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In Vitro Study of Alginate/Poly-*L*-Arginine Microcapsules as a Protein or Anticancer Drug Carrier

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Abstract — **It is known that drug carriers are widely used for controlling the administration of drugs *in vivo*. Since Lim and Sun invented the first APA microencapsule in 1980s, different microencapsulating crafts have been developed for the application of drug delivery. However, the search of new materials for the preparation of biocompatible, stable, biodegradable microcapsules with high pharmacological efficacy and well release behavior is still ongoing. Poly amino acids have attracted considerable attentions in recent years due to their non-toxicity, biocompatibility, and nutritional function. Poly-*L*-arginine, whose degradation product can inhibit tumor growth, enhance drug delivery across biological membranes and possess antimicrobial activity, has come into our sight.**

In this present work, we attempted to prepare a biodegradable and intervening therapeutic drug carrier and focused on its *in vitro* slow-release capacity of protein and anticancer drug.

Keywords — **poly-*L*-arginine, microcapsules, protein, anti-cancer drug, slow-release**

I. Indroduction

Due to their biocompatibility and biodegradability, Poly amino acids have been the ideal candidates for applications in drug delivery systems [1]. In this study, poly-*L*-arginine microcapsules were studied as drug carriers for its nutritional function and therapeutic efficacy [2-4]. Cytarabine (Ara-C) and Fluorouracil (5-Fu) were selected as the model drugs in this study because they are widely used in the clinical anticancer treatment while Hemoglobin (Hb) has already been screened out as the modal protein drug in the previous research [5-12]. We managed to manufacture Alginate/Poly-*L*-Arginine Microcapsules and proved them as a potential protein or anticancer drug carrier.

II. Materials

Hemoglobin (Hb)	BR	Cytarabine (Ara-C)	AR
Fluorouracil (5-Fu)	BR	Sodium alginate	AR
Calcium chloride	AR	poly-*L*-arginine(PLA)	AR

III. Methods

Microcapsules Formation

Sodium alginate was dissolved in distilled water and filtered through 0.22 μm membrane filters. Alginate solution under certain concentration was then pumped through a syringe into an aqueous solution of calcium chloride. The calcium alginate beads were formed, collected, washed with distilled water and then transferred to aqueous solution of the bovine hemoglobin, cytarabine, fluorouracil for drug loading. The microcapsules were finally obtained by suspending the beads in an aqueous solution of poly-*L*-arginine to form the polymeric membrane.

In Vitro Release Studies

Experiments were performed in a dissolution apparatus at 37°C. The drug loaded alginate/poly-*L*-arginine microcapsules were suspended in 200mL of a phosphate buffered saline solution (PBS, pH 6.8) under a stirring speed of 50rpm. The amount of drug releasing from the microcapsules was indirectly measured under the wavelength of 405nm (Hb), 272nm (Ara-C) and 265nm (5-Fu) by UV-visible spectrophotometer.

IV. Results and discussion

Optimized Condition of Beads Formation

The alginate beads were prepared using a high voltage electrostatic droplet generator. The optimized experimental conditions for the preparation of beads with a narrow size distribution were as follows: electric voltage 6.3 kV; pump rate 50 mm/h; distance between needle tip and gelling solution 20 mm; $7^{\#}$ flat needle.

Calcified alginate Beads with different sizes were obtained by changing the concentration of alginate or calcium chloride. When the concentration of alginate was too low (<1.8%), only fragments were formed; a higher concentration (>2.6%) resulted in nothing more than oval particles. The ultimate spherical and homogeneous beads

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were obtained when the concentration of alginate was 1.8% and with the corresponding 4.5% (w/v) concentration of calcium chloride.

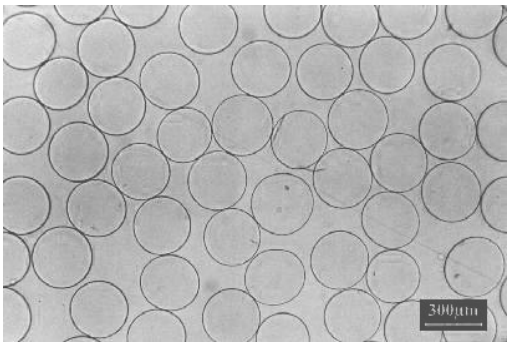

Fig.1 Morphology of Ca-Alginate beads (×40)

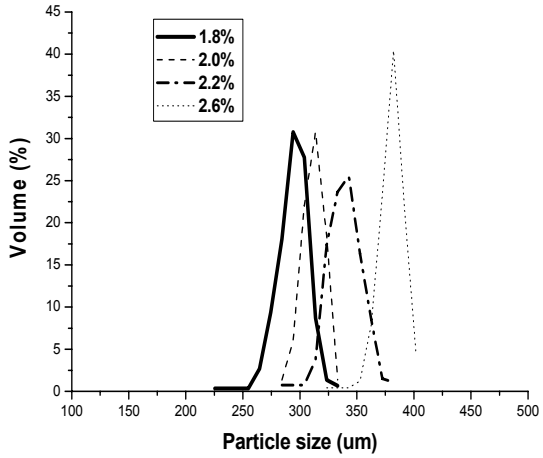

Fig.2 Particle size distribution with different concentrations of Na-Alginate

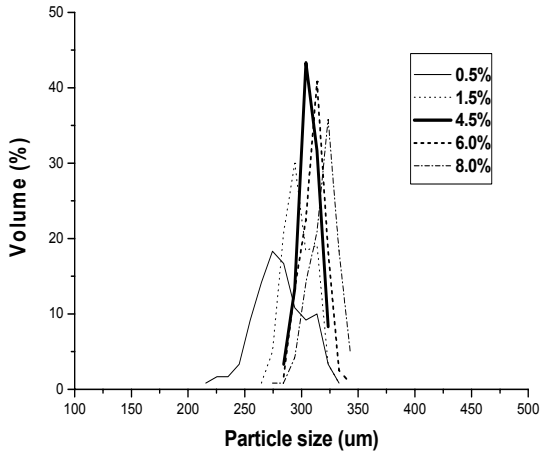

Fig.3 Particle size distribution with different concentrations of $CaCl_2$

Encapsulation Efficiency of microcapsules

Hb was loaded into the microcapsules by the suspension of beads in Hb solution under a shaking speed of 200rpm for 16h and successful encapsulation at 10℃ in a shaking container for 10 minutes to accomplish the electrostatic interaction sufficiently. Effects of molecular weights and concentrations of poly-*L*-arginine on encapsulation efficiency were considered dissectively, so did the effect of membrane-forming time. Results showed encapsulation efficiency were all beyond the specified value given in the ‘‘Pharmacopoeia of People’s Republic of China’’ (2005).

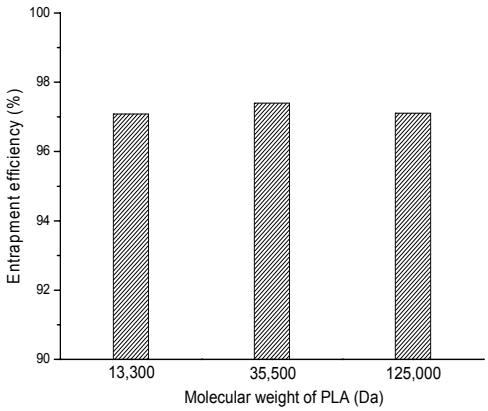

Fig.4 Entrapment efficiency of microcapsules with different molecular weights of PLA

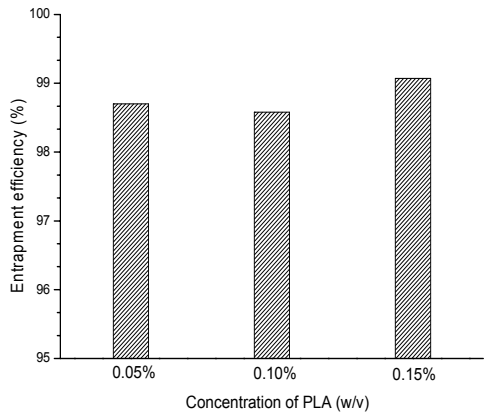

Fig.5 Entrapment efficiency of microcapsules with different concentrations of PLA

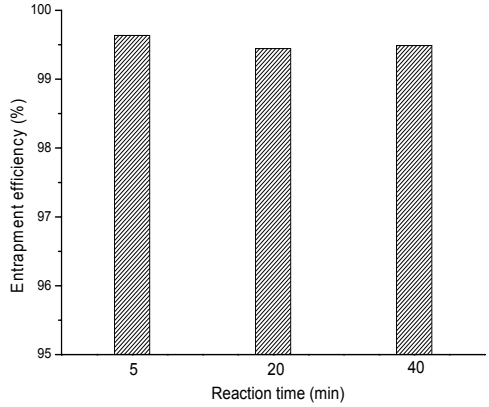

Fig.6 Entrapment efficiency of microcapsules with different reaction time of PLA

In Vitro Release Studies

In vitro release behavior of Hb from the microcapsules was studied. The release profile of microcapsules based on different molecular weights and concentrations of poly-*L*-arginine shared the same trend, Hb was slowly released from the microcapsules and the final cumulative release reached 98% and 95% respectively. However, the profile also showed microcapsules prepared under 5 minutes membrane-forming time got a preferable slow-release performance.

In vitro release behaviors of Ara-C and 5-Fu from the microcapsules were not so ideal. Cumulative release of Ara-C and 5-Fu reached 63% and 68% at the end of 0.5h, both were far higher than the specified value given in the ‘‘Pharmacopoeia of People’s Republic of China’’ (2005) of microcapsules and indicated an obvious burst release.

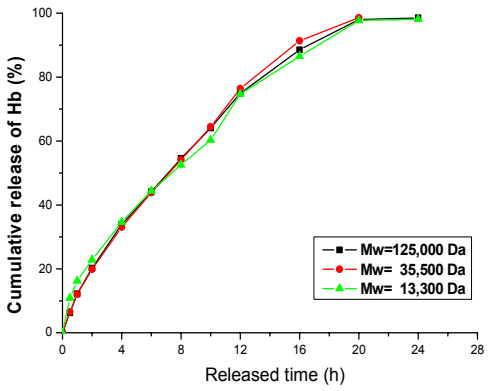

Fig.7 The cumulative released of Hb from microcapsules with different molecular weights of PLA

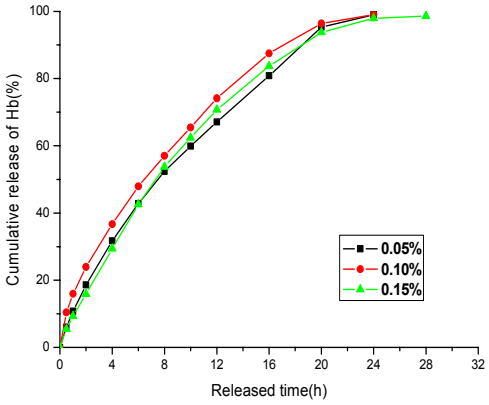

Fig.8 The cumulative released of Hb from microcapsules with different concentrations of PLA

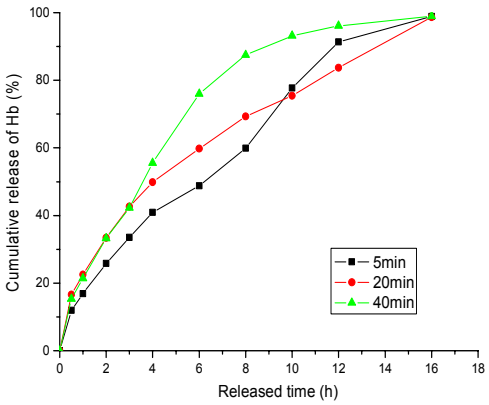

Fig.9 The cumulative released of Hb from microcapsules with different reaction time of PLA

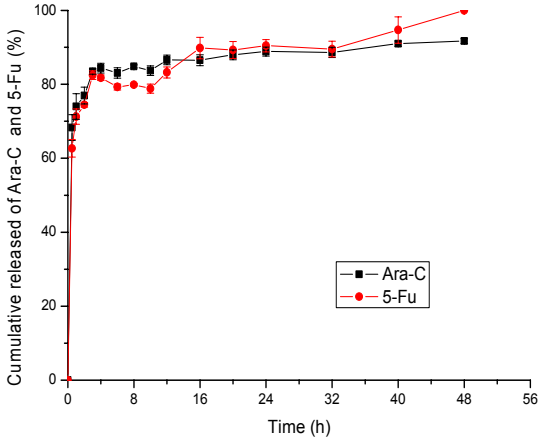

Fig.10 The cumulative released of Ara-C and 5-Fu from microcapsules

V. Conclusions

We reached a conclusion that the protein Hb was encapsulated with a high efficiency (>90%), *in vitro* release profiles showed that Hb was slowly and completely released from the microcapsules at pH 6.8 within 16 to 28h. Due to the molecular weights and chemical structures, the anticancer Ara-C and 5-Fu contained in alginate/poly-*L*-arginine microcapsules bursted out at first and turned to slow diffuse after.

These results implied that the alginate/poly-*L*-arginine microcapsules may be used for some further applications in the encapsulation of protein-based drugs since their stable and complete release *in vitro* studies. We also believe through better crafts, they can be a potential carrier of anticancer drugs as well.

Acknowledgements

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