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Microspheres of corn protein, zein, for an ivermectin drug delivery system

Xinming Liu[a], Qingshen Sun[b], Huajie Wang[b], Lei Zhang[a], Jin-Ye Wang[a,b,*]

[a] *Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China*
[b] *Biomedical Engineering, Shanghai Jiao Tong University, 1954 Huashan Road, 200030, China*

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Abstract

A novel microsphere drug delivery system of ivermectin (IVM) using hydrophobic protein zein was prepared by the phase separation method and characterized by a scanning electron microscope and laser light scattering particle size analyzer. Releases of model drug IVM from zein microspheres, tabletted microspheres and pepsin degradation of tabletted microspheres were also performed in vitro to investigate the mechanism of model drug release. The results show that the zein microspheres and tabletted microspheres are suitable for use as a sustained-release form of IVM. The microspheres may also be useful in drug targeting system since the diameter of the microspheres is appropriate for phagocytosis by macrophages. Moreover, the release of IVM from enzymatic degraded tabletted microspheres shows a zero-order release, implying a potential application in tissue engineering for preparing scaffold, which is composed of microspheres encapsulating bioactive components for stimulating cell differentiation and proliferation.

Keywords: Biodegradation; Controlled drug release; Drug delivery; Electron microscopy; Microspheres

1. Introduction

In recent years, microspheres have been proposed to treating many diseases needing a constant drug concentration in blood or drug targeting to specific cells or tissues [1,2]. Various synthetic or natural biodegradable polymers have been developed as materials of microspheres for drug delivery system [3]. Synthetic polymers such as poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) are widely studied because of their superior biodegradability and regulatory physiochemical properties [4]. However, PLGA undergoes bulk erosion that produces a very low pH environment within PLGA matrices, which may adversely affect sensitive therapeutic agents such as proteins [5,6]. As to natural polymers, natural proteins such as gelatin, albumin, casein and soluble monomolecular collagen represent good raw materials since they have the advantages of synthetic polymers own and the advantages of absorbability and low toxicity of the degradation end products. In spite of these possible advantages, they present a main drawback of a rapid solubilization in aqueous environments, thus resulting in fast drug release profiles. In order to overcome this problem, chemical cross-linking procedures (e.g. glutaraldehyde and formaldehyde treatment) have been considered [7–10]. Unfortunately, the presence of residual cross-linking agents could lead to toxic side effects, in addition, unwanted reactions between the drug and cross-linker could result in the formation of toxic or inactivated derivatives [11,12]. Furthermore, as a hydrophilic polymeric system, these protein microspheres have difficulties to achieve sustained drug release. When the system absorbs water and swells, drugs will rapidly diffuse out [7–9]. In contrast, the hydrophobic polymeric system has the capability of yielding sustained drug release [4,5].

As to drug targeting, many works focus on the macrophage drug delivery, which means using the

Abbreviations: IVM, ivermectin; SEM, scanning electron microscope; SR, sustained-release; PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic acid)

*Corresponding author. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China. Tel.: +86-21-64163300x1225; fax: +86-21-64376697.
E-mail address: jywang@mail.sioc.ac.cn (J.-Y. Wang).

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macrophage as a vehicle for the drug carrying device [13]. Uptake of microspheres by the macrophage cells heavily depends on the nature of the microspheres such as size, hydrophobicity, and charge. The size plays a great role in controlling drug delivery to the target tissues and the subsequent uptake of drugs into tissues; microspheres with an average diameter of 1 μm are most effectively taken up by macrophages. In addition, a more hydrophobic microsphere surface is desirable for better uptake by macrophages [14–17].

Microspheres with an average diameter of 1 μm have been developed for the i.v. or oral administration of drugs [18]. For per oral delivery, the microspheres could be administered in the form of an aqueous dispersion. However, poor stability of the drug or polymer in an aqueous environment or poor taste of the drug may require the incorporation of the microspheres into solid dosage forms, e.g. tablet. Tablet still accounts for more than 80% of all dosage forms administered to man. The principal reasons for its continued popularity include its ease of manufacture, its convenience of dosing, its stability and the release mechanism compared with liquid and semi-solid presentations [19].

In order to overcome the disadvantages of drug delivery system using hydrophilic protein and offer a drug targeting system, we chose a hydrophobic protein zein to prepare microsphere drug delivery system with an average diameter of 1 μm which can be most effectively taken up by macrophages, and then we used the microspheres to prepare tablet which can obtain zero-order release, an often desired property of a controlled release device. Ivermectin (IVM) was chosen as a model drug.

Zein is a major storage protein of corn. As indicated by SDS-PAGE, zein with biochemical purity used in the present study is mainly composed of two distinct bands, with molecular weight of 23 and 21 kDa. The minor bands are 13 and 9.6 kDa. The high proportion of non-polar amino acid residues is responsible for its solubility behavior. The molecular structure is helical wheel conformation that nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds [20]. Zein can form tough, glossy, hydrophobic coatings and has anti-bacterial activity, which has been used in food industry [21]. Furthermore, zein can also prevent avermectin from photo-degradation [22]. Zein has also been used to form microspheres by cross linking a zein solution containing the drug, but it is quite heterogeneous in size [23,24]. In our group, zein has been used to prepare films composed of microspheres with good biocompatibility [25].

IVM, a semi-synthetic derivative of avermectin B1 produced by the soil-dwelling actinomycete *Streptomyces avermitilis*, is a highly effective parasiticide that belongs to the macrocyclic lactone class of compounds. It has a broad spectrum of activity against endoparasites and ectoparasites of sheep, cattle, pigs and dogs [26–28]. Its low toxicity, high efficiency and safety also make it for treating onchocerciasis of human [29]. Many liquid oral, topical and injectable formulations of IVM are currently available for use in cattle. But these formulations have the disadvantages of injury to user or multiple injection or significant systemic side effects. Pour-on formulations of IVM are particularly convenient for single-dose applications in cattle. Unfortunately, assuming total bioavailability of subcutaneous formulations, the highest relative bioavailability for a pour-on formulation of IVM does not exceed 15% [30]. IVM sustained-release (IVM SR) bolus has been shown to have excellent anti-parasitic activity with a steady-state delivery rate over 6 months, and offer an excellent alternative to multiple injections [31,32]. But the IVM SR bolus was regarded as potentially more ecotoxic than the more rapidly excreted oral formulations for horses and sheep [33]. Furthermore, IVM SR bolus was prepared from polyvinyl chloride that cannot be degraded. So modification of the dosage form is expected to increase the therapeutic effect of IVM.

2. Materials and methods

2.1. Microsphere preparation

IVM-loaded microspheres were prepared using a phase separation procedure. Typically, 600 mg zein (Wako Pure Chemical Industries LTD., Japan) and 60 mg IVM (Tongren Drug Company, Shanghai, China) were dissolved in 12 ml of 66.7% ethanol. Then, 8 ml of ultrapure Milli-Q water was immediately added with vigorous mixing using an agitator (IKAMAG RCT basic, IKA, Germany) set at 9 at room temperature. The resulted microspheres were lyophilized overnight before use.

2.2. Tabletted microsphere preparation

Tabletted microspheres, in diameters of 10 mm, were prepared by compressing 220 mg of microspheres containing IVM with a mold, and then the tabletted microspheres were placed into wet box at 37°C for 3 days to acquire certain toughness.

2.3. Morphology analysis

The morphologies of the microspheres and tabletted microspheres were observed using a scanning electron microscope (SEM, S-450, Hitachi, Japan). The zein microspheres and tabletted microspheres were vacuum-dried at room temperature, mounted onto brass stubs and sputter-coated with gold in an argon atmosphere prior to examination under SEM.

2.4. Particle size distribution

The mean diameter and particle size distribution of the microspheres were measured using a laser light scattering particle size analyzer (Zetasizer 3000HS, MALVERN, UK) after the microspheres were well dispersed in ultrapure Milli-Q water containing 40% ethanol to dissolve unencapsulated IVM. Average particle size was expressed as intensity mean diameter.

2.5. Ivermectin loading and encapsulation efficiency

The amount of IVM entrapped within the microspheres was determined using the following method. Lyophilized microspheres (10 mg) were washed with 1 ml × 3 ethyl acetate and vacuum-dried. The microspheres and the substances extracted by ethyl acetate were dissolved in 66.7% ethanol separately and analyzed using an ultraviolet–visible spectrophotometer (UNICAM UV500, UK) at 245 nm. IVM encapsulation efficiency and IVM loading were determined by Eqs. (1) and (2), respectively:

$$\text{encapsulation efficiency (w/w\%)} = \frac{\text{amount of IVM in microspheres}}{\text{IVM initially added}}, \quad (1)$$

$$\text{IVM loading (w/w\%)} = \frac{\text{amount of IVM in microspheres}}{\text{amount of microspheres}}. \quad (2)$$

2.6. In vitro release of ivermectin from zein microspheres

IVM microspheres (110 mg) were washed with ethyl acetate to remove unencapsulated IVM, vacuum-dried, and then suspended in 100 ml PBS (pH 7.4) containing Tween 20 (0.5% w/v) to increase the IVM solubility, incubated at 37°C. Medium from each sample was periodically removed and replaced with fresh PBS (pH 7.4) containing Tween 20. The IVM content in the medium was analyzed using an ultraviolet–visible spectrophotometer at 245 nm. The percentage of IVM cumulative release (% w/w) was investigated as a function of incubation time. Each experiment was performed in six replicates.

2.7. In vitro release of ivermectin from tabletted microspheres and from pepsin degraded tabletted microspheres

Tabletted microspheres (220 mg) were placed in 200 ml PBS buffer (pH 7.4) containing Tween 20 (0.5% w/v) or 200 ml citric acid-NaH_2PO_4 buffer (pH 2.2) containing pepsin (zein:pepsin = 10:1 in w/w) and Tween 20 (0.5% w/v), then incubated at 37°C. Medium from each sample was periodically removed and replaced with fresh PBS buffer (pH 7.4) containing Tween 20 or citric acid-NaH_2PO_4 buffer (pH 2.2) containing pepsin and Tween 20. The IVM content in the release medium and the degradation rate of the tabletted microspheres were analyzed using an ultraviolet–visible spectrophotometer at 245 and 280 nm, respectively. The percentage of IVM cumulative release (% w/w) and zein degradation rate were investigated as a function of incubation time. Each experiment was performed in six replicates.

2.8. Statistical analysis

All the data were analyzed by one-way factorial ANOVA and multiple comparisons. Significant effects of treatment were defined using Scheffe's method as post hoc test. The results were expressed as mean ± standard error of the mean.

3. Results and discussion

3.1. Microsphere characterization

The scanning electron micrographs of the microspheres before and after lyophilization prepared by the phase separation method are shown in Fig. 1. The microspheres after lyophilization did not aggregate, and could be easily dispersed in distilled water. The surface of both microspheres observed by SEM was spherical and smooth. Particle size analysis showed that the microspheres were rather homogeneous in size, which distributed between 0.3 and 1.2 μm. It is well known that microspheres with an average diameter of 1 μm are most effectively taken up by macrophages, and the size plays a great role in controlling drug delivery to the target tissues [17]. In addition, zein is a hydrophobic protein and the microspheres prepared from it have a more hydrophobic surface which is desirable for better uptake by macrophages [14–17]. So the microspheres prepared in our study are appropriate for the phagocytosis by macrophages and delivering the drug to the target tissues.

3.2. Tabletted microsphere characterization

The outward and the morphology of the internal structure of tabletted microspheres are shown in Fig. 2. The direct compressing method is applicable for unstable drugs such as peptides because of no heating, and the release rate can be controlled easily by appropriate selection of polymer species and formulations [34]. However, the tabletting process represents the potential risk of damaging microspheres by the mechanical load during compaction [35]. In our method, the microspheres after direct compressing and shaping for 3

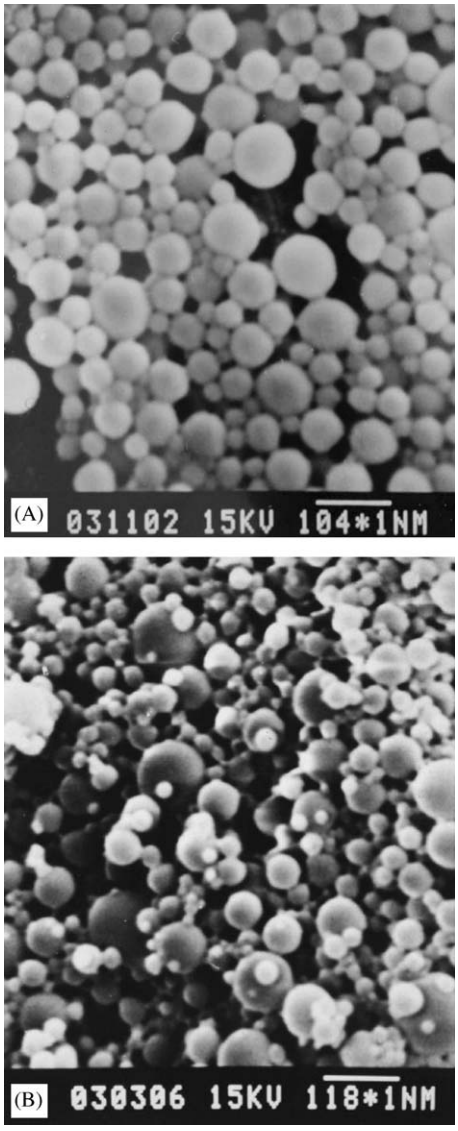

Fig. 1. Scanning electron micrographs of IVM-loaded zein microspheres prepared by the phase separation method. (A) Before lyophilization, the scale bar is 1.04 μm; (B) after lyophilization, the scale bar is 1.18 μm.

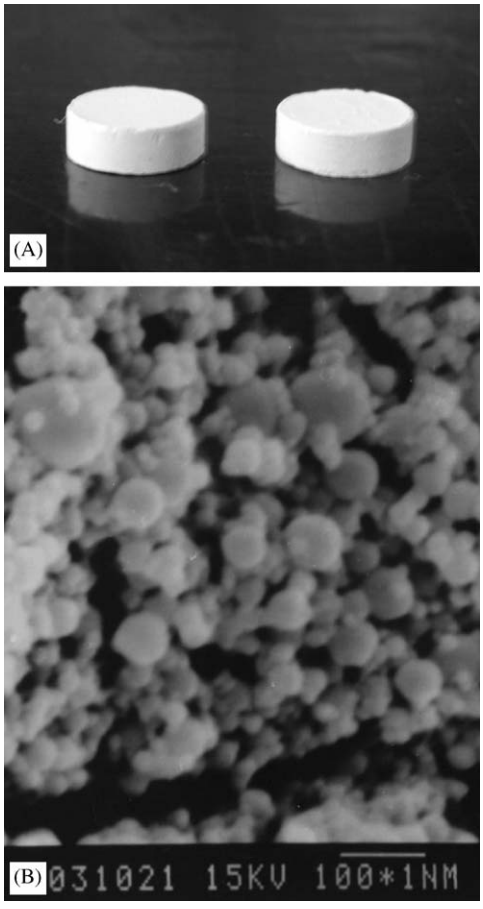

Fig. 2. Outward of the tabletted microspheres (A) and scanning electron micrograph of the internal structure (B) after compressing and shaping in wet box at 37°C for 3 days, the scale bar is 1 μm.

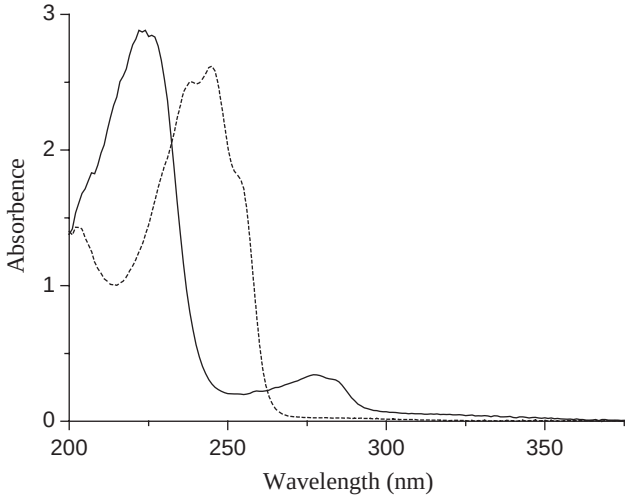

Fig. 3. Ultraviolet–visible spectra of IVM (- - -) and zein (—) in 66.7% ethanol. The coefficients of light extinction of IVM and zein at 245 nm are 351.5 and 7.1, respectively.

days are still spherical and smooth without any damage. In our previous study, the film composed of microspheres is suitable for culturing cells in vitro [25], so the microspheres preloaded with various bioactive components can be included into scaffold in any desired shapes without damage to microspheres. The scaffold can sustainedly release bioactive components for stimulating cell differentiation and proliferation in tissue engineering.

3.3. Ivermectin loading and encapsulation efficiency

The ultraviolet–visible spectra of IVM and zein are shown in Fig. 3. IVM and zein in 66.7% ethanol were quantified at OD_{245} and OD_{280} (nm), respectively. The standard curves of IVM and zein obtained by plotting the concentration vs. absorbance were highly reproducible. It was also confirmed that the presence of zein did

Table 1
IVM encapsulation efficiency and IVM loading in zein microspheres ($n = 3$)

Preparation concentrations of IVM and zein	IVM encapsulation efficiency (w/w%)[a]	IVM loading (w/w%)[b]
IVM 7.5 mg/ml, zein 30 mg/ml	57.01±0.85	17.16±0.50
IVM 5 mg/ml, zein 20 mg/ml	50.86±1.28	14.86±0.23
IVM 2.5 mg/ml, zein 10 mg/ml	47.02±0.43	13.62±0.33
IVM 1.25 mg/ml, zein 5 mg/ml	38.50±0.31	11.01±0.23
IVM 10 mg/ml, zein 20 mg/ml	24.73±0.29	15.56±0.18
IVM 7.5 mg/ml, zein 20 mg/ml	39.86±0.05	16.90±0.56
IVM 2.5 mg/ml, zein 20 mg/ml	63.02±0.84	9.07±0.24
IVM 1 mg/ml, zein 20 mg/ml	68.51±0.48	4.05±0.07

[a] Encapsulation efficiency (w/w%) = amount of IVM in microspheres/IVM initially added.
[b] IVM loading (w/w%) = amount of IVM in microspheres/amount of microspheres.

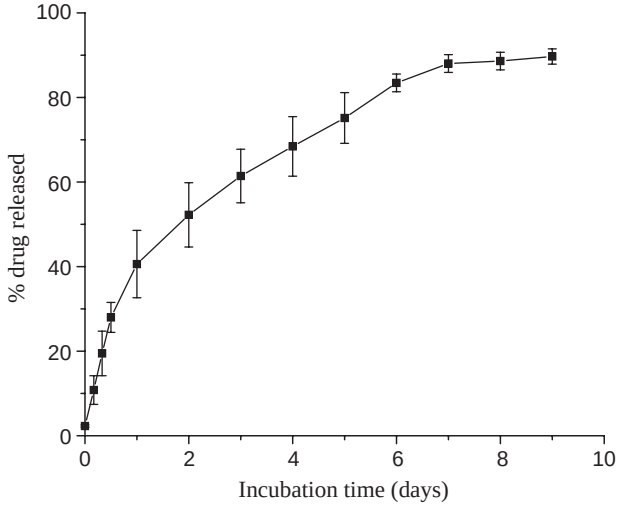

Fig. 4. In vitro release profile of IVM as a function of time from zein microspheres in PBS, pH 7.4, 37°C. Each point represents the mean±SD ($n = 6$).

not interfere with the spectrophotometric determination of IVM at 245 nm because the coefficient of light extinction of IVM at 245 nm is 351.5, while that of zein at 245 nm is just 7.1. As can be seen in Table 1, IVM loading and encapsulation efficiency depended on the concentration and the ratio of IVM to zein. Both IVM loading and encapsulation efficiency increased with the increase of IVM and zein concentrations at a given ratio of them; the IVM encapsulation efficiency increased while IVM loading decreased with the decrease of the ratio of IVM to zein. Satisfactory result was obtained when the concentration of IVM and zein was 7.5 and 30 mg/ml, respectively, in which the IVM encapsulation efficiency was nearly 60% and IVM loading was nearly 20%.

3.4. In vitro release of ivermectin from zein microspheres

In an attempt to use IVM microspheres as potential therapy against endoparasites and ectoparasites, we have examined the in vitro release profile of IVM encapsulated in microsphere formulation, which is shown in Fig. 4. The releasing curve indicated a sustained-release pattern during 9-day test and it appeared biphasic. At the initial stage, the burst effect is usually attributed to the drug entrapped near the surface of the microspheres, and this was followed by a very slow release stage. After 1 day, about 40% of the entrapped IVM was released, but the release rate tended to tail off in the subsequent 'slower' phase. Indeed, about 90% of the entrapped IVM was released after 9 days. The slowing of the release rate probably represents the long diffusion route of IVM that was entrapped deeper in the zein matrix. Parameters corresponding to the drug release kinetics from microspheres are the rate of water uptake, drug dissolution/diffusion rate and the size of the microspheres including matrix erosion/degradation rate [36–39]. The hydrophobic property of zein causes the delay of water penetration, thus the diffusion of the drug into the release medium may be retarded; IVM is a poorly water-soluble drug, only insufficient release rate may be obtained. On the other hand, the IVM microspheres with an average diameter of 1 μm are so small which maybe lead to a fast release because of the increased ratio of surface area to volume as the microspheres decrease in size. Combining all factors, the size may play a great role in the IVM release, as the total surface area of small IVM microspheres is much larger, the fraction of IVM near the surface is higher, resulting in an initial burst release.

3.5. In vitro release of ivermectin from tabletted microspheres and from pepsin degraded tabletted microspheres

Fig. 5A shows the release of IVM from non-degrading tabletted microspheres and degrading tabletted microspheres. Significant difference was observed in release behavior of IVM between tabletted microspheres with and without pepsin. The tabletted microspheres without pepsin yielded an IVM release platform during the first 4 days. This may be due to the hydrophobic nature of zein and IVM, and the internal structure with a thick and dense wall. Normally, it needs longer time for water to penetrate through more hydrophobic zein matrix and across the thick wall to let IVM diffuse out. For the enzymatic degraded tabletted microspheres, the release of IVM was almost linear with the time (zero-order release), and continued for 11 days. It is generally recognized that the drug release from matrix tablet depends on diffusion which is influenced by degradation

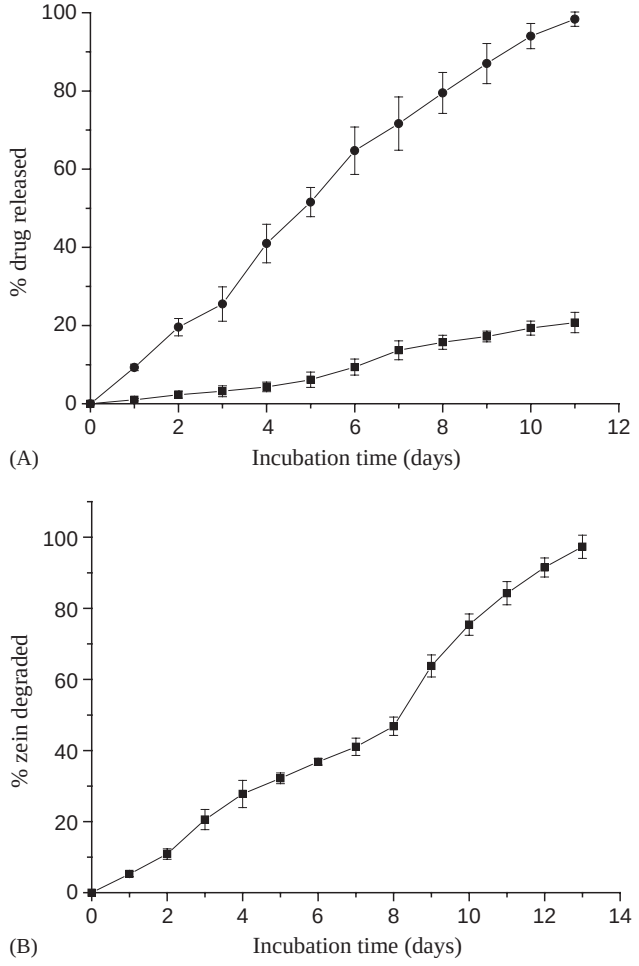

Fig. 5. (A) In vitro release profiles of IVM as a function of time from tabletted microspheres (■, in PBS, pH 7.4, 37°C) and from pepsin degraded tabletted microspheres (●, in citric acid-NaH_2PO_4 buffer, pH = 2.2, 37°C). Each point represents the mean ± SD ($n = 6$). (B) In vitro degradation of tabletted microspheres with pepsin in citric acid-NaH_2PO_4 buffer, pH = 2.2, 37°C. Each point represents the mean ± SD ($n = 6$).

of the matrix [40]. The release of IVM from the tabletted microspheres without pepsin maybe depend on the diffusion of IVM through the matrix, and have an IVM release platform during the first 4 days. The release of IVM from enzymatic degraded tabletted microspheres was controlled by both the diffusion and the matrix degradation. It needs a longer time for IVM diffusing out, which results in a zero-order release, a desired property of controlled release device. It can maintain a constant drug concentration in plasma and a constant pharmacological effect in vivo. Fig. 5B shows pepsin degraded rate of tabletted microspheres. The best correlation between drug release rate and degradation rate of the tabletted microspheres was found. This implies that the release mechanism is mainly controlled by the degradation of tabletted microspheres [38].

After 8 days incubation, about 50% of the tabletted microspheres were degraded, which reached to 100% after 13 days. So it is possible that the microspheres and tabletted microspheres can endure the gastric degradation after oral administration. This drug delivery system may also be used to administer drugs topically in most excessive environment, such as ruminal bolus or subcutaneous implant. The drug release from it depends on degradation and diffusion, which can achieve zero-order release. The advantage of using this biodegradable matrix over that of IVM SR is that it eliminates the need for surgery to remove the device after drug releasing [31,32].

4. Conclusion

Zein microspheres and tabletted microspheres described here are suitable for use as a sustained-release form of IVM. The microspheres could also be useful in drug targeting system, since the diameter of the microspheres is appropriate for phagocytosis by macrophages. Moreover, the release of IVM from enzymatic degraded tabletted microspheres is zero-order release; this formulation can be used to administer drugs orally as ruminal bolus or as a subcutaneous implant with which to maintain a constant plasma drug concentration in vivo. Furthermore, this system could also be useful for preparing scaffold composed of the microspheres, which can sustainedly release bioactive components for stimulating cell differentiation and proliferation in tissue engineering.

Acknowledgements

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References

[1] Arshady R. Albumin microspheres and microcapsules: methodology of manufacturing techniques. J Control Release 1990;14:111–31.
[2] Lee KC, Lee YJ, Kim WB, Cha CY. Monoclonal antibody-based targeting of methotrexate-loaded microspheres. Int J Pharm 1990;59:27–33.
[3] Peppas N, Langer RS. Biopolymers I, Advances in Polymer Science, vol. 107. Berlin: Springer; 1993.
[4] Wu XS. Preparation, characterization, and drug delivery applications of microspheres based on biodegradable lactic: glycolic acid polymers. In: Wise DL, Trantolo DJ, Altobelli DE, Yaszemski MJ, Gresser JD, Schwartz ER, editors. Encyclopedic handbook of biomaterials and bioengineering. Part A: materials, vol. II. New York: Marcel Dekker; 1995. p. 1151–2.

[5] Park TG, Lu WQ, Crotts G. Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly(D,L-lactic acid-*co*-glycolic acid) microspheres. J Control Release 1995;33:211–22.
[6] Fu K, Pack DW, Laverdier A, Son R, Langer R. Visualization of pH in degrading polymer microspheres. Proc Int Symp Control Release Bioact Mater 1998;25:150–1.
[7] Vandelli M A, Rivasi F, Guerra P, Forni F, Arletti R. Gelatin microspheres crosslinked with D,L-glyceraldehyde as a potential drug delivery system: preparation, characterization, in vitro and in vivo studies. Int J Pharm 2001;215(1–2):175–84.
[8] Sahin S, Selek H, Ponchel G, Ercan MT, Sargon MA, Hincal A, Süheyla Kas H. Preparation, characterization and in vivo distribution of terbutaline sulfate loaded albumin microspheres. J Control Release 2002;82:345–58.
[9] Latha MS, Rathinam K, Mohanan PV, Jayakrishnan . Bioavailability of theophylline from glutaraldehyde cross-linked casein microspheres in rabbits following oral administration. J Control Release 1995;34:1–7.
[10] Digenis GA, Thomas BG, Shah VP. Cross-linking of gelatin capsules and its relevance to their in vitro–in vivo performance. J Pharm Sci 1994;83:915–21.
[11] Speer DP, Chvapil M, Eskelson CD, Ulrich J. Biological effects of residual glutaraldehyde in glutaraldehyde—tanned collagen biomaterials. J Biomed Mater Res 1980;14:753–64.
[12] Ben-Slimane S, Guidoin R, Marceau D, Merhi Y, King MW, Sigot-Luizard MF. Characteristics of polyester arterial grafts coated with albumin: the role and importance of the cross-linking chemicals. J Eur Surg Res 1988;20:18–28.
[13] D'Souza MJ, DeSouza P. Site specific microencapsulated drug targeting strategies-liver and gastro-intestinal tract targeting. Adv Drug Deliver Rev 1995;17:247–54.
[14] Norris DA, Sinko PJ. Effect of size, surface charge, and hydrophobicity on the translocation of polystyrene microspheres through gastrointestinal mucin. J Appl Polym Sci 1997;63: 1481–92.
[15] Kidane A, Guimond P, Ju TR, Sanchez M, Gibson J, North A, HogenEsch H, Bowersock TL. Effects of cellulose derivatives and poly(ethylene oxide)–poly(propylene oxide) tri-block copolymers (Pluronic surfactants) on the properties of alginate based microspheres and their interactions with phagocytic cells. J Control Release 2002;85:181–9.
[16] Ashen F, Rivas IP, Khan MA, Suarez AT. Targeting to macrophages: role of physicochemical properties of particulate carriers—liposome's and microspheres—on the phagocytes by macrophages. J Control Release 2002;79:29–40.
[17] Makino K, Yamamoto N, Higuchi K, Harada N, Ohshima H, Terada H. Phagocyte uptake of polystyrene microspheres by alveolar macrophages: effects of the size and surface properties of the microspheres. Colloid Surface B 2003;27:33–9.
[18] Kreutzer J. Nanoparticles. In: Kreuter J, editor. Colloidal drug delivery systems. New York: Marcel Dekker; 1994. p. 219–342.
[19] Jivraj M, Martini LG, Thomson CM. An overview of the different excipients useful for the direct compression of tablets. Pharm Sci Technol Today 2000;3:58–63.
[20] Argos P, Pedersen K, Marks MD, Larkins BA. A structural model for maize zein proteins. J Biol Chem 1982;257(17):9984–90.
[21] Shukla R, Munir C. Zein: the industrial protein from corn. Ind Crop Prod 2001;13:171–92.
[22] Demchak RJ, Dybas RA. Photostability of abamectin/zein microspheres. J Agric Food Chem 1997;45:260–2.
[23] Suzuki T, Sato E, Matsuda Y, Tada H, Unno K, Kato T. Preparation of zein microspheres conjugated with antitumor drugs available for selective cancer chemotherapy and development of a simple colorimetric determination of drugs in microspheres. Chem Pharm Bull 1989;37(4):1051–4.
[24] Mathiowitz E, Bernstein H, Morrel E, Schwaller K. Method for producing protein microspheres. WO Patent No. 91/06286, 1991.
[25] Dong J, Sun Q, Wang JY. Basic study of corn protein, zein, as a biomaterial in tissue engineering, surface morphology and biocompatibility. Biomaterials, in press.
[26] Campbell WC, Fisher MH, Albers-Schönberg G, Jacob TA. IVM: a potent new antiparasitic agent. Science 1983;221(4613): 823–8.
[27] Shröder J, Swan GE, Soll MD, Hotson IK. Efficacy of IVM against ectoparasites of cattle in South Africa. J S Afr Vet Med Assoc 1985;56:31–5.
[28] Campbell WC, editor. IVM and abamectin. New York, USA: Springer; 1989. p. 1–23, 363.
[29] Basáñez M-G, Ricárdez-Esquinca J. Models for the population biology and control of human onchocerciasis. Trends Parasitol 2001;17:430–8.
[30] Alvinerie M, Dupuy J, Eeckhoutte C, Sutra JF. Enhanced absorption of pour-on ivermectin formulation in rats by co-administration of the multidrug-resistant-reversing agent verapamil. Parasitol Res 1999;85:920–2.
[31] Hsu TTP, Michaels AS. Controlled release drug delivery device comprising a polymer matrix and a plasticizer. EP Patent No. 0537998 A1, 1992.
[32] Zimmerman GL, Mulrooney DM, Wallace DH. Efficacy of IVM administered via sustained-release bolus against gastrointestinal nematodes in cattle. Am J Vet Res 1991;52:62–3.
[33] Herd RP, Stinner BR, Purrington FF. Dung dispersal and grazing area following treatment of horses with a single dose of IVM. Vet Parasitol 1993;48:229–40.
[34] Aso Y, Yoshida S, Po ALW, Terao T. Effect of temperature on mechanisms of drug release and matrix degradation of poly(DL-lactide) microspheres. J Control Release 1994;31:33–9.
[35] Kühl P, Mielck JB. Tabletted of pellet–matrix systems: ability of parameters from dynamic and kinetic models to elucidate the densification of matrix formers and of pellets. Int J Pharm 2002;248:101–14.
[36] Oneda F, Re MI. The effect of formulation variables on the dissolution and physical properties of spray-dried microspheres containing organic salts. Powder Technol 2003;130: 377–84.
[37] Franssen O, Stenekes RJH, Hennink WE. Controlled release of a model protein from enzymatically degrading dextran microspheres. J Control Release 1999;59:219–28.
[38] Brazel CS, Peppas NA. Modelling of drug release from swellable polymers. Eur J Pharm Biopharm 2000;49:47–58.
[39] Berkland C, Kim KK, Pack DW. Fabrication of PLG microspheres with precisely controlled and monodisperse size distributions. J Control Release 2001;73:59–74.
[40] Zuleger S, Lippold BC. Polymer particle erosion controlling drug release. I. Factors influencing drug release and characterization of the release mechanism. Int J Pharm 2001;217:139–52.