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In Vitro Release Studies of Levonorgestrel Loaded Biodegradable Microspheres

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Levonorgestrel (LNG) loaded biodegradable microspheres, with different drug loading and polymer composition, were prepared by emulsion solvent evaporation technique. *In vitro* release profiles of these microspheres were studied in ethanolic phosphate buffered saline pH 7.4 (EPBS). The effect of different percentages of ethanol in the dissolution media on the release profiles of LNG from the micropheres, with varying drug load and polymer composition, was determined. The release kinetics of LNG from the microspheres was evaluated by fitting the release data to the zero order, first order, Higuchi, Korsmeyer-Peppas and Baker-Lonsdale equations. Initial examinations revealed that the release data followed more than one kinetic model. Diffusional rate treatment was used to distinguish between different kinetic models. The release kinetics of LNG from the microspheres was observed to be dependant on the ethanol content of the dissolution media.

Parenteral controlled-release systems are of considerable interest for drugs which either require daily administration, or have high toxicity, or a very low oral bioavailability. Their potential is greatly increased if the dosage form can be injected to the patient. In addition, the use of totally bioabsorbable polymers for the realisation of these systems avoids the need for surgical residue removal. Microspheres, which consist of a drug dispersed in a spherical polymer matrix, have already extensively been evaluated for the administration of a wide range of drugs[1-3]. Various dissolution methods have been used, in absence of pharmacopoeial guidelines, to evaluate the *in vitro* release properties of long acting microparticulate parenteral dosage forms[4-9]. However, the data generated during *in vitro* release studies can be meaningful provided the methodology adopted for the dissolution study and the components of the dissolution method such as; composition and volume of dissolution media, pH, rate of agitation and temperature have been selected such that they do not influence physico-chemical property of the dosage form[10].

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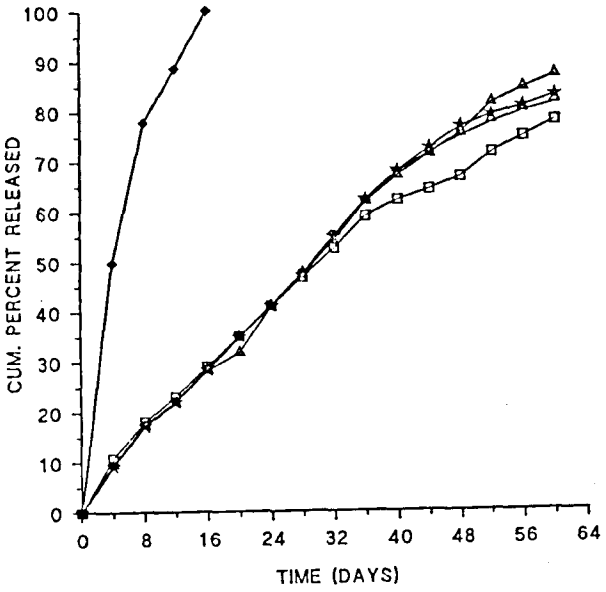
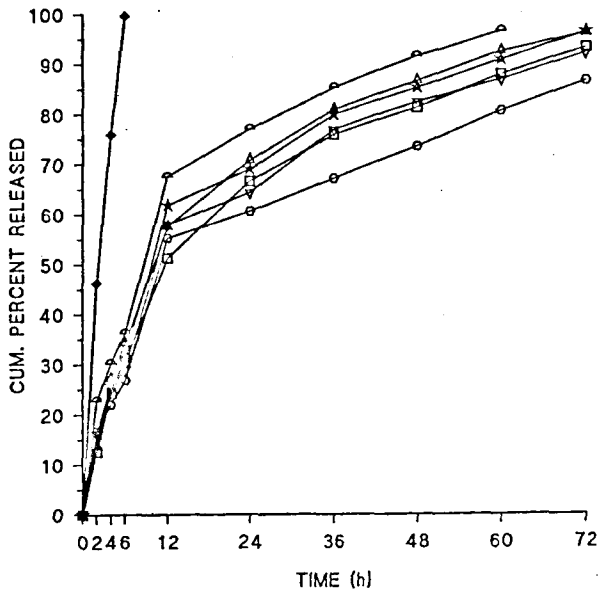
The objective of this investigation was to develop biodegradable microspheres of LNG. Several parameters such as drug content, polymer composition, molecular weight of the polymer and manufacturing process are known to have dramatic influence on the *in vitro* release rates of active component from the biodegradable microspheres. Therefore, selection of a dissolution method which could differentiate the influence of these parameters on the *in vitro* release properties of the LNG loaded microspheres was a part of this research investigation. Hence, the microspheres with different drug loading and polymer composition were prepared. Their *in vitro* release rates and mechanism of drug release were studied in the dissolution medium containing different proportions of ethanol. Dissolution media containing 10% v/v and 50% v/v of ethanol were used to evaluate the influence of alcohol content on the release mechanism and approximate duration of drug release.

The analysis of the mechanism of drug release from pharmaceutical dosage forms is an important but complicated problem and is particularly evident in case of microsphere dosage form. Drug release from microsphere dosage forms has been described by either a zero order kinetics or first order kinetics or square-root of time relation or Baker-



◆ indicate dissolution of LNG whereas □, △, ○ and ★ indicate release profile of LNG from microspheres batch dlm-1, dlm-2, dlm-3 and dlm-6 respectively

Fig 1: Dissolution and Release profile of LNG in 10% EPBS



◆ indicate dissolution of LNG whereas □, △, ○, ★, ▽ and ○ indicate release profile of LNG from microspheres batch dlm-1, dlm-2, dlm-3, dlm-4, dlm-5 and dlm-6 respectively

Fig 2: Dissoluton and Release profile of LNG in 50% EPBS

Lonsdale kinetics. Adherence of release data data to more than one kinetic model has also been reported. In such cases, data analysis using differential rates has been reported to identify the release mechanism[11-13].

EXPERIMENTAL

Materials

Levonorgestrel B.P./U.S.P. (Wyeth Lab. Ltd. Mumbai), Poly-ε-caprolactone (PCL) (Sigma chemicals, USA), Poly (dl-lactide-co-glycolide) (PLGA) (Birmingham Polymers, USA), methylene chloride (analytical grade), fused calcium chloride (laboratory grade), poly vinyl alcohol (S-D Fine chemicals), di-sodium hydrogen orthophosphate, potassium dihydrogen orthophosphate and sodium chloride (analytical grade) and absolute alcohol I.P. were used in the preparation and dissolution study.

Preparation of Levonorgestrel loaded microspheres

Levonorgestrel loaded biodegradable microspheres were prepared by an emulsion solvent evaporation method as reported previously[14]. The composition of different batches of microspheres is shown in Table 1.

Table – 1 Composition of LNG loaded microspheres

B.No.	LNG content (% w/w)	PCL content (% w/w)	PLGA content (% w/w)
DLM-1	05	95	—
DLM-2	10	90	—
DLM-3	20	80	—
DLM-4	10	72	18
DLM-5	10	54	36
DLM-6	10	36	54

In vitro release studies

Ethanolic phosphate buffered saline pH 7.4 (EPBS), with ethanol content 10% v/v or 50% v/v, was used as a dissolution medium in the present investigation. Microspheres, equivalent to 5 mg of LNG, were tied in small pieces of nylon cloth (400 mesh). These were immersed in

Table - 2 : Fit of different kinetic models and Comparison of coefficients of determination (r2) for the differential plots to *in vitro* release data using 10% EPBS as dissolution medium

Microsphere Batch No	Kinetic Model					Differential Rate Model		
	Zero-Order	First-Order	Higuchi	Baker-Lonsdale	Korsmeyer-Peppas	Zero-Order dq/dt v/s t	First-Order dq/dt v/s q	Higuchi dq/dt v/s 1/q
DLM-1 (m)	0.063	-0.010	0.562	0.0028	0.701	-	-	-
(r2)	0.982	0.994	0.991	0.980	0.992	0.219	0.196	0.257
DLM-2 (m)	0.074	-0.014	0.694	0.0040	0.783	-	-	-
(r2)	0.994	0.969	0.987	0.922	0.991	0.026	0.022	0.025
DLM-3 (m)	0.071	-0.013	0.622	0.0035	0.770	-	-	-
(r2)	0.984	0.988	0.971	0.952	0.991	0.001	0.001	0.001
DLM-4 (m)	0.068	-0.012	0.599	0.0032	0.758	-	-	-
(r2)	0.983	0.994	0.977	0.960	0.990	0.016	0.012	0.015
DLM-5 (m)	0.071	-0.013	0.628	0.0032	0.794	-	-	-
(r2)	0.985	0.986	0.970	0.947	0.989	0.019	0.024	0.087
DLM-6 (m)	0.061	-0.009	0.537	0.0225	0.735	-	-	-
(r2)	0.984	0.992	0.982	0.951	0.992	0.121	0.105	0.019

m=slope

100 ml of 10% v/v EPBS or 50 ml of 50% v/v EPBS (pre warmed to 37 ±1°), in screw capped bottles. These bottles were maintained at 37 ±1° and agitated (100 strokes per minute) on a horizontal shaker water bath, during the entire period of the study, i.e. 60 days or 7 days respectively. At periodic intervals of time 25 ml or 1 ml of the dissolution medium, from each bottle containing 10% v/v or 50% v/v EPBS respectively, was collected in tubes and replaced by an equal volume of the fresh dissolution medium. The drug concentrations in the periodically sampled solutions were determined, after suitable dilution, by measuring their absorbance at 247 nm (10% v/v EPBS) or 244 nm (50% v/v EPBS) against suitably prepared blanks.

Analysis of release data

The data obtained was fitted to zero order, first order, Higuchi, Baker-Lonsdale and Korsmeyer-Peppas equations to understand the mechanism of LNG release from the microspheres. A more rigorous test, based on the differential rates was applied in cases where release data fitted more than one kinetic models to distinguish the actual mechanism of drug release. [i.e. graphs of release rates (dq/dt) V/S time (t); dq/dt V/S the percentage of drug released (q) and dq/dt V/S 1/q were plotted]

RESULTS AND DISCUSSION

A common problem in the dissolution testing of an insoluble drug is the attainment of sink conditions. The use of surfactants and co-solvents such as polyethylene glycol, alcohol, benzalkonium chloride (BKC) have been reported for dissolution studies of steroid drugs (e.g. progesterone, estrone, norethisterone)7,9,15,16. Ethanolic phosphate buffered saline pH 7.4 was used in the present study to provide sink solubility condition and to inhibit bacterial growth. The aqueous component was isotonic to simulate any osmotic effects *in vivo*. Dissolution media containing

Table - 3 : Fit of different kinetic models and Comparison of coefficients of determination (r2 for the differential plots to *in vitro* release data using 50% EPBS as dissolution medium

Microsphere Batch No	Kinetic Model					Differential Rate Model		
	Zero-Order	First-Order	Higuchi	Baker-Lonsdale	Korsmeyer-Peppas	Zero Order dq/dt v/s t	First-Order dq/dt v/s q	Higuchi dq/dt v/s1/q
DLM-1 (m)	1.604	-0.015	11.36	0.004	0.456	-	-	-
(r2)	0.854	0.986	0.974	0.996	0.971	0.687	0.878	0.970
DLM-2 (m)	1.690	-0.018	11.77	0.005	0.429	-	-	-
(R2)	0.829	0.989	0.962	0.996	0.962	0.676	0.866	0.963
DLM-3 (m)	2.043	-0.022	12.68	0.006	0.427	-	-	-
(r2)	0.804	0.980	0.949	0.987	0.931	0.781	0.974	0.998
DLM-4 (m)	1.675	-0.018	11.60	0.005	0.445	-	-	-
(r2)	0.822	0.975	0.955	0.988	0.934	0.629	0.856	0.986
DLM-5 (m)	1.602	-0.014	11.11	0.004	0.450	-	-	-
(r2)	0.832	0.977	0.960	0.989	0.941	0.575	0.750	0.931
DLM-6 (m)	1.473	-0.011	10.13	0.003	0.452	-	-	-
(r2)	0.834	0.958	0.957	0.981	0.928	0.622	0.873	0.969

m = slope

different percentages of ethanol were used to evaluate the influence of alcohol content on the release mechanism and approximate duration of drug release.

The dissolution profile of uncoated drug and release profile of drug from the microspheres (B. No. 1,2,3 and 6), in 10% v/v EPBS, are as shown in Figs. 1. The uncoated drug dissolved slowly and complete dissolution was observed after 15 days. This may be attributed to the low solubility of the drug in the dissolution medium (0.1-0.11 mg/ml). All the batches of microspheres released about 80% of drug over a period of 60 days. The slope and the corresponding coefficients of determination (r2) for zero-order, first-order, square-root of time, Baker-Lonsdale and Korsmeyer-Peppas plots are listed in Table 2. The slopes indicate that the release rates of LNG from the microspheres,in 10% v/v EPBS, were not influenced by the drug loading or polymer composition. The coefficients of determination indicate that each of these models adequately fits the release data. However, the coefficients of determination for the plots of differential rate (dq/dt) against time (t) and percentage of drug released (q) indicated that the release rates were independent of time or drug concentration, suggesting the unfit of release data to zero-order and first-order kinetics (Table 2). Further, the coefficients of determination for the plot of differential rate (dq/dt) against time (1/q) indicated that the drug release does not follow Higuchi kinetics. The slope (n) of Korsmeyer-Peppas plot is release exponent, indicative of the drug release mechanism. The value of 'n' varies with the geometry, and porosity of the dosage form. The 'n' value of 0.5 or 0.43 is indicative of drug release by Fickian diffusion for the homogeneous planar or spherical matrices respectively. The higher values of 'n' (0.7 to 0.8) for release of LNG in this dissoution media indicates the non-fickian diffusion of drug.

The dissolution profile of uncoated drug and the different microspheres, in 50% v/v EPBS, are as shown in Figs. 2A and 2B. The uncoated drug dissolved completely within 6 h. All microspheres showed initial burst release, releasing about 40-60% of the drug in the first 12 h. The initial rapid release of LNG in 50% v/v EPBS may be attributed to the small quantity of free drug that might be present, smaller mean particle diameter of the microspheres and high solubility of drug in the dissolution media (0.9-1.0 mg/ml). An increase in the release rate was observed with increased drug loading. Release of levonorgestrel decreased with increasing PLGA ratio. This could be explained by the low permeability of PLGA to steroids. Nuessle et al[9] have also reported burst release of oestrone, in 50% v/v methanolic phospate buffer solution pH 7.4, from poly (1-lactic acid) microspheres with average diameters of 125-160 mm.

This was attributed to the presence of free drug and/or drug on the surface of microspheres. A burst release of norethisterone enanthate, was observed by Beck et al.[7], also, from the drug containing poly (dl-lactide) microcapsules. The dissolution medium used in their study was 27.5% w/w aqueous ethanol. The slopes and the corresponding coefficients of determination (r2) for zero-order, first-order, square-root of time, Baker-Lonsdale and Korsmeyer-Peppas plots are listed in Table 3. The coefficients of determination indicated that the release data did not fit adequately to the zero-order kinetics, whereas it fitted adequately to the other kinetic models. Lack of fit of the release data to the zero-order kinetics was further confirmed by coefficients of determinations of the plots of differential rate (dq/dt) VS time (t) as shown in Table 3. In addition, the coefficients of determinations of the differential plots for the first-order (i.e. dq/dt VS q) or square-root of time (i.e. dq/dt VS 1/q) models suggested diffusion controlled release of LNG from the microspheres in 50% EPBS (Table 3). This was further confirmed by the adequate fitting of the release data to the Baker-Lonsdale kinetic model (equation-1), which is more specific for the diffusion controlled release from spherical matrix systems.

$$3/2\ [(1-F)^{2/3}]\ F = kt \text{——} 1$$

Where F=fraction of drug released; t=time and k=rate constant.

Additional evidence for the diffusion controlled release mechanism was obtained by fitting the Korsmeyer-Peppas equation to the release data. The 'n' value ranged from 0.42 to 0.46 for different batches of the Microspheres prepared with different drug loading and ploymer composition, indicating Fickian diffusion of drug through microspheres.

This study demonstrated the suitability of 50% v/v EPBS as the dissolution medium for the *in vitro* release studies of LNG loaded biodegradble microspheres. Further, the results presented indicate that the mechanism of release of LNG from microsphers is influenced by the ethanol content of the dissolution medium.

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