Recognition and Absorption of the Water-soluble X-ray Contrast Medium Iodixanol using Molecularly Imprinted Polymers for Biomedical Applications

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ABSTRACT

This work presents the study on the recognition and absorption of the water-soluble X-ray contrast medium iodixanol in aqueous solution using synthetic molecularly imprinted polymers (MIPs). A non-covalent imprinting technique was applied to prepare iodixanol-imprinted polymers using 4-vinylpyridine as the functional monomer and ethylene glycol dimethacrylate as the cross-linker. The effects of quantity of iodixanol templates, the crosslink density, and the solvent were studied in terms of the binding capacity and imprint effect of the polymers. UV-vis spectrometric analysis shows that the highest binding capacity achieved is 284 mg iodixanol per gram of dry polymer, which is 8.8 times higher than the binding capacity of the non-imprinted control polymers (NIPs). SEM and BET surface analysis have also been performed to investigate the effect of morphology and porosity on the binding capacities of polymers.

INTRODUCTION

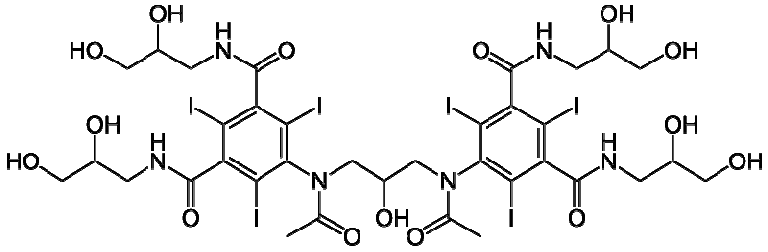

Figure 1. The molecular structure of iodixanol.

Iodixanol has been widely used as a radiographic contrast agent, Figure 1. It is normally administered by intravascular injection and ultimately excreted by the kidneys. However, clinical studies show that renal clearance of iodixanol might lead to acute kidney injury, especially for at-risk patient populations [1]. A nano-functional material that is able to molecularly recognize and selectively absorb iodixanols could greatly ease the renal burden on these patients. To achieve this we propose the use of iodixanol-imprinted polymers. Within the context of molecular imprinting [2, 3], properly imprinted polymers can exclusively detect and effectively capture iodixanols by non-covalent interactions. Hence various biomedical applications based on such

iodixanol-imprinted polymers can be envisaged including hemodialysis, diagnostic devices, and molecular specific sensors.

The molecular target of the imprinting in this study is iodixanol, which is a water-soluble macromolecule. Imprinting of biomacromolecular templates has challenging issues associated with their low solubility in apolar or weak polar monomer/crosslinker solutions and their diffusion limitations, resulting in a lack of specific imprinting and recognition [4, 5]. To this end, polar solvents are proposed, but the presence of polar solvents, e.g. aqueous solvents, are capable of destabilizing non-covalent associations between templates and monomers [4]. Therefore only a limited amount of research on non-covalently imprinting water-soluble, macromolecular templates has been undertaken.

In this work, aqueous solutions were used in non-covalent imprinting of iodixanols in a crosslinked polymer matrix of poly(4-vinylpiridine-*co*-ethylene glycol dimethacrylate). This paper presents details of the preparation and characterization of such iodixanol-imprinted polymers.

EXPERIMENTAL DETAILS

Iodixanol was obtained from Sigma-Aldrich Chemical Company as an aqueous solution (600 mg/ml). The 4-vinylpyridine (4-VP) monomer, ethylene glycol dimethacrylate (EGDMA), 2,2'-azo-bis(isobutyronitrile) (AIBN) and all solvents were purchased from Sigma-Aldrich Chemical Company as A.C.S. grade.

A typical synthesis procedure to imprint iodixanol is as follows: iodixanol (template), 4-VP (monomer), EGDMA (crosslinker) and AIBN (initiator) were weighed and added to 10 ml ethanol/DI water (5:1) solvent mixture with the specific ratios listed in Table I. The solution was stirred for 1 hour to ensure equilibration 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 hours during which time free radical thermal polymerization of the monomers occurred. Non-imprinted polymers (NIPs) were prepared using the same procedure but without the addition of iodixanol templates.

Table I summarizes the details of the various experimental conditions used to generate the various MIP and NIP polymers. Each sample is labeled with a prefix of either M or N to represent MIP and NIP materials, respectively. The first series of polymers (M1~M3) were prepared with an equimolar ratio of [monomers]:[crosslinkers] and variable ratio of the template, *x*. The second series of polymers (M21, M2, M22) were prepared using a constant [templates]:[monomers] ratio of 0.026:1 and a variable cross-linker ratio, *y*. Moreover dimethyl sulfoxide (DMSO) solvent was also examined with samples of M23 and N4, replacing the ethanol-water solvent mixture in the method described above.

The resultant bulky solid polymers were powdered and sieved through a 25 μm mesh, with the powder size verified by SEM. The polymer powders were washed in tetrahydrofuran (THF) for 24 hours using Soxhlet extraction to remove residual components in polymer matrix including monomers, crosslinkers, and linear oligomers. The powders were then rinsed in DI water for 12 hours at room temperature to extract the imprinted iodixanol templates and then filtered. 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.

Table I. Details of sample codes and molar feed ratios of MIPs and their control samples NIPs.

Polymer Code No.	Iodixanol Templates	4-VP Monomers	EGDMA Cross-linkers	Solvents
M1	0.167	1	1	aqueous ethanol
M2	0.026	1	1	
M3	0.006	1	1	
N1	0	1	1	
M21	0.026	1	0.333	aqueous ethanol
N2	0	1	0.333	
M2	0.026	1	1	
N1	0	1	1	
M22	0.026	1	1.667	
N3	0	1	1.667	
M23	0.026	1	1	DMSO
N4	0	1	1	

Recognition and absorption tests were performed both on MIPs and on NIPs. In a typical procedure, 50 mg of dry polymers were added to a vial of 10 ml iodixanol aqueous solution followed by magnetic stirring for 24 hours. The initial iodixanol solution concentration is 15 mg/ml which mimics a typical iodixanol concentration in the blood after administration for medical imaging experiments. Subsequently, the solution was centrifuged and analyzed by UV-vis spectroscopy (at 245 nm) [6] to characterize the concentration of remaining iodixanol in the solution. The binding capacity (BC) and imprinting effect (IE) were calculated using Equations 1 and 2. The mean value was determined from three independent tests.

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

$$IE = \frac{BC_{MIPs}}{BC_{NIPs}} \quad (2)$$

where C_i and C_f are the initial and final iodixanol solution concentrations, respectively, V is the solution volume, Δm_t is the amount of iodixanol bound in the polymers, m_p is the mass of polymer, and 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 imprint effect, IE, quantifies the improvement of absorption efficacy of imprinted polymers relative to non-imprinted polymers.

RESULTS AND DISCUSSION

The measured binding capacities (BCs) and imprint effects (IEs) of the polymers in aqueous solutions are listed in Table II. Results show that all of MIPs can bind iodixanol in larger amounts than the analogous control polymers NIPs, giving IE values exceeding unity. The

highest BC obtained was 284 mg/g from sample M2, which has an IE = 8.8, indicating that a strong imprint effect was achieved.

Table II. BCs and IEs of polymers in aqueous solutions.

Polymer Code No.	BC (mg /g)	IE
M1	128 ± 22.4	4.0
M2	284 ± 49.7	8.8
M3	178 ± 31.15	5.6
N1	32 ± 1.6	-
M21	26 ± 4.55	2.2
N2	12 ± 0.35	-
M2	284 ± 49.7	8.8
N1	32 ± 5.6	-
M22	190 ± 33.25	6.8
N3	28 ± 4.9	-
M23	192 ± 35.2	2.7
N4	72 ± 6.8	-

Effect of molar ratios

It was found that [template]:[monomer] and [monomer]:[crosslinker] ratios were important factors affecting the BCs and IEs values of the MIPs. For these samples there was found to be an optimal value of [template]:[monomer]:[crosslinker] ratio (*i.e. x*:1:*y*) that produces a maximum in the binding capacity (BC) with a good corresponding imprint effect (IE). Figure 2 represents the results graphically. From the trends shown in Figures 2a and 2b, the most efficient system has a [template]:[monomer]:[crosslinker] molar ratio of 0.026:1:1.

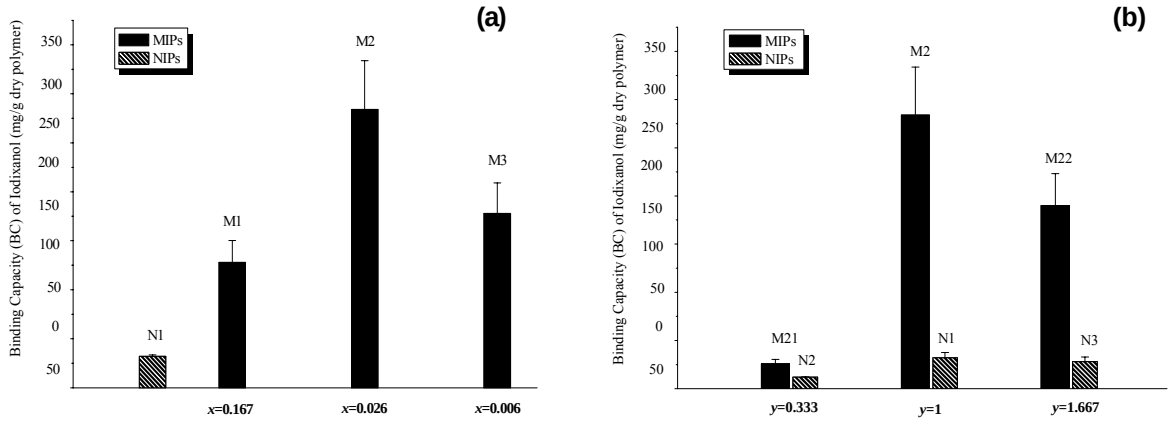

Figure 2. BCs of polymers (a) with a molar ratio of *x*:1:1, and (b) with a molar ratio of 0.026:1:*y*.

From Figure 2a, it can be observed that at low *x* ([template]:[monomer] ratio of *x* is less than or equal to 0.026) the binding capacity (BC) of MIPs increases with increased number of imprint sites, *i.e. x*. Once the ratio exceeds 0.026 the BC of MIPs decreases with increasing *x*.

This could be caused by the interference of excess iodixanol templates with the formation of sites of templates [7], or binding site heterogeneity [8] which could occur when excess templates self-assemble to form clusters. Further experiments are required to unambiguously differentiate between these or other mechanisms to explain the observed [template]:[monomer] ratio dependence.

The [monomer]:[crosslinker] ratio, *y*, also plays an important role by affecting the crosslink density and consequently the BCs of polymers. If the crosslink density is too low, then the network is too flexible to retain the sites that the templates induced. However, if the crosslink density is too high, then diffusion of templates or target molecules within the network would become significant [4]. That is to say, high crosslink density might prevent templates from diffusing out of the network in the extraction step or prevent iodixanol target molecules from diffusing into the binding sites during absorption. As shown in Figure 2b the ideal ratio of *y* is 1:1.

Effect of the solvent

Selection of solvents is challenging, especially for water-soluble, medium or large size templates. It has been proposed that the presence of polar solvents can interfere with the monomer-template associations, however, in this work the extremely low solubility of iodixanol in apolar or weak polar solvents requires the use of polar solvents, sucah as. ethanol/DI water (5:1) and DMSO. Comparison in the effects of these two solvent systems was performed in samples M2 and M23 together with the control samples N1 and N4.

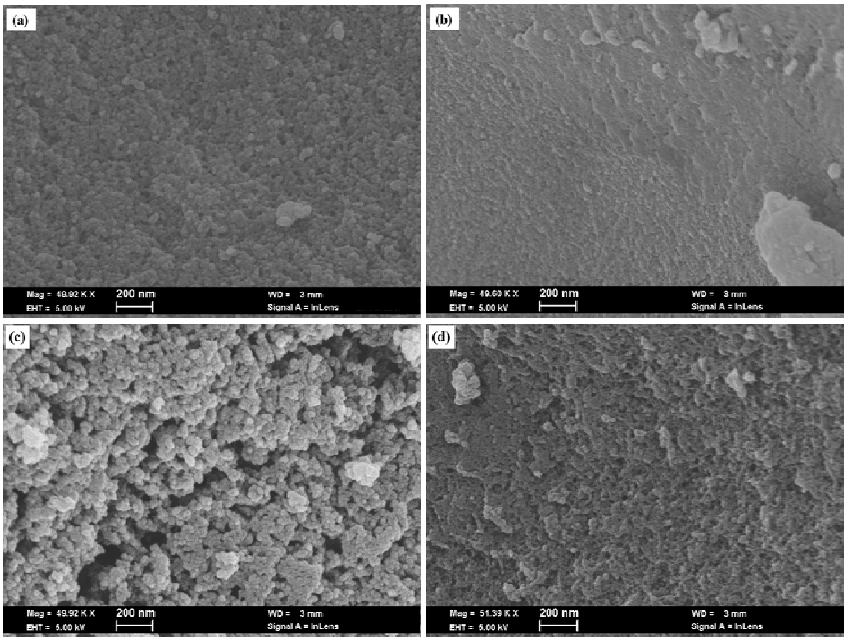

Figure 3. SEM images of prepared polymers with a scale bar of 200 nm, (a) M2, (b) N1, (c) M23, and (d) N4.

The measured BC and IE values for M23 (BC=192 mg/g, IE= 2.7) produced from DMSO is much lower than those of M2 (BC=284 mg/g, IE=8.8) synthesized from aqueous ethanol solvents (see Table II). SEM results (see Figure 3) indicate that the samples prepared in DMSO

(M23 and N4) have similar but slightly more porous morphology than those prepared in aqueous ethanol (M2 and N1). BET surface analysis measurements were further used to explore the impact of the solvent on the porosity of polymers. Sample M23 (S=53.86 m^2/g and V_p=0.221 cm^3/g) and its control polymer N4 (S=17.64 m^2/g and V_p=0.071 cm^3/g) were found to be more porous than M2 (S=6.83 m^2/g and V_p=0.019 cm^3/g) and N1 (S=3.59 m^2/g and V_p=0.012 cm^3/g). SEM and BET results indicate that DMSO is a better porogenic solvent than aqueous ethanol leading to a higher S and V_p, which is associated with the solvent quality compared to the aqueous ethanol. However based on the BC and IE values aqueous ethanol is a better media than DMSO for imprinting iodixanol. It is hypothesized that due to the higher solvent polarity of DMSO, DMSO could interfere with monomer-template associations more than aqueous ethanol. This polarity effect is however moderated since DMSO is a better porogenic solvent enhancing the surface porosity of produced polymers.

CONCLUSIONS

This work summarizes molecular non-covalent imprinting of the water-soluble X-ray contrast medium iodixanol in aqueous solvents using 4-vinylpyridine as the functional monomer, and ethylene glycol dimethacrylate as the crosslinker. Recognition and absorption of template molecules by imprinted polymers is demonstrated in aqueous solution. Results demonstrate good binding capacity and imprint efficiency have been achieved. The best binding capacity achieved from optimized imprinted polymers in this study is 284 mg/g, 8.8 times higher than that of the control polymers. Thus this work has proved the feasibility of molecularly imprinting iodixanol. Such imprinted polymers can be applied to hemodialysis as well as other biomedical applications.

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REFERENCES

1. S. D. Weisbord, *Reviews in Cardiovascular Medicine*. **9**, S14 (2008).
2. K. Mosbach and O. Ramstrom, *Biotechnology*, **14**, 163 (1996).
3. K. Haupt, A. Dzgoev, and K. Mosbach, *Anal. Chem.* **70**, 628 (1998).
4. N. M. Bergmann and N. A. Peppas, *Progress in Polymer Science*, **33**, 271 (2008).
5. L. Ye and K. Mosbach, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, **41**, 107 (2001).
6. P. B. Jacobsen, A. Larsen, R. Konarboland, and T. Skotland, *Drug Metabolism and Disposition*, **27**, 1205 (1999).
7. W. J. Wizeman, P. Kofinas, *Biomaterials*, **22**, 1485 (2001).
8. L. E. Bromberg and A. M. Klibanov, *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 1262 (1995).