Scientific paper

Synthesis of Glucose/Fructose Sensitive Poly(ethylene glycol) Methyl Ether Methacrylate Particles with Novel Boronate Ester Bridge Crosslinker and their Dye Release Applications

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Received: 03-18-2021

Abstract

In this study, it is aimed to develop glucose/fructose sensitive poly(ethylene glycol) methyl ether methacrylate (PEGMA) particles which can be employed in controlled drug delivery applications. For this purpose, a boric acid based crosslinker was synthesized using 4-vinylphenylboronic acid (VPBA) and its formation was confirmed by ^{1}H-NMR and FT-IR analyses. Sugar-sensitive polymeric particles were then achieved using this crosslinker and PEGMA monomer in single step and surfactant free emulsion polymerization technique. Polymeric particles were characterized by DLS, SEM, and TEM in terms of size and morphology. In order to determine the sensitivity of the particles to sugar molecules, first Rhodamine B dye (as a model drug) loading experiments were performed. Then, the particles were subjected to glucose/fructose rich media and dye release was monitored as a function of time using UV-Vis spectrophotometry. The results of the current study revealed that the PEGMA particles were more sensitive to fructose (~39% release) compared to glucose (~25% release) at pH 7.4 and 310 K.

Keywords: Controlled drug release, fructose sensitivity, phenylboronic acid, smart polymers, crosslinker

1. Introduction

Smart polymers are a group of materials which can modify some of their physical/chemical properties upon exposure to external stimuli such as temperature,[1] pH,[2] light[3], and magnetic/electric field.[4] They may also show response to variety of organic compounds. Examples of these compounds include carbohydrates, enzymes, acids, and sugar molecules.[5] Due to this unique behavior, these polymers are highly promising in various innovative applications such as biomedical and bioengineering studies.[6] Among these applications, non-invasive biosensors have attracted great attention as these sensors provide the opportunity to detect the level of glucose or fructose molecules in the metabolism without disturbing the patients.[7]

Glucose-sensitive biosensors can be classified into three types according to their chemical make-up and sensing mechanism.[8] These are known as glucose-oxidase, protein, and phenyl boronic acid (PBA) based systems. Glucose-oxidase sensors operate via the enzymatic oxidation of glucose molecules, whereas glucose-binding proteins are functioning through the binding of glucose molecules with glycol polymer-lectin complexes. These compounds are natural biological proteins which makes them intolerant to several environmental factors such as high temperature and pH. Further, the instability of these materials limits their widely use as glucose-sensitive systems. On the contrary, PBA is known to be the synthetic derivative of boronic acid with good stability and easy preparation.[7,9] Boronic acid can bind to diol and polyol species of saccharides with high affinity through reversible boronate ester

formation.[1a,7,10] For example, cis-1,2 and cis-1,3 diols of the sugar molecules are able to link reversibly with boronic acid. The details of this binding mechanism were well established in literature.[6]

PBA compounds exhibit an equilibrium between the charged and uncharged forms in aqueous media.[11] These two forms of PBA can react with the cis-1,2 diols of sugar molecules. While the uncharged PBA is hydrophobic in nature, the complex of this form with glucose is not stable in aqueous media due to hydrolysis. On the other hand, the complex of charged PBA-glucose can form hydrophilic phenyl borate which is highly stable. Moreover, the indicated reaction can shift the equilibrium towards the hydrophilic state[12] which in principle increase the swelling ratio of the polymer network. This provides an opportunity to use polymeric particles as sugar sensitive systems in glucose or fructose containing environments for controlled insulin release.[10] However, PBA-based glucose sensitive systems cannot function effectively at physiological pH due to the high pKa value (~9) of this compound.[13] This is due to low ionization of phenyl-boronic acid at pH = 7.4 which decrease the solubility of polymer in water and also its affinity to glucose. Among the proposed methods to reduce the pKa of PBA, using of alkaline solutions was suggested as a promising route which increase the binding between the boronic acid and sugar molecules.[6,14]

Up to now, many forms of PBA based system, such as hydrogels,[15] multi-layered films,[16] nanofibers,[17] and nanoparticles (NPs)[18] have been studied for insulin release applications. Chen et al., reported that a polymer network can be obtained by crosslinking the boronate ester of two separate polymers containing boronic acid functional PBA based system, such as hydrogels,[15] multi-layered films,[16] nanofibers,[17] and nanoparticles (NPs)[18] have been studied for insulin release applications. Chen et al., reported that a polymer network can be obtained by crosslinking the boronate ester of two separate polymers containing boronic acid functional groups and diols.[14a] With a similar approach, it has been shown that polymeric NPs can be synthesized using a polymer containing PEG (polyethylene glycol)-based boronic acid and another polymer containing diol groups.[14b] Further, the NPs synthesized with the surfactant free emulsion polymerization method were reported to exhibit smooth surface and high stability.[6,19] In addition, these samples were successfully employed in drug delivery and glucose/fructose sensing applications. However, only a few studies have been reported in literature which focused on the synthesis of a crosslinker with boronate ester bridge and using the surfactant free polymerization method in the production of stimuli responsive polymeric particles.[2a,14]

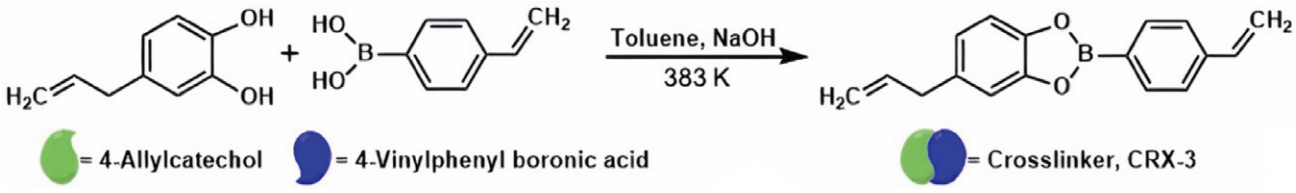
Here, we have synthesized PEGMA particles which are sensitive to glucose/fructose molecules similar to those mentioned above albeit using a novel crosslinker containing boronate ester bonds for the first time. The PEGMA particles were achieved using this novel crosslinker in one step and surfactant free emulsion polymerization method. These particles were loaded with a model drug (Rhodamine B dye) during the polymerization reaction and the amount of dye release was monitored carefully upon exposing the particles to glucose or fructose rich media and interpreted as the sensitivity level of the particles to sugar molecules.

2. Experimental

4-vinylphenyl boronic acid (VPBA, 97%, Sigma Aldrich), 4-allylatechol (%95, Sigma Aldrich), toluene (99.8%, Sigma Aldrich), and sodium hydroxide (NaOH, 97%, Sigma Aldrich) were used in the synthesis of the novel crosslinker. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, Mn of 300 g/mol, 97%, Sigma Aldrich), acetone (99.8%, Sigma Aldrich) and 2,2'-azobis 2-methylpropinamide dihydrochloride (AMPDH, 97%, Sigma Aldrich) were employed in the production of PEGMA particles. Toluene and deionized water (18.2 MΩ.cm) were used as catalyst and for cleaning purposes where necessary.

2. 1. Synthesis of Boranate Ester Bridge Containing Crosslinker

The crosslinker was synthesized according to Scheme 1 given below.[20] The samples were obtained by mixing 1 mmol (0.150 g) of 4-allylcatechol and 1 mmol (0.147 g) of VPBA in 10 mL of toluene in a glass beaker equipped with a reflux system. The solution pH was adjusted to 8.2 using NaOH and continuously stirred at 450 rpm for 72 h at a temperature of 383 K. After the reaction completed, the system was cooled down to room temperature and brown colored precipitates were collected and washed with fresh toluene for 3 times and then dried in a vacuum oven for 24 h at 348 K. Hereafter, this novel boronate ester bridge containing crosslinker is referred as CRX-3.



Scheme 1. Schematic representation of boronate ester bridge containing crosslinker (CRX-3) synthesis

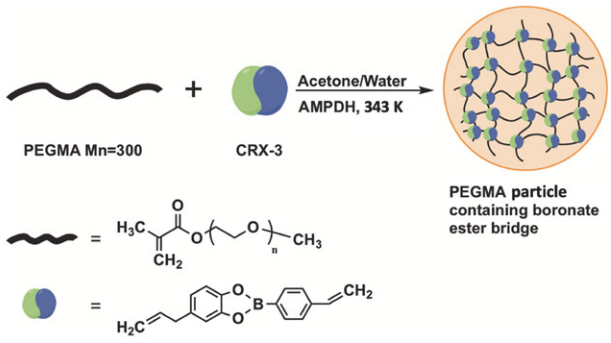
2. 2. Synthesis of PEGMA Particles

PEGMA particles were obtained by mixing poly(ethylene glycol) methyl ether methacrylate monomer (Mn of 300 g/mol) with various amounts of CRX-3, as summarized in Table 1. Scheme 2 shows the emulsion polymerization reaction between the two precursors. For the synthesis of the particles; 300 μL of PEGMA monomer and proper amount of crosslinker were dissolved in 30 mL of acetone:water mixture (1/29, V/V). The amount of the crosslinker was calculated based on the mole % of the monomer. The reaction flask was first purged with ultra-high purity N_2 gas for 30 min to remove any dissolved oxygen. Polymerization process was carried out in this flask which was placed in a water bath on a magnetic stirrer at 343 K. 10 mg of AMPDH (free radical initiator) was added to the medium to initiate the polymerization reaction. The solution was stirred for 3 h at 400 rpm. After cooling to room temperature, pale orange color suspension was centrifuged at 7000 rpm for 10 min to collect the final PEGMA particles. Any unreacted monomer or reactant were then removed by rinsing the product for 3 times with distilled water. Finally, the particles were suspended in ultra-pure water for further use.

Table 1. Synthesis conditions for PEGMA particles.

Sample	PEGMA 300 (μL)	CRX-3 (%)[a]	AMPDH (mg)	Acetone (mL)	DI water (mL)
P-P-1	1				
P-P-2	3				
P-P-3	300	5	10	1	29
P-P-4	7.5				
P-P-5	10				

[a] According to the mole of the monomer.



Scheme 2. Schematic representation of PEGMA particle synthesis using poly(ethylene glycol) methyl ether methacrylate monomer (Mn of 300 g/mol) and boronate ester bridge containing crosslinker

2. 3. Synthesis of Dye Loaded PEGMA Particles

In order to achieve dye loaded PEGMA particles, 1 mg of Rhodamine B (in powder form) was added to the reactants at the initial stage of polymerization reaction. Thereby, the dissolved dye molecules were forced to be trapped in the polymer network during polymerization. After the reactions completed, non-trapped dye molecules were removed by washing the particles in phosphate buffered saline (PBS) solution for 3 times. Then, certain amount of PEGMA particles were dispersed in 30 mL of PBS and poured into 3 separate vials with identical volumes i.e., 10 mL. 10 mg of fructose was added to the first vial, 10 mg of glucose was added to the second vial and the third vial left as it is, as the control sample. The vials were placed on a magnetic stirrer and heated to a constant temperature of 310 K. Finally, the absorbance data were recorded using a UV-Vis spectrophotometer after 0.5, 1, 2, 4, 6, 12, 24, and 48 h.

2. 4. Sample Characterization

Attenuated total reflectance/Fourier transform infrared (ATR/FT-IR) spectra of the samples were collected using a Bruker VERTEX-70 spectroscopy over the range of 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} and averaging 10 scans for each measurement. Solid-state ^{1}H-NMR spectrum was recorded on a Varian 400 MHz spectrometer with a 5 mm double-resonance probe, sample spinning rate of 8.0 kHz, contact time of 0.002 s, and a pulse delay of 5 s to verify the formation of the material. The following numbering scheme was used to determine the sample; 400 MHz, DMSO-d6, ppm, δ = 8.79–8.67 (Ar-OH), 8.07 (B-OH), 7.25–7.23 (d), 7.18–7.16 (c), 6.60 (b), 6.36–6.28 (f,e,g), 5.92 (a2), 5.88–5.85 (i), 5.04–5.00 (a1), 4.99–4.95 (j), 3.18–3.17 (h). The conversion efficiency for the crosslinker was estimated using an integration on the reduction of H peaks in ^{1}H-NMR spectra of CRX-3 compared to the one belonging to the precursors. The calculations were performed using MestraNova software.

The morphology of the PEGMA particles was investigated in as-centrifuged state using SM Zeiss LS-10 scanning electron microscope (SEM). The surfaces of the particles were coated with gold for 30 s prior to SEM analysis using a Cressington Sputter Coater system. For transmission electron microscope (TEM) examinations, the particles were first washed with DI-water for 3 times to remove any unreacted species and then mixed with 2 mL of fresh DI-water to obtain a dispersion in an ultrasonic bath. This dispersion was then dropped on a carbon-coated copper grid and dried for an overnight at room temperature. A JEOL 2100F model TEM was used to determine the size and morphology of the synthesized particles. The size of PEGMA particles were further verified using Malvern ZetaSizer Nano ZS dynamic light scattering (DLS) system. The polydispersity index (PDI) and average hydrodynamic diameters (dH) of the particles were measured after dispersing in 2 mL KCl solution (10 mM). The change in the absorbance of the samples during dye release was recorded via Biochrom Libra S22 UV-Vis spectrometer in the wavelength range of 450–650 nm.

3. Results and Discussion

3. 1. Structural and Morphological Evaluation of the Prepared Materials

^{1}H-NMR spectra of the synthesized crosslinker (Fig. 1a) and the precursors (4-allylcatechol (Fig. 1b) and VPBA (Fig. 1c)) are presented together for comparison. The spectra of VPBA and 4-allylcatechol exhibits (-OH) groups in Ar-OH, (δ= 8.79–8.67 ppm) and in B-OH, (δ= 8.07 ppm) peaks, respectively with high intensity. On the other hand, these peaks were almost disappeared in the spectrum of CRX-3. According to the integration calculations, the reduction of H peaks of Ar-OH groups in 4-allylcatechol molecule and B-OH groups in VPBA is more than 80% which also suggests that the product was obtained with a yield of more than 80%. A detailed ^{1}H-NMR spectrum for the crosslinker (Fig. S1) and the integration steps can be followed from the supplementary information.

The formation of the crosslinker was further verified by comparing the FT-IR spectra of CRX-3 and VPBA. The results are presented in Fig. 2. In the FT-IR spectrum of boronate esters, bands at 1220 and 1250 cm^{-1}, 1000 -1090 cm^{-1}, and 500–750 cm^{-1} are generally assigned to C–O stretching,[21] B–C stretching,[21b,21c,22] and out-of-plane vibrations,[21b,23] respectively. On the contrary, in some other studies, one can find that the assigned wavenumbers for C–O and B–C stretching were replaced; i.e., 1200 and 1270 cm^{-1} for B-C stretching[24] and 1100 and 1200 cm^{-1} for C-O stretching.[24a,25] In addition, bands around 1300 cm^{-1} are mostly attributed to B-O stretching in boronate esters.[26] The spectrum for our sample displayed 2 sharp peaks in this region at 1330 cm^{-1} and 1366 cm^{-1}. However, boronic acid have also been reported to exhibit stretching

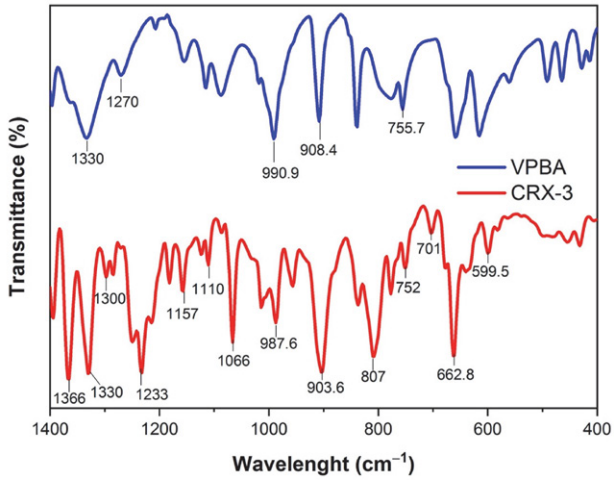


Figure 2. FT-IR spectrum of 4-vinylphenyl boronic acid (VPBA, top) and CRX-3 (bottom).

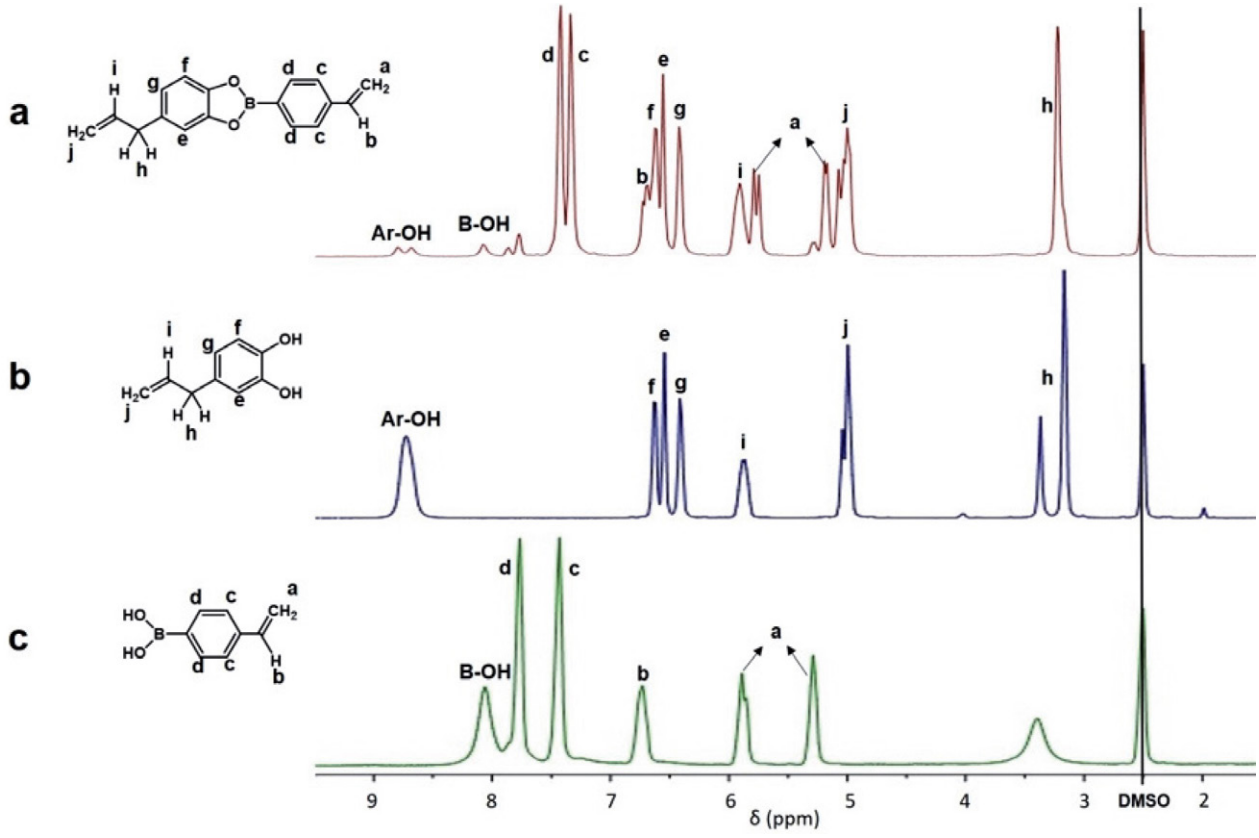


Figure 1. ^{1}H-NMR spectrum of a) CRX-3 b) 4-allylcatechol c) 4-vinylphenyl boronic acid.

here which makes the distinction between the boranate ester and boronic acid disputable.[27] As discussed above, peaks observed in the spectrum of the crosslinker at 1233 cm^{-1} and 1050 cm^{-1} are probably due to C–O and B-O stretching. The presence of these peaks was evaluated as the characteristic of boronate ester formation.[21a] Further, the vibrational mode at 662.8 cm^{-1} also designates that the boronic acid was consumed during the reaction in order to form the boronate ester bridge containing crosslinker.[27] These findings show that the 4-allylcatechol and 4-vinylphenyl boronic acid precursors almost reacted successfully to form the crosslinker under the experimental conditions applied in the current study.

As discussed in the experimental section, PEGMA particles were obtained by mixing constant amount of PEGMA 300 monomers and different amounts of CRX-3 (1, 3, 5, 7.5, and 10% mole of the monomer) in the presence of AMPDH. Further, the emulsion polymerization process yielded PEGMA including suspensions and the polymer content of these suspensions was collected by centrifugation. Morphological examination of these as-collected samples was carried out via SEM analysis. Fig. 3 (a and b) demonstrates SEM images of as-centrifuged P-P-3 sample. The product consists of uneven shaped and large sized (20–30 μm) individual agglomerates. Fig. 3a shows only a portion of the surface of a random agglomerate. As it is clear from this image, under the experimental conditions applied, the polymerization reaction ended-up with the formation of spherical shaped PEGMA (indicated by red arrows) embedded in an un-reacted matrix. Fig. 3b provides a closer view of the surface belonging to one of the PEGMA spheres. It is obvious that the large spheres are made-up of much smaller particles. As this image refer to unwashed state, it is not easy to determine the size and size distribution of the particles using the SEM micrograph. Yet, it can be stated that the particles exhibit a distinct spherical morphology, and the diameter of the largest particles can reach up to ~194 nm.

The size and morphology of cleaned PEGMA particles were investigated using DLS and TEM techniques. The average hydrodynamic diameter (dH) and polydispersity index (PDI) of the particles were extracted from DLS measurements. The results are demonstrated in Fig. 4. In addition, the numerical values can be followed from Table 2. No data are presented for P-P-1 and P-P-2 samples, since PEGMA formation could not be achieved when the amount of the crosslinker used was 1 or 3% mole of monomer probably due to insufficient linking. On the other hand, in case of CRX-3 additions at 5, 7.5, and 10% mole of monomer, PEGMA particles were successfully obtained. The values given in Table 2 states that the dH and PDI of the particles are directly proportional to the amount of the crosslinker used during synthesis. In consistent with the SEM examinations given above, the dH value of P-P-3

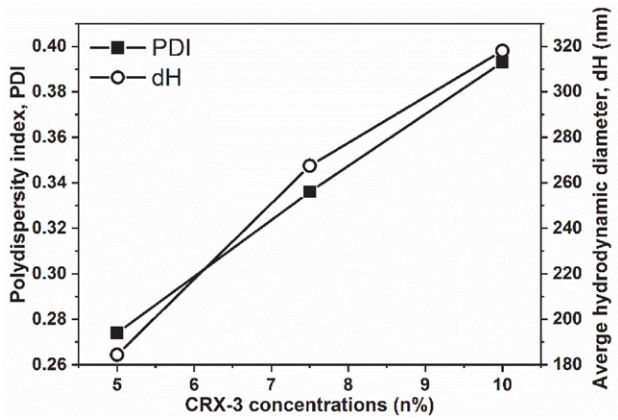


Figure 4. Polydispersity index and average hydrodynamic diameter values of the synthesized PEGMA particles as a function of the amount of CRX-3 used during synthesis

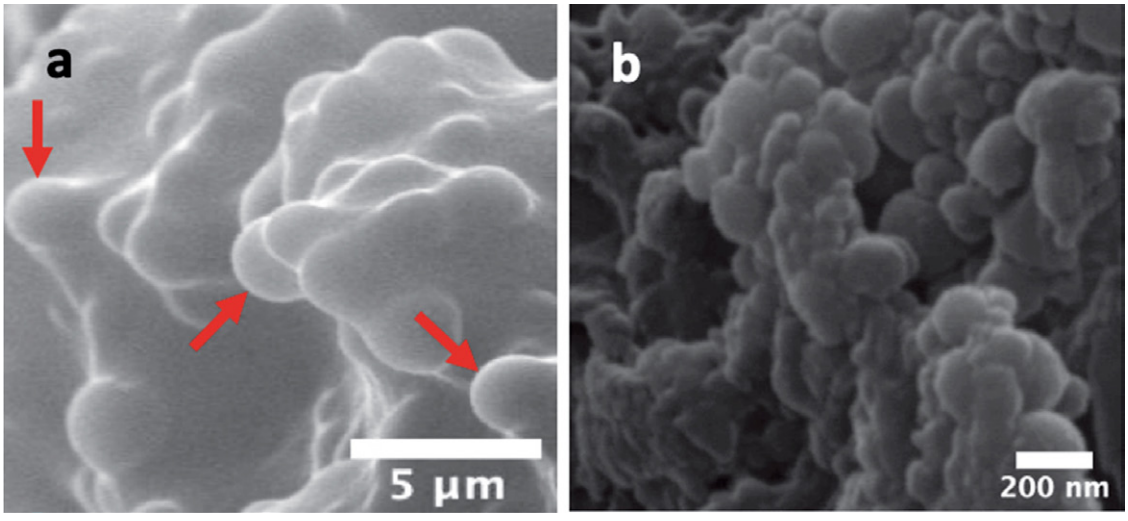


Figure 3. SEM images of P-P-3 sample in as-centrifuged state a) general view of the surface of a large sized agglomerate, 10k magnification, b) a closer view of the surface of a random sphere seen in (a), 100k magnification

sample was measured as 184.4 nm. In addition, dH values of 267.5 nm for 7.5% mole and 318 nm for 10% mole of monomer crosslinker additions were identified. Further, PDI values were obtained as 0.274, 0.336, and 0.393 for P-P-3, P-P-4, and P-P-5, respectively.

Above-mentioned findings show that the amount of CRX-3 used during synthesis is a significant parameter such that the formation of particles directly depends on the amount of crosslinker present in the reaction environment. Moreover, the amount of the crosslinker not only affects the size but also the size distribution of the PEGMA particles. In a similar study, the acetone/water ratio was reported as an another important parameter in terms of controlling the average hydrodynamic diameter of Poly methyl methacrylate (PMMA) particles.[6] It was stated that increasing the fraction of acetone in the solvent could be used as an effective way to reduce the size of the particles. On the other hand, for the current study, a constant value of acetone to water ratio (1/29 V/V) was used for all experiments. Increasing the amount of acetone in the solvent mixture led to the formation of strong agglomerates which are not dispersible by washing/ultrasonic treatments. Therefore, the observed difference in the diameter of the particles here can be ascribed to the change in the amount of CRX-3 used during the synthesis reactions.

As the size of the particles dictate a specific value for the surface area of the samples and further the reaction with sugar molecules was expected to proceed from boronate ester bridges exposed to the surface; the synthesis condition which provides the lowest hydrodynamic diameter for the particles was selected for further investigation. As seen from Fig. 4 and Table 2, the P-P-3 (synthesized by using CRX-3 at an amount of 5% mole of monomer) sample exhibited the lowest size with dH value of 184.4 nm. In addition, the PDI (0.274) of this sample is the lowest among others which indicate that the synthesized particles exhibit acceptable narrow size distribution.

Table 2. Average hydrodynamic diameters and polydipersity index of the synthesized PEGMA particles

Sample	dH (nm)	PDI[a]
P-P-1	–	–
P-P-2	–	–
P-P-3	184.4	0.274
P-P-4	267.5	0.336
P-P-5	318	0.393

[a] Multi-distribution indicator, unitless.

Fig. 5 (a and b) shows low-resolution TEM images of P-P-3 sample. PEGMA particles are highly dispersed after the cleaning procedure and exhibit almost perfectly spherical morphology. Fig. 5a illustrates an array of PEGMA particles on holey carbon coated Cu grid with various sizes. In addition, two random spherical PEGMA particle with similar diameters can be seen in Fig. 5b. According to the measurements conducted using TEM images, the particles have sizes in the range of 90 to 186 nm. This result agrees well with SEM and DLS measurements given above. In addition, these observations reveal that the PEGMA particles were successfully formed by the surfactant free emulsion polymerization reaction using the boranate ester bridge containing crosslinker, exhibiting a particle size below 200 nm with a narrow size distribution.

3. 2. Dye Loading and Sugar Sensitivity Experiments

Rhodamine B dye with specific absorbance in the visible region (λ_{max} = 554 nm) was used as a model drug in this study. Scheme 3a summarizes the dye loading process into the network of a PEGMA particle. In addition, the proposed dye release mechanism is presented in Scheme 3b. According to this mechanism, in case of the presence of sugar molecules in the environment, the trapped dye is expected to be released due to the high sensitivity of CRX-3 to these surrounding molecules. While the sugar molecules can bind to boronic acid, this simply breaks the bor-

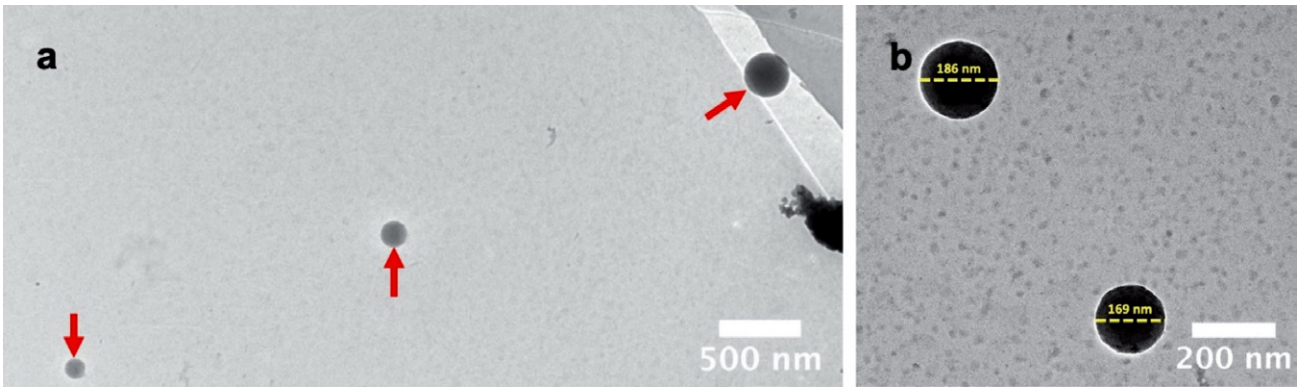
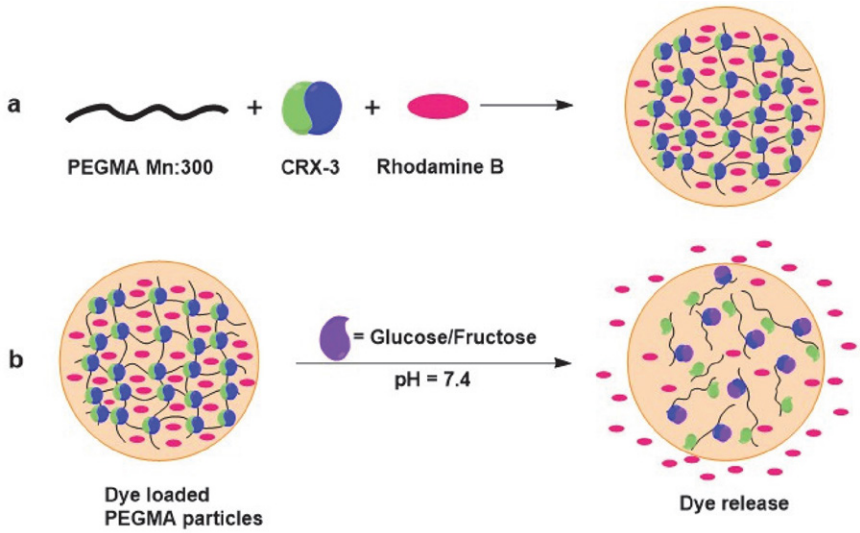


Figure 5. Low resolution TEM images of P-P-3 sample, a) an array of particles with different sizes b) two random PEGMA particles with similar diameters.



Scheme 3. Schematic representation of a) dye loading into PEGMA particles, b) dye release in the presence of glucose/fructose.

onate ester bridge between the monomers. Thus, the reaction will collapse the network of the polymer. Therefore, the amount of the liberated dye to the surrounding can be considered as a measure of drug released by the polymeric particles.

Fig. 6a shows the absorbance spectra of P-P-3 sample (1.25 10^{-2} g) dispersed in PBS, PBS + glucose, and PBS + fructose environments. These spectra were recorded after 2 h of the dispersion process. Absorbance behavior of the pure PEGMA particles (synthesized without dye loading) in the range of 450 to 650 nm was also presented for comparison. As seen, pure particles displayed no absorbance in this region. Therefore, any measured absorbance for other cases can be considered as due to the Rhodamine B dye molecules released from the network depending on the surrounding media. According to the measured values after 2 h, the lowest amount of dye was released in PBS environment, which also means that the particles are releasing a certain amount of dye (16.7 %) even in sugar free environment. In case of PBS with 10 mg/mL glucose, the released amount of dye (18.2 %) was only slightly increased compared to PBS environment, which suggests that the particles with boronate ester bridge containing crosslinker is only partially sensitive to glucose molecules. On the other hand, in case of PBS + 10 mg/mL fructose environment, the amount of the released dye increased substantially and reached to 24.5 % after 120 min.

Fig. 6b demonstrates time dependent increase of dye concentration in PBS+10 mg/mL fructose medium for a total holding duration of 48 h. The absorbance increases continuously as a function of time which means the quantity of the liberated dye is increasing in the environment

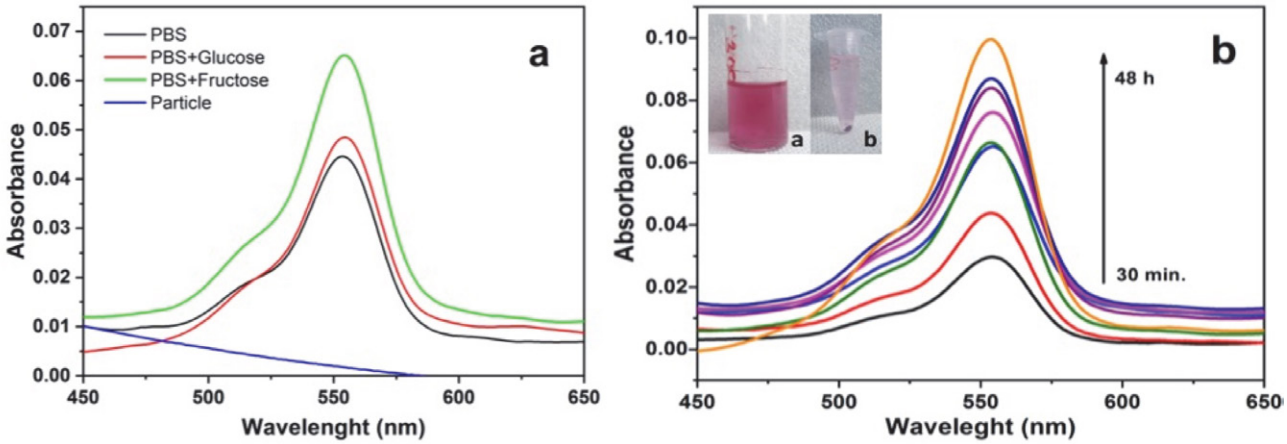


Figure 6. Absorbance spectra of P-P-3 sample after 2 h in a) PBS, PBS+10 mg/mL glucose, and PBS+10 mg/mL fructose environments, b) time dependent increase of dye release in PBS+10 mg/mL fructose environment (insets in (b) shows the digital images of the solution after releasing of Rhodamine B dye by the particles (left) and the collected particles with centrifuging (right)).

with time. After 48 h, a total of 39 % dye release was achieved. The inset (a) shows the deep pink color of the solution because of Rhodamine B dye that was released to the environment in the presence of fructose after 48 h by the P-P-3 sample. In addition, the inset (b) presents these PEGMA particles collected from the pink colored solution, which indicates that the particles can be separated from the solution via a simple centrifugation.

The cumulative release of the dye (after 48 h) by the P-P-3 sample dispersed in PBS, PBS + glucose, and PBS + fructose environments are presented in a comparative base in Fig. 7. This figure implies that the dye release by the particles reached a constant value of ~25 % in PBS or PBS + glucose environments within the first 6 h. After this period, the recorded amount of dye lost by the network was ascended only slightly. On the other hand, in PBS + fructose medium, dye release by the particles increased rapidly up to 28.52 % in 6 h. After this point, the concentration of Rhodamine B gradually continued to increase in the solution, but a decline in the slope of the curve is obvious. At the end of 48 h, total amount of the released dye was recorded as 39 %. As these dispersions were prepared by dividing a single and homogenous 30 mL PBS-PEGMA particles dispersion into three identical vials for dye release experiments (see section 2.3), the quantity of the particles in each vial and the amount of initially trapped dye by these particles can be assumed similar for each condition. As other parameters such as temperature, pH, time, etc. were all kept constant, the total amount of released dye for each case can be correlated to the presence of PBS, glucose, or fructose in these environments. The highest amount of dye was released in fructose environment. Therefore, it can be stated that the boranate ester bridge network is much more sensitive to external fructose molecules compared to glucose molecules.

In a recent study, Wu et. al., discussed on an amphiphilic boronic acid glucose sensor, where the hydrophobic group in glucose and hydrophilic boronic acid attached to each other with a dynamic covalent linkage, preferably an imine bond.[28] According to the proposed mechanism, the presence of glucose can induce aggregation of simple boronic acids due to its ability to crosslink the two boronic acid molecules.[29] On the other hand, experiments with fructose showed no sign of turbidity in the solutions which indicate little or no amphiphile aggregation.[28] Therefore, they stated that the binding of glucose may lead to formation of "*Gemini-type*" amphiphiles, which have a higher ability of aggregation compared to "*single-tail*" amphiphiles formed with boronic acid and fructose. Based on the dye release values for the current experiments, it is believed that the boranate ester bridge in the network has opened in the presence of glucose, but due to a similar mechanism discussed above or a competition between the network disintegration and amphiphile aggregation, the dye release by the particles was inhibited. On the contrary, fructose molecules probably have disintegrated the network to form a single tail amphiphiles, which allowed the liberation of the trapped dye in the network. As a result, higher number of dye molecules were released to the environment for fructose containing experiment. Of course, this speculation needs to be verified by further experiments, which will be considered in future studies. In addition, it is clear from the earlier studies that the affinity of samples to external molecules and the amount of the model drug released by the particles may scatter widely depending on various parameters, such as temperature,[1] time,[30] pH,[2] glucose/fructose concentration,[5,31] and etc. On the other hand, the current study aimed to propose a simple preparation route for the polymeric particles using a novel crosslinker. Therefore, the pH, polymeric particle dosage or sugar molecule concentration were kept constant in all experiments. And the amount of the dye liberated by the samples were measured as a function of time only. This also implies that the dye release performance of the particles can be improved by applying the optimized conditions.

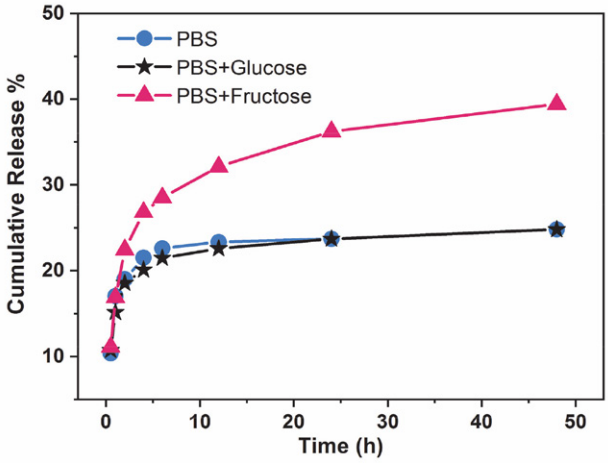


Figure 7. Time dependent Rhodamine B dye release % of P-P-3 sample dispersed in different environments

4. Conclusions

In this study, PEGMA particles were synthesized successfully in one step with surfactant free emulsion polymerization method using a novel boronate ester bridge containing crosslinker. The characterization studies have revealed perfectly spherical morphology for the particles. Further, the study has shown the tunability of the process in terms of achievable particle sizes. It was observed that PEGMA particles could be synthesized in various sizes depending on the amount of the crosslinker added to the polymerization reaction. The particles with the lowest average diameter and the narrower size distribution were then examined for controlled drug release application using their sensitivity against glucose or fructose molecules. The

total amount of dye release in glucose and fructose environments were recorded as 25% and 39%, respectively. Therefore, the experiments have revealed that the affinity of PEGMA particles with boronate ester bonds was 56% higher relative to fructose compared to glucose molecules. Finally, this study demonstrated that it is possible to synthesize a variety of polymeric particles with improved sensing ability by designing a suitable crosslinker.

Acknowledgement

Support for this work is provided by the Scientific Research Foundation (BAP) of Selçuk University (Project Number 191019020) which authors gratefully acknowledge.

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Povzetek

Namen študije je bil razvoj delcev metil eter poli(etilenglikol) metakrilata (PEGMA), občutljivih na glukozo/fruktozo, ki jih je mogoče uporabiti pri izdelavi kontroliranih dostavnih sistemov zdravil. V ta namen smo z uporabo 4-vinilfenilboronske kisline sintetizirali zamreževalec, osnovan na borovi kislini, ter njegovo tvorbo potrdili z ^{1}H-NMR in FT-IR analizama. Polimerne delce, občutljive na izbrana sladkorja, smo nato pripravili z uporabo tega zamreževalca in PEGMA monomera v enostopenjski emulzijski polimerizaciji brez uporabe površinsko aktivnih snovi. Morfologijo in velikost polimernih delcev smo določili z DLS, SEM in TEM. Za analizo občutljivosti delcev na molekule sladkorja smo najprej izvedli poskuse polnjenja z barvilom rodamin B (kot vzorčno zdravilo). Nato smo delce izpostavili medijem, bogatim z glukozo/fruktozo, sproščanje barvila pa smo spremljali z UV-VIS spektrofotometrijo v odvisnosti od časa. Rezultati študije so pokazali, da so delci PEGMA pri pH 7,4 in 310 K bolj občutljivi na fruktozo (~39 % sproščanje) kot na glukozo (~25 % sproščanje).