Novel Glycerol-Crosslinked Poly(acrylic acid) Hydrogel for Encapsulation and Release of Benzocaine

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Drug delivery by a controlled-release mechanism offers several advantages over traditional delivery systems such as improved therapeutic activity, reduced number of drug administrations, as well as minimizing the risk of over-dose or under-dose. In this study, attempt has been made to develop a novel controlled release device from glycerol crosslinked poly(acrylic acid) hydrogels. Glycerol crosslinked poly(acrylic acid) was fabricated by polymerizing acrylic acid and crosslinking the polymer with glycerol in the same reaction pot in the presence of benzoyl peroxide and Novozym 435. The fabricated hydrogel swelled more than 100% in neutral, acidic and basic buffer solutions. Swelling was pH and temperature dependent. The hydrogel encapsulated the drug benzocaine; the amount encapsulated was dependent on time, temperature and initial benzocaine concentration. The hydrogel also released the drug at a controlled rate.

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INTRODUCTION

Glycerol, generated by the hydrolysis of triglycerides, is mainly produced in the process of saponification or biodiesel production. Global glycerol production has grown in recent years, particularly as a byproduct of biodiesel production. Glycerol has been extensively explored as an emulsifier (Piao and Adachi 2006), (Wilde et al. 2009), stabilizer (Brougham and Johnson 1981) and plasticizer (Anker et al. 2001) in the food industry and as a humectant in cosmetic formulations (Pedersen and Jemec 1999). It is also used for the generation of hyperbranched structures (Zhou et al 2011), as reaction solvent (Fang et al. 2009) and as plasticizer (Lavorga et al. 2010, Avella et al. 2007), however, glycerol as a crosslinker for the polymeric network has hardly been explored.

Polyacrylic acid (PAA) in the form of hydrogel has been extensively studied for controlled drug delivery applications (Tu et al. 2010, Devine et al. 2006, Huang et al. 2007). Some of the crosslinkers that have been used for the formulation of PAA hydrogel are N,N′-methylene-bis-acrylamide (Jin et al. 2006), 4,4′-divinylazobenzene (Kakoulides et al. 1998), ethylene glycol dimethacrylate (Chansai et al. 2009) and sucrose (García-González et al. 1993) . Even photocrosslinked (Onuki et al. 2008, Kadłubowski et al. 2007) and electron beam crosslinked

(Sheikh et al. 2010) PAA hydrogels have been fabricated; however, glycerol crosslinked PAA hydrogel has not been explored as a delivery vehicle. Glycerol crosslinked PAA is expected to generate the PAA network to encapsulate drugs without compromising the hydrophilicity of the polymer.

Mouth ulcers are often treated by application of local anesthetics or topical steroids. Most of the formulations available are rapidly cleared from the site of action and hence repeated applications are necessary. Repeated application can be minimized by the use of a controlled release device for sustained release of drug over a prolonged period of time. The polymeric networks that have been studied for the controlled release of drug to treat mouth ulcers are gelatin (Po and Mhando 1984), hyaluronan (Saxen et al. 1997) and chitosan along with PAA Ahn et al. 2002) and methylpyrrolidinone (Rossi et al. 2010).

In this study, we explored the feasibility of using glycerol as the crosslinker for poly(acrylic acid) (PAA) to develop a hydrogel that can be investigated for the controlled release of a mouth ulcer drug. Polymeric network of PAA with glycerol can be considered as an ideal vehicle for drug delivery in the buccal cavity due to its inherent mucoadhesive property and flexibility (Salamat-Miller et al. 2005, Chun et al. 2002). In addition, glycerol owing to its humectant effect, will minimize the burning sensation of the wound. Benzocaine (BZC) is the prototype drug selected for the study. It is the active ingredient in several commercially available ointments for oral ulcers.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were of analytical grade, purchased from Aldrich Chemical Co. Inc., except for acrylic acid which was purchased from Rohm and Hass and glycerol purchased from Merck Chemicals Ltd. These were used as received unless otherwise noted. Novozym 435 was obtained from Sigma Aldrich Chemical Co. Inc and was dried over P_2O_5 *in vacuo* (16 h, 25 °C, 0.5 mmHg) prior to use. Molecular sieves (4 Å mesh size) were purchased from Aldrich Chemical Co. Inc. and dried for 24 h at 160 °C prior to use.

^{1}H NMR spectroscopy

The ^{1}H NMR spectra were recorded at 25°C on a JEOL Lambda 400 MHz NMR spectrometer located in NCIC at Ateneo de Manila University. The chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS). To perform the ^{1}H NMR experiments, 8.0 wt% of samples were dissolved in $CDCl_3$. In order to quantify the amount of unreacted glycerol, the NMR samples were spiked with a known quantity of bromothiophene.

UV-Vis spectroscopy

The amount of drug loaded into the hydrogel was determined using UV-Vis spectroscopy. A calibration curve was first prepared using 1, 2, 4, 5, 6, 8, 10, and 15 ppm of benzocaine solution. Then, the amount of drug loaded into the hydrogel was calculated by subtracting the amount of drug left in the supernatant solution determined via UV-Vis absorbance at 294 nm from the initial amount of drug added. The amount of loaded drug was quantified as the percentage ratio of the mass of drug loaded to the mass of the initial drug concentration. The amount of drug released was also quantified from the UV-Vis spectroscopy of the supernatant. A calibration curve of benzocaine in pH 7 phosphate buffer was made to relate UV-Vis absorbance with the concentration of the drug released from hydrogel. Percent of drug released was calculated as the ratio of amount (mass) of drug in supernatant versus the amount (mass) of loaded drug in the hydrogels. Shimadzu 2401 PC UV Spectrophotometer was used for the UV-Vis analysis.

Synthesis of glycerol-crosslinked polyacrylic acid

Acrylic acid (AA) (6.9 mL, 0.1 mol) and acetonitrile (3.80 mL) were transferred to a 30 mL round-bottom flask. Glycerol

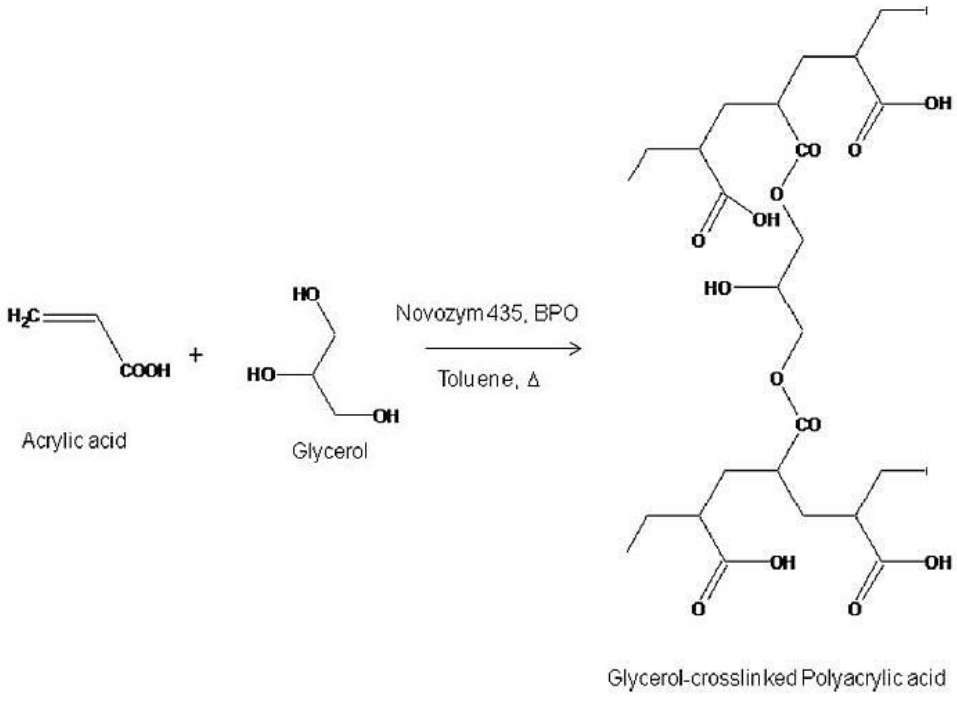

Figure 1. Synthesis of glycerol-crosslinked polyacrylic acid from acrylic acid and glycerol

(0.69 mL, 9.4x10^{-3} mol) was added to the flask followed by addition of Novozym 435 (0.09 g) and benzoyl peroxide (BPO) (0.07 g). The flask was sealed using a rubber stopper. The reaction mixture was purged with nitrogen for 5 min before performing the reaction at 60°C for 14 h under constant magnetic stirring in the presence of molecular sieves. To terminate the reaction, the product was first diluted with 5 mL of acetonitrile and filtered to remove the enzyme and molecular sieves. The filtered product was precipitated in excess of ethyl acetate. The precipitate was collected by filtration and air-dried.

To remove unreacted glycerol trapped inside the polymeric network, the product was swelled in deionized water for 48 h, the water was discarded and the swelled product was freeze-dried at 475-550 x10 $^{-3}$ mbar, -50^{0}C for 48 h. Controlled experiments were performed in two sets. In set one, all the ingredients were placed in the round-bottom flask except for Novozyme 435, and in set two, all the ingredients were placed in the round-bottom flask except for glycerol.

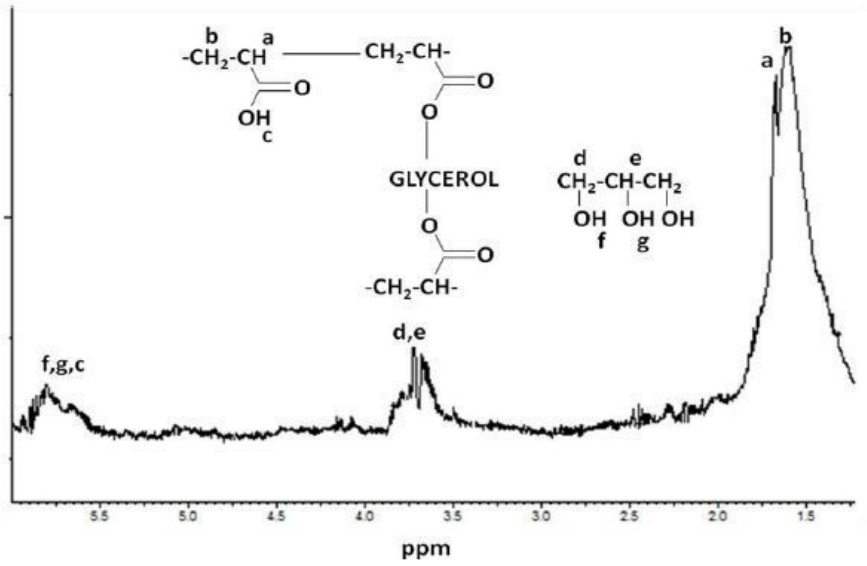

Figure 2. ^{1}H NMR spectrum of glycerol-crosslinked poly(acrylic acid)

Swelling study

Glycerol-crosslinked PAA polymers that were subjected to swelling were dried in an oven at 30□C until a constant weight was reached. This was done to ensure complete removal of moisture from the polymeric network. Dry polymers (0.10 g) were completely submerged in aluminum pans containing 15 mL of pH 7, pH 4 and pH 10 buffer solutions. After a certain period of time, buffer solution was decanted out of the pan, and the hydrogels were pat-dried to remove excess water on their surface. Swelling percentage (% swelling) was determined using the formula

$$\% \text{ swelling} = \frac{(\text{weight of swelled hydrogel} - \text{weight of dry hydrogel})}{\text{weight of dry hydrogel}} \times 100\%$$

Drug loading

Seventeen (17) mg of hydrogel was swelled in 1 mL of ethanol. After 30 min, 1mL of stock solution of BZC in ethanol was added to the hydrogel. Stock solutions were prepared by dissolving 100 mg, 200 mg and 400 mg of BZC in 1L of ethanol. The system was allowed to equilibrate under low-speed stirring. After a desired interval of time, BZC-loaded hydrogel was rinsed once with ethanol to remove the free drug. The ethanol wash was collected together with the residual drug solution after the removal of hydrogel and was subjected to UV analysis to determine the concentration of the residual drug. The amount of drug loaded was studied as a function of time, temperature and the initial drug concentration.

Drug release

BZC loaded hydrogels were equilibrated in 1 mL of pH 7 phosphate buffer at 37°C. The hydrogels were removed from buffer after a certain period of time and were rinsed once with water. The buffer and the wash collected together were subjected to UV analysis to determine the amount of drug released. Swelling, drug loading and drug release experiments were performed in duplicate and the mean values were reported.

RESULTS AND DISCUSSION

Synthesis of glycerol crosslinked PAA

Simultaneous polymerization of acrylic acid to PAA and crosslinking of PAA with glycerol was performed in one step. Addition polymerization of AA was initiated by BPO, whereas the condensation reaction between glycerol and PAA was catalyzed by Novozym-435. Use of Novozym 435 enabled the reaction to be performed at relatively lower temperature of 60ºC. Chemical catalysts need much higher temperature to catalyze such reaction. The reaction scheme and one of the probable structures of the products is shown in Figure 1.

The product was characterized by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of the product is shown in Figure 2. and the data are as follows: ^{1}H NMR in $CDCl_3$ (in ppm): 1.5-1.9 [a: C<u>H</u> of PAA], 1.3-1.9 [b: C<u>H</u>$_2$ of PAA], 3.7-3.9 [d: C<u>H</u>$_2$ of glycerol; e: C<u>H</u> of glycerol], 5.5-5.9[c: O<u>H</u> of PAA; f,g: O<u>H</u> of glycerol].

Presence of unreacted monomer and glycerol was

determined from the ^{1}H NMR analysis of the crude product, spiked with a known concentration of bromothiophene. After 12h of reaction, no peak of ($C\underline{H}_2$=$C\underline{H}_2$) was detected between 6.1 to 6.4 ppm confirming complete polymerization of AA. After 14 h of reaction, the presence of unreacted glycerol was detected from the appearance of (-$C\underline{H}$, $C\underline{H}_2$) peaks of glycerol between 3.8 to 4ppm. The amount of unreacted glycerol was determined by comparing the intensity of the glycerol peaks with the peak of bromothiophene of known concentration at 7.1 ppm, the amount corresponds to 1.2 x 10^{-3} mol which is equal to 12.8% of added glycerol.

The ^{1}H NMR spectrum of the product shown in Figure 2 indicates the feasibility of polymerization and crosslinking of PAA in one pot as a one step synthesis. Since almost 12 h was required for the complete consumption of AA, it can be assumed that at the initial state of the reaction, condensation of –OH of glycerol with the -COOH was predominant, resulting in the formation of the gelled networks which hindered the diffusion of AA and glycerol, thus slowing down the progress of the reaction. Also the gelled network prevented diffusion of glycerol and its contact with catalytic enzyme, thereby retarding the rate of condensation. This interpretation can be corroborated by the observation of a rapid increase in viscosity in the first 1h of the reaction. Gel formation was not observed for two sets of controlled reactions when the reaction was performed under similar conditions in the absence of glycerol and Novozym 435 which indicates that glycerol crosslinks PAA and this reaction takes place only in the presence of Novozym 435.

Swelling behavior of hydrogel

As shown in Figure 3, in its uncrosslinked form, PAA dissolves in water; however, when PAA is crosslinked with glycerol, the

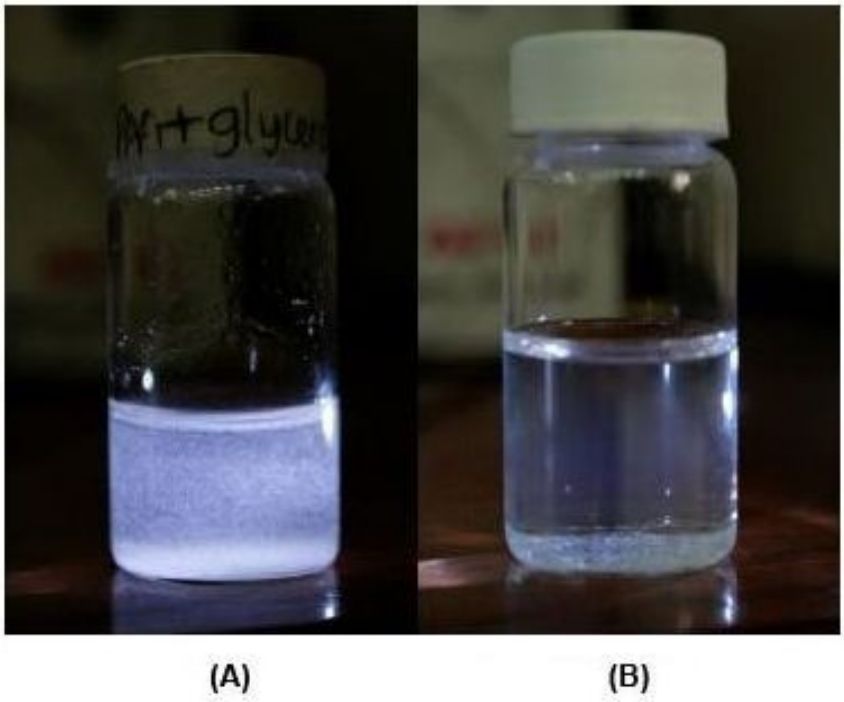

Figure 3. (A) Glycerol-crosslinked poly(acrylic acid) dispersed in water; (B) poly(acrylic acid) solution in water.

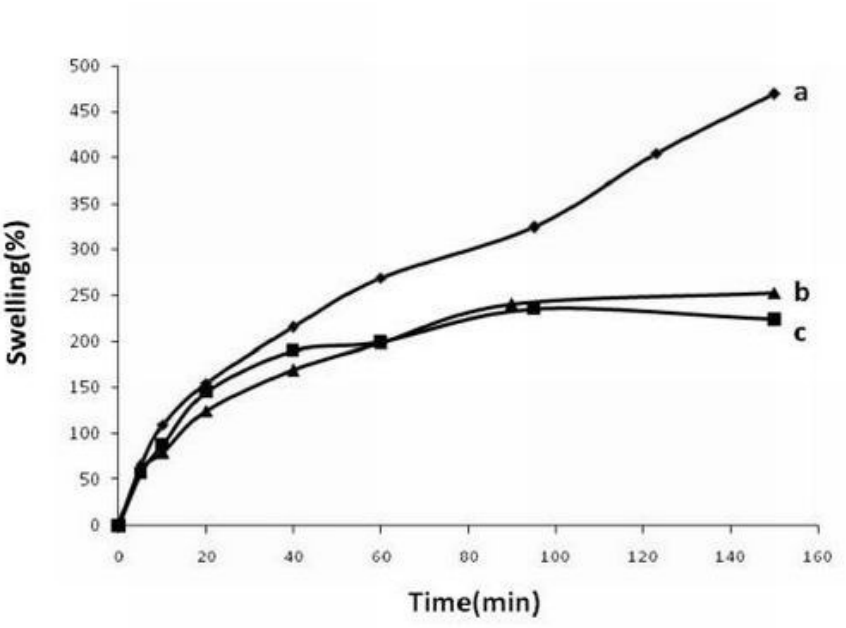

Figure 4. Swelling of glycerol-crosslinked PAA hydrogel in a) pH 10, b) pH 7, c) pH 10.

crosslinked polymer swelled in water and in buffer solutions.

The extent of swelling is shown in Figure 4.

The hydrogels swelled more than 100% in less than 20 min. Swelling was more prominent in basic pH over neutral and acidic pH due to the repulsion between the carboxylate ions generated along the PAA chains in basic pH. Swelling also increased with time. In 100 min, hydrogel in pH 10 swelled 470% whereas hydrogels at pH 7 and 4 swelled up to 250% and 220%, respectively.

Figure 5 shows the effect of equilibration temperature on swelling. There was also an increase in the swelling capacity of the PAA hydrogels from 250% to 300% after 150 min with an increase in temperature from 30ºC to 50°C at pH 7.

It has also been observed that PAA hydrogel makes the media slightly acidic. When glycerol-crosslinked PAA was swelled in a buffer solution of pH 7, the pH of the solution went down to 6.3 from 7.

Drug loading

Benzocaine might have been incorporated inside the glycerol-crosslinked PAA hydrogel primarily due to the hydrogen bonding between the hydrogel and the drug as shown in Figure 6.

Table 1 summarizes the effect of various parameters on drug loading.

Entries 1 – 3 illustrate that increasing the equilibration time increases drug incorporation into the hydrogel. In 10 min and 30 min, 4% and 9.5% of the drug was incorporated, respectively. The effect of temperature is demonstrated in Entries 3-5. Increase of temperature from 30□C to 50□C increased the drug loading from 9.5 to 12%. However, further increase of

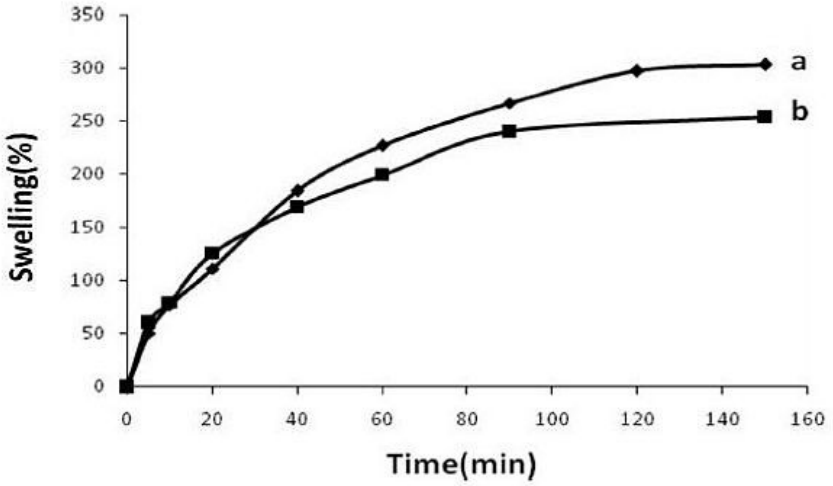

Figure 5. Swelling of glycerol-crosslinked PAA hydrogel in pH 7 at a) 50°C, b) 30°C

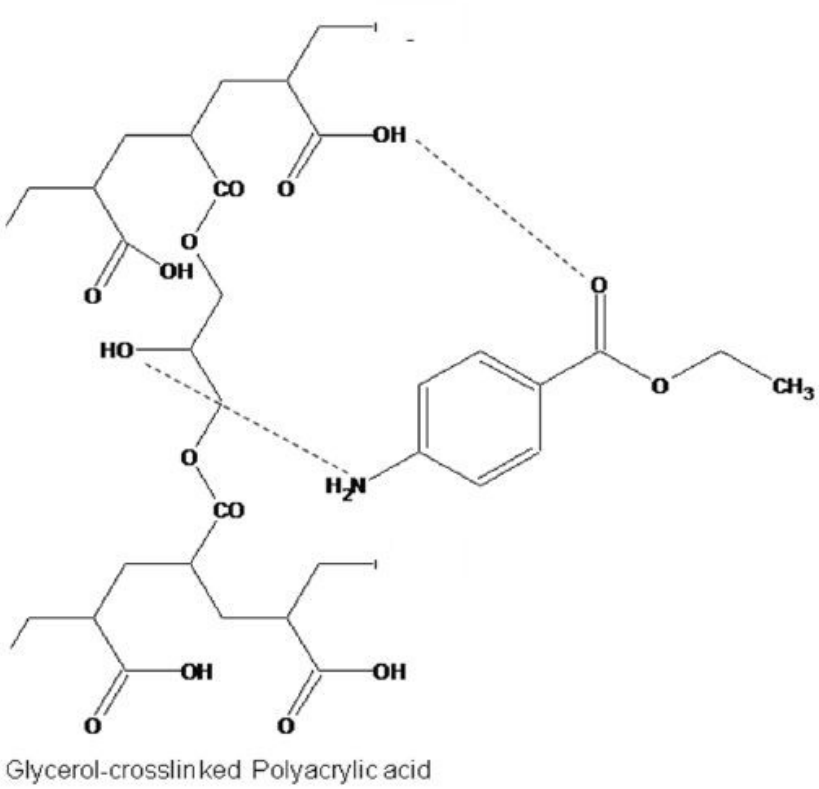

Figure 6. Interaction between glycerol-crosslinked PAA and benzocaine through hydrogen bonding

temperature to 70□C decreased the drug loading to 3%. Improved drug loading upon increase of temperature from 30 to 50ºC can be attributed to the loosening of the polymeric network at higher temperature which is also seen in Figure 3. Increase of temperature beyond 50ºC loosens the network to the extent that the pores of the hydrogel become too large to entrap the drug molecules.

Entries 3, 6-8 compare the effect of initial drug concentration on drug loading. There was a marked increase in drug incorporation as initial drug concentration was raised from 100 to 200 mg/L. However, beyond this concentration, there was no significant increase in drug loading as evidenced from entry 6 to 8. It can be hypothesized that saturation of the surface pores of the hydrogel occurs at 200mg/L of initial drug concentration, thus drug loading did not improve above this initial drug concentration.

Drug release

Since the pH of human saliva ranges between 6.2 to 7.4, the release of benzocaine from the hydrogels was monitored by dispersing the BZC loaded hydrogels in buffer at pH 7. Table 2 indicates that as the equilibration time of BZC- loaded hydrogel in the buffer was enhanced, more BZC was released. In 10 min (Entry 1) and 30min (Entry 2), 1% and 7% of the incorporated BZC was released. The release rate was also affected by the concentration of encapsulated BZC. Entries 2 and 4 show that as the concentration of BZC inside the hydrogel increased from 0.01 mg/ml to 0.04 mg/ml, the amount of drug released also increased from 7 to 11%.

Table 1. Effects of various parameters on BZC encapsulation by glycerol-crosslinked PAA hydrogel

Entry	Initial BZC(mg/mL)	Time(min)[a]	Temp(ºC)	Amount of BZC incorporated (%)[b,c]
1.	0.1	10	30	4
2.	0.1	15	30	5
3.	0.1	30	30	9.5
4.	0.1	30	50	12
5.	0.1	30	70	3
6.	0.2	30	30	15
7.	0.3	30	30	14.5
8.	0.4	30	30	16

a. Equilibration time of hydrogel with BZC; b. Amount of BZC incorporated w.r.t. the initial BZC
c. Mean of two readings

Table 2. Effect of various parameters on release of BZC from glycerol-crosslinked PAAhydrogel

Entry	BZC inside the hydrogel(mg/mL)	Time(min)[a]	BZC released(%)[b,c]
1.	0.01	10	1
2.	0.01	30	7
3.	0.04	30	11

a. Equilibration time of BZC loaded hydrogel with buffer; b. release w.r.t. BZC inside the hydrogel;
c. Mean of two readings

CONCLUSION

The study established that PAA can be synthesized as well as crosslinked with glycerol in one step in the presence of BPO and Novozym 435. The formation of gels due to the condensation of glycerol with AA reduces the diffusion of glycerol and monomer into the gel, thereby slowing down the reaction. Glycerol-crosslinked PAA swells to a very large extent and can serve as a controlled release device.

It was observed that the crosslinked network could encapsulate the drug benzocaine. Encapsulation efficacy depends on the temperature and initial drug concentration. Encapsulated BZC was released at a controlled rate. Since the glycerol-crosslinked PAA swells retain moisture and can encapsulate and release BZC at a controlled rate, this hydrogel has the potential to serve as a mouth ulcer healing patch. Further investigation is required to improve the diffusion of glycerol in the hydrogel by determining its relationship with reaction temperature, solvent concentration, and glycerol concentration. Also the encapsulation and release of BZC has to be monitored for a longer period. As a future study, it is also recommended that bioassay experiments could be performed to compare the analgesic effect of the formulation over the commercially available formulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INDIVIDUAL CONTRIBUTION OF AUTHORS

Soma Chakraborty: Supervised the conduct of research activities, helped in data analysis and prepared the manuscript.

Gayzen A. Ordoñez: Performed 30% of the bench experiments and analyzed the data generated from her experiments.

Angela Lisandra S. Lee: Performed remaining 70% of the bench experiments and analyzed the data generated from her experiments.

REFERENCES

Anh J-S, Choi H-K, Chun M-K, Ryu J-M, Jung J-H, Kim Y-U, Cho C-S. Release of triamcinolone acetonide from mucoadhesive polymer composed of chitosan and poly(acrylic acid) in vitro. Biomaterials 2002; 23:1411–1416.

Anker M, Stading M, Hermasson A. Aging of whey protein films and the effect on mechanical and barrier properties. J Agric Food Chem 2001; 49(2): 989-995.

Avella M, Pace ED, Immirzi B, Impallomeni G, Malinconico M, Santagata G. Addition of glycerol plasticizer to seaweeds derived alginates: Influence of microstructure on chemical–physical properties, Carbohydr Polym 2007; 69(3):503-511.

Brougham MJ, Johnson DB. Glycerol, α-glycerophosphate and other compounds as stabilizers of alcohol dehydrogenase from yeast. Enzyme Microb Technol 1981; 3(3): 225-228.

Chansai P, Sirivat A, Niamlangs S, Chotpattnanont D, Viravaidya-Pasuwat K. Controlled transdermal iontophoresis of sulfosalicylic acid from polypyrrole/poly(acrylic acid) hydrogel. Int J Pharm 2009; 381(1):25-33.

Chun M-K, Chob C-S, Choi H-K. Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. J Controlled Release 2002; 81(3):327-334.

Devine DM, Devery SM, Lyons JG, Geever LM, Kennedy JE, Higginbotham CL. Multifunctional polyvinyl pyrrolidinone-polyacrylic acid copolymer hydrogels for biomedical applications. Int J Pharm 2006; 326(1-2):50-59.

Fang Y, Yu H, Chen Li, Chen S. Facile Glycerol-Assisted Synthesis of N-Vinyl Pyrrolidinone-Based Thermosensitive Hydrogels via Frontal Polymerization. Chem Mater 2009; 21(19):4711–4718.

Garcia-Gonzalez N, Kellaway IW, Anguiano-Igea HS, Delado-Charro B, Oteo-Espinar FJ, Blanco-Mendez J. Design and evaluation of buccoadhesive metoclopramide hydrogels composed of poly(acrylic acid) crosslinked with sucrose. Int J Pharm 1993; 100(1-3):65-70.

Huang Y, Yu H, Xiao C. pH-sensitive cationic guar gum/poly (acrylic acid) polyelectrolyte hydrogels: Swelling and in vitro drug release. Carbohydr Polym 2007; 69(4):774-783.

Jin S, Liu M, Zhang F, Chen S, Niu A. Synthesis and characterization of pH-sensitivity semi-IPN hydrogel based on hydrogen bond between poly(N-vinylpyrrolidone) and poly(acrylic acid). Polymer 2006; 22:1526-1532.

Kadlubowski S, Henke A, Ulanski P, Rosiak JM, Bromberg L, Hatton A. Hydrogels of polyvinylpyrrolidone (PVP) and poly(acrylic acid) (PAA) synthesized by photoinduced crosslinking of homopolymers. Polymer 2007; 48(17):4974-4981.

Kakoulides EP, Smart JD, Tsibouklis J. Azocrosslinked poly(acrylic acid) for colonic delivery and adhesion specificity: in vitro degradation and preliminary ex vivo bioadhesion studies , J. Controlled Release 1998; 54(1): 95-109.

Lavorgna M, Piscitelli F, Mangiacapra P, Buonocore GG. Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films. Carbohydr Polym 2010; 82(5): 291-298.

Onuki Y, Nishikawa M, Morishita M, Takayama K. Development of photocrosslinked polyacrylic acid hydrogel as an adhesive for dermatological patches: Involvement of formulation factors in physical properties and pharmacological effects. Int J Pharm 2008; 349(1-2):47-52.

Pedersen LK, Jemec GBE. Plasticising effect of water and glycerin on human skin in vivo. J Dermatol Sci 1999; 19(1):48-52.

Piao J, Adachi S. Stability of O/W emulsions prepared using various monoacyl sugar alcohols as an emulsifier. Innov Food Sci Emerg 2006; 7(3):211-215.

Po ALW, Mhando JR. Formulation of sustained-release dissolution and diffusion-controlled gelatin films. Int J Pharm 1984; 20:87-98.

Rossi S, Marciello M, Bonferoni MC, Ferrari F, Sandri G, Dacarro C, Grisoli P, Carmella C. Thermally sensitive gels based on chitosan derivatives for the treatment of oral mucositis. Eur J Pharm Biopharm 2010; 74: 248–254.

Salamat-Miller N, Chittchang M, Johnston TP. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Delivery Rev 2005; 57:1666–1691.

Saxen MA, Ambrosins WT, Rehemtula Al-K F, Russella AL, Eckert GJ. Sustained relief of oral aphthous ulcer pain from topical diclofenac in hyaluronan, : a randomized, double-blind clinical trial. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 84(4): 356-361.

Sheikh N, Jalili L, Anvari F. A study on the swelling behavior of poly(acrylic acid) hydrogels obtained by electron beam crosslinking. Radiat Phys Chem 2010; 79(6): 735-739.

Tu H, Qu Y, Hu X, YIin Y, Zheng H, Xu P, Xiong F. Study of the sigmoidal swelling kinetics of carboxymethylchitosan-g-poly(acrylic acid) hydrogels intended for colon-specific drug delivery. Carbohydr Polym 2010; 82(2):440-445.

Wilde PJ, Ridout MJ, Mackie AR, Wickham MSJ, Faulks RM. Modulating Lipid Delivery in Food Emulsions In: Huang Q, Given P, Qian M, ed. Micro/Nanoencapsulation of Active Food Ingredients Washington D.C:ACS publication, 2009:67-88.

Zhou H, Steinhilber D, Schlaad H, Sisson AL, Haag R. Glycerol based polyether-nanogels with tunable properties via acid-catalyzed epoxide-opening in miniemulsion, React Funct Polym 2011; 71(3): 356-361.