www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1271-1278, Oct-Dec 2009

EVALUATION OF TERBUTALINE SULPHATE ENCAPSULATED ETHYLCELLULOSE MICROSPHERES: A FACTORIAL APPROACH

Rajendra Kotadiya[1]*, Vishnu Patel[2], Harsha Patel[1], Bhavram Salaniya[2]
[1]Indukaka Ipcowala College of Pharmacy, New V V Nagar, Gujarat, India
[2]A.R. College of Pharmacy, V V Nagar, Gujarat, India

EMAIL: rajlec_qa@yahoo.com

ABSTRACT : The aim of this study was to formulate and evaluate microspheres of Terbutaline sulphate (TBS) by water-in-oil-in-water (w/o/w) double emulsion solvent diffusion method using ethyl cellulose. The influence of formulation factors viz. drug content and polymer content on physicochemical properties of the microspheres was investigated by using 3^2 factorial design to investigate the joint influence of two variables: the Drug content (X_1) and Polymer content (X_2) on the Encapsulation efficiency (EE), time for 50% drug dissolution (t_{50}) and time for 70% drug dissolution (t_{70}). The microspheres were found to be comparatively smooth, spherical in shape and free flowing with particle size ranges from 17 µm to 24 µm. The yield was found to be in the range of 48 – 76 %. The results of multiple linear regression analysis revealed that for obtaining controlled drug release with high EE, the microspheres should be prepared using relatively lower levels of drug and higher levels of polymer.
Key words: Factorial design, Encapsulation efficiency, t_{50}, t_{70}, surface plot

INTRODUCTION AND EXPERIMENTAL

Advancement in drug delivery could come from innovative improvement to existing drug delivery system. Because of reduce frequency of administration, sustained release dosage form enjoy convenience and ambulatory patient compliance. Terbutaline sulphate (TBS), a selective β_2 adrenergic agonist, is widely used in the treatment of bronchial asthma, chronic bronchitis and emphysema. Because of its short biological half life and dosing schedule, a long acting TBS formulation is required to improve patient compliance. One of the common methods of controlling the rate of drug release is micro encapsulation using encapsulating materials.

Ethyl cellulose (EC10), a non-biodegradable and biocompatible polymer, one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals, was selected as the retardant material for drug. Several researchers have investigated the utilization of EC10 as a polymer to microencapsulate a drug by coacervation phase separation technique (1-4), emulsion solvent evaporation technique (5,6) and spherical crystallization technique (7). The use of w/o/w double emulsion solvent evaporation method (8) to microencapsulate drug using poly (lactide/glycolide, PLGA) as the polymer was reported with entrapment efficiency of only 5%. The maximum entrapment of only 17% after modifying the secondary aqueous phase was also reported for drug (9).

Thus, the purpose of the present work was to prepare and evaluate oral controlled release microparticulate drug delivery system of drug using EC10 by w/o/w double emulsion solvent diffusion method with high entrapment capacity and extended release. In addition, a 3^2 full factorial design was employed to study the effect of independent variables, drug content (X_1) and polymer content (X_2) on dependent variables, drug entrapment efficiency, time required for 50 % drug release (t_{50}) and time required for 70 % drug release (t_{70}).

Corresponding author:
Rajendra M. Kotadiya
Lecturer
Indukaka Ipcowala College of Pharmacy,
New Vallabh Vidyanagar-388121,
Dist: Anand, Gujarat, India
Mob. 09427529509

EXPERIMENTAL

MATERIALS

Terbutaline Sulphate: Themis Pharmaceutical, Vapi.
Ethyl cellulose (viscosity-10cps): Samir Tech-chem. Pvt Ltd, Baroda
Light liquid paraffin: Allied chemical corporation, Baroda
Heavy liquid paraffin: Chiti-chem Corporation Ltd, Baroda
Polyvinyl alcohol: Allied Chemical Corporation, Baroda

Dichloromethane: A.R. Allied Chemical Corporation, Baroda
Others: AR grade

FOURIER TRANSFORMS INFRARED RADIATION MEASUREMENT (FT-IR)

The FT-IR spectra acquired were taken from dried samples. A FT-IR (Shimadzu IR spectrophotometer, model 840, Japan) was used for the analysis in the frequency range between 4000 and 205 cm^{-1} (8 cm^{-1} resolution & 0.2 cm^{-1} rate). A quantity equivalent to 2 mg of pure drug and physical mixture of drug and polymer were selected separately (10).

VISCOSITY MEASUREMENT

Viscosity of the various organic polymeric solutions (1%, 1.5% and 2% wt/vol) were determined using the Brookefield viscometer (Brooke-field Engineering Laboratories, Stoughton, MA).

PREPARATION OF MICROSPHERES

All microspheres were prepared by the w/o/w double emulsion solvent diffusion method (11). The effect of drug content and polymer content on microspheres characteristics were investigated by using 3^2 factorial design. The initial w/o emulsion was formed by adding drug-deionized water solution to the polymer-Dichloromethane solution with constant stirring at 500 rpm for 5 min. The w/o primary emulsion was then slowly added to aqueous solution of 1 % PVA with continues stirring at maximum speed on a magnetic stirrer (Remi equipment, Mumbai) to get a w/o/w emulsion. The stirring was continued at maximum speed for 1.5 h at room temperature to allow solvent evaporation and microspheres were prepared. The dispersion of microsphere was centrifuged (Ultrasonic processor Centrifuge Remi laboratory centrifuge, Model-R8C UV) at 2000 rpm for 10min. The resulting microspheres were separated by decantation and traces of non-encapsulated drug remaining in the palette was removed by re-suspending in 25 mL of distilled water and centrifuged at 2000 rpm for 10min. This procedure was repeated two times; finally the palette was transferred to what man filter paper and subjected to drying in an incubator at 37° for 16 h. Microsphere were preserved in desiccators until the time of evaluation.

FACTORIAL DESIGN

Well established statistical tools such as factorial designs are very essential to understand the complexity of pharmaceutical formulations. The number of experiment required for these studies is dependent on the number of independent variables selected by the formulator. For simplicity, it was decided to perform a two variable study as three experimental levels to achieve the set objectives efficiently The Drug content (X_1) and the Polymer content (X_2) were selected as independent variables. The Encapsulation efficiency (EE), time for 50% drug dissolution (t_{50}) and time for 70% drug dissolution (t_{70}) were selected as dependent variables. Various compositions of formulations as per the 3^2 factorial design were shown in Table 1. The statistical analysis of the factorial batches was performed by multiple regression analysis using Microsoft Excel followed by ANOVA. The results are depicted in Table 2.

Table1 Formulation summary as per 3^2 factorial design

Batch no.	X_1	X_2	EE %	t_{50} min	t_{70} min
B1	-1	-1	58.56	218	346
B2	0	-1	42.30	240	345
B3	1	-1	35.70	300	329
B4	-1	0	49.70	183	356
B5	0	0	40.16	200	346
B6	1	0	33.46	230	346
B7	-1	1	39.48	120	398
B8	0	1	36.43	153	372
B9	1	1	30.61	170	358

Coded value	Actual values	
	X_1	X_2
-1	50	150
0	75	200
1	100	250

X_1 = Drug content (mg)
X_2 = Polymer content (mg)

Table 2 Summary results of Analysis of Variance for measured responses*

Parameters	df	SS	MS	F	Significant F
For EE					
regression	**5**	**7225.333**	**1445.067**	**61.34717**	**0.003208**
residual	**3**	**70.66667**	**23.55556**		
total	**8**	**7296**			
For t_{50}					
regression	**5**	**860.477**	**172.0954**	**139.611**	**0.000947**
residual	**3**	**3.698033**	**1.232678**		
	8	**864.175**			
For t_{70}					
regression	**5**	**2977.361**	**595.4722**	**9.425619**	**0.047061**
residual	**3**	**189.5278**	**63.17593**		
total	**8**	**3166.889**			

*** df indicate degree of freedom; SS, sum of square; MS, mean sum of square; and F, Fischer's ration.**

PHYSICAL CHARACTERIZATION OF MICROSPHERES

PERCENTAGE YIELD

Percentage yield was calculated for each batch using following equation.

$$\% \text{ Yield} = \frac{\text{Weight of microspheres}}{\text{Weight of polymer + drug}} \times 100$$

PARTICLE SIZE

The simple optical microscope (Light Microscope Metzer optical instruments Pvt Ltd, Mathura) was used for particle size measurement to measure particle size of individual microspheres; optical micrometer was calibrated using standard stage micrometer. According to microscopic method of particle size analysis, slides of various batches of microspheres were prepared using dilute suspension of microspheres in liquid paraffin. Particle size of 100 microspheres from each batch was measured for calculating size distribution and average particle size.

ENCAPSULATION EFFICIENCY (EE)

To determine drug entrapment within the microspheres, 25 mg of microspheres was dissolved in dichloromethane to prepare 10 mL solution. The drug was extracted three times from dichloromethane using 25 mL of Distilled Water. Each time extraction was carried out using separating funnel with shaking time of 15 min. After complete extraction of drug, the amount of drug was quantified using a spectrophotometric method (Spectrophotometer Shimadzu, model-UV1601) at 272.0 nm in the presence of a blank prepared from microspheres containing all materials except the drug.

Efficacy of the microspheres preparation method was determined by dividing the amount of the prepared microspheres to the initial amount of the applied material.

SURFACE MORPHOLOGY

The shape and surface morphologies of the microspheres were investigated using Scanning Electron Microscope (Philips, FE1 Company, ESEM-XL-30 TMP) at 20 kV. Prior to examination samples were gold coated under vacuum (Fine coat, Ion Sputter, JFC-1100) to render them electrically conductive.

IN VITRO DRUG RELEASE STUDY

The United States Pharmacopoeia dissolution rate test apparatus type I (USP dissolution apparatus Scientific USP standards, Model-DA60) was used for all the *in vitro* release studies. A weighed quantity of the microspheres (50 mg) was suspended in 900 mL of dissolution medium (distil water). The dissolution medium was stirred at 100 rpm and maintained at constant temperature ($37\pm1^{O}C$). At preset time intervals 5 mL aliquots were withdrawn and replaced by an equal volume of fresh pre warmed dissolution medium maintaining sink condition throughout the experiment. After suitable dilution, the samples were analyzed for drug quantification at 272.0 nm using UV-VIS spectrophotometer (Spectrophotometer Shimadzu, model-UV1601).

RESULTS AND DISCUSSION

The microspheres were found to be comparatively smooth, spherical in shape and free flowing with particle size ranges from 17 µm to 24 µm. The yield was found to be in the range of 48 – 76 %.

FTIR STUDY

It is clear from FTIR results (Table 3) that peaks of different functional groups of drug in physical mixture of drug and polymer are not much deviated from peaks of standard sample of drug, so we can conclude that ingredient and condition used for preparation of microspheres are compatible with drug.

VISCOSITY DETERMINATION

The viscosities of various polymer concentrations were depicted in the Table 4. It was found that as the polymer concentration increases (1%, 1.5%, 2%) viscosity of EC10 polymer would also increased. Concentration increased from 1% to 1.5%, viscosity increased 1.5 times greater than 1% EC10 concentration. Concentration increased from 1% to 2%, viscosity increased 2 times greater than 1% EC10 concentration.

SURFACE MORPHOLOGY (FIG 1)

The surface morphological study was summarized in tabular form in Table 5. The microspheres were found to have fairly porous rough surface and the sphericity would be deviated at lower concentration of polymer.

FACTORIAL DESIGN

A statistical model [Eq. (1)] incorporating interactive and polynomial terms was used to evaluate the selected responses.

$$Y=b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_1b_2X_1X_2 \quad \text{..... (1)}$$

where Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing 1 factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity.

The fitted equation relating the response (EE) to the transformed factors is shown in Eq. (2)

$$EE= 28.8+3.5X1+11.43X_2+0.42X_1^2+0.945X_2^2+0.025X_1X_2 \quad \text{..... (2)}$$

(R=0.995721)

The EE value for the nine batches show a wide variation i.e. the response ranges from a minimum of 15 to a maximum of 45 %. The data indicates that the EE value is dependent on the factors.

The significant level (p value) of coefficients of b_1^2, b_2^2 and b_1b_2 were found to be more than 0.05 and hence they were removed from regression Eq. (2) to generate the reduced model. The coefficients b_1 and b_2 showed significant values (p value) of less than 0.05 and hence they were retained in the reduced model. [Eq. (3)]

$$EE = 28.8+3.5X_1+11.43X_2 \quad \text{.....(3)}$$

It may be concluded that the high levels of X_1 (drug content) and X_2 (polymer content) appear to favor the preparation of microspheres with better EE. The Eq. (3) is presented in the form of a response surface plot in Fig 2 to visualize the impact of changing independent variables on EE.

It is evident from results that when drug content was increased there was an increase in encapsulation efficiency of EC10 microspheres. Increase in viscosity of internal phase of primary emulsion will reduce the mobility of drug material from internal phase of primary emulsion to external phase of secondary emulsion during preparation of secondary emulsion. This in turn will reduce the rate of drug loss. It is evident from results that when polymer content was increased there was an increase in encapsulation efficiency because of increase in viscosity of the dispersed phase. Similarly this higher viscosity effectively slows down the drug dissipation into the continuous phase and result in a better drug encapsulation with an increased loading efficiency. The polymer content in external phase of primary emulsion increases which in turn may reduce the mobility of drug solution from internal phase of primary emulsion to external phase of secondary emulsion. And also this will increase the viscosity of primary emulsion increases the EE by similar reason of drug loading.

The fitted equation relating the response (t_{50}) to the transformed factors is shown in Eq. (4)

$$t_{50} = 254-12.16 \ X1+31.16X_2+2.5X_1^2+2.5X_2^2-11X_1X_2 \quad \text{.....(4)}$$

(R= 0.990314)

The t_{50} value for the nine batches show a variation i.e. the response ranges from a minimum of 230 to a maximum of 284 min. The data clearly indicates that the t_{50} value is dependent on the factors.

The significant levels (p value) of coefficients of $b_1^2$and b_2^2 were found to be more than 0.05 and hence they were removed from regression Eq. (4) to generate the reduced model. The coefficients b_1, b_2 and b_1b_2 showed significant values (p value) of less than 0.05 and hence they were retained in the reduced model. [Eq. (5)]

$$t_{50} = 254-12.16 \ X_1+31.16X_2-11X_1X_2 \quad \text{.....(5)}$$

It may be concluded that the low level X_1 (drug content) and high level of X_2(polymer content) appear to favor the preparation of controlled release microspheres. The Eq. (5) is presented in the form of a response surface plot in Fig 3 to visualize the impact of changing independent variables on t_{50}.

The fitted equation relating the response (t_{70}) to the transformed factors is shown in Eq. (6)

$$t_{70}=348.55-11.16\ X_1+18X_2+1.16X_1^2+8.66X_2^2-5.75X_1X_2 \quad \text{.....(6)}$$

$(R^2=0.940153)$

The t_{70} value for the nine batches show a variation i.e. the response ranges from a minimum of 329 to a maximum of 398 min. The data clearly indicates that the t_{50} value is dependent on the factors.

The significant levels (p value) of coefficients of b_1^2, b_2^2 and $b_1b_2$were found to be more than 0.05 and hence they were removed from regression Eq. (6) to generate the reduced model. The coefficients b_1 and b_2 showed significant values (p value) of less than 0.05 and hence they were retained in the reduced model. [Eq. (7)]

$$t_{70}=348.55-11.16\ X_1+18X_2 \quad \text{……..(7)}$$

It may be concluded that the low level X_1 (drug content) and high level of X_2 (polymer content) appear to favor the preparation of controlled release microspheres. The Eq. (7) is presented in the form of a response surface plot in Fig 4 to visualize the impact of changing independent variables on t_{70}.

The sustained drug release profiles from all prepared batches (B1 to B9) were found over a longer period of time. The release was in the order of B1>B2>B3>B4>B5>B6>B7>B8>B9 as shown in Fig 5.

However, the effect of drug content and polymer content on drug release by using T_{50} and T_{70} was studied. It was found that higher drug levels in the microsphere formulation produced a higher drug concentration gradient between the microspheres and dissolution medium, thus drug release rate was increased. With higher drug loading, more drug molecules are available at the surface of microspheres, leading to higher initial release (12). Also, by increasing the amount of drug loading, a point will be reached when the solid drug particles will begin to form continuous pores or channels within the matrix. Under these circumstances, the path of least resistance for drug molecules will be diffusion within the channels formed from areas where drug has previously leached out from the matrix (13,14). Therefore, as the amount of drug content is increased and drug leaches out from the polymer, the matrix becomes more porous and a faster drug release rate occurs.

Also it was found that with increasing polymer concentration, the drug release rate from microspheres decreased. The cumulative release of drug significantly decreased with increasing ethyl cellulose concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.

CONCLUSION

In conclusion, the attempt to prepare controlled release microspheres of TBS was successful and the outcomes of factorial design were interesting will be used for further study, even though the entrapment efficiency was still lower compared to the same process reported for other hydrophilic drugs. Further studies are required to find out the exact cause for the difference and to improve the entrapment efficiency.

Figure 1 SEM photomicrographs of EC10 microspheres Batch B7

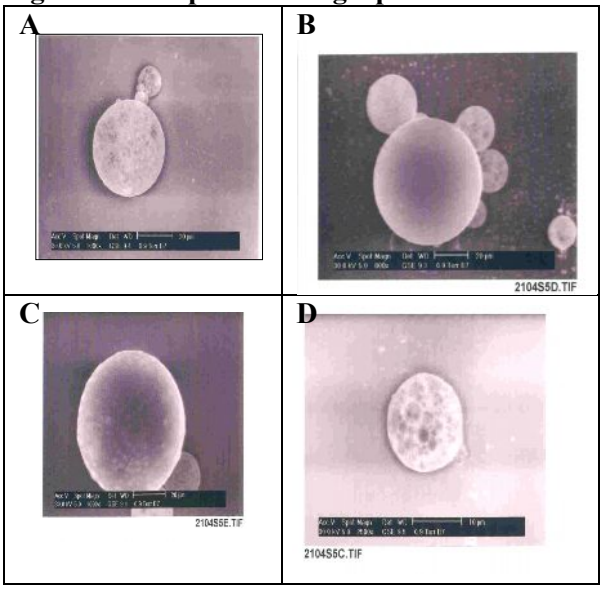


Figure 2 Response surface plot of EE

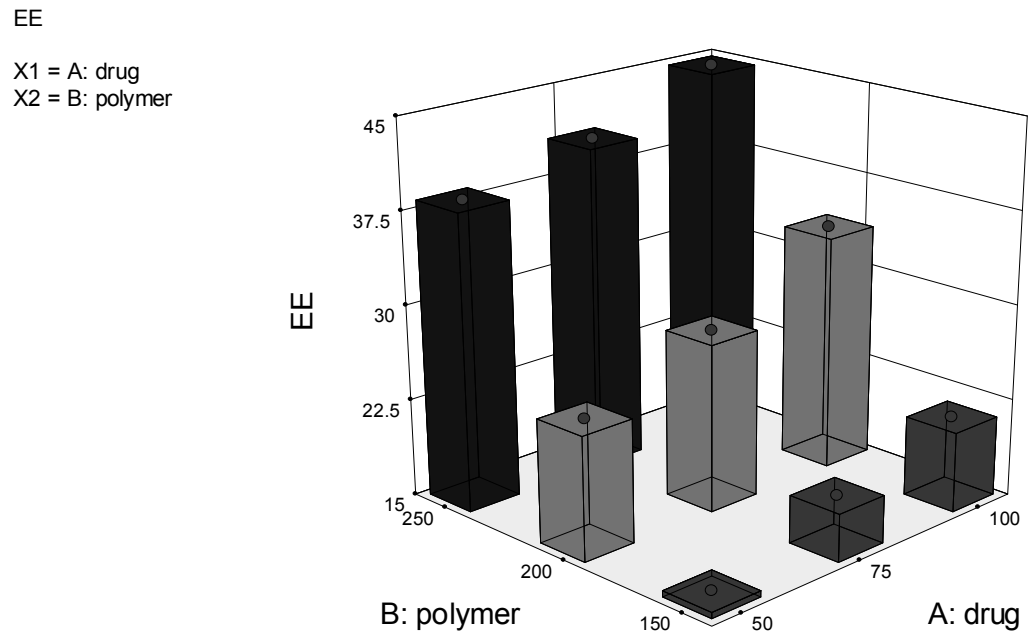


Figure 3 Response surface plot of t_{50}

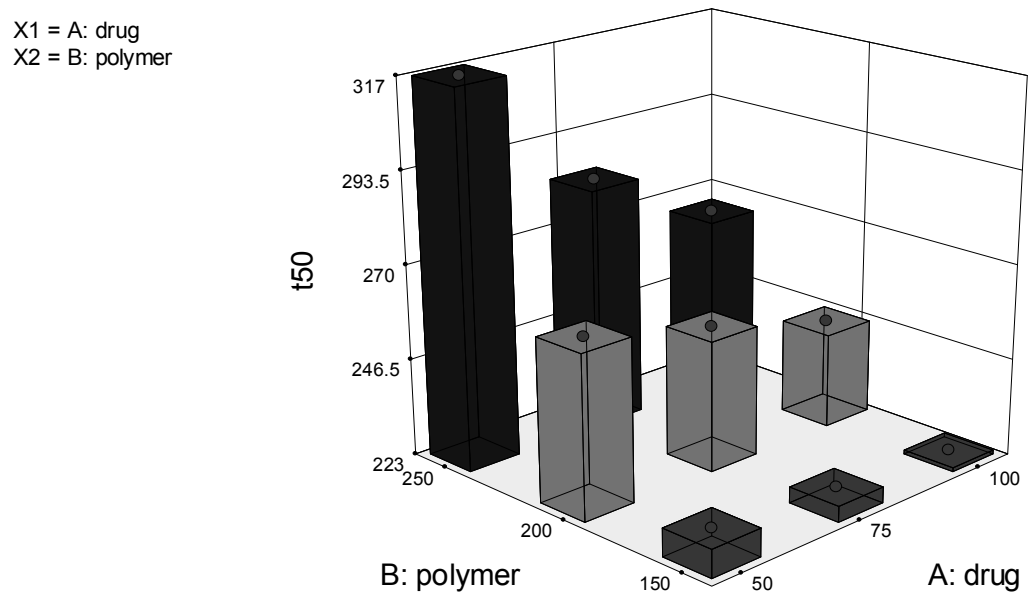


Figure 4 Response surface plot of t_{70}

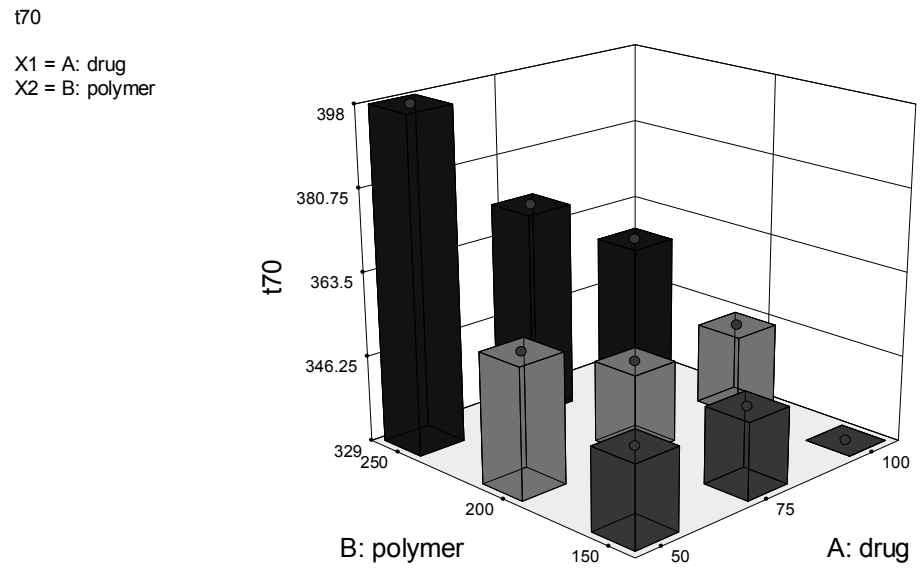
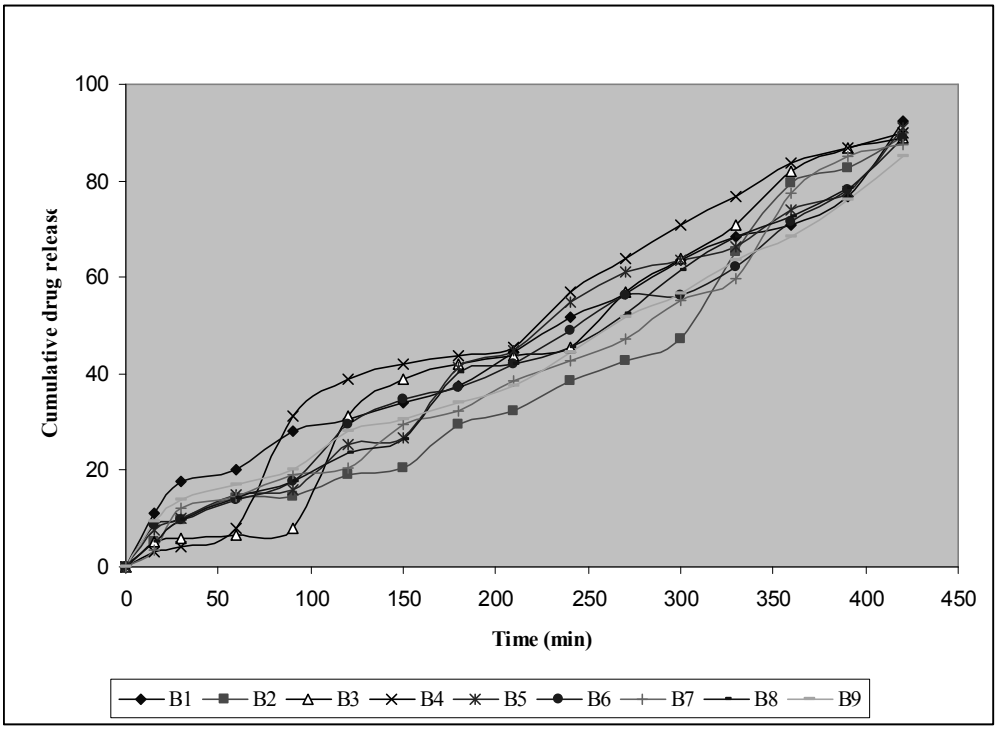


Figure 5 Cumulative drug releases



REFERENCES

1. Sajeev C, Vinay G, Archna, R, and Saha, R.N., Oral controlled release formulation of diclofenac sodium by microencapsulation with ethyl cellulose, J Microencapsulation, 2002, 19(6), 753-760.
2. Wu J.C, Su S.G, Shyu S.S and Chen H., Effect of the solvent-non-solvent pairs on the surface morphology and release behaviour of ethylcellulose microcapsules prepared by non-solvent-addition phase separation method, J Microencapsulation, 1994, 11(3), 297-308.
3. Tsai Y.L, Jong C.C. and Chen H., Preparation of double-encapsulated microcapsules for mitigating drug loss and extending release, J Microencapsulation, 2001, 18(6), 701-711.

4. Singh J. and Robinson D.H., Controlled release kinetics of. captopril form tableted microcapsules, Drug Dev. Ind. Pharm., 1988, 14*(4),* 545-*560.*
5. Saravanan M., Bhaskar K., Srinivasa Rao G., Dhanaraju M.D., Ibuprofen-loaded ethylcellulose/polystyrene microspheres: an approach to get prolonged drug release with reduced burst effect and low ethylcellulose content, J Microencapsulation, 2003, 20(3), 289-302.
6. Zinutti C., Kedzierewicz F, Hoffman M. and Maincent P., Preparation and characterization of ethylcellulose microspheres containing 5-fluorouracil, J Microencapsulation, 1994, 11(5), 555-563.
7. Julide A., Furosemide-loaded ethyl cellulose microspheres prepared by spherical crystallization technique: Morphology and release characteristics, International Journal of Pharmaceutics, 1991, 76(3), 193-198.
8. Mandal T.K., Shekleton M., Onyebueke E., Washington L. and Penson T., Effect of formulation and processing factors on the characteristics of biodegradable microcapsules of zidovudine, J Microencapsulation, 1996, 13(5), 545-557.
9. Tarun K. and Srini T., Preparation of biodegradable microcapsules of zidovudine using solvent evaporation: Effect of the modification of aqueous phase, International Journal of Pharmaceutics, 1996, 137(2), 187-197.
10. Bhumkar D.R, Maheshwari M., Patil V.B. and Pokharkar V.B., Studies on effect of variables by response surface methodology for naproxen microspheres, Indian Drugs, 2003, 40, 455-461.
11. Fan T.M., Guang H.M., Wei Q.Z. and Guo S., W /O/W double emulsion technique using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of microparticles, Journal of Controlled Release, 2003, 91, 407–416.
12. Ravivarapu H.B., Lee H., DeLuca P.P., Enhancing initial release of peptide from poly(d,l-lactide-co-glycolide) (PLGA) microspheres by addition of a porosigen and increasing drug load, Pharm Dev Technol., 2000, 5, 287-296.
13. Cardinal J.R., Matrix systems. In: Langer RS, Wise DL, eds. Medical Applications of Controlled Release Systems. Vol 1. Boca Raton, FL: CRC Press Inc; 1984:43-44.
14. Song S.Z., Cardinal J.R., Kim S.H. and Kim S.W., Progestin permeation through polymer membranes, V: progesterone release from monolithic hydrogel devices, J Pharm Sci., 1981, 70, 216-221.

*****