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Research Article

Microencapsulation of Diclofenac Sodium by Non-solvent Addition Technique

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Abstract

Purpose: *To prepare, using non-solvent addition technique, diclofenac sodium-ethylcellulose microparticles with modified drug release properties.*
Methods: *Microparticles were prepared by non-solvent addition phase separation method and characterized by micromeritics, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), dissolution test and thermal analysis.*
Results: *The microparticles were whitish, irregular, aggregated, and in the size range of 390 - 442 µm size. Drug embedment efficiency was 89 - 91 %. Characterisation studies indicate that there was no strong chemical interaction between the drug and the polymer in the microparticles. Polymer concentration and sustained release behavior were directly proportional.*
Conclusion: *Non-solvent addition phase separation is a suitable method for preparing diclofenac sodium-ethylcellulose multi-unit controlled release drug delivery system.*

Keywords: *Phase separation, Diclofenac sodium, Ethylcellulose, Non-solvent addition, Characterisation.*

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INTRODUCTION

Chronic diseases require drugs with extended therapeutic effect and this can be achieved either by the development of drugs with long half-life or sustained release drug delivery systems. Several approaches have been employed to prepare systems for the sustained delivery of drug. Microencapsulation is one of the techniques that has been used to develop multi-unit sustained release dosage forms which are regarded as more reliable for the uniform distribution of drug in the gastrointestinal tract [1].

Diclofenac sodium (DS), a phenylacetic acid derivative, is an NSAID with a pK_a of 4.0. It exists in acidic form in acidic solutions such as gastric juice, and is practically insoluble in water but soluble in intestinal fluid [2]. It is rapidly absorbed when given orally and is used for the management of pain and inflammation in various musculoskeletal and joint disorders. Its plasma half-life is about 1 - 2 h and its usual oral dose is 75 to 150 mg daily in divided doses [3]. On the basis of the pharmacokinetic properties of diclofenac sodium, it is a suitable candidate for microencapsulation.

Ethylcellulose (EC) with complete ethoxyl substitution (DS = 3) is $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_n C_{12}H_{23}O_6$ where "n" can vary to provide a wide variety of molecular weights. Ethylcellulose, an ethyl ether of cellulose, is a long chain polymer of β-anhydroglucose units joined together by acetal linkages. It is generally considered a non-toxic, biocompatible and non-biodegradable polymer. These characteristics are the reasons for its extensive use in the development of oral dosage forms, especially sustained release formulations, including oral multi-unit dosage forms (i.e., microparticles) [4].

The literature contains some citations on microencapsulation of diclofenac sodium using various techniques [5,6]. None of these, to the best of our knowledge, described the characterisation of the microparticles by micromeritics, scanning electron microscope, FTIR, thermal analysis and X-ray diffractometry. Furthermore, no record was found of the microencapsulation of diclofenac sodium with non-solvent addition phase separation method. Consequently, and in view of the pharmacokinetic profile of DS, the objective of this study was to prepare and evaluate DS microparticles formulated with ethylcellulose.

EXPERIMENTAL

Materials

Diclofenac sodium (DS) was donated by Sami Pharmaceuticals, Pakistan. Ethyl cellulose (22 cp, EC) was purchased from Sigma, USA. All other chemicals (analytical grade) were purchased from various commercial sources.

Preparation of microparticles

EC (1 g) was dissolved in 20 ml of toluene containing polyisobutylene (6 %w/w) in a closed beaker with magnetic stirring (Velp, Europe) at 500 rpm for 6 h, followed by dispersion of DS in it. After stirring the system for 15 min, phase separation was induced by adding petroleum benzin (non-solvent). The product was transferred to an ice bath to solidify the microparticles. The microparticles were treated with chilled petroleum benzin five times while still stirring, washed with n-hexane and dried in air for 2 h followed by drying in an oven (Memmert, Germany) at 50 ℃ for 4 h. Three batches of microparticles, viz, M_1, M_2 and M_3, were prepared with drug:polymer ratio of 1:1, 1:2 and 1:3, respectively.

Embedment efficiency

A quantity (50 mg) of the microparticles was added to methanol (15 ml) and sonicated for 3 min to remove EC. Sufficient amount of water (50 ml) was added, the system heated to evaporate methanol and then filtered. After

suitable dilution, the resultant solution was analysed spectrophotometrically at 276 nm (predetermined maxima for DS) using a UV-Vis spectrophotometer (model 1601, Shimadzu, Japan). The concentration of DS was obtained from the calibration curve[5] and DS loading (%) and embedment efficiency (%) were calculated using the Eqs 1 and 2, respectively.

Drug loading (%) = $W_1/W \times 100$..... (1)

where W_1 is among of drug in microcapsules and W is weight of microcepsules

Embedment efficiency (%) = $L_a/L \times 100$ ..... (2)

where L_a is the actual drug loading and L is the theoretical drug loading

Yield efficiency

The yield (%) of microparticles was calculated as shown in Eq 3.

Production yield (%) = $\frac{M}{Mt} \times 100$ ..... (3)

where, M and M_t are the weights of microparticles and starting materials (M_t, total weight of DS plus EC), respectively[7,8].

Microparticle size

The mean diameter of the microparticles was determined using a light microscope equipped with a microscope stage and a digital camera connected to a computer. The microparticles were suspended in water, placed on a glass slide, and observed through the microscope. Photomicrographs were taken with the camera and the microparticle diameter determined by image analysis.

Scanning electron microscopy (SEM)

The morphology of the microparticles was examined by scanning electron microscopy (SEM, Shimadzu, Japan) after coating with palladium [9].

Fourier Transform Infrared Spectroscopy (FTIR)

DS, EC and DS-EC microparticles were studied by Fourier Transform Infrared (FTIR) spectroscopy (MIDAC M2000, USA) after first incorporating them in KBr disc in order to study DS-EC interaction.

X-ray diffractometry

X-ray diffractometry of DS, EC and DS-EC microparticles was conducted to evaluate the effect of microencapsulation technique on the crystallinity of DS using D8 Discover (Bruker, Germany). The samples were scanned in the 8 to 70° diffraction angle range under the following conditions: Cu-K∞ radiation 1.5406 A° (source), 4°/min scan speed, scintillation detector, primary slit 1 mm, secondary slit 0.6 mm.

Thermal analysis

Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and differential thermometric analysis (DTA) (TA Instruments, USA) of 10 mg powdered samples of DS-EC microparticles as well as DS and EC were carried out to determine DS-EC interaction. The samples were placed in an alumina pan at a heating rate of 10 °C/min under a nitrogen flow of 40 ml/min.

Micromeritic properties

Micromeritic properties of microparticles were also studied. Bulk and tapped densities were assessed by the conventional tapping method (see Eqs 4 and 5) using a 10 ml graduated measuring cylinder as a measure of packability of microparticles.

Bulk density = W/V ..... (4)

where W and V are the weight and volume, respectively.

Tapped density = Wt/Vt ..... (5)

where Wt and Vt are the weight and volume of particles, respectively, after 100 tappings

Compressibility index (Ci) and Hausnner's ratio were also determined as indices of microparticles flowability as in Eqs 6 and 7, respectively.

$Ci = \{(Vo - Vf)/Vo\} \times 100$ …….. (6)

Where Vo and Vf are the initial and finaly volume of the particles, respectively.

Hausnner's ratio = V/Vt …….... (7)

where V and Vt are the volume before and after tapping respectively

Angle of repose (θ) of microparticles was measured using the fixed-base cone method and calculated as in Eq 8 [5,10].

$\theta = \tan^{-1}.h/r$ …….. (8)

where r is the radius of cone base and h is the height of the cone.

Stability studies

Microparticles (500 mg) were sealed in an air-tight amber colour glass vial and stored at 20°C/60% RH, 30°C/65% RH and 40°C/75% RH. The microparticles were evaluated for drug content and release profile spectrophotometrically (as described above) weekly for eight weeks. One vial was used in each evaluation.

In vitro dissolution studies

In vitro drug release evaluation of accurately weighed DS microparticles, containing 100 mg DS, was carried out in 900 ml of double distilled water maintained at 37 ± 0.7 °C and stirred at 50 rpm, using a USP XXIV Type II dissolution test apparatus (Pharmatest, Germany). Aliquots (5 ml) were withdrawn and filtered automatically at various time intervals, replenishing the medium with 5 ml of dissolution medium after each withdrawal. The samples were analysed spectrophotometrically at 276 nm. The concentration of DS was obtained from the calibration curve generated [11, 5].

Assessment of release mechanism

In the model-dependent approach, various kinetic models were applied to the release profiles, as in Eqs 9 – 13.

Zero order [12]: $M_t = M_o + K_o t$ …….... (9)
First order [12]: $\ln M_t = \ln M_o + K_1 t$ …… (10)
*Higuch[*13]*i*: $M_t = M_o + K_H t^{1/2}$ ……. (11)
Hixson-Crowell [14]: $M_o^3 - M_t^3 = K_{HC} t$ .... (12)
Korsmeyer-Peppas [15]: $M_t/M_\alpha = K_k t^n$…. (13)

where, M_t is the cumulative amount of drug released at any specified time point and M_o is the initial amount of drug in the formulation. K_o, K_1, K_H, K_{HC} and K_k are rate constants for zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models, respectively. In Eq 13, M_t/M_α is the percentage of TH released at time t and n is the release exponent that characterises different release mechanisms.

In the model-independent approaches, ANOVA-based and pair-wise procedures were applied to the release data. One way ANOVA plus Post-Hoc analysis (Duncan and Tukey H.S.D.) for significance at $P < 0.05$ was conducted for whole release profiles using SPSS version 12.0.[16] Pair-wise procedures include the difference factor (f_1), as shown in Eq 14, and the similarity factor (f_2) as indicated in Eq 15. According to FDA guidelines, values of f_1 between 0 and 15 and of f_2 between 50 and 100 ensure sameness or equivalence of the two dissolution profiles. In both equations, R_t and T_t represent dissolution measurements at P time points of the reference and test, respectively. [17]

$$f_1 = \{[\sum_{i=1}^{P} |R_t - T_t|] / [\sum_{i=1}^{P} R_t]\} \quad \ldots\ldots\ldots (14)$$

$$f_2 = 50 \log \{[1 + (1/P) \sum_{i=1}^{P} (R_t - T_t)^2]^{-1/2} * 100\} \ldots (15)$$

Estimation of swelling and erosion of microparticles

The microparticles were also evaluated for their swelling and erosion behavior to determine anomalous mode of diffusion of drug during dissolution[18]. The microparticle formulation (100 mg) was placed in 900 ml of double distilled water for 12 h and then weighed again after blotting out excess fluid on the surface of microparticles. It was dried at 40 ℃ for 48 h and then weighed. Swelling (%) and erosion (%) were determined using Eqs 16 and 17, respectively.

$$\text{Swelling (\%)} = S/R \times 100 \quad \ldots\ldots\ldots\ldots\ldots \quad (16)$$

$$\text{Erosion (\%)} = (T - R)/T \times 100 \quad \ldots\ldots\ldots \quad (17)$$

where T is the initial weight of the microparticles; S is the weight of the microparticle matrix after swelling; and R is the weight of the eroded matrix.

RESULTS

Embedment efficiency and Microparticle size

Microparticle drug embedment efficiency increased as drug:polymer ratio was varied from 1:1 to 1:2 to 1:3. Microparticles prepared with drug:polymer ratio of 1:3 showed optimum drug embedment (88.9, 90.0 and 90.9 % for M_1, M_2 and M_3, respectively). No significant change ($p < 0.05$) in drug embedment efficiency was observed with further increases in EC concentration, as shown by the data in Table 1.

Yield efficiency

Good yield efficiency (~ 90 %) was achieved for all batches. Increased polymer concentration did not increase yield significantly ($p < 0.05$) (see Table 1) [5].

Scanning electron microscopy

From the SEM results, the microparticles were irregular in shape with deep cracks on their rough surface (Figure 1).

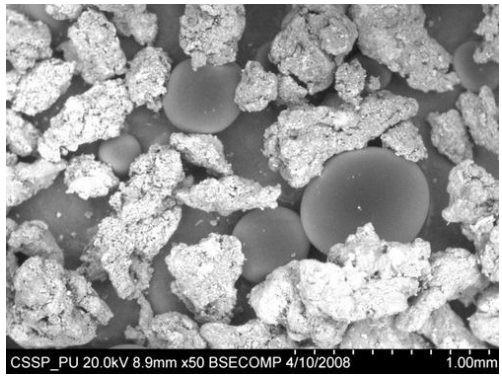

Figure 1: Scanning electron micrographs of diclofenac sodium microparticles with drug:polymer ration of 1:2 (M_2)

Fourier transform infrared spectroscopy

FTIR spectra in Figure 2 showed no degradation of drug during microencapsulation. Amino peak (N-H stretching) and C-Cl stretching appeared at 3320 and 768, respectively, representing DS in EC. Aromatic peak (C-H stretching) at 3020 confirmed the presence of EC. The nature of the peaks did not vary for pure drug or polymer and microparticles, indicating that there was no interaction between the drug and the polymer in the microparticles.

X-ray diffractometry

X-ray powder diffraction patterns of DS revealed its crystalline form. DS crystallinity decreased following its incorporation in the microparticles as indicated by the diffractograms shown in Figure 2.

Thermal analysis

The results of the thermal analysis evaluations are shown in Figure 3. They show that the thermograms of the pure drug and M_2 exhibited identical peaks (endotherms) at about 75 ℃, indicating that the drug was largely intact in the microparticles. This finding is essentially supported by the DTA thermograms. However, the slope is slightly more curved in

the M_2 endotherm, probably due the loss of drug crystallinity during microencapsulation as explained in the section on x-ray diffraction.

The DSC thermograms for for DS alone and DS-EC show an endothermic peak at 70 ℃ but with reduced peak sharpness for the latter, indicating slight variation in the crystallinity of the drug in the microparticles.

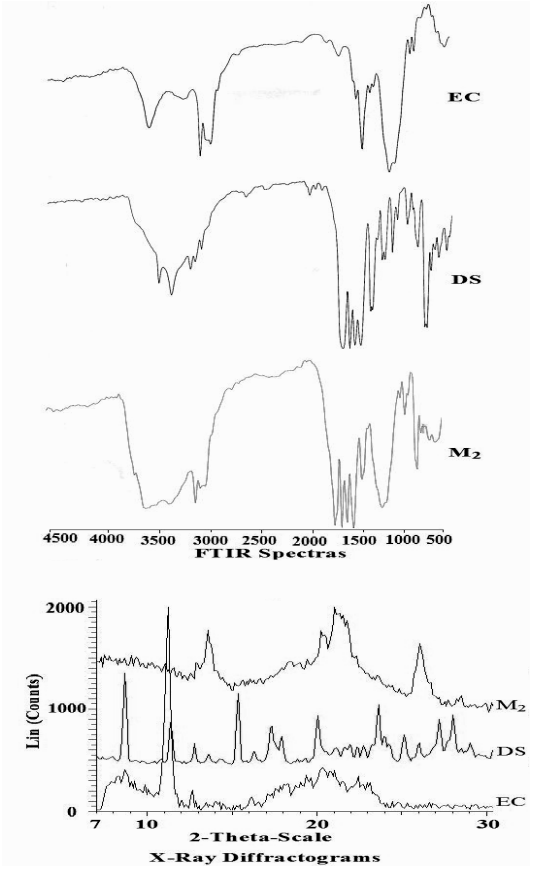

Figure 2: FTIR spectra and x-ray diffractograms of diclofenac sodium (DS), ethyl cellulose (EC) and microparticles with drug:polymer ratio of 1:2 (M2).

Micromeritic properties

Micromeritic data for the microparticles are listed in Table 1. Pure DS and the microparticles showed almost identical bulk and tapped densities. However, angle of repose and compressibility data indicate that the microparticles exhibited better flow properties the than the drug alone.

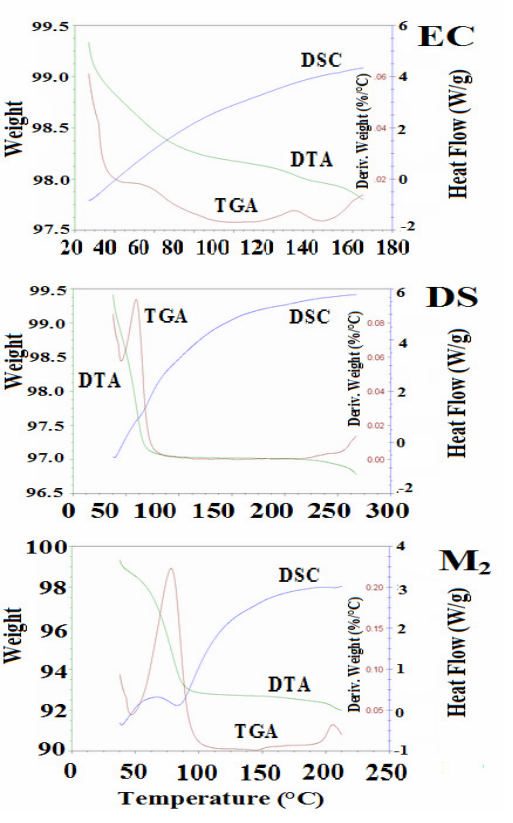

Figure 3: Thermograms of diclofenac sodium (DS), ethyl cellulose (EC) and microparticles with drug:polymer ratio of 1:2 (M_2).

In vitro dissolution studies

Figure 4 shows the *in vitro* release results for DS and microparticle formulations. Polymer concentration influenced drug release from the microparticles. Sixty percent drug release from pure DS, M_1, M_2 and M_3 was achieved in 0.12, 1.70, 2.06 and 2.33 h, respectively, thus indicating that the microparticle formulations resulted in sustained release of the drug.

Swelling and erosion of microparticles

Formulation M_2 undergoes swelling (101 to 138 %) and erosion (2.47 to 6.28%)

Table 1: Physicochemical and rheological properties of diclofenac sodium microparticles

		Pure diclofenac sodium	M_1	M_2	M_3
1	Drug: Polymer ratio	-	1:1	1:2	1:3
2	Embedment efficiency (%)	-	88.94	90.04	90.89
3	Yield efficiency (Mean ± SD) %	-	89.87±1.29	90.57±1.15	90.23±1.24
4	Size (Mean Diameter) (Mean ± SD) µm	-	389.62±14.15	410.94±09.64	442.19±18.15
5	$t_{60\%}$ (Mean ± SD) (h)	.12	1.70	2.06	2.33
Rheological properties of diclofenac sodium and its microparticles					
6	Bulk density (g/ml)	0.25	0.30	0.24	0.28
7	Taped density (g/ml)	0.22	0.24	0.29	0.27
8	Compressibility index (%)	9.72	12.43	11.09	12.93
9	Hausner's ratio	1.03	1.22	1.15	1.19
10	Angle of repose (θ°)	11.65	19.78	22.47	21.72

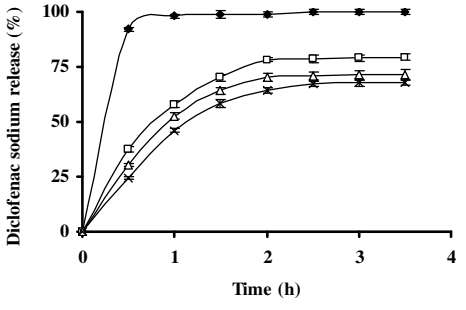

Figure 4: The dissolution profiles of diclofenac sodium microparticles showing the effect of polymer concentration. Each data point is a mean of three values. (-♦- = Pure diclofenac sodium; -□- = M_1; -Δ- = M_2; -x- = M_3)

continuously with time (0 to 12 h) after putting into dissolution apparatus.

Stability studies

No significant variation in the drug contents of the microparticles stored in 20 ℃/60% RH, 30℃/65% RH and 40 ℃/75% RH, was observed for eight weeks. Drug embedment efficiency and the release profiles did not vary during this period. Thus DS-EC microparticles showed good stability in these conditions. After 8 week storage, the drug contents of microparticles stored at 25 ℃/60% RH, 30 ℃/65% RH and 40 ℃/75% RH were 99.12±0.86%, 99.43±0.86% and 98.91±0.86%, respectively.

DISCUSSION

The results indicate that embedment efficiency varied with microparticle size. Large size microparticles had higher embedment efficiency. This may be due to increase in system viscosity with increase in EC concentration. Increased viscosity would cause adherence of a higher number of individual particles, thus resulting in larger microparticles. These results are, therefore, comparable with previous findings which also certify that there is an increase in embedment efficiency and particle size with the increase in EC concentration [5,6]. Magnetic stirring speed and its duration did not affect embedment efficiency. However, prolonged stirring at higher speed produced microparticles with low size.

The virtual lack of change in the thermal behavior and FTIR spectra of DS after inclusion in the microparticles indicates probable absence of drug-polymer interaction. This finding therefore, indicates good stability of DS in DS-EC microparticles. Furthermore, formulation of the drug in microparticle form improved its packability and flow characteristics, which in turn should facilitate handling.

Microparticles with low polymer concentration released drug quickly probably because they

were smaller in size and, therefore, provided a larger surface area for drug release based on the modified Noyes-Whitney equation18 shown in Eq 18

$(dc/dt) = kS (Cs – Ct)$ …… (18)

where dc/dt is the rate of dissolution, k is the dissolution rate constant, S is the surface area of the dissolving body and Cs – Ct is the concentration gradient.

Drug release from the microparticles were also evaluated by mathematical kinetic models. From Duncan test, $t_{60\%}$ of all microparticle batches was in the same homogenous group (M_1=M_2=M_3) whereas Tukey H.S.D. similarized $t_{60\%}$ of M_1 and M_2, and differentiated them from that of M_3 but not significantly ($p > 0.05$). Based on the difference factor (f_1) and similarity factor (f_2), the release profiles of the following microparticle formulations were different from each other: M_1 vs M_3 with $f_1 > 15.0$ and $f_2 < 50.0$, while M_1 vs M_2 and M_2 vs M_3 have $f_1 < 15.0$ and $f_2 > 50.0$ which indicates the mutual similarity of the compared release profiles but to a much less extent. With a decreasing core to wall ratio, the velocity of drug release would decreases since it can be assumed that with decreasing core to wall ratios, wall thickness of microparticles increases which then slows down the diffusion of the dissolution medium into the microparticles. This is because the number of surface pores would decrease with increasing polymer concentration [9,19].

The Higuchi model best explained the drug release pattern, i.e., release of drug is directly proportional to the square root of time with R^2 value of 0.973. Anomalous diffusion was the mechanism of drug release from microparticles. Subjection of the release data to the Hixson-Crowell equation indicated a change in surface area and diameter of the formulations with progressive dissolution of the matrix as a function of time. The slight swelling and moderate erosion of the microparticles is likely to be responsible for the slow release of drug from the matrix of the micropparticles. It also supports anomalous diffusion of DS from the microparticles.

CONCLUSION

This study demonstrates that non-solvent addition phase separation is a useful technique for the microencapsulation of diclofenac sodium with ethylcellulose for sustained release. Characterization studies indicate that interaction of the components of the microparticles was physical, and that there was no chemical interaction between the drug and the polymer.

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