Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

QUALITY BY DESIGN APPROACH IN FORMULATION OF BIOADHESIVE LEVODOPA MICROSPHERES

V. CHANDRAKALA*, A. MARY SARAL,

School of Advanced Sciences, Vellore Institute of Technology, Vellore, Tamil Nadu, India. Corresponding author – V.Chandrakala, East Point College of Pharmacy, Bidrahalli, Bangalore, Karnataka, India. Email -chandra_kalav@gmail.com

Received: 06 Aug 2013, Revised and Accepted: 27 Sep 2013

ABSTRACT

Objective-The present study deals with a case study to understand the effect of formulation variables of microspheres of a model drug, levodopa (LD). Methods-A three-factor, three-level design of experiment (DOE) with response surface methodology (RSM) was run to evaluate the main and interaction effect of several independent formulation variables that included ratio of drug: polymer (X1), ratio of HPMC:carbopol 934p (X2) and agitation speed (X3) in LD microspheres. The dependent variables included swelling index (Y1), drug content (Y2), time of drug release (Y3), size (Y4) and percentage bioadhesion (Y5). A desirability function was used to minimize lag time and to maximize the other dependent variables. A mathematical relationship, for each of the above responses was obtained to explain the effect of all factors and their colinearities on formulation of microspheres. Results- The optimized microspheres were predicted to yield Y1, Y2, Y3, Y4 and Y5 values of 9.41, 99.58%, 6.5hrs, 23µm, 77% bioadhesion respectively, when X1, X2, and X3 values were1:2.81, 1:1.79 and 2200 rpm, respectively. A new batch was prepared with these levels of the independent variables to yield Y1-Y5 values that were remarkably close to the predicted values. Conclusion-This investigation demonstrated the potential of QBD in understanding the effect of the formulation variables on the quality of levodopa microsphere formulations.

Keywords: Levodopa, HPMC, Carbopol934p, Shear stress, Box Behnken design.

INTRODUCTION

Quality by design (QBD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QBD is the understanding of factors and their interaction effects by a desired set of experiments. To understand the variables and their interactions, many statistical experimental designs have been recognized as useful techniques [1]. Response surface methodology (RSM) is used when only a few significant factors are involved in optimization. The main advantage of RSM is to reduce the experimental runs required than would be needed in a full factorial design and it is already widely applied to optimize formulation design in pharmaceutics studies. Box Behnken design (BBD) is a popular form of RSM and is more effective than other response surface designs, which is acknowledged as one of the best statistical and analytical models [2].

Drug delivery systems that can previously control the release rates or target the drugs to a specific body site have had an enormous impact on health care system. However the success of these novel drug delivery systems is limited due to their short residence time at the site of absorption [3]. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery systems with absorbing membrane. It can be achieved by coupling mucoadhesion characteristics to microspheres and developing novel delivery systems referred to as mucoadhesive microspheres [4, 5].

Over the last two decades mucoadhesion has become one of the interests for its potential to optimize localized drug delivery by retaining a dosage form at the site of action or systemic delivery by retaining a formulation in intimate contact with the absorption site [6]. The nasal cavity offers a large, highly vascularised subepithelial layer for efficient absorption. Blood is drained directly from nose to systemic circulation, thereby avoiding first pass effect. Use of bioadhesive drug delivery system increases the residence time of formulation in nasal cavity thereby improving absorption of drugs [7].

Nasal delivery of drugs targeting the CNS is currently an area of great interest. In addition to "nose to brain delivery" intranasal drugs can enter via a "nose to systemic circulation to brain" pathway. In this case, it is necessary for the drug to readily permeate the BBB from the circulation. In order for this to be achieved the drug must exhibit satisfactory passive or active transport across the light junction barriers of the BBB [8]. The clinical failure of much potentially effective therapeutics is often not due to lack of drug potency but rather to shortcoming in the method by which the drug is delivered. By localizing drugs at the desired site of action one can reduce toxicity and increase treatment efficiency [9].

Levodopa provides the most robust relief of the motor signs and symptoms of Parkinsons disease and is considered the gold standard of treatment because of its therapeutic success and lack of toxicity in clinical and experimental research. Levodopa is being studied with the hope of developing a formulation that provides stable and sustained blood levels throughout the day. Such a medication would be expected to provide sustained benefit for patients with Parkinsons disease and avoid the development of motor fluctuations possibly dyskinesias. Various alternative levodopa formulations such as intravenous formulation, levodopa prodrugs, sustained release levodopa, intraduodenal levodopa and orally disintegrating levodopa are available [10]. In the present study an attempt is made to formulate and evaluate intranasal levodopa microspheres by using bioadhesive polymer to minimize peripheral decarboxylation of levodopa and increasing levodopa concentration in brain and thus minimizing dose frequency and increasing patient compliance.

MATERIALS AND METHOD

Materials

Levodopa was received as gift sample from Divis Lab, Hyderabad, Andhra Pradesh, India. Polymers Hydroxypropyl Methylcellulose (HPMC), Carbopol 974p, Sodium Carboxymethyl Cellulose (Na CMC) from Apotex Research Pvt. Ltd, Bangalore, India. Carbopol 934p from Strides Acrolabs Bangalore, India. Liquid paraffin, Span 60 and all other chemicals were of analytical grade.

Methods

Evaluation of mucoadhesive property of various polymers by shear stress measurement

Screening of the various polymers, HPMC, CMC and carbopol 974p and carbopol 934p was carried out by shear stress measurement, two smooth polished glass slides were selected one of which was fixed with an adhesive onto a fixed surface, the second slide was tied with a thread which was then passed over a pulley and tied to a pan, the weight of the pan and frictional force of the upper slide was nullified by putting a weight on the pan such that the upper slide moves freely after infinitesimal small increase of weight in the pan. One drop of each polymer (3% aqueous solution) was placed at the centre of the fixed slide and then a second slide was pressed down with a weight (100) for fixed intervals of 5,10,15 and 30 min after

which weights were added to the pan. The load required to pull the upper slide or cause it to slide down from the fixed slide represents adhesion strength [11].

Following this screening, the best two bioadhesive polymers, HPMC and Carbopol 934p, were selected for the formulation of microspheres.

Preparation of microspheres

Microspheres were prepared by the w/o emulsification solvent evaporation technique. The drug was dissolved in warm water and this solution was added in aqueous solution of polymeric mixture containing HPMC and carbopol 934p. This solution was stirred for 30min to homogenize. The above solution was added drop wise into mineral oil containing 0.5% w/v span 60 as emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in a beaker. Constant agitation at various RPM was carried out using a homogenizer stirring rod and stirring motor. The flask and its contents were heated by an electrothermal isomantle at 80^0C. Stirring and heating were maintained for 1hr until the aqueous phase was completely removed by evaporation.

The light mineral oil was decanted and filtered using vacuum, the collected microspheres were washed 3 times with 100ml aliquots of petroleum ether. Filtered through whattman filter paper (grade 1) dried in an oven at 50^0C for 2hr and stored in a desiccators at room temperature [12].

HPMC and carbopol 934p (polymer:polymer) were used in different ratios according to Box Behnken Design (table 1) other independent parameters chosen were rpm and drug:polymer ratio.

Particle size, entrapment ratio, swelling, extent of dissolution and extent of bioadhesion were the dependent variables and their levels were investigated in the preparation of microspheres (table 2).

Box Behnken Design

A three-factor, three-level Box–Behnken design was applied for the optimization procedure using Design-Expert software (Trial Version 7.1.6, Stat-Ease Inc., MN). The ratio of drug:polymer, polymer: polymer and agitation speed used to prepare each of the 14 formulations are given in Table 1. These high, medium, and low levels were selected from the preliminary experimentation. After generating the polynomial equations relating the dependent and independent variables, the process was optimized [13]. Optimization was performed using a desirability function to obtain the levels of X1, X2 and X3, which maximized Y1, Y2, Y3 and Y5 while minimizing Y4.

Experimental Design

Box–Behnken design was applied to reduce the number of experiments and to optimize the range of variable concentrations needed to obtain maximum responses [14].

A three factor, three-level Box–Behnken design was used for the optimization of levodopa microspheres with drug:polymer ratio, polymer:polymer ratio and agitation speed (rpm) as the independent variables (Table 1).

Table 1: Box Behnken design

Formula	RPM	Drug:Polymer	Polymer:Polymer
B1	2000	1:4	1:1
B2	2000	1:1	1:1
B3	1800	1:2.5	1:1
B4	2200	1:4	1:1.5
B5	1800	1:4	1:1.5
B6	1800	1:2.5	1:2
B7	2000	1:4	1:2
B8	2000	1:2.5	1:1.5
B9	1800	1:1	1:1.5
B10	2200	1:2.5	1:1
B11	2200	1:2.5	1:2
B12	2000	1:2.5	1:1.5
B13	2000	1:1	1:2
B14	2200	1:1	1:1.5

Preliminary studies provided a setting of the levels for each formulation variable. The factors and levels of these three parameters were determined from preliminary studies.

Statistical experimental design of three factors at three different levels was used to evaluate the influence and interactions of these factors to the final responses tested. In order to minimize the formulation run three level-BBD was chosen.

This design is suitable for exploration of quadratic response surfaces and constructs a second order polynomial model, thus helping in optimizing a process using a small number of experimental runs.

The model constructed is as follows: -

$$Y=b0+b1X1+b2X2+b3X3+b4X1X1+b5X2X2+b6X3X3+b7X1X2+b8X1X3+b9X2X3 \quad \text{.....(1)}$$

Where $b0$–$b9$ is the regression coefficients, $X1$–$X3$ are the factors studied and Y is the measured response associated with each factor level combination.

The Box-Behnken design was specifically selected since it requires fewer treatment combinations than a Central Composite Design in cases involving three or four factors. The Box-Behnken design is also rotatable and contains statistical "missing corners" which may be useful when the experimenter is trying to avoid combined factor extremes. This property prevents a potential loss of data in those cases.

A design matrix comprising of 16 experimental runs was constructed. An interactive second order polynomial model was utilised to evaluate both the response variables:

Five key responses as mentioned earlier in the text were selected to derive the mathematical models for evaluating relevant factors. The experimental levels of the variables (maximum and minimum) boundary of each response variables are defined in the optimization technique [15].

Design-Expert software (v.7.0 Stat-Ease Inc., Minneapolis, USA) was used for the generation and evaluation of the statistical experimental design. Table 1&2 summarizes the design matrix with the experimental runs, factor levels and combinations and the measured responses.

Characterization of Microspheres

Particle size analysis

Particle size analysis was carried out using the optical microscopic method with the help of a calibrated eye piece micrometer. The size of around 100 particles was measured and median diameter was calculated [16].

Drug loading (entrapment efficiency) [17]

A weighed amount of microsphere were crushed into powder and added to 0.1N HCl, mixture was allowed to stand for 30min. then the solution was filtered and drug content was estimated by UV spectrophotometer at 280nm. The drug entrapment efficiency was determined by using the formula,

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \quad \text{.....(2)}$$

Percentage yield

The percentage yield of microsphere was also calculated based on the quantity of polymer and drug used in microsphere

Swelling studies of microspheres

A known weight of microspheres were placed in a glass vials containing 10ml phosphate buffer pH 7.4 at 37 ± 0.5^0C with occasional shaking. The microspheres were periodically removed, blotted with filter paper and their changes in weight were measured during the swelling until equilibrium was attained. Finally the weight of swollen microspheres was recorded after a time period of 4hr and the swelling ratio (SR) was then calculated using the formula [18].

$SR=W_R-W_0/ W_0$

W_0= Initial weight of drug microspheres

W_R= Weight of swollen microspheres at equilibrium swelling in medium

***In vitro* Bioadhesion [19]**

Bioadhesive properties of microsphere were evaluated using everted sac technique. Unfasted Albino rats were nourished and grown in normal lab conditions were sacrificed and intestinal tissue was excised and flushed with 10ml ice cold isotonic phosphate buffer pH 7.2 containing 2mg/ml glucose. Segment (6 cm) of jejunum was everted using a glass tube and one end was tied. Through the opposite end of the tube 1-1.5ml of isotonic phosphate buffer was poured until the sac was filled thereafter the segment end was tightly tied. The intestinal tissue was maintained at 4^0C prior to incubation. The sacs were introduced into 15ml glass tube containing 60mg of microsphere and shaken end over end. After 30min the sacs were removed then the unattached microspheres were removed by centrifugation and dried. The percentage of the attached microspheres was calculated by the difference between the initial amount of microspheres and amount of unattached microspheres before and after incubation were calculated using formula

% of attached microspheres = Initial amount of microspheres-Unattached microspheres×100

***In vitro* drug release**

To carry out the *in vitro* drug release accurately weighed drug loaded microspheres were dispersed in 400 ml of phosphate buffer (pH 6.6) USP Paddle type dissolution test apparatus. At selected time interval samples were withdrawn and replaced with the same volume of pre warmed fresh buffer solution to maintain a constant volume. The samples were analysed spectrophotometrically at 280nm. The released drug content was determined from the standard calibration curve of drug.

Data analysis

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. %CDR of optimized formulation C0 were fitted into zero order, first order, higuchi Korsmeyer and Peppas and Hixon Crowell cube root release model [20, 21].

Zero-order release kinetics:

The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration[22]. To study the zero-order release kinetics the release data was fitted into the following equation:

$F=K.t$……(3)

Where, 'F' is the fraction of drug release, 'K' is the release rate constant and 't' is the release time.

First-order release kinetics

The first order Eq. (2) describes the release from system where release rate is concentration dependent [23]. To study the first-order release kinetics the release rate data are fitted into the following equation:

$F=100*(1- e^{-Kt})$……..(4)

Where, 'F' is the fraction of drug release, 'K' is the release rate constant and't' is the release time and 'e' is the exponent coefficient.

Higuchi release model

Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (3).

$F=K.t^{1/2}$ ……(5)

Where, 'F' is the fraction of drug release, 'K' is the release rate constant and 't' is the release time.

Krosmeyer and Peppas release model

By incorporating the first 60% of release data mechanism of release can be indicated according to Korsmeyer where n is the release exponent, indicative of mechanism of drug release. To study the Krosmeyer and Peppas release model the release rate data are fitted to the following equation.

$M_t / M_\infty = K.t^n$ ……..(6)

Where M_t / M_∞ is the fraction of drug release, 'K' is the release rate constant and 't' is the release time and 'n' is the diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.

Hixon-Crowell cube root release kinetics

The Hixson-Crowell cube root law Eq. (5) describes the release from systems where there is a change in surface area and diameter of particles. To study the hixon cube root release model, release rate data are fitted to the following equation.

$W_t^{1/3}=W_0^{1/3}-Kt$……..(7)

Where W_t = weight of microspheres at time t

W_0= initial weigh of microspheres

RESULT AND DISCUSSION

Shear stress measurement

The shear stress of the four polymer tested is depicted in fig 1. As the time of contact with mucus is increased the shear force of the polymers also increased almost linearly except for carbopol 947p which initially showed a rapid increase of shear stress and thereafter a steady state or plateau was observed.

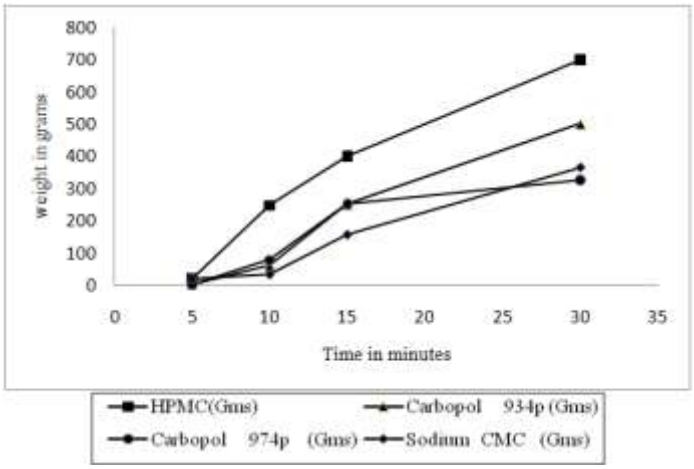

Fig. 1: Shear stress measurement of various polymers

Shear stress in terms of weights (grams) verses time (min) of contact with the mucus showed almost linear relationship with lag time for all the polymers tested. However carbopol974p showed an exceptional irregularity from linear behaviour with increase in the shear stress initially followed by decreased shear stress (fig 1). Highest shear stress was shown by 3% solutions of HPMC and carbopol934p. This may be attributed to the moderate swelling of the polymers which lead to greater entanglement of polymeric chains due to controlled hydration. Thus, shear stress increases with time as a result of the controlled rate of hydration and swelling.

Mechanism of coacervation method to produce levodopa microspheres

The production of microspheres intended for nasal administration of levodopa was tailored as per the emulsification solvent evaporation technique described in various literatures [24]. The study deals with the mechanism of microsphere formation by emulsion solvent evaporation and the principle behind incorporation of levodopa into these microparticulate systems. In this process, sorbitan monostearate (Span 60), a non-ionic surfactant was selected as the emulgent in the formulation of microspheres. In this process, span 60, a non-ionic surfactant selected as the lipid matrix in the formulation of microspheres, could be self-dispersed in water to form spherical micelles above the Krafft temperature.

Span 60 was used to decrease the surface tension of the aqueous phase in the oil phase so that aqueous phase can be uniformly dispersed in the oil phase. Initially an emulsion was formed by adding drug and polymer (HPMC and carbopol 934p) solution in liquid paraffin in presence of emulgent span 60 at 80^0C. If the temperature was reduced lesser than 80^0C there was formation of lumps since aqueous phase does not evaporate completely and if the temperature was above 80^0C the formed microspheres started charring. Agitation speed helped in dispersion of the aqueous phase in oil phase as well as controlled the size of the globules. When agitation speed was less than 1800, large microsphere formed which was unsuitable for nasal administration and when speed was higher than 2200 very small microspheres resulted which caused microsphere to deposit in trachea. Stirring and heating were maintained until the aqueous phase was completely removed by evaporation. Microspheres were washed with petroleum ether to remove traces of oil. Microspheres were dried in an oven at 50^0C for 2hr and stored in desiccators at room temperature to increase flow.

Statistical analysis of experimental data by Design-Expert Software

The results of the experimental design indicated that this system was highly influenced by the glutaraldehyde concentration, agitation speed and drug: polymer ratio. The best model fit for each of the responses Y1 (Swelling index), Y3 (dissolution time) and Y4 (size) were found for quadratic models, and Y2 (% drug content) were found for two factor interaction and Y5 (bioadhesion) were found for linear model.

In order to evaluate the significance of the models on the responses and their quantitative effects, analysis of variance (ANOVA) was carried out. At a 95% confidence level, a model was considered significant if the p value < 0.05. The sign and value of the quantitative effect represent tendency and magnitude of the term's influence on the response, respectively. A positive value in the regression equation exhibits an effect that favours the optimization due to synergistic effect, while a negative value indicates an inverse relationship or antagonistic effect between the factor and the response surface analyses were also plotted in three-dimensional model graphs for optimization of microparticles with suitable and satisfied physicochemical properties.

Swelling index

Quadratic model was chosen to describe the effects of the variables on swelling index. Each experimental response could be represented by polynomial equation obtained after regression model fit.

The regression Eq. (7) of the fitted model constructed for SI was presented below:

$$SI=72.21306-0.08678*X1+5.9388*X2+10.28833*X3-.0009*X1*X2-0.00245*X1*X3+0.066667*X2*X3+2.47e-05*X1^2-0.63444*X2^2-2.05*X3^2 \quad (8)$$

Were X1=Agitation speed (rpm), X2= ratio of drug:polymer (µl) and X3= ratio of polymer:polymer

All the three factors affected the swelling indices of microspheres. The magnitude of coefficient X1 (Eq. 6) is -0.08678 which concludes that antagonistic effect of rpm on SI is also to a lesser extent. Generally increase in agitation speed reduced size which in turn increases surface area and hence swelling but if the size is too small (at higher rpm) SI was less resulting in dissolution of polymer due to large surface area available for the media.

Increase in the ratio of drug:polymer (X2) increases swelling index. If concentration of drug is higher than polymer, drug dissolves in the media creating pores by which media (buffer pH 6.4) penetrates and dissolves the polymer hence lesser amount of drug, lesser numbers of pores limits dissolution of the polymer and increases SI. Swelling index increases when concentration of the drug is less than the polymeric concentration and vice versa. SI was higher for B4 and B10 where drug:polymer ratio was found to be 1:4 and 1:2.5 respectively. Swelling index (SI) of formulation B2, B9, B13 and B14 were less compared to other formulations (table 2) which may be due to equal concentration of drug and polymer.

Table 2: The dependent variable and their levels

Formula	Size µm	Drug content %	yield %	Swelling index	Bioadhesion %	Drug Dissolution Time(hr)	%
B1	21	95	92	7.4	73	6.5	100
B2	25	82	88	3.92	60	4	92.5
B3	40	97	97	7.48	76	6.5	97.83
B4	10	99.6	94	9.88	92	6.5	97.7
B5	42	71	93	7.04	80	6	99.7
B6	50	80.6	85	7	73	5.5	99.8
B7	33	83	89	7.28	70	6.5	95.3
B8	30	99	92	7.46	75	5.5	95.81
B9	45	82	86	3.68	53	4.5	97.73
B10	10	72	82	9.42	90	6.5	99.16
B11	20	99.15	99	7.96	84	6.5	97.23
B12	30	98.5	91	7.52	80	5.5	96.29
B13	35	88.3	95	3.6	54	4.5	94
B14	15	90	78	7.6	50	4.5	95.5

Increase in polymer:polymer increases SI according to eq (7) but not only polymer:polymer ratio, agitation speed and drug:polymer ratio also has an effect on SI. Only when polymer concentration was optimum swelling increased due to increased concentration of polymer. Swelling at certain level is directly proportional to the bioadhesion, increasing rate of swelling attributed to the higher

flexibility of polymer chain. However too much swelling resulted in separation of monomers leads to poor polymer chain entanglement and hence bioadhesion.

Drug content

Since drug content is not a critical factor for evaluating the formulation, a temporal relationship with independent variables to be established in order to understand content uniformity. To evaluate various independent variables to the drug content in the loaded microsphere by BBD, the drug contents data from designed formulations were transformed to log scale and fitted to the polynomial regression equations. Based on the fitted equation, it was found that agitation speed played a negligible role in drug loading of the microsphere however it helped in uniform mixing of polymer with drug.

Whereas drug:polymer ratio had negative impact on DC. Maximum drug content was obtained from the formulation having drug: polymer ratio 1:1 or close to 1, probably because of uniform mixing.

Increases in polymer:polymer ratio had antagonistic effect on response DC. It was observed that higher concentration of polymer killed the loading efficiency due to increased viscosity of the dispersed fluid.

Final Equation in Terms of Actual Factors:

Ln (DC) =8.766598289+0.00211*X1-0.39483*X2-2.44056* X3+0.000196* X1* X2+ 0.001212* X1*X3-0.00689* X2 * X3…… (9)

Time of Drug Release

Since formulation is intended for the extended release of drug, time required for maximum percentage of drug release is considered as an important response variable in the formulation. In order to extend the release of drug for a long period of time various rational combination of drug to polymer, polymer to polymer or agitation time were investigated using BBD. How these three factors influencing the time of drug release can be explained by polynomial equation below obtained from polynomial regression of a Quadratic model.

Dissolution time = 50.59028-0.04135*X1+1.194444*X2-9.20833*X3+0.000417*X1* X2+0.0025*X1*X3-0.16667*X2*X3+9.38e^{-06}*$X1^2$-0.22222* $X2^2$+1.5* $X3^2$…… (10)

It can be concluded from above equation that drug:polymer has synergistic effect on response dissolution time, which may be attributed to polymer concentration as the concentration of polymer increased there was a delay in release of drug into the media prolonging dissolution time whereas agitation speed and polymer:polymer showing antagonistic effect on dissolution time studied. As the rpm increased dissolution time decreases, due to decreases in size of microspheres. As the polymer to polymer concentration increases dissolution time decreases this may be due to increased concentration of carbopol 934p which inhibits dissolution of the drug into the media. Only when HPMC and carbopol 934p concentration was optimum dissolution was time also was optimum.

Size of microspheres

The mean size range of the all 14 BBD formulations of microspheres was estimated between 10-50 µm with very narrow size distribution [Table 2], which is suitable for intranasal administration. Microspheres size of all Individual formulations was analyzed by ANOVA and the independent variables were fitted to various models using restricted maximum likelihood estimation as a covariance structure. The best fit for the response size was found for the Quadratic model. Therefore the quadratic model incorporating interactional and quadratic terms was chosen to describe the effects of the variables.

The regression Eq. (3) of the fitted model constructed for response, size was presented below

SIZE=136.2222-0.04708* X1+5.055556* X2+5.833333* X3--0.00167 * X1 * X2+5.55E-17* X1 * X3+0.666667* X2 * X3-6.25e^{-06} * $X1^2$-0.77778* $X2^2$+1* $X3^2$…… (11)

It was observed from the equation that size of the microsphere depends not only on agitation speed but also on viscosity of polymer solution due to ratio drug: polymer and polymer:polymer. Negative coefficients of the agitation speed (rpm) signify inverse relationship of the size and positive coefficients of drug to polymer ratio and polymer:polymer ratio signify proportional change of size. Hence an optimum combinations of these variables are needed to get desired microspheres size.

Bioadhesion

The best fit for the response bioadhesion was found for the linear model. Therefore the linear model incorporating only the factors was chosen to describe the effects of the variables. The regression Eq. (3) of the fitted model constructed for bioadhesion was presented below:

Bioadhesion=16.11905+0.02125*X1+8.16667*X2-4.5*X3…… (12)

Increase in agitation speed reduces size and therefore has a larger surface area for bioadhesion, which can be observed in formulation B4 and B10. Bioadhesion increases with increase in drug:polymer concentration. polymer:polymer ratio has antagonistic effect since optimum concentration of both the polymer is necessary for bioadhesion increase in concentration of polymer causes reduced wetting and chain flexibility which may cause decreased bioadhesion.

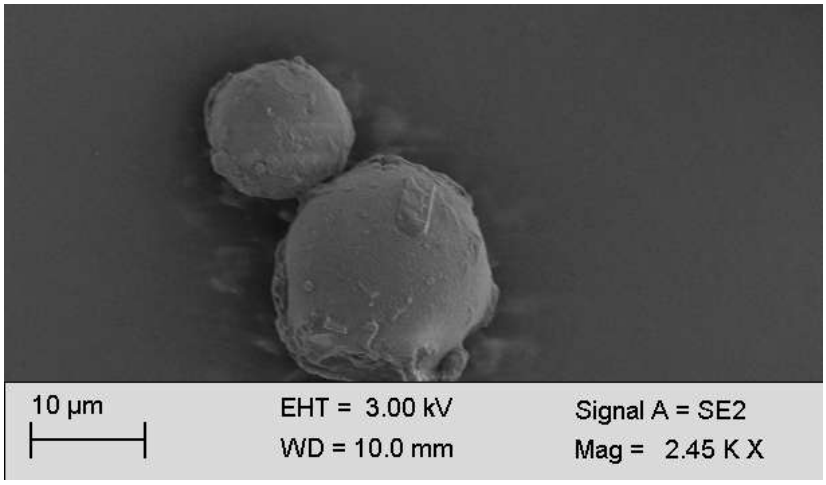

Fig. 2: SEM of the final optimized formulation B0

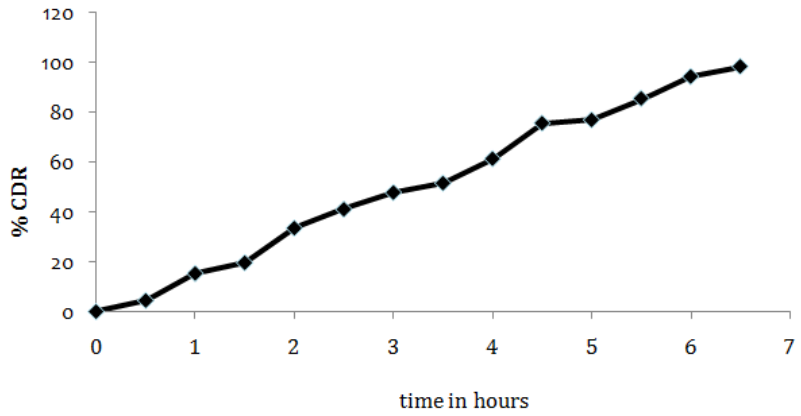

Fig. 3: Dissolution profile of formulation B0

Optimization and validation

After analyzing the polynomial equations depicting the dependent and independent variables, a further optimization and validation process by means of the design expert software was undertaken with desirable characteristics to probe the optimal formula solution of microspheres which depended on the prescriptive criteria of studied responses. The composition of optimum formulation was determined as 2200 rpm, 1:2.81 drug:polymer and 1:1.79 polymer:polymer, which fulfilled the requirements of optimization. At these levels, the predicted values of swelling index, drug content, dissolution time, size and bioadhesion were 9.41, 99.58%, 6.5hrs, 23µm, 77% bioadhesion respectively. Therefore in order to confirm the predicted model, a new batch of microspheres according to the optimal formulation factors levels was prepared.

The observed optimized formulation had swelling index of 9, drug content 99%, dissolution time of 6.5hrs, size of 20 µm (fig. 1) and bioadhesion of 75% which were in good agreement with the predicted values. The microsphere shape and morphology of was investigated using scanning electron microscopy. Prior to examination, the samples were gold coated under vacuum to render them electrically conductive. A comparison between these observed results and mathematical predictions indicates the reliability of BBD used in predicting a desirable microsphere formulation.

Release kinetics of optimized formula B0

To analyze the *in vitro* release data (fig. 2) various kinetic models were used to describe the release kinetics of optimized formulation B0.

Various kinetic models showed linear relationship. R^2 value for Zero order, First order, Higuchi, Hixon Crowell and Korsmeyer-Peppas were found to be 0.994, 0.8148, 0.9224, 0.9152 and 0.9758 respectively. R^2 value for zero order was found to be highest and therefore it was concluded that the mechanism of drug release from the bioadhesive drug delivery system followed controlled release.

CONCLUSION

Box-Behnken design was used to statistically optimize the formulation parameters and evaluate the main effects and interaction effects of the independent variables on the particle size, loading efficiency, bioadhesion, swelling index and *in vitro* drug release from microspheres. Levodopa-microspheres as reservoirs for nasal delivery were successfully formulated and optimized by using experimental design. Upon trading of various response variables and comprehensive evaluation of the feasibility search, the formulation composition with 2200 rpm, 1:2.81 of drug:polymer and 1:1.79 polymer:polymer was determined to fulfil requisites of an optimum formulation.

ACKNOWLEDGEMENT

Authors wish to thank Vellore Institute of Technology, Chennai and East Point College of Pharmacy, Bangalore for constant support and given research laboratory to carry out this project work. We also thank Divis laboratories, Hyderabad for providing gift sample of levodopa. We also acknowledge the help provided by our fellow colleagues in completion of the project.

REFERENCE

1. Ahmed ZS , Omaima SA, Mohammed HA, Nagia MA, Muhammad HJ, Mansoor KA. Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function. Int J Pharm 2007;332:55–63.
2. Kincl M, Turk S, Vrecer F. Application of experimental design methodology in development and optimization of drug release method. Int J Pharm 2005; 291:39–49.
3. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Int J Pharm 2004;282:1–18.
4. Madhusmita M, Brahmeshwar M. Mucoadhesive microparticles as potentialcarriers in inhalation delivery of doxycyclinehyclate: a comparative study. Acta Pharm Sin B 2012;2(5):518–26.
5. Chowdary KPR, Srinivasa RY. Mucoadhesive Microspheres and Microcapsulses:Current Status. Indian J Pharm Sci 2005;67(2):141-150.
6. John SD. The Basics Underlying Mechanisms of Mucoadhesion. Adv Drug Deliv Rev 2005;57:1556-68.
7. Jaspreet KV, Kaustubh T, Sanjay G. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
8. Henry CR, Lisbeth Illum, Gordon B, Paul JH, Steven QC. Intranasal delivery: physicochemical and therapeutic aspects. Int J Pharm 2007;337: 1-24
9. Jain NK. Progress in controlled and novel drug delivery systems. 1st edition.Delhi publishers; 2010.
10. Lauren SC , Robert HA. Optimizing bioavailability in the treatment of parkinson's disease. Neuro pharmacol 2007;53:791-800.
11. Chandrakala V, Srinath MS, Saral MA and Kumar US. Formulation and Evaluation of BioadhesiveCyproheptadine Tablets. Trop J Pharm Res 2011;10 (4):365-73.
12. Abd el-Hameed, Kellaway IW. Preparation and i*n vitro* characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. *Eur J Pharm Biopharm* 1997;44:53-60.
13. Anees KA, Mansoor KA. Box-behnken design for the optimization of formulation variables of indomethacin coprecipitates with polymer mixtures. Int J Pharm 1996;131:9-17.
14. Shuoye Yang, Jiayin Chen, Di Zhao, Deen Han, Xijing Chen Comparative study on preparative methods of DC-Chol/DOPE liposomes and formulation optimization by determining encapsulation efficiency. Int J Pharm 2012;434:155–160.
15. David QG, David TE, Adriana GQ, Eric A, Eric D. Adaptation and optimization of the emulsification-diffusion technique to prepare lipidic nanospheres Eur J Pharm Sci 26 (2005) 211–18.

16. Chelladurai S, Brahmeshwar M. Development and *in vitro* evaluations of gelatin A microspheres of letorolac tromethamine for intranasal administration. Acta pharm 2003;53:101-10.
17. Vidyavathi M, nirmala D, asha S, joginapalli S. design and evaluation of niacin microspheres. Indian j. Pharm. Sci 2009; 12:663-69.
18. Jain SK, Nitin Jain K , Gupta Y, Jain A, Jain D, Chaurasia. Mucoadhesive chitosan microspheres for non-invasive and improved nasal delivery of insulin. Indian j. Pharm. Sci 2007;7:498-504.
19. Dandagi PM, Mastiholimath VS, Gadad AP, Ililger SR. Mucoadhesive microspheres of propranolol hydrochloride for nasal delivery. Indian j. Pharm. Sci 2007;5:402-07.
20. Appa Rao. B, Shivalingam MR, Kishore RYV, Somesekhara Rao, Rajesh K, Sunitha N. Formulation and Evaluation of Aceclofenac Solid Dispersions forDissolution Rate Enhancement. Int. J. Pharma. Sci. Drug Res 2010;2(2):146-150.
21. Durrani MJ, Andrews A, Whitaker R, Benner SC. Studies on drug-release kinetics from carbomer matrices, Drug Dev.Ind. Pharm. 20 (1994) 2439–47.
22. Karunakar S, Singh J. Preparation, characterization and *in-vitro* release kinetics of salbutamol sulfate loaded albumin microspheres. Drug Dev. Ind. Pharm. 1994:20(8): 1377-99.
23. Stuart CL, Stephen HW and Larry AL. Formulation and Characterization of a Compacted Multiparticulate System for Modified Release of Water- Soluble Drugs—Part II Theophylline and Cimetidine. Drug Dev. Ind. Pharm., 2009; 35:568–82.
24. Nurhan E, Nevin C. Factors influencing release of salbutamol sulphatre from poly(lactide-co-glycolide) microspheres prepared by water-in-iol-in-water emulsion technique. Int J Pharm. 1996;137:57-56.