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CONTROLLED-RELEASE FRUSEMIDE MICROCAPSULES: PREFORMULATION STUDIES

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ABSTRACT

Microencapsulation of plain frusemide or its solid-dispersion with PEG 6000 was achieved by phase-separation coacervation. Formulations showed reasonable in-vitro dissolution behaviour were assessed for their absorption rates by LD_{50} testing in mice. Toxicity studies showed close agreement between the increase in lethal dose and the decrease in dissolution rate and revealed that the formulation containing frusemide as fused mixture with PEG 6000 and microencapsulated with polystyrene, in frusemide-PEG 6000-polystyrene weight ratio of 2:2:1, was the formula of choice for prolonging the absorption, hence, the action of frusemide.

INTRODUCTION

Frusemide is an extremely potent high-ceiling diuretic. It has prompt onset of action and effects a

[1] Correspondence

peak diuresis far greater than that observed with other agents (1). The major side effects are related to the electrolyte imbalance induced by diuresis (2). By far the most frequently encountered problem is excessive depletion of blood volume, which can lead to profound shock, frequently complicated by hypokalemia, and ending in death (3).

Beermann (4) found that the conventional frusemide tablets induced a brief, intense diuresis and excretion of Na^+, K^+, and Cl^-, while there was no such peak after the sustained-release preparation.

The objective of this study was to microencapsulate frusemide using polystyrene as the wall-forming polymer, in an attempt to prepare a prolonged-release formulation which would offer the advantage of avoidance the short period of peak diuresis (5).

MATERIALS

All drug, chemicals and solvents were analytical grade.

PROCEDURES

Preparation of Frusemide-PEG 6000 Solid-Dispersions

The solid-dispersions were prepared adopting the fusion method.

Microencapsulation

Phase-separation coacervation was achieved by adding petroleum ether (the non-solvent) to a suspension of frusemide or frusemide-PEG 6000 solid-dispersion in a solution of polystyrene in cyclohexane.

Frusemide Determination

A standard calibration curve for frusemide in phosphate buffer, pH 7.4 (6), was constructed at the

wavelength of maximum absorption (277 nm) using Beckman spectrophotometer (Type DU_7, U.S.A.). Polystyrene and PEG 6000 in the concentration present in the assay sample were found not to interfer with the spectrophotometric determination of frusemide at 277 nm.

Dissolution Studies

The dissolution studies were carried out employing Erweka Automatic Dissolution Tester (Type DT, West Germany), using 400 ml of deaerated phosphate buffer (pH 7.4), equilibrated at $37\pm0.5^{\circ}$, as dissolution medium for an amount of the microcapsules containing 100 mg of the drug placed in the apparatus basket.

LD_{50} Studies

Formulations showed reasonable dissolution behaviour, viz. microcapsules containing frusemide as fused mixture with PEG 6000 in frusemide-PEG 6000-polystyrene weight ratios of 2:1:0.75 (II) and 2:2:1 (III), were selected along with pure drug (I) to assess the relative rate of drug absorption. Formulae I, II, and III were suspended in 2% gum acacia solution so as to contain 20% frusemide. Mice, each weighing 18-22 g were used. The doses were administered orally by intubation to 6 mice per dose level. The LD_{50} values were calculated adopting Litchfield and Wilcoxon method (7).

Oral Toxicity of Polymers

The polymers present in the formulations were evaluated in the same manner as the drug and were found to have no effect on the LD_{50} values in the maximum amount used in the formulation (Table 4).

RESULTS AND DISCUSSION

Dissolution Studies

A preliminary dissolution studies have been done using HCl buffer (pH 1.2, U.S.P. XX), as dissolution

medium, resulted in a distinct slow or even negligible frusemide release from the investigated microcapsules. This is not the case when using phosphate buffer (pH 7.4, U.S.P. XX). This finding is in agreement with that of Prasad et al. (6).

The data in Table 1 revealed undue prolongation of drug release on microencapsulating plain frusemide with polystyrene in the proposed drug/polymer ratios. Thus, while non-microencapsulated frusemide granules released all the drug within 30 minutes, plain frusemide microcapsules released only 15.96-45.04% of the contained drug after 6 hours. On the other hand,Table 2 shows that 79.11-100% of frusemide was released, after 2.5 hours of dissolution, from the microcapsules containing its solid-dispersion with PEG 6000.

The increase in the dissolution rate of frusemide from its microcapsules containing the drug as solid-dispersion with PEG 6000 may be attributed to the change in the physical state of the drug during the preparation of the solid-dispersion. Thus, when quenching a melted solid-dispersion, the solute molecule is arrested in the solvent matrix by the instantaneous solidification process (8,9). Under such condition, a much finer dispersion of drug crystallites is obtained, thus, optimizes the effective surface area of the drug particles. Meanwhile, PEG 6000, when dissolved will go into solution leaving behined pores and channels in the solid-dispersion matrix, thus, aid in the penetration of the dissolution medium inside the microcapsule core. This, in turn, will increase the hydrostatic pressure inside the microcapsule, thus, rupture the microcapsule wall or even forming channels in it.

LD_{50} Studies

Toxicity studies have been used as *in-vivo* method of demonstrating duration of effect (10-12). Dittert

TABLE 1

Release of Frusemide in Phosphate Buffer (pH 7.4) from its Microcapsules

Drug/ Polymer Ratio	Mean Values (%) of Frusemide Released after the Following Time Intervals (Hours)											
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
2:1	9.27	15.17	17.32	20.00	21.26	23.51	24.53	27.15	28.70	29.17	30.01	31.86
3:1	5.37	9.80	12.95	15.76	18.05	20.85	23.11	26.48	27.96	30.10	31.54	33.28
4:1	8.91	16.41	19.50	22.25	24.97	29.27	32.97	33.72	35.67	36.70	42.09	45.04
5:1	10.65	13.95	16.38	17.81	18.91	20.13	21.20	25.11	27.07	28.39	30.00	31.06
6:1	7.83	13.70	15.58	16.69	18.27	20.15	21.22	22.15	22.84	23.22	24.05	25.01
7:1	5.63	7.60	8.89	9.93	10.71	11.63	11.94	14.08	14.75	15.32	15.82	16.20
8:1	6.10	8.27	9.30	10.00	10.79	12.43	12.82	13.62	14.10	15.02	15.26	15.96
9:1	7.95	9.95	11.07	12.01	12.78	13.64	14.10	14.52	15.00	15.54	15.81	16.70
10:1	8.10	11.90	14.30	15.20	16.70	17.30	17.80	18.20	18.60	19.00	19.40	19.90
12:1	9.60	12.32	14.18	15.72	17.14	18.56	20.57	20.93	21.21	22.00	22.58	23.59
14:1	10.80	13.40	15.30	16.60	17.80	19.70	21.02	21.50	21.70	22.10	22.50	22.80
16:1	13.27	15.14	16.78	17.80	18.07	21.23	21.50	22.00	22.10	22.30	22.50	23.50

N.B.: Non-microencapsulated frusemide, granulated with alcohol to 16 mesh (U.S.S.), released all the drug within 30 minutes.

TABLE 2

Release of Frusemide in Phosphate Buffer (pH 7.4) from its Microencapsulated Solid-Dispersion with PEG 6000

Batch No.	Drug: PEG 6000: Polystyrene	Mean Values (%) of Frusemide Released after the Following Time Intervals (Hours)														
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
I	2:2:1	38.7	53.2	66.1	73.7	79.1	85.5	86.6	87.3	88.5	89.5	91.1	92.4	93.9	94.2	95.7	97.4
II	3:3:1	43.2	63.1	74.9	77.0	81.3	84.9	85.9	86.2	86.9	87.3	88.9	90.5	91.5	92.7	93.2	94.3
III	4:4:1	52.5	72.3	82.5	83.6	84.5	85.2	86.0	86.4	87.0	88.1	89.3	90.4	90.8	91.1	91.5	92.2
IV	3:1:1	33.0	55.7	65.2	73.8	86.0	92.1	95.8	100								
V	2:1:0.75	46.0	67.1	75.2	84.2	89.1	93.4	95.3	96.3	100							
VI	3:1:1.25	50.6	74.4	84.1	98.8	100											

TABLE 3

Acute Toxicity of Frusemide in Mice

Formulation	Dose mg/kg	Number Dead/ Number Dosed	LD_{50} (mg/kg) Mean (95% Confidence Limits)
I	2200	6/6	1300 (970 - 1742)
	2000	5/6	
	1800	4/6	
	1500	3/6	
	1200	3/6	
	1000	2/6	
	800	1/6	
	600	0/6	
II	2800	6/6	1800 (1463 - 2214)
	2500	5/6	
	2200	4/6	
	2000	4/6	
	1800	3/6	
	1500	2/6	
	1200	1/6	
	1000	1/6	
	800	0/6	
	600	0/6	
III	2800	6/6	2150 (1720 - 2688)
	2500	5/6	
	2200	4/6	
	2000	3/6	
	1800	2/6	
	1500	2/6	
	1200	1/6	
	1000	0/6	
	800	0/6	
	600	0/6	

TABLE 4

Acute Toxicity of Polymers Used in Frusemide Formulations

Formulation	Polymers	Dose (mg/kg)		Number Dead/Number Dosed	
		Drug	Polymers	Polymers and Drug	Polymers Alone
II	PEG 6000+ Polystyrene	2800	1400 +1050	6/6	0/6
III	PEG 6000+ Polystyrene	2800	2800 +1400	6/6	0/6

et al. (13) considered the LD_{50} values as an index of relative absorption rate.

Table 3 shows that Formulae II and III have significantly high lethal doses than Formula I. The increase in lethal dose was attributed to slower absorption of the drug from the microencapsulated forms. Thus, one may conclude that Formula III has more prolonged action than Formulae II and I, and would be a formulation of choice for further pharmacokinetic and pharmacodynamic studies in human.

The results of this in-vivo investigation, thus, proved to be in agreement with those obtained from the in-vitro dissolution studies, as Formulae I and II released 100% of frusemide in 30 minutes and 4.5 hours, respectively, while Formula III released 97% of the drug in 8 hours.

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