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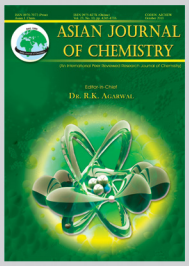

Preparation of Cross-linked Starch Microparticles by a Water-in-oil Emulsion Solvent Diffusion Method for Use as Drug Delivery Carriers

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Cross-linked starch microparticles containing model drug were prepared *via* a water-in-oil emulsion solvent diffusion method. Aqueous cross-linked starch solution and emulsifier mixture in ethyl acetate were used as water and oil phases, respectively. Sodium trimetaphosphate and methylene blue was used as cross-linker and water soluble model drug, respectively. The starch microparticles prepared with different starch/methylene blue and starch/sodium trimetaphosphate ratios resulted in nearly spherical in shape with fine dispersibility. From *in vitro* release test, the cross-linked starch microparticles showed sustainable release profiles. Cumulative drug released from starch microparticles decreased as decreasing of methylene blue ratio and increasing of sodium trimetaphosphate ratio.

Key Words: Starch, Microparticles, Cross-linking, SEM, Controlled release.

INTRODUCTION

Starch is an abundant, inexpensive, edible and biodegradable polysaccharide that has been widely investigated for biomedical[1,2] and pharmaceutical[3-5] applications. Starch-based microparticles have been prepared for use as drug delivery carriers by various methods such as spray drying[6], emulsification-cross-linking[7,8] and emulsification-solvent evaporation[9,10]. However, a suitable method for fabricating the starch-based microparticles remains to be identified. Poorly water-soluble starch microparticles can be prepared by cross-linking that are suitable for controlled release of entrapped bioactive molecules. Sodium trimetaphosphate has been reported to be an effective low toxicity cross-linker for starch[11,12].

Our previous works have shown that water-in-oil (W/O) emulsion solvent diffusion method was a simple and rapid method for preparing hydrophilic polymeric microparticles such as silk fibroin[13,14], chitosan[15,16] and starch[17]. The aim of this study is to develop cross-linked starch microparticles containing water soluble model drug by water-in-oil emulsion solvent diffusion method. Influences of starch/drug and starch/cross-linker ratios on morphology, size and drug loading of starch microparticles were studied. Drug releasing behaviour of drug-loaded starch microparticles was also investigated.

EXPERIMENTAL

Water soluble starch (AR) was purchased from BDH. mehylene blue (MB, Carlo Erba), ethyl acetate (AR grade, lab scan) and sodium trimetaphosphate (STMP, Sigma) were used without further purification. Span80 (Merck) and Tween80 (Acros organics) were used as received.

Preparation of starch microparticles: The 20 % (w/v) starch solution was prepared by using 2 % (w/v) NaOH solution as the solvent. Starch microparticles were prepared by the water-in-oil emulsion solvent diffusion method. For cross-linking, the starch solution was reacted with sodium trimetaphosphate for 15 min before dissolving mehylene blue and microparticle formation. The 1 mL of cross-linked starch and mehylene blue solution was added drop-wise into 100 mL of ethyl acetate containing 5 % (w/v) of Span80 and Tween80 mixture (Span80/Tween80 = 90.65/9.35, w/w)[7] with a stirring speed of 900 rpm for 1 h. The beaker was tightly sealed with aluminum foil to prevent evaporation of ethyl acetate during the emulsification-diffusion process. The starch microparticles suspended in ethyl acetate were collected by centrifugation before rinsing with fresh ethyl acetate for three times and drying in a vacuum oven at room temperature for overnight. Influences of starch/mehylene blue ratios (10/0.1, 10/0.3 and 10/0.5 w/w) and starch/sodium trimetaphosphate ratios (10/0.1, 10/0.5 and 10/1 w/w) on microparticle and drug release characteristics were determined.

Morphology and size of starch microparticles: The morphology of the starch microparticles was investigated by scanning electron microscopy (SEM) using a JEOL JSM-6460LV SEM. The microparticles were coated with gold for

enhancing conductivity before scanning. Average particle size and size distribution of microparticles were determined by light-scattering (LS) technique using a Coulter LS230 particle size analyzer at 25 ºC.

Drug loading of starch microparticles: The drug-loaded starch microparticles were dissolved in 2 % (w/v) NaOH solution before adjusting to pH 7 with HCl solution. The amount of mehylene blue in clear solution was calculated from absorbance at λ_{max} = 668 nm by a UV-VIS spectrophotometry compared to standard curve of mehylene blue. Theoretical drug loading content ($DLC_{theoretical}$), actual drug loading content (DLC_{actual}) and drug loading efficiency (DLE) were calculated from eqns. (1) - (3), respectively. The DLC_{actual} is an average value from three measurements.

$$DLC_{theoretical}\,(\%) = \frac{\text{Weight of feed MB}}{\text{Weights of feed MB and starch}} \times 100 \quad (1)$$

$$DLC_{actual}\,(\%) = \frac{\text{Weight of MB in microparticles}}{\text{Weight of drug - loaded microparticles}} \times 100 \quad (2)$$

$$DLE\,(\%) = \frac{DLC_{actual}}{DLC_{theoretical}} \times 100 \quad (3)$$

***In vitro* drug release test:** *In vitro* drug release tests were performed in phosphate buffer (1 mM; pH 7.4) at 37 ºC. The drug-loaded starch microparticles (20 mg) were dispersed in 1 mL of buffer solution. The suspension was gently shaken. At desired times, the drug solution was removed after centrifugation at 5,000 rpm for 5 min. The 1 mL of fresh buffer was returned to the container. Amount of mehylene blue was determined as described above. Cumulative drug release was calculated from the ratio of cumulative mass of mehylene blue released from microparticles at a given time and total loading amount of mehylene blue in microparticles. *In vitro* BSA release tests were tested in triplicate (n = 3).

RESULTS AND DISCUSSION

In this study, the starch microparticles were formed and solidified after diffusion out of water from dispersed water-in-oil emulsion droplets to continuous phase, ethyl acetate. For drug-loaded cross-linked starch microparticle preparation, the starch solution was first cross-linked with sodium trimetaphosphate before mehylene blue dissolving and microparticle formation. The sodium trimetaphosphate shows effective cross-linking the starch by reacting with hydroxyl groups of starch molecules.[7]

Morphology and size of microparticles: Morphology of microparticles was determined from the SEM images, as example of which is shown in Fig. 1. The microparticles had nearly spherical in shape with good dispersibility. This suggests that the mehylene blue loading and sodium trimetaphosphate cross-linking did not affect the microparticle shape.

Surface of microparticles was investigated from the expanded SEM images. Fig. 2 shows microparticle surfaces of drug-loaded microparticles with different mehylene blue ratios. It can be seen that the microparticles contained rough surfaces. The drug-loaded microparticles prepared with different sodium trimetaphosphate ratios showed also rough surfaces. The some surface collapse of microparticles may occur during the solvent diffusion out. Then, microparticle matrix was self-condensed in the solidification process. Microparticle matrix contained closed voids, as shown in Fig. 3. These voids may form from the solidification of microparticle matrix when the water was diffused.

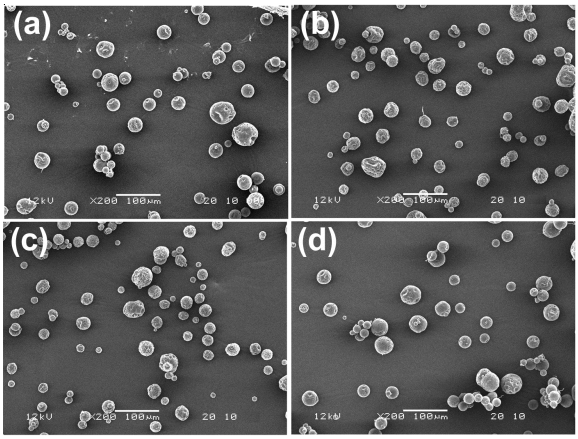

Fig. 1. SEM images of (a) drug-free cross-linked starch microparticles and drug-loaded cross-linked starch microparticles prepared with starch/MB ratios of (b) 10/0.1, (c) 10/0.3 and (d) 10/0.5 (w/w) for 10/1 (w/w) starch/STMP ratio. All bars = 100 μm.

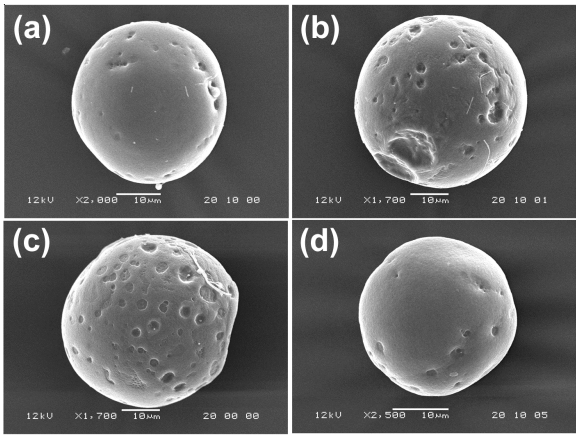

Fig. 2. Expanded SEM images of (a) drug-free cross-linked starch microparticles and drug-loaded cross-linked starch microparticles prepared with starch/MB ratios of (b) 10/0.1, (c) 10/0.3 and (d) 10/0.5 (w/w) for 10/1 (w/w) starch/STMP ratio. All bars = 10 μm.

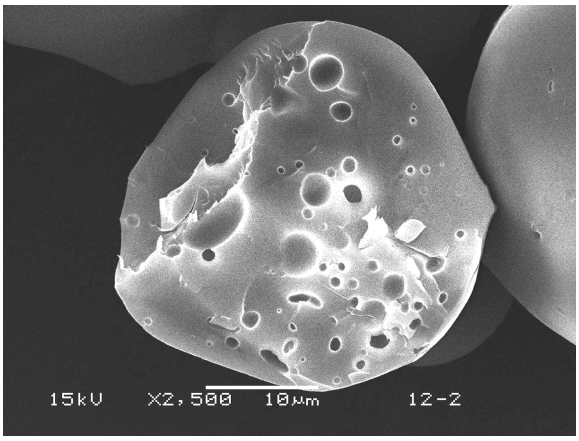

Fig. 3. SEM image of cross-section of drug-loaded cross-linked starch microparticles prepared with 10/0.5 w/w starch/MB and 10/1 (w/w) starch/STMP ratios. Bar = 10 μm.

TABLE-1
SIZE AND DRUG LOADING OF STARCH MICROPARTICLES

Starch microparticles		Average size (μm)	$DLC_{theoretical}$ [a] (%)	DLC_{actual} [b] (%)	DLE [c] (%)
Starch/STMP ratio (w/w)	Starch/MB ratio (w/w)				
10/1	10/0.1	32 ± 8	0.99	0.15	15
10/1	10/0.3	36 ± 5	2.91	0.58	20
10/1	10/0.5	32 ± 6	4.76	0.95	20
10/0.5	10/0.3	34 ± 8	2.91	0.47	16
10/0.1	10/0.3	38 ± 5	2.91	0.47	16

[a] calculated from equation (1); [b] calculated from equation (2); [c] calculated from equation (3)

Average size and distribution of starch microparticles determined from light-scattering analysis are summarized in Table-1. The results suggest that the average sizes did not significant change when the mehylene blue and sodium trimetaphosphate ratios were increased. The average sizes are in range of 32-38 μm.

Drug loading of microparticles: The influences of mehylene blue and sodium trimetaphosphate ratios on the loading mehylene blue of starch microparticles were determined. Table-1 reports drug loading content (DLC) and loading efficiency (DLE) of starch microparticles. It was found that the DLC_{actual} increased steadily from 0.15 to 0.95 % as the $DLC_{theoretical}$ increased from 0.99 to 4.76 %. However, the DLC_{actual} slightly decreased from 0.58 to 0.47 % when the sodium trimetaphosphate ratio was decreased from 1 to 0.5 and 0.1. The loading efficiency values are in range of 15-20 %.

***In vitro* drug release:** The influences of mehylene blue and sodium trimetaphosphate ratios on drug release behaviours are illustrated in Figs. 4 and 5, respectively. The drug release profile is plotted between cumulative mehylene blue release and release time. The higher drug loading content gave a faster drug release rate (Fig. 4). The higher level of loaded drug leads to a wider concentration gap between the starch microparticles and the released medium, phosphate buffer. This induces a higher drug diffusion or release rate. The total cumulative drug release at 24 h decreased significantly from 72 to 61 % when the starch/sodium trimetaphosphate ratio changed from 10/0.1 to 10/1 (w/w), as shown in Fig. 5. This indicated that the cross-linked starch matrices resulted from sodium trimetaphosphate-cross-linking can reduce drug release rate.

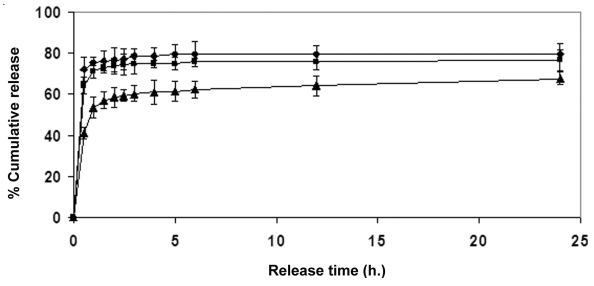

Fig. 4. Cumulative MB released from cross-linked starch microparticles prepared with starch/MB ratios of (▲) 10/0.1, (■) 10/0.3 and (◆) 10/0.5 (w/w) for 10/1 (w/w) starch/STMP ratio

Conclusion

The drug-loaded starch microparticles cross-linked with sodium trimetaphosphate were successfully prepared by the water-in-oil emulsion solvent diffusion method. All drug loaded starch microparticles had nearly spherical in shape. The drug release rate decreased with the mehylene blue ratio.

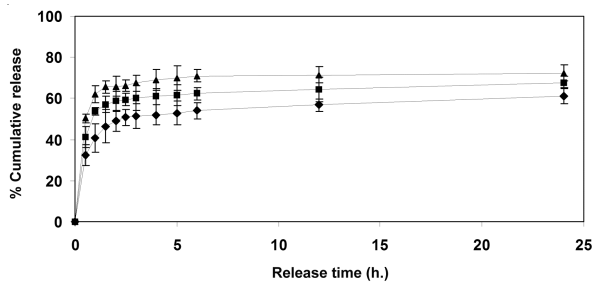

Fig. 5. Cumulative MB released from cross-linked starch microparticles prepared with starch/STMP ratios of (▲) 10/0.1, (■) 10/0.5 and (◆) 10/1 (w/w) for 10/0.3 (w/w) starch/MB ratio.

The starch microparticles with higher sodium trimetaphosphate ratio showed slower drug release than the lower sodium trimetaphosphate ratio. These data suggest that the water-in-oil emulsion solvent diffusion can be used to prepare cross-linked starch microparticles for use in controlled release drug delivery applications. The drug release rate can be controlled by adjusting the drug and cross-linker ratios.

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REFERENCES

1. M.E. Gomes, A.S. Ribeiro, P.B. Malafaya, R.L. Reis and A.M. Cunha, *Biomaterials*, **22**, 883 (2001).
2. J. Nakamatsu, F.G. Torres, O.P. Troncoso, Y. Miin-Lin and A.R. Boccaccini, *Biomacromolecules*, **7**, 3345 (2006).
3. K.P.R. Chowdary and G.V. Radha, *Asian J. Chem.*, **23**, 502 (2011).
4. A.K. Bajpai and S. Bhanu, *J. Mater. Sci. Mater. Med.*, **18**, 1613 (2007).
5. R.C. Mundargi, N.B. Shelke, A.P. Rokhade, S.A. Patil and T.M. Aminabhavi, *Carbohyd. Polym.*, **71**, 42, (2008).
6. K.G.H. Desai, *AAPS Pharm. Sci. Tech.*, **6**, E202 (2005).
7. Y.Y. Fang, L.J. Wang, D. Li, B.Z. Li, B. Bhandari, X.D. Chen and Z.H. Mao, *Carbohyd. Polym.*, **74**, 379 (2008).
8. B.Z. Li, L.J. Wang, D. Li, B. Bhandari, S.J. Li, Y. Lan, Y. Lan, X.D. Chen and Z.H. Mao, *J. Food Eng.*, **92**, 250 (2009).
9. K.P.R. Chowdary and M.S. Rao, *Asian J. Chem.*, **20**, 5383 (2008).
10. K.P.R. Chowdary, P. Seenivasan and C.U. Reddy, *Asian J. Chem.*, **22**, 1723 (2010).
11. K. Woo and P.A. Seib, *Carbohyd. Polym.*, **33**, 263 (1997).
12. T. Kasemsuwan, T. Bailey and J. Jane, *Carbohyd. Polym.*, **36**, 301 (1998).
13. Y. Baimark, P. Srihanam, Y. Srisuwan and P. Phinyocheep, *J. Appl. Polym. Sci.*, **118**, 1127 (2010).
14. T. Imsombut, Y. Srisuwan, P. Srihanam and Y. Baimark, *Powder Technol.*, **203**, 603 (2010).
15. N. Kotsaeng, J. Karnchanajindanun and Y. Baimark, *Particul. Sci. Technol.*, **28**, 369 (2010).
16. J. Karnchanajindanun, M. Srisa-ard, P. Srihanam and Y. Baimark, *Nat. Sci.*, **2**, 1061 (2010).
17. Y. Baimark, M. Srisa-ard and P. Srihanam, *Express Polym. Lett.*, **4**, 781 (2010).