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Studies in the Preparation of Diclofenac Sodium Microspheres by Emulsion Solvent Evaporation Technique using Response Surface Analysis

M.C. GOHEL* AND AVANI F. AMIN
L.M. College of Pharmacy, Navrangpura, Ahmedabad-380 009
e-mail : lmcp@ad1.vsnl.net.in

The objective of this investigation was to study the factors influencing the characteristics of modified release microspheres of diclofenac sodium. A series of batches were prepared by solvent evaporation technique using ethyl cellulose as a matrixing agent. The study revealed that for obtaining discrete microspheres optimum quantity of Span 60, a surfactant, is necessary. The stirring speed, polymer to drug ratio, concentration of ethyl cellulose solution and type of solvent were found to influence the *in vitro* drug release from the microspheres. Sigma Plot® software was used to generate interpolated values of the percent drug dissolved in 360 min (Y_{360}). The response surface plot showed that the effect of polymer to drug ratio is more predominant than the concentration of ethyl cellulose solution. The argument is further supported by modeling the data using regression analysis. The kinetics of drug release was studied using non-linear regression analysis in EXCEL® spreadsheet. The Korsmeyer and Peppas model fitted well to the data. The drug was released by non-Fickian diffusion. The performance of the formulated product is compared with a commercial product. Comparison showed that the effect of the formulated product may last for longer time duration. The microspheres exhibited poor compressional characteristics.

Diclofenac sodium, a potent non-steroidal anti-inflammatory drug with pronounced analgesic properties, is used in the long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Its biological half-life has been reported as 1-2 h and gastrointestinal side effects such as bleeding, ulceration or perforation of intestinal wall are commonly seen[1]. Due to short biological half life and associated adverse effects, it is considered as an ideal candidate for controlled drug delivery. In pharmaceutical sustained release preparations, small pellets and microspheres quickly disperse throughout the gastrointestinal tract. Hence, the absorption of drugs is improved and side effects related to localized build-up of irritating drugs are reduced[2].

Cellulose ether polymers have a wide diversity of applications ranging from organic soluble thermoplastic products to water soluble food additives. The importance of cellulose ethers has increased in recent years because of economic factors that have adversely affected the supply and pricing of natural gums and low viscosity products such as starch derivatives. The rising prices of petroleum based polymers and chemicals engender interest in cellulose as a renewable source. Ethyl cellulose has been earlier used at our center for preparing microspheres of diclofenac sodium by the emulsion diffusion technique[3]. Sodium alginate has been used as a matrixing agent for the preparation of microspheres of diclofenac sodium by our team[4]. The emulsion solvent evaporation technique has been applied to polymers such as ethyl cellulose[5,6] and Eudragit[7,8].

The purpose of this study was to investigate the influence of processing variables in the preparation of diclofenac sodium microspheres by the emulsion solvent evaporation technique using ethyl cellulose as a matrix forming polymer.

*Author to whom correspondence should be addressed

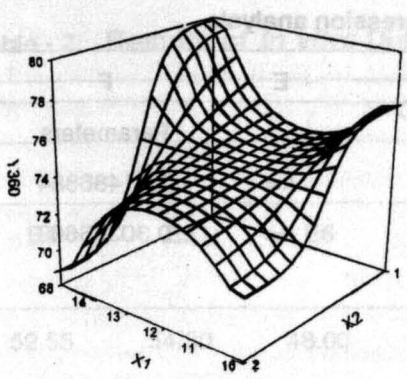

Fig. 1 : Response Surface Plot

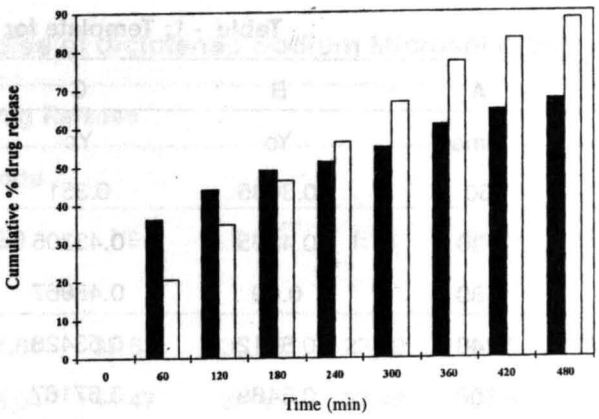

Fig.2: *In Vitro* dissolution profile of Batch E12 and a commercial product. Release of diclofenac sodium was studied for 8h from the batch E12 ■ and Voveran - SR tablets (100mg) □

MATERIALS AND METHODS

Diclofenac sodium (JP) was received as a gift sample from Sharda drugs, Chhatral. Ethyl cellulose, was purchased from Laser Laboratories, Ahmedabad. The viscosity of a 5% w/v solution of the sample in a blend of toluene and ethanol (80:20) at 25° measured on a Brookfield viscometer, was found to be 14 cps. Span 60 was obtained from Robert Johnson Co., UK. All the other solvents and chemicals were of analytical grade and were used without further purification.

Preparation of Diclofenac Sodium Microspheres

The microspheres were prepared by emulsion solvent evaporation technique. Ethyl cellulose (10 g) was dissolved in acetonitrile or in a blend of acetone and acetonitrile (1:1, 100 ml). A known quantity of diclofenac sodium (3 to 10 g) was dissolved in the solution and it was kept in an ultrasonifier for 10 min. The drug-polymer solution was then added dropwise to the dispersion medium (500 ml) consisting of a mixture of heavy and light liquid paraffin in a ratio of 1:1, Span 60 (2 g) was used as a surfactant in the dispersion medium. The emulsion was stirred using a propeller stirrer at 1500 RPM. The stirring was continued for 2.5-3.0 h at ambient conditions. The microspheres were collected by filtration and washed with petroleum ether (100*3 ml). Finally, the microspheres were washed with distilled water and vacuum dried. The microspheres were stored in glass-bottles before being assessed.

Size Distribution of Microspheres

The separation of the microspheres, into various size fractions, was achieved using a mechanical sieve shaker (Industrial Combustion Ltd., UK). A series of six standard stainless steel sieves (100-720 μm) were arranged in the order of decreasing aperture size. Ten g of drug loaded microspheres were shaken for a period of 15 min and the microspheres retained on each sieve were weighed.

Drug Content

Drug loaded microspheres (100 mg) from each batch were finely powdered in a glass mortar. Hot water was added to the powder and the resultant dispersions were exposed to ultrasonic treatment (Vibrionics, Bombay) for 20 min. The ultrasonic treatment was repeated three times with a resting period of 30 min between the treatments. The drug content was determined after filtering the dispersion through 0.45 μ filter. The absorbance measurements were done on a Hitachi double beam U-2000 spectrophotometer at 276 nm[9]. Corresponding concentrations in the samples were calculated from the standard plot generated by fitting weighted linear regression model[10] to the data obtained in triplicate (Absorbance =0.029* Concentration-0.0062).

Dissolution Study

Microspheres containing 100 mg of diclofenac sodium were filled in hard gelatin capsules. The dissolution study was performed using USP XXII basket apparatus at a rotational speed of 50 RPM at 37° in 900 ml phosphate buffer (pH 7.2). Samples (10 ml) were withdrawn at predetermined time intervals and filtered through 0.45 μ membrane filter. The same volume (10 ml) of the dissolution

Table - 1: Template for Non-Linear regression analysis

	A	B	C	D	E	F	G
1	Time	Yo	Yc	SSR		Parameters	
2	60	0.3635	0.351	0.00016	k=	10.1486884	
3	120	0.4395	0.43305	4.2E-05	n=	0.30306663	
4	180	0.49	0.48967	1.1E-07			
5	240	0.5112	0.53428	0.00053			
6	300	0.5489	0.57167	0.00052			
7	360	0.607	0.60414	8.2E-06			
8	420	0.6451	0.63304	0.00015			
9	480	0.6723	0.65918	0.00017			
10							
11							
12			sum=	0.00157			
13							

medium was replenished immediately. The drug content was determined in the filtrate.

Kinetics of Drug Release

Lu and co-workers[11] used a custom made (MSFIT) computer programme in C language for nonlinear fitting of release data from controlled release devices. The goal of this work is to emphasize the capacity of EXCEL[R] 5.0 in solving nonlinear equations. The procedure is discussed in detail because it has not been hitherto reported by researchers engaged in formulation development. The aim of non-linear regression is to minimize the residual sum of squares (SSR, Eq. 1).

$$SSR=\sum_{i=1}^{n} [y_{oi}-y_{ci}]^2 \quad -(1)$$

where y_{oi} and y_{ci} represents the observed and calculated data respectively and n is the total number of data points.

The Korsmeyer and Peppas[12], Weibull[13] and Higuchi[14] equations were fitted to the data of batch E12. The critical steps for creating a worksheet for the Korsmeyer and Peppas model are shown below.

(i) The data of time (x) and fraction of drug released (y_o) are entered in columns A and B of the spreadsheet respectively (Table 1).

(ii) Temporarily assign the value 1 to k and in the cells F2, and F3.

(iii) Enter the Korsmeyer and Peppas equation in column C, [=(K*t^n)].

(iv) Enter the formulae to calculate SSR in column D [=(B2-C2)^2].

(v) In cell D12, compute the sum of the squares of residuals [= SUM (D2 D9)].

(vi) In EXCEL[R] (version 5.0), Solver is found under the Tools menu. After invoking the Solver, a screen appears. the value of the sum of the square of residuals which is to be changed i.e. D12 is entered in Set Cell box. Because we wish to minimize the value in cell D12, click "Min" on the second line beside "Equal to". Finally write "F2, F3" in the dialog box labelled "By Changing Cells". Now click the "Solve" button to fit data to the equation. The Solver finishes its task in a few seconds.

(vii) The procedure may be repeated with the new starting

Table - 2 : **Results of *In vitro* Dissolution Studies of diclofenac Sodium Microspheres**

	Cumulative% Drug Release									
	Batch Code									
Time in min.	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13
60	52.55	54.90	48.00	62.50	47.65	39.63	35.26	23.69	36.35	33.13
120	55.52	58.52	52.37	68.47	53.04	44.47	39.48	37.40	43.95	38.90
180	59.45	65.49	56.61	71.23	57.37	47.82	42.56	45.14	49.00	40.68
240	64.22	72.17	61.37	73.46	61.08	52.52	48.99	49.60	51.12	43.15
300	67.13	74.96	65.22	76.66	64.17	56.61	53.66	53.87	54.89	46.83
360	71.24	77.19	68.47	79.85	68.83	58.98	58.74	56.60	60.70	51.05
420	75.46	78.86	71.91	83.02	73.53	62.03	61.44	61.09	64.51	53.75
480	78.21	88.89	74.22	87.46	77.61	65.54	64.23	63.55	67.23	58.35

Note : See text for batches E1 to E3

values in cells F2 and F3. The process can be repeated till the lowest value for SSR is obtained. Worksheets were also created for the Weibull and the Higuchi models. The authors may be contacted for obtaining detailed write-up of the procedure.

RESULTS AND DISCUSSION

The microspheres of diclofenac sodium were prepared by emulsion solvent evaporation technique using ethyl cellulose as a polymer and acetonitrile (100 ml) as a solvent. Span 60 was tried as a surfactant at different levels, i.e., from 500 mg to 5 g, in the dispersion medium. The amount of surfactant was found to influence the characteristics of the microspheres. Aggregation of the microspheres was noticed when less than 1.5 g of the surfactant was used. Discrete Microspheres were obtained when 2 g of Span 60 was used. On increasing the amount of Span 60 to 3 g, the dispersion turned milky white in colour and aggregated product was obtained. The product could not be recovered when 5 g of Span 60 was used as lump formation was noticed. From the results, it may be concluded that 2 g of Span 60 seems to be the optimum amount and therefore, all the batches were prepared using Span 60 at this level. The surfactant may have prevented coalescence of the droplets by forming a film on the globules. The dispersion medium, liquid paraffin, was replaced by arachis oil in a trial batch keeping the other conditions constant. An aggregated product was obtained, indicating that the physico-chemical characteristics of the dispersion medium also plays a vital role in obtaining satisfactory microspheres. Aggregation of microspheres was observed in a batch of microspheres that were prepared using a magnetic stirrer instead of a propeller stirrer. The results underscore the importance of type of the agitating device.

In an attempt to investigate the effect of stirring speed on the particle size distribution and drug release profile from the microspheres, batches E1 and E2 were prepared at 1500 and 2000 RPM, respectively. At higher stirring speed, smaller microspheres were obtained as anticipated. The drug was released at a relatively faster rate from the microspheres of batch E2, probably because of increased surface area. A total of 50% of the microspheres of batch E2 were smaller than 180μ. About 90% of the Microspheres of batch E1 were found to be in the size range of 180-355 μ. The drug was released at an uniform rate from the microspheres of batch E1. Thus the speed of rotation was kept constant at 1500 RPM in the remaining batches. The initial drug release was however very high i.e, 60% in 1 h in batch E1.

In an attempt to modify the drug release pattern, batch E3 was prepared using light to heavy liquid paraffin ratio of 1:3. The initial drug release was not much affected but slower *in vitro* drug release was observed after the second hour. The drug release rate was also not uniform and hence the ratio of light to heavy liquid paraffin was kept at 1:1 in the remaining trials.

To investigate the influence of solvent on the drug release, batch E4 was prepared using 1:1 solvent blend of acetonitrile and acetone. The polymer to drug dispersion was found to be more translucent in nature with this solvent blend. The microspheres of batch E4 were found to be discrete in nature. The drug release in the first hour from the microspheres of batch E4 reduced to 52% as compared to 60% from batch E2. The results reveal that the change in solvent blend did have significant influence on the burst effect. The probable reason is the difference in solubility of the drug in the two solvent systems.

In order to investigate the effect of polymer to drug ratio, the performance of batches E4 (2:1), E5 (1:1) and E6 (3:1) was compared. The drug was released at a faster rate from the microspheres of batch E5 (1:1) and at a relatively slower rate from the microspheres of batch E6 (Table 2). The probable reason for slower drug release is the change in the tourtuosity of the matrix. It may be concluded that by choosing an appropriate polymer to drug ratio, one can engineer the release of drug. However, burst effect was noticed in batches E4, E5 and E6.

The following criteria were used for selecting a good sustained released formulation:

20%<**Y60**<40% ; 50%<**Y360**<70%;65%<**Y480**<80%

Batches E4, E5 and E6 did not satisfy the above mentioned criteria. An attempt was made to further control the burst effect by increasing the concentration of the ethyl cellulose solution from 10 to 15%. Batches E7, E8, E9 and E10 were prepared using 15% ethyl cellulose solution at polymer to drug ratios of 1:1, 2:1, 3:1 and 4:1 respectively. Batches E7 and E8 did not satisfy the requirements of Y_{60} Table 2. The microspheres of batch E10 satisfied the criteria for Y_{60} and Y_{360} but not for Y_{480}. Slow dissolution of the drug in the terminal phase could be attributed to high polymer to drug ratio. The microspheres of batch E9 satisfied all the three criteria, (Y^{60} =39.6; Y^{360} = 58.9; Y^{480} = 65.5). However, the results reveal that batch E9 is a border line case. In batch E11, the polymer concentration was increased to 25% and the polymer to drug ratio was tried at 5:1. The criteria for Y_{480} was not satisfied as only 63% of drug was released in eight hours. The results supplement our earlier claims for sustaining the drug release by using concentrated solution of ethyl cellulose and high polymer to drug ratio.

A response surface plot was generated in Sigma Plot[R] using the settings of batches E4, E5, E7 and E8 to study the effect of concentration of ethyl cellulose solution (X_1) and the polymer to drug ratio (X_2) on the amount of drug released in 360 min (Y_{360}). Fig. 1 depicts than the effect of polymer to drug ratio is more predominant than the effect of the concentration of ethyl cellulose solution on the Y_{360}. The interpolated values were subjected to multiple regression analysis. The effect of interaction between the two factors on Y_{360} was found to be insignificant when students 't' test was employed. The statistical valid reduced model is shown below;

$$Y_{360}=63.86+1.55X_1+6.73X_2$$

The equation may be used for calculation of Y_{360}. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficients for the main effects (X_1 and X_2) carries positive sign. Therefore, it is concluded that Y_{360} can be increased either by increasing X_1 or X_2. From the values of the coefficients, it is further concluded that an unit change in X_2 will give approximately a four fold increase in Y_{360} as compared to that of X_1.

To achieve the set criteria, an attempt was made to modulate the drug release by using a combination of microspheres of batches E9 and E11 in the ratio of 1:1 (batch E12). The microspheres of batch E12 satisfied all the three set criteria (Y_{60}=36.3; Y_{360}=60.7; Y480-67.2).

In an attempt to further decrease the initial drug release from batch E12, the microspheres were compressed into tablets containing microspheres equivalent to 100 mg of diclofenac sodium, 100 mg of methyl cellulose and 12 mg HPMC K15M. The blend was not found to be directly

compressible in nature. Moreover, capping was also noticed. The probable reason could be poor bonding between the particles of methyl cellulose and the microspheres. Therefore, granules of methyl cellulose were prepared using polyvinyl pyrollidone as a dry binder and absolute alcohol as a granulating agent. The granules were mixed with the microspheres in the same size range of 250 - 355 μ. Acceptable tablets were not obtained from this blend too. The problem of hardness and capping could not be resolved.

An attempt was made to prepare batch E13 by coating the microspheres of batch E12 with a beeswax solution (20 ml of 5% beeswax solution in n-hexane). The initial drug release decreased to 33% from the microspheres of batch E13 but the criteria for Y_{480} could not be fulfilled as only 58% of the drug was released in the eight hours period (Table 2). Thus, it may be concluded that batch E12 satisfies all the set criteria of a good sustained release formulation. The drug release profile of batch E12 was compared with the release profile of a commercial product (Voveran-SR tablet 100 mg) (Fig. 2). The commercial product did not show burst effect however, more than 85% of the drug was released in 8 h. Batch E12 showed 67% drug release in 8 h and hence it can be concluded that Batch E12 may show pharmacological effect for longer duration of time as compared to that of the commercial product.

Kinetics of Drug Release

The kinetics of drug release is best explained by the Korsmeyer and Peppas model as minimum SSR (0.00157) was obtained with the model. The optimized value of the coefficient "n" was 0.3. Thus, one can conclude that the drug was released by non-Fickian diffusion ($0 < n < 0.5$).

In summary, the modified release ethyl cellulose microspheres with good spherical geometry and surface characteristics were prepared by emulsion solvent evaporation technique. The presence of Span 60 was found to be essential for reducing aggregation of the microspheres. The stirring speed, polymer to drug ratio and concentration of ethyl cellulose solution were found to be important parameters affecting the drug release profile of the microspheres. The type of solvent blend exhibited significant influence on the drug dissolution. The desired release pattern was achieved by combining microspheres of two batches. The Korsmeyer and Peppas model fitted well to the *in vitro* dissolution data of batch E12. The microspheres exhibited poor compressional characteristics.

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