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Formulation of prolonged release lipid micropellets by emulsion congealing: Optimization of ketoprofen entrapment and release

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Abstract

An emulsion-congealing technique was used to prepare prolonged release lipid micropellets containing ketoprofen. The lipid matrix consisted of cottonseed oil/beeswax mixture and was emulsified at 70° C into 0.1 N HCl containing Tween 60 and gelatin. The studied formulation factors were the amounts of drug, Tween 60 and gelatin, in addition to the matrix composition and rate of cooling. The optimization protocol required preliminary formulation studies (11 batches) to help choose suitable levels for formulation factors, and a central composite design (17 batches) to correlate simultaneously some of these factors to measured variables, e.g., drug entrapment, particle size and dissolution parameters. The adopted optimization strategy led to formulation of micropellets of about 1.5 mm in diameter and 25% w/w drug loading with prolonged release characteristics. The study design offers adequate prediction of micropellet characteristics and provides a basis for development of other micropellet systems.

Keywords: Lipid micropellet; Emulsion congealing; Ketoprofen; Prolonged release; Optimization

1. Introduction

Ketoprofen is an effective nonsteroidal anti-inflammatory agent, whose short half-life (1 h) and irritating effect on gastric mucosa favor its formulation into sustained release (SR) dosage forms (Teule, 1986). Ketoprofen has been formulated into SR granules (Kohri et al., 1989, Giunchedi et al., 1991), microcapsules (Hagan, 1982), hard gelatin capsules filled with semisolid wax matrix (Dennis et al., 1990), extended release solid dispersion (Ho and Hwang, 1992) or as non-disintegrating tablets (El Khodairy et al., 1992). Among these dosage forms, multiparticulate drug delivery systems, e.g., granules and microcapsules up to 2–3 mm in diameter, are more advantageous since local accumulation of the drug onto gastric mucosa is less probable. Moreover, multiparticulate systems give less variable absorption profiles as their residence time in the stomach is usually short and less influenced by the always-changing gastric emptying time.

Preparation of SR micropellets is commonly achieved using spray drying (Masters, 1985; Eldem et al., 1991) or the emulsion/solvent evaporation technique (Bodmeier and McGinity, 1987;

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Table 1
Preliminary formulations of plain micropellets

Formula	Examined variables				Particle size analysis	
	Lipid matrix		Tween (% w/v)	Gelatin (% w/v)	Modal size [a] (μm)	Weight fraction (% w/w)
	Oil (% w/w)	Wax (% w/w)				
F1	10	90	0.2	1.5	500–800	55.0
F2	20	80	0.2	1.5	800–1250	40.7
F3	30	70	0.2	1.5	1600–2000	43.6
F4	40	60	0.2	1.5	1600–2000	43.4
F5	10	90	0.2	2.5	500–800	28.0
F6	20	80	0.2	2.5	800–1250	39.9
F7	30	70	0.4	1.5	315–500	38.4
F8	30	70	0.6	1.5	315–500	45.1
F9	40	60	0.6	1.5	500–800	40.1
F10 [b]	30	45	0.4	1.5	1250–1600	37.7
F11 [b,c]	30	45	0.4	1.5	90–315	67.7

[a] Most prevalent particle size (modal size).
[b] Oily phase contained 25% (w/w) ketoprofen.
[c] Micropellets were recovered by rapid cooling.

Das, 1991; Jeffery et al., 1991) which necessitate the use of hazardous organic solvents, and/or heating for a prolonged time. An alternative technique to overcome these limitations is the emulsion/congealing technique (Adeyeye and Price, 1991), which utilizes fats of animal and/or vegetable origin as the matrix. The wide variety of available fats provide broad flexibility in choosing matrices with tailored controlled release characteristics of various drugs (Kagadis and Choulis, 1985; Benita et al., 1986). Despite the great interest in multiparticulate formulations, few reports consider statistical experimental design for product optimization (e.g., Mcleod et al., 1988; Hassan et al., 1992).

In this work, we describe an optimization protocol for preparing ketoprofen-containing micropellets prepared from beeswax-cottonseed oil mixtures, employing the emulsion/congealing procedure.

2. Experimental

2.1. Materials

Ketoprofen was supplied courtesy of Amriya Pharm. Ind. (Alexandria, Egypt), cottonseed oil was purchased from Alexandria Oils and Soap Co. (Alexandria, Egypt) and Tween 60 was obtained from Fluka AG, Buchs, Switzerland. Gelatin (240 Bloom) was a gift from Arab Caps (Alexandria, Egypt). All other chemicals were of analytical grades.

Table 2
Factors and levels tested during the optimization of ketoprofen-containing micropellets according to the central composite design

Factors	Levels				
Ketoprofen concentration (% w/w) [a]	13.3	20	30	40	46.7
Cottonseed oil concentration (% w/w) [a]	11.6	15	20	25	28.4
Tween 60 concentration (% w/v)	0.23	0.3	0.4	0.5	0.57

[a] Matrix was completed to 100% w/w with beeswax.

2.2. Experimental design

2.2.1. Preparation of micropellets

Preparation of plain and ketoprofen-loaded micropellets was based on a hot emulsion/congealing technique (Adeyeye and Price, 1991). As a preliminary study, 11 micropellet formulations (Table 1) were prepared to help choose suitable levels for formulation factors. For factor optimization, another set of formulations was prepared according to a central experimental design (Bayne and Rubin, 1986) to correlate percent of each of cottonseed oil and ketoprofen in the lipid matrix as well as Tween 60 in the aqueous phase to certain response variables (indicated in section 2.2.2). The basic procedure for preparing micropellets was as follows: the melted fatty phase, 6.5 g of different ratios of beeswax, cottonseed oil and ketoprofen (Table 2) was emulsified into 100 ml of 0.1 N HCl containing 1.5–2.5% (w/w) gelatin and 0.2–0.8% (w/w) Tween 60. Both aqueous and fatty phases were preheated to 70° C prior to emulsification with a mechanical stirrer (VEB MLW Pruefgeraete Werk, Mendingen/Sitz Freital, Germany) at 600 rpm for 3 min. The formed emulsion was gradually cooled (while stirring was continued) using a circulating water bath to reach a temperature of 40° C. A final cooling step was conducted by adding 100 ml of ice-cold 0.1 N HCl. Micropellets were separated from the aqueous phase by filtration through a filter paper followed by successive washing with 0.1 N HCl and distilled water before drying at room temperature.

2.2.2. Analysis of micropellets

Preliminary formulations (Table 1) were analyzed for particle size distribution only, whereas for formulation of the central composite design, particle size, % yield, % drug loading and drug release were determined as follows:

2.2.2.1. Particle size. Micropellet size analysis was accomplished by sieving through a nest of seven sieves covering the size range of 90–2000 μm, followed by weighing each retained fraction.

2.2.2.2. Percent yield. Yield of micropellets (% w/w) was calculated as the weight of the dried micropellets recovered from each batch divided by 6.5, the sum of dry weights of the internal phase components, multiplied by 100.

2.2.2.3. Percent drug loading (E). Percent drug loading was assessed as follows; 25 mg of a micropellet sample was extracted with 20 ml of 0.1 M phosphate buffer (pH 7.4) at 70° C for 10 min, with intermittent shaking. After extraction, samples were cooled to room temperature and filtered through a 0.2 μm membrane filter. Drug content in the filtrate was measured spectrophotometrically (Pye Unicam SP 1800, Cambridge, UK) at 262 nm. The micropellet matrix did not interfere with the assay, and extraction was complete as demonstrated by analysis of drug-free micropellets, and matrix components, spiked with known amounts of the drug, respectively.

2.2.2.4. Drug release studies. Drug release studies were performed for the size fraction of 800–1600 μm from batches which gave more than 50% of micropellets over this particle size range. Dissolution runs (in triplicate) were carried out at 37° C in a standard USP XXII dissolution apparatus with teflon-coated paddles driven at 50 rpm. About 50 mg samples of micropellets (containing a maximum of about 12.5 mg of ketoprofen) were introduced in 1 l of USP simulated gastric (SGF; pH 1) or intestinal (SIF; pH 7.2) fluids without enzymes. 5 ml aliquots were withdrawn at specific time intervals and analyzed spectrophotometrically for ketoprofen content at 262 nm. Withdrawn samples were replaced immediately with fresh dissolution medium. Exact conditions were applied for testing dissolution of triplicate samples of ketoprofen powder (12.5 mg each) in SGF or SIF.

2.2.3. Data analysis

2.2.3.1. Parameters. For quantitative comparison between different formulations, the following parameters were considered: (a) PS 800–1600, % weight fraction of micropellet yield having a particle size range of 800–1600 μm; (b) DRa, % (w/w) of ketoprofen remaining unreleased after 120 min of dissolution in SGF; (c) DRb, % (w/w)

of ketoprofen remaining unreleased after 15 min of dissolution in SIF; (d) D, overall desirability function:

$$D = \frac{E + (\mathrm{PS}\,800 - 1600) + (\mathrm{DRa}) + (\mathrm{DRb})}{4} \quad (1)$$

2.2.3.2. Regression analyses. For the central composite design, a multiple regression procedure was utilized to fit full second-order polynomial equations correlating responses such as E, PS 800–1600 and D to the examined variables; amount of oil, surfactant and ketoprofen. A backward regression procedure was further applied in an attempt to simplify full second-order polynomial equations primarily generated from the multiple regression procedure (Microstat-II Version 1.01, 1988, Ecosoft Inc., USA)

3. Results and discussion

For the preliminary studies, changes in particle size range of the most prevalent size, modal size (MS), of the micropellets as a function of the amount of each component, presence of drug, as well as the cooling process are illustrated in Table 1. Cottonseed oil was included in the matrix as a matrix modifier to facilitate micropellet formation. In one factor at a time fashion (Table 1), an increase in concentration of cottonseed oil (F1 and F4), inclusion of ketoprofen (F7 and F10), or gradual cooling (F10 and F11) resulted in a larger particle size range of the final product. On the other hand, increasing Tween concentration (F3, F7 and F8) or applying sudden cooling (F10 and F11) produced finer micropellets. In the emulsion congealing technique, the particle size is determined by the emulsification and solidification steps. Thus, reduction of the congealing point of the matrix by increased cottonseed oil levels or by drug incorporation increases the chance of globule coalescence, leading to final product of larger particle size. Similarly, the gradual cooling process favoured the formation of larger particles. On the other hand, higher surfactant concentration or sudden cooling produced finer micropellets. An emulsion stabilizer is usually used to prevent phase separation by increasing emulsion

Table 3
Response variable data following preparation of micropellets containing ketoprofen

Batch no.	Entrapment (% w/w)	Yield (% w/w)	% WF [a]	Formation of micropellets [c]	% drug remaining		D [b]
					pH 1	pH 7.2	
K1	31.6	80.5	7.9	–	–	–	0
K2	28.4	90.2	4.1	–	–	–	0
K3	16.8	88.4	16.6	+	59 ± 1.8	62 ± 2.3	50
K4	12.5	96.2	0.8	–	–	–	0
K5	12.2	82.1	57.8	+	48 ± 4.3	60 ± 7.2	43
K6	18.5	84.7	3.94	–	–	–	0
K7	24.1	80.1	2.71	–	–	–	0
K8	17.6	83.2	69.5	+	57 ± 0.5	70 ± 3.3	54
K9	24.4	89.5	12.8	–	–	–	0
K10	11.6	80.5	4.7	–	–	–	0
K11	26.8	79.4	83.5	+	63 ± 1.3	60 ± 4.4	58
K12	32.8	78.6	23.0	–	–	–	0
K13	12.4	83.2	2.8	–	–	–	0
K14	12.0	83.7	89.8	+	50 ± 1.5	74 ± 1.8	57
K15	23.4	81.5	57.7	+	74 ± 2	74 ± 5.0	57
K16	25.6	90.5	57.1	+	60 ± 3.4	68 ± 0.0	53
K17	26.0	85.5	52.2	+	66 ± 2.4	66 ± 1.7	50

[a] WF, % of weight fraction between 800 and 1600 μm.
[b] D, overall desirability function.
[c] (+) Indicates formation of spherical micropellets; (–) corresponds to formation of irregular clumps (> 2 mm).

viscosity and stabilizing the surfactant sheath around the internal phase. The tested narrow range of gelatin concentration provided no significant change in particle size. Gelatin effect on emulsion viscosity was also minimal at the relatively high temperature of emulsification.

Data from micropellet analysis of the batches prepared according to the central composite design (Table 2) are summarized in Table 3. The yield of micropellets ranged from 78.6 to 96.2%. Nine out of the prepared 17 batches exhibited a spherical form, with the maximum yield fraction having the size range of 800–1600 μm. The other batches produced irregular particles (> 2.0 μm). Particle size analysis of a selected formula (batch K15, Table 3) is illustrated in Fig. 1. Unsuccessful production of micropellets was consistently observed for batches prepared from matrices containing $> 30\%$ (w/w) of the drug. At high drug concentration, ketoprofen was incompletely soluble in the lipid matrix, providing greater potential for bridging between particles and leading to clump formation. The percent of drug loading ranged between 12.2 and 26.8.

Ketoprofen dissolution profiles as pure powder and from batch K15 are shown in Fig. 2. The percent of ketoprofen remaining undissolved in each formulation was calculated at 15 min in SIF and after 2 h in SGF, the corresponding times for 100% dissolution of drug powder, to indicate prolonged release efficiency of each formulation relative to the drug powder. All tested formulations exhibited a significant delay in drug release showing DRa and DRb of 48–74 and 60–74%, respectively. D is a single parameter which facilitates the selection of optimum formulation conditions. D is usually calculated as the geometric mean of desirability parameters (range between 0 and 1) calculated for each of the contributing responses (Mcleod et al., 1988; Hassan et al., 1992). In this report, D was calculated as the arithmetic mean of actual values of contributing responses. This approach simplified calculations and kept equal weights for all responses as they inherited the range of 0–100%. Accordingly, the results of the optimization procedure gave ranges for factor levels (Table 4) as follows: cottonseed oil 20–26% (w/w), ketoprofen 20–30% (w/w), Tween 0.4–0.57% (w/v) and gelatin at 1.5% (w/v).

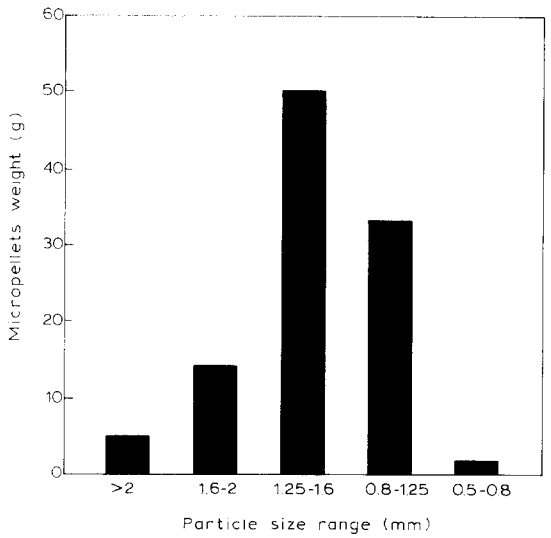

Fig. 1. Particle size distribution of micropellets prepared under optimum conditions (20% cottonseed oil, 50% beeswax, 30% ketoprofen; emulsified in 0.1 N HCl containing 0.4% Tween 60 and 1.5% gelatin).

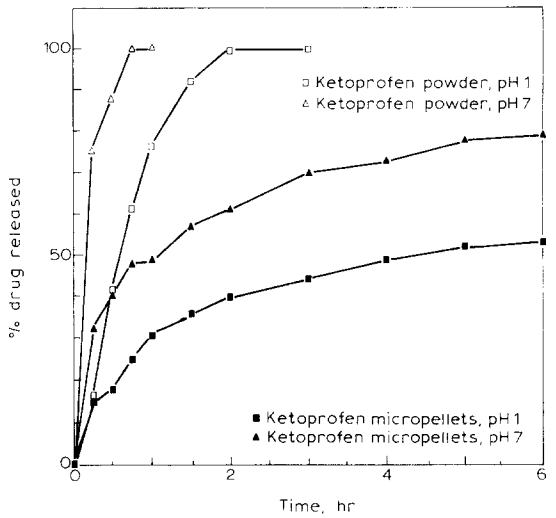

Fig. 2. Dissolution profiles of ketoprofen powder and ketoprofen-loaded micropellets prepared under optimum conditions (20% cottonseed oil, 50% beeswax, 30% ketoprofen; emulsified in 0.1 N HCl containing 0.4% Tween 60 and 1.5% gelatin).

Table 4
Selected optimal conditions of formulation factors for production of ketoprofen-loaded micropellets

Variables	Concentrations (%)
Gelatin (w/v)	1.5
Cottonseed oil (w/w)	20–25
Tween 60 (w/v)	0.4–0.57
Ketoprofen (w/w)	20–30

3.1. Regression analysis

Full-second order equations relating different responses (e.g., PS 800–1600, D and E) to the studied formulation factors resulted in good correlation ($r > 0.85$). However, each of these equations includes 10 parameters. Thus, a model simplification was undertaken to contain only parameters of the highest significance ($P > 0.05$), without much effect on the regression coefficient. The simplification procedure produced the following equations:

$$E = 0.624(\text{drug}) + 2.15\,[r = 0.827] \quad (2)$$

$$\text{PS}\,800 - 1600 = 47.27(\text{oil}) - 1.05(\text{oil})^2 - 0.107(\text{oil})(\text{drug}) - 402.94\,[r = 0.895] \quad (3)$$

$$D = 39.62(\text{oil}) - 0.886(\text{oil})^2 - 0.09(\text{oil})(\text{drug}) - 340.48\,[r = 0.887] \quad (4)$$

Prediction of response variables using full second-order equations and simplified equations (Eq. 2–4) is shown in Table 5, which indicates similar results. Eq. 2–4 demonstrate that the concentrations of oil and drug are most significant factors correlated to the measured responses, E, D and PS 800–1600. The concentration of Tween was less significant when related to measured responses using multiple regression analysis, although its effect on particle size was more obvious when tested separately in the preliminary studies. This observation reflects the importance of simultaneous investigation of different formulation factors. Conclusions from one factor at a time studies may not be fully informative especially when measured responses are sensitive to changes in other contributing factors. As stated earlier, batches containing $> 30\%$ ketoprofen in the applied matrix failed to form a spherical product, regardless of the Tween concentration. The selection of optimal formulation(s) was based on the D function, with preference for those formulations having the highest drug entrapment (e.g., batches K11 and K15 in Table 3).

In this work, a two-step optimization protocol was adopted so that preliminary studies were performed to help choose factors' ranges within which micropellets of suitable particle size range could be prepared. Based on these preliminary studies, a central composite experimental design was applied for optimization of other relevant micropellet characteristics, in addition to particle size. The adopted protocol led to successful formulation of SR ketoprofen micropellets and satisfactory prediction of their in vitro properties. The optimal in vitro performance of the prepared micropellets encourages future in vivo investigation.

Table 5
Observed and model-predicted values of tested variables for micropellets prepared under optimal conditions (20% w/w cottonseed oil, 30% w/w ketoprofen, 1.5% w/v gelatin and 0.4% w/v Tween 60)

Variables	Actual values	Model-predicted values	
		a	*b*
Drug entrapment (% w/w)	25.0 ± 1.4	21.9	20.9
(WF) 800–1600 μm (% w/w)	55.7 ± 3.0	57.4	54.6
Desirability function (D)	55.3 ± 3.7	48.2	43.3

[a] Using full second-order equation.
[b] Using simplified equations; see text.

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