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International Journal of Pharmaceutics 144 (1996) 241–245

international journal of pharmaceutics

Preparation and in-vitro evaluation of nifedipine loaded albumin microspheres cross-linked by different glutaraldehyde concentrations

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Received 1 February 1996; revised 23 August 1996; accepted 9 September 1996

Abstract

Nifedipine-loaded albumin microspheres were prepared by a chemical cross-linking method to develop a sustained release form. The effects of cross-linking agent (glutaraldehyde) on the percentage of drug loading, biodegradability of albumin microspheres and drug release kinetics were investigated in this study. Moreover, the kinetics of nifedipine released from different albumin microspheres were analysed using four different theoretical models, that is, zero order, first order, planar matrix and spherical matrix model. Albumin microspheres prepared with different amounts of glutaraldehyde indicated different release kinetics. Increasing the glutaraldehyde concentration decreased the release rate of nifedipine from albumin microspheres as a result of formation of greater structural strength and more tightly texture. Besides, albumin microspheres gave an adequate fit to either zero order or spherical matrix model, depending on the extent of cross-linking reaction. Copyright © 1996 Elsevier Science B.V.

Keywords: Nifedipine; Microspheres; Bovine serum albumin; Glutaraldehyde; Release models

Nifedipine, one of the most potent calcium antagonists in clinical use for its antihypertensive effects (Henry, 1980; Braunwald, 1982; Scheinman and Hess, 1983), has also shown an improvement on the survival of rat ischaemic skin flap (Pal et al., 1991). Several studies reported that the absorption of nifedipine is inferior when administered orally in solid dosage form because of its poor water solubility (Sugimoto et al., 1982; Kohri et al., 1987). Moreover, because of short elimination half-life of nifedipine, the plasma nifedipine levels fluctuate markedly following ad-

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PII S0378-5173(96)04742-4

Table 1
The effect of the amount of glutaraldehyde on the drug loading content percentage and incorporation efficiency of the albumin microspheres

Albumin concentration (mg/ml)	Amount of glutaraldehyde (ml)	Drug content percentage (mg/100ml albumin)	Incorporation efficiency (% recovery ± S.D.)
250	0.4	3.907 ± 0.107	48.060 ± 1.317
250	0.45	3.556 ± 0.114	34.561 ± 1.115
250	0.5	2.669 ± 0.056	30.219 ± 0.637

ministration of the conventional capsule form of nifedipine (Foster et al., 1983) and its antihypertensive effect lasts only for a few hours (Echizen and Eichelbaum, 1986). It is, therefore, important to develop a sustained release form of nifedipine to improve the therapeutic efficacy.

In the present study, the preparation of nifedipine-loaded albumin microspheres was investigated. Many process variables can influence the characteristics of the resultant albumin microspheres, we studied the effects of four variables (drug/albumin ratio, amount of glutaraldehyde, amount of surfactant, stirring speed) on the percentage of drug incorporated in the albumin microspheres by the method of experimental design and carried out statistical comparison using analysis of variance (ANOVA) (Abdullah and Al-Khamis, 1993; Chuo et al., 1996). It got definite results that increasing the drug/albumin ratio and stirring speed accompany with the decreasing extent of glutaraldehyde and surfactant may obtained albumin microspheres with higher percentage of drug loading. In this study, drug-loaded albumin microspheres cross-linked by different amount of glutaraldehyde were investigated, as far as the pharmaceutical aspects are concerned.

Nifedipine-loaded microspheres were prepared by a method in our previous study (Chuo et al., 1996). Three formulations listed in Table 1 were prepared using different amounts of glutaraldehyde (0.4, 0.45 and 0.5 ml). The ratio of the concentration of glutaraldehyde to the amount of albumin was 0.1, 0.09 and 0.08%, respectively. All experiments were carried out under subdued light conditions to prevent photodegradation of nifedipine. The resultant microspheres were stored in a desiccator at room temperature prior to further investigation.

The loading percentage and the incorporation efficiency of the three albumin microspheres cross-linked with different glutaraldehyde amounts are listed in Table 1. The resultant range in loading percentage and incorporation efficiency of nifedipine ranged from 2.67 to 3.91% (W/W) and 30.22 to 48.06%, respectively. The loading percentage and incorporation efficiency of albumin microspheres cross-linked with 0.5 ml glutaraldehyde was higher than the percentages cross-linked with 0.45 and 0.4 ml glutaraldehyde. This result is identical to our preliminary finding (Chuo et al., 1996)—increasing the extent of glutaraldehyde leads to an increase in the drug loading percentage.

Albumin microspheres prepared using the higher concentration of glutaraldehyde have a slower digestion with trypsin than those made using the lower concentration of glutaraldehyde. But when the experiment was completed after 72 h, all microspheres prepared under the three different amount of glutaraldehyde showed no precipitation after centrifugation. That is, the nifedipine-loaded albumin microspheres prepared under the three different conditions were completely digested with trypsin and exhibited biodegradable characteristics.

To observe the shape of nifedipine-loaded albumin microspheres prepared using 0.4, 0.45 and 0.5 ml glutaraldehyde, both optical and scanning electron microscopy were carried out. The resultant microspheres showed spherical shapes with optical microscopy, but with the scanning electron microscopy, albumin microspheres prepared using 0.4 and 0.45 ml glutaraldehyde were not quite

spherical, especially the microspheres cross-linked by 0.4 ml glutaraldehyde. The phenomena were also noted by Benita et al. (1990) and was attributed to the existence of internal void volume of the albumin microspheres. The nifedipine microspheres which comprise internal void volume may be deflated by the vacuum during the gold coating process of SEM evaluation. Furthermore, albumin microspheres prepared using higher amounts of glutaraldehyde exhibit greater structural strength and durability (Sheu et al., 1986) and may maintain the shape integrity of the albumin microspheres.

In vitro release studies were carried out using a modification of the method of Vural et al. (1990). Drug-loaded albumin microspheres (50 mg) were taken in 10 ml, pH 7.4, phosphate buffer containing 1% Tween 80 and 0.002% trypsin in a screw tube, shaken at 150 rpm in a horizontal laboratory shaker (shaker, Shin-Kwang, Taiwan). At various time intervals, the samples were centrifuged and 1.0 ml of the supernatant were taken and replaced by an equal volume of dissolution medium. All the samples collected were assayed by HPLC (HPLC, Type *p*-1500, UV-2000, Spectra-Physics, CA, USA).

The dissolution profiles of nifedipine from albumin microspheres cross-linked with different amount of glutaraldehyde for various times are shown in Fig. 1. The amount of glutaraldehyde markedly affected the drug release properties of the albumin microspheres. It is notable that the release rate of nifedipine from albumin microspheres were inversely related to the amount of glutaraldehyde. The release rate of nifedipine from albumin microspheres cross-linked by 0.5 ml glutaraldehyde was discovered to be slower and considerably linear when compared to the albumin microspheres prepared with 0.4 and 0.45 ml glutaraldehyde. Furthermore, the dissolution pat-

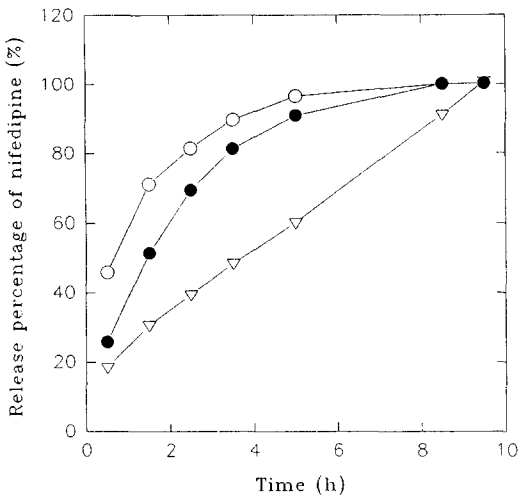

Fig. 1. Effect of glutaraldehyde amount on the drug release profile from the albumin microspheres: ○, 0.4 ml; ●, 0.45 ml and ▽, 0.5ml

Table 2
In vitro release kinetics of nifedipine from albumin microspheres cross-linked with different amount of glutaraldehyde

Model employed	Function	Amount of glutaraldehyde (ml)	Correlation coefficients
Zero-order	$Q_0 - Q = f(t)$	0.5	0.9985
		0.45	0.7988
		0.4	0.7174
First-order	$\ln(Q_0 - Q) = f(t)$	0.5	0.6319
		0.45	0.6573
		0.4	0.9249
Spherical matrix	$3/2[1-(1-Q)^{2/3}]-Q = f(t)$	0.5	0.9047
		0.45	0.9986
		0.4	0.9627
Planar matrix	$Q_0 - Q = f(t^{1/2})$	0.5	0.9761
		0.45	0.9299
		0.4	0.8744

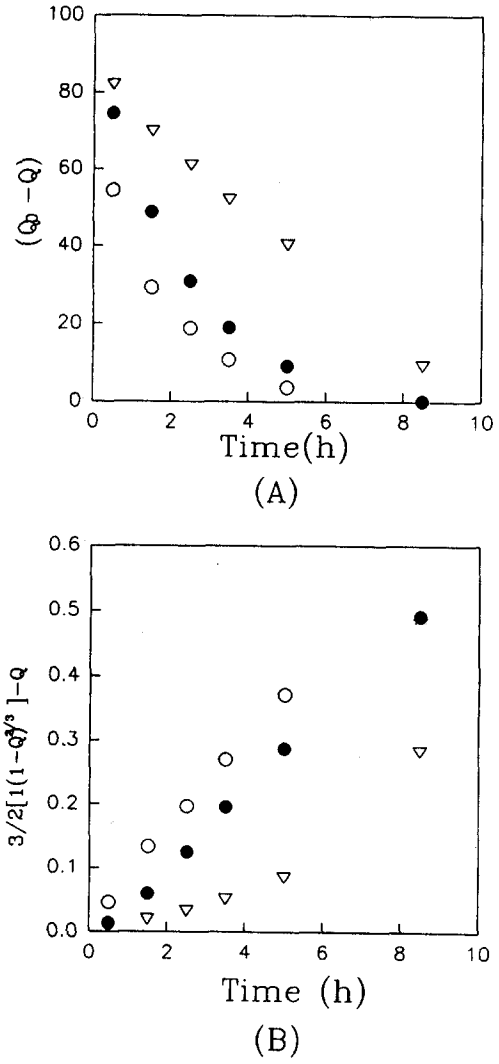

Fig. 2. Plots of nifedipine release from albumin microspheres: ○, 0.4 ml; ●, 0.45 ml; ▽, 0.5 ml. (A) $(Q_0 - Q)$ vs t (B) $3/2[1-(1-Q)^{2/3}]-Q$ vs t.

tern of nifedipine from the albumin microspheres prepared with 0.4 and 0.45 ml glutaraldehyde was similar—they exhibited an initial burst release followed by a constant release of nifedipine from the albumin microspheres over the 10 h period.

The release kinetics of nifedipine from different albumin microspheres were analysed using four different models and the linear correlation coefficients of the slopes is shown in Table 2. It can be observed from the data that the zero order model provided a good fit to the release profile of albumin microspheres cross-linked with 0.5 ml glutaraldehyde, and those microspheres which were prepared with 0.4 and 0.45 ml glutaraldehyde showed an adequate fit to the spherical matrix model. It had been reported that the albumin microspheres prepared using greater amount of glutaraldehyde may result more extent cross-linking reaction which made the resultant albumin microspheres exhibited a tighter and firmer structure (Sheu et al., 1986). Therefore, drug release from the albumin microspheres was mainly controlled by the permeability of the albumin microspheres and exhibited a zero-order character (Leucuta, 1990). However, albumin microspheres cross-linked with less amounts of glutaraldehyde resulted in a looser texture, which may swell faster in buffer causing, first, a burst release, and second, a constant release of drug diffusion from the microspheres (Chandy and Sharma, 1992).

Comparing with the plotting method in Fig. 2, the linearity was obtained for albumin microspheres prepared with 0.5 ml glutaraldehyde when $(Q_0 - Q)$ was plotted as a function of t (Fig. 2A), and for albumin microspheres prepared with 0.4 and 0.45 ml glutaraldehyde when $3/2[1-(1-Q)^{2/3}]-Q$ was plotted as a function of t (Fig. 2B).

In conclusion, nifedipine-loaded albumin microspheres prepared using cross-linking methods are suitable for a controlled release system. The in-vitro release kinetics of nifedipine from albumin microspheres exhibited either zero-order or spherical matrix models, depending on the amount of glutaraldehyde added for the cross-linking reaction. Thus, by varying the extent of the cross-linking reaction, it is feasible to obtain albumin microspheres with different in-vitro release kinetics depending on the different requirements.

Acknowledgements

We are grateful to the Royal Chem. and Pharm. Co., Ltd for a gift of nifedipine and to Sheng Chung Tang Pharma. Ind. Co. for financial aid in part. We would like to thank the Department of Electrical Engineering, National Sun Yat-Sen University, for the loan of the micronizer.

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