PREPARATION AND *IN VITRO* EVALUATION OF DICLOFENAC SODIUM LOADED ETHYL CELLULOSE MICROCAPSULES BY EXPERIMENTAL DESIGN

P.AMARESHWAR*, V.HANUMATH SASTRY**, AND P.CHAKRAVARTI*

*UNIVERSITY COLLEGE OF TECHNOLOGY, OSMANIA UNIVERSITY, HYDERABAD

**MESCO COLLEGE OF PHARMACY, MUSTAEEDPURA, KARWAN, HYDERABAD

ABSTRACT

The primary goal of the present investigation was to study the main and interaction effects of selected process and formulation variables on the response variable(s) in the coacervation phase separation induced by non-solvent addition using experimental design and thereby identifying the global solution to optimize the response variable(s) for sustaining the drug release. For this a model a drug Diclofenac Sodium (DFS) was microencapsulated with Toluene-Ethylcellulose (EC)-Petroleum ether system. A 2-level-3-factor (2^3) full factorial experimental design with 3 replications was used to prepare the microcapsules with five different coat: core ratios (ECMC-1, ECMC-2, ECMC-3, ECMC-4, and ECMC-5) selecting solvent to non-solvent ratio, stirring speed, and stirring time as independent variables (predictors) and time taken for 50% release (T_{50}) as dependent variable (response). The results revealed that, within the gamut of the levels of predictors studied, the global solution to maximize this response variable is: Toluene: petroleum ether ratio 1:2, stirring speed 200 rpm, and stirring time 30 minutes.

KEY WORDS : Coacervation-phase separation, Full Factorial Experimental Design, Replications, Variables.

1.INTRODUCTION

In pharmaceutical technology, simple coacervation, alike other coacervation types, is very frequently used to entrap drugs into microcapsules. Simple coacervation in organic and aqueous systems depends on molecular interactions of the materials involved at a given temperature, the presence of solid or liquid surfaces with a high affinity for the coacervate phase, the rate of polymer desolvation and fluid dynamic processes, e.g., diffusion, laminar or turbulent movements. In the coacervation process phase equilibrium is never reached. Therefore, the formulation and process variables significantly affect the kinetics of the entire process and ultimately the characteristics of microcapsules (Gander,2007). The methodology of Design of Experiments (DoE) provides proven strategies and methods of experimental design for performing and analyzing test series in a systematic and efficient way. All experimental parameters are varied in an intelligent and balanced fashion so that a maximum of information is gained from the analysis of the experimental results. In most cases, the time and money spent on the experimental investigation will be greatly reduced (Montgomery,1976).To the best of our knowledge, influence of process variables on microencapsulation by coacervation-phase separation induced by non-solvent addition has not been reported. Hence, in the present investigation a model drug Diclofenac Sodium (Adeyeye,Li,1990) (DFS) was microencapsulated with Toluene-Ethylcellulose (EC)-Petroleum ether system by selecting three variables, stirring speed, stirring time, and solvent to non-solvent ratio as 3 factors (independent variables), each at 2 levels- low and high; using full factorial experimental design. The objective was to study the magnitude of main and interaction effects of the predictors and to rank their contribution on response variable- time taken for 50% of the drug dissolution (T_{50}).

2.MATERIALS AND METHODS

Materials

Diclofenac Sodium (gift sample from Amoli Organics, Ahmadabad), Ethylcellulose (EC) (BDH) (having an ethoxyl content of 47.5% weight and a viscosity of 22 cp in a 5 % concentration, by weight, in a 80: 20 toluene-ethanol solution at 25^{0}C), Toluene (BDH) (109^0-112^{0}C), Petroleum ether (Glaxo) (60^0-

** Corresponding Author:
e-mail: vhsastry@rediff.com

80^{0}C) were used. All other chemicals and reagents used were of analytical grade.

Preparation of Microcapsules

DFS was microencapsulated with EC by coacervation-phase separation induced by a non-solvent addition method reported earlier (Al-omran,2002), with minor modifications. Microcapsules were prepared at five coat: core ratios of 1:9 (ECMC-1), 1:4 (ECMC-2), 2:3 (ECMC-3), 3:2 (ECMC-4), and 3:1(ECMC-5). A 2^3 full factorial experimental design with 3 replications was used to prepare the microcapsules with each of the coat: core ratio. In this experimental design, solvent to non-solvent ratio, stirring speed, and stirring time were selected as independent variables (predictors) and time taken for 50% of the drug dissolution (T_{50}) was chosen as dependent variable (response). The coded and actual values of the independent variables are shown in the Table 1.

Table 1. Coded and Actual Values of the Independent Variables

Variable	Low level (-1)*	High level (+1)*
Solvent to non-solvent ratio	1:1	1:2
Stirring speed	200 rpm	300 rpm
Stirring time	15 minutes	30 minutes

* Coded values are indicated in the parentheses

EC was dissolved in a one liter beaker containing toluene (solvent) for EC. DFS powder in a 1:9, 1:4, 2:3, 3:2, and 3:1 coat: core ratio was added to EC solution with stirring at for a uniform distribution. Petroleum ether (non-solvent to EC) was gradually added with continuous stirring. The microcapsules were filtered and air dried for 24 h to obtain discrete microcapsules. This procedure was followed with each of the coat: core ratio, varying the combinations of the independent variables, as per the experimental design matrix shown in Table 2.

The amount of EC precipitated with different ratios of toluene: petroleum ether was determined by the following procedure (Al-omran,2002): Fifty millilitres of toluene were introduced into a set of (500ml) flasks, together with 1.5 g of EC, and allowed to dissolve. Varying volumes of petroleum ether (10, 15, 25, 50, 75, 100 and 150ml) were added at a constant rate (2 ml.min^{-1}) and the flasks were equilibrated for 24 h at room temperature The supernatant liquid and the colloidal phases were separated by decantation and evaporated. The amount of EC in each phase was weighed.

Characterization of Microcapsules

Drug Content: An accurately weighed thoroughly ground DFS loaded microcapsules was pulverized and digested in pH 6.8 buffer. The drug was extracted with the solvent overnight; filtered using 0.45 im filter and the amount of medicament in the filtrate was assayed after appropriate dilution by measuring the absorbance at 276 nm in Shimadzu UV- 2550 UV/ Visible Spectrophotometer. The drug content was estimated in triplicate.

Microencapsulation Efficiency: Microencapsulation efficiency was calculated using the following formula:

Microencapsulation efficiency = (estimated percentage drug content/theoretical percentage drug content) × 100. (1)

Yield: The percentage yield of microcapsules was calculated using the following formula:

% yield= weight of microcapsules (g)/initial weight of DFS (g) + initial weight of EC (g) × 100 (2)

Morphology and Size Distribution: The morphology of microcapsules was evaluated by the scanning electron microscopy (SEM) (JSM-6510LV).

Particle size range and distribution of microcapsules were determined using optical microscopy.

Measurement of Micromeritic and Packing Properties of Microcapsules

The angle of repose of microcapsules was determined by the fixed- funnel and free-standing cone method. Hausner ratio and Carr's Index were determined from bulk density measurements (Amareshwar,2010).

Statistical Diameters: The following statistical diameters (Parrott) were calculated:

Geometric mean diameter $d_{geo} = \text{Antilog}\ \{\sum(n \log d)/\sum n\}$ (3)

Arithmetic mean diameter $d_{ave} = \sum nd / \sum n$ (4)

Mean surface diameter $d_s = \sqrt{\sum nd^2 / \sum n}$ (5)

Mean volume diameter $d_v = \sqrt[3]{\sum nd^3 / \sum n}$ (6)

Mean volume surface diameter $d_{vs} = \sum nd^3 / \sum nd^2$ (7)

Weight mean diameter $d_w = \sum nd^4 / \sum nd^3$ (8)

Sphericity: Ten microcapsules were observed by optical microscopy, fitted with a camera lucida connected to a grabbing board. The size and shape descriptors characterizing each particle as in its silhouette were calculated. Two parameters, the silhouette breadth (*B*, smallest dimension) and length (*L*, largest dimension), were noted, and the degree of sphericity (Xinde,2007) ø is defined as: $ø = \frac{L}{B}$

Porosity: The porosity of the microcapsules was calculated from the density values using the following equation (Ozyazici,1996):

$$å = 1- \{(C_{EC} \times ñ_{DFS} + C_{DFS} \times ñ_{EC})\, ñ_{M}\} / ñ_{DFS} \times ñ_{EC}$$

where: å, porosity of the microcapsules; C_{EC}, weight per cent of EC; C_{DFS}, weight per cent of DFS; $ñ_{DFS}$, density of DFS particles; $ñ_{EC}$, density of the EC particles; $ñ_{M}$, density of the microcapsule particles.

Thickness: The thickness of the microcapsule wall was calculated using the following equation (Luu and Cartier,1973):

$$h = r/3 \times \{(1-P) \times d_1 / (P \times d_2) + (1-P) \times d_1\}$$

where h is the thickness of the envelope (in µ); r is the value of the average radius (in µ); P is the ratio of the mass of the nucleus to the total mass of the microcapsules; d_1, is the density of the material of the nucleus (in g/cm^3); d_2 is the density of the material of the envelope (in g/cm^3).

Release Rate Studies

Microcapsules equivalent to 100 mg of DFS were filled in hard gelatin capsules and were evaluated for in-vitro release studies. The study was carried out in USP XXII basket apparatus at a rotational speed of 50 rpm at 37 ± 0.5° C in 900 ml phosphate buffer (pH 6.8). Samples (10 ml) were withdrawn at regular time intervals and filtered through 0.45 µm membrane filter. The drug content was determined in the filtrate at 276nm either directly or after appropriate dilution with the dissolution medium.

To find out the kinetics and mechanism of drug release, data obtained from release rate studies was fitted to both linear and non-linear kinetic models (Zhang,2010) (Table 3).

Table 3. Fitting of Data from Release Rate Studies to Linear and Non-Linear Models

Linear Models		Non- linear Models	
Zero-order	$F = k_0.t$	Gompertz	$F = F_{max}.e^{-e^{k_G(t-\gamma)}}$
First-order	$F = 100.(1-e^{-k_1.t})$	Logistic	$F = F_{max} \cdot \frac{1}{1+e^{-k_L(t-\gamma)}}$
Higuchi	$F = k_H.t^{0.5}$	Quadratic	$F = 100.(k_1.t^2 + k_2.t)$
Korsmeyer-Peppas	$F = k_{KP}.t^n$	Weibull	$F = F_{max}.(1-e^{-\frac{(t-\gamma)^{\beta}}{\alpha}})$

F denotes fraction of the drug released at time t; k stands for release rate constant in respective models; n indicates the exponent- If the n value is 0.5 or less, the release mechanism follows Fickian diffusion, and the values 0.5 < n < 1 indicate a non-Fickian release (anomalous/zero order release). The drug release follows zero-order and case-II transport if the n value is 1. For the values of n higher than 1, the mechanism of drug release is regarded as super case-II transport. F_{max} is the maximum fraction of the drug released at infinite time; k stands for release rate constant in respective models; á, â, and ã denote scale factors in respective models. The criteria considered for selecting the best fit model were: the adjusted coefficient of determination ($R^2_{adjusted}$) and standard error of mean (SEM) (Costa and Sousa,2001).

To characterizing drug release curve, area under the release curve (AUC), mean residence time of the drug substance molecules in the dosage form (MRT), mean dissolution time (MDT), and dissolution efficiency up to 30 minutes (DE_{30}) were also calculated (Zhang,2010).

Further, data obtained from release rate studies was fitted for the following probability distributions using Anderson-Darling test statistic (De muth,2006)[12] as the criterion: normal, log-normal, exponential, Weibull, logistic, and log logistic.

3.RESULTS AND DISCUSSION

For microencapsulation with ethyl cellulose a non-aqueous phase separation method was employed. Five ratios of EC and DFS, namely, 1:9 (ECMC-1), 1:4 (ECMC-2), 2:3 (ECMC-3), 3:2 (ECMC-4), and 3:1 (ECMC-5) were used to prepare microcapsules using 2^3 full factorial experimental design with 3 replications with each of the coat: core ratio. Replicate trials were performed to obtain an estimate of the standard error (deviation) associated with the effects. Without this estimate, it is often difficult to determine

whether a variable actually affects the measured response or whether the value obtained is simply due to experimental error. To illustrate the main and interaction effects of the predictors on the response, the results of DoE in the preparation of ECMC-5 are shown in Figs.1-3.

In addition to these, contour plots were also shown, which indicate the combined effect of toluene: petroleum ether ratio and stirring speed on the T_{50} values holding stirring time both at low (Fig.4) and high (Fig.5) levels. These results indicate that:

- Increasing the toluene: petroleum ether ratio increases T_{50}
- Increasing the stirring speed decreases T_{50}
- Increasing the stirring time increases T_{50}

The toluene: petroleum ether ratio influenced the amount of EC precipitated from EC solution. As the ratio increased, T_{50} values increased because more amount of EC was precipitated and was available for coat formation on the drug. To corroborate this, the amounts of EC precipitated with varying toluene: petroleum ether ratios were determined (Fig.6). The results are in agreement with that reported earlier (Al-omran,2002).

The effect of stirring speed can be attributed to a higher percentage of smaller microcapsules. A higher speed gives fewer aggregates by promoting better deposit of the coacervates, which prevents the bridging of microcapsules. This yields higher percentage of smaller microcapsules, which increases the surface area available for dissolution, thereby promoting higher percentages of drug dissolved (Chemtob,1986).

The effect of stirring time can be attributed to the more uniform deposition of the coat on the drug particles. Extended time of stirring might had resulted in better dispersion of the coat material precipitated during phase separation, and subsequent uniform coating on drug particles.

Results of ANOVA indicated that: With all batches of EC microcapsules prepared, toluene: petroleum ether ratio and stirring time exerted a positive influence and stirring speed exerted a negative influence on T_{50} values. Toluene: petroleum ether ratio* stirring speed (interaction) exerted a negative effect on T_{50} values. In the case of ECMC-1, Toluene: petroleum ether ratio* stirring time (interaction) exerted a negative effect and in the case of ECMC-2, a positive effect on T_{50} values. This may be attributed to the high proportion of EC in the latter. With still higher proportions of EC (ECMC-3, ECMC-4, and ECMC-5), the interaction was insignificant ($p>0.05$) and most probably the proportion of EC was the major controlling factor for drug release rate (and hence T_{50} values). Similarly, stirring speed* stirring time (interaction) was significant and positive when the proportion of EC was low (ECMC-1 and ECMC-2), and insignificant in the case of other batches of microcapsules. Here also, the proportion of EC might be the major controlling factor for drug release rate (and hence T_{50} values). Similarly, the three way interaction Toluene: petroleum ether ratio*Stirring speed* stirring time was significant only in the case of ECMC-1 and ECMC-2.

Since the target is to maximize T_{50} values for sustained release, it may be concluded that the global solution to maximize this response variable is: Toluene: petroleum ether ratio 1:2, stirring speed 200 rpm, and stirring time 30 minutes.

Since 24 formulations were prepared as per the experimental design matrix shown in Table 2, a formulation as per the global solution to maximize the response variable is further studied. The results are presented in the following paragraphs.

To characterize the microcapsules, three parameters were calculated: the drug content, the microencapsulation efficiency, and the weight yield. Low s.d values in the mean percent drug content ensured uniformity of drug content in each batch of pellets. Also, loading efficiency and weight yield are satisfactory (Table 4). SEM picture of the selected ECMCs indicated spherical nature and uniform coating of the microcapsules.

Angle of repose, Hausner ratio and Carr's Index values indicate good flow characteristics of microcapsules (Amareshwar,2010) (Table 5). Sphericity values (Table 5) indicate that pellets are of uniform coat thickness and have good flow properties. Size distribution was of log-normal. Porosity values ranged from 12.3-16.3%. Thickness ranged from 0.25-0.90ìm (Table 5). The statistical diameters calculated are shown in Table 6. Among them Mean volume surface diameter d_{vs} influences the release rate of drug (Parrott).

Further, from Table 4 and Table 5 the following information may be obtained:

- There is a direct relationship between % EC and microencapsulation efficiency (and inverse relationship between % DFS and microencapsulation efficiency)
- T_{50} values of EC microcapsules are directly related to % EC and square of their diameters
- There is a direct relationship between % EC and microcapsule wall thickness

The release profile of drug from microcapsules is shown in Fig.7. The differences in DFS release rate from microcapsules may be attributed to varying coat thickness. Drug release decreased when the proportion of EC increased. However, drug release from microcapsules was rapid, perhaps due to finer size of microcapsules. The calculated values of Dissolution Efficiency upto 30 minutes (DE_{30}), Mean Dissolution Time (MDT), Mean Residence Time (MRT), Area under the Release Curve (AUC), and T_{50} values were calculated and shown in Table 7. It is evident that, dissolution efficiency decreased from ECMC-1 to ECMC-5, mean dissolution time increased from ECMC-1 to ECMC-5, mean residence time increased from ECMC-1 to ECMC-5, AUC values increased from ECMC-1 to ECMC-5, and T_{50} values increased from ECMC-1 to ECMC-5. The reasons for these observations may be attributed to the differences in the proportion of EC in microcapsules as discussed in the above paragraph.

When the data from release rate studies was fitted to linear models, it was observed that the drug release may be described by first order kinetics. 'n' values in Peppas equation ranged from 0.109 to 0.378 suggesting that Fickian diffusion is involved in drug release. When the data was fitted to non-linear models (Table 8), it was observed that they could also be used to fit the data. But, however, convergence problem may occur. This is in agreement with the literature report (Adams,2002). Among the probability distributions verified, it was observed that normal distribution fitted well (based on high value of Anderson-Darling test statistic for normal distribution).

4.CONCLUSIONS

Diclofenac Sodium (DFS) was microencapsulated with Toluene-Ethylcellulose (EC)-Petroleum ether system. A 2-level-3-factor (2^3) full factorial experimental design with 3 replications was used to prepare the microcapsules with five different coat: core ratios (ECMC-1, ECMC-2, ECMC-3, ECMC-4, and ECMC-5) selecting solvent to non-solvent ratio, stirring speed, and stirring time as independent variables (predictors) and time taken for 50% of the drug dissolution (T_{50}) as dependent variable (response). The results revealed that, within the gamut of the levels of predictors studied, the global solution to maximize this response variable is: Toluene: petroleum ether ratio 1:2, stirring speed 200 rpm, and stirring time 30 minutes. Drug release from microcapsules depended on the proportion of EC; however, the release was rapid. Both linear and non-linear models are suitable to fit the data obtained from release rate studies.

Table 2. 2^3 Full Factorial Experimental Design Matrix with 3 Replications

RunOrder	Solvent to Non-Solvent Ratio	Stirring Speed	Stirring Time
1	-1	-1	1
2	1	-1	1
3	-1	-1	-1
4	-1	-1	1
5	-1	1	1
6	-1	1	1
7	-1	1	-1
8	-1	1	-1
9	1	-1	-1
10	1	1	-1
11	1	-1	-1
12	1	1	1
13	1	1	-1
14	1	-1	1
15	1	1	1
16	1	1	1
17	-1	-1	-1
18	-1	1	1
19	-1	-1	-1
20	-1	1	-1
21	1	-1	-1
22	1	-1	1
23	-1	-1	1
24	1	1	-1

Table 4. Drug Content, Microencapsulation Efficiency, and Yield of EC Microcapsules

Formulation	Drug Content (mg/100 mg MC)		Microencapsulation Efficiency	Yield %
	Theoretical	Estimated		
ECMC-1	90.00	60.23 (0.48)	66.92 (0.32)	65.53 (1.28)
ECMC-2	80.00	60.15 (0.34)	75.18 (0.12)	72.59 (1.29)
ECMC-3	60.00	48.46 (0.76)	80.77 (0.16)	80.56 (1.18)
ECMC-4	40.00	35.45 (0.23)	88.63 (0.61)	82.32 (1.01)
ECMC-5	25.00	24.21 (0.47)	96.48 (0.41)	89.51 (1.14)

Table 5. Sphericity, Thickness, Flow, and Packing Properties of EC Microcapsules

Formulation	Angle of Repose (°)	Hausner Ratio	Carr's Index	Sphericity	Thickness µm
ECMC-1	31.32 (0.05)	1.31 (0.21)	24.0 (0.05)	0.55 (0.15)	0.25 (0.99)
ECMC -2	30.12 (0.62)	1.38 (0.42)	27.6 (0.39)	0.69 (0.11)	0.57 (1.13)
ECMC-3	29.34 (0.53)	1.25 (0.71)	20.1 (0.09)	0.78 (0.31)	0.69 (0.99)
ECMC-4	26.56 (0.54)	1.11 (0.36)	09.5 (0.16)	0.89 (0.53)	0.80 (2.23)
ECMC-5	25.78 (0.15)	1.18 (0.48)	15.4 (0.53)	0.96 (0.18)	0.90 (0.99)

*** Values in parentheses are standard deviations**

Table 6. Statistical Diameters of EC Microcapsules (in µm)

Diameter	ECMC-1	ECMC-2	ECMC-3	ECMC-4	ECMC-5
Average	51.02	51.28	51.44	51.86	52.50
Geometric	51.30	51.01	51.08	51.51	52.16
Mean surface	51.33	51.55	51.79	52.21	52.84
Mean volume	51.65	51.81	52.14	52.55	53.17
Mean volume surface	52.29	52.35	52.85	53.24	53.84
Weight mean	52.91	52.89	53.52	53.90	54.48

Table 7. Dissolution Parameters of EC Microcapsules

Formulation	Coat: Core Ratio	DE_{30} (%)	MDT (min)	MRT (min)	AUC (%.min)	T_{50} (min)
ECMC-1	1:9	85.8 (0.21)	4.25 (0.15)	6.61 (0.32)	2575 (0.76)	3.7 (0.16)
ECMC-2	1:4	83.6 (0.65)	7.35 (0.13)	10.05 (0.34)	3765 (0.64)	6.9 (0.31)
ECMC-3	2:3	79.9 (0.51)	12.06 (0.21)	15.19 (0.56)	4794 (0.98)	11.6 (0.38)
ECMC-4	3:2	81.5 (0.95)	22.24 (0.87)	27.71 (0.75)	9776 (0.72)	20.3 (0.87)
ECMC-5	3:1	79.0 (0.47)	31.48 (0.62)	36.97 (0.74)	11853 (0.69)	29.4 (0.39)

Table 8. Fitting of Data from Release Rate Studies to Non-linear Models

Formulation	Gompertz	Logistic	Quadratic	Weibull
ECMC-1	R^2 - 0.9828 R^2_{adj} - 0.9655 SEM- 7.5498	R^2 - ** R^2_{adj} - ** SEM- **	R^2 - 0.6992 R^2_{adj} - 0.3983 SEM- 15.4348	R^2 - 0.1550 R^2_{adj} - 0.0000 SEM- 74.7663
ECMC-2	R^2 - 0.9702 R^2_{adj} - 0.9503 SEM- 8.3991	R^2 - 0.9991 R^2_{adj} - 0.9986 SEM- 1.4289	R^2 - 0.8210 R^2_{adj} - 0.7017 SEM- 20.5727	R^2 - 0.6035 R^2_{adj} - 0.0087 SEM- 37.5012
ECMC-3	R^2 - 0.9277 R^2_{adj} - 0.8915 SEM- 11.6540	R^2 - 0.9992 R^2_{adj} - 0.9988 SEM- 1.2283	R^2 - 0.8760 R^2_{adj} - 0.8141 SEM- 15.2584	R^2 - 0.7684 R^2_{adj} - 0.5368 SEM- 24.0832
ECMC-4	R^2 - 0.9460 R^2_{adj} - 0.9280 SEM- 9.2131	R^2 - 0.9975 R^2_{adj} - 0.9966 SEM- 1.9911	R^2 - 0.8839 R^2_{adj} - 0.8452 SEM- 13.5080	R^2 - 0.9996 R^2_{adj} - 0.9994 SEM- 0.8688
ECMC-5	R^2 - 0.9475 R^2_{adj} - 0.9326 SEM- 8.4543	R^2 - 0.9988 R^2_{adj} - 0.9984 SEM- 1.2853	R^2 - 0.9187 R^2_{adj} - 0.8954 SEM- 10.5264	R^2 - 0.9961 R^2_{adj} - 0.9942 SEM- 2.4894

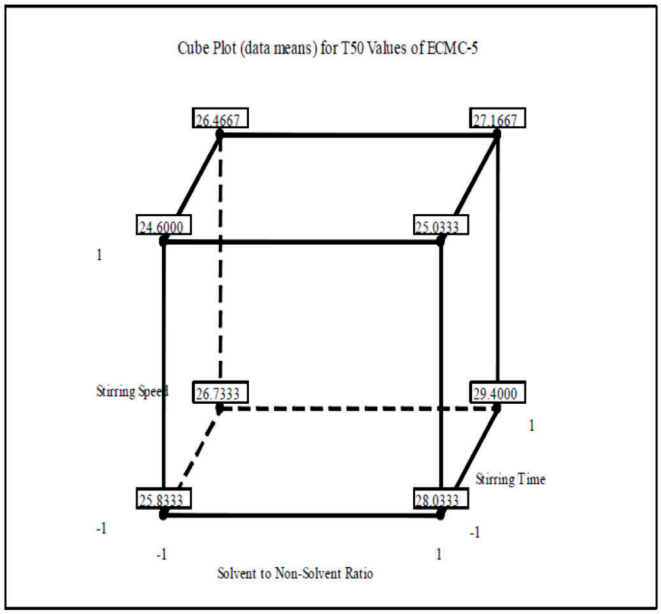


Fig 1. Cube plot for T_{50} Values of ECMC-5

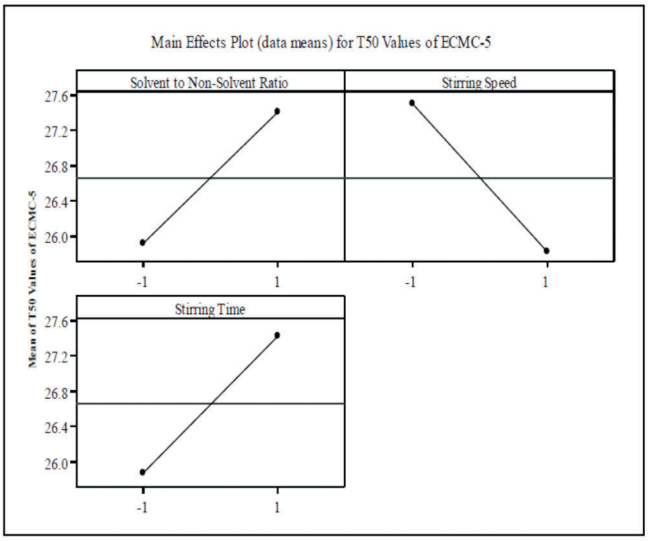


Fig 2. Main Effects Plot for T_{50} Values of ECMC-5

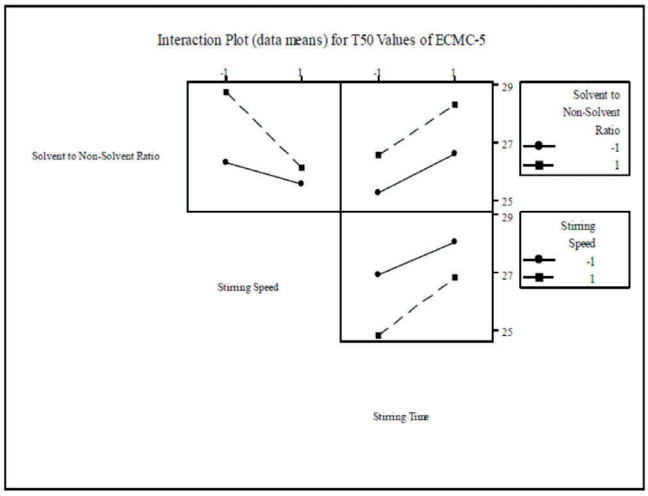


Fig 3. Interaction Plot for T_{50} Values of ECMC-5

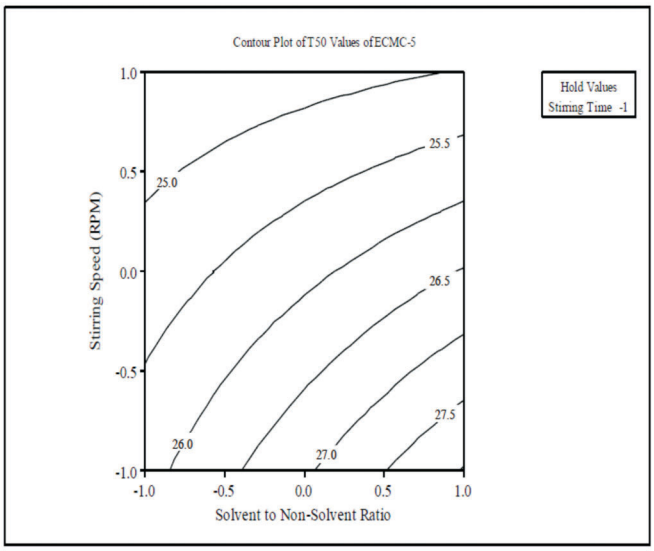
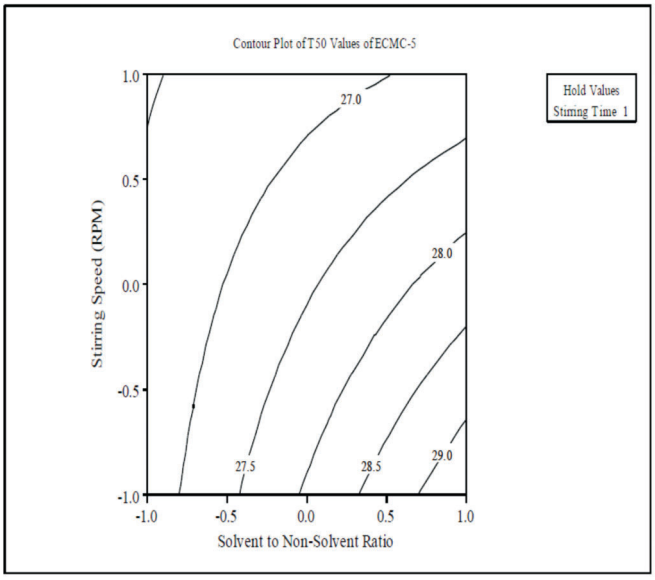


Fig 4. Contour Plot of T_{50} Values of ECMC-5



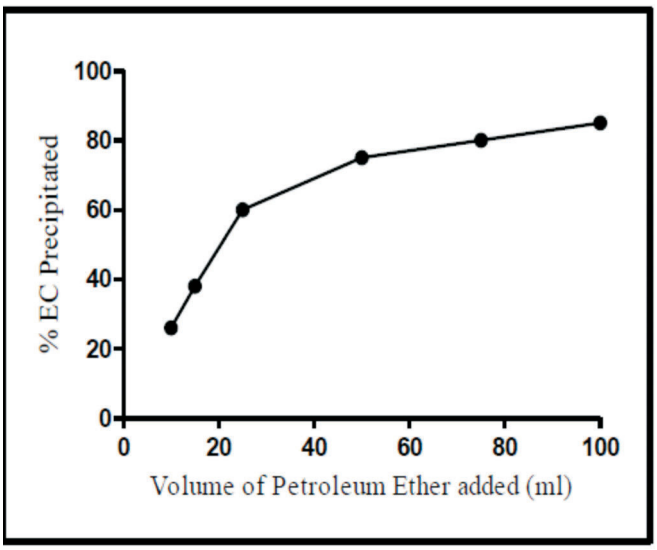


Fig.6. Effect of Non-solvent Quantity on % EC Precipitated

REFERENCES

Adams E, et al., Int.J.Pharm., 240, 2002, 37-53.

Adeyeye C.M, Li P.K, In Florey K, (Ed.), Analytical Profiles of Drug Substances, vol.19, Academic Press, New Jersey, 1990, 123-144.

Al-Omran M.F, Al-Suwayeh S.A, El-Helw A.M, Saleh S.I, J.Microencapsul., 19(1), 2002, 45-52.

Amareshwar P, Hanumath Sastry V, Nivethithai P, Chakravarthi P, J.Chem.Pharm.Sci., 3(1), 2010, 62-66.

Chemtob C, Chaumeil J.C, N'Dongo M, Int.J.Pharm., 29, 1986, 1-7.

Costa P, Sousa Lobo J.M, Eur.J.Pharm.Sci., 13, 2001, 123-133.

De Muth J.E, Basic Statistics and Pharmaceutical Statistical Applications, 2nd edition, Chapman & Hall, Florida, 2006, 582-583.

Gander B, et al., In Swarbrick J, (Ed), Encyclopedia of Pharmaceutical Technology, 3rd edition, Informa Healthcare USA, Inc., New York. 2007, 600-614.

Luu Si-Nang P, Cartier P.F, J.Pharm.Sci., 62, 1973, 452-455.

Montgomery D.C, Design and Analysis of Experiments, 5th edition, Wiley, New York, 1976, 14.

Ozyazici M, Sevgi F, Ertan G, Int.J.Pharm., 138, 1996, 25-35.

Parrott E.L, In Lachman L, Libermann H.A, Kanig J.L, (eds), The Theory and Practice of Industrial Pharmacy, 3rd ed., Varghese Publishing House, Mumbai, India, 26.

Xinde Xu, et al., Chin.J.Chem.Eng., 15(4), 2007, 579-585.

Zhang Y, et al., The AAPS Journal, 12(3) , 2010, DOI: 10.1208/s12248-010-9185-1.