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Short communication

Development of nifedipine-loaded albumin microspheres using a statistical factorial design

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Abstract

Nifedipine-loaded albumin microspheres were prepared by a chemical cross-linking method to develop a sustained-release form. The qualities of the nifedipine-loaded albumin microsphere products were affected by several variables. In this study, the effects on the percentage of nifedipine incorporated into albumin microspheres of four different variables (i.e. drug/albumin ratio, amount of glutaraldehyde, amount of sodium dodecyl sulfate and stirring speed) at two levels were studied according to a factorial design of experiments.

Keywords: Nifedipine; Microspheres; Bovine serum albumin; Factorial design; Sustained release

Recently, albumin microspheres have received wide attention (Vural et al., 1990; Karunaker and Singh, 1994) because of their specificity (Kramer, 1974), biodegradability (Lee et al., 1981) and other desirable characteristics such as non-toxicity and biocompatibility (Ratcliffe et al., 1984) with an ideal drug carrier. Most studies involving the preparation of nifedipine sustained release systems were by employing other microencapsulation materials, such as chitosan (Chandy and Sharma, 1992), hydroxypropyl-β-cyclodextrin and hydroxypropyl-cellulose (Wang et al., 1993), but none of the previous studies has employed bovine serum albumin (BSA) in the preparation of the sustained release form of nifedipine. Therefore, the aim of the present work was to investigate the effects of four important variables (drug/albumin ratio, amount of glutaraldehyde, amount of surfactant, stirring speed) on the percentage of nifedipine incorporated into the albumin microspheres. A 2^4 full factorial design was employed and the data compared statistically using analysis of variance (ANOVA) (Abdullah and Al-Khamis, 1993).

Nifedipine-loaded microspheres were prepared by a modification of the methods described by Longo et al. (1982) and Goosen et al. (1983). Micronized nifedipine powders were suspended in 3.0 ml 0.1 M sodium phosphate buffer, pH 7.4, containing the desired amount of sodium dodecyl

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sulfate. BSA (500 mg) was then dissolved in the suspension. After rapid mixing, the suspension was added dropwise to a mixture of 75 ml of a stirred (either 600 or 1200 rev./min) olive oil and petroleum ether solution (3:2 by vol.) (stirrer, 3000H, Heidon, Tokyo, Japan). After addition of the aqueous glutaraldehyde (5%, w/w) which was used for cross-linking, the dispersion was continuously stirred at 25°C for 1 h. The required amount of 2-aminoethanol then was added to cap any free aldehyde groups. After a 1 h reaction time, the residual solvent was decanted and the albumin microspheres were washed with petroleum ether (three times) followed by ethanol (three times). The washed microspheres were dried by freeze-drying. All experiments were carried out under subdued light conditions to prevent photodegradation of nifedipine.

The factorial design experiments were performed in random order and the response was measured as a percentage of the drug in the albumin microspheres. The design matrix and data obtained from a duplicate of the 2^4 experiments are given in Table 1. The effects of the individual variables (variables *A*, *B*, *C* and *D*) and their interactions between the four variables in the factorial design of experiments can be calculated using a so-called table of contrast coefficients (Montgomery, 1991). From these contrasts, we may estimate the 15 factorial effects as well as the normal probability plot of these effects as shown in Fig. 1. The important effects which influence the percentage of nifedipine in albumin microspheres seem to be the main effects of *A*, *B*, *C* and the *AC*, *AD*, *BC*, *BD*, *CD* and *ABC* interactions which are far from the line. The statistical differences were assessed using the ANOVA test to prove these results (Daniel, 1987) (see Table 2).

By the manner described by Montgomery (1991), the response value of the effects could be calculated. The values showed that two effects (*A* and *B*) are positive, and one effect (*C*) is negative. However, main effects alone do not have much meaning when they are involved in significant interactions. Those interactions are the key to getting the optimal conditions. The results obtained from the interactions are shown in Fig. 2 and can be described as follows.

(1) From the *AC* interaction, the data showed that the drug/albumin ratio (variable *A*) has little effect when the amount of surfactant (variable *C*) at the high level is in contrast to the low level. Increasing variable *A* at a low level of variable *C* tended to increase the drug loading percentage, but the influence was minor compared with that at a high level of variable *C*. Moreover, the variable *C* effect is very small for the variable *A* at the low level but will be very large when it is at a high level.

(2) By the manner described previously, it is concluded from Fig. 2 (b–e) that a lower amount of both glutaraldehyde (variable *B*) and surfactant (variable *C*) indicates a better response. Decreasing the amount of glutaraldehyde at a low level of surfactant leads to an increase in the drug loading percentage; besides, the variable *D* that exhibited no significant effect individually shows the best result at the high level accompanied by the high level of the drug/albumin ratio (variable *A*), the low level of glutaraldehyde (variable *B*) and surfactant (variable *C*).

The correlation between the effective experimental variables (*A*, *B*, *C*, *AC*, *BC*, *AD*, *BD*, *CD* and *ABC*) and the dependent variable (the percentage of nifedipine in albumin microspheres) could be calculated by multiple linear regression (Montgomery, 1991). The method of least squares (Montgomery, 1991) is used to estimate the regression coefficients and the polynomial equation obtained is as follows:

$$\begin{aligned} y = {} & 3.2011 + 0.2138x_1 - 0.1335x_2 - 0.2640x_3 \\ & - 0.2305x_1x_3 + 0.1663x_1x_4 + 0.2578x_2x_3 \\ & - 0.2250x_2x_4 - 0.3925x_3x_4 + 0.1693x_1x_2x_3 \end{aligned} \quad (1)$$

Where the coded variables x_1, x_2 and x_3 represent variables *A*, *B* and *C*, respectively.

The results comparing the experimentally obtained and model-predicted values of response

Table 1
A design matrix and the results of the 2^4 factorial design number

Random order	Independent variables				Independent variables				Order	Drug content[a] (%)
	A	*B*	*C*	*D*	Amount of nifedipine (mg)	Amount of glutaraldehyde (ml)	Amount of surfactant (mg)	Stirring speed (rev./min)		
1	+	−	+	+	60	0.6	60	1200	4	2.9613 ± 0.0199
2	−	+	+	+	30	1.0	60	1200	5	2.2861 ± 0.0054
3	−	−	+	−	30	0.6	60	600	14	2.9358 ± 0.0071
4	+	−	−	+	60	0.6	45	1200	9	5.5743 ± 0.1889
5	−	−	+	+	30	0.6	60	1200	11	2.8942 ± 0.0006
6	−	−	−	−	30	0.6	45	600	16	2.5462 ± 0.0164
7	+	+	−	+	60	1.0	45	1200	3	3.7530 ± 0.0386
8	−	+	+	−	30	1.0	60	600	8	3.7016 ± 0.2719
9	+	+	+	−	60	1.0	60	600	2	3.2601 ± 0.0009
10	+	−	+	−	60	0.6	60	600	7	2.4603 ± 0.0047
11	−	+	−	−	30	1.0	45	600	13	2.4717 ± 0.0019
12	−	+	−	+	30	1.0	45	1200	10	3.2953 ± 0.0246
13	+	−	−	−	60	0.6	45	600	12	3.5359 ± 0.0499
14	−	−	−	+	30	0.6	45	1200	15	3.7713 ± 0.1206
15	+	+	+	+	60	1.0	60	1200	1	2.9958 ± 0.0287
16	+	+	−	−	60	1.0	45	600	6	2.7752 ± 0.0026

[a]The percentage of nifedipine in the albumin beads (w/w) for each combination.

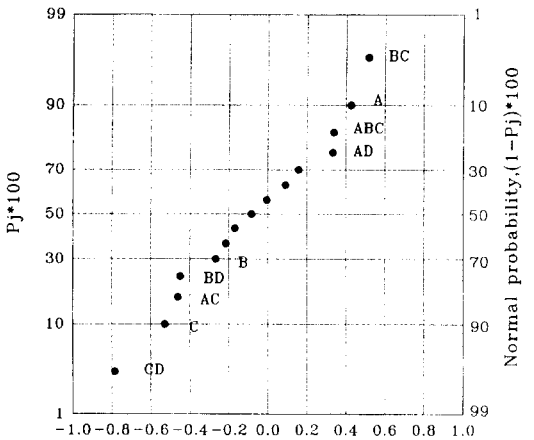

Fig. 1. Plot of effects on normal probability paper of nifedipine-loaded albumin microspheres produced by the chemical cross-linking method.

are calculated and the predicted values demonstrate a good agreement with the experimental data ($r = 0.993$), lending support to our results that the *A*, *B*, *C*, *AC*, *BC*, *AD*, *BD*, *CD* and *ABC* are the only significant effects in this study.

In conclusion, the preparation of nifedipine-loaded albumin microspheres using a chemical cross-linking method is feasible. Moreover, we may prepare albumin microspheres with maximum loading percentage of nifedipine by adjusting the effective experimental variables obtained from experimental design. In this study, we may conclude that a higher extent of drug/albumin ratio and stirring speed accompanied by a lower amount of glutaraldehyde and surfactant is the optimal condition for the preparation of nifedipine-loaded albumin microspheres.

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Table 2
Summary of the analysis of variance for the 2^4 factorial design

Effect	Sum of squares	df	Mean square	F_o
A	1.4566	1	1.4566	13.379 [a]
B	0.5727	1	0.5727	5.2602 [a]
C	2.2340	1	2.2340	20.519 [a]
D	0.3740	1	0.3740	3.4353
AB	0.2294	1	0.2294	2.1070
AC	1.7057	1	1.7057	15.666 [a]
AD	0.8852	1	0.8852	8.1313 [a]
BC	2.1265	1	2.1265	19.532 [a]
BD	1.6212	1	1.6212	14.890 [a]
CD	4.9379	1	4.9379	45.354 [a]
ABC	0.9166	1	0.9166	8.4193 [a]
ABD	0.0003	1	0.0003	0.0029
ACD	0.0659	1	0.0659	0.6054
BCD	0.0573	1	0.0573	0.5264
ABCD	0.2009	1	0.2009	1.8457
Error	1.7419	16	0.1088	
Total	19.126	31		

[a]Significant at the 1% level ($P < 0.01$).

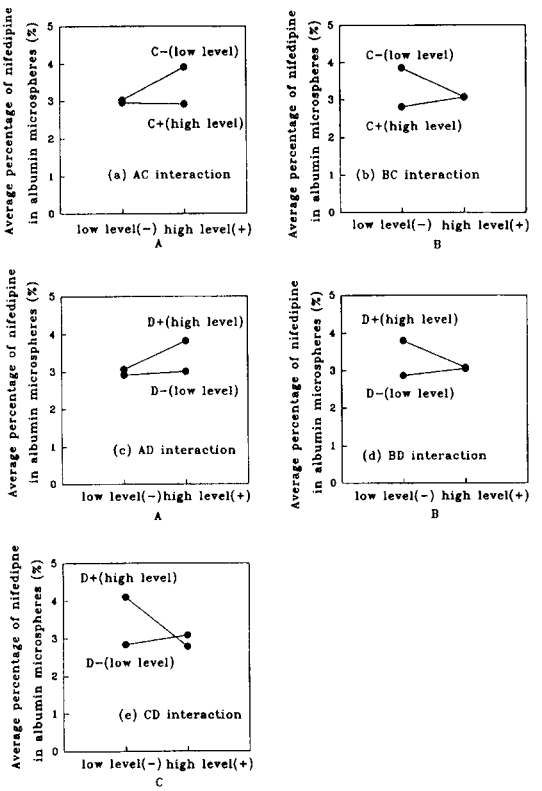

Fig. 2. Plots of *AC*, *BC*, *AD*, *BD* and *CD* interactions. (a) *AC* interaction plot. (b) *BC* interaction plot. (c) *AD* interaction plot. (d) *BD* interaction plot. (e) *CD* interaction plot. *A*: drug/albumin ratio; *B*: amount of glutaraldehyde; *C*: amount of surfactant; *D*: stirring speed.

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