FORMULATION DEVELOPMENT AND EVALUATION OF COLON TARGETING NANOSPONGES OF DEFLAZACORT USING BOX BEHNKEN DESIGN

ABSTRACT

Deflazacort is a glucocorticoid used as an anti-inflammatory, immunosuppressant and commonly prescribed for the patient in disease condition, Inflammatory Bowel Disease such as ulcerative colitis (UC) and Crohn's disease (CD). IBD cannot be controlled effortlessly and the reappearance is the most challenging issue for the physicians. There are various controlled and colon targeted drug delivery systems available for the treatment with limited success rate. The objective behind the present study was to develop nanosponges loaded with deflazacort by using quasi-emulsion solvent diffusion method using Eudragit S-100 and investigating the effect of process variables on the response using Box-Behnken design. Effect of three independent factors that is, Eudragit S100, PMMA and PVA was studied on two dependent responses, that is, particle size and % drug entrapment. Seventeen nanosponge formulations were prepared by quasi-emulsion solvent diffusion method using Eudragit S-100 (0.2% to 0.5%w/v), PMMA (0.2% to 0.5%w/v), and PVA (0.5%-1.5%w/v) applying Box-behnken design. The nanosponge formulations were characterized for particle size, % drug entrapment, shape and surface morphology, determination of drug content and *in vitro* drug release behaviour. The developed nanosponge showed nearly spherical in shape and spongy in nature with particle size 170.45 nm and % drug entrapment of 73.42%. *In vitro* drug release of optimized formulations was found to show the maximum drug release of 90.3±3.3% in colonic fluid with 4%w/v caecal content over a period of 24 hr. The values of various evaluation parameters observed were found to be in close concurrence with the values predicted employing the Design expert software. The nanosponge formulation obtained using Eudragit S-100 in low concentration, optimum concentration ratio of eudragit: PVA along with low stirring speed showed desired features. The mathematical models were further designed to develop nanosponge with required characteristics.

Keywords: Box-behnken design, Deflazacort, Nanosponges, Eudragit S-100, Quasi-emulsion solvent diffusion method.

INTRODUCTION

Colon delivery of a therapeutic drug may reduce the systemic side effects and provide effective and safe therapy that may reduce the dose and duration of therapy when compared with the conventional treatment. However, various strategies have been used for targeting colon, such as pH-sensitive polymers, coating with biodegradable polymers, fabrication of pro-drugs, timed release systems, embedding in biodegradable matrices and hydrogels [1, 2]. There is a growing interest in multiparticulate modified release drug delivery systems especially for site specific targeting within the gastrointestinal tract. *Asghar and Chandran* [3] provided a multiparticular formulation for colon delivery of drugs with more uniform *in vivo* dissolution performance compared to single unit dosage forms. It resulted in more uniform inter-individual bioavailability and clinical effects. However, these systems are comparatively complex and their large-scale manufacturing requires many skills and technological development. Among the different types of multiple-unit dosage forms, nanosponges appear as one of the most attractive dosage forms from the economic, process development and scale-up points of view. Nanosponges (NS) are a novel formulation, a sponge-like structure used to encapsulate nanoparticles with a non-collapsible and porous structure. It is primarily used for pharmaceutical and cosmeceutical approaches, as it blends the advantages of microsponges and nanosized vesicular structure. The porous structure not only enables us to entrap a wide range of active ingredients but also modulates the release pattern. NS, if incorporated in hydrogel offers remarkable perks, the most important being improved skin retention [4, 5]. NS offers remarkable advantages including higher entrapment efficiency, improving the drug profile, economical method of preparation, and ease of drug release owing to three-dimensional porous structures. Different preparation methods are used: solvent method, ultra-assisted synthesis, emulsion solvent diffusion method, and melting method to formulate stable NS in different categories. Software-based optimization techniques are employed to derive optimized product of superior attributes and quality. Moreover, 3D printing techniques are now being considered to ease the production of NS. Different routes and modes of drug administration e.g., aerosols, capsules, parenteral, tablets, topicals are now being exhausted for NS delivery [6]. Deflazacort (1-(1, 16)-21-(acetyloxy)-11-hydroxyl-2-methyl-5H-pregna-1,4-dieno[17,16-d] oxazole-3, 20-dione) is a synthetic glucocorticoid and an oxazoline derivative of prednisolone. It has potent anti-inflammatory activity and immunosuppressive action [7, 8], which is quite similar to prednisolone. Deflazacort is a prodrug and is used in Duchenne muscular dystrophy (DMD), Polymyalgia rheumatic, Drug-resistant epilepsy of childhood, Idiopathic nephrotic syndrome (INS), renal

transplant and Asthma [9]. Box-Bhenken factorial design is an optimization technique that is used to develop designs of acceptable formulations in a manner that save time, effort and chemicals. Factorial design is a disciplined technique of studying the virtual significance of variables and their combined effect on different responses. Moreover, the response surface characterization is an effective method for attaining a proper model with no need for long time of trial. In this present study, Deflazacort nanosponges formula optimized by factorial design software. The optimized formula is subjected to scale up process. The bioavailability and therapeutic efficacy could be improved by sustained release formulations. In these research deflazacort nanosponges preparation was applied Box-Behnken design model to obtain the optimal formula.

EXPERIMENTAL

Materials

The materials required for the present work were procured from diverse sources. Deflazacort was obtained as a gift sample from Torrent Pharmaceuticals (India). and Eudragit S‑100 was provided as gift sample by Evonik Pharma, Mumbai, India. Polyvinyl alcohol (PVA) and PMMA were procured from Central Drug House Pvt. Ltd., Mumbai, India and Qualigens Fine Chemicals, Mumbai, India, respectively. All the other ingredients used were of analytical grade and were used as procured. Demineralised and double distilled water was prepared freshly and used whenever required. All other reagents and chemicals used were of analytical grade.

Preparation of nanosponges

Formulation Design

Regular 3 level factorial designs for 2 factors was employed for screening of significant formulation and process variables involved in the development of nanosponges. Table showed high and low levels of various variables screened for their influence in the development of nanosponges of deflazacort. Optimization of all process and formulation variables was carried out by 3^2 levels factorial design using Design of expert 12 software (DOE 12 trial version) in the nanosponge's formulations. For the optimization, 17 run was designed by Quadratic randomized, Box-Benkon response surface method. The prepared formulations were characterized for particle Size and Entrapment efficiency Table 1.

Table 1: List of variables employed in 3^2 factorial designs

Factor	Name	Units	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Eudragit S100	%w/v	0.2000	0.5000	-1 ↔ 0.20	+1 ↔ 0.50	0.3500	0.1061
B	PMMA	%w/v	0.2000	0.5000	-1 ↔ 0.20	+1 ↔ 0.50	0.3500	0.1061
C	PVA	%w/v	0.5000	1.50	-1 ↔ 0.50	+1 ↔ 1.50	1.0000	0.3536

Method of preparation

The Nanosponges containing deflazacort were fabricated by quasi-emulsion solvent diffusion method using an inner phase comprising Eudragit S-100 (0.2% to 0.5%w/v) and PMMA (0.2% to 0.5%w/v) dissolved in 5 ml of ethanol:dichloromethane (1:1). Further deflazacort was put in and dissolved through ultrasonication at 35°C. This mixture was then poured into an aqueous solution of PVA (outer phase) with stirring rate 500 rpm for 60 min. Next on, Nanosponges were formed due to the removal of dichloromethane and ethanol from the system by evaporation. Prepared Nanosponges were then filtered, washed with distilled water and subjected to drying at 40°C for 12 h in hot air oven. Finally, microsponges were weighed to determine production yield. Various formulation batches are prepared as per Table 2.

Table 2: Formulation Design

Std	Run	Eudragit S-100 (%w/v)	PMMA (%w/v)	PVA (%w/v)
9	1	0.35	0.2	0.5
8	2	0.5	0.35	1.5
6	3	0.5	0.35	0.5
16	4	0.35	0.35	1
7	5	0.2	0.35	1.5
13	6	0.35	0.35	1
2	7	0.5	0.2	1
12	8	0.35	0.5	1.5
3	9	0.2	0.5	1
14	10	0.35	0.35	1

15	11	0.35	0.35	1
1	12	0.2	0.2	1
4	13	0.5	0.5	1
17	14	0.35	0.35	1
5	15	0.2	0.35	0.5
11	16	0.35	0.2	1.5
10	17	0.35	0.5	0.5

Characterization of nanosponges

Particle size

Average particles size, of prepared nanosponges was determined using zetasizer (Malvern Zetasizer). The nanosponge's formulation was diluted with deionized water and analysed for average size and PDI.

Entrapment efficiency

20 mg of deflazacort loaded nanosponges was diluted up to 10 ml with 7.4 pH buffer and kept for overnight. The shocked solution was centrifuged at 5000 rpm for 10 minutes. Supernatant was than filtered by 0.2µ membrane filter and analyzed by UVVIS spectroscopy at 242nm^2.

Shape and Surface Morphology, Determination of Drug Content and *In Vitro* Drug Release

The shape and surface morphology of the nanosponges were investigated using scanning electron microscopy (IISER, Bhopal). The nanosponges were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the Scanning Electron Microscope at 10 kV.

Determination of Drug Content

The amount of drug entrapped in the nanosponges was determined using a UV spectrophotometer. The weighed amount of the nanosponges was incubated with PBS, pH 7.4, for 48 h. It was centrifuged at 10,000 g for 30 min and the supernatant was diluted 10 times before analysis into the UV spectrophotometer system at λmax 242 nm.

***In Vitro* Drug Release from Nanosponges**

The drug release study of nanosponges was carried out in sealed glass vials at 37±0.1^{o}C. The weighed amount of nanosponges (10mg) were filled in gelatin capsules and taken in a beaker containing 100 mL of dissolution media (PBS of pH 7.0 containing 1%, 2%, and 3% rats

caecal contents). Simultaneously, similar experiment was performed containing simulated colonic fluid without enzyme induction. The samples (1 mL each) were withdrawn periodically for 24 hr and the withdrawn volume was replaced immediately with fresh and respective simulated colonic media containing rabbit caecal content. Later, the samples were centrifuged at 2000 rpm for 10 min and supernatant was filtered through Whatman filter paper. The filtrate was analysed using UV spectrophotometer.

RESULTS AND DISCUSSIONS

The calibration curve of deflazacort was found to be linear in the concentration range of 10-30 µg/ml at 242 nm. Total17 confirmatory runs with 2 centre points were developed by Box-Behnken design for optimization of polymeric NPs keeping 3independent and 2dependent variables. All developed NPs were subjected for characterization, that is, average particle size and percentage drug entrapment. The effect of independent variables on dependent variables was investigated and contour plots were developed (Table 3 Figure 1-8). Results of *In-vitro* drug release from optimized formulation are given in table & figure was found after 24 hrs.

Final Equation in Terms of Actual Factors

Particle Size =+364.46564-55.75667 Eudragit S100-472.02056 PMMA-226.53350 PVA+222.00000 Eudragit S100 * PMMA-143.40000 Eudragit S100 * PVA-13.50000 PMMA * PVA+225.03333+658.25556 $PMMA^2$+136.70300 PVA^2

%EE=-23.90961+243.42167 Eudragit S100 +207.99944 PMMA+33.98533 PVA+80.44444 Eudragit S100 * PMMA-15.96667 Eudragit S100 * PVA-6.70000 PMMA * PVA-357.28889 Eudragit $S100^2$-321.73333 $PMMA^2$-13.40600 PVA.

Table 3: Results of Particle Size and Entrapment Efficiency of formulation F1 to F17

F. Code	Particle Size (nm)	EE (nm)
F1	201.41	60.24
F2	195.64	59.42
F3	235.27	64.32
F4	**170.23**	**73.41**
F5	205.51	62.11
F6	**170.21**	**73.39**
F7	190.33	58.43
F8	235.33	64.39
F9	180.22	54.21

F10	**170.55**	**73.38**
F11	**170.45**	**73.42**
F12	190.32	58.45
F13	200.21	61.43
F14	170.54	73.44
F15	202.12	62.22
F16	205.47	62.23
F17	235.32	64.41

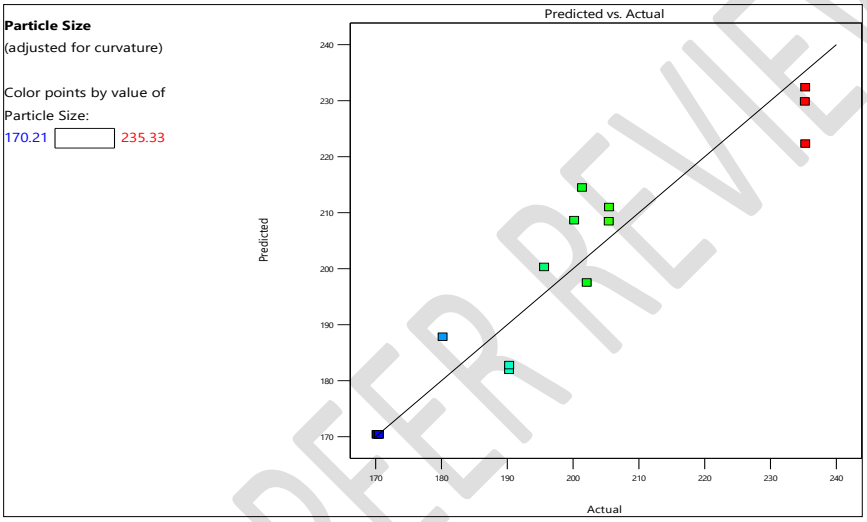

Fig. 1: Graph of Particle Size (Predicted vs Actual)

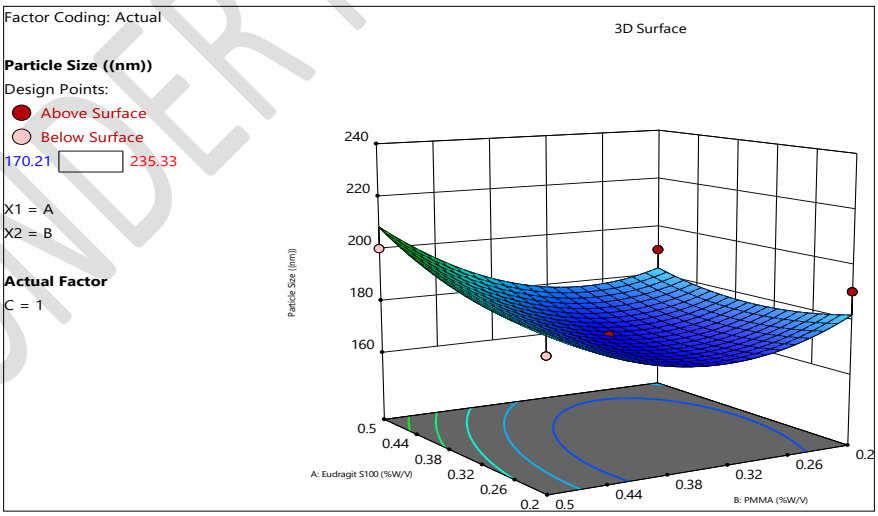

Fig. 2: 3 D Surface Graph of Particle Size (Eudragit S 100 and PMMA)

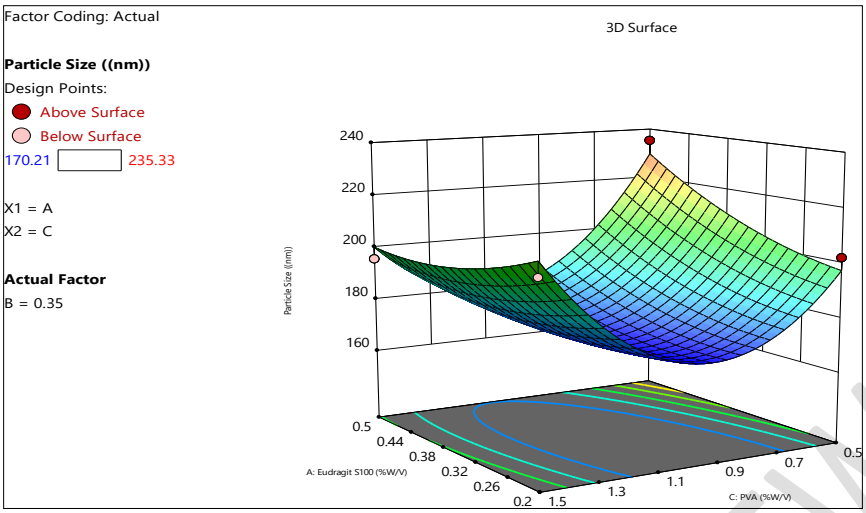

Fig. 3: 3 D Surface Graph of Particle Size (Eudragit S 100 and PVA)

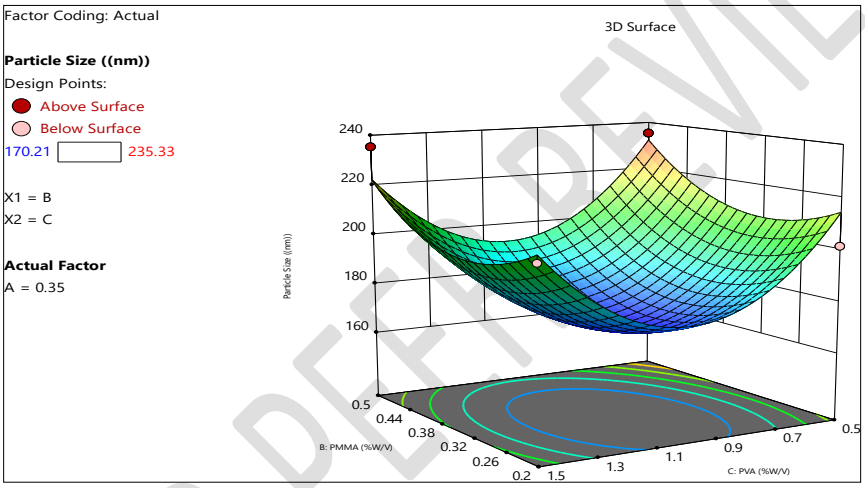

Fig. 4: 3 D Surface Graph of Particle Size (PMMA and PVA)

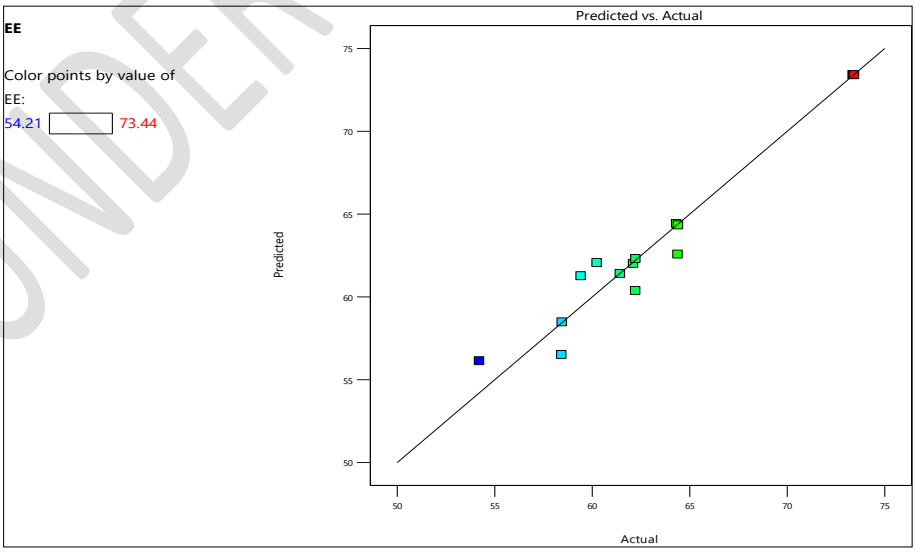

Fig. 5: Graph of entrapment efficiency (Predicted vs Actual)

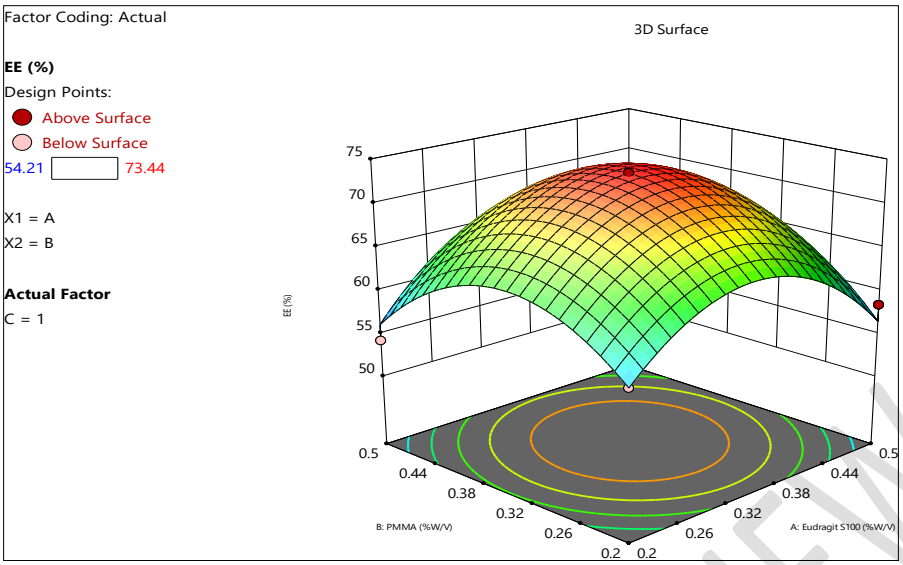

Fig. 6: 3D Surface Graph of entrapment efficiency (Eudragit S 100 and PMMA)

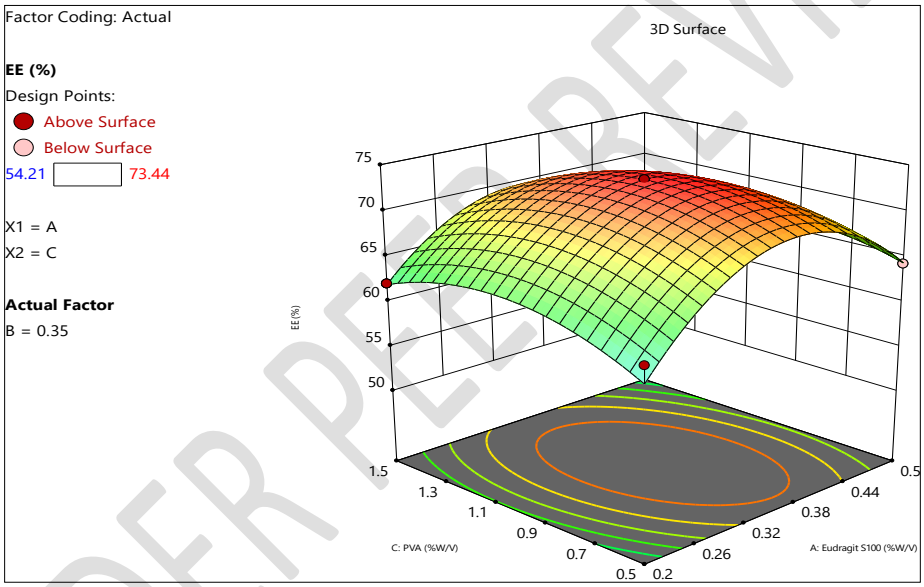

Fig. 7: 3D Surface Graph of entrapment efficiency (Eudragit S 100 and PVA)

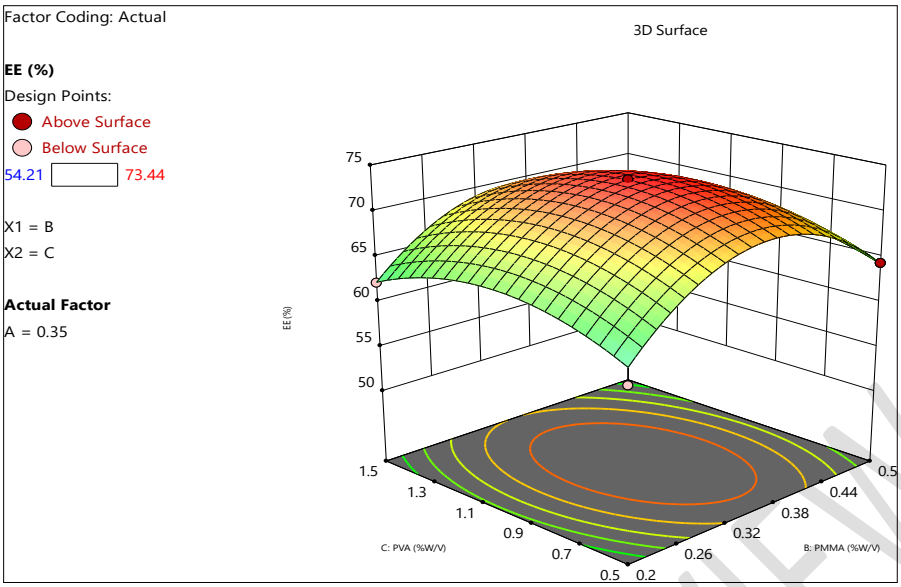

Fig. 8: 3D Surface Graph of entrapment efficiency (PMMA and PVA)

Table 4: ***In vitro*** **drug release studies of optimized formulation F4**

S. No.	Time (h)	Cumulative % drug release				
		Plain drug	Nanosponges in Colonic fluid without enzyme induction	Nanosponges in Colonic fluid with 1%w/v caecal content	Nanosponges in Colonic fluid with 2%w/v caecal content	Nanosponges in Colonic fluid with 4%w/v caecal content
1	0.5	36.65	8.45	11.12	13.32	14.45
2	1	52.23	11.32	14.45	17.78	18.89
3	2	65.58	26.65	25.65	32.25	38.85
4	3	98.85	36.23	38.85	41.15	45.56
5	4		45.65	48.85	53.32	56.65
6	5		52.23	56.65	62.25	67.78
7	6		65.56	69.98	72.32	74.45
8	8		73.32	76.65	81.15	83.32
9	12		78.85	82.23	89.98	92.23
10	24		89.98	92.25	96.65	99.12

CONCLUSION

Optimization of a colon targeted formulation is a complex process that requires a large number of variables and their interactions to be considered. The present study conclusively demonstrates the use fullness of a Box-Behnken design in optimization of colon targeted formulations. The derived polynomial equations and contour plots aid in predicting the values of selected independent variables for preparation of the optimum controlled release colon targeted formulation of Deflazacort with desired properties.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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