

Effect of Phthalate Buffer on Drug Released from Chitosan Microspheres Cross-linked by Triphosphate

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Abstract. Controlled release experiments involve release of drugs from biodegradable polymers. Paracetamol loaded chitosan microspheres were prepared using triphosphate as the crosslinking agent. Complete release of paracetamol was observed in less than six hours in simulated gastric fluid having pH of 4 prepared with phthalate buffer. This observation is in contrast to negligible release from simulated fluids prepared with other buffer solutions, indicating strong interaction between phthalate ion and the protonated amine group of chitosan, leading to significant microspheres degradation and release of paracetamol. Phthalate-induced degradation of chitosan microspheres was also observed in the release of paracetamol from chitosan-triphosphate-alginate hydrogel beads. Our results suggest that the use phthalate ion in release profile experiments from cross-linked chitosan should be avoided.

Keywords: Chitosan; biopolymer; controlled release; phthalate

Introduction

Most of the drugs in the clinical phase fail to achieve clinical outcomes due the absence of ability to reach the target site. An effective approach to overcome this failure is by the improvement of controlled drug release systems that release drugs or bioactive compounds at the targeted sites. One of the issues to be solved in is the extreme changes of pH in the human digestive system. Drugs that do not survive the changing pH along its path to reach the targeted site would not produce effective medications [1]. A controlled drug release system consists of three components: a therapeutic agent, a targeting moiety, and, a carrier system. A wide range of materials such as natural or synthetic polymers, lipids, and surfactants have been employed as drug carriers [2]. Among the natural polymers used as drug carriers, chitosan has received significant attention due to its abundant availability, unique mucoadhesivity, biodegradability, non-toxicity, low-immunogenicity, and biocompatibility [3,4]. Chitosan, a linear amino polysaccharide, is obtained by deacetylation of chitin, a natural polysaccharide found in the exoskeleton of crustaceans

such as crab and shrimp.

Chitosan beads or microspheres prepared using the complexation reaction between anions and chitosan has been developed by many researchers due to its relatively easy process [5]. This anion complexation, usually called ionotropic gelation method, used a wide variety of anions to crosslink chitosan to produce chitosan-drug matrix in the form of microspheres or nanospheres. Among the anions that can be used in this method, tripolyphosphate (TPP) produces chitosan-drug microspheres with the strongest mechanical resistance, as well as release profile curves approaching the zero-order curve [5]. The non-toxic multivalent tripolyphosphate forms a gel with chitosan due to ionic interactions between the positively charged amino groups on chitosan and a negatively charged TPP anion. This ionic interaction is dependent on the pH of the solution [6]. The aims of this study are to obtain chitosan-drug microspheres with high drug loadings and encapsulation efficiencies, as well as having release profiles suitable for drug release targeted in the digestive system, specifically in the colon area. Digestive system condition is represented by using synthetic gastrointestinal fluids which are buffer solutions with pH values of 1.2, 6.8 and 7.4 to simulate conditions in the stomach, small intestine, and large intestine or colon, respectively. Paracetamol was used as a model drug because it has a relatively high solubility in water. The effect of enzymes existing in the digestive system on drug release profile was also observed by the addition of two enzymes, α -amylase and β -glucosidase, into the buffer solutions. The release of paracetamol from chitosan microspheres was determined using UV-Vis spectrophotometry method and sampling after immersing the microspheres in buffer solution at predetermined intervals in 24 h duration.

Experimental

Materials

Chitosan was obtained from Biotech Surindo (Cirebon, Indonesia) as a medical grade powder with degree of N-deacetylation of 93.6%. Paracetamol with purity >99% was obtained from Mallinckrodt Pharmaceuticals. Sodium tripolyphosphate (STTP) with a purity of 90% was obtained from Bratachem Chemical. KCl-HCl buffer solution (pH 1.2), KH_2PO_4 -NaOH buffer solution (pH 6.8), and KH_2PO_4 -NaOH buffer solution (pH 7.4) were prepared using the standard USP. The enzymes α -amylase and β -glucosidase were obtained from Clarichem Indonesia and Sigma Aldrich, respectively.

Preparation of chitosan-paracetamol microspheres

A solution of 1% (w/v) acetic acid-chitosan mixture was prepared by dissolving chitosan in 2.5% acetic acid solution (w/v). Then, paracetamol was dissolved in the aqueous chitosan solution with drug to chitosan weight ratio of 1:5. The mixture was homogenized at 400 rpm for 15 min. Various amounts of STPP were dissolved in 100 ml of distilled water to form 2, 4, 6 and 8% (w/v) solutions. The drug-chitosan mixture was injected and slowly dripped into the STPP solution as it was homogenized at 400 rpm. After all of chitosan mixture has been added into STPP solution, it was left for 30 min to let TPP anions crosslink the chitosan beads. The produced solid particles were filtered, washed repeatedly, and freeze dried. The filtered solution was analyzed for the amount of residual drug to determine the total drug loading and the encapsulation efficiency. The dried particles were ground to form TPP-chitosan-drug microspheres and the resulting microspheres were sieved through a wire mesh screen.

Preparation of gastrointestinal synthetic fluids

Gastrointestinal synthetic fluid was used as media for drug release. The three synthetic fluids employed are simulated gastric fluid (SGF) as pH 1.2 solution, simulated intestinal fluid (SIF) as pH 6.8 buffer, and, simulated colonic fluid (SCF) as pH 7.4 buffer. Separate synthetic fluids were also made by mixing these buffer solutions with enzymes, α -amylase in pH 6.8 and β -glucosidase in pH 7.4.

Determination of drug loading and encapsulation efficiency

The solutions obtained from microsphere filtration and washing was diluted 100 times and the absorbance of paracetamol was measured using a UV spectrophotometer at 243 nm. Using the standard curve, the amount of paracetamol encapsulated can be calculated based on the drug concentration before and after microsphere formation. The following equations are used to calculate percentages of drug loading and encapsulation efficiency:

$$\% \text{ drug loading} = \frac{m(\text{encapsulated drug})}{m(\text{microspheres})} \times 100\% \quad (1)$$

$$\% \text{ encapsulation efficiency} = \frac{m(\text{encapsulated drug})}{m(\text{initial drug used})} \times 100\% \quad (2)$$

Release profile determination

Batches of accurately weighed microspheres, approximately 25 mg each, were placed into test tubes. Synthetic gastrointestinal fluids prepared earlier were added into each test tube. Samples were taken from the release solution every hour until 8 h and the last sample was

taken after 24 h. After each sampling, the same volume of fresh solution was added to replace the sample taken. Drug presence in each sample taken from the synthetic fluids was analyzed using UV/VIS Spectrophotometer at 243 nm.

Results and Discussion

Particle size

Micrographs of the TPP-chitosan-drug particles confirmed that microspheres having diameter less than 100 μm were obtained.

Drug loading and encapsulation efficiency

Microsphere of chitosan-paracetamol is formed due to crosslinking that takes place during its preparation. The more crosslinking occurred, the stronger the microstructure of chitosan-drug formed. It is noted that not all paracetamol, which was previously interacting with chitosan by hydrogen bonding, was entrapped into the chitosan-tripolyphosphate matrix. Varying TPP concentration used during microsphere preparation resulted in drug loadings and encapsulation efficiencies given in Table 1.

Table 1. Paracetamol loading and encapsulation efficiency in chitosan microspheres for various TPP concentrations.

TPP concentration (%, w/v)	Encapsulation efficiency (%)	Paracetamol loading (%)
2	30.3	4.5
4	43.5	3.0
6	42.4	6.2
8	50.1	7.8

The data given in Table 1 indicated that the increased TPP concentration correspond to the increased value of drug loading and encapsulation efficiency. The ionic crosslinking caused by TPP anions increased the strength of the microsphere networks and more paracetamol is entrapped within the microspheres. The large amount of microspheres formed using the 4%-w/v TPP solution caused a decline in the drug loading.

In-vitro drug release

The release profile of paracetamol was obtained in simulated gastrointestinal fluids having pH values of 1.2 (SGF), 6.8 (SIF), and, 7.4 (SCF). The release experiments were repeated using buffer pH 6.8 containing α -amylase and buffer pH 7.4 containing β -glucosidase to see the effect of enzymes on chitosan degradation. Fig. 1 shows that the paracetamol

release profile in simulated gastric fluid is increasing with time, up to 8 h. The highest amount of drug released decreases with increasing TPP solution concentration and up to 30% paracetamol was released from microspheres prepared using the 2% TPP solution. In ionically crosslinked TPP-chitosan microspheres, the pH of the release fluids affects the crosslinking density, degree of swelling, and release of paracetamol. If the pH decreases, the protonation of the amino groups of chitosan leads to chain repulsion and polymer network swelling. This explains the relatively high drug release in acidic media of pH 1.2.

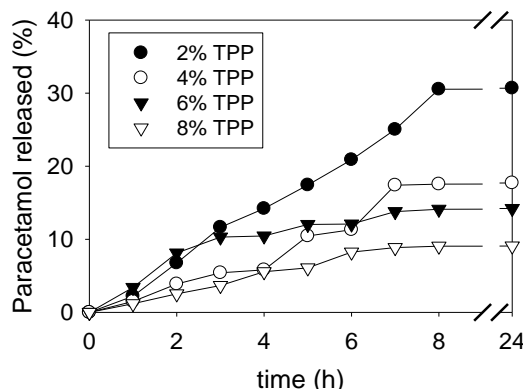


Figure 1. Paracetamol released into the simulated gastric fluid of pH 1.2.

Paracetamol profile release from chitosan microspheres in simulated intestinal fluid is shown Fig. 2a for release without enzyme and Fig. 2b for containing α -amylase enzyme (b). The release profiles in SIF show similar trends as those in SGF, except that the amount of paracetamol released is much lower. This result indicated that in buffer pH 7.4 chitosan microspheres were only slightly swelled due to electrostatic repulsion between the ionized groups in chitosan chain.

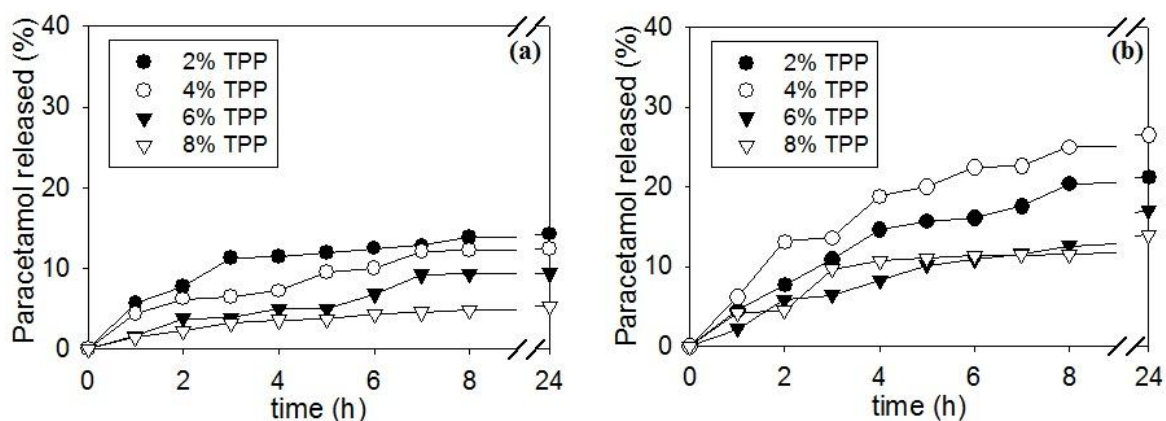


Figure 2. Paracetamol released into the simulated intestinal fluid of pH 7.4 without enzyme (a) and with α -amylase enzyme (b).

The addition of α -amylase enhanced the release of paracetamol up to twice the amount

released without added enzyme. The enzyme was found to be effective to hydrolyze β -1,4 linkages in chitosan in pH of 7.4 although it was reported to be most effective in pH of 5.0 [7,8]. It is noted that the presence of α -amylase changed the position of the release profiles in Fig. 2 (b) relative to those in Fig. 2 (a).

Paracetamol release profiles obtained in simulated colonic fluid shown in Fig. 3 (a) closely matched those shown in Fig. 1 for release in SGF where up to 30% of the paracetamol in the microspheres prepared with the 2% TPP solution were released.

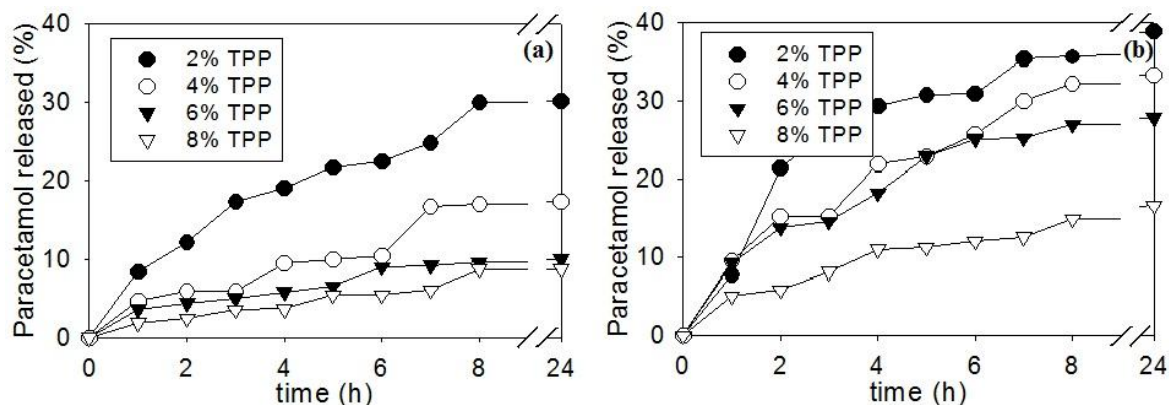


Figure 3. Paracetamol released into the simulated colonic fluid of pH 6.8 without enzyme (a) and with β -glucosidase enzyme.

Fig. 3 (b) shows paracetamol release from microspheres immersed in SCF containing β -glucosidase enzyme. In the presence of this enzyme, the maximum drug release of 39% was observed in this study. This observation indicates that β -glucosidase, which pH optimum is 5.0 [9], is still active accelerating the scission of β -glucosidic link in chitosan at pH 6.8. The paracetamol release profiles in SCF without β -glucosidase is similar to those in SGF, and they are significantly higher than those in SIF without α -amylase. The difference is due to the pH of the release media where an acidic media facilitates swelling and more extensive drug release. It is noted that while pH of the SCF and the SIF were 6.8 and 7.4, respectively; the difference of only 0.6 unit was sufficient to double the amount of drug released in the lower pH media.

In general, the release profiles obtained in this study show zero-order release behavior up to about 8 h. Afterwards, the release profiles become flat up to 24 h indicating that no more drug is released during this period. The maximum paracetamol release observed in this study was only 39% of the amount loaded in the microspheres and the rest of the drug was entrapped in the partly degraded chitosan microparticles. The incomplete drug release indicated that strong crosslinking between chitosan and TPP anions were formed. To increase the percentage drug release, an additional polymer whose hydrophilicity is

different from chitosan could be incorporated into the microspheres to perturb the ionic crosslinking between chitosan chains, decreasing the crosslinking density and making available more protonable amino groups [10].

Conclusion

The results indicated that increasing concentration tripolyphosphate solutions increased the loading of paracetamol in chitosan microspheres, while the least extensive crosslinks formed at lower concentrations of tripolyphosphate facilitated more drug to be released. The release profiles showed zero-order release behavior up to 8 hours where the highest drug release was 39% of the paracetamol loaded in the chitosan microspheres, indicating a strong crosslinking between chitosan and TPP anions. The relatively low accumulated drug release could be compensated by employing suitable enzymes, lower TPP solution concentration, and other biodegradable polymer to reduce the crosslink due to TPP. In conclusion, this study indicated that the chitosan microspheres prepared by this method can be developed for preparing controlled drug release formulation.

Acknowledgments

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