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Adsorption of indomethacin onto chemically modified chitosan beads

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Abstract

Macroporous chitosan beads used for the immobilization of an anti-inflammatory drug were prepared by the wet phase-inversion method. There are two stages of phase-inversion observed from the cast of chitosan droplet in tripolyphosphate (TPP) aqueous solution. The first stage of phase-inversion is dominated by liquid–liquid demixing and the morphology of the freeze-dried chitosan bead shows a bundle-like porous structure. The following stage of phase-inversion is attributed to the solid–liquid demixing and the morphology of the freeze-dried chitosan bead changes to an interconnected porous structure comprising particulates around the pores. The pore size and porosity of the bead can be varied by altering synthesis conditions, such as initial polymer concentration, and the pH value and concentration of the casting agent (TPP aqueous solution). Quaternary ammonium, and aliphatic and aromatic acyl groups were introduced into the porous chitosan beads to interact with an anti-inflammatory drug, indomethacin, through the electrostatic interaction and hydrophobic interaction. The results indicated that chemical modification of the porous chitosan beads have obvious effect on the adsorption of indomethacin. © 2001 Published by Elsevier Science Ltd.

Keywords: Chitosan; Macroporous; Phase-inversion

1. Introduction

Chitosan is a biopolymer comprising D-glucosamine and D-acetyl-glucosamine, and is readily processible into films, beads and sponges from aqueous acid solution. Chitosan beads were developed for controlled drug release, removal of heavy metal ions from waste water, such as Hg(II), UO_2(II), Cd(II), Zn(II), Cu(II) and Ni(II) ions [1,2], and were also applied to immobilize biological agents, such as yeast cells, *Escherichia coil*, protease, lipoprotein lipase and bovine serum albumin [3–8]. Recently, macroporous chitosan scaffolds were explored as a material used for tissue engineering. Due to its wide application in chemical, biochemical and biomedical fields of researches, chitosan became an important biomaterial in the last years.

Porous drug delivery device, have received much attention to deliver anti-cancer drug such as cisplatin, adriamycin, and peptide-based therapeutic agents [9–11]. Macroporous hydrogel implants prepared from poly(vinylpyrrolidone), poly(acryl amide), poly(methacrylic acid), poly(acrylic acid) or poly(vinyl alcohol) and poly(hydroxyethyl methacrylate) have been reported to be useful for long-term or pH-sensitive delivery of proteins, such as insulin, γ-globulin and gene etc [12–17]. Furthermore, macroporous sponges were also prepared for the controlled delivery of various growth factors for guided tissue engineering, for example, wound and bone healing, and liver tissue regeneration [18–20].

Macroporous polymeric structures can be derived by various methods. They can be prepared by generating gas bubbles within the material during synthesis, leaching of soluble particles form within a polymer, copolymerization of a bicontinuous emulsion, or copolymerization/cross-linking of monomers in a poor solvent for the polymer [21–25]. Porous structures are also easily obtained using phase separation technique by inducing phase separation of a polymer solution followed by solidification of the polymeric microstructure [26–28]. In the past works, porous chitosan materials have been prepared using macromolecular organic compound — poly(ethylene glycol) as porogen [29], or prepared by casting chitosan–silica particles mixed solution and removing the silica particles by immersing the membrane into alkaline solution [30]. However, the preparation of porous chitosan materials via wet phase-inversion method has not been discussed.

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Wet phase-inversion (immersion precipitation induced phase-inversion) method is a suitable technique to prepare macroporous gels with desired morphology and pore size [31,32]. In the present study, a porous chitosan bead is prepared by a wet-phase inversion process for the immobilization of an anti-inflammatory drug. Tripolyphosphate (TPP) aqueous solution was used as a cast solution. Both of the liquid–liquid and solid–liquid phase separation processes are responsible for the formation of chitosan beads with high porosity. Following phase-inversion, the porous chitosan beads are chemically modified with reagents to introduce quaternary ammonium, aliphatic and aromatic acyl groups using 3-chloro-2-hydroxypropyltrimethylammonium chloride, benzoic and octanoic anhydride. A non-steroid anti-inflammatory drug, indomethacin, used for the treatment of arthritis, is immobilized onto the porous chitosan beads through different types of intermolecular interaction, such as electrostatic attraction or hydrophobic interaction. These results are evaluated for the possibility of using such a porous chitosan bead as a potential drug delivery system in future.

2. Materials and method

2.1. Materials

Chitosan was purchased from Fluka Co. Ltd, Switzerland. Molecular weight and deacetylation degree is 70,000 and 87%, respectively. Sodium TPP and lysozyme were derived from Sigma (St Louis, MO, USA). 3-chloro-2-hydroxypropyltrimethylammonium chloride, benzoic and octanoic anhydride were all purchased from Tokyo Chem. Ind., Japan. All other materials used were of analytical reagent grade.

2.2. Preparation of macroporous chitosan beads

Chitosan solution was prepared by dissolving 1.5 g of chitosan in 100 ml, 1%(v/v) acetic acid. This solution was dropped through a burette into 1–10%(w/v) sodium TPP aqueous solution. The TPP solution was prepared by dissolving 1–10 g of sodium TPP in 100 ml of deionized water. The pH values of prepared TPP solutions were adjusted from basic (8.9) to acidic (4.0) using hydrochloric acid. Chitosan solution was dropped into TPP solution and the gelled spheres formed instantaneously. The formed chitosan beads were remained in this solution for 1 h and washed with deionized water. Then, the beads were cross-linked chemically with 5% of ethylene glycol diglycidyl ether (EGDE) to glucose residue for 12 h in water–ethanol mixing solution. The cross-linked chitosan beads were washed with deionized water repeatedly, and finally dried using a freeze dryer (Eyela, FD-5N, Japan) to produce porous chitosan beads.

2.3. Electron scanning microscopy

The freeze-dried chitosan beads were gold coated to about 500×10^{-8} cm thickness using an Hitachi coating unit IB-2 coater under a high vaccum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Coated samples were examined using Hitachi S-2300 electron scanning microscopy.

2.4. X-ray analysis of crystallinity

The freeze-dried chitosan beads prepared from different stages of phase separation were all ground to powder. The crystalline forms of the chitosan beads were all determined by an X-ray powder diffractometer (Simadzu, XD-5, Japan).

2.5. Modification of porous chitosan beads

Macroporous chitosan beads were modified chemically with reagents, such as 3-chloro-2-hydroxypropyltrimethylammonium chloride, benzoic and octanoic anhydride to introduce three types of functional groups; aliphatic and aromatic acyl or quaternary ammonium group for the improvement of drug immobilization. Each 500 ml of chitosan beads was reacted with 68 or 55 g of octanoic or benzoic anhydride, respectively, in 500 ml of ethanol, 94 g of 3-chloro-2-hydroxypropyltrimethylammonium chloride in 500 ml dioxane at 70°C. The modified chitosan beads are rinsed with acetone and water to remove residue reagents.

2.6. Degrees of cross-linking and chemical modification

The degree of cross-linking and chemical modification determined by ninhydrin assay, was defined as the percentage of free amino groups in chitosan beads reacted with each test cross-linking agent subsequent to cross-linking. In the ninhydrin assay, the cross-linked and chemically modified chitosan beads were heated with ninhydrin solution for 20 min. After heating with ninhydrin, the optical absorbance of the solution was recorded with a spectrophotometer (Model UV-150-02; Shimadzu Corp., Kyoto, Japan) using *N*-glucosamine at various known concentrations as standard. It is known that the amount of free amino groups in the cross-linked and chemically modified chitosan beads, after heating with ninhydrin, are proportional to the optical absorbance of the solution. The degrees of cross-linking and chemical modification are evaluated from the remaining amino groups of cross-linked and chemically modified chitosan beads.

2.7. Adsorption of indomethacin

The adsorption of indomethacin was performed using a stirred cell apparatus. Indomethacin (0.5 g) was dissolved in 500 ml of deionized water to prepare dissolved indomethacin/water solution. The medium was placed in a 1 l round flask and stirred with a mechanical stirrer at a rate of 50 rpm at room temperature. The porous quateraminate chitosan beads or aliphatic and aromatic acylchitosan were added to the aqueous indomethacin solution for the adsorption experiment. An adsorption experiment was continued until the indomethacin concentration was constant. The adsorption capacity is calculated as follows:

$$Q = [(A_0 - A)V/W]M$$

where A_0 and A are the concentrations of indomethacin medium before and after the adsorption of indomethacin by chemically modified chitosan beads, respectively, V is the volume of indomethacin medium, and M is the molecular weight of indomethacin. W is the weight of porous chitosan beads used in the adsorption experiment.

3. Results and discussion

In our previous study, preparation of membranes with a variety of structures was accomplished by using different phase-inversion methods [33–36]. In this method, the cast of chitosan droplets in TPP aqueous solution is a convenient and effective procedure to tailor bead's structure. Control of the morphology of polymeric beads by altering the immersion time and the nature of TPP aqueous solution, such as its concentration and pH value, are discussed here.

3.1. Phase-inversion of chitosan beads

The phase-inversion of chitosan beads in TPP solution is time-dependent. In Fig. 1, the surface morphology of freeze-dried beads formed by immersing a chitosan droplet (1.5 wt%) in a TPP solution (10 wt%) within 10 s–5 min is shown. As can be seen in Fig. 1(a), cellular pores are formed on the surface of freeze-dried beads prepared by immersing chitosan droplet in TPP solution. The porous structure is possibly formed on the surface of a chitosan bead via the instantaneous mechanism of liquid–liquid phase separation. Fig. 1(b)–(d) shows the variation of porous structure on the surface of a freeze-dried chitosan bead with time. The pores gradually disappear after 5 min of immersion, indicating the variation of porous structure on the surface of a chitosan bead during this stage (the initial stage) of phase-inversion was time-dependent. Fig. 2(a) shows the overall cross-section of the freeze-dried chitosan bead prepared by immersion for 1 min. The remarkable parallel bundle-like structure (pore size 50–100 μm) of

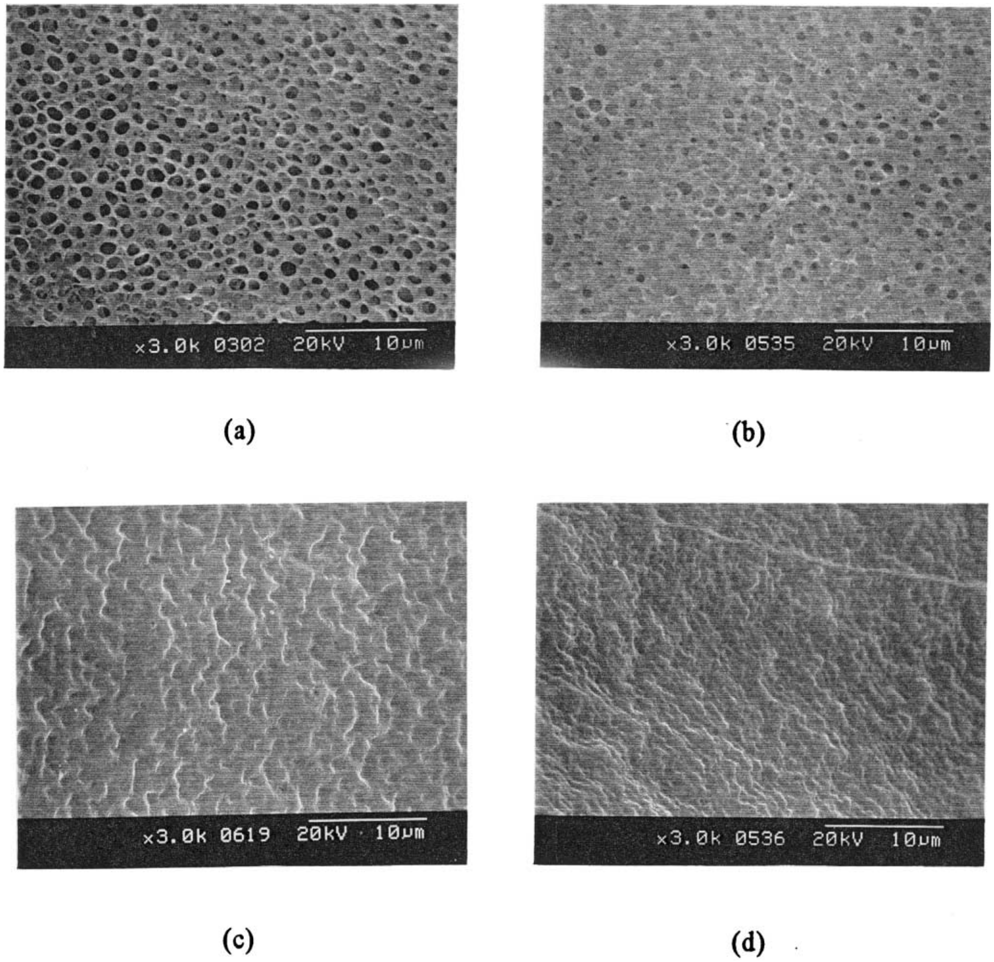


Fig. 1. SEM pictures of surface morphology of the phase inversed chitosan bead (initial stage of phase inversion). Immersion time: (a) 10 s; (b) 20 s; (c) 1 min; (d) 5 min.

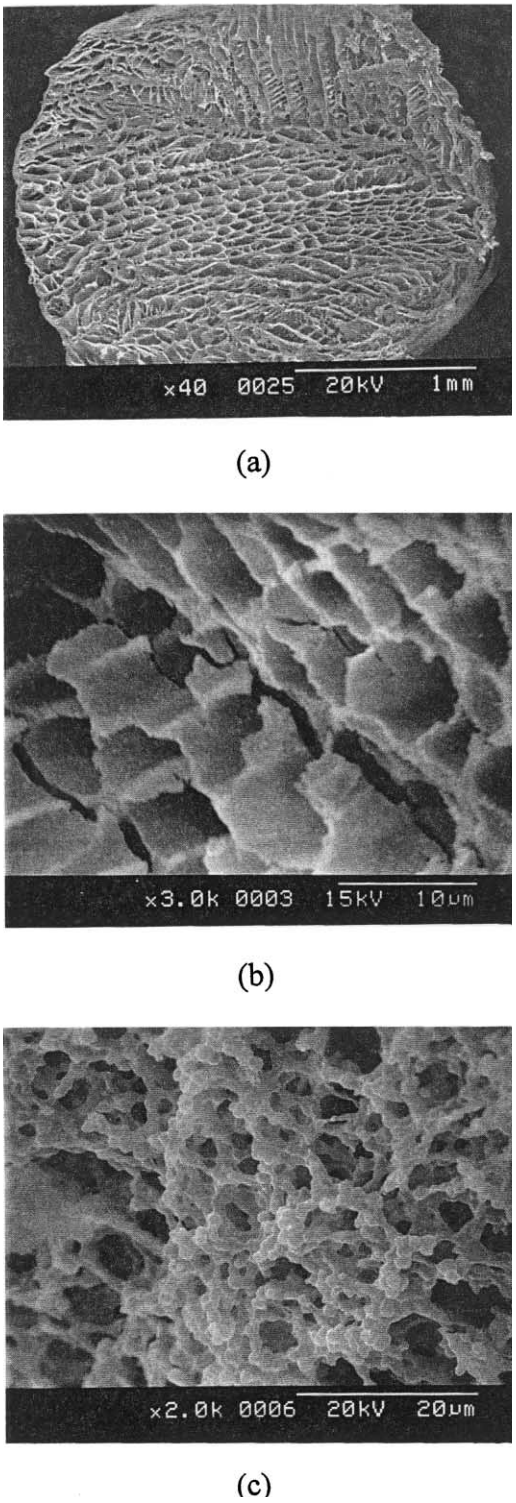


Fig. 2. SEM pictures of cross-section of the phase inversed chitosan beads. Immersion time: (a) 1 min (the initial stage of phase inversion); (b) 10 min (the initial stage of phase inversion); (c) 30 min (the following stage of phase inversion).

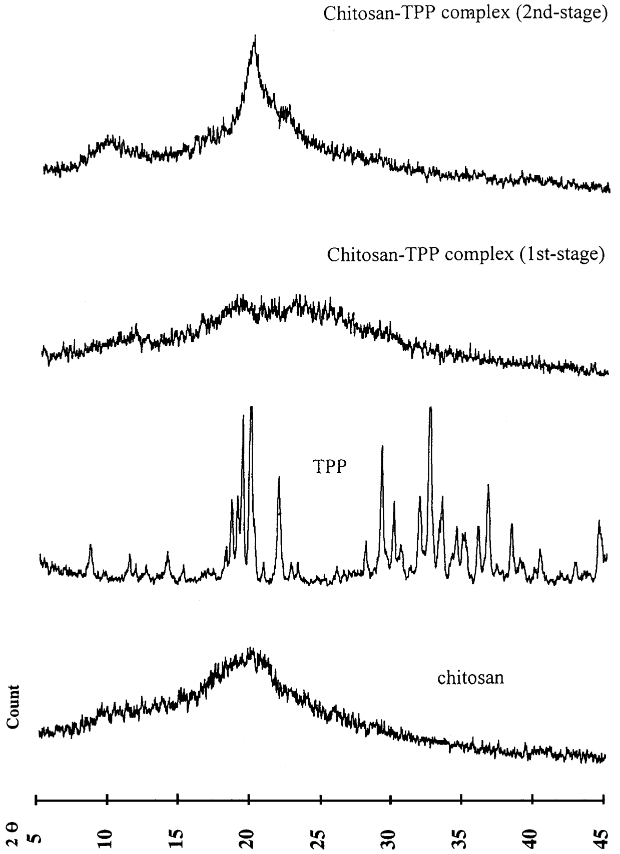


Fig. 3. X-ray diffraction analysis the crystallinity of the initial and following stage of phase inversed chitosan beads.

the internal matrix is observed, ascribed to partial crystallization of ice, which is clearly favorable to a higher degree of order and orientation. This result suggests that less liquid–liquid demixing occurs in the region of internal matrix, and the phase separation may be induced by the sublimation of ice through a freeze-drying process. Fig. 2(b) shows the structure of freeze-dried chitosan bead prepared by immersion for 10 min. The structure of the beads is also bundle-like, but has a much smaller pore size (~10 μm).

Fig. 2(c) shows the cross-section morphology of chitosan bead obtained from the following stage of phase-inversion. After 30 min of inversion, the structure of the chitosan bead varies from a bundle-like structure to interconnected structure comprising particles of uniform size. From the observation of the micrographic pictures of the chitosan beads, it can be found that the phase-inversion of this bead may be either due to the onset of liquid–liquid demixing or solid–liquid demixing. The particulates around the pores in the internal matrix of the beads are due to nucleation and growth of a polymer poor phase. Most likely after liquid–liquid demixing, the solid–liquid demixing process is responsible for the fixation of the structure. From a kinetic consideration, the crystallization process needs a longer induction period for the growth of crystalline. The suggested mechanism of liquid–liquid demixing followed by solid–liquid demixing is supported by the rather long inversion time.

The X-ray diffraction patterns of the beads prepared at the initial stage of phase-inversion is shown in Fig. 3, together with the beads prepared at the following stage of phase-inversion. The crystallinity of chitosan beads prepared at the following stage appears to be obviously higher than the beads prepared at the initial stage. These results suggest that crystallization occurs at the following stage of phase-inversion, wherein liquid–liquid phase separation has completed and the bundle-like structure was ever formed. As described in our previous studies [37], TPP dissolved in water to dissociate both OH^- and tripolyphosphoric ions in the TPP aqueous solution and could competitively diffuse into chitosan droplet, and electrostatically attracted to the NH_3^+ groups in chitosan, which resulted in the phase-inversion of chitosan droplets. The OH^- reacted with chitosan by deprotonation; however, TPP ions reacted with chitosan by electrostatic attraction likely to induce nucleation. Due to the fact that molecular size of TPP is much larger than that of OH^-, OH^- can diffuse faster than TPP ions into chitosan droplets. One can conclude that OH^- induced phase-inversion of chitosan droplet is like a liquid–liquid demixing; while, TPP ions induce solid–liquid demixing to form particulate structure. The transformation of chitosan bead's structure depends on the competition between OH^- induced liquid–liquid phase separation and TPP ion induced solid–liquid phase separation.

3.2. Factors influencing the structure of chitosan beads

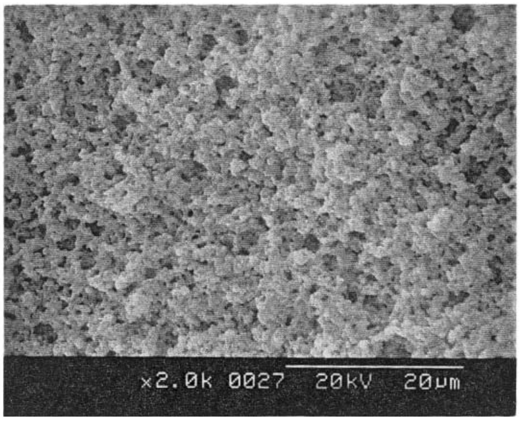
Molecular weight is one of the major factors to influence the phase-inversion. The beads prepared from high molecular weight of chitosan are covered with a dense layer on its surface. The chitosan solution (1.5 wt%) degraded by lysozyme (1000 U/ml) can be concentrated (1.5–8.0 wt%) to prepare chitosan bead consisting of interconnected pores throughout the entire bead. Chemically cross-linking should be another important factor to influence the microstructure of chitosan beads. However, the factor of cross-linking is controlled (all beads are cross-linked at the same condition) in this study to examine the effect of phase-separation on the variation of structures of chitosan bead. Chitosan beads prepared by such a process are very brittle. The pore size and effective porosity of the bead can be varied by altering synthesis conditions, such as initial polymer concentration, and the pH value and concentration of TPP solution. The key factors affecting the microstructural characteristics of the beads are discussed below.

3.2.1. Effect of pH value of TPP solution

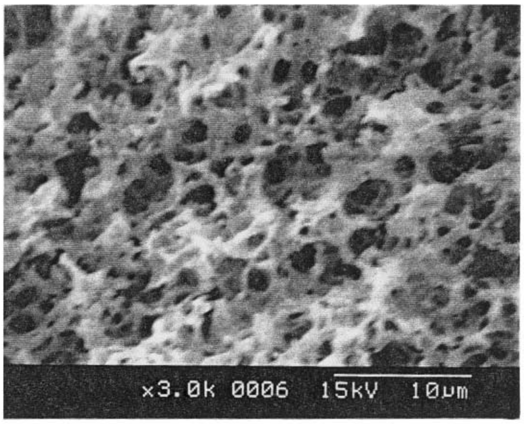
As described in our previous study, the solidification of chitosan beads in acidic TPP solution was attributed to electrostatic attraction like ionic cross-linking [37]. None of pores, but particulates, can be observed from the chitosan beads. However, the phase-inversion of chitosan droplets in basic TPP solution was dependent on the competition between OH^- induced deprotonation and TPP ions induced ionic cross-linking. The deprotonation may reduce chitosan solubility due to the transformation of more hydrophilic $-NH_3^+$ to hydrophobic $-NH_2$, and then induces phase separation. Whereas, the TPP ions are electrostatically attracted to chitosan, which results in the solid–liquid demixing. The competed liquid–liquid and solid–liquid phase separations promote the formation of interconnected porous structure with particulates surrounding the pores. The ionic cross-linking of linear polymer chain by TPP ions locks the three-dimensional network, which could help to fix the phase separated structure. From the SEM micrograph of the chitosan gel beads, displayed in Fig. 4(a) and (b), it is evident that the chitosan bead prepared in acidic TPP aq. solution is non-porous; however, the beads prepared in basic TPP aq. solution are macroporous.

3.2.2. Effect of polymer concentration

The initial polymer concentration plays a crucial role in the development of gel microstructure, since it governs the



(a)



(b)

Fig. 4. Effect of pH of TPP aqueous solution on the morphology of chitosan–TPP gel beads: (a) prepared from pH 8.9 of aqueous TPP (×2000) (b) prepared from pH 4.0 of aqueous TPP (×2000).

Table 1
Effect of chitosan concentration on chitosan bead's morphology (the concentration and pH of TPP aq. solution is 10 wt% and 8.9, respectively; the chitosan used is hydrolyzed by lysozyme)

Chitosan concentration (%)	Average pore size (μm)	Effective porosity (%)	Pore morphology
10.0	0.98	74.38	Interconnect/particulate
8.0	1.37	80.75	Interconnect
5.0	3.92	84.19	Interconnect
1.5	5.85	88.06	Interconnect

relative amounts of polymer-dilute and polymer-rich phases produced upon phase separation, which can affect both the liquid–liquid or solid–liquid demixing. The synthesis parameters examined in this study and the microstructural characteristics of the gel beads obtained are summarized in Table 1. Representative micrographs of these gel beads are provided in Fig. 5(a)–(d). An increase in the initial polymer concentration induces a significant decrease in the effective porosity of these macroporous chitosan beads. The reduction in effective porosity of macroporous gel beads accompanied by an increase in the initial polymer concentration can be readily explained according to the variation of concentration of polymer-rich and polymer-lean phase. Reducing the initial polymer concentration consequently leads to a greater percentage of the dilute phase. Since the polymer-dilute phase leads to pores and the concentrated phase forms continuous structures, the effective porosity of macroporous gel beads prepared by the phase-inversion technique is expected to decrease with an increase in the initial polymer concentration.

3.2.3. *Effect of TPP concentration*

Only a little change of the porosity is observed in macroporous beads upon changing the TPP concentration from 5

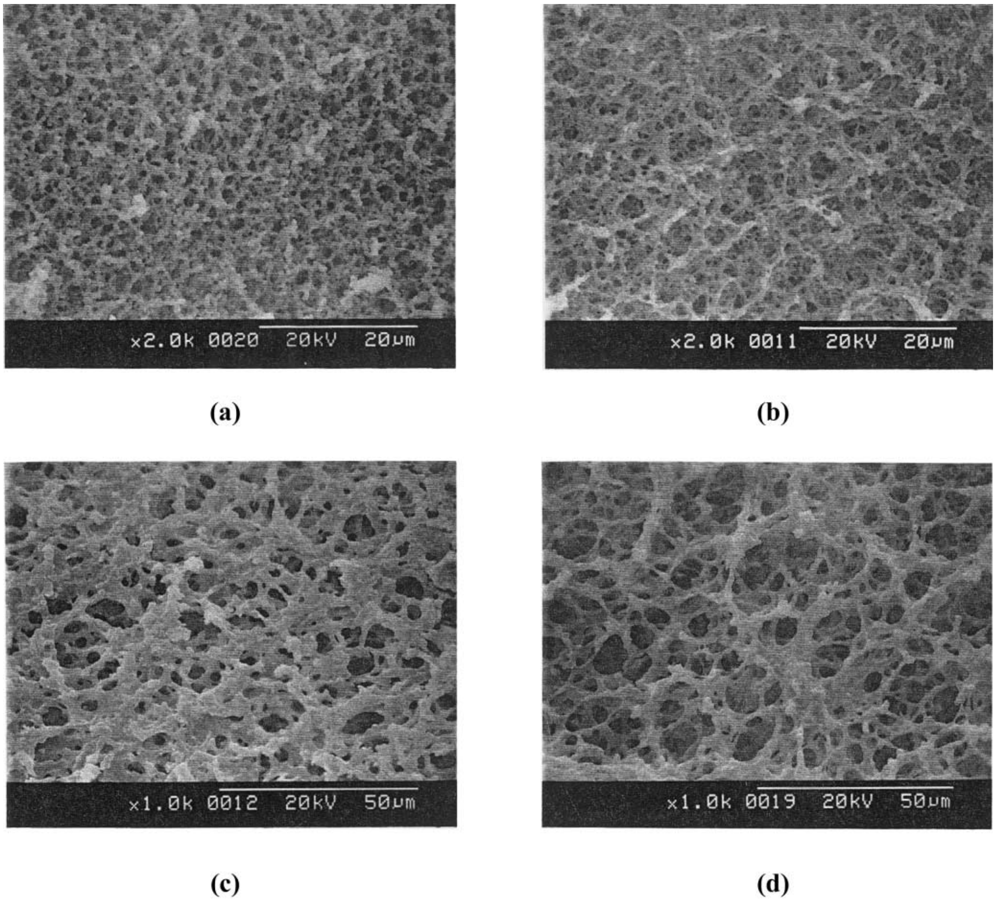


Fig. 5. Effect of polymer concentration on the morphology of chitosan–TPP gel beads: (a) 8 wt% of aqueous chitosan solution (b) 5 wt% of aqueous chitosan solution (c) 2.5 wt% of aqueous chitosan solution (d) 1.5 wt% of aqueous chitosan solution.

Table 2
Effect of TPP concentration on chitosan bead's morphology (the concentration of chitosan is 1.5 wt% and pH of TPP aq. solution is 8.9 respectively; the chitosan used is hydrolyzed by lysozyme)

TPP concentration (%)	Average pore size (μm)	Effective porosity (%)	Pore morphology
8.0	2.93	87.45	Interconnect/particulate
5.0	2.17	82.64	Interconnect
3.0	1.04	78.32	Interconnect
1.0	0.157	67.97	–

to 8 wt% (Table 2). But as TPP concentration is lower than 3 wt%, porosity of chitosan beads significantly decreases (Fig. 6). If porous structure is dominated only by liquid–liquid demixing, the pore size and porosity should not be affected by TPP concentration. However, as described in the previous study, the early stage of phase-inversion of the chitosan beads were dominated by neutralization induced liquid–liquid demixing, but the final stage of phase-inversion of these chitosan beads were affected by the TPP ions induced solid–liquid demixing. In the liquid–liquid demixing process, nucleation of the liquid micelles composed of acetic acid and OH^- commenced when the droplet solution entered the binodal phase envelop. These micelles are in equilibrium at their interface with the surrounding polymer-rich gel phase. Radial growth of the micelles continues until the polymer-rich phase fused and solidified. The

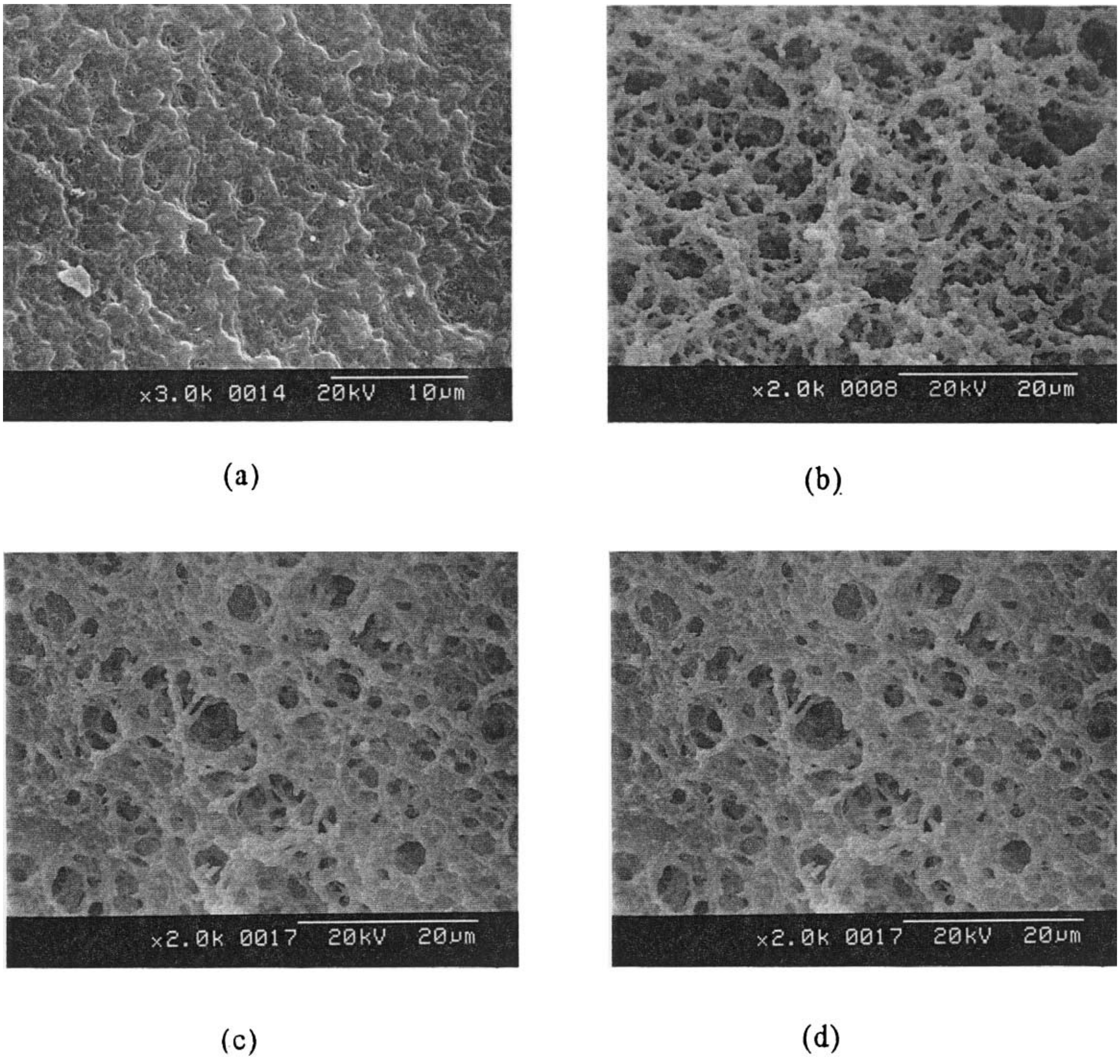


Fig. 6. Effect of TPP concentration on the morphology of chitosan–TPP gel beads: (a) 8 wt% of aqueous TPP solution (b) 5 wt% of aqueous chitosan solution (c) 3 wt% of aqueous chitosan solution (d) 1 wt% of aqueous chitosan solution.

supersaturated polymer-rich gel eventually crystallizes into the solid matrix in contact with TPP ions. It is during this stage that the bicontinuous network is actually formed.

3.3. Adsorption of indomethacin onto porous chitosan beads

The cross-linked porous chitosan beads are chemically modified by reagents to introduce quaternary ammonium, and aliphatic and aromatic acyl groups, the reaction degrees of modification are 37, 43 and 39%, respectively. After chemical modification, the beads are rinsed in acetone and water to remove residue reagents without further purification. Due to the reason that the phase-separated structure of chitosan beads is fixed by EGDE, the microstructure of porous chitosan beads doesn't obviously change after modification. The equilibrium adsorption quantities of various chemically modified chitosan beads are shown in Fig. 7. The quateraminated chitosan (QC) beads display the highest quantity of indomethacin adsorption in all samples. This is due to the fact that the negatively charged $-COO^-$ of indomethacin could form strong interaction with $-N^+(CH_3)_3$ group of the quateramminated chitosan based on electrostatic interaction.

Indomethacin can also be effectively recovered from deionized water using a hydrophobic chitosan bead with benzoyl acyl groups or aliphatic acyl groups. The sorption of dye by chitosan gel with hydrophobic groups has been investigated [38,39]. The dye can be adsorbed onto acylchitosan gel by hydrophobic interaction. It was also reported that indomethacin could be successfully incorporated into the hydrophobic inner core of methoxy poly(ethylene glycol)/ϵ-caprolactone amphiphilic block copolymeric micelle through lipophilic interaction [40,41]. Similarly, the porous acylchitosan beads can interact with indomethacin through different hydrophobic interactions. The benzoyl chitosan (BC) bead has higher indomethacin affinity than that of octanoyl chitosan (OC) beads. This indicates that the presence of aromatic amide group substituted on chitosan shows a significantly higher binding affinity for the adsorption of indomethacin due to their aromatic similarity. The schematic chemical interaction between indomethacin and the chemically modified porous chitosan beads are shown in Fig. 8.

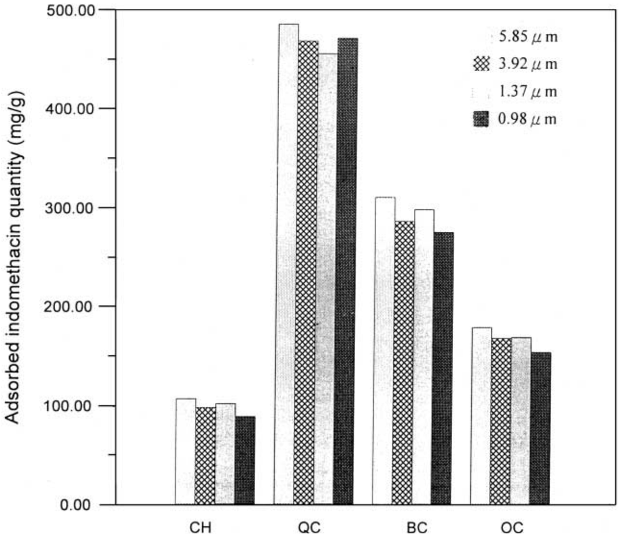


Fig. 7. Equilibrium quantities of indomethacin adsorbed onto different pore size and chemically modified chitosan beads; CH: original chitosan, QC: quateraminated chitosan, BC: benzoyl chitosan, OC: octanoyl chitosan.

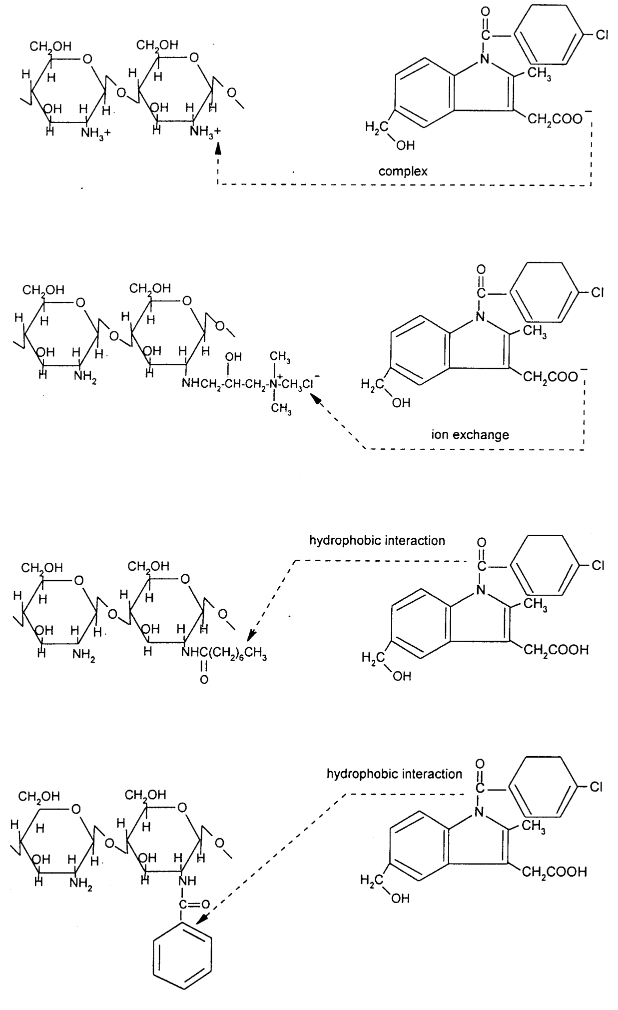


Fig. 8. Shematic chemical interaction between indomethacin and chemically modified porous chitosan beads.

From the result of the indomethacin adsorption study, it is demonstrated that indomethacin can be successfully immobilized to the porous and chemically modified chitosan beads. The factors dominating the immobilization of drug are the electrostatic attraction and hydrophobic interaction between indomethacin and the chemically modified chitosan.

4. Conclusion

In the present study, a novel phase-inversion process is developed for the preparation of porous drug delivery system based on chitosan. Variation of porous structure of the chitosan beads is achieved by altering the chitosan concentration, and the pH value and TPP concentration. Aliphatic and aromatic acyl groups and quaternary ammonium groups are introduced into the porous chitosan beads. The efficiency of the adsorption of indomethacin onto the porous chitosan beads is obviously dependent on the chemically modified functional groups of the beads. In conclusion, indomethacin could be effectively adsorbed onto the porous chitosan bead prepared in this study. Such a drug carrier may be used for the delivery of protein-based therapeutic agents, such as growth factor, in future.

Acknowledgements

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