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Absorption performance of iodixanol-imprinted polymers in aqueous and blood plasma media

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ABSTRACT

This paper presents the preparation and absorption performance of iodixanol-imprinted polymers in aqueous and blood plasma media in vitro for biomedical applications. The imprinted polymers were prepared by non-covalent imprinting of iodixanol in a matrix of poly(4-vinylpyridine) crosslinked by ethylene glycol dimethacrylate. The binding capacities (BCs) were investigated as a function of template-to-monomer, as well as monomer-to-crosslinker, ratios in the polymerization, and the solvent type. The highest BC of iodixanols achieved from the optimized imprinted polymer in the aqueous solution is 284 mg g^{-1} dry polymer with an imprinting effect (IE) 8.8 times higher than that of the non-imprinted polymer. In blood plasma, the BC of this polymer is slightly reduced to 232 mg g^{-1} with a smaller IE 4.3 times higher than that of the control polymer. The BCs of molecularly imprinted polymers as a function of the initial assay solution concentration as well as the examination time are also addressed. Surface analyses were additionally performed to characterize the surface morphologies and porosities of synthetic polymers. This work has demonstrated the feasibility of molecular imprinting of iodixanol, and the observed absorption performance of the imprinted polymers is encouraging for biomedical applications.

1. Introduction

Most countries are facing high and increasing rates of cardiovascular disease. Each year, it kills more Americans than cancer [1]. In the clinic, radiological (X-ray) examination is used as a reliable method to help diagnose cardiovascular disease by imaging certain disorders, and the biomedical molecule iodixanol (sold commercially under the trade name Visipaque) (Fig. 1) has been widely used as a water-soluble, iodinated X-ray contrast agent [2]. Normally iodixanol is given in hospital, administered by injection, and ultimately excreted by the kidneys. Although clinical trials demonstrated that iodixanol has lower rates of adverse renal events than other available iodinated contrast agents, it has still been linked to high rates of acute renal failure, so-called contrast-induced nephropathy (CIN) [3]. Moreover this risk becomes much higher in patients with cardiovascular disease. In a study of Swedish cardiac patients, 1108/47,543 patients (2.3%) who used iodixanol were rehospitalized with renal failure [4]. In addition, iodixanol was evaluated as no less toxic than any other iodinated contrast agent in patients with ongoing kidney disease [5]. Apparently CIN leads to increased incidence of mortality. Thus increasing attention and efforts have been paid to such at-risk patients. Nevertheless clinical practices have not found desirable solutions to prevent CIN associated with iodixanol. Current methods are simply to limit the dosage, hospitalize patients for observation after iodixanol administration, or not use any contrast agent for patients with serious diseases.

The existing techniques to remove iodixanol from blood have inherent problems and cannot completely alleviate the incidence of CIN. For example, dialysis is feasible in principle to protect kidneys by partially extracting iodixanol [6,7]. However, current dialysis methods are based on a physical/mechanical filtration using commercially available hollow fiber membranes, such as polysulfone and cellulose triacetate. These filtration materials have neither specificity nor high binding capacity (BC) for iodixanol. As a result, this approach results in low extraction efficiency with additional complications associated with removing considerable amounts of certain blood constituents which should preferably be retained in the blood. Therefore a highly efficient filtration material that can selectively capture significant quantities of iodixanol from the blood is required.

Molecular imprinting has become increasingly recognized as a powerful technique to produce synthetic polymers that contain tailor-made recognition sites for binding specific target molecules. The non-covalent imprinting and recognition principle is based on

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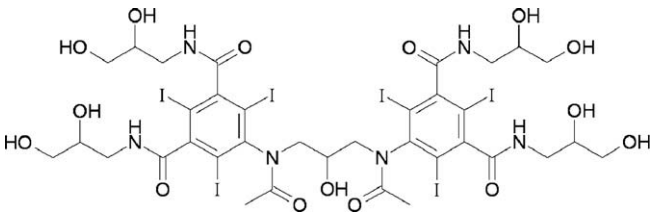


Fig. 1. The molecular structure of iodixanol.

the concepts of molecular "keys" and polymeric "locks" mimicking the recognition between antibodies and antigens shown in Fig. 2. In principle, the imprinted sites would specifically recognize only the template molecules [8–10]. Consequently a number of biomedical applications in the life sciences have been enabled by molecularly imprinted polymers (MIPs), including chromatographic separation, drug delivery, solid-phase extraction, diagnostic devices and biosensors [8,9,11–16]. In fact, for the last several years a large number of publications have reported imprinting of relatively small molecules such as sugars [17,18], steroids [19], pesticides [10], pigments [20] and certain drugs [16]. By contrast non-covalent imprinting of macro- and biomolecules has not been adequately investigated because it involves a more complicated mechanism with various experimental issues. Although the development of template imprinting is currently still at the incipient stage, its great potential for biomedical applications has led to an increasing number of studies and efforts [15,21–26].

We hypothesize that molecular imprinting of suitable polymers can be used to enhance their absorption capacity for iodixanol to the point where clinically useful amounts of this contrast agent can be absorbed. We further hypothesize that the specificity of the imprinting procedure will allow this enhanced absorption to be maintained even in the presence of a variety of molecules extant in blood plasma. The proposed biomedical applications based on such imprinted polymer particles can be used either in vivo or in vitro for healthcare treatments as illustrated in Fig. 3, indicating the potential for reliable, predictable and efficient solutions to remove iodixanols from the blood. In vitro application could include dialysis treatment using MIP-packed filtration columns to exclusively absorb and efficiently excrete previously administered contrast agents from the bloodstream like an artificial kidney. In vivo applications could, for example, be implantable wireless microsensors or nanomedicines which involve MIP materials encapsulated into or coated onto microelectromechanical-based microfabricated "vascular stents" or "stent grafts". These devices could then be inserted into the bloodstream, and guided to specific sites inside the body to monitor contrast concentration as well as to facilitate contrast filtration.

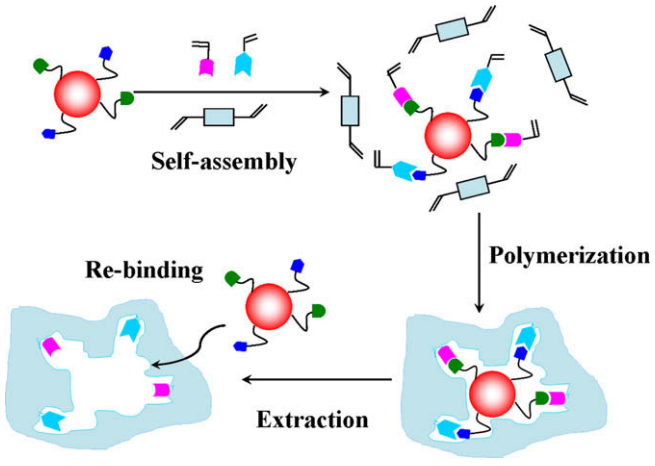


Fig. 2. The principle of non-covalent molecular imprinting and recognition.

To date, attempts to molecularly imprint water-soluble biomedical molecules have achieved only limited success [21–27], primarily because template molecules have complex conformations, low solubilities in organic monomers/crosslinkers solutions, slow diffusion rates, and suffer from interference with other components in the imprint systems [11–13]. A recent notable development in non-covalent bulk imprinting in aqueous solutions is the work of Kofinas et al., who studied various templates and achieved increased imprinting factors, with viruses (imprinting factor of 2.3) [21,25], antigens (imprinting factor of ~5) [27] and proteins (imprinting factor of 4.5) [28] in addition to glucoses (imprinting factor of 2.9) [17]. In practice, many parameters involved in the imprinting system and preparation process can affect the information associated with the binding sites, such as functional monomers/polymers, crosslinkers and solvents/porogens [11,29]. Thus both the feasibility of imprinting and proper preparation conditions need further exploration for the iodixanol template.

In this work synthetic iodixanol-imprinted polymers were prepared by a non-covalent imprinting approach in aqueous solution using 4-vinylpyridine (4-VP) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the crosslinker. The binding performance of these polymers was investigated in aqueous and plasma media. Several factors, including the quantity of iodixanol templates, the crosslink density and the solvents/porogens, were studied to optimize the BC and imprinting effect (IE) of synthetic MIPs. In addition, the BCs of MIPs as a function of the initial assay solution concentration as well as the examination time are also addressed. Details of the preparation and characterization of iodixanol-imprinted polymers for biomedical applications are presented.

2. Materials and methods

2.1. Materials

Iodixanol was obtained from Sigma–Aldrich Chemical Company as an aqueous solution with a concentration of 600 mg ml^{-1}. This concentration is a typical clinically relevant concentration. The 4-VP monomer, EGDMA, 2,2′-azo-bis(isobutyronitrile) (AIBN) and all solvents were purchased from Sigma–Aldrich Chemical Company as ACS grade. Sterile filtered sheep plasma was purchased from Hemostat Laboratories (Dixon, CA, USA). AIBN was used after recrystallization. Other chemicals and solvents were used without further purification.

2.2. Synthesis of iodixanol-imprinted polymers

A typical iodixanol-imprinted polymer, poly(4-VP-*co*-EGDMA), was synthesized using the following procedure. Iodixanol (template), 4-VP (monomer), EGDMA (crosslinker) and AIBN (initiator) were weighed and added to 10 ml ethanol/deionized (DI) water (5:1) solvent mixture at the specific ratios listed in Table 1. The solution was stirred for 1 h to ensure equilibration of non-covalent associations between templates and monomers followed by nitrogen gas bubbling to remove oxygen. The solution was then placed in an oven at 60 °C for 6 h during which time free radical thermal polymerization occurred. Non-imprinted polymers (NIPs) were prepared following the same procedure described above but without the addition of iodixanol templates.

Table 1 summarizes the details of the various experimental conditions used for each MIP and NIP material synthesis. Each sample is labeled with a prefix of either M or N to represent MIP and NIP

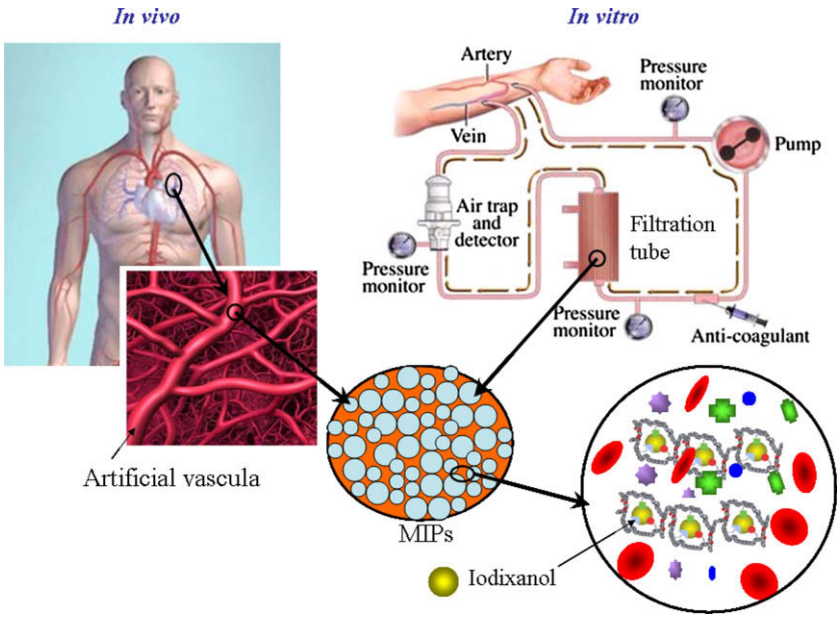


Fig. 3. Conceptual illustration of applying MIPs in health care, adapted from 3DScience.com and nucleusinc.com.

Table 1
Details of sample codes and molar feed ratios of iodixanol-imprinted polymers and their control samples.

Polymer code no.	Molar ratio of components			Solvents	Category
	Iodixanol templates	4-VP monomers	EGDMA crosslinkers		
M1	0.167	1	1	Aqueous ethanol	First series *x*:1:1
M2	0.077	1	1		
M3	0.038	1	1		
M4	0.026	1	1		
M5	0.018	1	1		
M6	0.006	1	1		
N1	0	1	1		
M41	0.026	1	0.333	Aqueous ethanol	Second series 0.026:1:*y*
N2	0	1	0.333		
M42	0.026	1	0.667		
N3	0	1	0.667		
M4	0.026	1	1		
N1	0	1	1		
M43	0.026	1	1.333		
N4	0	1	1.333		
M44	0.026	1	1.667		
N5	0	1	1.667		
M45	0.026	1	2		
N6	0	1	2		
M46	0.026	1	1	DMSO	Third series
N7	0	1	1		

materials, respectively. The first series of polymers (M1–M6) were prepared with an equimolar ratio [monomers]:[crosslinkers] of 1:1 and variable ratio, *x*, of the template. Since the [monomers]:[crosslinkers] ratio for this series was kept constant, only one type of NIP control polymer was prepared; this was named N1. The second series of polymers (M41–M45 and M4) were prepared using a constant ratio [templates]:[monomers] of 0.026:1 and a variable crosslinker ratio, *y*. The analogous control polymers (N2–N6) were synthesized and numbered correspondingly. Moreover dimethyl sulfoxide (DMSO) solvent was also examined, replacing the ethanol/water solvent in the method described above, and the MIP and NIP produced using DMSO were labeled as M46 and N7.

The resulting solid polymers were powdered and sieved through a 25 μm mesh, and the powder size was verified by scanning electron microscopy (SEM). The polymer powders were washed in tetrahydrofuran (THF) for 24 h using Soxhlet extraction to remove residual monomers, crosslinkers and linear oligomers from the polymer matrix. After filtering, the powders were dried in a vacuum at room temperature for 24 h. The powders were then rinsed in DI water for 12 h at room temperature to extract the iodixanol templates and then filtered to obtain the solid powders. This washing and filtration cycle was typically repeated 9–10 times to remove most of the iodixanol templates. The powders were finally dried to a constant weight in vacuum at 60 °C. The removal of imprint template molecules was verified by monitoring the amount of iodixanol in the rinse water using UV–vis spectrophotometric measurements at 245 nm [21]. This analysis indicated that about 95% of the template molecules were removed with respect to the total amount used in the polymer preparation.

2.3. Binding tests

Binding tests were performed on both imprinted and non-imprinted polymers. In a typical procedure, 50 mg of dry polymers were added to a vial of 10 ml iodixanol aqueous solution with an initial concentration of 15 mg ml^{-1}. The use of a concentration of 15 mg ml^{-1} mimics a typical iodixanol concentration in blood after administration of iodixanol for angiography. This calculation is based on an average adult, with a blood volume of 5 l, being administered an injection dose of 450 ml iodixanol. The test solution and powdered polymer was slowly stirred by magnetic stirrer for 24 h. The solution was then centrifuged, and diluted with DI water by 650 times, to ensure the UV absorbance in the spectra region is not saturated. A typical UV–vis spectrum of diluted iodixanol aqueous solution is shown in Fig. 4a. The binding performance of MIPs and NIPs in the aqueous media is evaluated by the BC and IE, calculated as follows:

$$\mathrm{BC} = \frac{\Delta m_t}{m_p} = \frac{(C_i - C_f)V}{m_p} \quad (1)$$

$$\mathrm{IE} = \frac{\mathrm{BC}_{\mathrm{MIPs}}}{\mathrm{BC}_{\mathrm{NIPs}}}, \quad (2)$$

where C_i and C_f are the initial and final concentrations of iodixanol in the solution, respectively, V is the solution volume, Δm_t is the

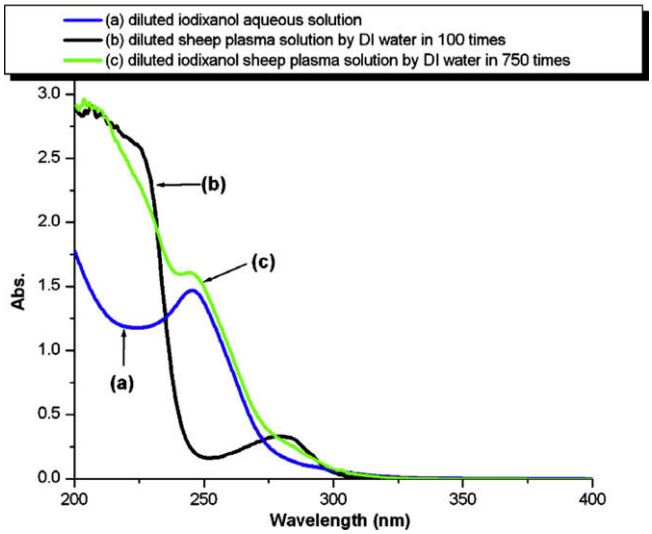


Fig. 4. UV–vis spectra of diluted iodixanol aqueous solution (a), diluted sheep plasma solution (b), and diluted iodixanol sheep plasma solution (c).

amount of iodixanol bound to polymers, and m_p is the mass of polymers. BC_{MIPs} and BC_{NIPs} are the binding capacities of MIPs and NIPs. Using these definitions, the binding capacity, BC, represents the total mass of iodixanol absorbed per mass of polymer, while the imprinting effect, IE, quantifies the improvement of absorption efficacy of imprinted polymers relative to non-imprinted polymers. The mean value of these values was determined from three independent tests.

The absorption profile of MIPs in iodixanol aqueous solutions with different initial concentrations from 0.15 to 25 mg ml^{-1} was also tested, since the actual amount of iodixanol injected for radio-imaging is varied by physicians according to patient body conditions and their actual tolerances, which consequently require different initial iodixanol concentrations, C_i.

Subsequently sterile filtered sheep plasma media was examined using a similar test procedure as described above, where a series of 10 ml sheep plasma solutions with an initial iodixanol concentration of 15 mg ml^{-1} were tested and characterized by UV–vis spectroscopy (at 245 nm). Although the plasma components would have a UV–vis absorption at 280 nm [27] (see Fig. 4b), this absorbance becomes negligible after dilution with DI water by 750 times. By contrast, the UV absorbance at 245 nm associated with iodixanols is still clearly observed (see Fig. 4c). Therefore, after calibration of known concentrations of iodixanol solution, the UV absorbance intensity at 245 nm is used to determine the concentration of the remaining iodixanols in sheep plasma.

An equilibrium binding experiment was additionally performed to determine the time necessary for a complete absorption of iodixanol in plasma. For the first 36 h aliquots of the stirred sheep plasma were taken periodically and analyzed using UV–vis spectrometry. The BCs as a function of time were plotted to determine the equilibrium time. The total volume of the aliquots removed were less than 5% of the total test volume (10 ml), thereby minimizing the errors introduced in the test due to aliquot volume loss.

Moreover, in terms of the practical application of these imprinted polymers, few specific molecules are actually found in plasma or blood that can serve as theoretically ideal competitors with similar sizes, shapes and chemical functionalities to iodixanol for evaluating the selectivity of synthetic MIPs. Instead, various components in plasma or blood found in amounts far larger than that of iodixanol would spontaneously become strong competitors. Hence in this work plasma components, including dissolved proteins, amino acid residues, glucose and mineral ions, are native binding competitors. The difference of resulting BCs and IEs obtained from aqueous as well as plasma solutions would indicate the selectivity and binding capability of MIPs to iodixanol targets.

2.4. Characterization

Fourier transform infrared (FTIR) spectroscopy (Bruker Vector 22) was used to characterize the MIPs and quantitatively determine the actual copolymer composition ratios of monomers (4-VP) and crosslinkers (EGDMA) from the integrated area ratios of two characteristic peaks at 1600 cm^{-1} (4-VP) and 1727 cm^{-1} (EGDMA).

A UV–vis spectrophotometer (Shimadzu UV-160A) was used to determine the iodixanol concentration in both aqueous and sheep plasma solutions by measuring the UV absorbance at 245 nm. The calibrated molar absorptivity of iodixanol in aqueous solutions is 64.7 ± 0.3 ml mg^{-1} cm^{-1} calculated from the slope of a plot of absorbance vs. solution concentration in accordance with the Beer–Lambert law. This plot was obtained by measuring the absorbance of a series of iodixanol solutions with known concentrations. The molar absorptivity of iodixanol in diluted plasma solution was determined in a similar way and found to be 79.4 ± 0.5 ml mg^{-1} cm^{-1}.

The powder size and surface morphology were characterized by SEM (FEI Nova Nanolab 200 FIB/SEM). The surface porosity was determined by nitrogen absorption/desorption Brunauer–Emmett–Teller (BET) analysis (SA 3100 Surface Area & Pore Size Analyzer, Beckman Coulter, Inc.).

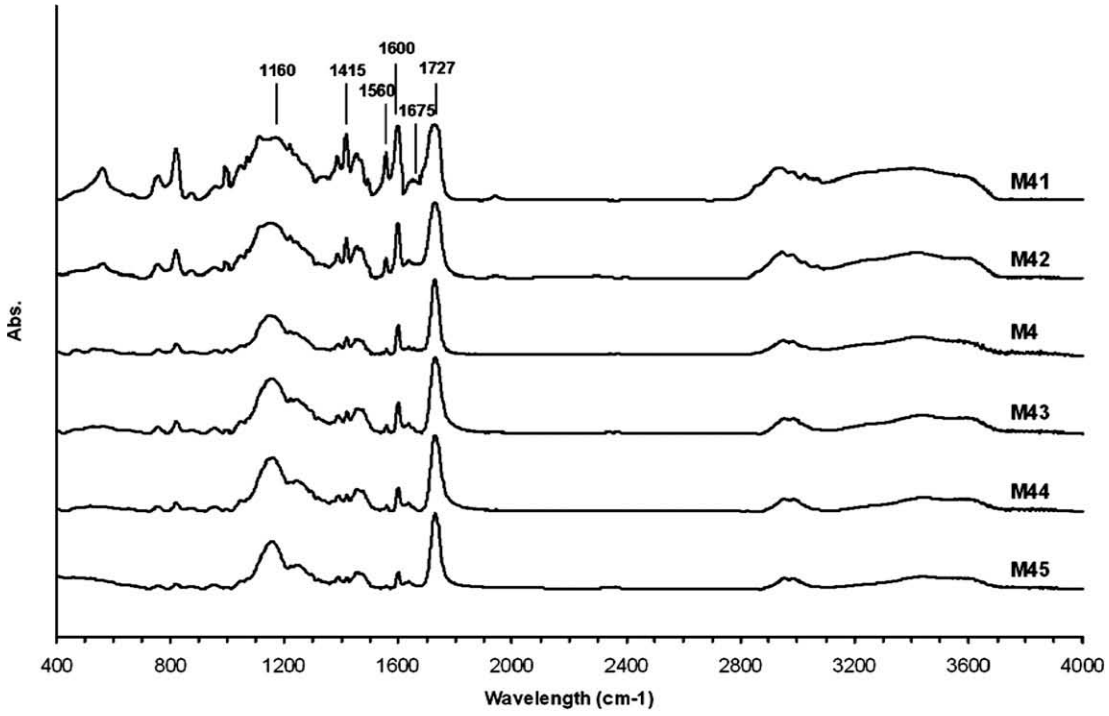
3. Results and discussion

3.1. FTIR study on the structural characteristics and compositions of MIPs

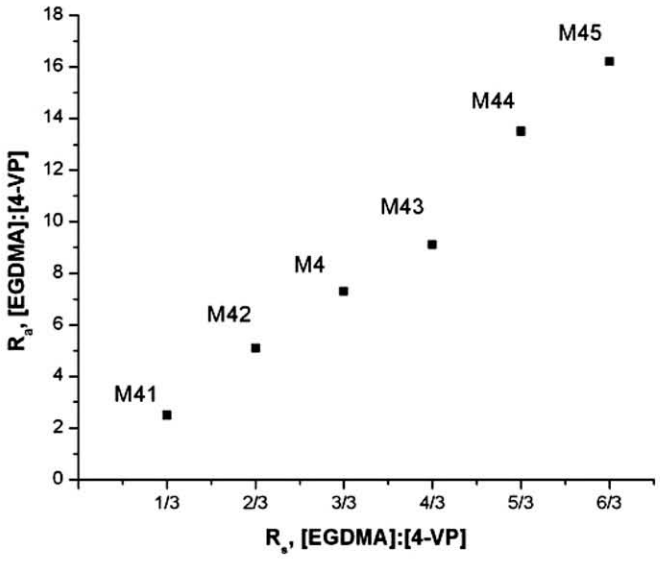
Fig. 5a shows a series of FTIR spectra of MIP samples M4, M41–M45 with various feed ratios, R_s, of EGDMA and 4-VP as listed in Table 1. The absorption bands observed at 1727 and 1160 cm^{-1} are associated with the C═O and –C–O–C– groups in EGDMA [30], and the bands at 1675, 1600, 1560 and 1415 cm^{-1} are attributed to the pyridine rings [31]. The peak area ratios of the two characteristic peaks at 1727 and 1600 cm^{-1} are utilized to quantitatively evaluate the actual ratios (R_a) of [EGDMA]:[4-VP] in the synthesized MIPs. The value of R_a in the copolymer is plotted as a function of R_s in Fig. 5b. This plot clearly shows that there is a linear relation between R_a and R_s, indicating the consistency of experimental results (R_a) with the feed ratios (R_s). In the following sections, the feed ratios will be mainly used for discussion, where *x* represents the feed ratio of [template]:[monomer], and *y* stands for the feed ratio of [crosslinker]:[monomer].

3.2. Binding capacities and imprinting effects of MIPs in aqueous and plasma media

The BCs and IEs of the polymers in aqueous solutions and sheep plasma for an initial iodixanol concentration of 15 mg ml^{-1} are listed in Table 2. The results show that all of the MIPs can bind iodixanol in larger amounts than the analogous NIP control polymers, giving IE values exceeding unity. This indicates that basic 4-VP is a proper functional monomer for imprinting iodixanol, forming appropriate non-covalent affinities. These affinities are attributed to a combination of various associations in the imprinting system, such as interactions between basic pyridines and acidic hydroxyl groups, π–π stacking between pyridine and benzene rings, as well as hydrophobic effects. Therefore the feasibility of non-covalent imprinting of iodixanol is achieved by using 4-VP as the functional monomer.



(a)



(b)

Fig. 5. (a) FTIR spectra of MIPs (M4, M41–M45) with different feed ratios of [EGDMA]:[4-VP] in polymerization; (b) the plot of the actual ratios (R_a) of [EGDMA]:[4-VP] vs. the feed ratios (R_s).

Furthermore, by careful comparison of the BCs and IEs achieved in aqueous solutions listed in the second column of Table 2, it is found that the highest BC obtained is 284 mg g^{-1} from M4 with an IE of 8.8. Although some other MIPs, such as M43 (BC = 198 mg g^{-1}, IE = 7.1) and M44 (BC = 190 mg g^{-1}, IE = 6.8), display good IEs (>5), their BCs are much lower than M4; thus they have poorer binding capabilities than M4. Meanwhile, a similar observation is also found in sheep plasma media from the results listed in the third column of Table 2. The highest BC obtained of 232 mg g^{-1} is still from M4 with an IE of 4.3. Although M45 has the highest IE (4.7) in plasma, its low BC (14 mg g^{-1}) is too small to be of practical use. Thus these binding results indicate that M4 is the most desirable MIP obtained in this work for potential biomedical applications by optimizing the preparation conditions, which will be discussed in detail below.

It can also be noticed, from a comparison of values summarized in Table 2, that all MIPs show higher BCs and IEs in aqueous than in plasma solutions. Among them, M4 is taken as an example for the following discussion. Fig. 6 shows the column plot of BCs of M4 and its control polymer N1 obtained in aqueous and plasma solutions. It apparently shows that when M4 is tested in plasma mixture, its BC and IE are approximately reduced by 20% and 50%, respectively, from those values obtained in aqueous solution. This is primarily due to the complex nature of the plasma mixture, which includes

Table 2
Binding capacities of MIPs and NIPs in aqueous and plasma solutions (at C_i = 15 mg ml^{-1}) and their corresponding imprint effects correspondingly.

Polymer Code	Aqueous Solution		Sheep Plasma	
Number	BC (mg /g)	IE	BC (mg /g)	IE
Ml	128 ± 22.4	4.0	90 ± 13.5	1.7
M2	152 ± 26.6	4.7	96 ± 14.4	1.8
M3	190 ± 33.25	5.9	178 ± 26.7	3.3
M4	284 ± 49.7	8.8	232 ± 34.8	4.3
M5	186 ± 32.55	5.8	154 ± 23.1	2.8
M6	178 ± 31.15	5.6	168 ± 25.2	3.1
Nl	32 ± 5.6	–	54 ± 8.1	–
M4l	26 ± 4.55	2.2	10 ± 1.5	5
N2	12 ± 0.35	–	2 ± 0.2	–
M42	212 ± 37.1	3.9	16 ± 2.4	4
N3	54 ± 9.45	–	4 ± 0.3	–
M4	284 ± 49.7	8.8	232 ± 34.8	4.3
Nl	32 ± 5.6	–	54 ± 8.1	–
M43	198 ± 34.65	7.1	84 ± 12.6	2.2
N4	28 ± 1.4	–	38 ± 5.7	–
M44	190 ± 33.25	6.8	68 ± 10.2	1.2
N5	28 ± 4.9	–	56 ± 8.4	–
M45	164 ± 28.7	6.8	14 ± 2.1	4.7
N6	24 ± 3.7	–	3.0 ± 1.2	–

a large number of components that could compete with iodixanol to occupy a certain percentage of the imprinted binding sites. In addition, it is also possible that plasma components might potentially form complexes with iodixanol, thereby affecting the conformations and chemical functionality of iodixanol, resulting in a reduction of BC and IE. From the current data it is not possible to distinguish which of these mechanisms, if any, caused the observed effects, and investigation of this aspect will form part of our future research. On the other hand, even if the achieved binding capability of iodixanol target is affected by the plasma components, M4 still shows high BC (232 mg g^{-1}) and IE (4.3), which indicates that M4 possesses an adequate binding selectivity in plasma media for practical requirements, and can bind well to iodixanol targets in the presence of competitors.

3.3. Effects of molar ratios

The efficiency of the studied MIP system is affected by several factors in polymerization, including [templates]:[monomers] (x) and [crosslinkers]:[monomers] (y) ratios, as well as the solvent used. Of these parameters, the molar ratios of components are found to be the most important in determining the BC and IE of MIPs as shown by analysis of the results of Table 2 in Figs. 7 and 8. Fig. 7 presents the variation of BCs for the various MIPs and NIPs as a function of x, at a constant y = 1. Fig. 8 similarly presents the variation of BC as a function of y, at constant x = 0.026. These results apparently indicate that the optimal [template]:[monomer]:[crosslinker] ratio (i.e. x:1:y) is 0.026:1:1, which produces a maximum in the BC in addition to a good IE. A typical FTIR spectrum of MIPs prepared using this feed ratio is shown in Fig. 5a for sample M4.

In terms of the [templates]:[monomers] ratio, functional monomers are usually used in an excess molar ratio to the template [11] (x < 1). In general, there is an optimized [templates]:[monomers] ratio, x_o, for a specific imprinting system. When $x < x_o$ the BC of MIPs would be expected to increase with increasing x until x_o because of the increase in the number of imprinting sites in the MIPs. However, when x exceeds x_o, the BC of MIPs decreases again, most probably due to aggregation-related phenomena of iodixanol. When excess templates aggregate and form clusters, the resulting imprinted cavities are shaped and functionalized with respect to the clusters rather than the isolated templates, leading to a poor recognition of, and binding to, template molecules [22,25]. Experimental results (see Fig. 7) show that the maximal BC appears when the ratio of [template]:[monomer] is 0.026; thus 0.026 is believed to be related to the optimized [templates]:[monomers] ratio, x_o, for this specific imprinting system, and is therefore used as the value of x_o.

The [crosslinkers]:[monomers] ratio, y, plays another important role that affects the crosslink density and consequently the BC of the polymers. MIPs are polymeric networks with an effective distance between crosslinks. If the crosslink density is too low, then the network is too flexible and dynamic, and consequently the shape or size of the sites that the templates induced may not be retained. However, if the crosslink density is too high, then template molecular diffusion within the network may be reduced [11], which would have the effect of preventing not only template extraction but also movement of the iodixanol targets into the binding sites. In addition, in this work it is found that an excess of crosslinkers significantly affected the solubility of iodixanol in the reactant monomer/crosslinker solution, which can change the

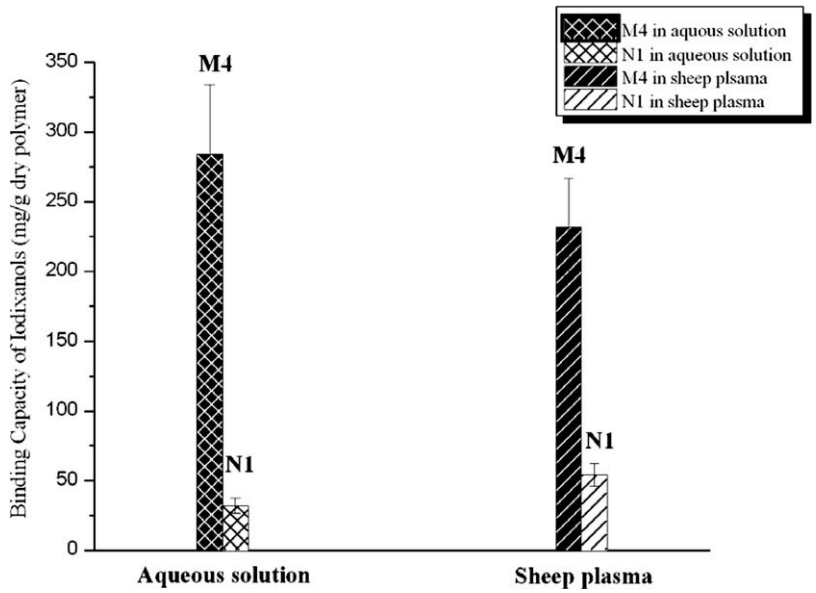


Fig. 6. Binding capacities (BCs) of MIPs and NIPs in aqueous and sheep plasma.

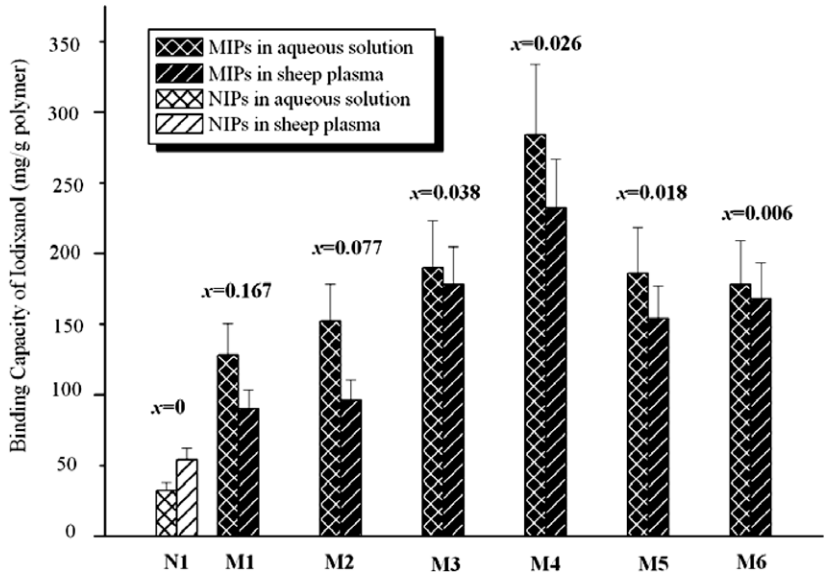


Fig. 7. Binding capacities (BCs) of polymers with a molar ratio of *x*:1:1, examined in aqueous solutions and sheep plasma.

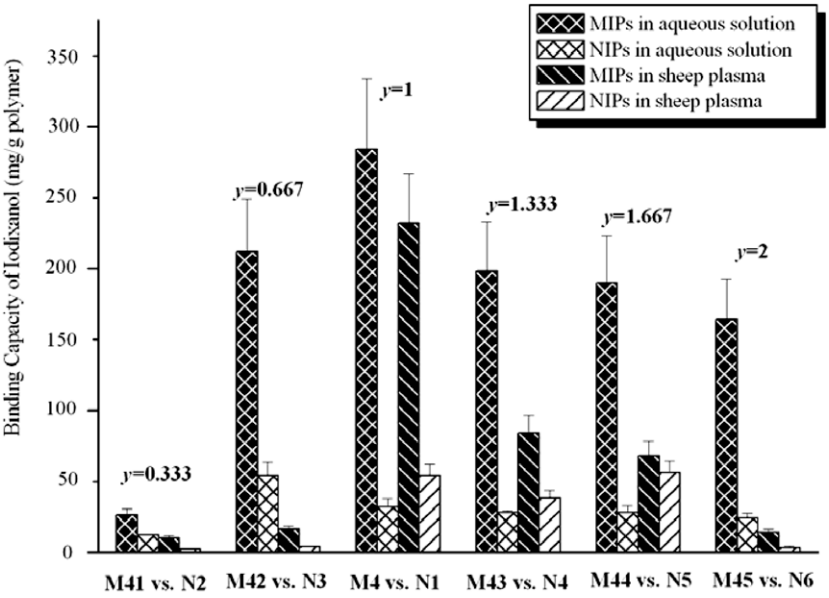


Fig. 8. Binding capacities (BCs) of polymers with a preparation molar ratio of 0.026:1:*y* examined in aqueous solutions and sheep plasma.

equilibration of non-covalent associations between monomers and templates. Fig. 8 clearly shows the resultant behavior, indicating the effects that these possible factors have on BCs; the proper [crosslinkers]:[monomers] ratio is taken as 1:1.

3.4. Effect of initial solution concentration on the binding capacity

In a clinical setting, the actual amount of iodixanol injected for radiological examination is adjusted depending on the condition of the patient and his/her actual tolerances; therefore patients will have different initial iodixanol concentrations, C_i, in their blood. It is necessary to study the absorption profile of MIPs in solutions with different initial concentrations. Fig. 9 shows absorption profiles for M4 and its control polymer N1 as a function of initial solution concentrations, C_i. The BC of MIPs is always a few times higher than that of NIPs, and both BCs increase with the rise of C_i. The theoretical BC (BC_t) of M4 is 132 mg g^{-1} and that of N1 is 0 mg g^{-1} based on the [templates]:[monomers]:[crosslinkers] ratio used in polymerization. This assumes that all template sites in the MIPs behave like antibodies to the iodixanol antigens where each binding site can properly absorb only one iodixanol molecule. The measured actual BC (BC_a) of MIPs is higher than its BC_t when C_i is larger than 5 mg ml^{-1}, while the BC_a of NIPs is never equal to zero. This is mainly attributed to the non-specific binding from "background" pores (non-template-induced pores present in the polymer matrix), which can be created by inherent local density fluctuations in the bulky polymer as well as porogenic solvents [32]. These "background" pores serve as binding sites to capture target molecules by non-specific, physical adsorption, leading to a higher value of BC_a than the predicted BC_t [33]. Thus non-specific absorption is the dominant effect on BC_a of NIPs. This effect increases when the number of available iodixanol molecules increases. In MIPs the BC_a

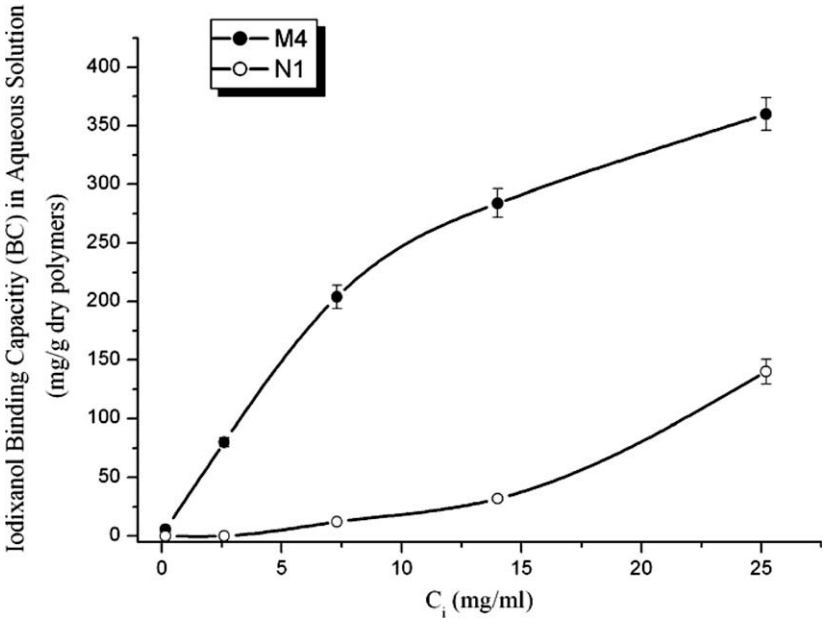


Fig. 9. Binding capacities (BCs) of polymers vs. the initial iodixanol concentration in aqueous solutions (C_i).

results from a combination of specific (molecular recognition) and non-specific absorption, and the specific absorption is the dominant effect. Thus the BC_a of MIPs is always a few times higher than that of NIPs for a range of solution concentrations. Additionally, when the number of available target molecules exceeds the number of accessible template-induced binding sites, the bound target molecules might serve as nucleation centers for adjacent extra targets to deposit, increasing the BCs of target molecules in MIPs compared to NIPs [34].

3.5. Binding equilibrium in plasma

It was noted above that the BC values of MIPs examined in aqueous solutions are slightly different from those in plasma because of the complex nature of the plasma media, which implies that variation in the media might impact the binding performance of MIPs as well as the binding equilibrium – for example, requiring longer time to reach equilibrium. Therefore the binding equilibrium profile of MIPs in plasma media was also studied, and M4 tested as a representative of those synthetic MIPs. Fig. 10 shows the time-dependent absorption profile of M4 performed in sheep plasma over 40 h (C_i = 15 mg ml^{-1}). It is observed that the initial binding rate of MIPs is the highest, ~30 mg g^{-1} h^{-1}, and then gradually decreases with time. After ~24 h the rate has reduced to zero, indicating the entire system has reached equilibrium. Therefore the binding equilibrium time of MIPs in plasma is taken as 24 h even though a small amount of iodixanols might be still absorbed afterwards due to non-specific binding or physical adsorption. Based on this characterization, all subsequent binding tests and BC measurements in plasma were determined within 24 h.

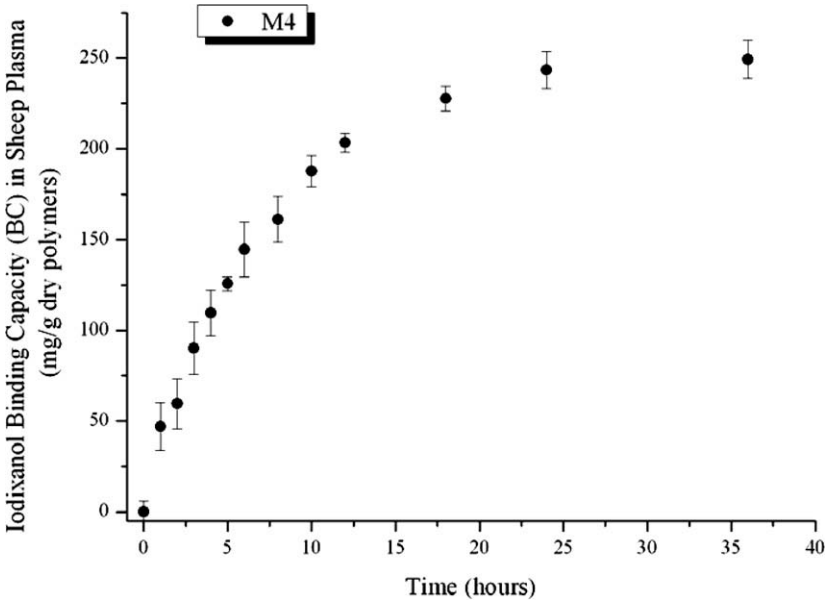


Fig. 10. Absorption time dependence of M4 in sheep plasma (C_i = 15 mg ml^{-1}).

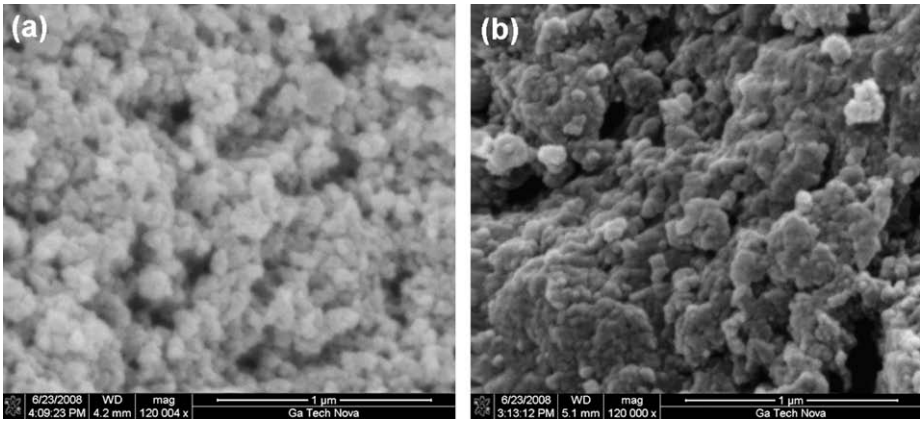


Fig. 11. SEM images of (a) M46 and (b) N7 polymers; scale bar = 1 μm.

3.6. Effect of the solvent

Selection of solvents is challenging, especially for medium or large water-soluble templates. It has been proposed that the presence of polar solvents could interfere with the monomer–template associations [11,29]; however, in this work the extremely low solubility of iodixanol in apolar or weakly polar solvents requires the use of polar solvents (ethanol:DI water (5:1), and DMSO). In practice, DMSO is a good solvent for all components in the imprinting system, including iodixanol, monomers and crosslinkers, which simplifies the preparation of a homogeneous solution before polymerization. However, the high polarity of DMSO may interfere with monomer–template association more than aqueous ethanol. Comparison of the effects of the solvent was tested in samples M4 and M46 together with control samples N1 and N7.

Table 3
BET surface analysis of polymers prepared in different solvents.

Sample code no.	Surface area, S ($m^2\ g^{-1}$)	Total pore volume, V_p ($cm^3\ g^{-1}$)	Solvent
M4	6.83	0.019	Aqueous ethanol
N1	3.59	0.012	
M46	53.86	0.221	DMSO
N7	17.64	0.071	

SEM images in Fig. 11 show the surface morphologies of samples M46 and N7 prepared in DMSO. Samples prepared in aqueous ethanol (e.g. M4 and N1) have similar surface morphologies to the equivalent structures in Fig. 11 and are therefore not shown. It is found that imprinted polymers are always slightly more porous than their non-imprinted control polymers, M46 vs. N7, which is attributed to the use of templates in preparing imprinted polymers, leading more pores to be formed in MIPs than in NIPs after template extraction. BET surface analysis is used to quantitatively check and measure the surface areas (S) and pore volumes (V_p) of M4, M46 and their control polymers N1, N7. The BET results are summarized in Table 3. It is shown that samples from the aqueous ethanol solvent system (M4 and N1) have much lower surface area and pore volume than samples from the DMSO solvent system (M46 and N7). Therefore BET results indicate that DMSO is a better porogenic solvent than aqueous ethanol, leading to higher values

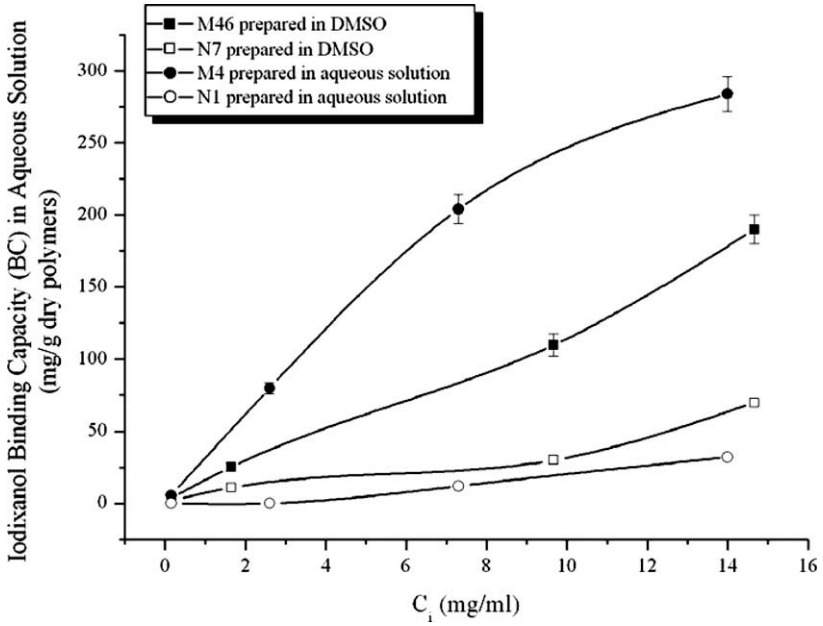


Fig. 12. Plots of the BCs of polymers vs. the initial iodixanol concentration (C_i).

of S and V_p due mainly to the nature of the solvent. With a high value of S and V_p the equilibrium time for absorption in plasma might be reduced from the expected 24 h (see Fig. 10).

However, the resultant BC values for more porous M46 are much lower than those of M4 over the entire concentration range (from 0.15 to 14 mg ml^{-1}) (see Fig. 12). Considering the results at C_i = 14 mg ml^{-1}, the BC for M4 is 275 mg g^{-1}, which is 8.8 times higher than its control polymer N1 (IE = 8.8). For M46 the BC is 190 mg ml^{-1} and only 2.7 times higher than that of its control polymers N7 (IE = 2.7). Clearly, although DMSO might be a better porogen, increasing the porosity of produced polymers as mentioned before, aqueous ethanol is a better solvent than DMSO for imprinting iodixanol mainly due to the effect of solvent polarity. In the literature it is reported that some biomacromolecules dissolved in polar organic solvents, i.e. DMSO, would exhibit distinctly different activity compared to that in aqueous media [35]. Hence it is possible that in our study the configuration of iodixanols in DMSO changed from that in aqueous solution, resulting in inferior imprinting.

4. Conclusions

This paper presents a molecularly non-covalent imprinting of the water-soluble X-ray contrast medium iodixanol in aqueous-based solvent using 4-VP as the functional monomer, and EGDMA as the crosslinker. Recognition and binding of iodixanol molecules by imprinted polymers were investigated in both aqueous solution and sheep plasma in vitro. The binding equilibrium measured for both media can be reached within 24 h. The best BC achieved from the optimized imprinted polymers in this study is 284 mg g^{-1} in aqueous solution (C_i = 15 mg ml^{-1}), 8.8 times higher than that of the control polymers, and 232 mg g^{-1} in sheep plasma (C_i = 15 mg ml^{-1}), 4.3 times higher than the control polymers. The slight reduction in BC of MIPs examined in plasma media is attributed to the effects of the plasma components. However, MIPs could still function well in plasma with satisfactory BCs for biomedical applications. Moreover, it is found that the BC of such synthetic MIPs is proportional to the initial solution concentration. With a higher initial solution concentration, more iodixanol molecules can be absorbed. Based on the above results, the feasibility of molecularly imprinting iodixanol has been demonstrated. The obtained binding results from the optimized MIPs are encouraging for biomedical applications in healthcare.

Acknowledgments

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figures 2–4, are difficult to interpret in black and white. The full color images can be found in the on-line version, at doi:10.1016/j.actbio.2009.11.007.

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