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Optimization and Characterisation of Nasal Microparticles for Levodopa Delivery to CNS

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ABSTRACT

The objective of this study is to formulate bioadhesive microparticulate to deliver levodopa into the brain which could significantly improve the bioavailability. The microspheres of gelatin were prepared by surface polymerization using glutaraldehyde as a crosslinking agent. Central composite design (CCD) was employed to optimize the formulation parameters of levodopa microsphere for maximum swelling index, drug content, time of drug release, percentage drug release, size of the microspheres and bioadhesivity by using polynomial regression model. The quantitative effect of the formulation factors (viz. drug:polymer ratio, agitation speed, glutaraldehyde concentration) at different levels on bioadhesion and drug release were predicted using polynomial equations. Following optimization a formulation comprising of levodopa: gelatin ratio 1:1.98 and glutaraldehyde 32.42µl at 2200rpm was identified for maximizing bioadhesivity and obtaining sustained drug release. The optimal microsphere preparation was subsequently characterized in terms of size (11 µm), drug loading (95%), swelling index (3.5), drug release (99.7% at 4.5hr), *in vitro* bioadhesion (95%) and release kinetics. Kinetic models revealed that drug release followed anomalous (non-Fickian) diffusion. Predicted values of final optimized formula were very close to actual values which confirmed practicability and validity of the model. Hence levodopa-microspheres as reservoirs for nasal delivery were successfully formulated and optimized CCD.

Key words: Bioadhesion, central composite design (CCD), parkinsons disease, gelatin microspheres, surface polymerization, intranasal drug delivery.

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INTRODUCTION

It is well known that the euphoria derived from the sniffing of cocaine in conscious subjects occurs rapidly (within 3-5 min). It has been suggested that the reason for such rapid effects is, apart from a rapid nasal absorption, the presence of a direct pathway from the nasal cavity to the CNS and the capacity of the drug to concentrate selectively in specific regions in the brain [1].

In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of great interest to the pharmaceutical industry, especially for systemically acting drugs that are difficult to deliver via routes other than injection. The nasal route could be important for drugs that are used in emergency treatments, such as for pain, and for centrally acting drugs where the putative pathway from nose to brain might provide a faster and more specific therapeutic effect. Nasal delivery can be used for local delivery like nasal allergy, nasal congestion and nasal infection systemic delivery like crisis treatment, long term treatment , peptide and protein delivery, vaccine delivery as well as to access the CNS so as to reach the local receptors and to circumvent BBB[2].

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 nasal 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 [3].

Drugs have been shown to reach the CNS from the nasal cavity by a direct transport across the olfactory region situated at the loft of the nasal cavity. It is the only site in the human body where the nervous system is in direct contact with the surrounding environment. The drug can cross the olfactory epithelium by one or a combination of mechanisms [4].

There is a transcellular route through the cells as well as a paracellular route between the cells, as is the case for the normal nasal epithelium. Furthermore, the drug can be transported through the olfactory neuron cells by intracellular axonal transport primarily to the olfactory bulbs [5]. The intracellular axonal pathway is a slow pathway that can take hours to deliver drugs to the CNS, whereas other two pathways are faster and enable transport of drug within few minutes. Thus, these last two pathways are often evident in the experimental settings. It is clear that, in many therapeutic situations where a rapid and/or specific targeting of drugs to the brain would be beneficial, such as for the treatment of Parkinson's disease, Alzheimer's disease or pain, these results, demonstrating direct nose to brain transport, are of great interest. Therefore, efforts are made to develop nasal delivery systems capable of increasing the fraction of the drug that will reach the CNS after nasal delivery [6].

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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 to 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 tight 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 therapeutic efficacy [9].

Various alternative levodopa formulations such as intravenous formulation, levodopa produdrugs, 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 by applying CCD to optimize formulation variables in order to minimize peripheral decarboxylation of levodopa and increasing levodopa concentration in brain and thus minimizing dose frequency and increasing patient compliance [11].

Response surface methodology (RSM), supported by statistical software, is a well-established approach for pharmaceutical formulation development and optimization allowing extraction of maximal information out of few well-designed experiments. Central composite design (CCD), one of the techniques in RSM, is suitable for pharmaceutical blending problems allowing investigation with the least number of experiments and selection of the optimal composition for achieving the presetting target. CCD has been extensively adopted to optimize the multiparticulate formulations. Those studies demonstrated the apparent advantage of CCD utilization in the formula optimization process for drug delivery system design [12].

The other aim of this study was to design and optimize a novel levodopa loaded microsphere carrier system composed of gelatin. The said microsphere was prepared form emulsion cross linking method using glutaraldehyde as a cross linking agent in presence of surfactant (span 60). After evaluating the main and interaction variables which affect drug loading, particle size and percent and time of drug release ,swelling index and bioadhesion , a three-factor three-level central composite design was employed to schedule and perform the experiments. Optimized microspheres formulation was prepared on the basis of the predicted optimum levels of the independent variables of the factorial design.

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MATERIALS AND METHOD

Materials

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

Methods

Preparation of Microspheres

Table 1: Agitation speed and composition of various microspheres based on central composite design

Formula	Gluteraldehyde (µL)	Rpm	Drug:Polymer
C1	58	2000	1:1.5
C2	50	2200	1:1
C3	30	2000	1:0.8
C4	1.72	2000	1:1.5
C5*	30	2000	1:1.5
C6	10	1800	1:1
C7	30	2000	1:2.2
C8	10	2200	1:2
C9*	30	2000	1:1.5
C10	30	2282	1:1.5
C11	50	1800	1:2
C12	30	1717	1:1.5

Agitation speed (rpm) , C5 & C9 are centre points

Gelatin microspheres were prepared by emulsion cross-linking method[13]. The drug was dispersed in an aqueous gelatin solution, which was preheated at 40^0C for 1 h. The solution was added drop wise to liquid paraffin 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 until the aqueous phase was completely removed by evaporation[14]. The washed microspheres were air dried and then dispersed in 5 ml of aqueous glutaraldehyde-saturated toluene solution at room temperature for 10 min to allow cross linking[15],[16]. The microspheres were washed with toluene and treated with 100 ml of 10mM glycine solution containing 0.1 % Tween 80 at 37° for 10 min to block unreacted gluteraldehyde. Microspheres were dried in an oven at 50^0C for 2hr and stored in a desiccators at room temperature[17],[18]. Gelatin was used in different concentration according to Central Composite Design (table 1) other independent parameters chosen were RPM and glutaraldehyde concentration.

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 3).

Central Composite Design

After opting for the most important factors influencing the physicochemical properties of the produced levodopa-loaded microspheres, a three-factor, three-level CCD was developed to explore the optimum levels of these variables. This methodology consisted of three groups of design points, including three-level factorial design points, axial or star points, and centre points. Therefore, three selected independent variables glutaraldehyde, rpm and drug:polymer ratio were studied at five different levels coded as -α, -1, 0, +1, and +α. The value for alpha (1.414) was intended to fulfill the rotatability in the design[19]. Physicochemical properties of the produced microspheres, was selected as dependent variables. According to the CCD matrix generated by Design-Expert software (Trial Version 7.1.6, Stat-Ease Inc., MN), a total of 12 experiments, including five factorial points, five axial points and two replicated centre points for statistical assessment of pure error sum of squares, were constructed.

When it came to the prediction of the best suitable formulation, the fitness of the model among the linear, two-factor interaction (2FI) , and quadratic model was assessed through p-value from analysis of variance and model maximizing multiple correlation coefficient r^2 (predicted and adjusted r^2) as quality indicators in the model summary statistic list. The probability value less than 0.05 was considered to be statistically significant[20]. Optimization was performed by using a desirability function to obtain the optimal points concerning the predetermined constraints in which the drug content, % drug release, time of drug release ,swelling index and bioadhesion at maximum level, particle size was at their minimum levels. The picked optimal formulation was prepared for further evaluation of the physico-chemical characteristics [21].

Experimental Design

Central Composite design was applied to reduce the number of experiments and to optimize the range of variable concentrations needed to obtain maximum responses. A three factor, three-level central composite design was used for the optimization of levodopa microspheres with glutaraldehyde, agitation speed (rpm) and drug:polymer concentration as the independent variables (Table 1).

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-CCD was chosen. The other important reason for selection of the CCD was based on the fact that the expected responses do not vary in a linear

manner with the selected variable and to enable the quantification of the prediction of the responses, a central composite plan was selected, where the response could be modelled in a quadratic manner since the error in predicting the response increases with the distance from the centre of the modelled region. It is advisable to limit the use of the models to an area bound by values corresponding to -α to +α limits [22].

The parameters were carefully selected to carry out composite factorial design based on codified values of -α, -1, 0, +1, +α. The value of alpha is chosen such that the variance of the response predicted by the model would depend only on the distance from the centre of the modelled region. The value of alpha was taken here as 1.414. Two replicate central points were prepared to estimate the degree of experimental error for the modelled responses.

Six 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 [23].

Table 2: Summary of statistical evaluation of experimental design

Name	Minimum	Maximum	Mean	SD	Ratio	Model*
SI	0.08	3.32	1.73	1.06	41.50	2FI
DC*	70	100	88.13	9.61	1.42	2FI
diss time	2	4.5	3.54	0.91	2.25	Linear
% DR	88.42	100.97	95.39	4.02	1.14	Quadratic
SIZE	10	35	23.75	7.13	3.50	Linear
BIOADHESION	54	92	78.50	11.39	1.70	Quadratic

*models were tested for significance using ANOVA and coefficient of polynomial equation under each model were calculated using multiple regression analysis

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.

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 a median diameter was calculated [24].

Drug Loading (Entrapment Efficiency)

A weighed amount of microsphere were crushed into powder and added to 0.1N HCl, mixture was allowed to stand for 30min. The solution was filtered and drug content was

estimated by UV spectrophotometer at 280nm [25]. The drug entrapment efficiency was determined by using the formula below,

Drug entrapment efficiency = Experimental drug content / Theoretical drug content

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 6.2 at 37±0.5^{0}C 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 [26].

$SR=W_R-W_O/ W_O$

W_O= Initial weight of drug microspheres
W_R= Weight of swollen microspheres at equilibrium swelling in medium

***In vitro* Bioadhesion**

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^{0}C 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 [27].

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

***In vitro* Drug Release**

To carry out *in vitro* drug release accurately weighed drug loaded microspheres were dispersed in 400 ml of phosphate buffer (pH 6.2) 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[28],[29].

Zero-order Release Kinetics

The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration [30]. To study the zero-order release kinetics the release data was fitted into the following equation:

$$F=K.t \quad \text{.....(1)}$$

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 [31]. To study the first-order release kinetics the release rate data are fitted into the following equation:

$$F=100*(1- e^{-Kt}) \quad \text{.....(2)}$$

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).

$$F=K.t^{1/2} \quad \text{.....(3)}$$

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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.

$$M_t / M_\infty = K.t^n \quad \text{.....(4)}$$

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.

$$W_t^{1/3} = W_0^{1/3} - Kt \quad \text{.....(5)}$$

Where W_t = weight of microspheres at time t
W_0= initial weigh of microspheres

RESULT AND DISCUSSION

Mechanism of Coacervation Method to Produce Levodopa Microspheres

The production of microspheres intended for nasal administration of levodopa was tailored as per the emulsion cross linking technique described in various literature. 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 monostrerate (Span 60), an nonionic surfactant was selected as the emulgent in the formulation of microspheres. In this process, span 60, a nonionic 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 gelatin 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 formed if agitation speed was less than 1800 there was very large microsphere formation which was unsuitable for nasal administration and if the speed was higher than 2200 very small microspheres resulted which causes microsphere to deposit in trachea. Stirring and heating were maintained until the aqueous phase was completely removed by evaporation. The washed microspheres were air dried and then dispersed in 5 ml of aqueous glutaraldehyde-saturated toluene solution (25% v/v) at room temperature for 10 min to allow cross linking for further hardening of the micorsphere. The microspheres were washed with toluene and treated with 100 ml of 10mM glycine solution containing 0.1 % Tween 80 at 37° for 10 min to remove unreacted toxic gluteraldehyde. Microspheres were dried in an oven at 50^0C for 2hr and stored in a 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. As shown in Table 3, the best model fit for each of the responses Y1 (Swelling index) and Y2 (drug content) were found for the two factor interaction, Y3 (dissolution time) and Y5 (microspheres size) were found for linear model, Y4 (% drug release), and Y6 (bioadhesion) were found quadratic models.

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 favors 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.

Table 3: Results of various parameters from Central Composite Design

Formula	Swelling Index (%)	Drug content (%)	Dissolution		Size	BA (%)	Yield (%)
			Time (hrs)	DR (%)	(µm)		
C1	3	98	4	96.85	25	85	90
C2	1.8	99	4	95.66	10	83	99
C3	0.08	95	3	97.03	20	54	95
C4	0.2	85	2	98.75	25	64	85
C5	2	86	4	99	25	72	86
C6	0.36	100	2	92.6	35	88	100
C7	3.32	80	4.5	88.99	28	90	70
C8	1.84	79.6	2.5	92.07	18	92	79.6

C9	1.93	87	4	98.3	25	70	87
C10	1.64	97	4	96	14	80	89
C11	2.84	70	4	100.98	30	80	80
C12	1.8	81	4.5	88.42	30	84	81

DR= Drug Release, BA= Bioadhesion

Swelling Index

The two factor interaction model incorporating interactional terms was chosen to describe the effects of the variables. Each experimental response could be represented by polynomial equation obtained after regression model fit.

The regression Eq. (6) of the fitted model constructed for SI was presented below:

SI=15.76001-0.18327*A-0.00985*B+0.480826*C+1.28878E-004*A*B-0.016657*A*C
+0.003799*B*C……..(6)

Were A=glutaraldehyde concentration (µl), B=agitation speed (rpm) and C=drug:polymer

All the three factors affected the swelling indices of microspheres. Increase in the concentration of glutaraldehyde decreases hydrophilicity which in turn reduces swelling. When the concentration of glutaraldehyde was very less the polymer dissolved entirely, hence concentration of glutaraldehyde is significant and must be optimum to produce ideal microshperes.

Since SI showed interaction with the individual factors and their combination, the polynomial regression obtained from the model is not significant hence SI cannot be included in the response variable.

The magnitude of coefficient B (Eq. 6) is -0.00985 which concludes that antagonistic effect of rpm on SI is also to an 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 higer rpm) SI was less resulting in dissolution of polymer due to large surface area available for the media. When polymer concentration was high swelling increased due to increased concentration of polymer (C7). Swelling at certain level is directly proportional to the bioadhesion, increasing rate of swelling attributed to the higher flexibility of polymer chain to tangle with the mucin polymer of the membrane and hence increase bioadhesion. 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 in presence of glutaraldehyde. To evaluate various independent variables to the drug content in the loaded microsphere by CCD, 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 and glutaraldehyde concentration had no effect on drug content where as polymer to drug concentration is critical attribute to the drug content of the formulation. However maximum drug content was obtained from the formulation having drug: polymer ratio 1:1 or close to 1, probably because of uniform mixing, Whereas C7 has the least drug: polymer ratio of 1: 2.21 and resulted poor drug content, only 70%.

Final Equation in Terms of Actual Factor

$$Ln(DC)=7.889039-0.04407*A-1.57775E-003*B-1.92304*C+2.07E-05*A*B +0.003412*A*C+0.00085*B*C \ldots\ldots (7)$$

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 cross-linker or hardening agents and blending or agitation time were investigated using CCD. How these three factors influencing the time of drug release can be explained by polynomial equation below obtained from polynomial regression of a linear model.

$$\text{Dissolution time}=1.630975+0.039553*A0.00013*B+0.65533*C \ldots\ldots (8)$$

It can be concluded from above equation that glutaraldehyde concentration and drug:polymer has synergistic effect on response dissolution time whereas agitation speed showing antagonistic effect on dissolution time studied. Glutaraldehyde concentration increased dissolution time due to reduced wetting and cross linking of polymeric chains. As the rpm increased dissolution time decreases, due to decreases in size of microspheres. As the drug to polymer ratio increased dissolution time also increased due to increase in polymer concentration in the microspheres.

Drug Release

Drug release from the microspheres depends on drug to polymer concentration, Size of the microsphere & polymer cross-linkers. To establish quantitative relationship of these independent variables to the drug release, % drug release from the CCD formulations were subjected to ANOVA and polynomial regression analysis. The best fit for percentage drug release was found by quadratic model. Therefore the quadratic model incorporating interactional and quadratic terms was chosen to describe the effects of the variables.

From quadratic polynomial equation it was found that the coefficient of glutaraldehyde is -1.27 which signifies that drug release has inverse relationship with glutaraldehyde concentration. The negative relationship of glutaraldehyde concentration to the drug release is attributed to increase cross-linking and thus reduced wetting of microspheres. A positive coefficient of agitation speed signifies proportional relationship with drug release. However 99% of drug was released within 4.5 hrs for C_0 formulation

The regression Eq. (3) of the fitted model constructed for %DR was presented below:

$$\%DR=-303.162-1.27078*A+0.315768*B-76.40396*C-0.00101*A*B+0.414124*A*C-0.03662*B*C+0.001557*A^2-5.43E-05*B^2-7.0878*C^2 \quad \text{.....(9)}$$

Size of Microspheres

The mean size range of the all 12 CCD formulations of microspheres was estimated between 10-35 µm with very narrow size distribution [Table 3], 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 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 response, size was presented below

$$\text{Microspheres size} = +95.35413-0.081250*A-0.037267*B+3.57843*C \quad \text{.....(10)}$$

It was observed from the equation that size of the microsphere depends not only on agitation speed but also on viscosity of polymer solution (drug: polymer) and on glutaraldehyde concentration to some extent. Negative coefficients of glutaraldehyde signify inverse relationship of the size, which may be attributed to shrinkage of microspheres due to gluteraldehyde. negative coefficients of the agitation speed (rpm) signify inverse relationship of the size and positive coefficients of drug to polymer concentration signify proportional change of size. Hence an optimum combinations of these variables are need to get desired microspheres size.

Bioadhesion

The best fit for response percentage drug release was found for 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 bioadhesion was presented below:

$$\textbf{Bioadhesion}=1374.677-6.0056*A+1.05911*B+232.544*C+0.003119*A*B-0.31642*A*C+0.116746*B*C+0.010208*A^2+0.000196*B^2+11.33333*C^2 \quad \text{.....(11)}$$

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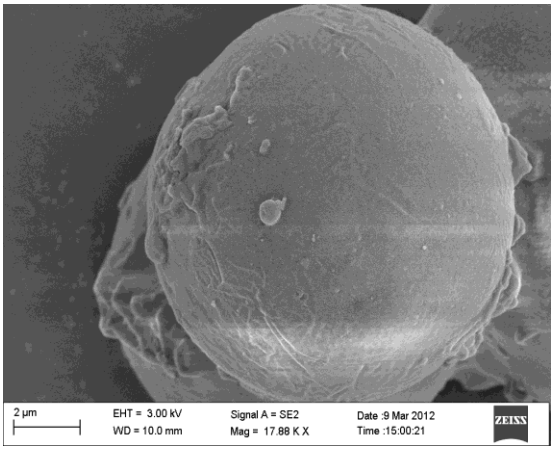
Glutaraldehyde concentration decreases bioadhesion due to decreased flexibility, hydrophilicity in polymeric chain.

Increase in agitation speed reduces size and therefore has a larger surface area for bioadhesion, which can be observed in formulation C9 and C10. Bioadhesion increases with increase in polymer concentration.

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 32.42 µl glutaraldehyde 2200 rpm, 1.98 drug: polymer, which fulfilled the requirements of optimization. At these levels, the predicted values of swelling index, drug content, dissolution time, % DR, size and bioadhesion were 3.32,99.57%, 4.5hrs,96%, 14µm and 81 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 3.5, drug content 95%, dissolution time of 4.5hrs, % drug release of 94%, size of 11 µm(fig. 1) and bioadhesion of 74 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 CCD used in predicting a desirable microsphere formulation.

Fig: 1 SEM of the final optimized formula C0



Release Kinetics of Optimized Formula C0

To analyze the *in vitro* release data (fig. 2) various kinetic models were used to describe the release kinetics of optimized formulation C0.

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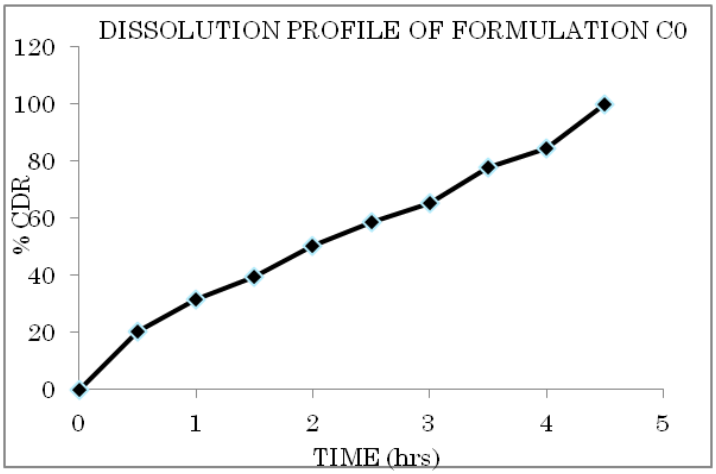
Various kinetic models showed linear relationship. R^2 value for Zero order, First order, Higuchi, Hixon Crowell and Korsmeyer-Peppas were found to be 0.986, 0.961, 0.954, 0.979 and 0.996.respectively. R^2 value for Korsmeyer-Peppaswas found to be highest and this value was obtained by incorporating the first 60% of release, n is the release exponent, indicative of mechanism of drug release its value was found to be 0.647 which showed that solute diffusion followed Anomalous (non-Fickian) diffusion [18].

Fig 2: Dissolution Profile of Formulation C0



CONCLUSION

Central Composite 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, 1.98 of drug:polymer and 32.42 glutaraldehyde concentration was determined to fulfill requisites of an optimum formulation.

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REFERENCES

[1] Chowdary KPR, SrinivasaRao Y. Indian J Pharm Sci 2005; 67(2): 141-150.
[2] John Smart D. Adv Drug Deliv Rev 2005; 57: 1556-1568.
[3] Goole J, Amighi K. Int J Pharm 2009; 380: 1-15.
[4] Tushar K Vyas, Aliasgar Shahiwala, Sudhanva Marathe and Ambikanandan Misra. Journal of Controlled Release 2003; 87: 187-198.
[5] Michael Ugwoke I, Remigius Agu U, Norbert Verbeke , Renaat Kinget. Advanced Drug Delivery Reviews 2005; 57: 1640-1665.
[6] JaspreetKaurVasir, KaustubhTambwekar, Sanjay Garg. Int J Pharm 2003; 255: 13-32.
[7] Henry Costantino R, LisbethIllum, Gordon Brandt, Paul Johnson H, Steven Quay C. Int J Pharm 2007; 337: 1-24.
[8] Jain NK. Progress in controlled and novel drug delivery systems. 1st edition.Delhi publishers; 2010.
[9] Lauren Seeberger C, Robert Hauser A. Neuropharmacol 2007; 53: 791-800.
[10] Barkai A, Pathak YV, Benita S. Drug Dev Ind Pharm 1990; 16: 2057-2075.
[11] Chueh HR, Zia H, Rhodes CT. Drug Dev Ind Pharm 1995; 21 (15): 1725-1747.
[12] NurhanErdern, NevinCelebi. Int J Pharm 1996; 137: 57-56.
[13] Shirui Mao, Jianming Chen, Zhenping Wei, Huan Liu, Dianzhou BI. Int J Pharm 2004; 272: 37-43.
[14] Pongpaibul Y, Price JC, Whitworth CW. Drug Dev Ind Pharm 1984; 10: 1597-1616.
[15] Conti B, Genta I, Modena T, Pavanetto F. Drug Dev Ind Pharmacy 1995; 21: 615-622.
[16] Abdel-Hameed, Kellaway IW. Eur J Pharm Biopharm 1997; 44: 53-60.
[17] SR Iliger and T Demappa. Journal of Pharmacy Research 2011; 4(1): 276-279.
[18] Siva Vaithiyalingam, Mansoor A Khan. International Journal of Pharmaceutics 2002; 234 179-193.
[19] A Billona, B Bataillea, G Cassanasb, M Jacoba. International Journal of Pharmaceutics 2000; 203: 159-168.
[20] Jifu Hao, Fugang Wang, Xiaodan Wang, Dianrui Zhang, Yanping B, Yunsheng Gao, ,Xuemei Zhao, Qiang Zhang. Eur J Pharm Sci 2012; 47: 497-505.
[21] Govender S, Pillay V ,Chetty DJ , Essack SY, Dangor CM, Govender. International Journal of Pharmaceutics 20005; 306 : 24-40.
[22] Kincl M, Turk S, Vrece RF. Int J Pharm 2005; 291: 39-49.
[23] ChelladuraiSankar, Brahmeshwar Mishra. Acta Pharm 2003; 53: 101-110.
[24] Vidyavathimaravajhala, Nirmaladasari, Ashasepuri, Joginapalli S. Indian J Pharm Sci 2009; 12: 663-669.
[25] Jain SK, Nitin Jain K , Gupta Y, Jain A, Jain D, Chaurasia. Indian J Pharm Sci 2007; 7: 498-504.
[26] Dandagi PM, Mastiholimath VS, Gadad AP, Ililger SR. Indian J Pharm Sci 2007; 5: 402-407.
[27] Appa Rao B, Shivalingam MR, Kishore Reddy YV, Somesekhara Rao, Rajesh K, Sunitha N. International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(2): 146-150.

ISSN: 0975-8585

[28] Durrani MJ, Andrews A, Whitaker R, Benner SC. Drug Dev Ind Pharm 1994; 20: 2439-2447.
[29] Karunakar S, Singh J. Drug Dev Ind Pharm 1994: 20(8): 1377-1399.
[30] Stuart Cantor L, Stephen Hoag W and Larry Augsburger L. Drug Dev Ind Pharm 2009; 35: 568-582.