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Chitosan and Quat-188 Modified Chitosan Poly(acrylic acid) Semi-Interpenetrating Network For Controlled Release of Drugs

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Semi-interpenetrating polymer networks (semi-IPNs) composed of polyacrylic acid (PAA), and chitosan or N-(3-chloro-2-hydroxypropyl)trimethylammonium chloride (Quat-188) modified chitosan were synthesized. To fabricate the semi-IPN, acrylic acid (AA) was polymerized and crosslinked in the presence of unmodified and Quat-188 modified chitosan. The wet strength of the semi-IPNs improved with the increase in molecular weight of chitosan, chitosan to PAA ratio and by Quat-188 modification. Both modified and unmodified semi-IPNs swelled in buffer solutions. Swelling was pH dependent. The mode of encapsulation and release of two different types of drugs from these semi IPNs was studied. Effects of various parameters on encapsulation and release of $AgNO_3$ and mafenide acetate from these semi-IPNs were investigated. The semi-IPN hydrogel encapsulated 10 mmol/L $AgNO_3$ (100% of added drug) in 5min and released 4% of it in 2h. In case of mafenide acetate, pH dependent encapsulation and controlled release was observed. It was observed that drug-semi IPN interaction strongly influences the encapsulation and release behavior. Freezable water associated with the hydrogels also played an important role for the encapsulation and release of drug.

Keywords: *chitosan; controlled drug delivery; hydrogel; Quat-188; poly(acrylic acid)*

INTRODUCTION

Chitosan, a derivative of chitin, due to its availability, biodegradability, and biocompatibility, has been utilized for numerous pharmaceutical and biomedical applications that includes controlled drug delivery (Park *et al.* 2010; Quiñones et al. 2011; Li *et al.* 2011), scaffolding (Li *et al.* 2010; Wu *et al.* 2011; Jaykumar *et al.* 2011) and as sensor (Odaci *et al.,* 2008; Njagi *et al.* 2010).

Some of the above mentioned applications use chitosan in semi-IPN form. Semi-IPN hydrogels are formed by the combination of two polymers. One forms a network due to crosslinking, the other polymer in linear form penetrates into the network of the first one.

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These polymers influence the properties of each other. Formation of semi-IPN and IPNs of chitosan with various synthetic polymers poly(methacryloylglycylglycine)(Dash *et al.,* 2012), poly(*N*-isopropylacrylamide)(Guo *et al.,* 2007; Henares *et al.*, 2010) and polyacrylamide(Dragan et al., 2013) has been reported.

Association of polyacrylic acid (PAA) with chitosan improves the swelling behavior and mucoadhesiveness of chitosan, hence chitosan in combination with PAA in the form of polyelectrolyte complex has been studied for various applications(Sailaja *et al.* 2006; Ahn *et al.* 2002; Hu *et al.* 2007).

N-(3-chloro-2-hydroxypropyl)trimethylammonium chloride (Quat-188) is a quaternary ammonium chloride that is used to modify polymers to produce quaternary ammonium side-groups. It is commonly used in cosmetics, the paper industry, the textile industry, and water management industries as a coagulant and an antistatic agent. Quaternary ammonium derivative of chitosan is used for various industrial and pharmaceutical applications (Jia and Wu, 2006). Quaternization helps chitosan to retain positive charge permanently and improves its interaction with water. Improvement of antimicrobial property of chitosan upon modified using Quat-188 has been reported by Qin *et al.*(Qin *et al.* 2004). In alkaline medium, Quat-188 is converted to glycidyltrimethylammonium chloride (GTMAC) which then reacts with chitosan.

Our project aimed to fabricate of chitosan and chitosan N-(2-hydroxypropyl)trimethylammonium chloride (modified chitosan) PAA semi IPN that can be and to study the impact of swelling, drug-polymer interaction and the size of the drug on the encapsulation and release behavior from Chitosan PAA matrix. $AgNO_3$ and mafenide acetate were used as the model drugs. The effect of freezable and non-freezable water on drug-polymer interaction has also been investigated using cryo DSC. Hydrogels reported in this study is intended for application as transdermal drug delivery patch.

EXPERIMENTAL

Chemicals. Medium and high molecular weight chitosan, with viscosity of 200-800 centipoise (cp) and 800-2000cp respectively and 75-85% deacylated, acrylic acid, N,N-methylenebisacrylamide and mafenide acetate were purchased from Sigma Aldrich. $AgNO_3$ was purchased from J.T. Baker. Quat-188 was obtained from Rohm and Haas Company. All chemicals were used without prior purification.

Instruments. *1H NMR Spectroscopy.* ^{1}H NMR spectra were recorded at 25°C on a JEOL Lambda 400 MHz. ^{1}H NMR chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS). 8.0 wt % of each sample was dissolved in deuterated water (D_2O). A trace amount of acetic acid was added to the sample to make chitosan soluble in D_2O.

Cryo Differential Scanning Calorimetry (Cryo DSC). Rigaku DSC 8230D was used to perform cryo DSC. The measurements were carried out from 100 °C to 20 °C at a cooling rate of 10 °C/min under a nitrogen flow. Gel was closely sealed in an aluminum pan to prevent the evaporation of water.

UV-Vis Spectroscopy. The amount of mafenide acetate loaded and release by the particles was quantified using UV-Vis analysis with a Shimadzu 2401 PC UV Spectrophotometer at 222 nm using calibration curve method.

A calibration curve was first prepared using 1, 2, 4, 5, 6, 8, 10, and 15 ppm of mafenide acetate solution in pH 7 and 5 buffer. 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 222 nm from the initial amount of drug added. Supernatant solution was diluted 3 times before subjecting the solution to UV-vis analysis. 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 mafenide acetate released was also quantified from the

UV-Vis spectroscopy of the supernatant. 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.

Inductively Coupled Plasma (ICP) Spectrometry. Inductively coupled plasma spectrometry was used to study the loading and release of $AgNO_3$. The supernatant liquid from the drug loading and release experiments were subjected to ICP-spectrometry using Shimadzu ICPS 7510 instrument. Supernatant liquid sample (2mL) was digested with 0.02 mL concentrated HNO_3 followed by adjustment with 5mL distilled water. Argon was used as the carrier gas with the flow rate of 0.7 L/min. It was also used as the cooling gas with the flow rate of 1.4 L/min. Silver ion was analyzed at 328.068 nm. Amount of silver ion was quantified using calibration curve method.

Swelling, drug loading and drug release experiments were performed in duplicate and the mean values are reported.

Methodology. *Modification of Chitosan with Quat-188*. To prepare Quat-188 modified chitosan, 3.75 mmol per unit chitosan was placed in a round bottom flask. To it 1.88 mmol of Quat-188 was added along with 10mL of deionized water. The pH of the mixture was adjusted to pH 9 by dropwise addition of 0.5M NaOH. The flask was sealed, purged with nitrogen and the reaction was performed at 70°C for 48h under constant stirring. After the reaction, the system was neutralized to pH 7, centrifuged and excess water was removed by decantation. Quat-188 modified chitosan was further freeze dried using Labconco Freeze Dry System/Freezone 45 freeze drier. The dried product was characterized using ^{1}H NMR spectroscopy. In past synthesis of Quat-188 derivative of chitosan has been reported using iodine (Sajomsang *et al.* 2009). 1M pH 9 buffer was prepared by mixing 0.477 moles of monosodium phosphate and 0.523 moles of disodium phosphate in 1L of water.

Synthesis of Chitosan-Polyacrylic Acid Semi-IPN. To synthesize the semi-IPN with 2:1 molar ratio of chitosan to PAA and 5mol% crosslinking, 0.146 mmol of acrylic acid (AA), 0.1400 g of chitosan and 5 mL of deionized water were transferred in a round-bottomed flask. The flask was sealed using a rubber septum and purged with N_2 gas. The system was stirred at room temperature for 1h and then N,N'-methylenebisacrylamide was added to the homogenized system using a syringe. The amount of N,N'-methylenebisacrylamide added was 5mol% with respect to the amount of AA. Polymerization was initiated by addition of 4.0 mg of potassium persulfate dissolved in 1mL of deionized water. The reactions were performed at 70°C for 6 hours. After 6h the product was transferred to a small aluminum mold and was oven dried until a constant weight was obtained. The final product was in the form of a very thin film. Above procedure was also adopted for the fabrication of semi-IPN of modified chitosan with PAA where Quat-188-modified chitosan replaced unmodified chitosan.

Swelling Study. The swelling behavior of the synthesized Semi-IPNs was studied by allowing the dry hydrogels (1g) to swell in 15mL of deionized water(pH 5.5) and pH 7 phosphate buffer solutions for certain period of time in aluminum mold. The hydrogels were then removed, pat dried and the weight of the swelled hydrogel was determined. 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 study. To load the drug, 2mL of buffer solution was added to semi-IPN hydrogels in the mold and equilibrated for 30min on a shaker then the aqueous solution of the drug of desired concentration (mg/mL) was added to the buffer. The systems were

further equilibrated on a shaker for certain period of time. The drug-loaded hydrogels were centrifuged at 10,000 rpm to allow them to settle down. The supernatant liquid was decanted and amount of residual drug present in the supernatant was quantified using UV-vis spectroscopy and ICP for mafenide acetate and $AgNO_3$ respectively.

Drug release study. Drug loaded semi IPN hydrogels were equilibrated in 2mL buffer solution for certain period of time. The hydrogels were then centrifuged at 10,000 rpm for 30 minutes to allow them to settle down. The supernatant liquid was decanted and the amount of drug present in the supernatant was quantified using UV-vis spectroscopy and ICP for mafenide acetate and $AgNO_3$ respectively.

RESULTS AND DISCUSSION

Synthesis of Quat-188 modified Chitosan. Chitosan was modified by reacting chitosan with Quat-188 in the presence of NaOH solution at pH 9. Chitosan was modified with Quat-188 to enhance its cationic nature for efficient interaction with polyanionic polyacrylic acid. After performing the reaction for 48 h, the product was characterized using ^{1}H NMR spectroscopy. Reaction scheme is shown in Figure 1.

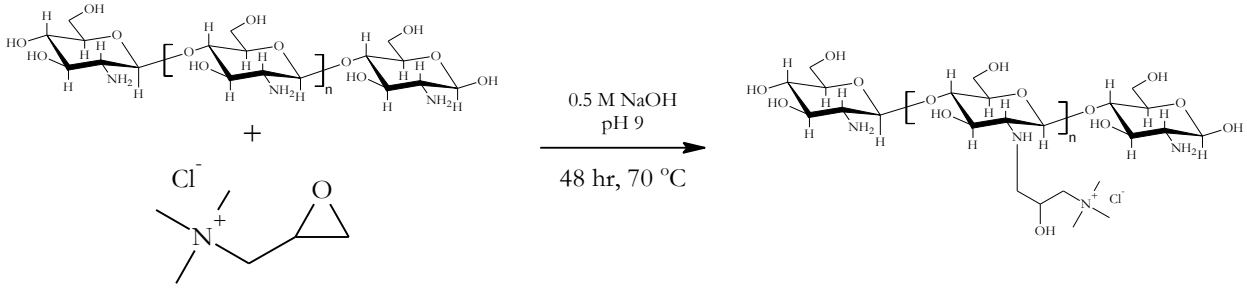

Figure 1. *Reaction between Chitosan and Quat-188 to form Quat-188 modified Chitosan.*

Figure 2 shows the ^{1}H NMR spectrum of Quat-188 modified chitosan in D_2O. ^{1}H NMR spectral data of the product are as follows: ^{1}H NMR in D_2O (in ppm): 4.3-4.25 [a: C<u>H</u>-O- of chitosan], 2.7-2.85 [b: C<u>H</u>-NH_2 of chitosan], 3.6-4.25 [c : C<u>H</u>-OH; d: -O-C<u>H</u>; e: C<u>H</u>-CH_2OH; f:CH_2OH of chitosan]: , 3.4-3.5[1:-NH-C<u>H</u>$_2$- of Quat-188], 4.3-4.25[2: C<u>H</u>-OH of Quat-188], 3.55-3.6[3: C<u>H</u>$_2$-$N^+(CH_3)$ of Quat-188],3.0-3.3[4: $(C\underline{H}_3)_3$-N^+ of Quat-188].

The peak position of 1:NH-C<u>H</u>$_2$ and 2: CH-OH of unreacted Quat-188 are 3.6ppm and 4.67ppm respectively which showed downfield shift when Quat-188 was reacted with chitosan.

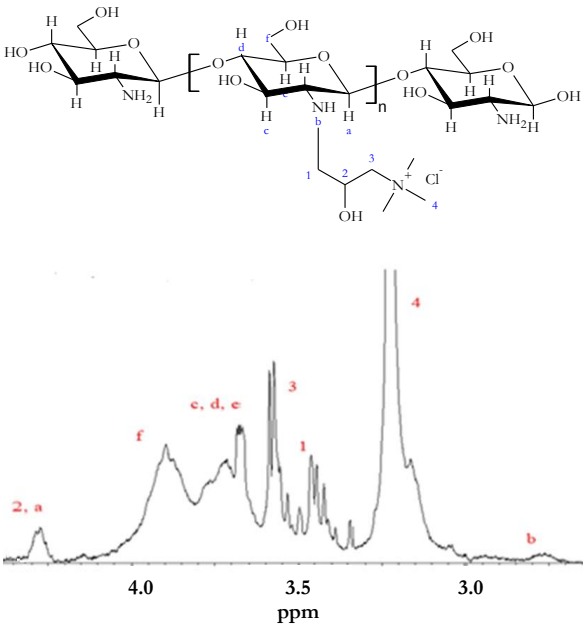

Figure 2. *^{1}H NMR spectrum of Quat-188 modified chitosan in D_2O.*

Swelling Behavior of the semi IPNs. Swelling determines the water retention capacity of the hydrogel and also the encapsulation and release efficiency. Hydrogels are required to swell to encapsulate the attributes, however excessive swelling causes the hydrogel to rupture. Hence the swelling behavior of the semi IPNs was studied to ensure that the gels are capable of drug encapsulation without rupturing and the effect of swelling on drug encapsulation and release. It was observed that molecular weight of chitosan, the amount of chitosan and

presence of Quat-188 had profound influence on the swelling behavior of the hydrogels. As summarized in Table 1, it was observed that when medium molecular weight chitosan was used the hydrogels swelled extensively and ruptured within a short period of time. Rupturing could not be prevented by enhancing the amount of crosslinker. However, increase in the molecular weight of the chitosan and the amount of chitosan increased the wet strength of the hydrogel, thus prevented rupturing of the hydrogels. Chitosan has the ability to form strong films thus addition of chitosan prevented the rupturing of the hydrogels. It was also noticed that modification of chitosan using Quat-188 also prevented rupturing. It can be hypothesized that when chitosan was modified with Quat-188, it induced more positive charge to chitosan through quaternary ammonium ion and enhanced the interaction between the poly anionic PAA and polycationic chitosan thus improved the wet strength of the hydrogels.

Table 1. Swelling behavior of Chitosan PAA semi IPNs.

Entry	Chitosan: AA (mol/mol)	Crosslinker (mol/mol) w.r.t. AA	Chitosan mol. weight	Time taken to rupture
1	1:1	5%	Medium	5 min
2	1:1	10%	Medium	30min
3	1:1	30%	Medium	2 h
4	1:1	30%	High	No rupture
5	2:1	30%	Medium	No rupture
6	1:1	30%	Medium*	No rupture

*Quat -188 modified medium molecular weight chitosan was used

The swelling behavior of semi-IPNs with 2:1 mol ratio of chitosan to PAA was studied systematically as function of time. As shown in Figure 3, the swelling was pH dependent. Chitosan semi-IPN swelled more in pH 7 than in pH solution. It swelled in acidic pH due to the repulsion between the ammonium ions of chitosan, in alkaline pH repulsion between the carboxylate ions of PAA swelled the semi-IPNs. Repulsion between the carboxylate ions was predominant over the repulsion between the ammonium ions, thus the hydrogels swelled more in pH 7. Reverse trend was observed for the Quat-188 modified chitosan semi IPN. Under acidic condition the repulsion between the ammonium ions of chitosan and quaternary ammonium of Quat-188 resulted in enhanced swelling. Modified chitosan semi-IPN swelled more than unmodified chitosan semi-IPN due to the presence of quaternary ammonium ion. A drastic decrease in %swelling was observed for the modified chitosan hydrogel after 1h of equilibration.

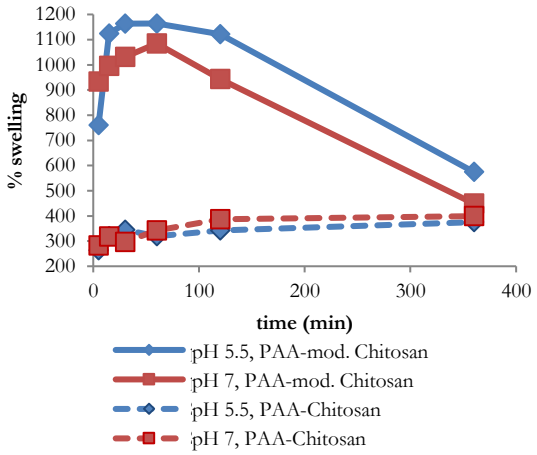

***Figure 3**. Swelling of chitosan and modified chitosan PAA semi IPNs as function of time and pH.*

Cryo DSC. In general, water in the hydrogel is classified into two states; freezable and non-freezable water. It has been established that non-freezable water has a strong interaction with polymer chain (Nakaoki et al 2008; Nakano et al 2011) and encapsulation and release of drugs is dependent on the amount of freezable water in the hydrogel. Therefore cryo DSC measurement was carried out to

estimate the amount of freezable water and its impact on drug encapsulation and release. Figure 4 shows the DSC thermogram of the melting process of water in the gel. The melting enthalpy of water in the hydrogel comes from the freezable water. The weight content of freezable water (w_F) was estimated by the following equation

$$w_F = \frac{\Delta H_{obs}}{\Delta H_{water}}$$

where ΔH_{obs} and ΔH_{water} denote the melting enthalpies of observed and pure water, respectively. The weight contents of freezable water for modified and native chitosan were 86 and 81 %, respectively. Modified chitosan contains larger amount of freezable water. Therefore it is expected that modified chitosan will allow more efficient encapsulation and release of drugs.

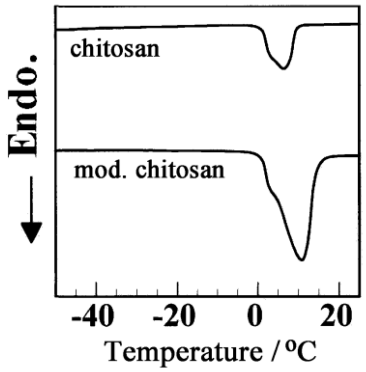

Figure 4. *DSC thermogram of the melting process of water in the chitosan and modified chitosan gels.*

Drug encapsulation and release. $AgNO_3$ and mafenide acetate were the two prototype drugs that were used for our study. These drugs are used to treat the wound due to injury from burn by topical application. Instead of their direct and repeated application, their controlled release at the site of injury from a hydrogel patch might serve as an efficient way of treating injury due to burn. Chitosan, owing to its strong film forming ability and haemostatic properties has also been explored as a primary component of wound dressing patch (Bochicchio *et al.* 2009).The group studied the encapsulation and release of these two drugs which differ in size and the nature of interaction with the semi IPN hydrogel of chitosan while both having the burn wound treatment application.

$AgNO_3$ encapsulation and release. A rapid incorporation of $AgNO_3$ inside the modified and unmodified chitosan hydrogels was observed within a short interval of time (Table 2). However, no noticeable release of $AgNO_3$ was observed from the hydrogel with 10mg of $AgNO_3$ even after 6h as shown in Table 3.

Table 2. Encapsulation of $AgNO_3$ by Chitosan PAA semi IPNs.

Entry	Initial $AgNO_3$ (mg)	Equilibration Time (mins.)	$AgNO_3$ incorporated (mg) (% incorporation)
Modified Chitosan PAA hydrogel			
1	10	10	10 (100%)
2	30	10	26.7 (89%)
3	30	120	29.1 (97%)
Unmodified Chitosan PAA hydrogel			
4	10	10	10 (100%)
5	30	10	26.1 (87%)
6	30	120	27 (90%)

Table 3. Release of $AgNO_3$ from Chitosan PAA semi IPNs.

Entry	pH	Equilibration Time (mins.)	$AgNO_3$ released (mg) (% release)
Modified Chitosan PAA hydrogel			
1	5.5	120	0.4 (4%)
2	5.5	360	0.4 (4%)
3	7	120	0.16 (1.6%)
Unmodified Chitosan PAA hydrogel			
3	5.5	120	-
4	7	120	-

Maximum release of 4% $AgNO_3$ in 2h was observed from modified chitosan PAA semi-IPN which can be attributed to immense swelling of the network and the presence of

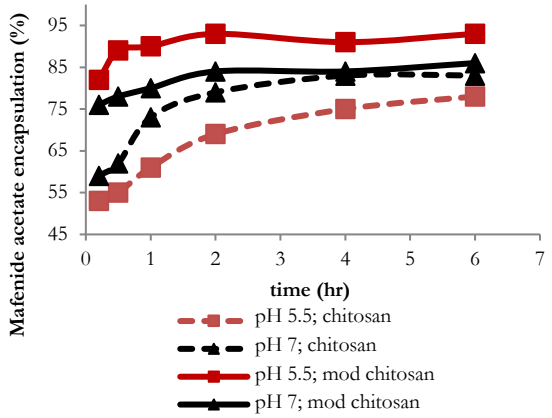

Figure 5. *Mafenide acetate encapsulation by chitosan and modified chitosan PAA semi IPNs as function of time and pH.*

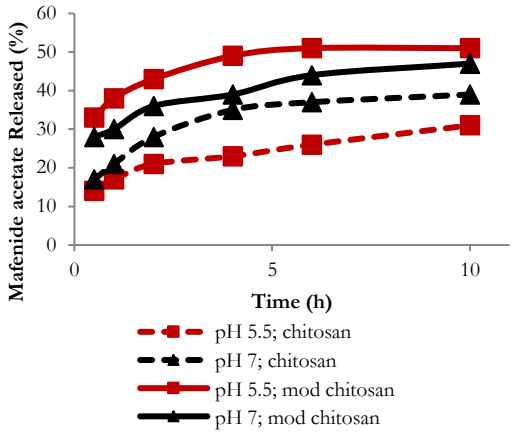

Figure 6*. Mafenide acetate release from chitosan and modified chitosan PAA semi IPNs as function of time and pH.*

freezable water. Efficient encapsulation is due to rapid diffusion of $AgNO_3$ into the swollen hydrogel owing to their small size and poor release of $AgNO_3$ from the hydrogel is attributed to strong chelation of silver ion with the –OH and -NH_2 of chitosan.

Mafenide acetate (MFA) encapsulation and release. As seen in Figure 5 and 6 encapsulation and release of MFA was pH dependent and followed the pH dependent swelling trend. Release was monitored using those hydrogels that encapsulated 26-28 mg of mafenide acetate. Encapsulation and release was most efficient for the Quat-188 modified chitosan at pH 5.5 (DI water), making it an ideal candidate to serve as controlled release vehicle for skin. Efficiency of encapsulation and release increased with the increase in the amount of freezable water and the extent of swelling. However, the rate of MFA incorporation was slower that $AgNO_3$ incorporation though the semi-IPNs swelled to the same extent for both these encapsulates. Smaller size and affinity of silver ion to chelate with chitosan might have aided $AgNO_3$ to diffuse faster into the hydrogel. It should also be noted that though the modified and the unmodified hydrogels were swollen more than 100%, complete release of mafenide acetate was not observed, which is probably due to the formation of hydrogen bonding between MFA and the hydrogel. Thus it can be stated that apart from swelling potential of the hydrogel, the interaction between the drug and the hydrogel and the amount of freezable water in the hydrogel also have profound influence on encapsulation and release behavior.

CONCLUSION

Semi IPN network of chitosan and PAA was synthesized in one step. Strength of the semi-IPN hydrogels increased with the increase in the amount of chitosan and upon modification of chitosan with Quat-188. Modification improved the pH dependent swelling behavior and showed better encapsulation and release potential. Encapsulation of $AgNO_3$ was much faster than mafenide acetate, however hardly any release of $AgNO_3$ was observed, whereas mafenide acetate got release at a controlled rate, proving that synthesized semi–IPN can be explored further as patch for encapsulation and release of mafenide acetate alone for topical application. Strong interaction between the drug and the matrix will facilitate efficient encapsulation but might adversely affect the release rate. Thus chitosan PAA semi-IPN hydrogel can serve as an efficient vehicle for the encapsulation and release of encapsulates such as MFA however it cannot be used as controlled release vehicle for drugs such as $AgNO_3$ which has strong affinity for the encapsulating vehicle.

This study has established that it is essential to consider drug-polymer interaction while

formulating a controlled release device. Encapsulation and release potential cannot be gauged from swelling profile alone.

The study reported is preliminary in nature. More experiments are required to be performed to establish the potential of chitosan poly(acrylic acid) semi-IPN serve as a wound dressing patch for topical delivery of the drug.

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