

# Biomimetic glucose recognition using molecularly imprinted polymer hydrogels

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## Abstract

Non-covalent molecular imprinting of poly(allylamine hydrochloride) (PAA · HCl) with D-glucose 6-phosphate monobarium salt (GPS-Ba) produced molecularly imprinted polymer hydrogels (MIP) having an affinity to glucose over fructose. The hydrogels were formed by ionic association of the template molecule, GPS-Ba, to the polymer, prior to covalent crosslinking using epichlorohydrin (EPI). The template was removed by an aqueous base wash. Batch equilibration studies using different MIP hydrogels and non-molecularly imprinted polymers (NIPs) were performed in aqueous and buffered media to determine the binding capacities and isomeric selectivities with respect to the sugars, glucose and fructose. MIP glucose hydrogels exhibited binding capacities in excess of 0.6 g of glucose per g of dry gel in a 100% DI H<sub>2</sub>O glucose solution, and in a 50–50% glucose–fructose solution mixture. Equilibrium binding capacities of fructose were lower than those observed with respect to glucose, indicating an isomeric preference for the binding of glucose over fructose. These hydrogels demonstrated a remarkable degree of biomimetic sugar recognition to specifically and selectively bind glucose in their swollen state in environments mimicking physiological conditions.

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## 1. Introduction

Molecular imprinting is an emerging technology, which allows the synthesis of materials containing highly specific receptor sites with an affinity for target compounds. MIPs can mimic some of the functions of enzymes, through the creation of three-dimensional cavities of specific size and shape for biomimetic recognition of bioactive compounds. Promising applications for MIPs include the development of assays and sensors, membranes, the production of polymers with special functions such as drug release matrices, separation materials, molecular recognition materials for biosensors, highly specific catalysts, and antibody mimics [1–8]. This research attempts to further expand the scope of applications of polymeric hydrogels, through the synthesis, characterization and elucidation of biomimetic sugar recognition in molecularly imprinted polymer (MIP) networks. The clinical relevance

of this research relates to the development of a pharmaceutical, which would aid in the treatment of type II diabetes and obesity. Such MIPs may also be employed in efforts to synthesize materials for biosensor applications, especially in quantitative glucose monitoring.

This paper deals with non-covalent molecular imprinting of poly(allylamine hydrochloride) (PAA · HCl) with a D-glucose 6-phosphate monobarium salt (GPS-Ba) in competitive environments to produce MIP hydrogels. The chosen polymer matrix, poly(allylamine), has good water solubility and presents a high density of amine groups. In its cross-linked form, this polymer possesses low toxicity [9,10]. We have previously demonstrated glucose imprinting from pure glucose solutions in DI water [11]. A novel characteristic of the imprinting technique presented in this paper is the choice of the monophosphate barium salt of glucose as the templated sugar, rather than pure unmodified glucose, which results in enhanced affinity and binding of the template to the primary amine groups of the polymer, and thus improved specificity in the imprinting procedure. These MIP hydrogels are formed by ionic

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association of the glucose analog, GPS-Ba, to the polymer, prior to covalent crosslinking using epichlorohydrin (EPI). Batch equilibration studies using different MIP hydrogels and similar non-molecularly imprinted polymers (NIPs) were performed to determine the binding capacities and isomeric selectivities of these polymers with respect to the sugars, glucose and fructose. The binding capacities were performed in aqueous and buffered media of pure and mixed sugar solutions to evaluate the effectiveness of the MIPs in competitive environments mimicking physiological conditions. These MIP hydrogels demonstrated a remarkable degree of sugar biomimetic recognition to specifically and selectively bind glucose in their swollen state.

While methods of template fixation vary among research groups, the majority of studies to date have concentrated on synthesizing MIPs from monomer in organic solvents. 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, starting from a readily available polymer, but both the MIP synthesis and subsequent imprint recognition are performed in aqueous solutions.

## 2. Experimental

A typical MIP hydrogel was synthesized as follows: 25% w/v aqueous PAA·HCl solution was mixed with GPS-Ba and allowed to stir for 2 h to ensure complete association of the imprint molecule with the polymer. A portion of the PAA·HCl amine sites were then neutralized by adding NaOH 10 M solution while stirring. The solution was allowed to stir continuously for 20 min before adding the crosslinker, EPI. The approximate time for gelation was 10–15 min, depending on the amount of crosslinker added. After gelation, the polymer was allowed to sit overnight to ensure complete crosslinking. The polymer was then cut into 4-mm squares and washed in 4 M aqueous NaOH solution for 24 h to remove the GPS-Ba imprint and any other unreacted reagents. The polymers were subsequently washed with deionized water to remove any remaining NaOH, while monitoring the effluent wash pH. When the effluent wash solution, after an overnight equilibration period with the gel, was no longer basic ( $\text{pH} < 6.5$ ), it was determined that the polymers were free of excess NaOH. Gels were finally dried under air in a 50°C oven.

Quantitative analysis was performed to ensure that the imprint binding and removal techniques were effective. In order to determine the effectiveness of the techniques, it was necessary to establish an accurate,

quantitative detection method for the glucose imprint, GPS-Ba. The total phosphorous concentration was determined spectrophotometrically, using a Hach D2010 spectrophotometer and Hach's method 8190 [12], which is an acid persulfate digestion method used to determine total phosphorus. During the total phosphorus test all of the phosphorus is converted to phosphate. The phosphate concentration is then determined, and is related to the template concentration by molar equivalence of phosphate to template, since 1 mol of GPS-Ba contains 1 mol of phosphate groups.

The presence or absence 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 deionized water and stirred for 24 h. A filtered aliquot was taken and diluted appropriately in a volumetric flask for the Hach total phosphorus test. The pH of the diluted sample was checked to ensure that it was between 6.5 and 7.5 and tested for total phosphorus. The phosphorus concentration in the water wash was found to be less than 7% of what would be expected if all of the GPS used in the synthesis were present in the DI water wash solution. This same polymer was then placed in 4 M NaOH solution and stirred for 24 h to remove the bound GPS. A filtered aliquot was then taken and the sample's pH was adjusted using HCl to be between 6.5 and 7.5. The polymer was placed in a fresh 4 M NaOH solution and was stirred for another 24 h. This solution was then tested for total phosphorus. The total phosphorus concentration in the base wash was determined to be 95% of what would be expected upon full GPS-Ba template removal from the imprinted gel.

Sugar binding capacities of hydrogels were determined via batch reactor studies as described in [11]. The initial sugar concentration in these experiments, chosen to mimic the consumption of a soft drink or other sugary snack, was 50 mg/ml. Dried polymers were added to aqueous or buffered media solutions of pure glucose or fructose, as well as to solutions consisting of a 50–50 mixture of the two sugars. The buffered media ( $\text{pH} = 7$ ) were prepared using BES (*N,N*-bis[2-Hydroxyethyl]-2-aminoethanesulfonic acid), potassium dihydrogenphosphate ( $\text{KH}_2\text{PO}_4$ ) and sodium chloride (NaCl). After combining the test solution and the MIP or NIP whose binding capacity was to be determined, the mixture was allowed to equilibrate while stirring for 4 h. Then, filtered aliquots of the test solution were removed to determine the remaining concentration of sugar in the test solution. Binding capacities were calculated as described in [11]. Glucose concentrations were determined colorimetrically using a Hach DR2010 spectrophotometer and Stanbio's enzymatic glucose reagent [12]. Fructose concentrations were determined colorimetrically using a procedure originally developed by Van Creveld and later modified by Oppel [11,13]. All

tests were performed in triplicate and average values were used to calculate the sugar binding capacity.

### 3. Results and discussion

The crosslinked, PAA·HCl networks were prepared by the aqueous reaction of 25% w/v solution of linear PAA·HCl chains with EPI, which served as the crosslinking agent. The crosslinking reaction used to synthesize the PAA·HCl MIPs using EPI as the crosslinker is shown in Fig. 1. The ionic association of an imprint molecule with the polymer prior to crosslinking led to molecularly imprinted hydrogels. In order to maximize the potential for additional future applications, the non-covalent approach to molecular imprinting was employed. The amine groups along the polymer have a net positive charge which enables the formation of an ionic bond with the negatively charged phosphate group of the glucose monobarium salt. The ionic interaction between the template and the polymer accomplishes the immobilization of the template prior to crosslinking. Cavities specifically designed to bind glucose over fructose are formed from the crosslinking of the polymer in the presence of an imprint molecule, which is a glucose analog. As the polymer crosslinks around the imprint molecule, specifically sized and shaped cavities are formed. The template is being removed by washing the hydrogel with a base and these cavities are emptied, able to rebind or allow the passage of molecules similar or identical to the glucose template. The polymers are subjected to an NaOH wash to remove the template and any unreacted reagents, followed by a deionized water wash to remove excess NaOH. The innovation of the technique presented in this work is that it not only employs the more flexible

non-covalent approach to imprinting, but begins with a polymer having an appropriate functionality, instead of functional monomer, and is performed in aqueous solution under air. The MIPs synthesized using PAA·HCl and GPS salts have shown considerable affinity and specificity for binding glucose.

MIP gels imprinted with 0.5% GPS-Ba were tested in buffered sugar solutions. Aqueous and BES-buffered solution mixtures consisting of 50% glucose and 50% fructose were also tested with MIPs and non-imprinted control samples to determine whether the imprinted polymers can discriminate one sugar from the other in competitive binding experiments conducted in mixed sugar media. Table 1 shows the binding capacities for the barium glucose imprinted hydrogels in DI H<sub>2</sub>O. A non-imprinted hydrogel, NIP, was used as a control specimen to determine the effectiveness of the imprinting procedure. Imprinted hydrogel polymers showed

Table 1  
Sugar binding capacity (mg of sugar bound/g of dry polymer gel) for GPS-Ba glucose imprints

	100% glucose	100% fructose	$\alpha$
Ba imprint	593±3	110±19	5.39
No imprint	139±15	105±3	1.32
$\alpha$	4.27	1.05	
50–50% glucose-fructose		$\alpha$	
		Glucose	Fructose
Ba imprint	601±32	84±2	7.15
No imprint	132±21	79±11	1.67
$\alpha$	4.55	1.06	

Sugar bindings were measured in standard solutions prepared in DI H<sub>2</sub>O.

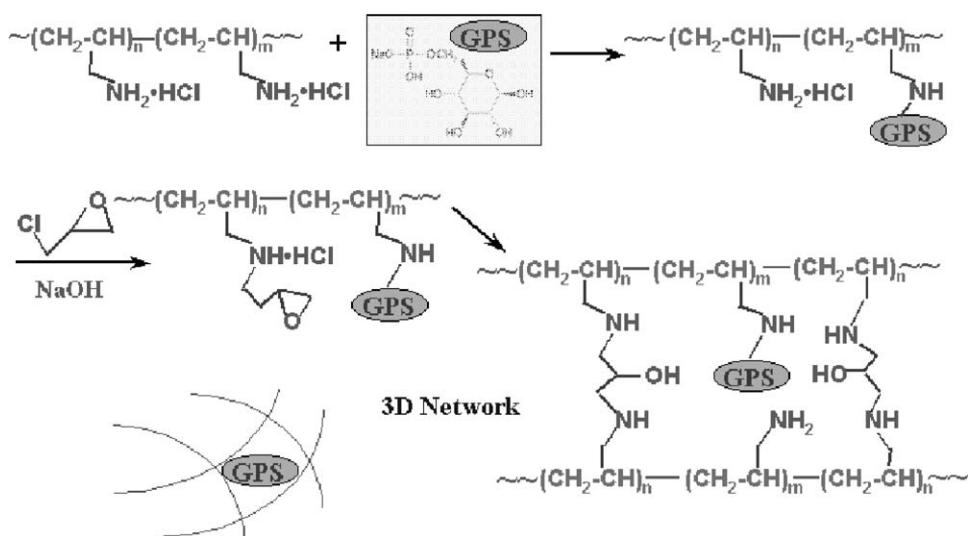


Fig. 1. Schematic representation of the crosslinking reaction using EPI.

Table 2

Sugar binding capacity (mg of sugar bound/g of dried polymer gel) for Ba glucose imprints

	100% glucose	100% fructose	$\alpha$
Ba imprint	530 ± 13	170 ± 10	3.12
No imprint	163 ± 36	156 ± 3	1.05
$\alpha$	3.25	1.09	
	50–50% glucose-fructose		$\alpha$
	Glucose	Fructose	
Ba imprint	561 ± 18	201 ± 7	2.79
No imprint	167 ± 29	161 ± 14	1.04
$\alpha$	3.36	1.25	

Sugar bindings were measured in pH = 7 BES buffer media solutions mimicking physiological conditions.

higher affinity for glucose over fructose. We also tested sugar binding capacities for GPS-Ba glucose imprinted polymer hydrogels in BES buffer media at pH = 7. Table 2 lists these sugar binding results. In all cases, GPS-Ba imprints show higher affinity for glucose over fructose.

Glucose binding for the GPS-Ba MIPs was shown as high as ~600 mg of glucose per g of dry polymer hydrogel in a DI water glucose solution and in 50–50% mixed sugar media. Fructose binding was considerably lower. The glucose imprinted gel bound only 110 mg of fructose per g of dry polymer hydrogel in a DI water fructose solution, and 84 mg of fructose per g of dry polymer hydrogel in 50–50% mixed sugar media. The non-imprinted hydrogels showed considerably lower binding capacities compared to their imprinted analogs. The separation factors,  $\alpha$  = glucose/fructose (shown at the end of each row in Tables 1 and 2), indicate that there is five- to seven-fold specificity for glucose over fructose in the imprinted gels, depending on the environment they are in, compared to the non-imprinted control polymers. The separation factors,  $\alpha$  = imprinted/control (shown at the bottom of each column in Tables 1 and 2), indicate that the imprinting procedure produces a three- to four-fold enhancement in glucose binding for the imprinted polymer, compared to a non-imprinted control sample with no significant increase in fructose binding. There is therefore significant specificity for glucose in the imprinted polymer gel. The experimental results shown in Table 2 demonstrate remarkable biosensing and specificity for glucose imprinted gels in physiological media. The data also indicate that the MIPs are also able to separate glucose out of a 50–50 glucose–fructose mixture, in both aqueous and buffered media, thus demonstrating their ability to function in competitive environments.

PAA · HCl chains have an HCl ionically associated with each of the side chain primary ( $\text{NH}_2$ ) groups along the polymer chain backbone, which enables template immobilization. However, before being able to ionically bond the template 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 free  $\text{NH}_2$  sites for the crosslinking reactions. In order to remove the imprint molecule from the MIPs after crosslinking, it is necessary to break the ionic interaction between the imprint and the amine groups of the polymer. This was accomplished by washing the hydrogel in an aqueous solution of 4 M sodium hydroxide.

Molecularly imprinted gels have cavities with an affinity for glucose through which glucose can pass. These cavities are correctly sized and shaped and appropriately functionalized to bind or allow the passage of the imprint or its analog and give rise to the variation in binding capacities and specificities. Both imprinted and non-imprinted control hydrogels exhibited a similar degree of swelling, and contain the same number of amine groups. Therefore, there should also be a similar number of accessible ionic interaction sites for the imprinted sugar. The higher glucose affinity of the MIP over the control non-imprinted polymer may thus be attributed to the generation of glucose selective, high affinity binding cites in the polymer matrix, during the crosslinking reaction with EPI. Besides electrostatic attraction, shape selective fitting of glucose into the complementary cavities created into the imprinted polymer matrix during the imprinting procedure may be the cause of the improved selectivity and affinity for glucose for the imprinted polymer.

Our experiments have shown that the proposed MIP gels have overcome the main problem of biomimetic recognition in a water-swollen state, i.e. the influence of flexibility of the polymer sequence between two cross-linking points. It is clear that the molecular imprinting procedure has produced recognizable cavities in a water-swollen state with an affinity for the imprint's analog, glucose. The shape and functional groups of these cavities give rise to the observed variation in binding capacities and specificities. A very key issue in MIPs is the conformational relaxation of the binding pocket that is left exposed when the template is dissociated from the matrix polymer. Although it is expected that the imprinted cavities will be distorted due to the swelling of the hydrogel in water, our experimental results show that even the swollen gels show remarkable biomimetic recognition for glucose over fructose.

#### 4. Conclusions

The experimental results presented in this paper demonstrated that the molecular imprinting procedure produced recognizable cavities in a water-swollen state with an affinity for the glucose imprint. Success has been demonstrated in discriminating between glucose and fructose under physiological conditions. The work presented here showed that the binding capacities of glucose in the molecularly imprinted hydrogels using GPS-Ba as a template were as high as 600 mg of sugar bound per g of dry polymer. The binding capacities in the non-imprinted control polymers were significantly lower in all cases. In addition, significant specificity for glucose was demonstrated by the MIPs using GPS-Ba, and this was demonstrated by seven-fold separation factors. The glucose and fructose binding capacities in mixed sugar media indicated that the glucose imprinted gels exhibited affinity and specificity for glucose over fructose in competitive binding environments. It is worthy to note that the prevalence of compounds capable of ionic interactions and hydrogen bonding with amines may allow an immense number of compounds to be imprinted using the polymer system proposed in this research.

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