Sustained Release Nimesulide Microparticles: Evaluation of Release Modifying Property of Ethylcellulose

[1]SHUJAAT ALI KHAN, [1]MAHMOOD AHMAD*, [2]GHULAM MURTAZA, [1]MUHAMMAD NAEEM AAMIR, [1]NISAR-UR-REHMAN AND [1]ASAD ULLAH MADNI
[1]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, 63100-Pakistan.
[2]Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan.

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Summary: Microencapsulated controlled-release preparations of nimesulide were formulated. Microparticles were prepared by modified phase separation (non-solvent addition) technique using different ratios of ethylcellulose. The microparticles (M_1, M_2, and M_3) were yellow, free flowing and spherical in shape with the particle size varying from 93.62 ± 14.15 to 104.19 ± 18.15 µm. The $t_{60\%}$ of nimesulide release from microparticles was found to be 3 ± 0.6, 5 ± 0.6 and 8 ± 0.8 h for formulations M_1, M_2, and M_3, respectively. FT-IR, XRD, and thermal analysis were done which showed that there is no interaction between the polymer and drug. The mechanism of drug release from nimesulide microparticles was studied by using Higuchi and Korsmeyer-Peppas models. The value of coefficient of determination (R^2) for M_1, M_2, and M_3 indicates anomalous and case-II transport release mechanism. The dissolution data of designed system verified its ability to maintain plasma concentration without the need of frequent dosing. The Nimesulide microparticles prolonged drug release for 12 hours or longer. Based on the results of release studies, M_3 was opted as a suitable microparticulate formulation allowing the controlled release of nimesulide over a prolonged period of time. Moreover, its encapsulation efficiency was also comparable to the other two formulations (M_1 and M_2). In conclusion, the influence of polymer concentration should be considered during formulation development.

Introduction

Nimesulide (Nim), chemically 4-nitro-2-phenoxy methane sulfonanilide, is a weakly acidic nonsteroidal anti-inflammatory drug. It differs from other nonsteroidal anti-inflammatory drugs (NSAIDs) in that its chemical structure contains a sulfonanilide moiety as the acidic group rather than a carboxylic group (Fig. 1). Nimesulide shows high analgesic, antipyretic, and anti-inflammatory activities in addition to low toxicity, a moderate incidence of gastric problems, and a high therapeutic index [1, 2]. *In vitro* studies showed that nimesulide is a poor inhibitor of prostaglandin synthesis and appears to exert its effect through various mechanisms including free radical scavenging, effects on histamine release, the neutrophil myeloperoxidase pathway, bradykinin activity, cartilage degradation, metalloprotease synthesis, phosphodi-esterase type IV inhibition, platelet aggregation, and synthesis of platelet activating factor. It also exhibits a significant selectivity toward cyclooxygenase-2 (COX-2) versus COX-1 inhibition, which may explain the lower incidence of gastric side effects. However, recent findings reported that nimesulide has a higher risk of hepatic toxicity when compared to other marketed NSAIDs. Like many non-steroidal anti-inflammatory drugs, nimesulide is very sparingly soluble in water (≈ 0.01 mg/mL) [3].

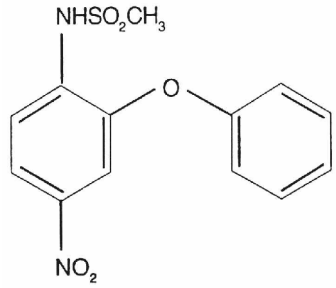

Fig. 1: Chemical structure of nimesulide.

Microencapsulation may be defined as the process of surrounding or enveloping one substance within another substance on a very small scale, yielding capsules ranging from less than one micron to several hundred microns in size. Microcapsules may be spherically shaped, with a continuous wall surrounding the core, while others are asymmetrically and variably shaped, with a quantity of smaller droplets of core material embedded throughout the microcapsule. All three states of matter (solids, liquids, and gases) may be microencapsulated. This allows liquid and gas phase materials to be handled more easily as solids, and can afford some measure of protection to those handling hazardous materials.

*To whom all correspondence should be addressed.

There are almost limitless applications for microencapsulated material. Micro-encapsulated materials are utilized in agriculture, pharmaceuticals, foods, cosmetics, and fragrances, textiles, paper, paints, coatings, and adhesives, printing applications, and many other industries.

In the present study we have used ethylcellulose (Fig. 2) as retardant material, because it is a compatible non-biodegradable polymer and widely used in pharmaceutical industry.

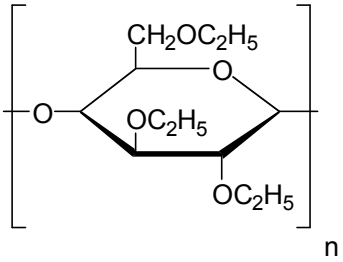

Fig. 2: Chemical structure of Ethylcellulose.

Results and Discussion

Evaluation of physical characteristics of nimesulide microparticles is presented in Table-1. The release studies of all microparticles (M_1 to M_3) were carried out by dissolution data in phosphate buffer pH 6.8. It is calculated as cumulative percent drug release and compared with conventional tablet (Fig. 3). The cumulative percentage drug release in the formulation M_1, M_2, M_3 and conventional tablet (Con. tab.) was found to be 97.70 ± 1.35, 92.85 ± 1.21, 87.24 ± 1.13 and 97.78 ± 0.52, respectively. The drug release from conventional tablet was exhausted almost completely within 4 h while microparticles sustained the drug more than 12 h. Formulation M_1 liberated 60% of its drug content within 2.73 hr, formulation M_2 in 3.82 hr ,formulation M_3 in 6.25 hr and conventional tablet in 2 hr.

Table-1: Evaluation of physical characteristics of nimesulide microparticles. Each measurement was carried out in triplicate.

Formulations	M_1	M_2	M_3
Drug:Polymer ratio	1:1	1:2	1:3
Entrapment (%)	96.48	96.92	97.48
Production yield (M ± S.D)%	96.24 ± 1.47	97.12 ± 1.47	97.95 ± 1.36
Size (Mean Diameter) (M ± S.D) µm	93.62 ± 14.15	97.94 ± 9.64	104.19 ± 18.15
$t_{60\%}$ (M ± S.D) (hrs)	2.73 ± 0.6	3.82 ± 0.6	6.25 ± 0.8

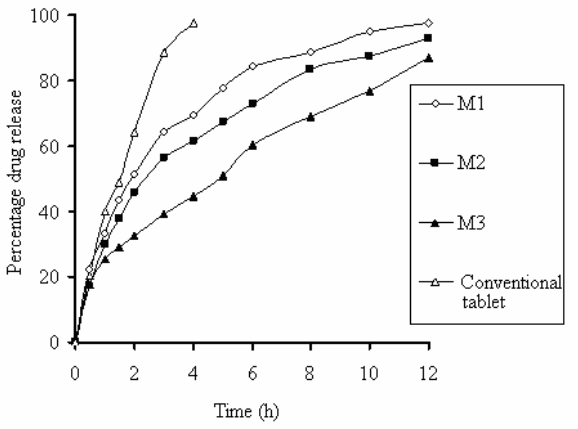

Fig. 3: Effect of various ethylcellulose concentrations on nimesulide release from micro-particles. Each data point is a mean of three values.

In order to describe the kinetics of drug release from controlled-release preparations, various mathematical equations have been proposed. The zero order model Eq. (i) describes the systems, where the drug release is independent of its concentration [4]. The first order equation Eq. (ii) describes the release from systems, where release rate is concentration dependent [5]. According to Higuchi model Eq. (iii), the drug release from matrix is directly proportional to a square root of time and is based on the Fickian diffusion [6]. The Hixson-Crowell cube root law Eq. (iv) describes the release from the systems, where it depends on the change in surface area and diameter of the particles or tablets with time and mainly applies in case of systems, which dissolute or erode over time [6]. A more comprehensive, but still very simple, semi-empirical equation to describe drug release mechanism from polymeric systems more precisely is the so-called Korsmeyer-Peppas power law, *i.e.* Eq. (v). Thus, drug release data were fitted to these kinetic models to explain the drug release kinetics and mechanism from the microparticles prepared. Criterion of selecting the most appropriate model was based on the best goodness of fit. The values of the release exponent (n), and correlation coefficient (R^2) as calculated from Eqs. (i-v) are presented in Table-2.

It was found that the *In vitro* drug release of M_1, M_2 and M_3 microparticles were best explained by Higuchi's equation, as the plots showed the highest linearity (R^2 = 0.9666, 0.983 and 0.9938, respectively, for M_1, M_2 and M_3), followed by zero order (R^2 = 0.8999, 0.8999 and 0.8999, respectively) and first order (R^2 = 0.3778, 0.4116 and 0.4641, respectively). This explains why the drug diffuses at

a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (or Higuchi's kinetics). However, drug release was also found to be very close to zero-order kinetics, indicating that the concentration was nearly independent of drug release.

Table-2: Release rate parameters for release data after fitting of the whole release profiles of nimesulide microparticles into different mathematical models.

Models	Formulations	M_1	M_2	M_3
Zero Order	Y-equation	7.1331x + 29.146	6.8763x + 24.03	6.3924x + 16.197
	R^2	0.8078	0.8513	0.9435
First Order	Y-equation	0.1999x + 2.8788	0.2058x + 2.7388	0.2078x + 2.5411
	R^2	0.3778	0.4116	0.4641
Higuchi	Y-equation	29.314x + 6.3748	27.76x + 3.002	24.647x +1.2052
	R^2	0.9666	0.983	0.9938
Hixson-Crowell	Y-equation	-0.2613x + 4.276	-0.2117x + 4.331	-0.1707x + 4.466
	R^2	0.9723	0.9708	0.9862
Korsmeyer-Peppas	Y-equation	0.5159x + 3.5508	0.4944x + 3.4362	0.4882x + 3.1751
	R^2	0.9868	0.9863	0.9828
	n	0.52	0.49	0.49

Where,
Y-equation = (Y=aX + b)
"a" and "b" is the Y-intercept and slope, respectively.
Y and X are Y-axis and X-axis, respectively.
R^2 = Determination co-efficient
N = Release exponent

The R^2 values for Hixson- Crowell model were slightly higher than the other models, showing a better conformance to this model. Applicability of the release curves to Hixson-Crowell model indicated a change in surface area and diameter of the microparticles, with a progressive dissolution of the matrix as a function of time.

The corresponding plot (log cumulative percent drug release vs time) for the Korsmeyer-Peppas equation (v) indicated a good linearity (R^2 = 0.9868, 0.9863, and 0.9828, respectively).The values of *n* determined for ethylcellulose microparticles studied ranged from 0.49-0.52 and confirmed that the formulations followed non-Fickian diffusion kinetics (anomalous transport), *i.e.* the release is ruled by both diffusion of the drug and dissolution of the polymer. They explained that increasing the polymer content decreases the release rate. This reduction of the release constant increases the time needed to release a given quantity of drug, allowing a greater hydration and relaxation of the polymer matrix before release, which in turn shift the release mechanism toward relaxation-erosion. This study has comparable results to the work done by Cheu *et al.* (2001).

The flow ability of microparticles was determined in terms of angle of repose and compressibility index (Table-3) for its application in fabrication of sustained products. The angle of repose was found to be in the range of 24.38° to 28.32° and compressibility indices in the range of 11-17% suggesting good flow ability of particles [9].

Table-3: Micromeritic studies of microparticles.

Formulations	M_1	M_2	M_3
Bulk density (g/mL)	0.265	0.279	0.310
Tap density (g/mL)	0.298	0.332	0.373
Hausner's ratio	1.123	1.190	1.204
Compressibility index (%)	11	16	17
Angle of repose (degree)	28.32	26.67	24.38

FT-IR studies indicate that bands present in the nimesulide spectrum, namely: C-H aromatic, S=O, C-O-C ether linkage, CH_3 C-H bending, NO_2, CH_3 C-H stretching, and N-H, respectively. The same bands were also found in the spectra of the formulations, showing that no drug-polymer interaction occurred (Fig. 4).

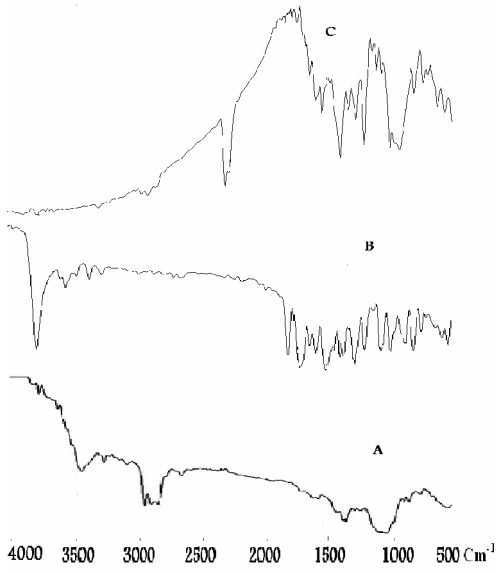

Fig. 4: FT-IR spectras of (A) EC, (B) nimesulide and (C) microparticles (M2).

X-ray diffraction patterns of nimesulide, ethylcellulose and nimesulide loaded microparticles in Fig. 5 confirm the presence of crystalline structure of Nimesulide in the microparticles. Nimesulide being entrapped into the ethylcellulose offers different preferred orientation resulting in a different pattern yet confirming its presence as a crystalline substance. The grinding of the drug loaded microparticles prior to the analysis during sample preparation may also have affected its XRD pattern.

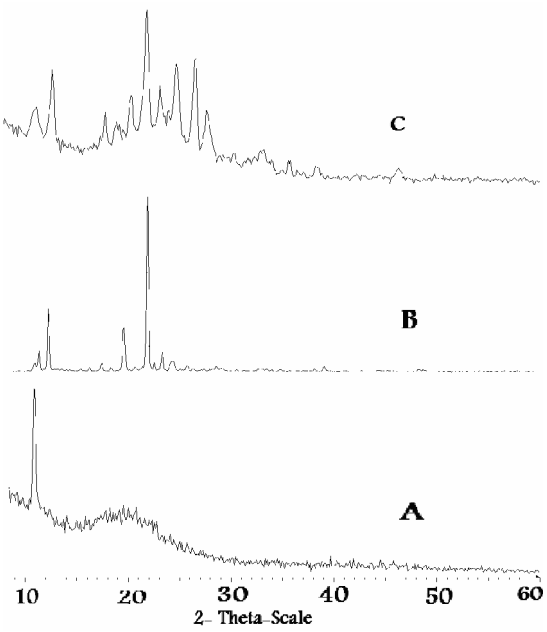

Fig. 5: XRD profiles of (A) EC, (B) nimesulide and (C) microparticles (M2).

DSC thermogram of pure nimesulide showed an endothermic depression between 145 to 150 °C. The thermograms of the microparticles M_1 to M_3 also showed similar endothermic depression between 145-150 °C. This further confirms that there was no drug polymer interaction (Fig. 6).

In order to make stable sustained product, the stability studies showed that microparticles are stable at 25 ± 1 °C (RT) and 40 ± 1 °C (HT). The percentage residual drug content of the M_1, M_2, and M_3 microparticles are shown in Table-4 at 25 ± 1 °C to 40 ± 1 °C after 1, 2, and 3 months of storage. Non-significant ($p>0.05$) differences in percent residual drug content were observed at ambient and accelerated storage conditions in the prepared formulations indicating all formulations were stable under the stability studies.

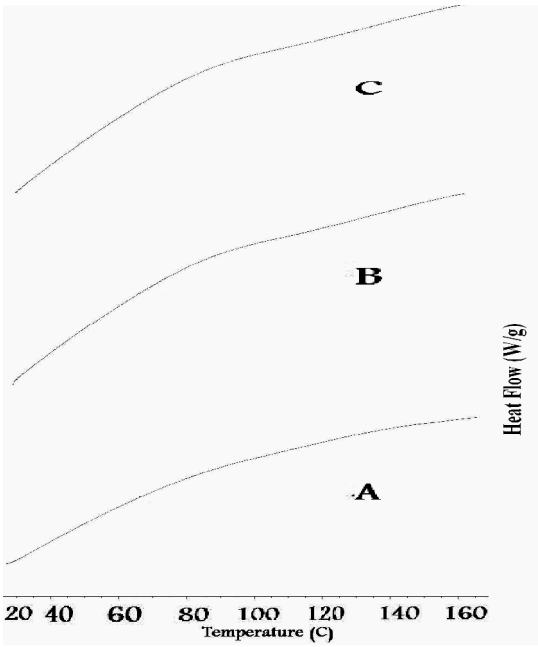

Fig. 6: Thermal analysis (DSC) of (A) EC, (B) nimesulide and (C) microparticles (M2).

Table-4: Percentage of nimesulide after stability studies of microparticles at 25 ± 1 (RT), 40 ± 1 (HT) °C.

Storage (Months)	Study at 25 ± 1 °C(RT) Percentage of Nimesulide			Study at 40 ± 1 °C(HT) Percentage of Nimesulide		
	M_1	M_2	M_3	M_1	M_2	M_3
0	100	100	100	100	100	100
1	98.74	98.20	98.40	98.51	98.10	98.26
2	98.48	98.43	98.14	98.21	97.79	97.85
3	97.75	98.00	97.40	97.58	97.59	97.59

Experimental

Material

Nimesulide B.P. received as gift sample from Pharmevo Pharma (Pvt) Ltd. Karachi Pakistan. Ethyl cellulose was purchased from Sigma, USA. The chemicals such as toluene, liquid paraffin (heavy and light) and *n*-hexane, were of analytical grade supplied by Merck, Germany.

Preparation of Microparticles

Microparticles of nimesulide with ethylcellulose were prepared by non solvent addition-phase separation technique with modification as proposed previously [10].

The specified amount of ethylcellulose was dissolved in 50 mL of toluene solution. Nimesulide was added into this solution. At a constant stirring speed of 700 rpm, a 100 mL of non-solvent liquid

paraffin was added drop wise slowly to the nimesulide and ethylcellulose solution in toluene. During the addition of liquid paraffin, nimesulide and ethylcellulose were co-precipitated out to form microparticles. At the end microparticles were filtered through vacuum pump using 0.45 micron filter disk, washed thrice with *n*-hexane (200 mL) and vacuum dried at room temperature to ensure complete removal of solvents used.

Characterization of Microparticles

In vitro Drug Release of Microparticles

In vitro drug release of various microparticles was determined using automatic apparatus II USP (Pharma Test, Germany) in 900 mL of phosphate buffer (pH 6.8). 5 mL of sample was collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, and 12 h with an automated sample collector after filtering through 10 μm Sinter filters. All samples were analyzed as such at 404 nm (11) using a UV-spectrophotometer (Shimadzu 1601, Japan). Percentage drug release at different sampling intervals was calculated.

Particle Size Analysis

Microparticles prepared were sieved through a combined sieve system (British Standard) and grouped based on particle size range. Selected fractions were used for *in vitro* dissolution.

Percentage Yield

The yield was calculated as the weight of the microparticles recovered from each batch divided by the initial total weight of drug plus polymer used to prepare that batch and multiplied by 100 [12, 13].

Drug Content Estimation

Drug loaded microparticles (100 mg) were powdered and suspended in 100 mL methanol. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 μm membrane filter. The drug content was determined spectrophotometrically (Shimadzu, 1601, spectrophotometer) at 404 nm using a regression equation derived from the standard graph ($r^2 = 0.998$) [12, 13].

Determination of Drug Entrapment Efficiency

A weighed quantity (100 mg) of microparticles was extracted with 50 mL methanol using sonication. The drug content was spectrophotometrically assayed at 404 nm [8] using a UV spectrophotometer (Shimadzu 1601, Japan). Each determination was carried out in triplicate. The encapsulation efficiency was calculated as percentage of the theoretical content.

Drug free microparticles, prepared exactly in the same manner, were used as reference.

The encapsulation efficiency (%) was determined by the following equation:

Encapsulation efficiency (%) = (Da/Dt) × 100
where Da = actual drug loading and Dt = theoretical drug loading

Micromeritics Studies

Tap Density

Tap density was measured by employing the conventional tapping method using 10 mL measuring cylinder and the number of tappings was reduced to 100 as it was sufficient to bring about a plateau condition. Tap density was calculated by the following formula:

$$T.D. = Wt / Vol.$$

where T.D. = Tap density, Wt = Weight of microparticles and Vol. = Volume of microparticles after 100 tappings.

Compressibility Index

Compressibility index (Ci) was calculated by the following formula:

$$Ci = \{(V_1 - V_2) / V_1\} \times 100$$

where V_1 = Initial volume and V_2 = Final volume

Hausner's Ratio

Hausner's ratio, another index of flowability of microcapsules, was calculated by the following formula:

$$\text{Hausnner's ratio} = V_1 / V_2$$

where V_1 =Volume before tapping and V_2 =Volume after tapping

Angle of Repose

Angle of repose was measured by passing microcapsules through a funnel on the horizontal surface. The height of heap formed (h) and radius of the cone base (r) were determined. The angle of repose (θ) was calculated by the following formula [13]:

$$\theta = \tan^{-1} h / r$$

Results are given in Table-1.

Swelling Studies

The swelling characteristics of microparticles were determined by immersing them in the dry state into a buffer solution at pH 6.8 (phosphate buffer) at a temperature of 37 °C. At specific time intervals, the samples were removed from the swelling medium and were blotted with a piece of paper towel to absorb excess water on the surface. The degree of swelling, S(t), at anytime was calculated using the following expression:

$$S(t) = (Wt - W_0)/W_0$$

where, Wt and W_0 are the sample weights at time t and in the initial dry state, respectively.

It is worth mentioning that preliminary experiments indicated that possible soluble fraction (sol) did not leach out of gels during swelling. It was confirmed by constant dried weight of samples before and after equilibrium swelling.

Drug Polymer Interaction Studies

Fourier Transform Infrared Spectrophotometer (FT-IR)

In order to study the interaction between nimesulide and ethylcellulose, FT-IR studies were performed using KBr pellet press method. Infrared spectra of Nim, ethylcellulose and nimesulide loaded microparticles were determined in the range of 4000 to 500 cm^{-1}using a Fourier-transform infrared spectrophotometer (FT-IR) (model FT-IR-8400 S Shimadzu, Japan) from KBr pellets.

Thermal Analysis

Thermal analysis [differential scanning calorimetry (DSC)] of microparticles and its individual components was conducted by using TA Instruments SDT Q600. Accurately weighed samples were heated on alumina pan at a constant rate of 20 °C/min under a nitrogen flow of 40 mL/min.

X-Ray Diffractometry Studies (XRD)

Samples of nimesulide, ethylcellulose and nimesulide loaded microparticles were prepared by pulverizing in a mortar. The XRD patterns of samples were recorded by using a Philips PW 1830 X-ray diffractometer to find out any change in the crystallinity of drug during microencapsulation.

Scanning Electron Microscopy (SEM)

The surface morphology of microparticles was performed by a scanning electron microscopy (Hitachi S-3000, Japan). Microparticles were mounted on stubs and coated for 120 s with a layer of gold using a sputter coater. The size of microparticles was determined by an optical microscopy using a calibrated ocular micrometer (Erma, Japan).

Stability Studies

Stability studies were conducted to find out stable product under storage. The microparticles were stored in amber glass bottles at 25 ± 1 °C (RT) and 40 ± 1 °C (HT) for a period of 3 months and observed for change in drug content and morphology, *i.e.* shape and structural integrity, by microscopic and scanning electron microscopy.

Release Kinetics

Data obtained from *in vitro* release studies were fitted to various kinetics equations [6] to find out the mechanism of drug release from microparticles. The kinetics models used were zero order, first order, Higuchi models, Hixson-Crowell and Korsmeyer-Peppas. The rate constants were also calculated for the respective models.

Zero Order Model [14]:

$$M_t = M_o + K_o t \quad \text{(i)}$$

First Order Model [14]:

$$\ln M_t = \ln M_o + K_1 t \quad \text{(ii)}$$

Higuchi Model [15]:

$$M_t = M_o + K_H t_{1/2} \quad \text{(iii)}$$

Hixson-Crowell Model [15]:

$$M_o^3 - M_t^3 = K_{HC} t \quad \text{(iv)}$$

Korsmeyer-Peppas Model [16]:

$$M_t/M_\alpha = K_k t^n \quad \text{(v)}$$

In these equations, 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 equation (v), M_t/M_α is the fraction of drug release at time t and n is the release exponent that characterizes different release mechanisms. The n-value is calculated from the slope of Korsmeyer-Peppas plot.

Conclusion

The nimesulide microparticles prolonged drug release for 12 h or longer. Based on the results of release studies, M_3 was opted as a suitable microparticulate formulation allowing the controlled release of nimesulide over a prolonged period of time. Moreover, its encapsulation efficiency was also comparable to the other two formulations (M_1 and M_2). In conclusion, the influence of polymer concentration should be considered during formulation development. No drug-polymer interaction was found as confirmed by FT-IR, XRD and thermal analysis. Nimesulide remained stable up to the duration monitored *i.e.* 3 months. The ethylcellulose could be a suitable polymer to give sustained release formulation of nimesulide. Such microparticles can also provide the flexibility to develop suitable dosage form for the desired period of drug delivery as compared to available sustained release formulation in the market. Thus, it may improve safety, efficacy and patient compliance as compared to the conventional tablets.

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