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Microspheres of Beclomethasone Dipropionate

Vishakha Vishwanath Doke*[1], Nilesh M. Khutle[2], Khemchand Gupta[3], Abhijeet Kunwarpuria[1]

1 Research Scholar (Ph.D), Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.
2 Assistant Professor (Pharmaceutics), Dr.L.H.Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India.
3 Professor (Pharmaceutics), Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

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For Correspondence:

Vishakha Vishwanath Doke

Research Scholar (Ph.D), Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India..

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ABSTRACT:

The research was done with the goal of development and evaluation of sustained release microsphere of Beclomethasone dipropionate (BDP) by microencapsulation process. Poly lactic glycolic acid (PLGA) was used as polymer and Polyvinyl alcohol (PVA) was used as emulsifying agent and concentration of both was optimized on the basis of entrapment efficiency. Microspheres were prepared using emulsion solvent evaporation method. Two different formulations were prepared using two different grades of PLGA i.e. PLGA 50: 50 and PLGA 75:25. It was found that 20 to 40 mg PLGA with 1-3% w/v PVA gives good entrapment efficiency. Optimized batch was selected using 32 factorial design. For BDP-PLGA 50:50 microspheres, batch containing 30 mg PLGA and 2% w/v PVA and for BDP-PLGA 75:25 microsphere, batch containing 20 mg PLGA and 2% w/v PVA was found to be best. A microsphere was filled in capsule shell size "2". Optimized batch was then evaluated for particle size which is in the range of 3000-6000 nm, zeta potential was found to be -10 to -19 mV, entrapment efficiency was found to be 97-98%. Microspheres had good flow properties, ex-vivo permeability study shows 40% release of BDP in 4 hrs. An optimized microsphere shows significant improvement in dissolution behaviour. It gives above 98% release in 24 hrs. DSC confirmed that there is entrapment of drug in polymer.

KEY WORDS: Beclomethasone dipropionate (BDP), Poly lactic glycolic acid (PLGA) and Polyvinyl alcohol (PVA).

INTRODUCTION

Oral delivery systems commonly formulated as solid dosage forms like tablets, capsules, micropellets, microsphere etc.[1] The solid dosage forms provide unique advantages over liquid dosage forms like excellent drug stability in solid state, less microbiological burden, ease of conveyance of dosage forms for patients etc. Depending on the dose and pharmacokinetics, the effective concentration of drug can be maintained systemically by frequent administration of dosage form. This frequent administration in many situations results in constantly changing, unpredictable and often sub-therapeutic plasma concentrations leading to marked side-effects.[2] Modified release drug delivery system divided into delayed release, sustained release, site specific targeting and receptor targeting.[3, 4] Sustained release preparation may provide an immediate dose required for normal therapeutic response followed by gradual release of drug in amounts sufficient to maintain the therapeutic response for extended period of time. The major advantage of this system is that along with reduced frequency of dosing, it provides blood levels devoid of peak and valley effect which is characteristic of conventional dosage regimen.[5]

Microspheres can encapsulate many types of drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time.[6] They are small particles of solids or small droplets of liquids surrounded by walls of natural & synthetic polymer films of varying thickness & degree of permeability

acting as a release rate controlling substance & have a diameter up to the range of 0.1µm-200µm.[5, 7]

Synthetic polymer (biodegradable and non biodegradable) [8] and natural polymers (protein and carbohydrates)[9, 10] are used to prepare microsphere.

Beclomethasone dipropionate is a prodrug of the free form, Beclomethasone (beclomethasone-17-monopropionate). It is an anti-inflammatory, synthetic corticosteroid. Beclomethasone dipropionate is also being investigated for oral treatment in mild-to-moderate Crohn's disease of ileal or ileal-right colonic localization and for mild-to-moderate graft versus host disease.[11] It has short half-life i.e 1-3 hrs. It has poor bioavailability (1-10%) and limited solubility i.e 49.39 mg/L. For overcoming this problem sustained release microsphere of Beclomethasone dipropionate can be formulated which improve its dissolution and leads to increase its bioavailability and gives sustained release and patient compliance.[12, 13]

MATERIALS AND METHODS

MATERIALS

Beclomethasone dipropionate was obtained from Yarrow chem. Products. PLGA 50:50 and PLGA 75:25 were received as gift sample from Purac biomaterials. PVA was procured from SD fine chemicals. Hard gelatin capsule obtained as gift sample from Associate capsules, Mumbai. Other analytical grades materials were used.

METHODS

Selection of Organic phase

Selection of organic phase was based on solubility of drug and polymer. The polymer and drug were dissolved in two different Organic phases (DCM and Acetone) and microspheres were made by emulsion solvent evaporation technique. The entrapment efficiency of microspheres of PLGA 50:50 and PLGA 75:25 made by using these two organic phases were compared.

Selection of emulsifying agent

Tween and Polyvinyl alcohol were evaluated as emulsifying agent using solvent evaporation technique. The effects of the different surfactants were seen on the Entrapment efficiency.

Selection of other factors

During Formulation many factors affecting the quality of formulation are observed which were listed in Table No. 1. Some factors like type of mixers, duration of mixing, type of centrifugation can be directly selected based on the comparison of quality of final formulations. While some other factors have a wide range of effects on the formulation, which can be optimized by Factorial design. Batches of PLGA 50:50 and PLGA 75:25 microspheres each containing 30 mg polymer was made with varying factors which are to be selected and optimized.

Table 1: Various factors affecting formulation and their types and ranges

Factors	Options
Concentration of polymer PLGA	Wide range of concentrations ranging from 10 to 80 mg were tried
Concentration of emulsifying agent PVA	0.5 to 4% Solution of PVA was used for making microspheres
Ratio of Volume of organic Phase and aqueous phase	1. 1:4 2. 1:5 3. 1:6 4. 1:7
Selection of equipment for mixing	1. Magnetic stirrer 2. High speed Homogenizer
Centrifugation type	1. Centrifuge 2. Cooling centrifuge
Evaporation time	1. 1-3 hr under stirring 2. Keeping overnight evaporation.
Washing liquid	1. Distilled water 2. Phosphate buffer pH 7.2

Method of preparation

PLGA microspheres were prepared using solvent evaporation method [14] with high speed homogenizer. PLGA was dissolved in organic phase and emulsifying agent was dissolved in aqueous phase. Organic phase was lowly injected into aqueous phase and stirred under high speed homogenizer over an ice bath. Emulsion was stands for 24 hrs to evaporate organic phase. microspheres were separated by centrifugation using Cooling centrifuge at 4^0C at 15000 rpm. The resultant microspheres were then lyophilized to obtain dry microspheres. Microspheres were diluted with lactose, talc and magnesium stearate to adjust dose and increase the bulk. Final formulation filled in a capsule of size "2".

Selection of concentration range for polymer

Different batches were formulated using 10 mg-60 mg concentration of polymer PLGA 50:50 and PLGA 75:25 and

2% w/v of PVA. All the other parameters were kept constant. The batches were made using High speed homogenizer over an ice bath, 5 mL aqueous phase, 1ml organic phase and the drug concentration was kept constant (10 mg). The range was selected based on the entrapment efficiency of batches.

Selection of concentration range for emulsifying agent

Different Batches of PLGA 50:50 and PLGA 75:25 microspheres with concentration of 0.5-3.0 % w/v PVA were made, keeping all other parameters constant. Drug concentration was 10 mg, Polymer concentration was 30 mg and high speed homogenizer was used for microspheres preparation. Entrapment efficiency of these microspheres was checked for selecting the range.

Optimization using 3^2 factorial design

Traditionally, pharmaceutical formulations were developed by changing one variable at a time. This method is time consuming and it is difficult to evolve an ideal formulation using this classical technique since the combined effects of the independent variables were not considered. It is thus important to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. [15, 16] After the selection of the parameters like Polymer, inner organic phase and surfactant; 3^2 full factorial design was employed to design the optimized batch as per Table No. 2 and Table No. 3. In this design 2 factors were evaluated each at 3 levels and the experimental trials were carried out with all possible 9 combinations.

Table 2: Levels of Independent variables for optimization

Factors	**Levels**		
	Low (-1)	**Medium (0)**	**High (+1)**
A: Concentration of PLGA (mg)	20	30	40
B: Concentration of PVA (%)	1	2	3
Response	**Goal**		**Acceptable range**
Y1: Entraptment efficiency	To achieve within limit		95.0-105.0%

All batches contained 10 mg Beclomethasone dipropionate, Organic phase used was 1 ml of Acetone in which drug and polymer were dissolved; external phase was 5 ml of PVA solution in water. Microspheres were made using Emulsion solvent Evaporation method using a homogenizer. The factorial design was studied using the contour plot and the 3-D response plot the interaction effect of the independent variable on the dependent variable was also observed.

Table 3: 3^2 Full factorial designs for optimization of BDP-PLGA 50:50 and BDP-PLGA 75:25 microspheres.

Factorial design for PLGA 50:50 Microspheres			**Factorial Design for PLGA 75:25 microspheres**		
Formulation Batch	Codes		Formulation Batch	Codes	
	A= PLGA concentration (mg)	B= PVA Concentration (%)		A= PLGA concentration (mg)	B= PVA Concentration (%)
F1	20	1	F1	20	1
F2	20	2	F2	20	2
F3	20	3	F3	20	3
F4	30	1	F4	30	1
F5	30	2	F5	30	2
F6	30	3	F6	30	3
F7	40	1	F7	40	1
F8	40	2	F8	40	2
F9	40	3	F9	40	3

Data analysis

Various computations for the current optimization study were carried out employing Design Expert Software (version 9.0.4.1; Stat-Ease Inc. And Minneapolis, MN) . Statistical second order model including interaction and polynomial terms were generated for all the response variables using multiple linear regression analysis. The general for of the model is represented as in equation.

$$Y = C_0 + C_1A + C_2B + C_3AB + C_4A^2 + C_5B^2$$

Statistical validity of polynomials was established on the basis of Analysis of variance (ANOVA) provision in the Design Expert® software. Level of significance was considered at $p< 0.05$. The 3-D response surface graphs and the 2-D contour plots were generated by the Design Expert® software. These plots are very useful to see the interaction effects of the factors on responses. Subsequently, the desirability approach was used to generate the optimum setting for the formulations.

Linear model: $Y = C_0 + C_1A + C_2B$

Quadratic model: $Y = C_0 + C_1A + C_2B + C_3A^2 + C_4B^2 + C_5A$

Evaluation of microsphere

Particle size

The microspheres were analyzed for their size and polydispersity index on Nano Particle SZ-100 series, Horiba

based on Dynamic Light Scattering (DLS) Technique, at a scattering angle of 90° and temperature of 25°.

Surface Charge (Zeta-Potential)

The surface charge of the microspheres was determined with Nano Particle SZ-100 series, Horiba based on Laser Doppler electrophoresis technique. The measurements were carried out in an aqueous solution of KCl 0.1N.

Particle Morphology by SEM

SEM studies were performed using FEI Quanta 200, Netherlands.

Measurement of Loading Efficiency of BDP in PLGA Microspheres

After preparation of emulsion, centrifugation was done for separating the microspheres. The microsphere was used to study the drug content. The concentration of Beclomethasone dipropionate content in supernatant was determined by $C_{18,}$ 5µm, 150 × 4.6 mm (Shodex) HPLC column was with mobile phase of Methanol: Water (90:10).

Evaluation of flow properties of powdered microsphere

Angle of repose

The blend was poured through a funnel and cone height (h) was measured. The radius of the heap (r) was measured and angle of repose was calculated.

$$\mathrm{Tan}\theta = \frac{h}{r}(\theta\text{- Angle of repose})$$

Where h and r are the height and radius of the powder heap respectively.

Bulk density and tapped density

The bulk density is the ratio of mass of an untapped sample and its volume including the contribution of the interparticulate void volume. Tap density is measured by tapping the Microsphere sample in a 10mL measuring cylinder till a constant level is reached.

$$\text{Bulk Density} = \frac{\text{Weight}}{\text{Bulk Volume}}$$

$$\text{Tapped Density} = \frac{\text{Weight}}{\text{Tapped Volume}}$$

Hausner's Ratio

Hausner's Ratio is an ease of index of powder flow. It is also known as the Packing factor. It is calculated by using the following formula-

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Compressibility /Carr's index

Based on the Bulk density and the Tap density, the percentage compressibility of the sample was calculated by using the following formula:

$$\text{Carr's Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100$$

Characterization of microspheres by DSC

The physical state of BDP in the microspheres was analyzed by Differential Scanning Calorimetry (DSC). The thermograms of BDP, BDP-PLGA microspheres were obtained at a scanning rate of 10^0C/minute conducted over a temperature range of 25-300°C, respectively.

Evaluation of capsules of BDP-PLGA microsphere

Final dried microspheres were filled in the capsule size "2". Capsules filled with microspheres were evaluated for following parameters-

Disintegration test of capsule

This study was carried out in disintegration apparatus i.e basket-rack assembly. 900 ml of phosphate buffer pH 7.2 was taken at temperature 37°C ± 0.5°C. Time was noted down when whole capsule get disintegrate.

Uniformity of weight

Final formulation was filled manually in hard gelatin capsules. An intact capsule was weighed. It was opened without losing any part of the shell and removed the contents as completely as possible. Then shell was weighed. The difference between the weighing gives the weight of the contents. Procedure was repeated for another 19 capsules. None of the value should deviate from 10% of average weight.

Uniformity of content

5 capsules were taken and content of drug in each unit dosage form was determined using HPLC method. %RSD was calculated.

In-vitro dissolution study [17]

Study was carried out in the USP dissolution test apparatus (Electro lab TDT - 08 L Dissolution testers USP) type 2 (paddle). 900 mL of the dissolution medium phosphate buffer pH 7.2 and 0.1 N HCl was taken in vessel and the temperature was maintained at 37 ± 0.5^0C. The speed of the paddle was set at 75± 2 rpm. Dissolution samples were

withdrawn at different time interval. The supernatant was then analyzed for drug content by using HPLC.

Ex-vivo permeability studies by non-everted sac technique [18]

The perfusion solution was prepared by dissolving 7.8 g sodium chloride, 0.35 g potassium chloride, 1.37 g sodium bicarbonate, 0.02 g magnesium chloride, 0.22 g sodium di-hydrogen phosphate and 1.48 g glucose in 100 mL of distilled water.

The BDP microsphere suspension was prepared by dissolving 60 mg of BDP microsphere in 10 mL of Kreb's Ringer's buffer solution. Mix it using cyclomixer to get uniform distribution of microsphere.

Ex-vivo permeability study of microsphere of BDP was carried out by using non-everted chicken intestinal sacs. Chicken was killed and the duodenal part of small intestine was isolated and washed with distilled water to remove the mucous and lumen content and then placed in cold KRPB (Krebs-Ringer's-Phosphate-buffer, pH 7.2) solution, continuously aerated with the help of electronic aerator. 5-6 cm long sacs were prepared by typing up the two ends of the sac either with cotton or silk thread. 2 mL of microsphere suspension was taken inside the sac. The sacs were then taken into different beakers containing 100 mL of KRPB solution, continuously bubbled with atmospheric air, maintained at 37±0.5^{o}C and stirred at 100 rpm. Aliquots were withdrawn at predetermined interval with a calibrated plastic disposable syringe. Each time an aliquot was withdrawn it was replaced by same quantity of fresh replenished media. The permeability study was carried out for about 4 hrs. The amount of BDP microsphere permeated across the intestinal sac was determined by measuring the area at 239 nm by HPLC.

RESULTS ANDDISCUSSIONS

Selection of Organic phase

It was observed in Table No. 4, that using Acetone the entrapment efficiency of the formulation increases. The volatility of Acetone was greater than DCM, Hence the time taken for Acetone to evaporate from the formulation was less. Thus the time of preparation of the formulation reduces. Hence Acetone was chosen as the Organic Phase.

Selection of emulsifying agent

The effects of the different surfactants were seen on the Entrapment efficiency. Batches for both PVA were selected for the preparation of optimized microspheres batch as they showed higher entraptment efficiency as compared to Tween which was shown in Table No.5.

Selection of other factors

A batch of 30 mg PLGA 50:50 and PLGA 75:25 were made varying the factors to optimize and select the level that gave higher entrapment efficiency. Criteria for selection factors given in Table No.6.

Table 4: Composition of results of batches for selection of organic phase

Ingredients	PLGA 50:50 Microspheres		PLGA 75:25 Microspheres	
	B1	B2	B3	B4
Drug Concentration (mg)	10	10	10	10
Polymer concentration (mg)	30	30	30	30
PVA concentration (%)	2	2	2	2
Volume of aqueous phase (mL)	5	5	5	5
Organic Phase and volume (mL)	1 ml DCM	1ml Acetone	1 ml DCM	1 ml Acetone
Entraptment efficiency (%)	**81.19**	**88.37**	**76.2**	**86.22**

Table 5: Composition and result of batches for selection of emulsifying agent

Ingredients	PLGA 50:50 Microspheres		PLGA 75:25 Microspheres	
	B5	B6	B7	B8
Drug Concentration (mg)	10	10	10	10
Polymer concentration (mg)	30	30	30	30
Organic phase volume (mL)	1 ml Acetone	1 ml Acetone	1 ml Acetone	1 ml Acetone
Emulsifying agent and concentration (%)	PVA (1%)	TWEEN 20 (0.1 %)	PVA (1%)	TWEEN 20 (0.1%)
Volume of aqueous phase(mL)	5	5	5	5
Entraptment efficiency (%)	**91.00**	**81.90**	**88.37**	**83.65**

Table 6: Criteria for selection of other factors

Factors	Options	Result	Conclusion
Concentration of polymer PLGA	Wide range of concentrations ranging from 10 to 60 mg were tried.	The concentration of polymer was seen to have a major effect on Entrapment efficiency of the microspheres.	Its concentration was optimized using factorial design.
Concentration of emulsifying agent PVA	0.5 to 4 % Solution of PVA was used for making microspheres	Concentration of PVA also majorly affected Entrapment efficiency of microspheres.	Its concentration was optimized using factorial design.
Ratio of Volume of organic Phase and aqueous phase	5. 1:4 6. 1:5 7. 1:6 8. 1:7	Highest entrapment efficiency was seen in the ratio 1:5	Ratio of 1:5 was selected
Selection of equipment for mixing	3. Magnetic stirrer 4. High speed Homogenizer	Highest entrapment efficiency was seen in the batches made with High speed Homogenizer	High speed homogenizer was selected
Centrifugation type	3. Centrifuge 4. Cooling centrifuge	Cooling centrifuge showed complete separation of microspheres from the supernatant	Cooling centrifuge was selected
Evaporation time	3. 1-3 hr under stirring 4. Keeping overnight evaporation.	Higher entrapment was seen in the batches that were kept overnight for evaporation of organic solvent	Overnight evaporation was selected
Washing liquid	3. Distilled water 4. Phosphate buffer pH 7.4	Higher entrapment efficiency was seen in batches which were washed with distilled water for second and third centrifugation cycle	Distilled water was selected as a washing liquid

Selection of concentration range for polymer

Polymer Concentration range for PLGA 50:50 microspheres and PLGA 75:25 microspheres was 20 mg to 40 mg as below 20 mg entrapment efficiency was very low and at 40 mg the entrapment efficiency was maximum and remained constant after increasing the polymer concentration. Entraptment efficiency was showed in Table No.7.

Table 7: Composition of batches for selection of PLGA concentration

Batch No.	PLGA concentration (mg)	PVA concentration (% w/v)	Entrapment Efficiency (%)	
			PLGA 50:50 Microspheres	PLGA 75:25 Microspheres
1	10	2	47.61	42.8
2	20	2	79.94	67.29
3	30	2	88.37	86.22
4	40	2	98.93	99.12
5	50	2	99.17	99.78
6	60	2	98.29	99.19

Selection of concentration range for emulsifying agent

Concentration range of emulsifying agent PVA was selected from 1% w/v to 3% w/v, as per Table No.8, this range showed maximum entrapment efficiency. The entrapment efficiency below and above these limits was found to be less. The entrapment efficiency decreased above 3% w/v PVA concentration.

Table 8: Composition of batches for selection of range of PVA

Batch No.	PLGA concentration (mg)	PVA concentration (%)	Entrapment Efficiency (%)	
			PLGA 50:50 Microspheres	PLGA 75:25 Microspheres
1	30	0.5	83.53	69.13
2	30	1.0	91.17	88.37
3	30	1.5	90.14	89.37
4	30	2.0	80.42	91.78
5	30	2.5	92.79	94.37
6	30	3.0	87.98	98.89
7	30	3.5	76.8	81.28
8	30	4.0	62.89	78.29

Optimization using 3^2 factorial design

Optimization of BDP-PLGA 50:50 microspheres

Factorial design trials were mentioned in Table No.9. The ANOVA of entrapment efficiency indicates PLGA concentration is the significant terms as the F values are above the critical F values and thus make the P values less than 0.05 (threshold level). The Model F-value of 6.86 implies the model is significant. There is only a 2.82% chance that a "Model F-Value" this large could occur due to noise. Thus, the factor significantly affects the entrapment efficiency of Beclomethasone dipropionate. F5 formulation was selected as optimized formulation. Figure 1 and 2 showed counter plot and 3D surface plot for percent entraptment efficiency.

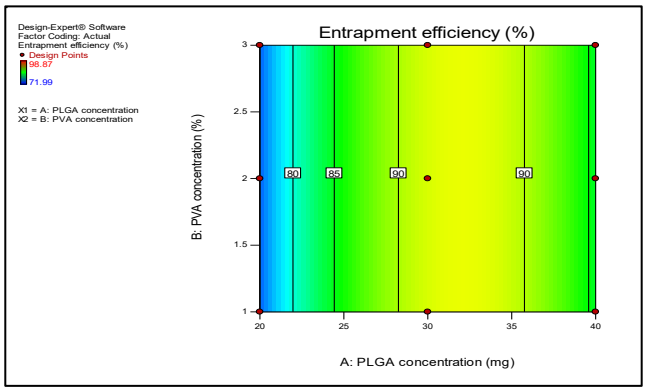

Figure 1: Contour Plot of percentage entrapment efficiency (BDP-PLGA 50:50)

In the present study when the concentration of was increased from 20 mg to 40 mg. There was increase in the entrapment from 20 mg to 30 mg and decrease when it was further increased to 40 mg. It was observed that maximum entrapment was observed when PLGA concentration was 30 mg.

Table 9: Formulation table for factorial design (BDP-PLGA 50:50 microsphere)

Formulation code	PLGA concentration (mg)	PVA concentration (% w/v)	Entraptment efficiency
F1	20	1	71.99
F2	20	2	72.12
F3	20	3	80.87
F4	30	1	84.45
F5	**30**	**2**	**98.87**
F6	30	3	90.16
F7	40	1	85.05
F8	40	2	86.55
F9	40	3	81.16

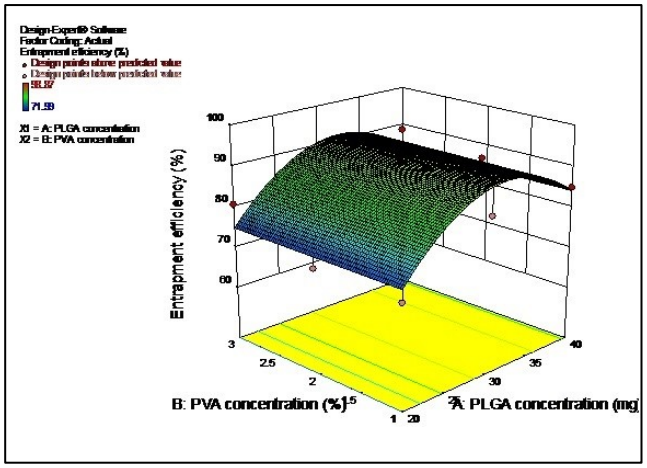

Figure 2: 3D surface graph of percentage entrapment efficiency (BDP-PLGA 50:50)

Optimization of BDP-PLGA 75:25 microspheres

Factorial design trials were mentioned in Table No.10. The ANNOVA of entrapment efficiency indicates both PLGA and PVA concentration are the significant terms as the F values are above the critical F values and thus make the P values less than 0.05 (threshold level). The Model F-value of 7.71 implies the model is significant. There is only a 2.74% chance that a "Model F-Value" this large could occur due to noise. Thus, both the factors significantly affect the entrapment efficiency of Beclomethasone dipropionate.

In the present study when the concentration of was increased from 20 mg to 40 mg. It was observed that maximum entrapment was observed when PLGA concentration was 20 mg. But with the initial trials it was also seen that above 40 mg there was decrease in the entrapment efficiency. Figure 3 and 4 showed counter plot and 3D surface plot for percent entraptment efficiency.

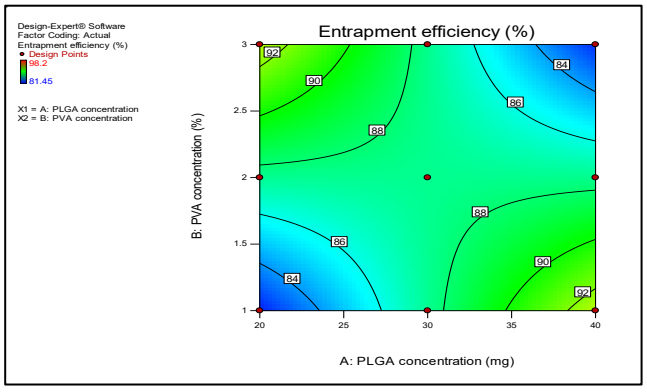

Figure 3: Contour Plot of percentage entrapment efficiency (BDP-PLGA 75:25)

Final optimized formulations

Final manufacturing formula was given in Table No. 11

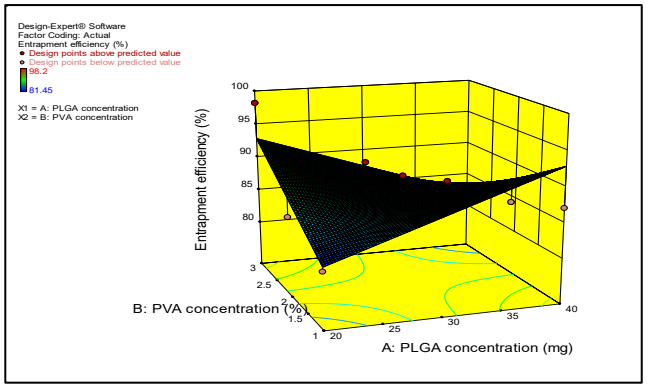

Figure 4: 3D surface graph of percentage entrapment efficiency (BDP-PLGA 75:25)

Table 10: Formulation table for factorial design (BDP-PLGA 75:25 microsphere)

Formulation code	PLGA concentration (mg)	PVA concentration (% w/v)	Entraptment efficiency
F1	20	1	81.45
F2	20	2	84.97
F3	**20**	**3**	**98.20**
F4	30	1	92.10
F5	30	2	89.40
F6	30	3	88.10
F7	40	1	87.10
F8	40	2	84.15
F9	40	3	82.20

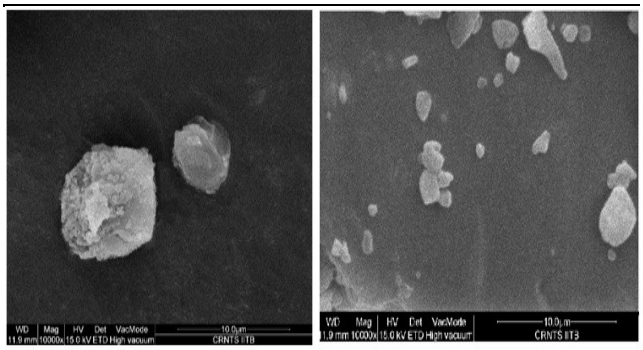

Figure 5 &6 : SEM Pictures of BDP-PLGA 50:50 microspheres & BDP-PLGA 75:25 microspheres at 10000× magnification

Table 11: Final manufacturing formula

Category	Ingredient	Quantity per unit	Ingredient	Quantity per unit
	Microsphere of PLGA 50:50		**Microsphere of PLGA 75:25**	
Drug	BDP	10 mg	BDP	10 mg
Polymer	PLGA 50:50	30 mg	PLGA 75:25	20 mg
Aqueous Phase	Water	5 ml	Water	5 ml
Organic Phase	Acetone	1 ml	Acetone	1 ml
Emulsifying agent	PVA	2%	PVA	3%
Lubricant	Talc	1%	Talc	1%
Lubricant	Magnesium stearate	2%	Magnesium stearate	2%
Diluent	Lactose	Adjust weight upto 60 mg	Lactose	Adjust weight upto 60 mg

Evaluation of microspheres

Particle size, Zeta potential and Polydispersibility index

Beclomethasone dipropionate-loaded PLGA microspheres were obtained in the size range around 4µm and good polydispersity index as shown in Table No.12. The size distribution of PLGA microspheres was evaluated before and lyophilisation. Lyophilized microspheres were mixed with water, vortexed for few minutes and then measured. Slight change in average particle size appeared after lyophilisation and resuspension.

Particle Morphology by SEM

The SEM images as per figure 5 and 6, showed that the microspheres had smooth surface with little surface irregularity. It exhibited a range of size within each batches.

Table No.12: Particle size, polydispersity index and zeta potential of final formulations

Formulation	Particle size (nm)		Polydispersity index		Zeta potential (mV)	
Lyophilisation	**Before**	**After**	**Before**	**After**	**Before**	**After**
BDP-PLGA 50:50 Microspheres	3729.7 nm	6440 nm	1.152	1.122	-19.6 mV	-19.8 mV
BDP-PLGA 75:25 Microspheres	2812.3 nm	4168.1 nm	1.154	1.252	-13.9 mV	-24.8 mV

Measurement of Loading Efficiency of BDP in PLGA Microspheres

Entrapment efficiency was measured before and after freeze-drying in order to evaluate the effect of this process on the drug retention. As can be seen below Table No.13, freeze-drying did not cause any leakage of the drug encapsulated in microspheres.

Table 13: Entrapment efficiency of optimized batches (before and after lyophilisation)

Batches	Entrapment efficiency before lyophilisation	Entrapment efficiency after lyophilisation (%)
BDP-PLGA 50:50 microspheres	98.87	97.72
BDP-PLGA 75:25 microspheres	98.20	98.82

Evaluation of flow properties of powdered microsphere

From Table No.14, It can be concluded that both microspheres were free flowing.

Table No.14: Powder characteristics of Microspheres

Microsphere Parameter	BDP-PLGA 50:50 Observation	Inference	BDP-PLGA 75:25 Observation	Inference
Angle of Repose	22°	Good flow	31^0	Good flow
Bulk Density	0.31	Good	0.39	Good
Tap density	0.36	Good	0.421	Good
Hausner's Ratio	1.16	Fair	1.079	Excellent
Compressibility Index	13.88%	Good	7.38%	Excellent

Table 15: Uniformity of weight of microspheres

BDP-PLGA 50:50 microsphere		BDP-PLGA 75:25 microsphere	
No. of capsule	Weight (mg)	No. of capsule	Weight (mg)
1	59.8	1	60.0
2	58.4	2	56.9
3	59.0	3	60.0
4	58.2	4	57.8
5	60.0	5	58.7
6	60.2	6	56.5
7	57.9	7	55.2
8	57.8	8	58.2
9	58.2	9	60.0
10	56.4	10	59.1

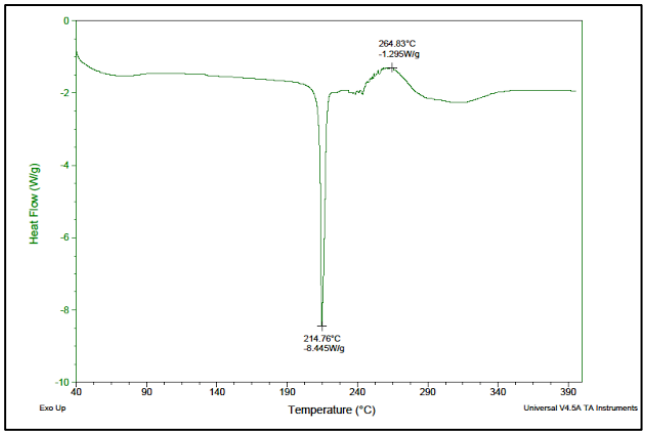

Figure 7: DSC thermogram of Beclomethasone dipropionate

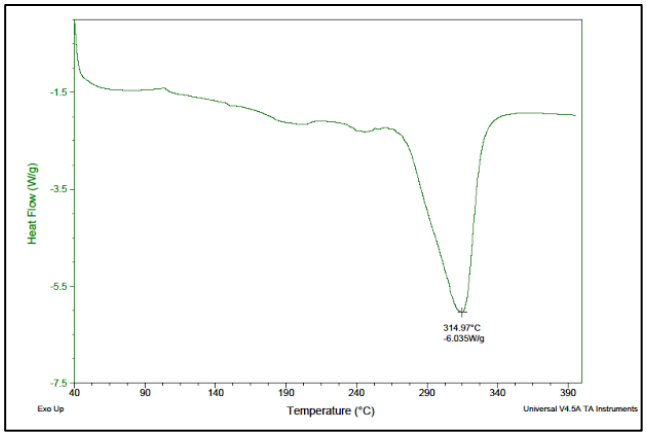

Figure 8: DSC thermogram of BDP-PLGA 50:50 microspheres

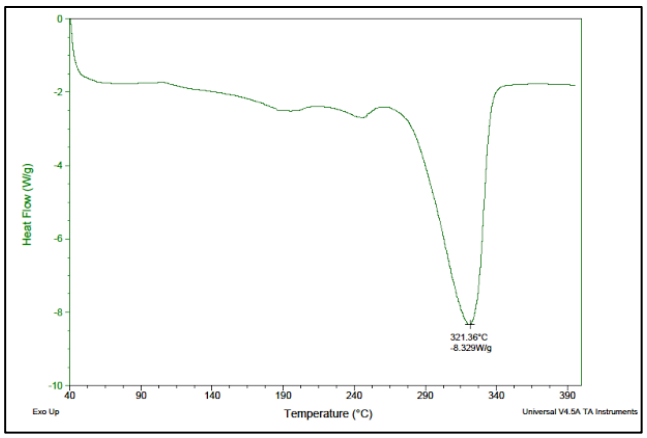

Figure 9: DSC thermogram of BDP-PLGA 75:25 microspheres

Characterization of microspheres by DSC

The DSC thermogram of pure drug Beclomethasone dipropionate showed one peak at 214.46°C as shown in Figure 7. This peak was not observed in the thermograms of BDP-PLGA 50:50 microspheres and BDP-PLGA 75:25 microspheres as shown in Figure 8 and 9. This indicates that the drug is completely entrapped within the PLGA 50:50 and PLGA 75:25 microspheres.

Evaluation of capsules of BDP-PLGA microsphere

Disintegration test of capsule

Disintegration of both capsules was found to be within 15 minutes.

Uniformity of weight

Average weight of BDP-PLGA 50:50 and BDP-PLGA 75:25 microspheres (final formulation) was found to be 58.59 mg and 58.24 mg respectively as given in Table No.15. Not a single value was found to be deviate from 10% of average value. Hence all capsules was complies within the limit and it passes uniformity of weight test.

Uniformity of content

As shown in Table No. 16, All the values were found to be in limits. Hence all capsules pass the content uniformity test. %RSD for BDP-PLGA 50:50 and BDP-PLGA 75-25 microsphere was found to be 2.368% and 2.912%.

Table No. 16: Content uniformity

BDP-PLGA 50:50 microsphere		**BDP-PLGA 75:25 microsphere**	
No. of capsule	Drug content (%)	No. of capsule	Drug content (%)
1	96.23	1	99.12
2	98.55	2	98.54
3	102.5	3	96.30
4	98.66	4	104.2
5	97.56	5	100.16

In-vitro dissolution study

Release studies were carried out by using release medium, phosphate buffer at pH 7.2 and 0.1 N HCl for lyophilized batch for both BDP-PLGA 50:50 microsphere and BDP-PLGA 75:25 microspheres is shown figure 10 and 11.

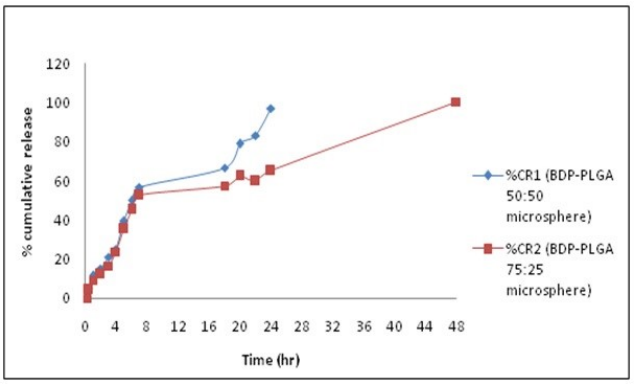

Figure 10: % Cumulative release of lyophilized microsphere BDP-PLGA 50:50 (%CR 1) and BDP-PLGA 75:25 (%CR 2) in buffer pH 7.2

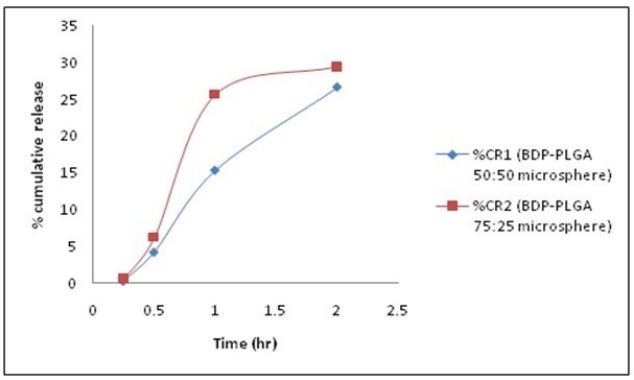

Figure 11: % Cumulative release of lyophilized microsphere BDP-PLGA 50:50 (%CR 1), BDP-PLGA 75:25 (%CR 2) in 0.1 N HCl

From release profile from Figure 10, BDP-PLGA 75:25 microsphere gives more sustained action as compare to BDP-PLGA 50:50 microspheres. It gives release of drug upto 48 hrs. BDP-PLGA 50:50 microsphere gives release of drug upto 24 hrs in buffer pH 7.2.

As seen from release profile shown in Figure 11, BDP-PLGA 50:50 gives 26.57% drug release in 2 hr and BDP-PLGA 75:25 microsphere gives 29.38% drug release in 2 hr in 0.1 N HCl.

Ex-vivo permeability studies by non-everted sac technique

From above release profile of Figure 12, BDP-PLGA 50:50 and BDP-PLGA 75:25 microsphere releases drug upto 23.61% and 39.09% respectively. It also shows that drug can easily permeate through the intestinal tissue and available for absorption at targeted site.

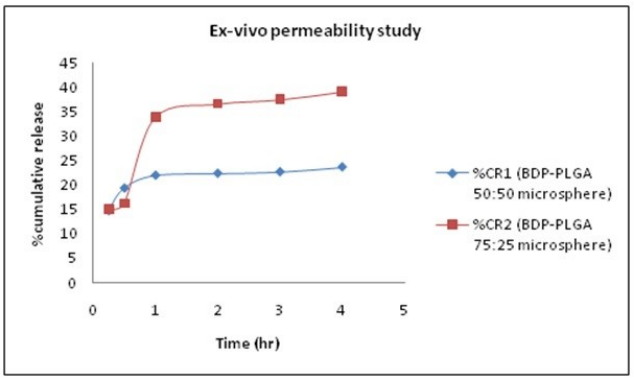

Figure 12: %Cumulative release of BDP-PLGA 50:50 (%CR 1) and BDP-PLGA 75:25 (%CR 2) microsphere in intestinal sac (buffer pH 7.2)

CONCLUSION

The present study has clearly indicated the potential advantages of microspheres for formulating BDP with permeability, stability and oral bioavailability. In the

formulated microspheres, the drug was entrapped in polymer and having globule size in micrometric range and a stable zeta potential value, thus presenting the drug in a form ready for its efficient absorption leading to significant improvement in permiability and rate and extent of dissolution in media.

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REFERENCES

1. Rathbone M.J, Hadgraft, Roberts M.S. Modified-Release Drug Delivery Technology, *Marcel- Decker, New York.* 2006; 123: 203-216.
2. Brahmankar DM, Jaiswal SB. Biopharmaceutics and pharmacokinetics: A treatise. Vallabh prakashan; 2005. 1st ed., *VallabhPrakashan*.1995; 16 (140): 335-37.
3. Lee V.H. Controlled Drug Delivery Fundamentals and Applications: Introduction, Marcel Dekker, (2nded) INC, New York. 1987:29.
4. Banker GS and Anderson NR. The Theory and Practice of Industrial Pharmacy: Tablet, Lachman, (3rded) *Varghese Publishing House, Bombay.* 1990; 293-303.
5. Dash S.K.; Khan A.S. Formulation and In-Vitro evaluation of sustained release Glibenclamide microsphere. *Int.J.of Pceutical Sci and Res.*2012; 3(5):1433-1443.
6. Keykyoon Kim and Daniel W Pack. Microsphere for drug delivery,University of Illinois at Urbana-Champaign. 20-33.
7. Garg A. and Upadhya P. Mucoadhesive microsphere: A short review. *Asian J. of Pceutical and clinical Res*, 2012; 5(3): 24-27.
8. AlagusundaramM.; Chetty C.; Umashankari K. Microsphere as a novel drug delivery system- A review. *Int. J. of ChemTech research*, 2009; 1(3): 526-534.
9. Vyas S.P. and Khar R.K., Targeted & Controlled drug delivery. 2007; 435.
10. Nixon J R.; Khalil S. and Carless J E. Gelatin coacervate microcapsules containing sulphamerazine: their preparation and the in vitro release of drug. *J. Pharm. Pharmacol*., 1968;20: 528–538.
11. Beclomethasone dipropionate http://www.drugs.com/ppa/beclomethasone-dipropionate.html.
12. Beclomethasone dipropionate http://en.wikipedia.org/wiki/Beclometasone_dipropionate.
13. Beclomethasone dipropionate rxlist http://www.rxlist.com/qvar-drug/patient-images-side-effects.htm.
14. Arshady, R. Preparation of biodegradable microspheres and microcapsules: 2. Polyactides and related polyesters*. J. Control. Release.*1991;17:1–21.
15. John E Freund.; Benjamin M.Peries. 8th Edition, “Statistics A First Course” Upper Saddle River, Nj07488; Pg. No. 406; Regression and Correlation.
16. Simo Bohanec.;Tanja Rozman. “Using Different Experimental Designs In Drug Excipient Compatibility Studies During The Preformulation Development Of A Stable Solid Dosage Formulation’; Acta Chim. Solv.2010; 57:895-903.
17. Modi F.; Patel P.; Priyal R. Fromulation, Optimization And Evaluation Of Fixed Dose Combination Of Moisture Barrier Film Coated Bilayer Tablet Of ArtesunateAnd Amodiaquine Hydrochloride. *Int. J. ournal Of Pharmtech Research*.2011; 4: 3.
18. Khutle N.; Chourasia K.; Kunwarpuriya A. Formulation and evaluation of self microemulsifying drug delivery system of Pravastatin sodium. *Int J of innovative pharm sci and res*.2015; 3(5): 527-5