Radiation Synthesis of Molecularly Imprinted Hydroxyethylmethacrylate-based Matrices for Glucose Recognition

Glikoz Tanıması için Hidroksietilmetakrilat Esaslı Moleküler Baskılı Matrislerin Radyasyon ile Sentezi

Research Article

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ABSTRACT

In this study, 2-hydroxyethyl methacrylate (HEMA) was used as functional monomer and diethylene glycol diacrylate (DEGDA) and polyethylene glycol (200) diacrylate (PEG(200)DA) were used as crosslinking agents to imprint D(+)glucose. D(+)glucose imprinted polymers were prepared in the presence of dimethyl sulfoxide (DMSO) /isopropyl alcohol (IPA) (3/1, v/v) at room temperature, in the air by radiation-induced polymerization/crosslinking. The control polymers were synthesized by the same procedure in the absence of D(+)glucose. In order to evaluate the recognition and separation properties of the imprinted system high performance liquid chromatography (HPLC) experiments were carried out where β (-) lactose, D(+)glucose and glycerol were used as analytes. To increase the affinity of the template to the stationary phase polarity of the mobile phase was decreased by the addition of acetonitrile into water. Optimum composition of acetonitrile/water (1/5 v/v) was determined according to the swelling experiments. The sizes of the cavities in the polymeric networks were determined by positron annihilation lifetime spectroscopy (PALS). The average radii of cavities were found as 0.254 and 0.279 nm for freeze-dried imprinted polymers prepared by using PEG(200)DA after swollen in water and acetonitrile/water mixture (1/5 by volume), respectively.

Key Words
Molecularly imprinted polymers, poly(2-hydroxyethyl methacrylate), glucose recognition, gamma irradiation.

ÖZ

Bu çalışmada D (+) Glikoz baskılamak amacıyla fonksiyonel monomer olarak 2-hidroksietil metakrilat (HEMA), çapraz bağlayıcı olarak dietilenglikol diakrilat (DEGDA) ve polietilenglikol (200) diakrilat (PEG(200)DA) kullanılmıştır. D (+) Glikoz baskılı polimerler dimetil sülfoksit (DMSO)/izopropil alkol (İPA) (hacimce, 3/1) varlığında oda sıcaklığında, havada radyasyonla başlatılan polimerizasyon/çapraz bağlanma ile hazırlanmıştır. Kontrol polimerleri D (+) glikoz yokluğunda aynı reçete ile sentezlenmiştir. Baskılı sistemin tanıma ve ayırma özelliklerini değerlendirmek amacıyla β (-) laktoz, D (+) glikoz and gliserolün analit olarak kullanıldığı yüksek performanslı sıvı kromatografisi (HPLC) deneyleri yapılmıştır. Hedef molekülün durgun faza olan ilgisini arttırmak amacıyla suya asetonitril eklenerek hareketli fazın polaritesi azaltılmıştır. Asetonitril/su (1/5, v/v) için optimum bileşim şişme deneylerine göre belirlenmiştir. Polimerik ağ yapı içindeki boşlukların boyutları positron yok olma ömrü spektroskopisi (PALS) ile hesaplanmıştır. PEG(200)DA kullanılarak hazırlanan suda ve asetonitril/su (hacimce, 1/5) karışımında şişirildikten sonra dondurularak kurutulmuş baskılı polimerlerin ortalama yarıçapları sırasıyla 0.254 ve 0.279 nm olarak hesaplanmıştır.

Anahtar Kelimeler
Moleküler baskılı polimerler, poli(2-hidroksietil metakrilat), glikoz tanıma, gama ışınlaması.

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INTRODUCTION

Molecular imprinting is a powerful tool to prepare synthetic receptors for recognition of various molecules such as drugs [1,2], herbicides [3], carbohydrates [4], amino acids [5], proteins [6], ions [7,8]. Imprinting method comprises the formation of a pre-polymerization complex between a template and functional monomer/monomers via covalent, non-covalent bonds or metal coordination interactions followed by polymerization/crosslinking reaction in the presence of a crosslinking agent to provide stability to the binding sites in the network. After removal of the template, specific binding sites that show chemical and physical complementarity towards the template molecule remain in the network. Combination of chemical affinity with size exclusion/inclusion towards a certain target molecule provides a big potential for use as intelligent drug delivery, catalytic and sensor systems.

Molecular imprinting can be categorized due to interactions between template and functional monomer/monomers as covalent and non-covalent imprinting. In covalent imprinting, the main forces between the template and the functional monomer/monomers are reversible covalent bonds such as boronic acid esters, acetals, ketals, Schiff bases, disulfide bonds, and coordination bonds [9]. This method provides more homogenous binding sites than non-covalent imprinting, however, there are some limitations for template and functional monomers selection based on the lack of functional moieties which result in formation of reversible covalent bonds. On the other hand, non-covalent imprinting is a simpler method and it facilitates to imprint a wide range of template molecules. The binding sites however, show more heterogeneity because of the uncertainty in the stoichiometry of pre-polymerization complex [10,11].

Since the beginning of the imprinting studies, sugars were most frequently used as template materials. The Wullf group was the first to prepare an imprinting system by using 4-vinylphenylboronic acid as functional monomer forming reversible covalent bonds with sugars [12].

There are several studies on imprinting of glucose [13]. A different method has been proposed which uses the hydrogels synthesized by crosslinking of poly(allylamine hydrochloride) with (±)-epichlorohydrin instead of methacrylate- or acrylate- based reagents. In this system template was D-glucose-6-phosphate monobarium salt instead of unmodified glucose. These hydrogels show preferential binding of D-glucose relative to D-fructose in deionized water [14]. Another approach to imprint glucose was by using coordination of metals to monomers [15]. These types of materials have the ability of binding of their templates in water, as well. However, these systems are not feasible for biological systems.

In our laboratory, several studies have been conducted on imprinting of glucose. Firstly, Bodugöz et al. [16] achieved to create three dimensional binding sites for glucose using 2-hydroxyethyl methacrylate and methacrylic acid as functional monomers and poly(ethylene glycol) (PEG) as the crosslinking agent. Then, Ates and Güven [17] showed how to control the size of the binding sites (cavities) for glucose to separate it from its structural analogues, galactose and fructose by using MIPs as support material in HPLC system.

The purpose of this study was to synthesize polymeric matrices with the capability of recognizing D(+)glucose by using radiation induced polymerization/crosslinking in a solvent mixture. For this purpose, 2-hydroxyethyl methacrylate (HEMA) was used as a functional monomer, diethylene glycol diacrylate (DEGDA) [17] and polyethylene glycol (200) diacrylate (PEG(200)DA) were used as crosslinking agents to imprint D(+)glucose. In this imprinting system, a solvent mixture composed of dimethyl sulfoxide (DMSO) and isopropyl alcohol (IPA) was used. In order to evaluate the separation performance of MIPs, β (-) lactose and glycerol were used as analogues of D(+)glucose. In order to evaluate the size of the binding sites of the MIPs, positron annihilation lifetime spectroscopy (PALS) experiments were conducted.

MATERIALS and METHODS

Experimental

Dimethyl sulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany). All the other chemicals, including template molecule, D(+) glucose and crosslinking agents, diethylene glycol diacrylate (DEGDA), polyethylene glycol(200) diacrylate (PEG(200)DA) and isopropyl alcohol were obtained from Sigma-Aldrich (Milwaukee, USA). Functional monomer, 2-hydroxyethyl methacrylate (HEMA) was purchased from Fluka (Buchs, Switzerland). All chemicals were used as received.

Synthesis of D(+)Glucose Imprinted Polymers

D(+)Glucose imprinted polymers were synthesized in the presence of dimethyl sulfoxide (DMSO) /isopropyl alcohol (IPA) (3/1, v/v) mixture as solvent in order to provide solubility and miscibility to glucose-monomer mixture (DMSO) and crosslinking agent (IPA) at room temperature, in air by free radical polymerization where γ-rays were used as initiator. Irradiation dose was selected as 15 kGy because no difference was observed in the crosslinking density at higher doses in HEMA-based MIPs [17], the mole ratio of functional monomer to target molecule was kept as 3/1 as determined before by Djourelov et al. [18] considering the hydrogen bonding interaction between the template molecule glucose and HEMA, and the amount of crosslinking agents used was 20 and 30%, by mole. In a typical synthesis, firstly glucose was dissolved in DMSO, and then HEMA was added to provide formation of the pre-polymerization complex between the template and functional monomer. The crosslinking agent was dissolved in IPA and then added to the mixture of HEMA and D(+)glucose. The solution was purged with nitrogen for 5 min. Free radical polymerization was carried out in glass vials in the air at room temperature in a Gammacell 220, ^{60}Co-γ irradiator (Nordion, Canada). The dose rate 0.101 kGy/h was measured by Fricke dosimetry. Upon completion of the polymerization, polymer monoliths were crushed, ground to particle sizes between 100-150 μm. In addition to the monoliths, to obtain matrixes in disc form for PALS experiments, some of the imprinted polymers were synthesized between the clamped glass slides (60 mm - 60 mm - 2 mm) separated from each other with natural rubber O-ring. Both type of imprinted polymers were washed by water to remove template molecule and unpolymerized material if any. The swelling solution was changed at every 12 h, and eluents were checked by UV-visible spectrophotometry (Varian, Cary100) and HPLC (Waters). After washing was completed the discs were dried at 40°C in an oven. The non-imprinted polymers (NIPs), without D(+)glucose, were synthesized following the same procedure.

Swelling Experiments

Swelling experiments were done by using water and water/acetonitrile mixtures with different compositions which were used as mobile phases in HPLC systems wherein synthesized MIPs was used as the stationary phase. Acetonitrile was used to increase the affinity of the template to the stationary phase by reducing the polarity of the mobile phase. Swelling curves were constructed by measuring the water/solvent uptake of gel particles gravimetrically with time.

High Pressure Liquid Chromatography (HPLC)

In order to evaluate recognition performance of MIPs as a function of some variables such as the composition of mobile phase, type and amount of crosslinker, HPLC experiments were carried out. In these experiments, β(-)lactose and glycerol were used as analytes together with D(+)glucose. All analyses were performed by Water HPLC system with a binary pump system and a refractive index detector. The ground MIP or NIP particles were filled into two commercial stainless steel HPLC columns (4.0 mm inner diameter, and 150 mm length) (TOSOH, Japan) and tested separately. Substrate solutions were prepared of each at a concentration of 500 mg/L and 200 μL of solution was injected for analysis. Each injection was repeated three times. All chromatographic analyses were carried out at 30°C.

Positron Annihilation Lifetime Spectroscopy (PALS)

The positron source, a droplet of ^{22}NaCl from a carrier-free neutral solution (activity: 35 μCi), was dried between two DuPont Kapton® foils (thickness 1.08 mg/cm^{-3}), which were afterward glued together. The source was inserted between two identical discs of MIPs or NIPs in a typical

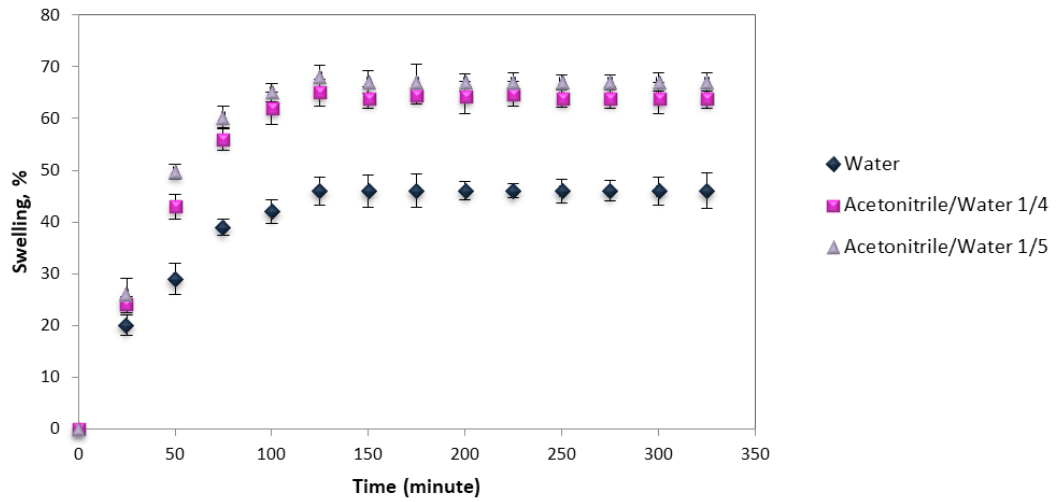

Figure 1. Swelling curves of MIPs prepared with HEMA/Glucose 3/1, and 20% PEG(200)DA as crosslinker in water (◆), acetonitrile/water 1/5 (v/v) (■) and acetonitrile/water 1/4 (v/v) (▲).

'sandwich' configuration. The discs of MIPs and NIPs were obtained by lyophilisation at -40°C and $1x10^{-3}$ mbar pressure after swelling in water and water/acetonitrile mixtures.

PALS experiments were carried out using a conventional fast - fast coincidence system having a time resolution (full width at half-maximum, FWHM) of about 312 ps. The measurements were carried out in the air at room temperature. The spectra were recorded at every 3 hours with total counts in each spectrum being $1.8x10^6$ Then, for each type of sample, five spectra were summed together resulting in statistics of $9x10^6$ counts. The spectra were evaluated by LT9 programme.

RESULTS AND DISCUSSION

Effect of Solvent Composition on Swelling Behaviour of MIPS

The swelling experiments were carried out for MIPs prepared by using PEG(200)DA and DEGDA as crosslinking agents. Maximum swelling in water was obtained as 26% and 49% for MIPs synthesized using DEGDA and PEG(200)DA as the crosslinking agent, respectively. The maximum swelling ratios in acetonitrile/water mixture (1/5 by volume) showed the same tendency and found as 58% and 65% for MIPs prepared with DEGDA and PEG(200)DA, respectively.

Higher swelling maximum values were obtained for PEG(200)DA that has a longer chain length with five ethylene oxide groups than the DEGDA in both water (49%) and acetonitrile/water mixtures with different composition (65% and 67% for acetonitrile/water 1/5 (v/v) and 1/4 (v/v), respectively) (Figure 1).

The maximum swelling of MIPs synthesized with DEGDA was not big enough to provide a selective binding [19], consequently, all the other evaluations were made for MIPs prepared with PEG(200)DA.

Acetonitrile was used to increase the affinity of the glucose to the stationary phase by decreasing polarity of the aqueous medium. Increasing the amount of acetonitrile in the solvent mixture caused an increase in swelling of MIPs, Table 1. Higher swelling of MIPs reduces the specific size selectivity for template [19,20]. Thus acetonitrile/water mixtures with 1/4 and 1/5 ratios by volume were tested as mobile phases for investigating swelling and chromatographic behaviour of this imprinting system.

Chromatographic Evaluation of MIPs

MIPs prepared with different type and amount of crosslinking agents were used as a stationary phase to evaluate their recognition properties. In addition to the template glucose, glycerol and lactose were used as the other competitive compounds due to their size differences. Also, it is possible for these three reagents to exist together in a biological sample, as glycerol 3-phosphate is a product of carbohydrate metabolism and

lactose is a disaccharide of glucose and galactose. Different mobile phases were used to see their effects on the performance of the imprinting systems in separation of these compounds.

Effect of the Type of Crosslinking Agent on Separation

In a previous MIP work from this laboratory, it was found that cavities of radiation crosslinked HEMA matrix were smaller than the cavities of the matrix prepared by using crosslinking agents [18]. Presence of a crosslinking agent prevents the formation of the denser crosslinked structure of pure HEMA by limiting the mesh size. Based on this result, MIPs were prepared using two different crosslinking agents with different ethylene glycol unit number on the chain in the present work.

There was no separation of the three analytes when DEGDA was used as crosslinking agent as seen in Figure 2a. By using columns filled with MIPs prepared with PEG(200)DA, a more effective separation was observed, Figure 2b. Because the size of the cavities inside the network that was prepared by using DEGDA were not big enough for an effective separation [18]. This result was supported by swelling experiments, as well.

Effect of the Amount of Crosslinking Agent on Separation

MIPs prepared by using two different amounts of PEG(200)DA (20 and 30%, by mole) were used as packing material in HPLC system. Ground MIP particles were filled into the columns. When these columns were employed in HPLC system the retention times of glucose were 9.64 and 9.37 min, for MIPs prepared using 20 and 30% crosslinking agent, respectively. This shows that increasing by 10 percent of the amount of PEG(200)DA did not affect the separation property of MIPs, because this increase in the amount of the crosslinking agent did not cause a change in the radius of the cavities which was found to be 0.254±0.001 nm, for both MIP systems, as determined by PALS.

Effect of the Mobile Phase on Separation

As seen in Figure 3a, a certain extent of separation was obtained in HPLC system wherein MIP particles used as stationary phase were prepared with 20% PEG(200)DA as crosslinking agent and ratio of HEMA/glucose 3/1, with a flow rate 0.30 mL/min for glycerol, glucose, and lactose whose hydrated diameters are 0.275, 0.425 and 0.525 nm, respectively [26]. The chromatograms show that separation by size of these three analytes was achieved. Considering the swelling experiments acetonitrile/water mixture with a ratio of 1/5 by volume was selected as the mobile phase for these imprinting systems. It can be seen from the result of swelling tests (Figure 1), the maximum swelling increases with increasing amount of acetonitrile which causes a change in polarity as well as in values of the solubility parameter of solvent mixtures (Table 1). This affects the separation property, due to the expansion of the cavity size according to the amount of acetonitrile. In this respect, in the HPLC system where acetonitrile/water was used as mobile phase, a better separation was obtained as seen in Figure 3b.

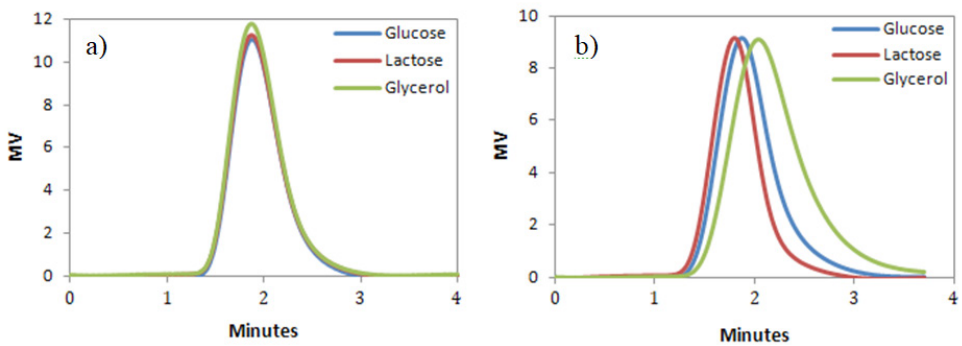

Figure 2. Chromatograms of D(+)Glucose, β(-)Lactose and Glycerol using MIPs synthesized with (a) 20% DEGDA and (b) 20% PEG(200)DA (mobile phase; water, flow rate 1.00 mL/min).

Table 1. The change in the maximum swelling values of MIPs prepared with 20% PEG(200)DA as a function of the solubility parameters; solubility parameter of acetonitrile is 11.9 $(cal/cm^3)^{1/2}$ [21].

Solvent/Solvent Mixture	Solubility Parameter $(cal/cm^3)^{1/2}$	Maximum Swelling %
Water	23.4	49
Acetonitrile/Water 1/5, v/v	21.5	65
Acetonitrile/Water 1/4, v/v	21.1	67

The solubility parameters of solvent mixtures were calculated by using Molar Volume Method [22].

Also, in order to optimize separation efficiency, it is necessary to maximize the number of theoretical plates (N), which requires reducing the plate height. Addition of acetonitrile into the mobile phase provides a certain increase in N value for each molecule (Table 2). The number of theoretical plates (N) was calculated using Equation 1, where t_R is the retention time and W is the peak width of the analyte tested. The numbers given in Table 2 show that by adding acetonitrile into the water the separation performance of the columns was improved for all three analytes as compared to using only water as the effluent.

$$N = 16\left(\frac{t_R}{W}\right)^2 \quad (1)$$

PALS Experiments

The sizes of the cavities in MIP and NIP networks were determined by using Equation (2) which is developed by Nakanishi and Jean according to the quantum-mechanical model of Tao [23] later developed by Eldrup et al [24].

$$\tau_{o-Ps} = 0.5\left[1 - \frac{R}{R+\Delta R} + \frac{1}{2\pi}\sin\left(\frac{2\pi R}{R+\Delta R}\right)\right]^{-1} \quad (2)$$

In this equation $\tau_{o\text{-}Ps}$ is the lifetime of the ***o***-Ps in ns, R is the radius of free volume holes in nm, and ΔR is a constant whose value is 0.1656 nm. The lifetime of ***o***-Ps, $\tau_{o\text{-}Ps}$ is determined by plotting of lifetime spectrum as typically shown in Figure 4 from the slope of the corresponding line. The results for MIPs prepared with different amounts of PEG(200) DA (20% and 30%) as the crosslinking agent are found to be nearly the same, 0.254 nm. This result is supported by swelling experiments, as well. The maximum swelling amounts of these MIPs in water are 49% and 50%, respectively.

The hole radius values of imprinted polymers synthesized with 20% PEG(200)DA as the crosslinking agent are 0.254 and 0.279 nm which

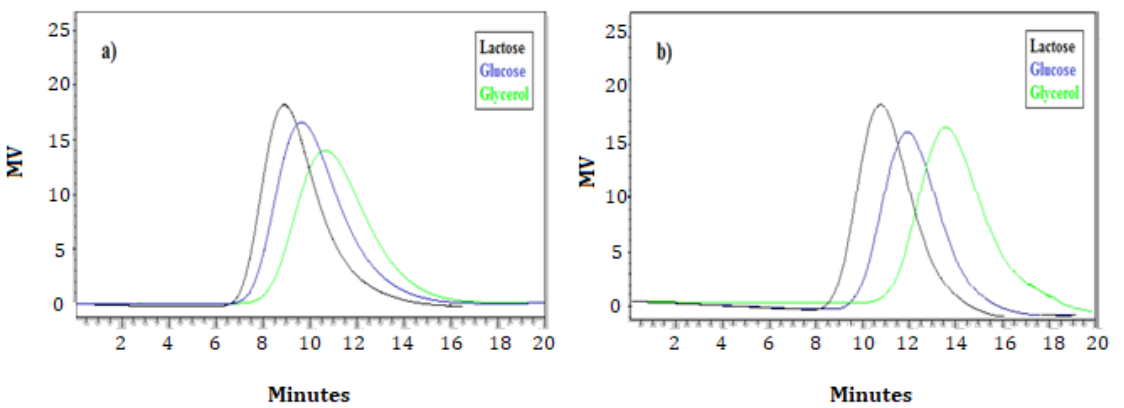

Figure 3. Chromatograms for D(+)Glucose, β(-)Lactose and Glycerol by using water (a) and acetonitrile-water (v/v, 1/5) as mobile phase (MIP particles used as stationary phase were prepared with 20% PEG(200)DA as crosslinking agent and ratio of HEMA/glucose 3/1, flow rate 0.30 mL/min).

Table 2. The number of theoretical plates (N) for glucose, lactose, and glycerol determined from the respective chromatograms of analytes.

	Water	Acetonitrile/Water (1/5)
Glucose, N_{Glu}	10.46	25.35
Lactose, N_{Lac}	11.27	28.44
Glycerol, N_{Gly}	9.29	33.52

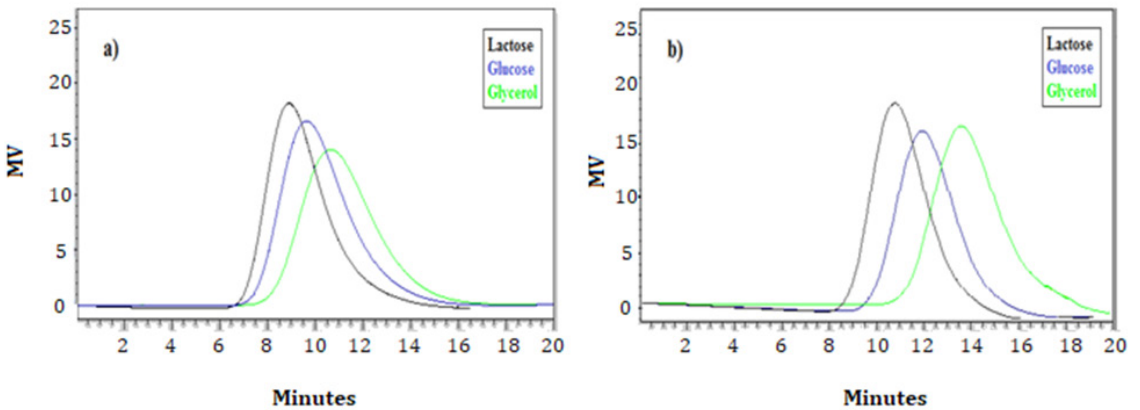

Figure 4. A typical lifetime spectrum of positron and positronium in a polymer with three lifetime components is indicated as (τ_1) the shortest lifetime p-Ps, (τ_2) lifetime of free positrons, and (τ_3 or $\tau_{o\text{-}Ps}$) the longest lifetime o-Ps [25].

were freeze-dried after swollen in water and water/acetonitrile (1/5 v/v) mixture, respectively.

This difference is also reflected in swelling results, Figure 1. The molecular diameter of hydrated glucose is known to be 0.425 nm [26] which shows a very good match with the cavity sizes obtained in this work 0.508nm.

CONCLUSION

In this study, molecularly imprinted polymers were prepared for glucose by using two different crosslinking agents in different amounts via radiation induced polymerization/crosslinking method. The advantages of this method are the following; no need for initiators since radiation chemical processes provide a continuous supply of free radicals in the system; conduction of the polymerization process at room or any desired temperature. It was tried to optimize the type and amount of the crosslinking agent and composition of the mobile phase by changing its polarity in the HPLC system in which MIPs prepared by using PEG(200)DA as crosslinking agent were used as stationary phase. The size of the free volume cavities was determined by using PALS. It was shown by this study that controlling of cavity size in the molecularly imprinted network by using proper crosslinking agent improves the size selectivity of the MIPs.

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