ELSEVIER

Biomaterials 22 (2001) 1485–1491

Biomaterials

www.elsevier.com/locate/biomaterials

Molecularly imprinted polymer hydrogels displaying isomerically resolved glucose binding

William J. Wizeman, Peter Kofinas*

Department of Materials and Nuclear Engineering, University of Maryland, College Park, MD 20742-2115, USA

Received 8 August 2000; accepted 2 October 2000

Abstract

Non-covalent molecular imprinting of poly(allylamine hydrochloride) (PAA·HCl) with glucose phosphate mono-sodium salt produced molecularly imprinted polymer (MIP) hydrogels capable of quantitative, isomerically specific binding of glucose. By ionic association of a template molecule, glucose phosphate mono-sodium salt, to the polymer prior to covalent crosslinking, MIP hydrogels were created with an affinity for binding glucose. In this study we have synthesized MIPs using epichlorohydrin, ethylene glucol diglycidyl ether, and glycerol diglycidyl ether as crosslinkers in order to evaluate their effectiveness with respect to molecular imprinting for glucose. MIP hydrogels were also synthesized with the different crosslinkers and varying amounts of the template molecule in an attempt to elucidate the impact of imprint quantities on the effectiveness of the imprinting technique. Batch equilibration studies, using each of the MIPs and similar non-molecularly imprinted polymers were performed to determine their binding capacities with respect to glucose and fructose. The binding capacity data are discussed and employed in the evaluation of the specificity imparted by the imprinting procedure. MIP hydrogels with binding capacities in excess of 0.5 g of glucose per gram of dried gel were synthesized. Isomeric specificity in hydrogels imprinted for glucose was demonstrated by higher binding capacities of glucose than those of fructose in the same polymers. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hydrogels; Molecular imprinting; Glucose; Diabetes; Poly(allylamine)

1. Introduction

The synthesis of highly specific molecularly imprinted polymers (MIPs) has been the goal of many research groups in the past decade. The methods of molecular imprinting and its potential applications have been recently reviewed [1,2]. Promising applications for these MIPs include tailor-made separation materials, molecular recognition materials for biosensors, highly specific catalysts, antibody mimics for quantitative assay and molecular recognition, and drug delivery. The application that promises to be of greatest industrial significance in the immediate future is that of separation materials. These materials have been used in conjunction with chromatographic techniques to separate amino acids, drugs, pesticides, sugar derivatives, and other compounds [3,4,6,7]. MIPs have also been employed in efforts to synthesize materials for biosensor applications. An especially interesting area of biosensors is quantitative glucose monitoring. This is because nearly 6% of the population in the United States suffers from diabetes and quantitative blood glucose monitoring is an essential part of the successful management of the disease. Arnold et al. [8] have used molecular imprinting to produce a glucose-sensitive polymer that exhibits a change in pH proportional to the glucose concentration of its environment. More recently, Zambonin et al. [9] have explored a new technique utilizing electrosynthesized MIPs in an effort to develop a glucose-sensitive material. Catalysts and antibody mimics stand to benefit in similar manners from the technique of molecular imprinting. The ability to construct complicated combinations of precisely placed functional groups in a mechanically stable and specifically shaped cavity allows the synthesis of materials capable of catalyzing complex reactions or of mimicking antibodies used in analytical reagents. Mosbach et al. [10] have shown promising results in the area of artificial antibodies. In the area of drug delivery Peppas et al. [11] have utilized the technique of molecular imprinting in the controlled delivery of proteins.

* Corresponding author. Tel.: + 1-301-405-7335; fax: + 1-301-314-2029.

E-mail address: kofinas@eng.umd.edu (P. Kofinas).

0142-9612/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0142-9612(00)00303-3

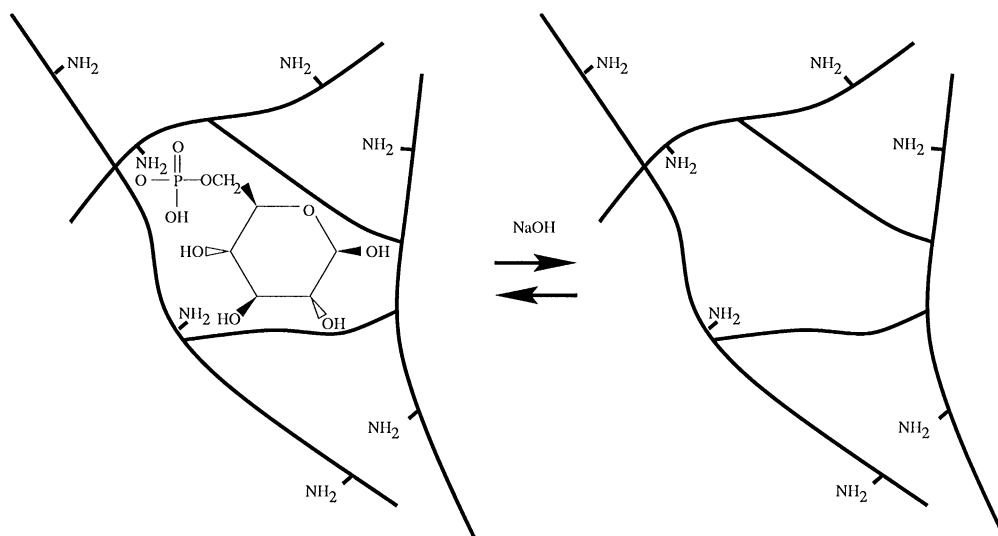

Fig. 1. Schematic representation of imprinting and removal procedures.

Molecular imprinting in polymers is achieved by incorporating a template, or imprint molecule into a highly crosslinked polymer matrix. The imprint molecule is identical or similar, in both size and functionality, to the molecule for which the polymer is being imprinted, the target molecule. Given a polymer matrix with sufficient mechanical stability, cavities with size, shape and functionality specific to the template molecule are created upon removal of the template. Fig. 1 is a schematic representation of the technique of molecular imprinting used in this study.

The template molecule is bonded to a polymerizable functional monomer or, as in this case, a polymer side group prior to crosslinking. The bond between the template and the functional monomer or polymer can be either covalent or non-covalent. Studies have shown successful results in the area of molecular imprinting via non-covalent interactions between template and functional monomer [3,6] as well as via covalent interactions [4]. The non-covalent imprinting approach seems to hold more potential for the future of molecular imprinting due to the vast number of compounds, including biological compounds, which are capable of non-covalent interactions with polymerizable monomers. These non-covalent interactions are easily reversed, usually by a wash in aqueous solution of an acid, a base, or methanol, thus facilitating the removal of the template molecule from the network after polymerization. Reversible covalent interactions with polymerizable monomers are fewer in number and often require an acid hydrolysis procedure to cleave the covalent bonds between the template and the functional monomer. For this reason, we have focused on the use of non-covalent interactions as a means to produce molecularly imprinted polymers. To the best of our knowledge, all of the molecular imprinting studies to date have focused on synthesizing imprinted polymers from monomer, rather than crosslinking an existing polymer with functionality which is conducive to a non-covalent imprinting technique. Molecular imprinting which utilizes a readily available polymer greatly simplifies the synthesis of the MIPs and may bring the technology closer to application in the aforementioned areas. Because of the environment in which they will be used, biosensor, pharmaceutical, and some chemosensor MIPs must be designed to function properly in aqueous environments. The technique presented here not only employs the more flexible non-covalent approach to imprinting, but begins with a polymer having amine functionality to bind the imprint, instead of a functional monomer, and both the MIP synthesis and testing are performed in aqueous solution under air.

We were able to synthesize MIPs which have a strong and specific affinity for glucose, using a template molecule derived from glucose. Refinement of these MIP hydrogels could result in a product to aid in the treatment of type 2 diabetes. Type 2 diabetes results from the inability of the pancreas to produce sufficient amounts of insulin. The treatment of type 2 diabetes typically includes diet control, exercise, home blood glucose testing, and, in some cases, oral medication and/or insulin. The MIPs presented here have potential as a drug capable of binding glucose in the stomach and small intestine and passing undigested through the body. The drug would simply be ingested along with foods that are high in glucose and would reduce the sharp rise in blood sugar associated with the ingestion of significant amounts of monosaccharides. In addition, this molecular imprinting technique may have application in the preparation of an MIP glucose sensor.

The system studied here begins with a polymer whose monomeric unit contains an amine group. This amine group imparts significant flexibility upon the system with

respect to the number of compounds for which the polymer can be imprinted, since amines are capable of ionic interactions with anionic species as well as hydrogen bonding. In this study the amine function is used to form an ionic association with the imprint molecule's phosphate group. The addition of glucose phosphate mono-sodium salt to a poly(allylamine hydrochloride) (PAA · HCl) solution thus results in a polymer solution that becomes an MIP hydrogel when crosslinked. The prevalence of compounds capable of ionic interaction with amines may allow a variety of compounds to be imprinted using the polymer system presented here.

2. Experimental

2.1. Materials

The MIPs were prepared using the polymer PAA · HCl with a weight average molecular weight of 15,000 g/mol and a polydispersity, determined by gel permeation chromatography, of 2.7. The imprint molecule was glucose phosphate mono-sodium salt (GPS). The crosslinkers used to synthesize the MIPs were epichlorohydrin (EPI), ethylene glycol diglycidyl ether (EGDE), and glycerol diglycidyl ether (GDE). All compounds were obtained from Sigma-Aldrich and were used without further purification. All experiments were performed in aqueous solution, using water from an Elix water purification system by Millipore, model # ZLXS6003Y.

2.2. MIP hydrogel synthesis

A typical MIP hydrogel was synthesized as follows: 25% w/v aqueous PAA · HCl solution was mixed with GPS and allowed to stir for 2 h to ensure complete association of the imprint molecule with the polymer. A portion of the PAA · HCl amines sites was then neutralized for crosslinking by adding NaOH under stirring. The neutralization of an amines site refers to the removal of the ionically associated HCl from the amine. The solution was allowed to stir continuously for 20 min before adding one of the three aforementioned crosslinkers. Upon addition of the crosslinker, the polymer solution was stirred until just before complete gelation in order to maximize the homogeneity of the polymers. Just prior to complete gelation the polymerizing solution was poured into a petri dish so that, upon gelation, the MIP hydrogel would set in the form of a slab. It is useful to note that the onset of gelation is sudden and often difficult to foresee. However, by carefully watching the decrease in height of the vortex of a stirring polymer solution one can roughly predict the time to complete gelation. After gelation, the polymer was allowed to stand overnight to ensure complete crosslinking. The polymer was then cut into 4 mm squares and washed in 1 M aqueous NaOH solution for at least 48 h to remove the GPS imprint. The polymers were repeatedly washed with de-ionized water to remove any remaining NaOH. The de-ionized water washes were performed in 1–2 h intervals, 3–4 times per day, while monitoring the pH of the effluent wash. When the effluent wash water, after an overnight equilibration period with the gel, was no longer basic it was determined that the polymers were free of excess NaOH. Typically, 5 days of washing the MIPs as described was required to remove all of the NaOH. Finally, the completely washed gels were dried under air in a 50°C oven. The molar ratio of compounds used in a typical MIP hydrogel synthesis is given by

$$\text{imprint:monomer:NaOH:crosslinker} = 3:200:100:26. \quad (1)$$

2.3. Verification of imprinting technique

Preliminary quantitative analysis was performed to ensure that the imprint binding and removal techniques were effective. The binding and removal of the GPS imprint was quantified using the following procedure: A freshly synthesized hydrogel, containing the GPS imprint, was placed in a known volume of de-ionized water and stirred slowly for 48 h. A filtered aliquot of the wash water was then taken. The quantity of phosphorus contained in this sample was then determined using Hach's total phosphorus test, method 8190 [5]. Prior to testing, the wash water sample was diluted so that the expected phosphorus concentration lay in the measurable range of the Hach test. In addition, the pH of the diluted sample was checked to ensure that it was between 6.5 and 7.5 and then tested for total phosphorus. A simple molar equivalence between phosphorus and GPS allowed the quantity of imprint present in the water wash to be determined. The phosphorus concentration was found to be less than 2% of what would be expected if all of the GPS used in the synthesis were present in the wash solution. This same polymer was then placed in a known volume of 1 M NaOH solution and equilibrated for 48 h to remove the bound GPS. A filtered aliquot was then taken, diluted appropriately for the Hach total phosphorus test. The sample's pH was then adjusted using HCl so that it was between 6.5 and 7.5. This solution was then tested for total phosphorus. The phosphorus concentration was 60% of what would be expected if all of the GPS used in the synthesis were present in the wash solution. Similar tests have shown removal concentrations as high as 99% of what would be expected upon full removal in the first NaOH wash. This test is useful to verify the absence of imprint removal in distilled water, indicating good template immobilization. It is also useful to verify, in the case of very high GPS concentrations (i.e. 99% of expected), complete GPS removal. However, in the case of lower GPS removal concentrations, without evaluating the phosphate concentration at subsequent wash steps it is

impossible to make an accurate quantitative statement about total GPS removal.

2.4. Binding experiments

Binding capacities of the hydrogels were determined via batch reactor studies. The dried polymers were added to a 50 mg/ml aqueous solution of either glucose or fructose. The 50 mg/ml concentration was intended to mimic the sugar concentration likely to be found in the stomach and duodenum after the consumption of a soft drink or sugary snack. This assumes a stomach volume of 1 l and a consumption of 50 g of sugar. The test solution and the MIP or non-imprinted polymer (NIP) being tested were then stirred slowly for 4 h, whereupon filtered aliquots of the solution were taken to determine the remaining concentration of sugar in the test solution. Binding capacities were calculated using the following equation:

$$\frac{g\text{ (sugar bound)}}{g\text{ (MIP)}} = \frac{(C_\mathrm{i} - C_\mathrm{f})V_\mathrm{s}}{m_\mathrm{MIP}}, \tag{2}$$

where C_i is the initial sugar concentration (mg/ml), C_f the final sugar concentration (mg/ml), V_s the volume of test solution (ml), and m_MIP the mass of dried polymer (mg).

In addition to the 4 h glucose binding tests, an equilibrium binding experiment was performed, using a different gel batch, to determine the time necessary for complete equilibration and the maximum glucose binding capacity of the MIP. In this experiment the test solution and the MIP were allowed to equilibrate under slow stirring for 36 h. Periodically, aliquots of the test solution were taken and the binding capacity was calculated. The total volume of the aliquots taken during this test was less than 4% of the total test volume, minimizing any effects that may have resulted from a significant decrease in the test volume.

Accurate quantitative determination of glucose and fructose concentrations was critical to the evaluation of the performance of the polymers. Glucose concentrations were determined colorimetrically using a Hach DR2010 spectrophotometer and Stanbio's enzymatic glucose reagent. The reported coefficient of variation for the Stanbio glucose test is 2.0–3.0%. This is in accordance with our findings. Fructose concentrations were also determined colorimetrically, using a procedure developed by van Creveld as modified by Oppel [12]. This fructose procedure reports to have an error of less than 10% associated with it. Using fructose standard solutions, we have shown coefficients of variation of 1.3–3.2% when each test is performed in triplicate and a mean value is obtained. The improvement in the precision of the fructose test is most certainly a result of the advent and implementation of newer, more accurate, volumetric measuring devices since the publication of the test. All compounds required for the quantitative fructose determination procedure were obtained from Aldrich and used without further purification.

3. Results

The glucose and fructose binding capacities of the MIPs presented here were determined using the equilibration tests previously described. Table 1 shows the binding capacities in four different polymers imprinted with GPS, using EGDE as the crosslinking agent. The first three MIPs listed are imprinted with increasing amounts of GPS. The imprint quantities given in the table are mole percents with respect to monomer units of the polymer. The last polymer listed is an NIP and was used as a control sample to evaluate the effectiveness of the imprinting procedure. Glucose binding in these MIPs was shown as high as 0.56 g of glucose per gram of dried polymer gel. Data from similar studies with MIPs synthesized using EPI and GDE as the crosslinking agents are also shown. Table 2 shows the glucose and fructose binding capacity in four different polymers imprinted against glucose, crosslinked with EPI. Again, the first three MIPs in the table contain different amounts of imprint while the last is an NIP control. These polymers were capable of the highest degree of glucose binding at 0.58 g of glucose per gram of dried MIP. Table 3 is organized in the same fashion as the preceding tables and summarizes the glucose and fructose binding in the MIPs imprinted against glucose and crosslinked with GDE.

The results of the 36 h equilibration test are given in Fig. 2. Glucose binding capacities were calculated at 1, 2, 4, 6, 10, 16, 24, and 36 h. The MIP employed in this test is a 1.0% imprinted, EGDE-crosslinked hydrogel. This

Table 1
Isomeric sugar binding in EGDE-crosslinked MIPs

% GPS	Glucose binding (g/g)	Fructose binding (g/g)
0.50	0.48 ± 0.03	0.34 ± 0.04
1.00	0.53 ± 0.03	0.21 ± 0.03
1.50	0.56 ± 0.01	0.23 ± 0.06
No imprint	0.18 ± 0.01	0.11 ± 0.02

Table 2
Isomeric sugar binding in EPI-crosslinked MIPs

% GPS	Glucose binding (g/g)	Fructose binding (g/g)
0.50	0.58 ± 0.02	0
1.00	0.54 ± 0.03	0
1.50	0.50 ± 0.01	0
No imprint	0.20 ± 0.01	0

Table 3
Isomeric sugar binding in GDE-crosslinked MIPs

% GPS	Glucose binding (g/g)	Fructose binding (g/g)
0.50	0.39 ± 0.02	0.22 ± 0.03
1.00	0.34 ± 0.06	0.18 ± 0.03
1.50	0.26 ± 0.03	0.12 ± 0.00
No imprint	0.13 ± 0.01	0.05 ± 0.03

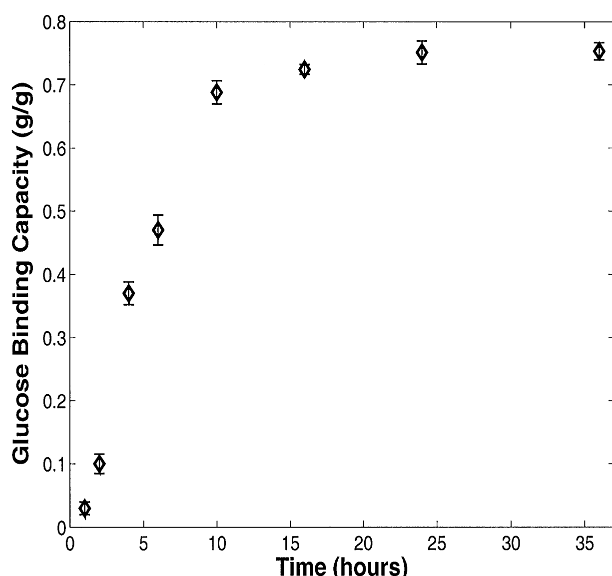

Fig. 2. 36 h equilibrium binding capacity test, 1% GPS imprinted, EDGE crosslinker.

same MIP was also employed in the 4 h tests of glucose and fructose binding capacities shown in Table 1.

3.1. Imprinting mechanism

Poly(allylamine hydrochloride) chains have an HCl ionically associated with each of the side chain primary (NH_2) groups along the polymer chain backbone. Before being able to ionically bond the imprint molecule or crosslink the polymer chains, amine sites must be freed of the associated HCl. In the case of imprinting, no extra steps were necessary since the phosphate group of the GPS is highly negatively charged. This allows the GPS to displace the previously associated HCl and bond to the amine itself. However, for the crosslinking step, a portion of the HCl groups of the PAA · HCL was neutralized with NaOH to provide available NH_2 sites for the crosslinking reactions. The completion of this neutralization reaction can be monitored by temperature since the neutralization of the HCl groups is an exothermic reaction. In other words, when the temperature of the mixture returns to room temperature, the reaction is complete. For total solution volumes of approximately 25 ml it was determined that 20 min is a sufficient reaction time for complete neutralization. For syntheses with total solution volumes of approximately 25 ml, the observed reaction times for complete gelation of EPI, EGDE, and GDE MIPs were 35, 8, and 6 min, respectively. The differing gelation times are functions of the reaction kinetics and conversion required for gelation for each crosslinker. In order to remove the imprint molecule from the MIPs after crosslinking, it is necessary to break the ionic interaction between the GPS and the amine groups of the polymer. This was accomplished by washing the hydrogel in a 1 M aqueous solution of sodium hydroxide for 48 h.

4. Discussion

4.1. Effects of imprinting on binding

It can be expected that a change in the quantity of imprint molecule will have a direct effect on the binding and specificity with respect to an analogue of the imprint molecule. This is due to the creation of binding sites, or cavities, which are correctly sized and shaped and appropriately functionalized to bind or allow the passage of the imprint or its analogue. Clearly, this assumption requires that the polymer network have an appropriate molecular weight between crosslinks to "encapsulate" a specific imprint molecule. It will be very difficult to impart any degree of specificity onto a system in which the effective length between crosslinks differs greatly from the size of the imprint and target molecules. In other words, if the crosslink density is too low then the effective length between crosslinks will be too large for the imprinting process to be successful. This creates an excessively large cavity and results in a non-specific imprint. An effective length between crosslinks that is too low may result in a network that traps the imprint molecule completely, resulting in very low split ratios and a totally ineffective MIP. The crosslinker is also key to forming a suitable imprint in a polymer network. Because of the amount of crosslinker used to synthesize these MIPs (13 mol% with respect to monomer), the crosslinker must be in intimate contact with the imprint molecule in the MIP network. It is likely that the crosslinker plays a key role in establishing the shape and dimensions of the resulting cavities. It is also expected that there should be some optimal imprint to crosslinker ratio for a given crosslinker, assuming all other parameters are held constant. Therefore, the choice of crosslinker greatly affects the establishment, or lack of establishment of suitable imprints, and is important in the design of an MIP. In these experiments it is clear that the molecular imprinting procedure has produced cavities with an affinity for the imprint's analogue, glucose. These cavities give rise to the observed variation in binding capacities and specificities.

The glucose binding observed in the MIPs crosslinked with EGDE behaves as one would expect if the factors affecting MIP design discussed previously are considered. As the amount of imprint present during synthesis increases, so does the binding capacity of the MIPs. This increase in binding capacity is a result of an increase in the number of cavities through which glucose can "pass freely". The first column of Table 1 clearly shows this trend. In addition to an increase in glucose binding with increasing imprint concentration, we also observed a decrease in the binding capacity of fructose. This trend is an indicator of specificity for glucose. Ideally, as the concentration of imprint increases, so does the number of cavities specific to glucose. As the number of cavities specific to glucose increases, the number of non-specific cavities large enough to allow fructose to pass decreases due to the addition of a glucose imprint molecule to the cavity during crosslinking. This trend is evident in the second column of Table 1 which shows a decrease in the binding capacity of fructose with increasing imprint concentration. It is also important to note that the binding capacities of the NIPs were significantly lower, confirming the effectiveness of the imprinting technique.

The MIPs using EPI as a crosslinker displayed some very interesting results. The first column of Table 2 shows that the glucose binding in these MIPs decreases with increasing concentration of imprint. One would expect the trend to be similar to that observed in the MIPs using EGDE as a crosslinker. In the second column of Table 2 the fructose binding data for the MIPs crosslinked with EPI are given. The binding capacity values are insignificant and are listed as zero. This degree of specificity has yet to be achieved using the MIP synthesis techniques presented here. The decreasing glucose absorption with increasing amount of imprint observed in the MIPs using EPI as a crosslinker is believed to be related to the size of the crosslinkers. EPI is a much smaller crosslinker than EGDE. This is evident in Fig. 3 where the structures of the crosslinkers employed in this study are shown. This makes EPI less able to form successful imprints. It is expected that there will be some optimal relative amounts of imprint and crosslinker, giving the highest binding capacity that will vary from one type of crosslinker to another. Excess imprint could actually hinder the imprinting procedure by interfering with the formation of cavities of other imprint molecules. This is believed to be the mechanism which results in a decrease in binding capacity with increasing imprint in the MIPs using EPI as a crosslinker.

MIPs and NIPs crosslinked with GDE do not follow either of the trends observed in the polymers crosslinked with EGDE or EPI. Table 3 shows that as the concentration of imprint increases, both the glucose and fructose binding capacities decrease. It is important to note that GDE-crosslinked polymers had very poor mechanical integrity when compared to the hydrogels produced by EGDE and EPI. Approximately 25% of each polymer was lost due to excessive fracture, giving the hydrogel a paste-like consistency. It was not possible to filter this

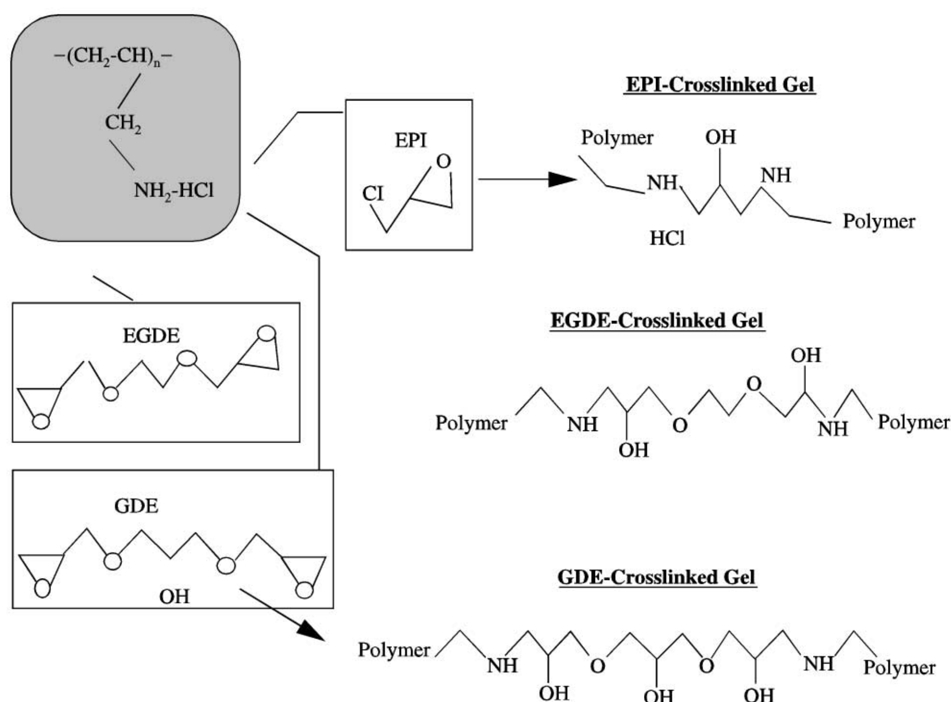

Fig. 3 . Crosslinking reaction and crosslinkers used in MIP synthesis.

hydrogel under vacuum. Therefore, a Buchner funnel without filter paper was used which allowed the highly fractured portion of the hydrogel to pass, resulting in the aforementioned loss of polymer. The networks produced by GDE were not mechanically stable and are broken by the washing, stirring, and swelling processes that the gel must endure during the synthesis procedures. During equilibration test the polymers also fractured appreciably, breaking up into what resembled an insoluble powder. Without question, as crosslinks are broken the number and effectiveness of the cavities established during the imprinting procedure would also be compromised, reducing the gels binding, specificity, and overall usefulness. For this reason the data obtained for the MIPs and NIPs crosslinked with GDE are presented here but are not discussed in depth.

In the 36 h equilibration test shown in Fig. 2 it is clear that initially the glucose binding capacity increases exponentially with positive curvature. However, the curvature of the trend becomes negative somewhere between 4 and 10 h, leading to the final equilibrium binding capacity conditions seen at 24 and 36 h. Consecutive equivalent binding capacities at 24 and 36 h were taken to be sufficient evidence of equilibrium. It is interesting to note that at 4 h, our chosen standard testing time, the binding capacity is approximately half of the observed maximum at 24 and 36 h. However, the application of the MIP hydrogels as a pharmaceutical most likely would not be able to take advantage of the increased binding capacities observed at longer times due to the relatively rapid rate of glucose absorption in the human body.

5. Conclusions

The novel molecular imprinting procedure presented in this work is the first attempt at molecular imprinting in aqueous solution using a polymer and crosslinkers rather than functional monomer. The isomeric selectivity demonstrated in this study is promising. However, there is still much work to be done in efforts to optimize the MIPs presented here and to further understand the factors affecting the behavior of these MIPs. Future work will include attempts at the synthesis of hydrogels imprinted specifically for fructose as well as further optimization to increase the binding capacities and selectivities for hydrogels imprinted against glucose.

Acknowledgements

This research has been supported from supplemental matching funds obtained from the National Science Foundation in support of CAREER Award CTS-9875001.

References

[1] Wulff G. Fitting molecules into polymeric receptors. Chemtech 1998;28:19–26.
[2] Mosbach K, Ramstrom O. The emerging technique of molecular imprinting and its future impact on biotechnology. Bio-Technology 1996;14:163–70.
[3] Mayes AG, Andersson LI, Mosbach K. Sugar binding polymers showing high anomeric and epimeric discrimination obtained by noncovalent molecular imprinting. Anal Biochem 1994; 222:483–8.
[4] Wulff G, Schauhoff S. Racemic resolution of free sugars with macroporous polymers prepared by molecular imprinting. J Org Chem 1991;56:395–400.
[5] Hach Company. DR/2010 Spectrophotometer Procedures Manual, 1996.
[6] Spivak D, Shea KJ. Molecular imprinting of carboxylic acids employing novel functional macroporous polymers. J Org Chem 1999;64:4627–34.
[7] Yu C, Mosbach K. Molecular imprinting utilizing an amide functional group for hydrogen bonding leading to highly efficient polymers. J Org Chem 1997;62:4057–64.
[8] Chen G, Guan Z, Chen CT, Fu L, Sundaresan V, Arnold FH. A glucose sensing polymer. Nat Biotechnol 1997;15:354–7.
[9] Malitesta C, Losito I, Zambonin PG. Molecularly imprinted electrosynthesized polymers: new materials for biomimetic sensors. Anal Chem 1999;71:1366–70.
[10] Ansell RJ, Ramstrom O, Mosbach K. Towards artificial antibodies prepared by molecular imprinting. Clin Chem 1996; 42:1506–12.
[11] Peppas NA, Keys KB, Torres-Lugo M, Lowman AM. Poly(ethylene glycol)-containing hydrogels in drug delivery. J Control Release 1999;62:81–7.
[12] Oppel. Diphenylamine method fructose. In: Browne CA, Zerban FW, editors. Physical and chemical methods of sugar analysis. New York: Wiley, 1941. p. 961.