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MICROPARTICULATE DRUG DELIVERY SYSTEM OF DICLOFENAC SODIUM; FORMULATION DEVELOPMENT AND IN-VITRO EVALUATION

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ABSTRACT

The aim of present study was to investigate the dissolution profile and release kinetics of modified release diclofenac sodium microspheres containing ethyl cellulose and eudragit L100. The influence of these polymers on drug release mechanism was characterized. Five formulations were prepared by using different amounts of polymers alone as well in combination by solvent evaporation method. FT-IR was used to detect interactions between polymers and active drug molecule. Scanning electron microscopy was used to observe the shape and surface morphology of the prepared microspheres. Sieving was used to find average particle size. Preformulation studies were conducted to find out the flow properties and finness of the developed formulations. Moisture content, drug entrapment efficiency and percent yield were evaluated of formulated microspheres. F1 formulated in the combination of Eudragit L100 and Ethyl cellulose showed better retarding effects during invitro drug release study. Upon application of statistical models, it was found that drug release mechanism was non Fickian diffusion following Korsmeyer peppas model. Finally, it was concluded that Eudragit L100 and Ethyl cellulose were appropriate polymers to prepare microspheres of diclofenac sodium as modified release drug delivery system.

Keywords: Diclofenac sodium, Ethyl cellulose, Eudragit L100, FT-IR, Scanning electron microscopy

INTRODUCTION

Microspheres or micro particles are small free flowing powders made up of drug molecule and suitable polymers and have size in the range of 50nm to 2mm or 1µm-1000µm. In microspheres, entrapped material is surrounded by a distinct capsule wall (1) Microencapsulation is a technique in which solid, liquid and gas particles are coated with a fine thin layer of polymeric material. Diclofenac sodium loaded microspheres are advantageous to obtain a steady state concentration of drug in blood, hence increasing patient compliance, reducing dose, toxicity and to give protection to entrapped drug from environmental and enzymatic degradation (2). Microspheres are taken as an excellent participant for controlled drug delivery system in the form of sustained release dosage forms. As compared to other oral pharmaceutical sustained release formulations the individuality of microspheres lies in the fact that these formulations are widely spread in the gastrointestinal tract thus minimizing damage to gastrointestinal mucosa due to localized accumulation of drugs. It is possible to deliver lower quantities of drug for diagnostic or therapeutic purposes entrapped within a polymeric structure for oral as well as intravenous delivery of drugs and prolong the retention time of drug at target site (3, 4). Diclofenac sodium was taken as a model drug and belongs to NSAIDS. It showed excellent oral absorption and plasma protein binding. It prevents production of prostaglandins and thus exerts its analgesic action and inhibits leukocyte formation to induce anti-inflammatory action. It is tremendously recommended to reduce and overcome signs and symptoms of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (5). A no of polymers are used in sustained release drug delivery system. Ethyl cellulose is advantageous because of its water retarding hydrophobic nature and antioxidant property. Eudragit L100 is an anionic polymer and has more swell ability than ethyl cellulose (6). The purpose of current study was to prepare controlled release microspheres of diclofenac sodium using ethyl cellulose and eudragit L100 by emulsion solvent evaporation method. Polymers were used alone as well as polymeric blends in different amounts. A single unit oral dosage form was of 250mg per day.

MATERIALS AND METHODS

Materials

Diclofenac sodium was used as model drug and gifted by Highnoon Pharmaceuticals Pvt. Lahore, Pakistan. Eudragit L100, Ethyl cellulose and Magnesium stearate were

obtained as gift from Nova med Pharmaceuticals Pvt. Lahore Pakistan. Methanol, Acetone, Chloroform, N-hexane, Span 20, Liquid paraffin and Petroleum ether were purchased from Aptcure Pharmaceuticals Pvt. Lahore, Pakistan. All chemicals used were of analytical grade.

Methods

Preparation of Diclofenac sodium loaded microspheres

Diclofenac sodium loaded microspheres were prepared using ethyl cellulose and eudragit L100 alone and in combination by emulsion solvent evaporation method. According to the formulation design, accurately weighed amounts of ethyl cellulose and eudragit L100 (1:1) were dissolved one after one in a mixture of acetone and chloroform (3:1) with continuous stirring. 1gm of pure Diclofenac sodium was dissolved in 10ml methanol. The drug mixture was then dispersed slowly in the polymer mixture with continuous stirring by using magnetic stirrer at room temperature. The drug polymer mixture was added drop wise with continuous stirring in 1000ml beaker containing 250ml light liquid paraffin mixed with 15ml n-hexane on silver son mixer at 500rpm. About 150mg magnesium stearate was sprinkled in the above mixture and speed of mixer increased up to 700rpm.The mixture was agitated for 3hours at 300—700 rpm. After evaporation of chloroform, the formed microspheres were filtered by Whatmann no.1 filter paper and washed 3 times with 30ml petroleum ether to remove oil from filtered microspheres. The collected microspheres were dried under shade at room temperature for 24 hours. The dried microspheres were evaluated for different tests (7).

Table 1: Composition of Diclofenac sodium loaded Microspheres

Serial No.	Formulations	Diclofenac sodium (gm)	Polymers (EudragitL100: Ethyl cellulose) (gm)	Drug:Polymer
1	F1	1	0:1	1:1
2	F2	1	1:0	1:1
3	F3	1	0.5:0.5	1:1
4	F4	1	1:1	1:2
5	F5	1	1.5:1.5	1:3

In-vitro evaluation of prepared microspheres containing Diclofenac sodium

Determination of calibration curve of Diclofenac sodium

In order to construct a calibration curve of Diclofenac sodium, a standard stock solution of Diclofenac sodium with concentration of 1mg/ml was prepared by dissolving drug in

distilled water. Using stock solution, serial dilutions of 5, 10, 15, 20, 25, 30 and 35 µg/ml were prepared and the analyzed by using UV-visible double beam spectrophotometer at 276nm.

Micromeritic Studies of the formulated Microspheres of Diclofenac sodium

Particle size analysis

The microspheres of each formulation were allowed to pass through a set of arranged sieves. The sieves were shaken manually for 10-15 minutes by side wise and bottom tapping so that all samples might transfer through all sieves. After complete shaking, the average particle size (µm) was determined by following formula: (8)

$$\text{Average size} = \frac{\text{Cumulative percent weight retained}}{100}$$

Bulk density

1.5gm of microspheres was taken in a graduated cylinder. Noted the volume and determined its bulk density by using following formula:

$$\text{Density} = \frac{\text{mass}}{\text{volume}}$$

Tap density

1.5gm of microspheres was taken in a graduated cylinder and tapped them 100 times. Noted the volume before and after tapping and calculate tapped density as following:

$$\text{Tap density} = \frac{\text{mass}}{\text{volume after tapping}}$$

Compressibility index

It is the parameter which is used to measure the flow ability of the material and can be calculated as follows

$$\text{C.I} = [\text{Vb} - \frac{\text{Vt}}{\text{Vb}}] \times 100$$

Where, Vb. = volume before tapping and Vt. = volume after tapping

Compressibility index is also called as Carr's index.

Angle of repose

A dry steel funnel was taken and accurately weighed 1gm microspheres were allowed to fall on a clean and dry paper. The keef was removed and determined height and diameter of the pile. Angle of repose was determined by following formula (9);

$$\text{Angle of repose} = 1/\tan(h/r)$$

Where h = height of heap and r = diameter of heap

Flow rate

1gm of microspheres after weighing were transferred in a dry funnel and noted the time of flow of microspheres from the funnel on a dry, clean paper and determined its rate of flow by following formula (10);

Flow rate: Mass/time

Coefficient of friction

Coefficient of friction was determined to observe flow ability of the microspheres. The value of tanθ was taken during measurement of angle of repose (11);

$$u = \tan\theta$$

Where, u < 1 indicated good flow and u > 1 indicated poor flow

Hausner's ratio

It is a ratio of tapped density and bulk density (12);

$$\textbf{Hausner's ratio} = \textbf{Tapped Density / Bulk Density}$$

Swelling index

100ml distilled water was taken in measuring flask. Weighed 15mg of formulated microspheres and transferred in beaker and kept it for 24 hours. After 24 hours, filtered the microspheres, kept for about 5 minutes and weighed them. Swelling index was determined as (13);

$$\textbf{Swelling index} = \frac{\textbf{Wet weight} - \textbf{dry weight}}{\textbf{dry weight}} \times 100$$

% Moisture content

1.5 grams of microspheres were placed in dry oven at a temperature of 105°C for 2 hours (14).

$$\%\ \textbf{Moisture content} = \frac{\textbf{Wi} - \textbf{Wf}}{\textbf{wi}} \times 100$$

Where, wi = initial weight and wf = final weight

Determination of drug content

100mg of microspheres were crushed by using pestle and mortar and was soaked in 60ml of phosphate buffer (pH6.8) for 24 hrs.to obtain a solution. The solution was sonicated for 10 minutes and filtered. Drug content was determined by observing absorbance on UV-Visible spectrophotometer [UV-1900, BMS, and Canada] against standard at 276nm. (15).

$$\%\ \textbf{drug content} = \frac{\textbf{Weight of drug}}{\textbf{Weight of microspheres}} \times 100$$

Drug entrapment efficiency

1gram of microspheres were weighed and ground in pestle and mortar. About 5 mg of the powder was dissolved in 50 ml of phosphate buffer pH 6.8. After complete one day, 1.2ml of filtered solution was pipette out and diluted to 12ml with phosphate buffer (pH 6.8). Absorbance was measured using UV-Visible spectrophotometry at 276nm.

$$\textbf{Drug entrapment efficiency} = \frac{\textbf{Act amount of drug}}{\textbf{Theoretical amount of drug}} \times 100$$

Percent yield

1gm prepared microspheres from each batch were weighed and percentage yield for each batch was determined by following formula.

$$\%\ \textbf{yield} = \frac{\textbf{Weight of microspheres}}{\textbf{total weight of drug and polymer in each batch}} \times 100$$

In-vitro drug release studies

In-vitro drug release was determined by using dissolution USP Type 1 Basket Apparatus at 37±5°C first in 900 ml of 0.1NHCl for 2 hours and then in 900ml pH 6.8 phosphate buffer as dissolution medium for 10hours. About 0.18g of microspheres (equivalent to 100 mg of Diclofenac sodium) were taken and placed in basket. The apparatus rotated at 100rpm for 12 hours. A sample of 5ml was taken after suitable time intervals and the 5ml of fresh medium was added to dissolution medium in order to maintain the total volume 900ml. The sample was filtered and diluted up to 25ml with dissolution medium and absorbance of the sample was measured at 276nm by using

UV-Visible spectrophotometer. Find out percent amount of drug release at each interval by comparing sample absorbance with standard (pure drug) using following formula (16);

$$\% \text{ drug release} = \frac{\text{absorbanc of sample}}{\text{absorbance of standard}} \times 100$$

Release kinetic modeling

Kinetic models were applied to predict in vivo performance of drugs by using in vitro dissolution data because quantitative and qualitative modifications in formulation may alter drug release and drug performance in body (17). For Controlled release formulations following models (curve fitting) are commonly used on in-vitro dissolution data.

Where Ft is the amount of drug dissolved at time t and k_o, Kt, K_H, Ks, a, is zero order, first order, Higuchi, Hixson, Korsmeyer rate constants respectively. 'F' is the fraction of the drug dissolved and W_o is the initial amount of drug and 'n' is the release coefficient from Korsmeyer Peppas demonstrating the drug release mechanism (18).

Scanning electron microscopy (SEM)

Scanning electron microscope with resolution power of (JSM 7500 S-JEOL JAPAN) was used to determine shape and surface morphology. It is an electron microscope that gives images of test sample after scanning with a preset beam of electrons. These detected signals are combined with beam position to give image (19).

Fourier transform infrared spectroscopy (FTIR)

Infra-red spectra of ethyl cellulose and eudragit L100 was determined by using KBr disc method (20).

RESULTS AND DISCUSSION

Determination of Calibration Curve

Calibration curve for Diclofenac sodium was constructed and it was found that value of R^2 was 0.9994 that was quite satisfactory (Figure 1).

Micromeritic studies of prepared microspheres of Diclofenac sodium

Micromeritic studies of formulated microspheres of all the formulations were conducted. F1 showed excellent results of micromeritics parameters with best flow properties amongst all the formulations all other formulations showed satisfactory results (Table 3).

Swelling index

Swelling index of F1 was 45%, for F2 showed greater swelling with 86%. Swelling index of F3 was 26%, of F4 was 76% and swelling index of F5 was maximum amongst all that was 94% (Table 4).

% Moisture contents

Moisture contents were greatly linked with the concentration of the polymer as showed in the table 4.

Determination of drug contents

All the formulation showed satisfactory drug contents. The range of the drug contents for all the formulation was found to be 83 to 96% (table 4).

Drug entrapment efficiency % Yield

In-vitro drug release studies

Invitro drug release studies were carried out for 9h in phosphate buffer of pH 6.8. From the results it was observed that concentration of ethyl cellulose has great impact on the release of Diclofenac sodium from prepared microspheres. F1 formulated with ethyl cellulose release 72% of the drug, F2 which was based upon eudragit L-100 released 55%, F3 with equal ratios of eudragit-L100 and ethyl cellulose showed 47% drug release, F4 and F5 with same ratios but comparatively high concentrations of both polymers released approximately 40% of the drug. Results were depicted by the figure 1.

FT-IR spectrum

Infra- red spectrum was used to prove interaction between diclofenac sodium and used polymers. In the following figures, the peaks at 1620 showed N-H deformation and peak at 1384 showed C-H stretching. A sharp peak at 613 was due to C-Cl group attached to aromatic ring. The peaks at 3610 showed O-H stretching and sharp peak at 3461 showed N-H stretching. Principle peaks of diclofenac sodium molecule appeared at 1393, 1384, 1382 and 1453 indicated that diclofenac sodium was successfully incorporated into the microspheres. There was no extra peak in the spectrum which indicated that there was no chemical reaction between diclofenac sodium and proposed polymers.

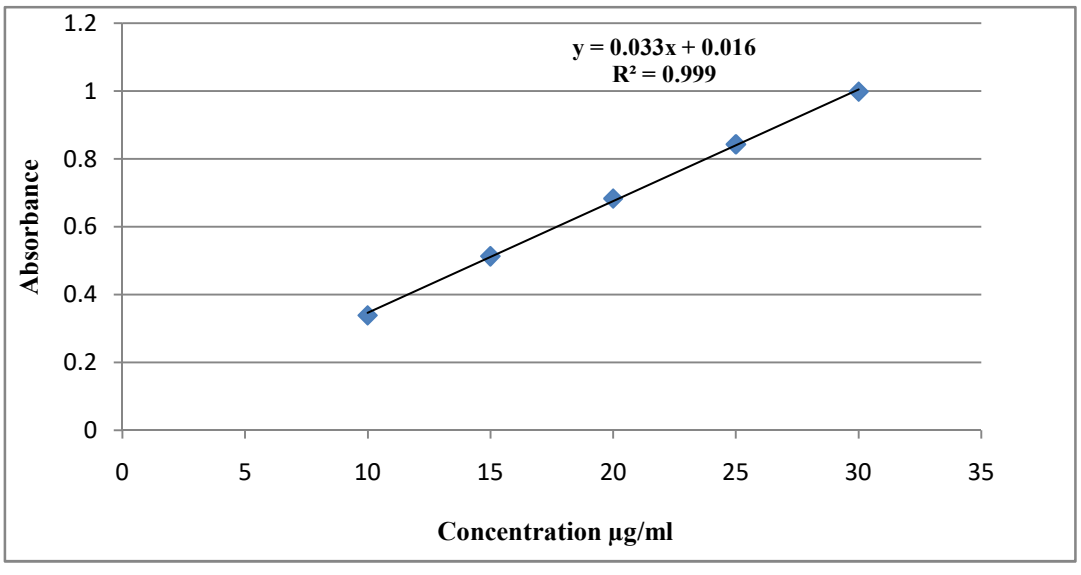

Figure 1: Calibration curve of Diclofenac sodium

Table 2: Drug Release Kinetics Models

Kinetic Models	Equations
Zero order(Cumulative percent drug released vs. time	$Ft = K_o t$
1st Order (log cumulative percent drug retained vs. time)	Log Qt = log Q_o+K_1t/2.303
Higuchi model(cumulative percent released vs. time)	F = $K_H t^{1/2}$
Hixson Crowell cube root model (percent retained⅓ vs. time	$W_o^{1/3} - Wt^{1/3} = Kst$
Korsmeyer Peppas (log of cumulative drug retained vs. time	$Ft = at^n$

Table 3: Micromeritics study of prepared microspheres of Diclofenac sodium

Formulations	Bulk Density	Tap Density	Carr's Index	Hausner's Ratio	Angle of Repose	Flow rate (g/sec)
F1	0.34	0.37	13.11	1.088	20.35	0.076
F2	0.28	0.31	16.54	1.107	25.35	0..08
F3	0.24	0.26	17.43	1.088	26.37	0.087
F4	0.17	0.21	16.16	1.23	25.47	0.083
F5	0.16	0.19	18.48	1.18	28.69	0.063

All the values are expressed as Mean±SD where n=3

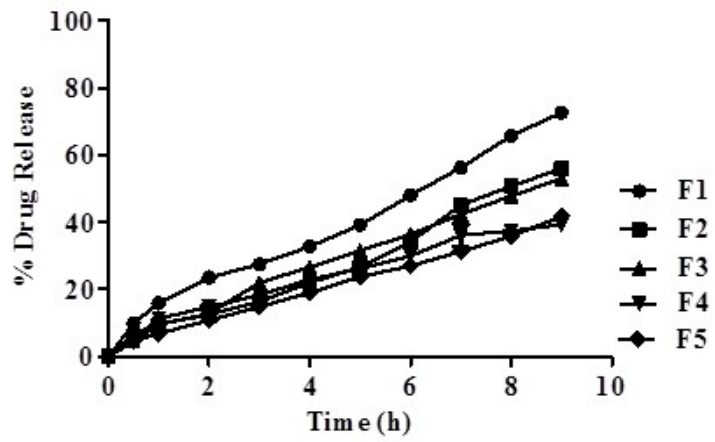

Figure 2: % Drug released from formulated microspheres (F1-F5) loaded with Diclofenac Sodium

Table 4: Kinetic modeling of drug released from formulated microspheres of Diclofenac sodium

Formulations	Zero order	1st Order	Higuchi	Hixson	Korsmeyer Peppas		Best Fit Model	Drug release mechanism
	R^2	R^2	R^2	R^2	R^2	Value of 'n'		
F1	0.966	0.954	0.8906	0.968	0.983	0.827	Korsmeyer peppas	Non Fickian Transport
F2	0.963	0.988	0.9146	0.987	0.993	0.781	Korsmeyer peppas	Non Fickian Transport
F3	0.979	0.995	0.9058	0.995	0.998	0.818	Korsmeyer peppas	Non Fickian Transport
F4	0.915	0.968	0.9504	0.955	0.995	0.686	Korsmeyer peppas	Non Fickian Transport
F5	0.990	0.989	0.8723	0.992	0.995	0.906	Korsmeyer peppas	Case ii Transport

Table 3: Micromeritics study of prepared microspheres of Diclofenac sodium

Formulations	Particle Size (µm)	Percent Yield	Percent Entrapment (%)	Moistyre Content (%)	Swelling Index	Friction Coefficient	Drug Content (%)
F1	135	95.7	73.44	35.9	0.45	0.41	96.8
F2	270	93.5	68.45	38.97	0.86	0.47	93.21
F3	350	89.9	49.03	42.21	0.26	0.54	89.14
F4	490	87.0	46.52	45.94	0.76	0.45	94.13
F5	540	86.6	51.44	47.91	0.94	0.56	83.13
*All the values are expressed as M ean±SD where n=3							

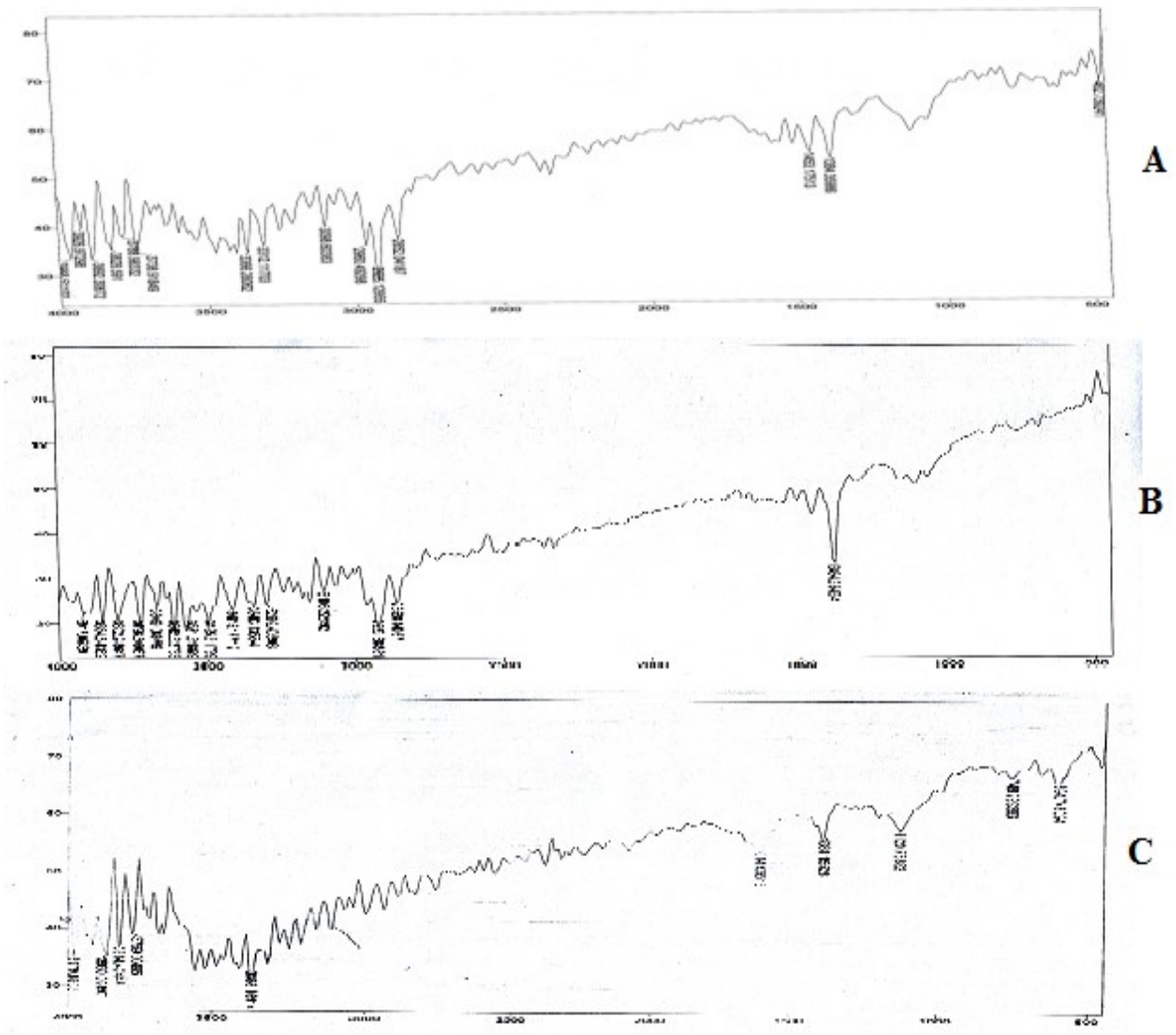

Figure 3: FTIR of Diclofenac sodium (A), ethyl cellulose (B) and Eudragit L-100 (C)

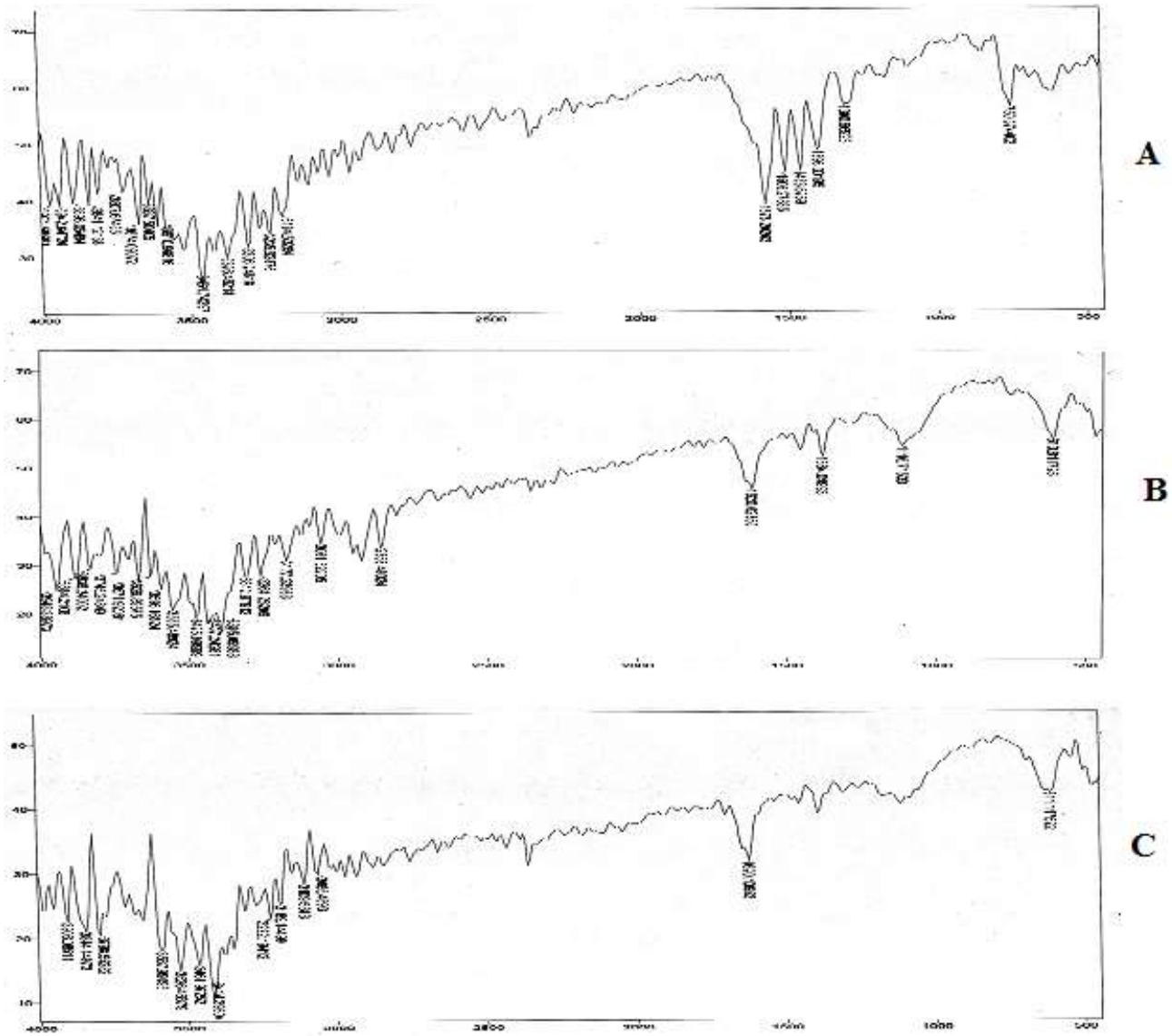

Figure 4: FTIR of F1 (A), F2 (B) and F3 (C)

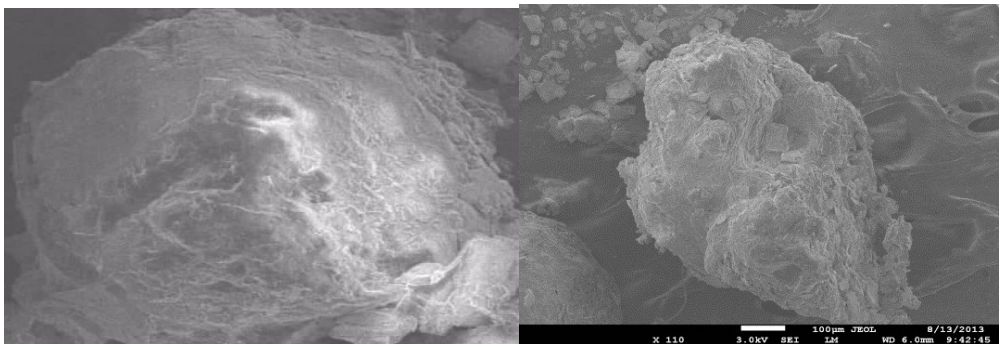

Figure 2: Scanning electron microscopy of formulated microspheres

DISCUSSION

Scanning electron microscopy images showed spherical particles with irregular surfaces. It was noticed that if stirring speed is kept below 500rpm, there was no formation of microspheres. This may be due to insufficient turbulence of the internal phase into the external continuous phase and material may settle down to the container bottom. A high stirring speed more than 1200rpm caused no spherical particles formation and particles stick to the container walls. An optimum speed of 500-900rpm produced desired microsphere. It was observed that with increase in polymer concentration particle size increased due to increase in viscosity of the polymers which facilitate particle coalescence (20). Particle size for all formulations was satisfactory. The average particle size ranged from 135µm for F1 to 460µm for F5. There was decrease in bulk density with increase in polymer concentration. Bulk and tap density showed good packing ability. Angle of repose ranged from 20 for F1 to 28 for F5 indicating good flow. Carr's index was also less than 30 for all formulations demonstrating good flow. Hausner's ratio was from 1.088 to 1.23 showing good flow (21). Coefficient of friction and flow rate were less than 1 demonstrating good flow properties. Minimum percent loss of moisture was observed in all formulations indicating good stability during prolong duration of time (22). It was observed that highest entrapment efficiency obtained with drug: polymer ratio 1:1 and drug content and production yield gradually decreases with increase in polymer concentration. Drug content ranges from 96.8% for F1 to 83.13% for F5. Drug entrapment ranges from 73.44% for F1 and 46.52% for F4. Production yield was from 95.7% for F1 to 86.67% for F5. An increase in span 20 (0.5% w/v) concentrations, there was decrease in drug content, entrapment efficiency and production yield. This could be because of solubilizing effects of span 20 on diclofenac sodium during production of microspheres. Diclofenac sodium microspheres showed good swelling ability which showed tendency of microspheres to release drug in continuous way (23). Drug polymer interaction was studied by FT-IR analysis. The IR spectra of pure drug and drug loaded microspheres were taken. The characteristic N-H stretching, C-H stretching, C-Cl stretching, O-H stretching were observed at 346cm^{-1},1384$cm^{-1,}$ 613$cm^{-1,}$ and 3610cm^{-1} respectively. Principle peaks confirmed the structure of diclofenac sodium. The same peaks were also observed in diclofenac sodium loaded microspheres.

There was no change or shifting of characteristic peaks in the diclofenac sodium loaded microspheres suggesting that there is no prominent drug polymer interaction demonstrating stability of the drug in all formulations (24, 25, 26, 27, 28).

Percent amount of drug release from diclofenac loaded microspheres decreased with increase amount of polymers (29). A rapid drug release was observed in first 30 minutes which was sufficient to produce initial therapeutic effect. This may be attributed to the amount of drug adhered to the surface of the microspheres during manufacturing. A gradual decrease in drug release after first 30minutes was observed which may be due to decrease amount of drug present near the surface and also due to the reason that the amount of uncoated drug decreases with increasing amount of polymers (30, 31). Dissolution study was performed in 900ml 0.1NHCl (pH 1.2) for 2 hours and drug release was negligible. This was due to insolubility of drug in acidic medium. In dissolution medium Phosphate buffer pH 6.8, the percent drug release after 10 hours of dissolution were; F1, 77.12%, F2, 57.23%, F3, 56.19%, F4, 43.35%, F5, 45.16%. All formulations gave best correlation for Korsmeyer Peppas suggesting that drug release mechanism is anomalous (diffusion plus erosion). The value of n was used to characterize release mechanism. The value of n was from 0.686 to 0.906 indicating that release mechanism was approximately non Fickian (32).

CONCLUSION

From the present study it can be concluded that diclofenac sodium microspheres using eudragit L100 and ethyl cellulose possess effective sustained and drug retarding effect. Microspheres prepared were spherical and have rough surfaces. The drug release showed initially burst release and then slow and constant rate of release for 9h. The results may suggest the successful encapsulation of diclofenac sodium in eudragit L100 and ethyl cellulose to produce a suitable sustained release drug delivery system. Thus ethyl cellulose and eudragit L100 are suitable candidates to prepare microspheres of diclofenac sodium as a single unit oral sustained release dosage form.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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