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The Sucrose Determination From Gummy Candy with Imprinted Polymer

Jelibon Şekerde Bulunan Sükrozun Baskılı Polimer ile Belirlenmesi

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Abstract

Sucrose is a valuable carbohydrate because of its broad usage in the chemical, microbiological, pharmaceutical, and food industries. The determination of sucrose content in all of these products is very desirable process. The molecularly imprinted polymers are candidate materials for analyzing carbohydrates without any derivatization. In this study; we synthesized 2,6-bis(acrylamido) pyridine as a designed monomer and also crosslinker. Spectroscopic characterization studies (FTIR and NMR) were carried out the monomer and for the sucrose-monomer complex. The sucrose imprinted polymer was used to determine sucrose from gummy candy. The 60 % of sucrose was recognized from gummy candy by sucrose imprinted polymer.

Keywords: 2,6-bis(acrylamido) pyridine, Gummy candy, Molecularly imprinted polymer, Sucrose

Öz

Sükroz kimya, mikrobiyoloji, farmakoloji ve gıda endüstrisinde yaygın kullanımından dolayı oldukça önemli bir karbohidrattır. Tüm bu alanlarda oluşturulan ürünlerden sükroz içeriğinin hassas tayini oldukça istenen bir prosestir. Bu yüzden baskılanmış polimerler karbohidratların türevlendirilmeden analizlenebilmesi için aday materyallerdir. Çalışmada 2,6-bis(akrilamido) piridin hem monomer hem de çapraz bağlayıcı gibi kullanılmak üzere sentezlendi. Monomer ve monomer-sükroz kompleksinin spektroskopik analizleri yapıldı (FTIR ve NMR). Hazırlanan sükroz baskılı polimer jelibon şekerden sükroz belirlenmesi için kullanıldı. Jelibon şekerden baskılı polimerin sükrozu tanıma kapasitesi % 60 olarak bulundu.

Anahtar Kelimeler: 2.6-bis(akrilamido) piridin, Jelibon şeker, Moleküler baskılanmış polimer, Sükroz

1. Introduction

Molecular imprinting has proved to be an effective technique for preparation of synthetic polymers. This technique is very simple that involves the construction of the sites of specific recognition. These sites are made in situ by co-polymerization of functional monomers and cross-linkers around the template molecules. The print molecules were extracted from the polymer, after then accessible binding sites with specific shape and functional group complementarily to print molecule in the polymer were existed. The usage of molecular imprinting polymer (MIP) is widely because of recognition of template by covalent or noncovalent interactions. The most area which MIP used is separation and the others are catalysis, sensors and chromatography (Bergmann et al. 2008, Kryscio et al. 2012, Okutucu et al. 2009).

Separation of carbohydrates from different matrices were still remains a challenge because of their wide variety of possible isomers, high polarity and similar chemical structures. There are many chromatographic and colorimetric techniques were usually used for analyzing carbohydrates. Due to their chemical properties that were given above, laborious derivatization procedures were needed for sensitive analysis. In order to overcome these techniques problems, it is necessary to establish a rapid, low cost and efficient method. Molecularly imprinted polymers can be used instead of these conventional techniques because of their easy preparation; no need of sample derivatization and low cost (Okutucu et al. 2009).

Sucrose is one of the problematical sugar that is hard to analysis from complex matrices. Sucrose is the principal

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product of the photosynthesis process and is the most widespread sugar in the plant and also renewable chemical resource and has considerable commercial value in the chemical, microbiological, pharmaceutical, and food industries. Not only is the efficiency of the method used to determine sucrose content, but also the delay and costs of the analyses are also important. The techniques which are used for determination of sucrose are the classical and physical refractrometric and polarimetric methods, HPLC, GLC, spectroscopic methods such as near-infrared or midinfrared can be easily adapted for routine usage (Fintey et al. 1973, Cadet et al. 2011). There are some papers that sucrose imprinted polymer was synthesized by conventional monomers and used for biosensor platform (Kirk et al. 2009, Shekarchizadeh et al. 2013).

The ultimate goal of our study is to prepare more effective imprinted polymer by using designed monomer for recognition of sucrose. Sucrose is used as a template molecule and designed functional monomer 2,6-bis(acrylamido) pyridine (BMP) is synthesized. The chloroform and dimethylsulfoxide were used as porogens and ethyleneglycoldimethacrylate as a crosslinker were used as a crosslinker. The sucrose imprinted polymer was tested with some other sugars to find out the polymer specifity. To investigate binding characterization of the adsoption experiments, Scatchard analysis were done. FTIR analysis of BMP, BMP-sucrose and imprinted polymer was done for characterization. The sucrose imprinted polymer was used to determine sucrose content of gummy candy. The 60 % of sucrose was recognized from gummy candy.

2. Material and Methods

2.1. Materials

Ethyleneglycoldimethacrylate (EGDMA), 2,6-diaminopyridine, methaacryloyl chloride, sucrose, raffinose, dimethylsulfoxide (DMSO), ethanol (EtOH), methanol, acetic acid were obtained from Sigma Chem. Co. (St. Louis, MO, USA). Azobisisobutyronitrile (AIBN) was purchased from Wako Pure Chem. Ind. (Osaka, Japan). TLC and column chromatography were performed on precoated aluminium plates (Merck 5554) and silica gel G-60 (Merck7734), respectively. All solvents were dried over molecular sieves, for at least 2h prior to use. When dry conditions were required, the reaction was performed under an argon atmosphere. All solvent removals were carried out under reduced pressure. All other chemicals and reagents were of the highest available purity and used as purchased.

2.2. Instrumentation

The FTIR (Fourier Transform Infrared Spectroscopy) spectra of the compounds were obtained using a Perkin Elmer 100 FT-IR Spectrometer (wave-numbers 400–4000 cm^{-1}) at room temperature and the KBr pellet technique. The NMR (Nucleer Magnetic Rezonans) spectra were recorded at 400 MHz (^1H) on a Varian Mercury FT NMR spectrometer at 300 K. Chemical shift values (δ) are reported in parts per million dowland field from TMS as an international standard: J values are given Hz.

2.3. Synthesis and Characterization of 2,6-bis(acrylamido) Pyridine (BMP)

Synthesis of BMP was done according to a previous report (Oikawa et al. 1993) with some adjustment. 2,6-Diaminopyridine was recrystallised in dichloromethane before using for synthesis. 1.09 g (10 mmol) light yellow crystal of 2,6-diaminopyridine and 2 ml of triethylamine were dissolved in 25 ml dry tetrahydrofuran in a 50 ml two necked flask. The reaction mixture was cooled at 0 °C with an ice bath. Afterwards, 2 ml (20 mmol) of acryloyl chloride was added to dropwise to the mixture over 1 h at 0 °C. The reaction mixture was refluxed for 2h under argon atmosphere then it was cooled at room temperature and white precipitates were filtrated off. 25 ml CH_2Cl_2 and 50 ml distilled water were added to the mixture. The organic layer was kept and washed with sodium carbonate solution (3x25 ml), sodium chloride solution (3x25 ml) and distilled water (3x25 ml). The organic layer was dried over sodium sulfate and after filtration the solvent was evaporated under reduced pressure. The yellow residue was purified by column chromatography (silica gel 60) with eluting solvent dichloromethane: methanol (100:1) to give pure yellow solid of BMP (250 mg, 20.4 % yield). ^1H NMR ($CDCl_3$, 400 MHz): δ 9.04 (2H, br s, NH), 7.94-7.70 (3H , m, Ar-H), 6.19 (2H, m, CH_2=CH-), 5.55 (2H, d, J = 16.0, CH_2=CH-), 5.27 (2H, m, CH_2=CH-) (Figure 1).

2.4. Preparation of Sucrose Imprinted Polymer

Sucrose imprinted polymer (SUC-MIP) was synthesized by non-covalent imprinting approach. 3.5 mmol sucrose 7 mmol synthesized monomer BMP (in appreciate amount of chloroform- 1ml) were dissolved in 1 ml of DMSO and stand up at room temperature for the formation of prepolymerization complex for 30 minutes. Then 14 mmol of EGDMA and appropriate amount of AIBN were added and incubated in ultrasonic bath until homogenous solution was obtained. The solution was purged with nitrogen.

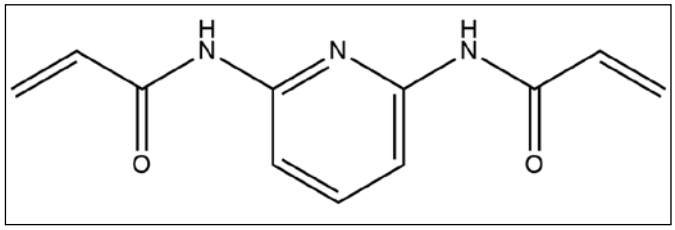

Figure 1. Structure of BMP.

The polymerization was done by thermal polymerization technique at 50°C for 12 h. Control polymer without sucrose (nonimprinted polymer; NIP) was prepared same identical conditions. The polymers (SUC-MIP and NIP) were dried in vacuum at 40°C for one day. The polymers were grounded to particles of 50 μm diameter. The template molecule (sucrose) were extracted by washing with methanol: acetic acid (4: 1, v/v) for three rounds and methanol from the imprinted polymer. The control polymer was also washed with same solutions at the same time. The amount of sucrose extracted from MIP was assayed by o-cresol-sulfuric acid method (Kumar et al. 1997).

2.5. Adsorption Experiments

The binding efficiency of polymer towards sucrose was assessed in the batch rebinding studies. Briefly, 10 mg of the each polymer SUC-MIP and NIP was placed in an eppendorf tube which is including a known concentration of sucrose (initial 10 μmol) in 1 ml of DMSO/ $CHCl_3$ (1: 0.1) and then it was shaken at room temperature for 4 h. After that, the polymers were centrifuged and the concentration of the substrate remaining in the solution (unbound sucrose) was determined by phenol-sulfuric acid method. Each test was carried out three times. The amount of sucrose bound to the polymers (Q; μmol/g) was calculated by Eq.1

$$Q = \frac{[(C_0 - C_t)V]}{W} \qquad \text{Eq. 1}$$

Where W(g) is the weight of the polymers, V (L) is the volume of solution, C_0(μmol/L) and Ct (μmol/L) are the initial concentration of sucrose and the concentration of sucrose at the time t, respectively.

Adsorption isotherms of polymers was graphed according to batch mode (Scatchard analysis- Eq 2). 10 mg of imprinted and non-imprinted polymers with different concentrations of sucrose (10-200 μmol) was shaken 4 hours. After reaching adsorption equilibrium the tubes were centrifuged and supernatant was analyzed by phenol-sulfuric acid method.

The scatchard equation is ;

$$\frac{Q_e}{C_e} = \frac{(Q_{\max} - Q_e)}{K_d} \qquad \text{Eq.2}$$

where K_d (μmol/mL) is the equilibrium dissociation constant, C_e (μmol/mL) is the equilibrium concentration of sucrose, Qmax (μmol/g) and Qe (μmol/g) are the apparent maximum adsorbed amount and the equilibrium adsorbed amount of sucrose, respectively.

2.6. Specific Adsorption

The specific adsorption involves selectivity adsorption with different concentrations the equimolar amount of carbohydrate moieties (galactose, glucose, fructose, raffinose). The selectivitiy adsorption of SUC-MIP was investigated by comparing adsorption capacity of these carbohydrates to polymer. The carbohydrates were incubated with 10 mg of polymer in 1 ml of DMSO/ $CHCl_3$ (1: 0.1) for 4 h at room temperature. After incubation time, the mixture was centrifuged and the concentration of the substrate remaining in the solution was determined. The galactose and glucose were determined with DNS assay (Miller 1959). The fructose and raffinose were assayed by o-cresol-sulfuric acid method.

2.7. The Determination of Sucrose From Gummy Candy

To analyze the effectivity of imprinted polymer sucrose content of gummy candy was tested. The gummy candy was taken from local market (Haribo; happy cola). 10 g gummy candy was extracted by Soxhlet with pure ethanol for 5 hours (Nojiri et al. 2000). The solvent was evaporated under reduced pressure. The residue was used as a sample. It was dissolved in 1ml EtOH and 1 ml DMSO and then applied to 500 mg sucrose imprinted polymer. The polymer and sample suspension was rotated for 2 hours and centrifuged at 5000 rpm for 20 minutes. The supernatant was measured by o-cresol-sulfuric acid method for finding the amount of sucrose.

3. Results and Discussion

3.1. Preparation of Sucrose Imprinted Polymer

There are two approaches were used for imprinting carbohydrates; covalent and noncovalent approach. The noncovalent molecular imprinting approach is much more useful in terms of preparation because of not very complicated chemistry. Also, there are many selections of functional monomers and possible target molecules are available. We prepared the sucrose imprinted polymer by

noncovalent approach. However, there are many advantages of noncovalent imprinting, the disadvantages are also existed. The binding sites are highly heterogeneous which limits the binding properties. To reduce the number of heterogenous binding, better defined template- monomers are needed. As known, functional monomer plays an important role in the adsorption performance of MIPs to template molecule. A designed functional monomer can create more specific recognition sites, and thus, increasing the specificity of MIPs. For this aim we synthesized a hydrogen bond donor acceptor-donor functional monomer BMP and used to prepare MIP. Many hydrogen bonds can be formed between BMP and sucrose molecule and this is the main advantage of using BMP as a designed monomer. The other advantage of BMP was its crosslinker effect. As mentioned papers, BMP can act like a monomer and also like a crosslinke (Kirkbride et al. 2008). Imprinting of sucrose was problematic because of its solubility, and also there are too many hydroxyl groups and their orientation change in the solvents. BMP can do hydrogen bonds with sucrose and also can form cavity. We used EGDMA as a crosslinker for more effective specific cavity (Gómez-Pineda et al. 2011, Toma et al. 2010). The difference and another important modification in our paper was the choice of solvent. As known; a solvent is used to produce pores in this structure, which significantly increase the mass transfer of the template molecule. The use of solvents with low polarity and hydrogen bonding strength is generally favored. Although polymers prepared by this route have displayed selective binding of their under aqueous conditions.. In our study we chose chloroform as a porogen because chloroform has been used as a thermodynamically good solvent (nonpolar and weak hydrogen bond donor) (Manesiotis et al. 2005, Athikomrattanakul et al. 2009, Tanabe et al. 1995).

3.2. Characterization of Sucrose Imprinted Polymer

3.2.1. FTIR Analysis

The spectroscopic characterization of the monomer BMP and sucrose-BMP complex was done with FTIR. By FTIR analysis, it is possible to determine the interactions of template- monomer interaction during the imprinting process (Zhang 2014). These results are valuable because it is the claim of binding between polymer backbone and template.

Representative FT-IR spectra of BMP and sucrose- BMP complex are provided in Figure 2. In the FT-IR spectrum of the BMP, the bands observed at approximately 3440 and 1727 cm^{-1} due to the characteristic amide (N-H) and carbonyl (C=O) vibrations of the acrylamide groups. The intensity of the vinyl linkage observed at 1608 cm^{-1} for BMP drastically decreased and shifted to a longer wave number after the reaction of sucrose, which was attributed to the absence of conjugation between C=O and $CH=CH_2$ linkages. On the other hand, the extended spectrum of polymers compared to monomers is also common for sucrose-BMP complex bearing vinyl groups and suggests the presence of the polymerized structure of BMP with broad chain dispersity. The band at 1608 cm^{-1} which is the most important and characteristic absorption for vinyl structure is also observed for monomer. However in the case of the reaction of sucrose and BMP, this band disappeared.

Concerning the FTIR spectra of sucrose imprinted polymer (Figure 3), the broad absorption is at 3441 cm^{-1} is attributed to the stretching vibrations of intra- and intermolecular H-bonded NH groups. The sharp absorption band at 2958 cm^{-1}, correspond to the stretching vibration of methylene

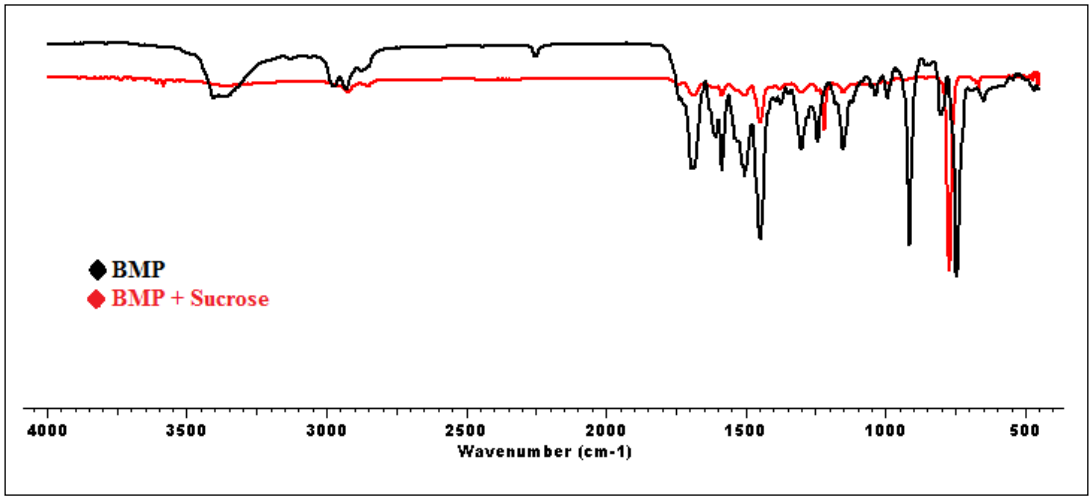

Figure 2. FTIR spectrums (BMP and sucrose- BMP complex).

groups. The band observed at approximately 1730 cm^{-1} due to the characteristic carbonyl (C=O) vibration of the acrylamide groups. In addition, the characteristic band due to the pyridine ring is observed at 1571 cm^{-1}. The other sharp absorption bands 1260 and 1160 cm^{-1} (asymmetric and symmetric C-O-C) support the existence of polymer. The band of characteristic absorption for vinyl structure is disappeared in the FTIR spectrum of polymer. These results are indicative that reaction between monomer and sucrose is completed.

According to these results; a sucrose-BMP complex can be used as a template-monomer couple for an effective imprinted polymer. Stable hydrogen bonds can form between NH groups of the monomer and different orientations of hydroxyl groups at the sucrose moiety. The intramolecular bonds of BMP were not destroyed during the polymerization process. The claim of this was the characteristic bands which were observed in the FTIR spectra.

3.2.2. Adsorption Kinetics (Scatchard isotherm)

The MIPs often exhibit higher imprinting effect in the solvent which is used as the porogen in the polymerization. In our study, we have chosen DMSO/ $CHCl_3$ as a rebinding solvent for more hydrogen bonding capacity cavities in imprinted polymer. The Scatchard plot is the easiest way to see multiple classes of binding sites. This is done by plotting the binding isotherm in a Scatchard format as bound/free template ratio (μmol/mM) versus bound template (μmol). Each linear region of the binding isotherm is fitted with

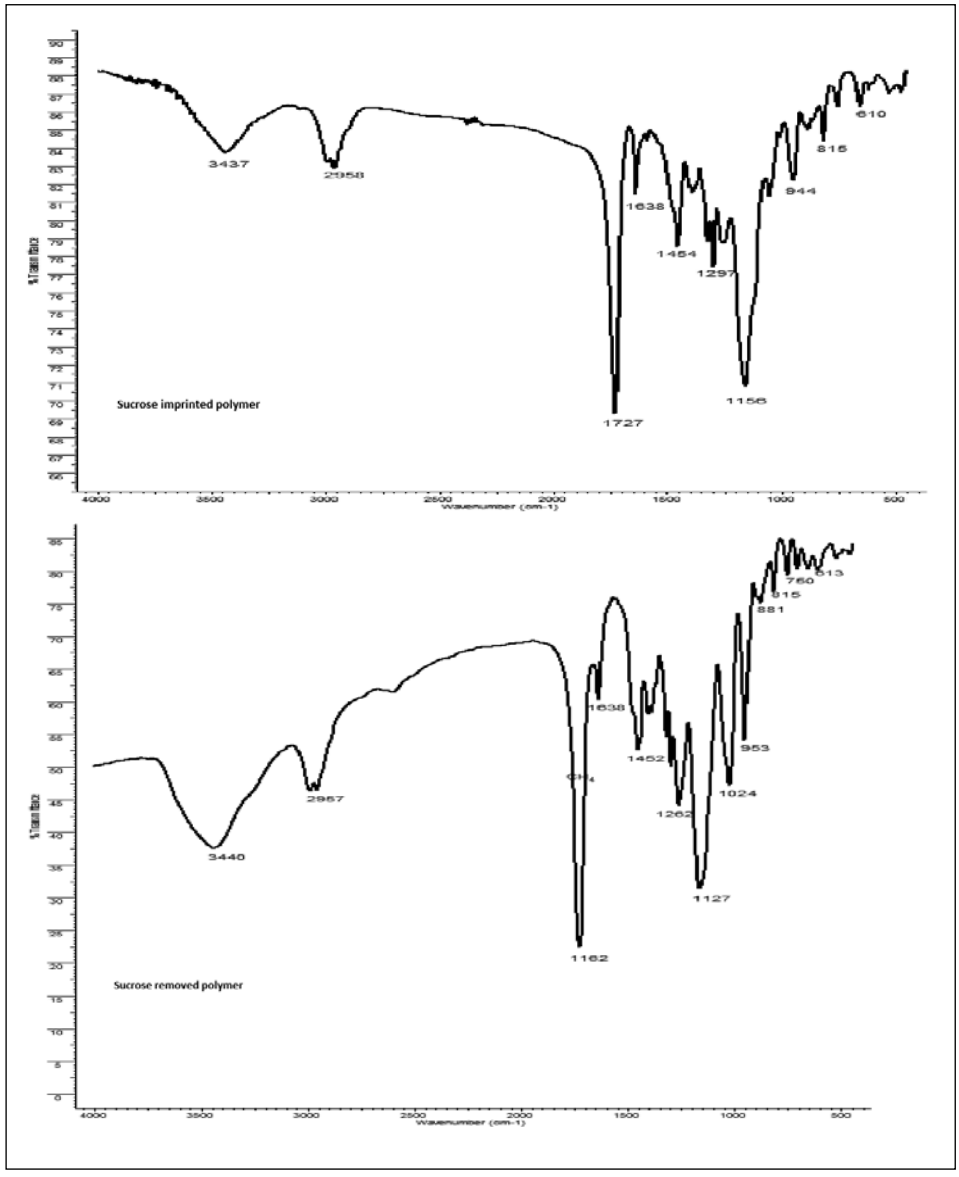

Figure 3. FTIR spectra of sucrose imprinted and sucrose removed polymer.

a straight line (Zhang 2014, Umpleby II et al. 2004). As seen in Fig. 4, there were two distinct linear sections that suggested two binding sites. The one is of high selectivity with a high binding energy and the other is low affinity with a low binding energy. The Q_{max} and K_D values were calculated from the slopes and intercepts of the two straight lines of Scatchard plot. The Q_{max} and K_D values of sucrose polymer were given, respectively. At low sucrose concentration K_{D1} was 0.016 µmol Q_{max1} was 0.7 µmol/ mM and at high sucrose concentration K_{D2} was 7.7x 10^{-3} µmol Q_{max2} was 0.44 µmol/ mM was found.

This curvature has been an evidence for binding site heterogeneity. The distribution of these heterogeneous binding sites is the result of the amorphous nature of the polymer matrix, stepwise complexation between the template and functional monomers. As seen at Fig. 4 the sucrose imprinted polymer have an ability to recognize sucrose at high concentration. The increasing of concentration was affected the recognition of imprinted polymer because the organization of stoichoimetric functional groups and sucrose was fitted into shape selective cavities very well.

3.2.3. The selectivity of sucrose imprinted polymer

The ability of discrimination of sucrose imprinted polymer was evaluated by template and structural analogue. The imprinting factor was calculated according to Eq.3

$$IF=K_{MIP}/ K_{NIP} \quad \text{Eq. 3}$$

Where k was the partition coefficient for each polymer and calculated in Eq.4

K= (bound template/ g polymer)/ [free] Eq. 4

Where [free] was the unbound carbohydrates in solution after equilibrium and bound carbohydrates was the amount of template bound to per gram dry polymer

The selectivity of sucrose imprinted polymers for other carbohydrates were given at Table 1. According to these results it can be said that, the raffinose has much more efficiency to sucrose imprinted polymer than other sugars. The reason of this can be the orientation of hydroxyl groups of raffinose similar to sucrose more than other sugars which were studied.

3.3. The recognition ability of sucrose imprinted polymer from gummy candy

Gummy candy is one of the sources of many sugars. As seen from our results, the other monosaccharides weren't recognized by sucrose imprinted polymer effectively. So that we can use sucrose imprinted polymer for sucrose

Table 1. The IF values for sucrose imprinted polymer.

Sugar	IF
Fru	0.15
Gal	0.18
Glc	0.12
Raf	0.55

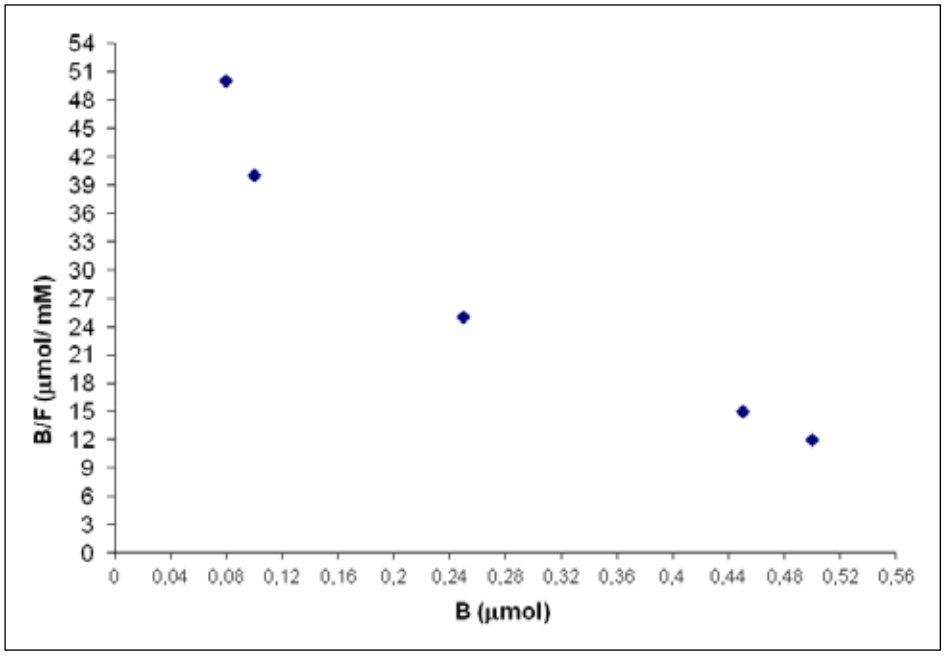

Figure 4. Scatchard graphic.

determination from gummy candy. The gummy candy solution was applied to sucrose imprinted polymer as explained in experimental section 2.7. The 60 % of the gummy candy sucrose extract was bound to the polymer.

4. Conclusion

To find out an imprinted polymer that has specific cavities and selective to the sucrose is aim of this study. BMP-sucrose complex formation is analysed by FTIR and the results showed that BMP was effective designed monomer for sucrose imprinting. Adsorption experiments revealed that sucrose imprinted polymer possessed strong affinity for the sucrose. The selectivity experimental results showed that the sucrose imprinted polymer exhibited less recognition selectivity and binding affinity for the sugars except sucrose. As seen form the results, imprinting sucrose could be done by non-covalent imprinting technique. The Scatchard analysis and selectivity characteristic of sucrose imprinted polymer showed that this polymer can be used for the analysis of sucrose from the various sources. The Scatchard analysis and selectivity characteristic of sucrose imprinted polymer showed that this polymer can be used for the analysis of sucrose from the various sources such as a mixed carbohydrate source, gummy candy.

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6. References

Athikomrattanakul, U., Katterle, M., Eichelmann, NG., Scheller, FW. 2009. Development of molecularly imprinted polymers for the binding of nitrofurantoin. *Biosens. Bioelectron*., 25: 82–87.

Bergmann, NM., Peppas, NA. 2008. Molecularly imprinted polymers with specific recognition for macromolecules and protein. *Prog. Polym. Sci.,* 33:271–288.

Cadet, F., Offmann, B. 1997. Direct spectroscopic sucrose determination of raw sugar cane juices. *J. Agric. Food Chem*., 45:166-171.

Finley, JW.,.Fellers, DA. 1973. Sucrose determination by a modified anthrone method. Application with sweetened wheat-soy blend and corn-soy-milk. *Cereal Chem*., 50:210-215.

Gómez-Pineda, LE., Pina-Luis, GE., Cuán, A., García-Calzón, JA., Díaz-García, ME. 2011. Physico-chemical characterization of flavonol molecularly imprinted polymers. *React. Funct. Poly.,* 71:402–408.

Kirk, C., Jensen, M., Kjaer, CN., Smedskjaer, MM., Larsen, KL., Wimmer, RD. 2009. Aqueous batch rebinding and selectivity studies on sucrose imprinted polymers. *Biosens. Bioelectron*, 25:623–628.

Kirkbride, KC., Townsend, TA., Bruinsma, MW., Barnett, JV., Blobe GC. 2008. Bone morphogenetic proteins signal through the transforming growth factor-beta type III receptor. *J Biol Chem,* 283:7628–7637.

Kumar, V., Pattabiraman, TN. 1997. Standardization of a colorimetric method for the determination of fructose using o-cresol: Sulphuric acid reagent. *Ind. J. Clin. Biochem.,* 12:95-99.

Kryscio, DR., Peppas, NA. 2012. Critical review and perspective of macromolecularly imprinted polymers. *Acta Biomaterialia*. 8:461–473.

Manesiotis, P., Hall, AJ., Courtois, J., Irgum, K., Sellergren, B. 2005. An artificial riboflavin receptor prepared by a template analogue imprinting strategy. *Angew. Chem. Int. Ed.,* 44:3902 –3906.

Miller, GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.,* 31:426-428.

Nojiri, S., Taguchi, N., Oishi, M., Suzuki, S. 2000. Determination of sugar alcohols in confectioneries by high-performance liquid chromatography after nitrobenzoylation. J Chromatogr A, 893:195–200.

Okutucu, B., Önal, S., Telefoncu, A. 2009. Noncovalently galactose imprinted polymer for the recognition of different saccharides. *Talanta,* 78:1190-1193.

Okutucu, B., Önal, S. 2011. Molecularly imprinted polymer for separation of various sugars from human urine. *Talanta,* 87:74-79.

Oikawa, E., Motami, KT., Aoki, O. 1993. Synthesis and properties of poly(thioether amide)s from 2,6-bis(acrylamido) pyridine and dithiols. *Polym. Sci. Part A: Polym. Chem.,* 31:457-465.

Shekarchizadeh, H., Ensafi, AA., Kadivari, M. 2013. Selective determination of sucrose based on electropolymerized molecularly imprinted polymer modified multiwall carbon nanotubes/glassy carbon electrode. *Mater. Sci. Eng. C,* 33:3553–3561.

Tanabe, K., Takeuch,i T., Matsui, J., Ikebukuro, K., Yano, K., Karube, I. 1995. Recognition of barbiturates in molecularly imprinted copolymers using multiple hydrogen bonding. *J. Chem. Soc. Chem. Commun.,* 24:2303-2304.

Toma, LA., Foster, N. 2010. Development of a molecularly imprinted polymer for the analysis of avermectin. *Anal. Chim. Acta,* 680:79–85.

Umpleby II, RJ., Baxter, SC., Rampey, AM., Rushton, GT., Chen, Y., Shimizu, KD. 2004. Characterization of the heterogeneous binding site affinity distributions in molecularly imprinted polymers. *J. Chromatogr. B*, 804:141-149.

Zhang H. 2014. Water-compatible molecularly imprinted polymers: Promising synthetic substitutes for biological receptors. *Polymer.,* 55:699-714.