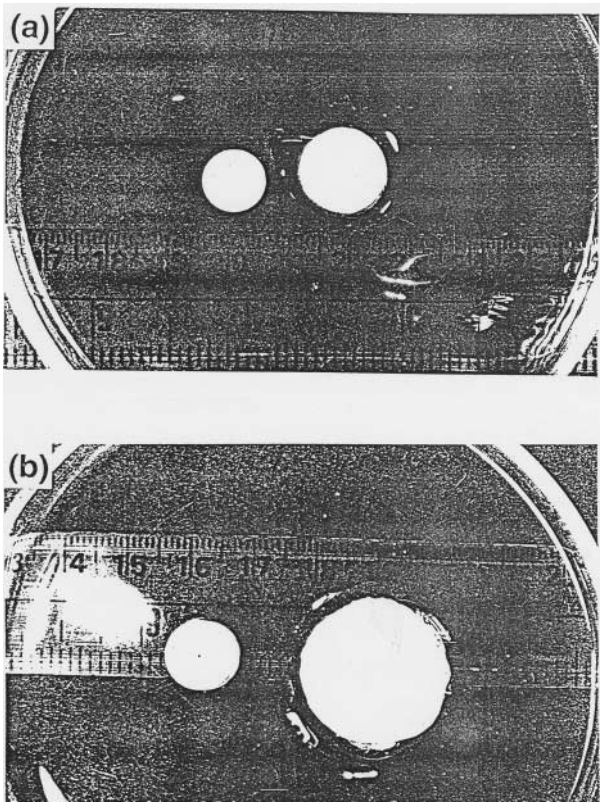

Figure 1. Photographs of the slow-swelling matrix (left) and the high-swelling matrix (right): (a) at day 3, (b) at day 7 after exposure to PBS pH 7.4.

hydration the tablets with the "slow" and "high"-swelling matrices had increased in diameter by 2 and 12 mm, respectively.

In Vitro Release Studies

The release of the propranolol enantiomers was determined from the tablets which incorporated either R- or S-propranolol-imprinted MIPs as pressed coats. The "low"-swelling matrices comprising either R- or S-propranolol-imprinted MIP and racemate of propranolol inserted in the core of tablet were used for the release studies. Figure 2 shows the release profiles of propranolol enantiomers from R-propranolol-imprinted matrices and S-propranolol-imprinted matrices, respectively. The release of propranolol enantiomers from either matrix could not be detected after 3 days of dissolution. After 3 days, the progressive release of propranolol enantiomers from R-enantiomer-imprinted matrices occurred, but only 15% of each of the

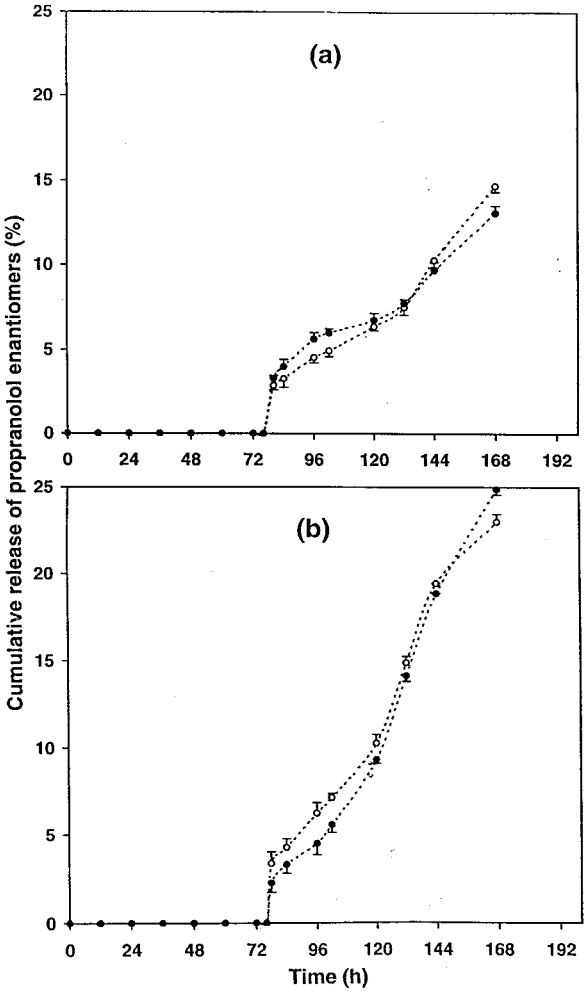

Figure 2. The cumulative percentage release of propranolol enantiomers from: (a) R-propranolol-imprinted matrices and (b) S-propranolol-imprinted matrices, applied as "low"-swelling pressed coats to racemic propranolol plotted as a function of time (mean ± SD, $n = 6$). (○) R-isomer, (●) S-isomer.

incorporated enantiomers was found in the dissolution media after 7 days (Fig. 2a). Over days 3–5, the matrices released the S-propranolol enantiomer ($k = 5.74 \times 10^{-2}$ day) faster than the R-propranolol enantiomer ($k = 6.77 \times 10^{-3}$/day). Beyond day 5, however, the release pattern changed with the R-enantiomer being released more rapidly than the S-enantiomer. For the S-enantiomer-imprinted matrices, both enantiomers of propranolol were gradually released and at day 7, 20–25% of each of the incorporated enantiomers was in solution (Fig. 2b). From day 3 to day 5 there was a clear

stereospecific trend in that significantly more of the R-enantiomer was released than the S-enantiomer. However, the selectivity for enantiomer release from these matrices was reversed after about 5 days of dissolution testing, similar to the R-enantiomer-imprinted matrices. The release rate constant during days 3–5 of the R- and S-propranolol enantiomers from the S-enantiomer-imprinted matrix were 1.25×10^{-3}/day and 6.22×10^{-5}/day, respectively.

The results indicate that for the release of propranolol from tablets utilizing the "low"-swelling matrices, the non-template isomer was released faster than the template isomer. However, as time progressed the difference in rates diminished and later reversed. The type of specificity demonstrated in the last phase of the dissolution process was similar to that obtained previously with "high"-swelling MIP matrices, presented as regular tablets (5). These results suggest that when swelling of the MIP matrix is limited, a degree of selectivity of enantiomer release can be obtained, presumably as a consequence of the enantiomeric recognition of the MIP.

Nevertheless, such selectivity of release was only evident over the first 5 days of dissolution testing. The change in selectivity later in the dissolution process might be due to the long period of time (7 days) over which the dissolution experiment was conducted. Even the "low"-swelling matrix swelled to some extent, and this conceivably modified the enantiomer recognition sites at longer times, possible leading to the enhanced release of the template isomer. These results, when compared with those obtained previously (5) where exactly the same MIP was employed but formulated such that the final matrix swelled more rapidly, indicate that the swellability of the matrix is a key factor in determining the selectivity of enantiomer release.

The mean ratio (R/S) of the cumulative percentage of R- and S-propranolol enantiomers released from the "low"-swelling matrix as a function of time is shown in Fig. 3. The mean ratio of R/S enantiomers from the S-propranolol-imprinted matrix decreased with time from 1.5 to 0.9 (Fig. 3a), while that from the R-propranolol-imprinted matrix increased with time from 0.8 to 1.16 (Fig. 3b). Thus, the maximum degree of stereoselectivity achieved at any time point for the amount of non-print to print isomer released was 1.5 (R/S ratio) and 1.25 (S/R ratio) from the S- and R-enantiomer-imprinted matrices, respectively. The

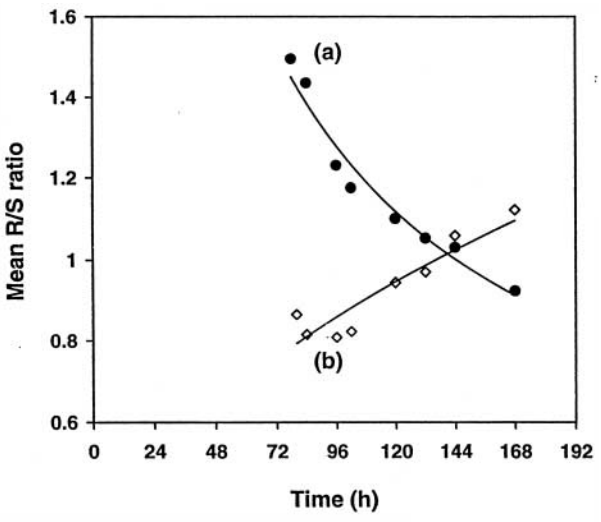

Figure 3. The mean ratio (R/S) of cumulative percentage of R- and S-propranolol enantiomers released from: (a) S-propranolol-imprinted matrices and (b) R-propranolol-imprinted matrices, applied as "low"-swelling pressed coats to racemic propranolol plotted as a function of time.

S-enantiomer-imprinted matrix exhibited a higher degree of enantioselectivity than the R-enantiomer-imprinted matrix. In fact, the enantioselectivity of these polymers should be identical. Since the evidence in the difference of pore volumes and surface areas of both MIPs was found, it is possible that the method of polymer preparation affects the efficiency of selectively regulating the release of enantiomer.

Microscopic Structure of the MIP Bead-Based Matrices

Cryo-scanning electron micrographs of the "low"-swelling matrices containing polymer imprinted with propranolol enantiomer were obtained only after 7 days' hydration, whereas drug release from the "high"-swelling matrix in the previous study was usually monitored up to a maximum of 24 hr. The micrographs indicated that the "low"-swelling matrix had relatively few surface pores (Fig. 4a), whilst the "high"-swelling matrix possessed numerous large surface pores (Fig. 4b), indicative of a more porous matrix. The pores were fewer in number in the "low"-swelling matrix and this presumably contributed to the slower drug release from formulations incorporating this matrix. In addition,

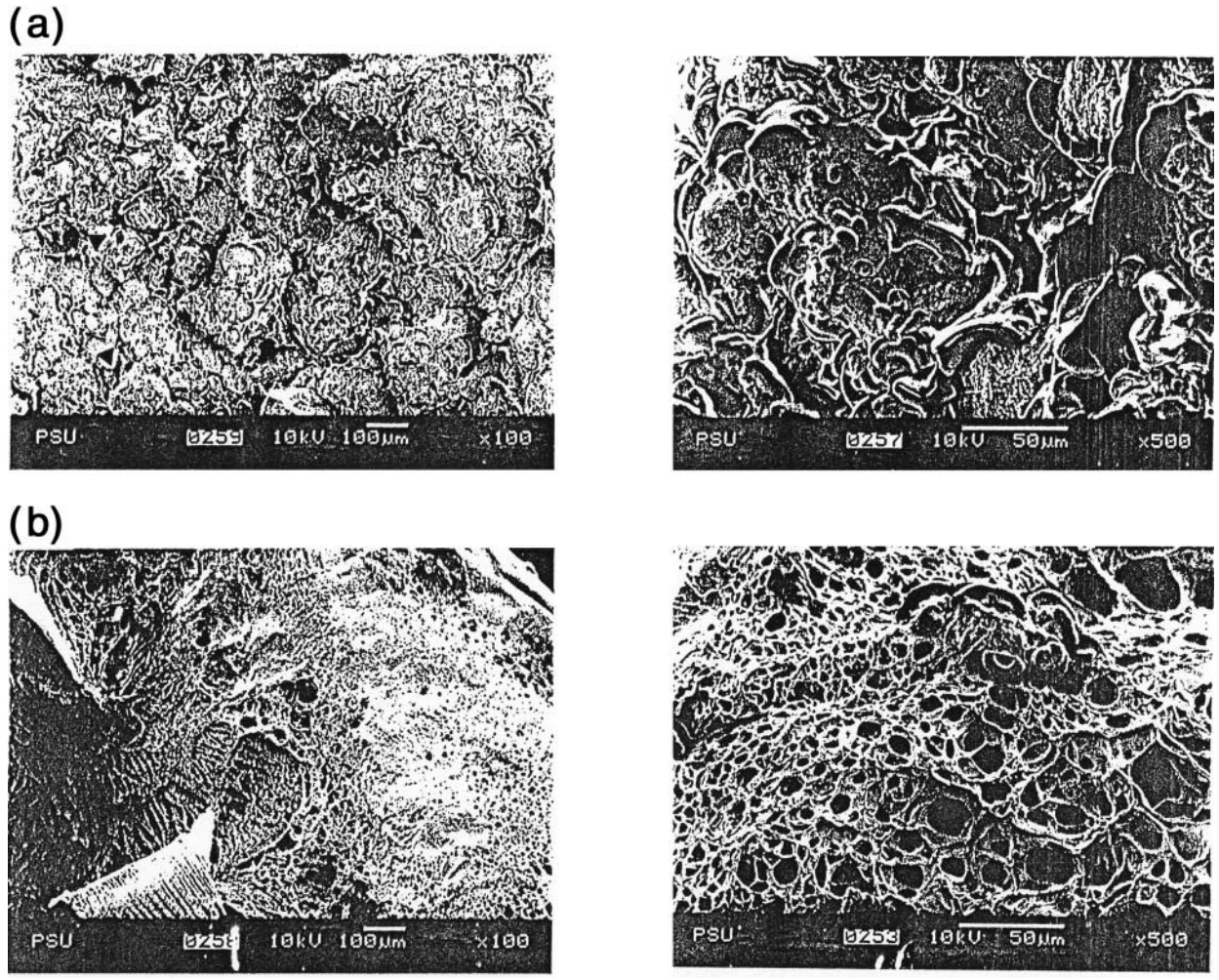

Figure 4. Cryo-scanning electron micrographs of (a) the "low"-swelling matrix and (b) the "high"-swelling matrix, imprinted with S-propranolol. The left micrographs employed a 100× magnification and the right micrographs a 500× magnification. Delta indicates pore.

drug diffusion through this matrix type will also be influenced by the hydrophobicity of the HVO incorporated within the polymer matrix. Moreover, interconnecting pores (or through-pores) were clearly apparent in the "high"-swelling matrix (Fig. 5).

DISCUSSION

The selective retardation for template release of the "low"-swelling MIP matrix, observed over the initial stages, might be explained by the affinity binding of the template enantiomer to the molecular recognition site, leaving the other enantiomer less strongly bound and therefore more readily released from the matrix into the bulk dissolution medium. As time progresses changes occur in the structure of the matrix, and the enantiomers closest to the boundary of the swelling matrix are released. This favors the release of the template molecule, which is concentrated within this region as a consequence of the previous selectivity of the positions within the "low"-swelling matrix.

The Enantioselective Release Mechanism of MIP Matrices

The enantioselective-controlled release of drugs from the MIP matrix partially depends upon the

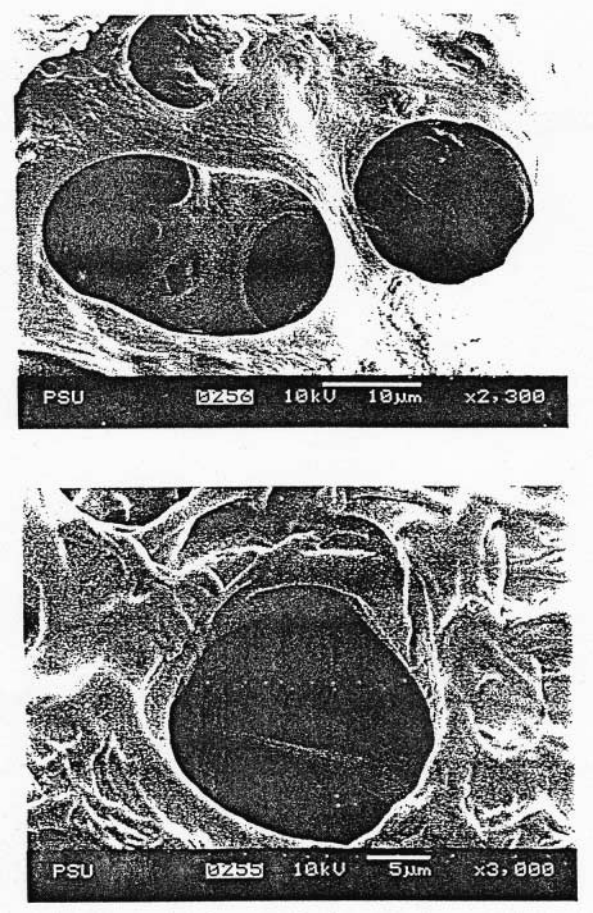

Figure 5. Interconnecting pores shown on a Cryo-SEM image of a "high"-swelling matrix imprinted with S-propranolol enantiomer. The upper micrograph employed a 2300× magnification and the lower micrograph a 3000× magnification.

specificity of MIP. Moreover, the transition of the original polymer from the dry to the swollen state, and possible loss of conformation at the active sites, would appear to be an important factor influencing the rate of drug release and selectivity of the MIP matrix. The type of interaction which occurs between the enantiomers and specific sites within the MIP matrix is dependent upon the formulation produced. For example, when the MIP particles are intimately integrated with racemic drug to generate a regular MIP matrix by a wet granulation technique, there is a greater opportunity of stereoselective interaction while preparing the matrix. The "recognized" enantiomer will preferentially occupy the selective "receptor" sites (8). The initial degree of porosity of the matrix would then appear to be a critical factor in controlling the rate of release, the template enantiomer being released more rapidly. However if, as in this study, the empty MIP matrix is applied as a pressed coat to a core containing racemic drug, the medium first needs to penetrate the preferably "non"-swelling MIP matrix to dissolve the drug. It is only then, as drug diffuses through the polymeric barrier, that the separate enantiomers can interact differentially with the "receptor" sites via specific sorption processes. Accessibility of these sites at the surface of pores within the matrix becomes a critical determinant of enantioselective release. Differential binding of stereoisomers at "receptor" sites leads to a more rapid diffusion of the "non-recognized" species, although release of any excess unbound "recognized" enantiomers via non-selective sites into the dissolution medium will also occur concomitantly. Swelling of the MIP could lead to a loss of structural recognition of the template enantiomer. The structure of the EDMA-MIPs typically consists of a set of highly cross-linked domains, interconnected by regions of lower cross-linking (9). The cross-links between the domains are flexible, since this polymer shrinks upon drying and swells to the original volume upon re-immersion in solvent, as observed previously (5,9,10). Thus, after hydration of the polymer matrix, the changes in swelling of polymer in the matrix can cause conformational reorganization of the polymeric structure. This may lead to the opening of the interconnecting pores, which in turn provides additional paths for the release of the enantiomers. Thus more "receptor" sites are likely to become more accessible as swelling continues. Therefore changes in the specificity of the MIP matrix as a function of time will occur, which might not be easy to predict. Specific isomers are released via selective pores or channels, which have accessible "receptor" sites on their surfaces. The release and rebinding of selective ligands, and the exchange of ligands at the binding sites, will occur. Previously, it was noted that the rebinding to MIPs was dependent on the template and also strongly upon the nature of the medium (11). For example, efficient imprint ligand rebinding can occur in aqueous buffers (12), and factors such as pH have been shown to have an influence (13).

It is of course feasible for the MIPs to act as a partial sieve, with the release of a particular enantiomer via selective sites being greater than that of

the other enantiomer via non-selective sites, as described in an earlier report (5) for regular MIP matrices containing dispersed drug. The relative importance of different mechanisms to the overall selectivity of release is likely to be different, depending upon the drug, the MIP employed, and the type of formulation used. Some of the mechanisms are analogous to those proposed for conferring selectivity of transport to MIP membrane pores and/or selective channels of the MIP matrix used in selecting only particular molecule releases. Indeed, the phenomena in the selectivity of the MIP matrix for enantiomer releases are analogous to those of the MIP membrane for selective transport (7,14). For example, the formation of polymer micropores in MIP membranes, which act as template-specific channels (specific ''gates'') between large pores, is proposed from an examination of porosity using nitrogen adsorption–desorption porosimetry (15). Alternatively, a change of the polymer pore structure induced by the interaction of template molecules with selective cavities has been suggested (16). Binding of the template could cause a shrinking of the receptor domains in the MIP, inducing an opening of ''gates'' for transport of molecules and ions through the membrane. Such template-induced changes in MIP membrane properties are supported by a number of previous studies (17–20). Mechanisms of this type involving selective pores or channels within the MIP matrix require experimental validation.

CONCLUSIONS

Generally, it has been demonstrated that the prominent mechanisms controlling the enantioselective release of drugs from MIP bead-based matrices are dependent upon the imprinting process as well as the nature of the affinity for the ligand. Moreover, the release of enantiomers is governed by the accessibility of the dissolution media to the drug within the matrix. The results obtained in this study show that the enantioselective release behavior of ''low''-swelling matrices is opposite to that of ''high''-swelling matrices. It is also found that the microscopic structure of the hydrated ''low''-swelling MIP matrix differs markedly from that of the hydrated ''high''-swelling MIP matrix. The difference in structural spacing of polymer in matrix, as modified by tablet formulation, is thought to be responsible for the reversed observation in enantiomeric release behaviors of those MIP matrices.

An understanding of the enantioselective-controlled release mechanism of MIP bead-based matrices is necessary in designing pilot formulations having the capacity to selectively regulate the release of specific enantiomers. Thus, further investigations into the mechanism of this novel device are certainly challenging.

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