Design, characterization and *in-vitro* evaluation of different cellulosic acrylic and methacrylic polymers loaded aceclofenac microspheres

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Abstract: The aspire of this attempt was to design and evaluate aceclofenac loaded sustained release microspheres by emulsion solvent evaporation method, using different polymers like Ethyl cellulose (EC), Kollidon SR (KSR), Eudragit RS 100, Eudragit RL 100 and Hydroxypropylmethyl Cellulose (HPMC K100M). Microspheres were prepared using different stirring rate (1200, 1500, 2000rpm) and larger microspheres were obtained with lower stirring rate. Performance of microspheres was evaluated in terms of in vitro dissolution study which was allowed according to USP paddle method using Phosphate Buffer (pH 6.8) for 8 hours. UV-spectrophotometric method was used to calculate the drug content and the maximum and minimum release of aceclofenac from microspheres was observed 96.08% and 46.41% for formulation F18 and F5 after 8 hours respectively. Dissolution data were fitted by different mathematical models such as the zero order plot, first order plot, Higuchi plot, Hixon-Crowel plot and korsemeyer plot. Korsemeyer model has found to best fitted with release data. Scanning electron microscopic technique was performed to obtain the particle size and morphological changes due to different polymers. Fourier Transform Infra-red (FT-IR) spectroscopy was performed to find out any interaction of drug with the polymers. The drug might be released by both diffusion and erosion as data were best fitted with Korsemeyer model. So it has been demonstrated that aceclofenac microspheres containing different cellulosic, acrylic and methacrylic loaded polymers may be excellent candidates for consideration in drug delivery systems.

Keywords: Aceclofenac, microspheres, emulsion solvent evaporation method, Korsemeyer model.

INTRODUCTION

Aceclofenac is superior form other NSAIDs as it has selectivity for COX-2, a beneficial COX inhibitor, well tolerated, better GI tolerability and improved cardiovascular safety when compared to other selective COX-2 inhibitors. It is an effective analgesic and anti-inflammatory agent with a good tolerability profile. Through its analgesic and anti-inflammatory properties, aceclofenac provides symptomatic relief in a variety of painful conditions like osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Aceclofenac is practically insoluble in water as the intrinsic solubility of ACL in pure water at room temperature is found to be 0.088 mg/ml with good permeability (calculated log P=2.170) and belongs to biopharmaceutics classification system (BCS) class II (low solubility, high permeability) (Tejal, 2008).

It should be administered frequently to maintain the preferred concentration due to its half life (3-4 hrs). For that reason, aceclofenac is the best candidate for sustained release formulation which is more reproducible and more reducing the risk of local irritations compared to conventional forms (Brogden and Wiseman, 1996). Micro encapsulation is a useful method which can be used to prolong the interval of drug effect significantly and enhances unwearied compliance. Ultimately total dose and undesirable effect may be reduced in sustain release microsphere due to the steady plasma concentration (Fu *et al*., 2005).

Microencapsulation process helps for converting the liquids to solids, changing the colloidal and surface properties, providing environmental protection and controlling the release characteristics of different coated materials. This has been done by developing the new drug entities, discovering of new polymeric materials that are suitable for prolonging the drug release, safety, improvement in therapeutic efficacy. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. Generally the size of the microencapsulated products (micro particles) is considered as larger than 1 micrometer and up to 1000 micrometers in diameter. Commercially available micro particles contained 10-90% w/w core (Gohel and Amin, 1998).

Among the several different methods of micro capsulation the most common are solvent evaporation, coacervation, coalescence and phase separation, interfacial polymerization, spray drying and ionotropic gelation. In case of micro encapsulation solvent-evaporation method engage of emulsification of a solution containing polymer and drug with an additional medium in which the drug and polymer cannot dissolve. The technique is relatively simple and has been used to prepare microcapsules of a

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variety of compounds using several different polymeric materials (Bolourtchian *et al.*, 2005).

As solvent evaporation technique may be a method of choice for the preparation of microspheres of water insoluble drugs (Obeidat and Price, 2006). In this method insoluble drugs and polymers are dissolved in an organic phase, emulsified to fabricate an oil in water emulsion, disappeared the organic part and finally formed microspheres are filtered and dried and kept for further experiment. Because aceclofenac is water insoluble drug, this technique is selected for the preparation of sustained release microspheres. In this current study, an experiment was made to develop sustained release aceclofenac microspheres using different polymers as a release retarded material by solvent evaporation technique (Perumal *et al*., 1996; Perez *et al.*, 2006). In this circumstance, the intend of this effort was to evaluate the effect of the addition of cellulosic polymers, polymethacrylic polymers on the drug content, particle size, morphology of aceclofenac microspheres and, consequently, on the release profile of aceclofenac (Khidr *et al*., 1998).

MATERIALS AND METHODS

Materials

Aceclofenac as an endowment sample from Beximco Pharmaceutical limited, Bangladesh, Eudragit- RS 100 (EVONIK, Germany), Eudragit-RS (EVONIK, Germany), Eudragit-RL (EVONIK, Germany), Dichloromethane (Merck, Germany), Ethanol (Merck, Germany), Light liquid paraffin (Merck, Germany), cyclohexane (Merck, Germany), Span 80 (Merck, Germany), distilled water.

Methods

Preparation of Aceclofenac Microspheres by Emulsion solvent evaporation method

Aceclofenac Microspheres were prepared with different polymer as per composition shown in table 1. At first polymer solution was prepared by using 5:5 ethanol and acetone which acts as internal phase. Then aceclofenac was suspended in light liquid paraffin oil along with 1% span 80 by stirring for 5-10 minutes which acts as external phase. Internal phase was incorporated to external phase drop wise and stirred for 2.5 hrs at different rpm (1200-2000). After 2.5 hours, prepared microspheres were washed with cyclo-hexane repeatedly and allowed to dry at room temperature. Finally the microspheres were transferred into glass vials after sieving and kept in the desiccators for further experiment. Different cellulose and acrylic polymers loaded aceclofenac microspheres were prepared by following fig.1.

In-vitro Release study of Aceclofenac Microspheres

In vitro dissolution study was performed in a paddle type dissolution Apparatus (Apparatus 2). 900 ml of Phosphate buffer (pH 6.8) was used as dissolution media, paddle speed was 100 rpm and temperature was maintained fixed at 37±0.5˚C. Then 20mg equivalent amount of aceclofenac microspheres from each batch was transferred in each dissolution basket. The dissolution process was carried out for 8hours and 10ml dissolution sample was withdrawn at predetermined intervals of 1 hour, 2hours, 3hours, 4hours, 5hours, 6hours, 7hours and 8hours. Each and every time 10ml dissolution sample was compensated by fresh 10ml of phosphate buffer. The samples were withdrawn with the help of 10ml syringe and were kept in test tube. The dissolution samples were then analyzed spectrophotometrically at a wavelength of 274 nm.

Study of release kinetics

To find out the mechanism of drug release, the controlled release Aceclofenac microspheres were treated in different mathematical models like Zero order (cumulative percentage of drug release versus time), First order (log percentage of drug remaining versus time), Higuchi model (cumulative percentage of drug release versus square root of time), Hixon-crowell model (cubic root percentage release versus time), Korsemeyer model (log cumulative percentage of drug release versus log time). The release data was plotted. From the linear portions of the curve slope correlation coefficients (R^2) were calculated (Costa and Lobo, 2001).

The data are summarized in table 3. The equation is as follows for Korsemeyer equation (Korsmeyer *et al.,* 1983)

$\text{Log } (Mt/mf) = \log K + \log t$

Where, Mt is the amount of drug release after infinite time, K is a release rate constant incorporating structural and geometric characteristics of the mechanism of drug release. To elucidate the release exponent batches of microspheres, the log value of percentage drug dissolved was plotted against log time for each batch according to the above equation. For the case of cylindrical tablets, $0.45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport that means both diffusion and erosion controlled drug release, $n=0.89$ to Case II (relaxation) transport, and $n > 0.89$ to super case II transport. The values of n depend upon the polymer concentration.

Solubility study

Solubility of Aceclofenac was determined in different media including distilled water, 0.1N HCL and Phosphate buffer pH 6.8. Excess amount of Aceclofenac was added into three different conical flask containing 100 ml of distilled water, 0 .1 N HCL and phosphate buffer pH 6.8. These solutions were shaken for 48 h at room temp on a magnetic stirrer. After equilibrium, the suspensions were filtered through 0.45 µm Millipore membrane filters. The filtrate was appropriately diluted and the concentration of the Aceclofenac in the filtrate was determined by UV spectrophotometer at 276 nm (shown in table 2).

A) Addition of drug to mixture of L.L.P and then add Polymer solution

B) Filtration of Microspheres

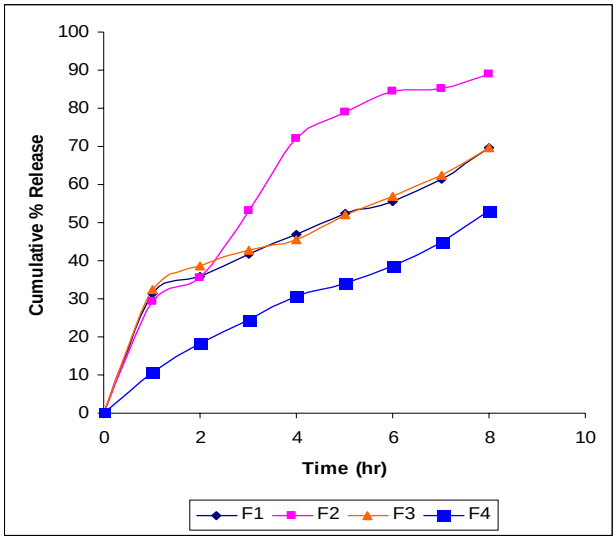
C) Micro spheres after drying

D) Storage in airtight container

Fig. 1: Schematically present the steps of microspheres prepared by emulsion solvent evaporation method.

Table 1: Formulation of Aceclofenac Microspheres prepared by Emulsion Solvent Evaporation method

Formulations	Drug (gm)	Polymers					Solvent
		Kollidon SR (gm)	Ethyl cellulose (gm)	Eudragit RS 100 (gm)	Eudragit RL 100 (gm)	HPMC K100M (gm)	Ethanol: Acetone
F1	1	-	1	-	-	-	5:5
F2	1	0.5	1	-	-	-	5:5
F3	1	-	2	-	-	-	5:5
F4	1		-	1	-	-	5:5
F5	1	-	-	2	-	-	5:5
F6	1	1	1	-	1	-	5:5
F7	1	1	1	-	-	0.5	5:5
F8	1	1	1.5	-	-	0.5	5:5
F9	1	1	-	1	-	1	5:5
F10	1	-	1	-	1	-	5:5
F11	1	-	1	-	-	1	5:5
F12	1	-	1.5	-	-	1	5:5
F13	1	1	-	-	-	1	5:5
F14	1	0.5	-	-	-	1	5:5
F15	1	-	-	-	1	1	5:5
F16	1	0.5	1	1	-	-	5:5
F17	1	1	1	-	-	0.5	5:5
F18	1	1	1	-	-	1	5:5
F19	1	-	-	1	-	1	5:5
F20	1	0.5	1.5	-	-	1	5:5



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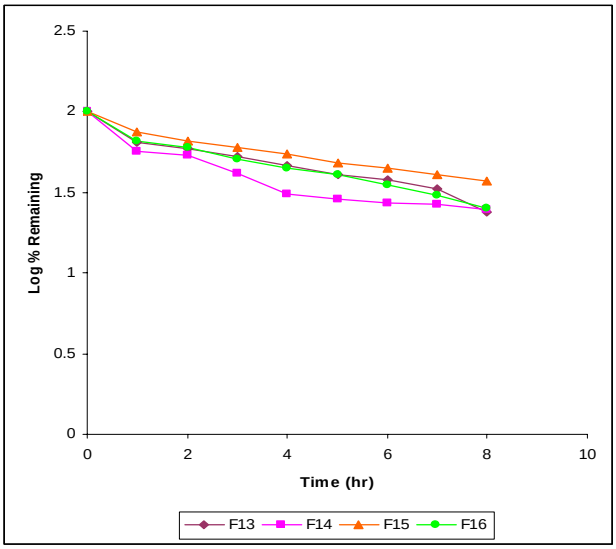

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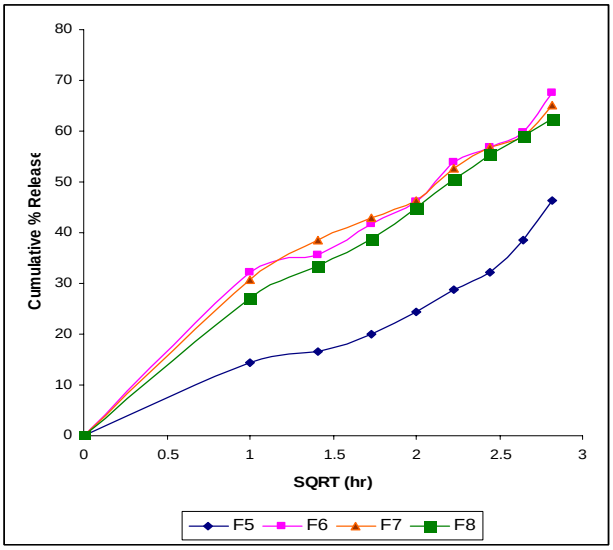

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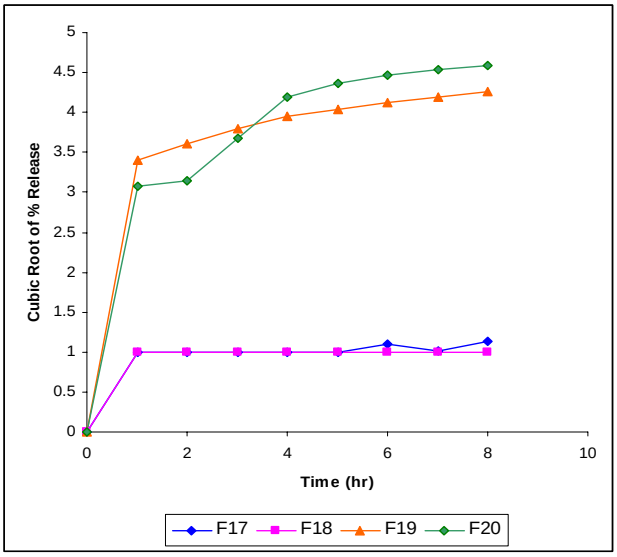

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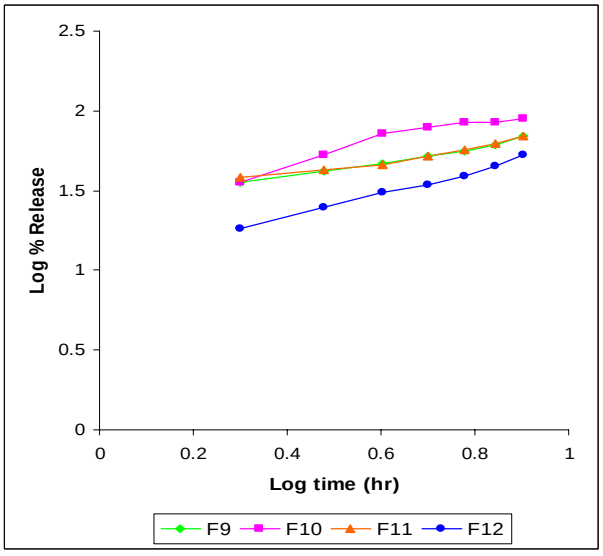

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Fig. 2: Release of aceclofenac from formulations F1 to F20 by Emulsion solvent evaporation method where A) Zero order plot for F1 to F4, B) Higuchi plot for F5 to F8 , C) Korsemeyer plot for F9 to F12 D) First order plot for F13 to F16 and E). Hixson-crowel plot for F17 to F20 respectively.

Fourier Transform Infrared (FT-IR) Spectroscopy studies

To study the interaction between drug and polymers used in the preparation of microspheres FT-IR spectroscopy was carried out. The pure drugs and best formulations (F2, F18) were subjected for FT-IR analysis. The samples were scanned over a range of 4000-400 cm^{-1} using Fourier transformer infrared spectrophotometer (8488S, Shimadzu, Kyoto, Japan). Spectra were analyzed for drug polymer interactions.

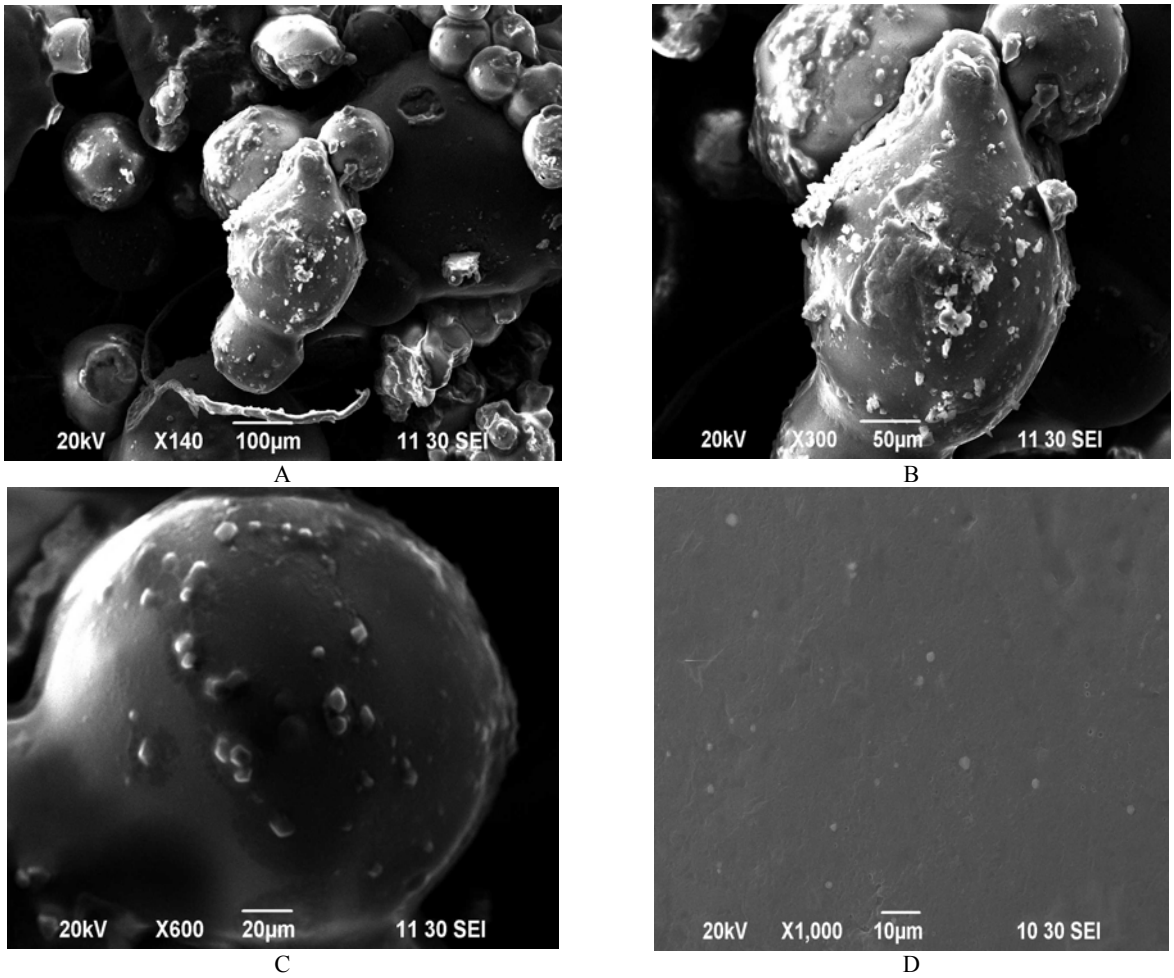

Fig. 3: Effect of polymers on surface Morphology of formulation F18 microspheres A) Magnification at X140 SE B) Magnification at X300 SE C) Magnification at X600 SE. and D) Magnification at X1000 SE.

Surface morphology study with the help of Scanning Electron Microscope (SEM) analysis

Surface nature of microspheres was examined with the help of Scanning Electron Microscope (JEOL, JSM-6490 LA, Japan). The microspheres were dried completely before examination. SEM were done at different magnifications of 20.0 kv X 140, 20.0 kv X 300, 20.0 kv X 600, 20.0 kv X 1000 SE to examine the surface picture and size of the microcapsules that changed from formula to formula. The working distance was 10 an 11 inches.

RESULTS

In vitro dissolution study of Aceclofenac microspheres

The *in-vitro* drug release study was performed using paddle type (USP type 2) dissolution apparatus in phosphate buffer (pH 6.8) at 37±0.5˚C up to 8 hours depending upon the formulation variables. Dissolution rate of Aceclofenac did not increase with the increase in the concentration of different polymers when the dissolution test was carried in phosphate buffer pH 6.8. This may be due to high solubility (1347.71 µg/ml) of Aceclofenac in phosphate buffer pH 6.8. These observations indicate the enhanced dissolution of microspheres with increase in the concentration of polymers possibly due to the increased wettability of the drug by the polymer, drug particle size reduction etc.

Table 2: Results of solubility study

Solution System	Absorbance	Solubility (µg/ml)
Distilled water	0.203	88.6
0.1 N H	0.082	33.6
Phosphate buffer pH 6.8	0.204	1347.71

Solubility Study

Solubility of Aceclofenac in distilled water, 0.1N HCL and Phosphate buffer pH 6.8 are shown in table 2. Solubility of Aceclofenac in water, 0.1N HCL and Phosphate buffer pH 6.8 were found to be 88.6, 33.6 and 1347.71µg/ml respectively.

Table 3: Interpretation of release rate constants and Correlation coefficient (R^2) values for different release kinetics of different formulations of Aceclofenac Microspheres using different polymers

Formulations	Zero order		First order		Higuchi		Korsmeyer		Hixon-Crowel	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2	K_{HC}	R^2
F1	5.754	0.892	-0.033	0.936	15.8	0.97	0.351	0.981	-0.133	0.539
F2	6.515	0.849	-0.04	0.914	18.26	0.973	0.475	0.993	-0.134	0.622
F3	6.595	0.839	-0.45	0.915	19.53	0.964	0.411	0.985	-0.135	0.528
F4	7.139	0.796	-0.049	0.892	20.3	0.939	0.456	0.983	-0.137	0.706
F5	8.538	0.824	-0.065	0.93	24.12	0.959	0.485	0.991	-0.135	0.654
F6	9.342	0.818	-0.081	0.931	26.28	0.944	0.271	0.964	-0.131	0.528
F7	10.66	0.752	-0.121	0.946	30.28	0.925	0.511	0.998	-0.121	0.515
F8	5.754	0.892	-0.033	0.936	15.8	0.98	0.451	0.982	-0.136	0.551
F9	6.515	0.849	-0.04	0.914	18.26	0.973	0.437	0.993	-0.144	0.611
F10	6.595	0.839	-0.45	0.915	19.53	0.964	0.311	0.985	-0.147	0.504
F11	7.139	0.796	-0.049	0.892	20.3	0.939	0.371	0.983	-0.143	0.502
F12	8.538	0.824	-0.065	0.93	24.12	0.959	0.495	0.992	-0.148	0.505
F13	9.342	0.818	-0.081	0.931	26.28	0.944	0.271	0.964	-0.149	0.526
F14	10.66	0.752	-0.121	0.946	30.28	0.925	0.462	0.998	-0.151	0.473
F15	6.327	0.766	-0.041	0.926	18.59	0.983	0.459	0.997	-0.152	0.557
F16	7.431	0.89	-0.049	0.948	20.42	0.979	0.352	0.983	-0.144	0.533
F17	8.205	0.876	-0.058	0.949	22.66	0.974	0.326	0.973	-0.155	0.497
F18	8.768	0.839	-0.068	0.94	24.62	0.964	0.372	0.985	-0.137	0.665
F19	9.151	0.801	-0.077	0.931	26.02	0.944	0.372	0.983	-0.142	0.611
F20	9.776	0.77	0.096	0.934	28.02	0.923	0.344	0.972	-0.131	0.491

DISCUSSION

Effect of different polymers (Kollidon SR, Eudragit RS 100, Eudragit RL 100, Ethyl cellulose and HPMC) on the release of Aceclofenac from microspheres prepared by Emulsion solvent evaporation method

Aceclofenac microspheres were prepared from single or combination of different polymer to find out their effect on the drug release from microspheres. Drug releases from different microspheres are shown in fig. 2. Formulation F1 to F4 was prepared using the polymers of kollidon SR, Eudragit RS 100, Ethyl cellulose and HPMC in different concentrations. The initial burst release of formulations F1, F2, F3 and F4 were about 31.41%, 10.61%, 32.26% and 29.29% respectively after 1 hour. After the end of 8 hours of dissolution, the release microspheres from F1, F2, F3 and F4 were 69.62%, 53.06%, 69.76% and 88.86% respectively. The addition of ethyl cellulose and increase in concentration of Kollidon SR in formulation F2 retards the rate of drug release. Good release retardant effect obtained from ethyl cellulose because of it is hydrophobic nature, less permeation of dissolution medium there by decrease of drug diffusion. Eudragit RS alone in formulation F4 could not able to control sustained release due to its nature of very low water solubility, low content of quaternary ammonium compound and reduced permeability.

Kollidon SR, Eudragit RL 100 and ethyl cellulose of different concentrations were used in formulations F5 to F8. After the end of 8 hours of dissolution, the release microspheres from F5 to F8 were 46.41%, 67.5%, 65.23% and 62.54% respectively. The addition of ethyl cellulose and increase in concentration of Kollidon SR in formulation F8 retards the rate of drug release.

Formulation F9 to F12 were prepared using the polymers of ethyl cellulose, Eudragit RS 100, kollidon SR and Eudragit RL100 and HPMC K100 in different concentrations. After the end of 8 hours of dissolution, the release of microspheres from F9, F10, F11 and F12 were 56.88%, 67.13%, 61.07% and 60.07% respectively. Formulation F9 has shown good sustained effect because HPMC K100 forms a strong viscous gel in contact with aqueous media which may be useful in controlled delivery of drug.

Formulation F13 to F16 were prepared using the polymers of Ethyl cellulose, HPMC, Eudragit RS 100, kollidon SR and Eudragit RL100 in different concentrations. The initial burst release of formulations F13, F14, F15 and F16 were about 31.09%, 34.73%, 41.61% and 26.61% respectively after 1 hour. The release of microspheres from F13, F14, F15 and F16 were 75.99%, 75.28%, 74.71% and 62.54% respectively found after 8 hours. Formulation F16 has shown good controlled release because of its kollidon SR. Kollidon SR has a unique character of maintaining geometric shape of dosage form until the end of dissolution test, this is mainly due to its major component; the water insoluble polyvinyl acetate,

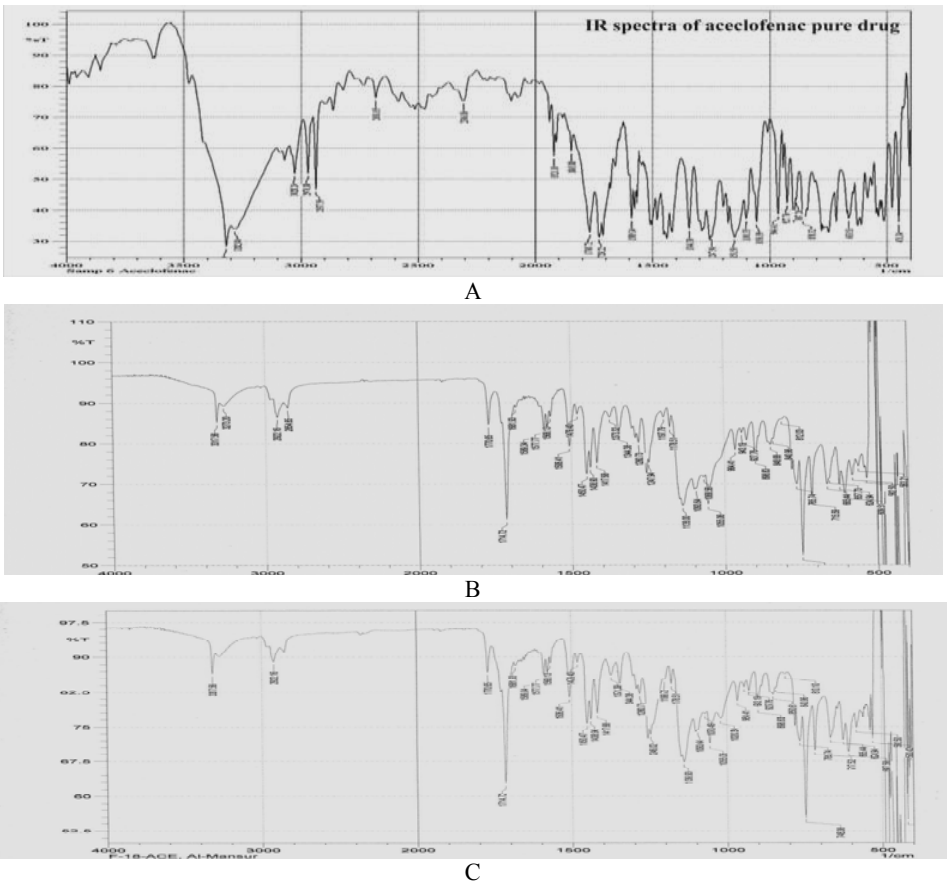

Fig. 4: FTIR spectrum of Aceclofenac microspheres A) pure drug B) formulation F2 C) Formulation F18

while the minor water soluble part; polyvinylpyrrolidone, is responsible for pore formation causing diffusion controlled release mechanism (Draganoiu *et al.,* 2001).

Formulation F17 to F20 were prepared using the polymers of Ethyl cellulose, Eudragit RS 100, HPMC, kollidon SR and Eudragit RL100 in different concentrations. After the end of 8 hours of dissolution, the release microspheres from F17 to F20 were 72.26%, 76.08%, 96.88% and 74.22% respectively. The addition of HPMC K100, kollidon SR and Ethyl Cellulose in formulation F18 retards the rate of drug release. The release mechanism of HPMC K100 from F17 can be described on the basis of it high water absorption, fast hydration and swelling to form an outer pseudo-gel layer controlling drug release from the inner to the outer side of the microspheres.

Thus the results showed that the release rate of aceclofenac from the microspheres can be modulated with adjusting the ratios of polymer/drug in the formulation. All the formulations were best fitted with Korsemeyer model as shown in table 3. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microspheres of different drug to polymer ratio was ranged between 0.27-0.51, indicating that the mechanism of the drug release was diffusion controlled. Comparative drug percent release of aceclofenac microspheres consisting of different polymers after 8 hours.

Effect of polymers on the surface morphology of Aceclofenac microspheres prepared by Emulsion solvent evaporation method

SEM study shows that particles made of Ethyl Cellulose, Kollidon SR and HPMC 50 cps in formulation F18 were quite spherical and aggregated with relatively tiny pores and rough surface. According to fig. 3, the surface of the drug loaded microspheres manifested the presence of drug particles, clearly visible from outside at high magnification. The surface morphology indicates that the mechanism of drug release mainly follows diffusion release although there are many possibilities of surface erosion.

Drug-polymer compatibility study by Fourier Transform Infrared (FTIR) spectroscopy

FT-IR spectra of aceclofenac alone and its combination with polymers are shown in fig. 4. An FT-IR spectrum of pure aceclofenac showed the peaks such as C=O stretch (1770.81, 1716.80 cm^{-1}), OH stretching (2970.64 cm^{-1}), CH stretching (2937.85 cm^{-1}), NH stretching (3319 cm^{-1}),

C-Cl stretch (669.50 cm^{-1}). These peaks can be considered as characteristic peaks of aceclofenac and were not affected and prominently observed in IR spectra of aceclofenac along with polymers as shown in the fig. 4. As there is no change in the positions of characteristic absorption bands and bonds of various functional groups present in the drug. This observation clearly suggests that the drug remains in its normal form with no prominent change in its characteristics even in its formulations.

CONCLUSION

The present study was performed to design aceclofenac sustained microspheres by emulsion solvent evaporation method. In this work, it has been demonstrated that aceclofenac microspheres containing different polymers may be excellent candidates for consideration in drug delivery systems. Specifically, the microspheres of the mentioned polymers can be used as a vehicle to modulate drug release for a sustain activity of up 8 hours. Microspheres were prepared by a method based on emulsion solvent evaporation technique which was found to be reproducible and also may be an ideal method to prepare microspheres in large sale. The microspheres were also examined and analyzed by SEM and FTIR. The SEM reports depict that the particle surface morphology changes drastically as the drug/polymer ratio changes. A high loading shows a high surface drug. The FTIR shows a successful formulation technique of drug and polymer by showing the presence drug in the microspheres. Further experiment should be performed to establish more sustained effect.

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