DESIGN AND PREDICTED OPTIMIZED EXTENDED RELEASE ACECLOFENAC MICROPARTICLE FORMULATIONS

B. Jayanthi[*]

Dr. B. Jayanthi[**]

ABSTRACT:

Optimization is a process of making a formulation as select as possible within a given physical, chemical and biological consideration. The final product must meet the requirement from a bioavailability, practical mass production and product reproducibility standpoint. Development of Extended release formulation employing optimization technique is based on many statistical experiments which are recognized as useful techniques to design an optimized formulation with desired properties wide. Particle size, entrapment efficiency and dissolution rate. In a short time period and with minimum number of trials. In the present study, two optimized formulations of Aceclofenac, employing different polymers (ethyl cellulose and eudragit RSPO), have been generated through use of software based optimization technique, response surface methodology (RSM). The predicted optimized formulations need validation to find their suitability for acceptance. Validation of predicted optimized aceclofenac formulations.

Keywords: Optimization, Response surface methodology, Extended release, Aceclofenac, Ethyl cellulose and Eudragit RSPO, Emulsion solvent evaporation technique.

[*] **Department of Pharmacy, Annamalai University, Annamalai Nagar – Chidambaram, Tamilnadu, India.**

[**] **Assistant Professor, Department of Pharmacy, Annamalai University, Annamalai Nagar –Chidambaram, Tamilnadu, India.**

Introduction

The widespread interest for microencapsulated drug can be explained by the aim to reduce drug oral administration gastric secondary effects. The short biological half-life of 1-4h following oral dosing makes aceclofenac, a non-steroidal anti-inflammatory drug (NSAID), an ideal candidate for microencapsulation [Saravana*et al* (2003)]. Several process parameters involved in the emulsion-solvent evaporation method, like nature and concentration of dispersing agents, aqueous and organic phase volumes, stirring rate of emulsion system, affect the final microcapsules properties (Pongpaibul et al 1988; Sanadrap and Moes 1993; Sanchez et al 1996).Dispersing agents have an important role in the production of lipophilic and hydrophilic phase of the emulsion (Lambert *et al* 2000).During the solvent evaporation process, the gradual removal of the solvent from the polymer droplets is accompanied by a corresponding decrease of the volume and the increase of the viscosity of the individual droplets. Highly viscous droplets particularly coalesce much faster than they can divide. Droplets coalescence and particle coagulation can usually be overcome by the use of a small amount of a suitable dispersing agent (Jalil and Nixon 1990).It provide a thin protective layer around the droplets and hence reduces the extent of their collision and coalescence.

A pharmaceutical optimization of formulation to has applying two objectives, (1) to determine and quantify the relationship between the formulation's response and the independent variables and (2) to select and quantify formulation and processing variables that produce the best response values. Response surface methodology (RSM) has proven to be a useful tool for achieving both the objectives (Khuri *et al* (1987); Hussain *et al* (1991). The procedure encompasses (a) designing a set of experiments that will reliably measure the response variables, (b) fitting a mathematical model to the data and conducting appropriate statistical tests to assure that the best possible formulation was chosen, and (c) Formation of the optimum levels of independent variables that produce the best response [Khuri *et al* (1987)].

One approach uses a constrained optimization procedure [Takayama *et al* (1983)]. This is done by optimizing one response variable while placing constraints on the remaining response variables to keep them within acceptable limits. However, this method does not really fulfill the goal of optimization, which is to attain the best balance among all the response variables

[Derringer *et al* (1980)]. The second method is to superimpose the contour diagrams of the different response variables [Lind *et al* (1960)]. The main drawbacks of this method are that the response factors may be too many to visualize the contour plots in higher dimensions (i.e., more than three dimensions) and that the scales of the different responses may be too different to be plotted on the same graph [Bayne *et al* (1986)]. Another approach to solving the problem of multiple responses is through the use of a desirability function that combines all the responses into one measurement. The advantages of using desirability functions include the following: (1) responses that have different scaling can be compared, (2) the transformation of different responses to one measurement is simple and quick, and (3) Both the effective and retarded responses can be used [Harrington *et al* (1965)]. The desirability function was first introduced by Harrington [Hassan *et al* (1992)]. Derringer and Suich later employed a different and simpler form of desirability function. This linear desirability function was later used by Hassan *et al* (1992) to optimize a magnetic micro particles formulation. In the present investigation, Response surface methodology (RSM) to design and optimize an extended release formulation of aceclofenac-loaded micro particles.

In the present study the Aceclofenac loaded extended release micro particles were prepared as per experimental design methodology using emulsion solvent evaporation technique. In this study, to design the extended–release micro particles prepared by solvent deposition system were combined into one step. Aerosil, an inert solid dispersing carrier, was introduced in this formulation to improve the dissolution rate of poorly water-soluble drug and the controlled release polymer Eudragit RSPO was employed to bind the inert solid dispersing carrier into micro particles and control the release rate. The significant responses were evaluated and optimized.

1. Preparation of predicted optimized formulations of Aceclofenacmicroparticles (AECM & AEUM)

1.1 Materials and Method

Materials: Aceclofenac was purchased from sigma Mumbai, India. Ethyl cellulose (EC having ethoxyl content of 48- 49.5 % by weight and viscosity of 18 - 22 cps), Eudragit RSPO (Mn=33800g/mol batch no.-5673) was obtained from Loba Chemie Pvt Ltd, Mumbai; Aerosil

200 (colloidal silicon dioxide), from Cobot Sanmer Ltd Mumbai. Dichloromethane AR & Acetonitrile (HPLC grade), Tween 80 (AR grade) were from Qualigens, Mumbai; Potassium Dihydrogen Orthophosphate AR, Sodium Chloride & Sodium Hydroxide AR from SD Fine Chemicals, Mumbai, India. All other reagents were of analytical grade.

Method: Aceclofenac microparticles using ethyl cellulose and eudragit RSPO as per the predicted optimized formulations (**Table 1**) were prepared by emulsion solvent evaporation technique employing a dispersing carrier, aerosil. Predicted optimized processing parameters were followed during preparation of microparticles.

Table 1. Predicted optimized formulae of Aceclofenac microparticles made with Ethyl cellulose and Eudragit RSPO (AECM&AEUM)

Composition	AECM	AEUM
Aceclofenc	200 mg	200 mg
Ethyl cellulose	600 mg	-
Eudragit RSPO	-	500mg
Aerosil	100mg	100mg
Emulsifying agent	Tween 80 (0.12%)	SDS (0.12%)

Step: 1 Preparation Aqueous Phase: 180 mg of emulsifying agent [Tween 80/ Sodium Dodecyl Sulphate (SDS)] was dissolved in 150 ml of milliQ water.

Step: 2 Preparation of organic phase: Required quantity of the polymer (ethyl cellulose or eudragit RSPO) was dissolved in 14 ml of dichloromethane. The required quantity of the drug, Aceclofenac (200 mg), was dissolved in 6 ml of acetone. The drug and the polymer solutions were mixed together and aerosil, a dispersing carrier (100 mg), was dissolved in the above solution.

Step: 3 Preparation of microparticles: The organic phase was added to the aqueous phase with stirring at predicted optimum speed (1100 rpm for AECM & 800 rpm for AEUM) using a medium duty mechanical stirrer (ROL 124, Remi Motors Ltd, and Mumbai) to get fine emulsion.

Stirring was continued for around 1 hour. The microparticles were recovered by filtration, washed with distilled water, air dried and stored in a desiccator containing fused calcium chloride as desiccant.

2. Evaluationof Aceclofenac ethyl cellulose microparticles (Final products)

Prepared Aceclofenac microparticle fornulations (AECM & AEUM) were evaluated for yield entrapment efficiency and drug release etc. various physic   co-chemical properties (yield entrapment efficiency and drug release)

1.   If the product made were of desired attributes were evaluated to assess.
2.   If the product performs as per desired responses drug release pattern.

2.1. Yield & Drug entrapment efficiency:

Yield and drug entrapment efficiency of the prepared microparticles were determined by the methods. The results are shown in Table.2*.*

Table.2.Entrapment efficiency of optimized Aceclofenac microparticles made with ethyl cellulose (AECM) and eudragit RSPO (AEUM).

Optimized Aceclofenac Microparticles formulation	Entrapment Efficiency (%)	% Yield
AECM	82.62	86
AEUM	83.37	84

2.2. Surface morphology

The surface morphology of the prepared microparticles was characterized by scanning electron microscopy (SEM). The SEM photographs of the microparticles are furnished in *Figure 1*. The microparticles were spherical to near spherical in shape with smooth surface in both the formulations (AECM & AEUM).
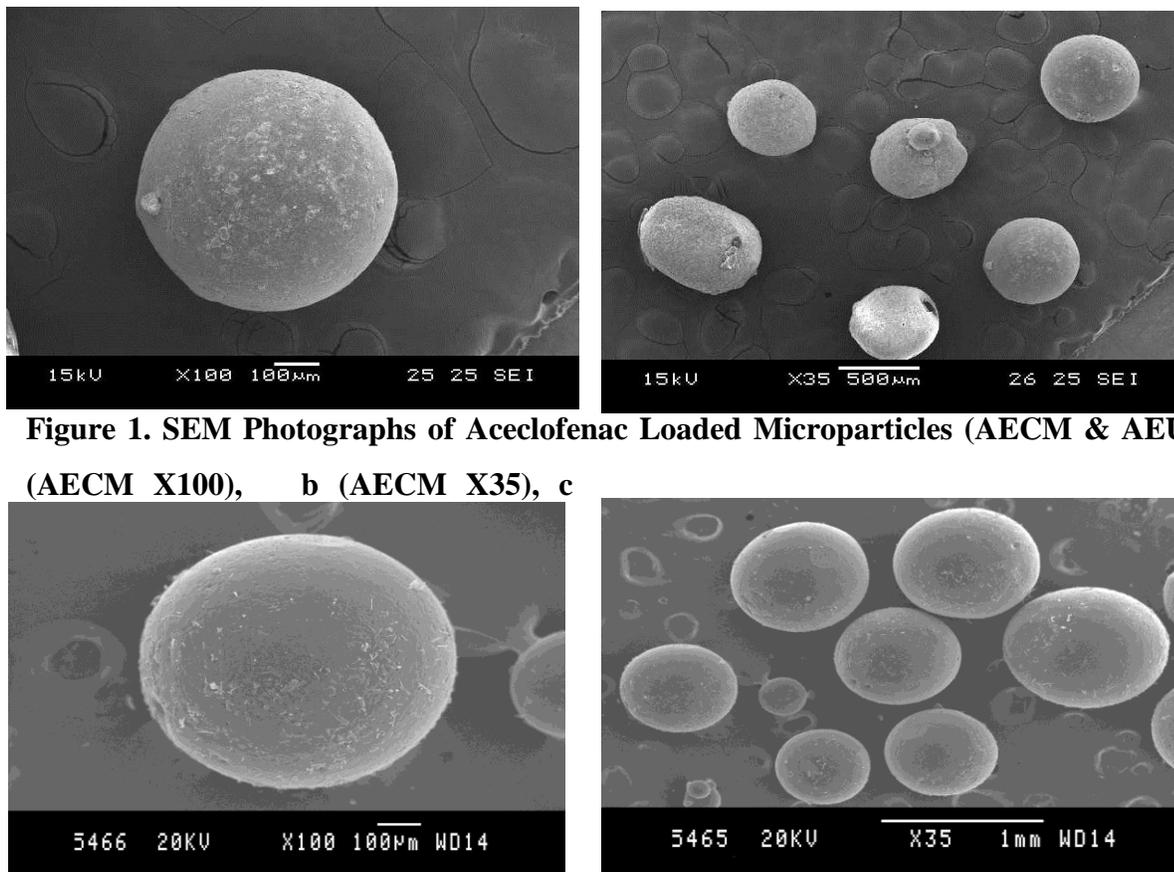
Figure 1. SEM Photographs of Aceclofenac Loaded Microparticles (AECM & AEUM): a (AECM X100),   b (AECM X35), c

(AEUM X100), & d (AEUM X 35),

2.3. MICROMERITICS:

Particle size analysis

Particle size analyses of aceclofenac micro particles were carried out using Malvern particle size analyzer (Malvern Instruments, UK) following the method of Ziyau*et al* 2006. About 10 mg of microparticles was suspended in 5 ml of purified water and analysed with an obscuration index of about 10% (measure of amount of light lost due to introduction of sample against light path). Particle size distribution curves (Volume % vs. size) were recordedand the results are shown in (Figs.2a and2b). The average particle size of microparticles was found to be 221.534 μm and 197.209 μm for AECM and AEUM respectively.

d(0.1): 107.619 um   d(0.5): 221.504 um   d(0.9): 727.851 um

:clof12,- Average, Thursday, August 12, 2010 12:45:22 AM

Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010		0.105		1.096		11.482		120.226		1258.925	
	0.00		0.00		0.00		0.21		2.26		0.00
0.011		0.120		1.259		13.183		138.038		1445.440	
	0.00		0.00		0.00		0.22		3.02		0.00
0.013		0.138		1.445		15.136		158.489		1659.587	
	0.00		0.00		0.00		0.25		3.95		0.00
0.015		0.158		1.660		17.378		181.970		1905.461	
	0.00		0.00		0.00		0.28		5.05		0.00
0.017		0.182		1.905		19.953		208.930		2187.762	
	0.00		0.00		0.08		0.33		6.18		0.00
0.020		0.209		2.188		22.909		239.883		2511.886	
	0.00		0.00		0.10		0.39		7.26		0.00
0.023		0.240		2.512		26.303		275.423		2884.032	
	0.00		0.00		0.12		0.46		8.12		0.00
0.026		0.275		2.884		30.200		316.228		3311.311	
	0.00		0.00		0.14		0.53		8.65		0.00
0.030		0.316		3.311		34.674		363.078		3801.894	
	0.00		0.00		0.16		0.61		8.74		0.00
0.035		0.363		3.802		39.811		416.869		4365.158	
	0.00		0.00		0.17		0.67		8.35		0.00
0.040		0.417		4.365		45.709		478.630		5011.872	
	0.00		0.00		0.17		0.74		7.53		0.00
0.046		0.479		5.012		52.481		549.541		5754.399	
	0.00		0.00		0.18		0.79		6.34		0.00
0.052		0.550		5.754		60.256		630.957		6606.934	
	0.00		0.00		0.18		0.86		4.94		0.00
0.060		0.631		6.607		69.183		724.436		7585.776	
	0.00		0.00		0.19		0.94		3.50		0.00
0.069		0.724		7.586		79.433		831.764		8709.636	
	0.00		0.00		0.19		1.09		2.05		0.00
0.079		0.832		8.710		91.201		954.993		10000.000	
	0.00		0.00		0.19		1.32		0.62		
0.091		0.955		10.000		104.713		1096.478			
	0.00		0.00		0.20		1.71		0.00		
0.105		1.096		11.482		120.226		1258.925			

Figure 2a. Volume (%) size distribution curve of aceclofenac ethyl cellulose microparticles (AECM).

| d(0.1): | 93.342 | um | d(0.5): | 197.209 | um | d(0.9): | 344.232 | um |
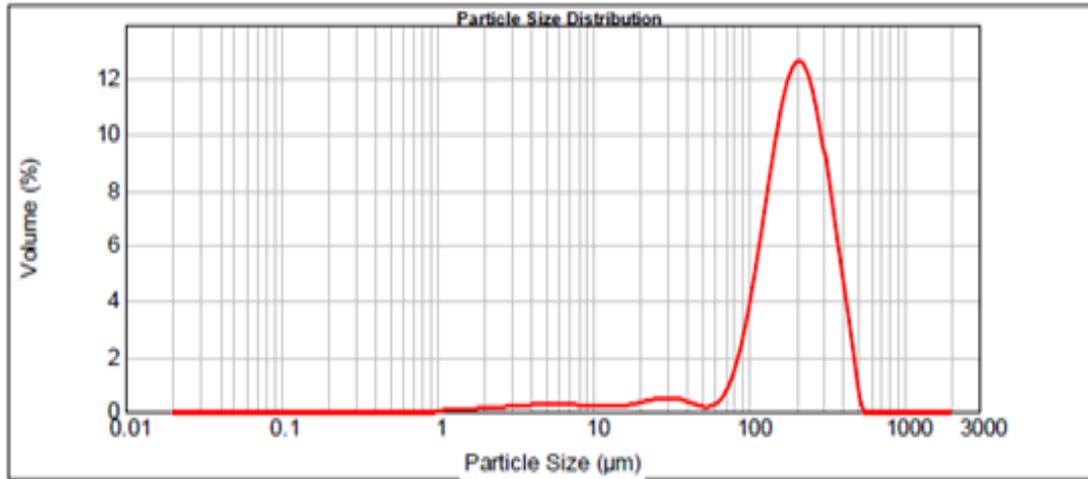
Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.08	11.482	0.20	120.226	6.78	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.10	13.183	0.20	138.038	8.72	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.12	15.136	0.23	158.489	10.29	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.14	17.378	0.28	181.970	11.23	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.16	19.953	0.35	208.930	11.30	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.18	22.909	0.41	239.883	10.47	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.20	26.303	0.45	275.423	8.88	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.22	30.200	0.45	316.228	6.76	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.24	34.674	0.40	363.078	4.59	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.26	39.811	0.30	416.869	2.50	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.27	45.709	0.20	478.630	0.38	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.28	52.481	0.18	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.28	60.256	0.36	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.27	69.183	0.84	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.25	79.433	1.74	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.03	8.710	0.23	91.201	3.08	954.993	0.00	10000.000	
0.091	0.00	0.955	0.07	10.000	0.21	104.713	4.63	1096.478	0.00		
0.105		1.096		11.482		120.226		1258.925			

Figure 2b. Volume (%) size distribution curve of aceclofenac loaded microparticles (AEUM).

Flow and compressibility characteristics:

Flow behavior and compressibility of the aceclofenac loaded microparticles were evaluated by methods and results are shown in *Table .3.*

Table 3. Flow and compressibility characteristics of final Aceclofenac loaded microparticles (AECM & AEUM).

S.No	Parameters	AECM*	AEUM*
1	Angle of Repose(^{o})	18.13±0.002	19.11±0.002
2	Bulk Density(g/cm^3)	0.627±0.004	0.562±0.004
3	True density(g/cm^3)	0.721±0.003	0.637±0.003
4	Carr's Index (%)	14.42±0.002	15.26±0.002
5	Hausner's Ratio	1.1±0.002	1.1±0.002

* Values areMean ± SD, for n=3

The angle of respose of microparticlre formulations were 18.13˚ (AECM) & 19.11˚ (AEUM) indicating their excellent flowability. This was further supported by the values of compressibility indices, Carr's index (14.42: AECM) & 15.26: AECM) & Hausner's ratio (1.1 for both the formulations) of the micropartcle formulations.

2.4.X-ray diffractometry

The *X*-ray diffractometric styudies conducted for both the optimized aceclofenac microparticle. *X*-ray diffraction is one of crystallography characterization tools. It is employed by researchers for a wide variety of applications – it is used as an analytical tool in identification of the constituents of mixtures of crystalline phases; and for the measurement of lattice parameters.Aceclofenac formulations prepared with polymer ethyl cellulose (AECM) and eudragit RSPO (AEUM) showed the presence of characteristic peaks of the drug, aceclofenac, in the formulationas (*Figure. 3*). These results indicated presence of the drug in the polymeric matrix of microparticle formulations either as dispersed / entrapped molecularly or in amorphous form.
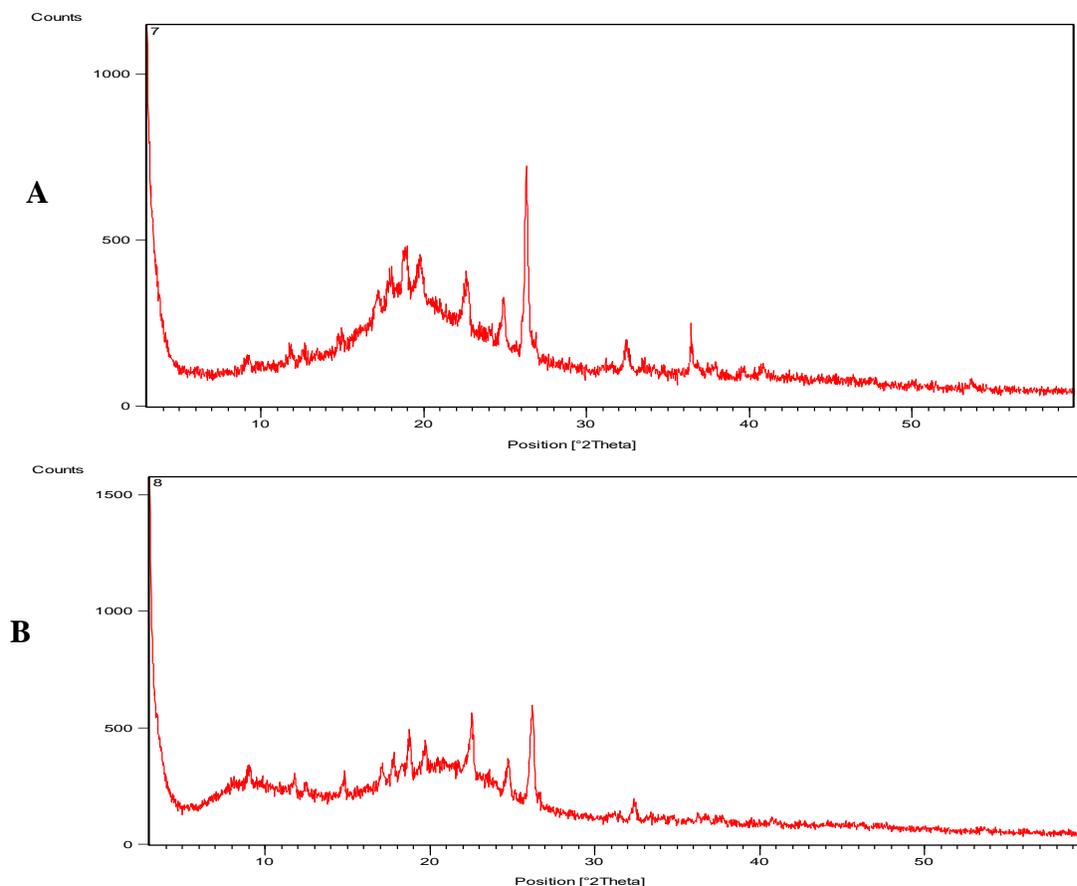
Figure 3. *X*- ray diffractogram (XRD) of final product of Aceclofenac loaded microparticles. (A. AECM, B. AEUM)

3. In vitro drug release of Final product of aceclofenac microparticles

In vitro drug release from the aceclofenac microparticles was carried out in USP Dissolution apparatus type I [Tablet Dissolution Tester, USP XVIII model, Electrolab, India] using 900 ml of phosphate buffer (pH 7.4) maintained at $37 \pm 0.5°C$ and 50 rpm.Microparticles, equivalent to 10 mg of aceclofenac, were used for the study. 10 ml of the sample solution were withdrawn at predetermined time intervals (viz. 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours). The samples were filtered through 0.45 μm membrane filter and analyzed in a UV-VIS spectrophotometer at 275 nm following this method of Rojnik*et al* 2005. Every time the test sample was withdrawn, it was replaced with an equal amount of fresh dissolution medium, maintained at 37°C.

In vitro drug release data were fitted into zero order and first order kinetic models to find the drug release kinetics of the aceclofenac loaded ethyl cellulose micro particles. The mechanisms of drug release were determined by fitting the data into Higuchi, Hixson- Crowell and Korsmeyer-Peppas equations. The results are furnished in **Tables 4 (**AECM)**&5**(AEUM) and Figures**4 (**AECM)**&5 to (**AEUM). Burst release during 1st hour was observed for both the formulations. It was 14% for the formulation AECM and 18% for the formulation AEUM. The drug release from the microparticles was slow and extended beyond 24 hours.
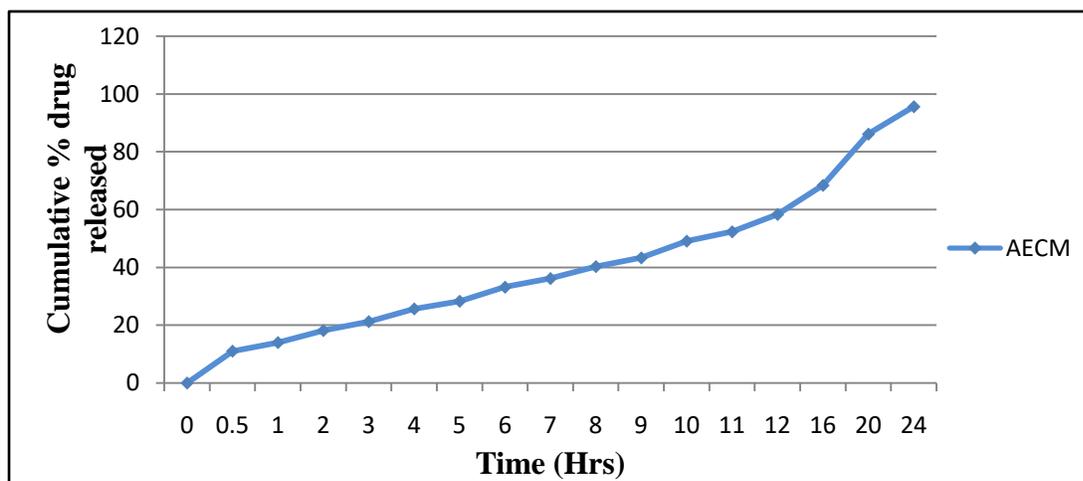
Figure 4.*In vitro* **drug release profile of Aceclofenac ethyl cellulose microparticles (AECM)**
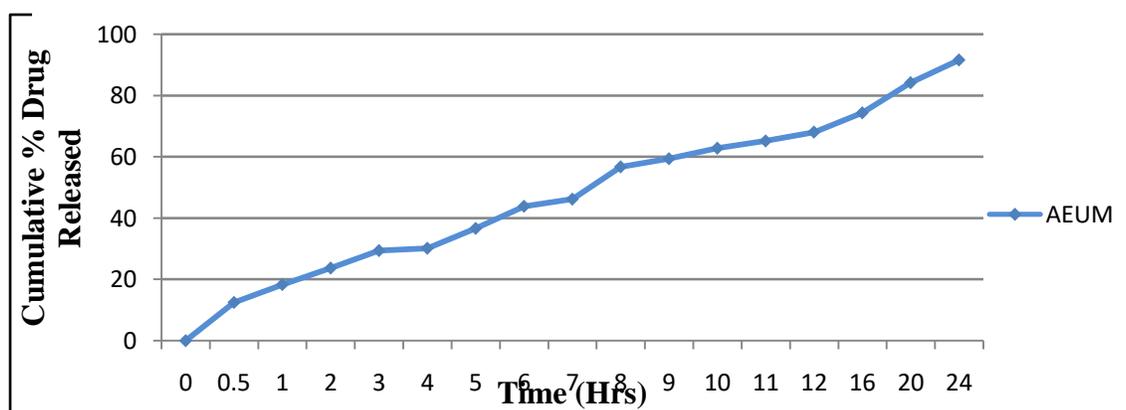
Figure 5.*In vitro* **drug release profile of Aceclofenac Eudragit RSPO microparticles (AEUM)**

In order to determine the release model which best describes the pattern of drug release, the *in vitro* drug release data of the microparticle formulations were fitted into various models.The results are furnished in ***Fig. 6 to15***.
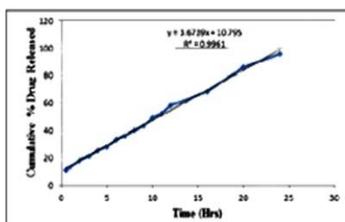
Fig:6
Zero Order Kinetic
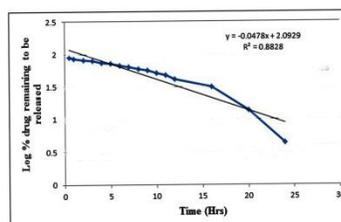
Fig:7
First Order Kinetic
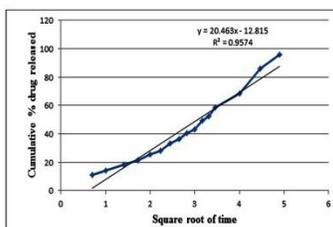
Fig:8
Higuchi Plot
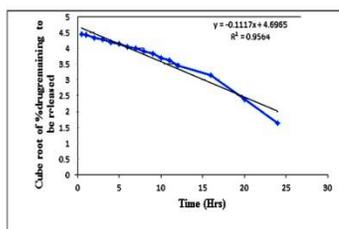
Fig:9
Hixson Crowell
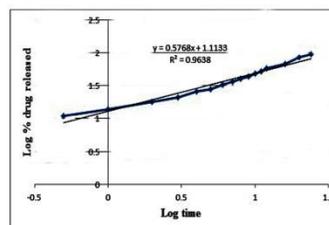
Fig:10
Korsmeyer Peppas

Figure 6 -10.Kinetics of*in vitro* drug release data of Aceclofenac ethyl cellulose microparticles (AECM)
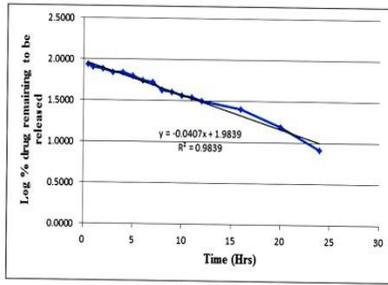
Fig:6
**Zero Order Kinetic**
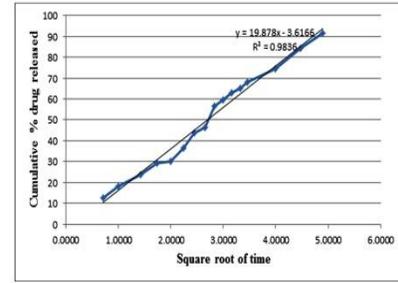
Fig:12
First Order Kinetic
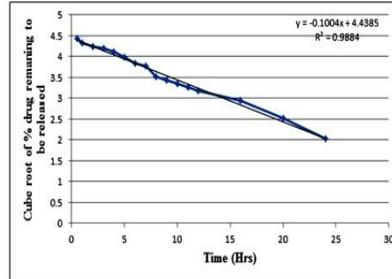
Fig:13
**Higuchi Plot**
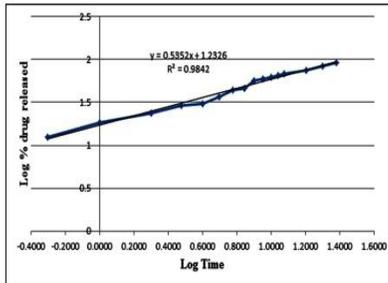
Fig:14
**Hixson Crowell**
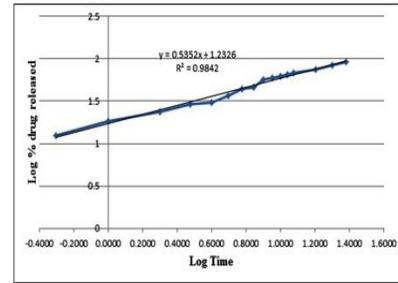
Fig:15
Korsmeyer Peppas

Figure 11 to 14 Kinetics of *in vitro* drug release data of Aceclofenac Eudragit RSPO microparticles (AEUM)

Table 4. Regression coefficients (R^2), rate constants (release kinetic plots) and n (slope of Korsmeyer-Peppas plot) values of various plots of drug release data of Aceclofenac loaded microparticles in Phosphate buffer pH 7.4.

Optimized Formulation	Regression coefficient (R^2) & Release rate constants									
	Zero order		First order		Higuchi		Hixson Crowell		Korsmeyer-Peppas	
	R^2	K_0	R^2	K_1	R^2	H	R^2	Hc	R^2	n
AEC	0.996	3.673	0.882	-0.047	0.957	20.46	0.956	-0.111	0.963	0.576
AEUM	0.927	3.397	0.983	-0.040	0.983	19.87	0.988	-0.100	0.984	0.535

The regression coefficient values (R^2) were taken into account to decide upon the relevance of the model/curve fit which will best describe the extent of fit. According to the coefficient values (R^2) (***Table.4***) drug release data fits well in Peppas (0.996 for AECM and 0.984 AEUM), Higuchi (0.957 for AECM and 0.983 AEUM) as well as Hixson-Crowell cube root (0.956 for AECM and 0.988 AEUM) models.

Zero- and First- order kinetic model fits of the drug release data exhibited good zero order fit (0.996)for AECM; while for AEUM it was first order (0.983) indicating that the amount of drug released is dependent on the matrix drug load

The *in vitro* release data fits into Higuchi model indicating drug release was by diffusion. The release profile of both the formulations displayed very good fitting with Hixson-Crowell cube root model as well indicating drug release by dissolution.

According to KorsmeyerPeppas fit, the release of the drug is decided upon the diffusion of the polymeric matrix. The factors which control this are diffusion coefficient and permeability

coefficient of the polymer at a constant temperature. The values of diffusion exponent, n, of the Korsmeyer-Peppas plots between log fraction drug released and log time ranged for optimized formulations between (0.576) for AECM, and (0.535) for AEUM indicating that drug release from both the formulations (*Table 4* & AECM *Figure 9*, & AEUM *Figure15*) followed anomalous (non-Fickian) diffusion mechanism,). According to Korsmeyer-Peppas equation, value of 'n' between $0.5 < n < 1.00$, is indication of anomalous (non-Fickian) diffusion.

4. Comparison of Predicted and Practical Response

The results of validation of the microparticle formulations (particle size, entrapment efficiency and drug release profile) were compared with those predicted through optimization technique. From the results (*Table 5*) it is evident that there were no significant differences (% deviation of < 2) between the values of the desired responses (particle size, entrapment efficiency and drug release profile) of the acelofenac microparticles (AECM and AEUM) obtained experimentally and those predicted through optimization technique.

Table 5. Predicted and practical responses of optimized Extended Release Aceclofenacmicroparticles made with Ethyl cellulose and (AECM) Eudragit RSPO (AEUM)

Response	AECM Predicted value	Practical value	% Deviation	AEUM Predicted value	Practical value	% Deviation
Particle size (μm)	225.813	221.534	1.89	194.770	197.209	-1.24
(%)Entrapment Efficiency	83.25	82.62	0.75	84.45	83.37	1.27
(%) Drug release in 24 hrs	97.25	95.66	1.63	92.35	91.64	0.76

Conclusion

Aceclofenac loaded microparticles were prepared successfully by emulsion solvent evaporation technique. With encapsulation efficiency increased from 82.62 % for AECM, 83.37 % for AEUM and were reasonably good (>80%). Microparticles prepared showed good flow properties as evidenced by Angle of Repose, Carr's Index and Hausner's Ratio.

In vitro drug release of the formulations depended on drug to polymer ratios, and formulations are above (95% for AECM & 91% for AEUM) at the end of 24 hrs, making them suitable for oral use and were selected for *in vivo* studies. The release from above formulations was slow, gradual, extended and expected to exhibit prolonged action anti inflammatory activity *in vivo*. Aceclofenac microparticle with desired responses were prepare with optimized formulation and processing parameters.The prepared aceclofenac microparticle were evaluated to assese if desired attributes are bultin the aceclofenac microparticle formulation.The evalution results indicates that aceclofenac microparticle formulation were having the desired responses(Particle size,drug entrapment efficiency,drug release pattern).Comparable with those predicted during optimization process with (% deviation < 2.0).Thus its inferred that optimization technique may be used as a useful tool in formulation development of aceclofenac microparticles.

REFERENCE

1. Higuchi, T., Mechanism of sustained action medication; theoretical analysis of rate release of solid drugs dispersed in solid matrices.J. Pharm. Sci., 1963; **52:** 1145.

2. Higuchi, T., Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci., 1961; **50:** 874.

3. Hixson, A.W. and Crowell, J., Dependent of reaction velocity upon surface and agitation. *Ind. Eng. Chem*., 1931; **23:** 923.

4. Jalil, R .andNixon, J.R.., Microencapsulation using poly (DL-lactic acid). IV: Effect of storage on the microcapsule Characteristics. J.Microencapsul., 1990; **7(3):** 375-383.

5. Kamlesh, D. Saraf, S. and SwarnlataSaraf,.Effect of processing variables on micro particulate system of Aceclofenac.Pak.J. Pharm Sci., 2006; **19(1):** 1-6.

6.   Kawashima, Y. Niwa, T. Handa, T. Takeuchi, H. Iwamoto, T. and Itoh,K., Preparation of controlled release microspheres of ibuprofen with acrylic polymers by a novel quassi-emulsion solvent diffusion method. J. Pharm. Sci., 1989; **78:** 68-72.

7.   Lambert, O. Nagele, O. Loux, V. Bonny, J.D. and Marchal, H.L., Poly (ethylene carbonate) microspheres: manufacturing process and internal structure characterization. J. Control. Release, 2000; **67:** 89-99.

8.   Lazarus, J.andCooper, J., Absorption testing and clinical evaluation of oral prolonged-action drugs. J Pharm Sci., 1961; **50:** 715-732

9.   Ming Shi yang, Fu de Cui, Ben-gang you, Yu ling Fan, Liang Wang, PengYue and He yang., Preparation of sustained release nitrendipine microspheres with eduragit RS and Aerosil using quasi emulsion solvent diffusion method. Int.J. Pharm., 2003; **259:** 103-113.

10.   Perumal, D., Microencapsulation of Ibuprofen &Eudragit RS 100 by the emulsion solvent diffusion technique. Int. J. Pharm., 2001; **218:** 1-11.

11.   Pongpaibul, Y. Maruyama, K.andIwatsuru, M., Formation and in-vitro evaluation of theophylline-loaded poly (methyl methacrylate) microspheres. J.Pharm.Pharmacol., 1988; **40(8):** 530-533.

12.   Ritger, P.L.and Peppas, N.A., A simple equation for description of solute release. I. Fickian and Non- Fickian release from non swellable devices in the forms of slabs, spheres, cylinder or discs. J. Control. Release, 1987; **5:** 23-36.

13.   Rojnik, T.M. RokFrlan, S. Bogataj, M. Peter, B. and Mrhar, A., Effect of temperature in solvent evaporation process on eudragit RS microsphere properties. Chem.Pharm.Bull, 2005; **53(1):** 143-146

14.   Saravanan, M. Dhanaraju, D. Sridhar, S.K. Ramachandran, S. Kishore Gran sam, S.Anand ,P. Bhaskar, K. and Srinivasarao, G., Preparation, Characterization and *in vitro* release kinetics of ibuprofen polystyrene microspheres. Ind J Pharm Sci 2004; **66(3):** 287-292.

15.   Pongpaibul, Y. Maruyama, K.andIwatsuru, M., Formation and in-vitro evaluation of theophylline-loaded poly (methyl methacrylate) microspheres. J. Pharm. Pharmacol. 1988; **40 (8):** 530-533.

16.   Sansdrap, P. and Moses, A.J., Influence of manufacturing parameters on the size characteristic and the release profiles of nifedipine from poly(-lactide-co-glycolide) microspheres. Int. J. Pharm 1993; **98:** 157-164.

17.   Sanchez, A. Gupta, R.K. Alonso, M.J. Siber, G.R.andLanger, R. Pulsed controlled-released system for potential use in vaccine delivery.J Pharm Sci. 1996; **85(6):** 547-552.

18.   Lambert, O. Nagele, O. Loux, V. Bonny, J.D. and MarchalHeussler, L. Poly (ethylene carbonate) microspheres: manufacturing process and internal structure characterization. J. Control. Rel. 2000; **67:** 89-99.

19.   Khuri, A.I. and Cornell, J.A., Response Surfaces: Designs and Analyses; Marcel Dekker: New York, 1987.

20.   Hussain, A.S. Yu, X. and Johnson, R.D., Application of Neural Computing in Pharmaceutical Product Development. Pharm. Res.1991; **8:** 1248-1252.

21.   Takayama, K. Nambu, N. Nagai, T., Computer optimization of formulation of flufenamic acid/polyvinyl poly pyrrolidone/methyl cellulose solid dispersions. Chem Pharm Bull.1983; **31:** 4496-4507.

22.   Takayama, K. Nambu, N. Nagai, T., Computer optimization of formulation of flufenamic acid/polyvinyl poly pyrrolidone/methyl cellulose solid dispersions. Chem Pharm Bull.1983; **31:** 4496-4507

23.   Derringer, G. Suich, R. Simultaneous Optimization of Several Response Variables. J. Qual .Technol. 1980; **12:** 214-219.

24.   Lind, .E.E. Goldin, J. Hickman, J.B., Fitting yield and cost response surfaces. ChemEngProg. 1960;**56:** 62-68.

25.   Bayne, C.K.and Rubin, I.B., Practical Experimental Design and Optimization Methods for Chemists. VCH Publisher, Deerfield Beach, FL, 1986.Hassan, E.E. Parish, R.C. Gallo,V., Optimized formulation of magnetic chitosan microspheres containing the anticancer agent, oxantrazole. J .M .Pharm. Res. 1992; **9:** 390-397.