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Formulation Development and Evaluation of Colon Targeting Nanosponges of Deflazacort Using Box Behnken Design

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Deflazacort is a glucocorticoid that is often used as an anti-inflammatory and immunosuppressant for patients suffering from Inflammatory Bowel Disease such as ulcerative colitis (UC) and Crohn's disease (CD). IBD is difficult to manage, and the most difficult issue for doctors is the recurrence. There are several regulated and colon focused medication delivery devices available for therapy, however they have a low success rate. The goal of this work was to create nanosponges loaded with deflazacort using the quasi-emulsion solvent diffusion technique using Eudragit S-100 and to investigate the influence of process factors on response using the Box-Behnken design. The effect of three independent parameters, Eudragit S100, PMMA, and PVA, on two dependent responses, particle size and percent drug entrapment, was investigated. Using the Box-behnken design, seventeen nanosponge formulations were created using the quasi-emulsion solvent diffusion technique and Eudragit S-100 (0.2 percent to 0.5 percent w/v), PMMA (0.2 percent to 0.5 percent w/v), and PVA (0.5 percent -1.5 percent w/v). Particle size, percent drug entrapment, shape and surface morphology, drug content determination, and in vitro drug release behaviour were all evaluated in the nanosponge formulations. The generated nanosponge was virtually spherical in form and spongy in character, with particle size 170.45 nm and a drug entrapment percentage of 73.42 percent. Over a 24-hour period, in vitro drug release of optimised formulations was shown to have a maximum drug release of 90.33.3 percent in colonic fluid with 4 percent w/v caecal content. The observed values of several assessment parameters were found to be in close agreement with

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the projected values using the Design expert programme. 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.

1. INTRODUCTION

When compared to traditional treatment, colon administration of a therapeutic agent may lessen systemic adverse effects and provide effective and safe therapy that may lower the dose and duration of therapy. However, several tactics for targeting the colon have been employed, including pH-sensitive polymers, coating with biodegradable polymers, creation of pro-drugs, timed release systems, and embedding in biodegradable matrices and hydrogels [1,2]. Multiparticulate modified release drug delivery methods are gaining popularity, particularly for site-specific targeting inside the gastrointestinal tract. Asghar and Chandran [3] developed a multiparticular formulation for drug administration in the colon that has more consistent in vivo dissolving performance than single unit dose forms. As a result, inter-individual bioavailability and clinical effects were more consistent. However, these systems are rather complicated, and large-scale production necessitates a wide range of talents and technical advancement. Among the several types of multiple-unit dosage forms, nanosponges appear to be one of the most appealing dosage forms in terms of economics, process development, and scale-up. Nanosponges (NS) are a unique formulation consisting of a sponge-like structure used to encapsulate nanoparticles in a non-collapsible and porous structure. Because it combines the benefits of microsponges and nanosized vesicular structure, it is predominantly employed in pharmaceutical and cosmeceutical methods. The porous structure not only allows us to entrap a diverse spectrum of active chemicals, but it also influences the release pattern. When NS is combined with hydrogel, it provides significant benefits, the most prominent of which is better skin retention [4,5]. Because of its three-dimensional porous structure, NS provides outstanding benefits such as increased entrapment efficiency, improved drug profile, cost-effective technique of manufacture, and simplicity of drug release. To synthesise stable NS in various categories, several preparation techniques are employed, including solvent method, ultra-assisted synthesis, emulsion solvent diffusion method, and melting method. To create an optimised product with improved qualities and quality, software-based optimization approaches are used. Furthermore, 3D printing techniques are being examined to help with the manufacture of NS. For NS delivery, many routes and modalities of drug administration, such as aerosols, capsules, parenteral, tablets, and topicals, are presently being explored [6]. Deflazacort (1-(1, 16)-21-(acetyloxy)-11-hydroxyl-2-methyl-5H-pregna-1,4-dieno-1-(1, 16)-21-(acetyloxy)-11-hydroxyl-2-methyl-5H-pregna-1,4-dieno-1-(1, 16)-21-(acetyl [17,16-d] oxazole-3, 20-dione) is a synthetic glucocorticoid and prednisolone oxazoline derivative. It has strong anti-inflammatory and immunosuppressive properties [7,8]. Deflazacort is a prodrug that is used to treat Duchenne muscular dystrophy (DMD), polymyalgia rheumatica, drug-resistant paediatric epilepsy, idiopathic nephrotic syndrome (INS), renal transplant, and asthma [9]. Box-Bhenken Factorial design is an optimization approach used to create designs of acceptable formulas while saving time, effort, and chemicals. Factorial design is a methodical approach to determining the virtual importance of factors and their combined influence on various responses. Furthermore, response surface characterisation is an efficient way for obtaining a correct model without requiring a lengthy trial period. In this work, the Deflazacort nanosponges formula was optimised using factorial design software. The improved formula is subjected to the scaling up procedure. 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.

2. EXPERIMENTAL MATERIALS AND METHODS

2.1 Materials

The materials needed for this project were obtained from a variety of sources. Torrent

Pharmaceuticals provided Deflazacort as a free sample (India). Evonik Pharma, Mumbai, India, gave Eudragit S100 as a free sample. PVA and PMMA (Poly(methyl methacrylate)) were obtained from Central Drug House Pvt. Ltd. in Mumbai, India, and Qualigens Fine Chemicals in Mumbai, India, respectively. All of the other components used were of analytical quality and were utilised exactly as they were purchased. Fresh demineralised and double distilled water was made and used as needed. The remainder of the reagents and chemicals utilised were of analytical grade.

2.2 Preparation of Nanosponges

2.2.1 Formulation design

For screening of relevant formulation and process variables involved in the creation of nanosponges, regular three-level factorial designs with two factors were used. The table displayed high and low levels of several factors that were examined for their effect in the development of deflazacort nanosponges. In the nanosponge formulations, all process and formulation variables were optimised using a 32-level factorial design with Design of expert 12 software (DOE 12 trial version). The Quadratic randomised, Box-Benkon response surface approach was used to create 17 runs for optimization. Table 1 shows the particle size and entrapment efficiency of the produced formulations.

2.2.2 Methods of preparation

The nanosponges containing deflazacort were created utilising a quasi-emulsion solvent diffusion process with an inner phase of Eudragit S-100 (0.2 percent to 0.5 percent w/v) and PMMA (0.2 percent to 0.5 percent w/v) dissolved in 5 ml of ethanol: dichloromethane (1:1). Deflazacort was then added and dissolved using ultrasonication at 35°C. This mixture was then placed into an aqueous PVA (outer phase) solution with a stirring rate of 500 rpm for 60 minutes. Following that, nanosponges were generated as a result of the evaporation of dichloromethane and ethanol from the system. The prepared Nanosponges were then filtered, rinsed with distilled water, and dried in a hot air oven at 40°C for 12 hours. Finally, the production yield was calculated by weighing the microsponges. Table 2 the preparation of several formulation batches.

2.3 Characterization of Nanosponges

2.3.1 Size of the particles

Using a zetasizer, the average particle size of produced nanosponges was measured (Malvern Zetasizer). The nanosponge formulation was diluted with deionized water and the average size and PDI were determined.

2.3.2 Efficiency of entrapment

20 mg of deflazacort-loaded nanosponges were diluted to a volume of 10 ml with 7.4 pH buffer and stored overnight. For 10 minutes, the shocked solution was centrifuged at 5000 rpm. The supernatant was then filtered through a 0.2 membrane filter and examined using UVVIS spectroscopy at 242 nm.

2.3.3 Shape and surface morphology, drug content determination, and in vitro drug release

Scanning electron microscopy was used to examine the form and surface morphology of the nanosponges (IISER, Bhopal). The carbon-glue-attached nanosponges were coated with gold using a gold sputter module in a vacuum chamber. Samples were then observed with the Scanning Electron Microscope at 10 kV.

2.3.4 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.

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

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

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

2.3.5 *In Vitro* drug release from nanosponges

The drug release of nanosponges was studied in sealed glass vials at 370.1oC. Weighed nanosponges (10mg) were placed in gelatin capsules and placed in a beaker with 100 mL of dissolving medium (PBS of pH 7.0 containing 1 percent , 2 percent , and 3 percent rats caecal contents). Simultaneously, a similar experiment with simulated colonic fluid without enzyme induction was carried out. The samples (1 mL each) were withdrawn at regular intervals during 24 hours, and the withdrawn volume was promptly replenished with new simulated colonic medium containing rabbit caecal material. After centrifuging the samples at 2000 rpm for 10 minutes, the supernatant was filtered using Whatman filter paper. A UV spectrophotometer was used to examine the filtrate.

3. RESULTS AND DISCUSSIONS

The calibration curve for deflazacort was found to be linear at 242 nm in the concentration range of 10-30 g/ml. The Box-Behnken design was used to create 17 confirmatory runs with two centre points for the optimization of polymeric NPs while keeping three independent and two dependent factors in mind. All produced NPs were characterised in terms of average particle size and % drug entrapment. The influence of independent factors on dependent variables was studied, and contour plots were created (Table 3 Figs 1-8). The results of in-vitro drug release

from improved formulation are shown in the table, and the figure was discovered after 24 hours.

3.1 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.

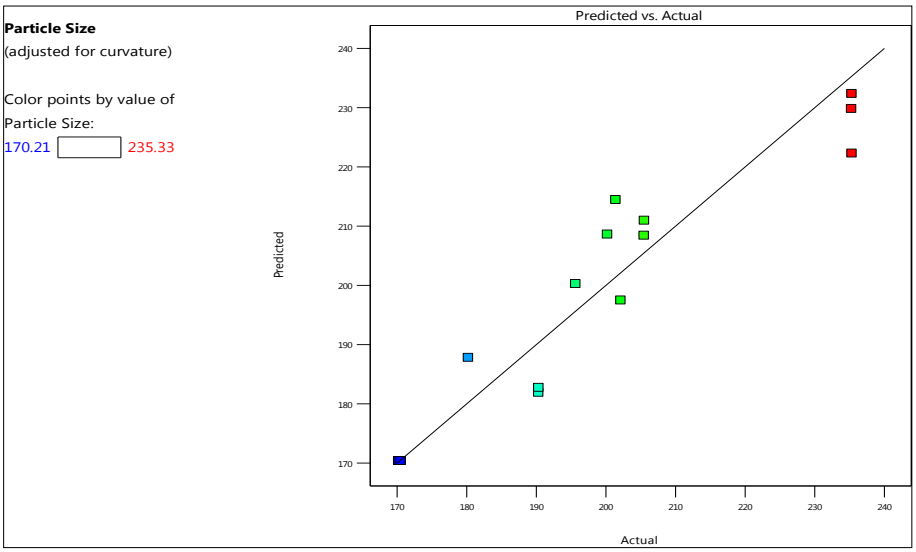

Fig. 1. Graph of Particle Size (Predicted vs Actual)

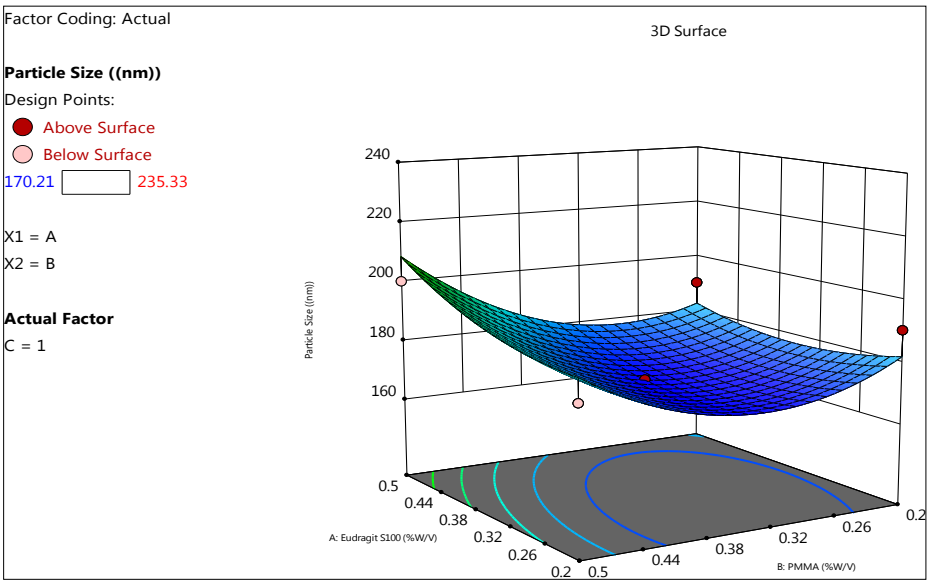

Fig. 2. 3 D Surface Graph of Particle Size (Eudragit S 100 and PMMA)

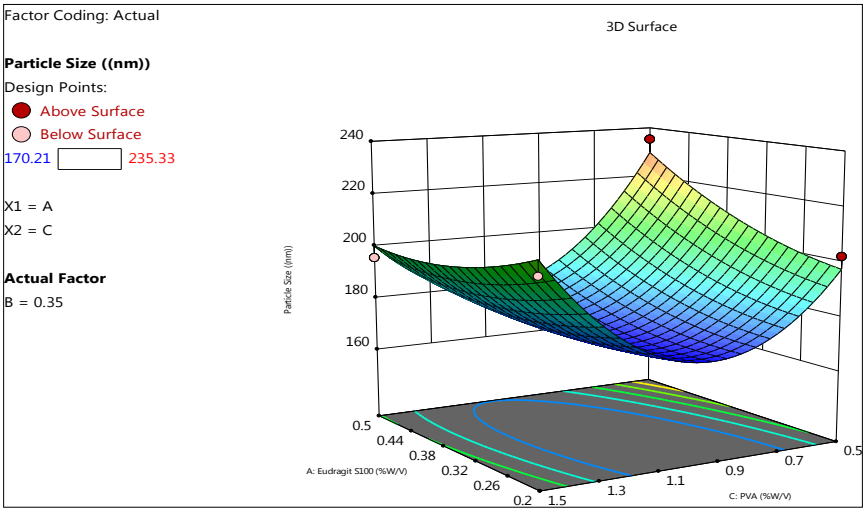

Fig. 3. 3 D Surface Graph of Particle Size (Eudragit S 100 and PVA)

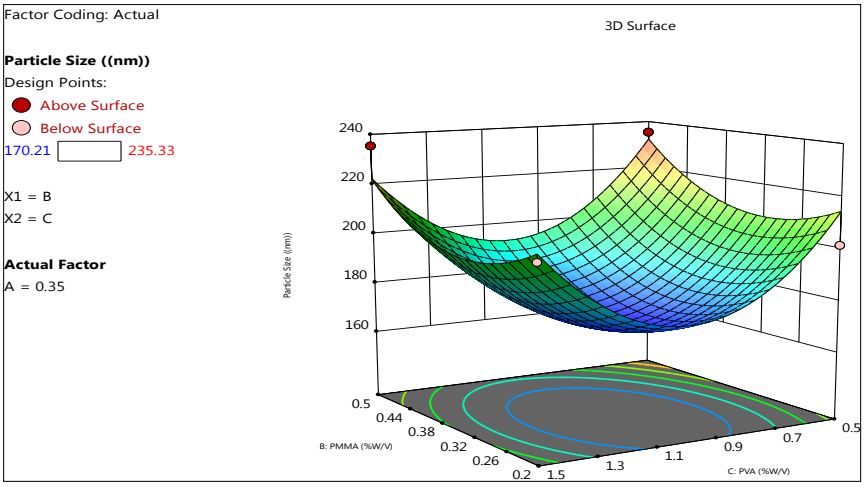

Fig. 4. 3 D Surface Graph of Particle Size (PMMA and PVA)

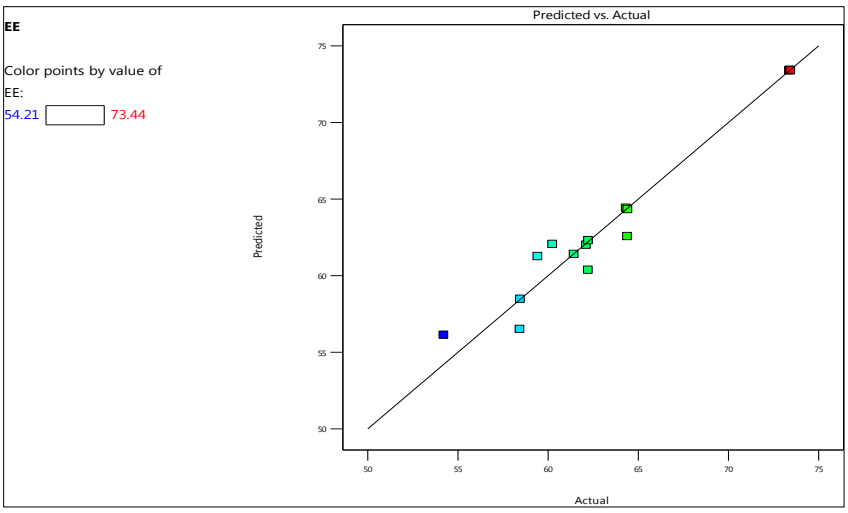

Fig. 5. Graph of entrapment efficiency (Predicted vs Actual)

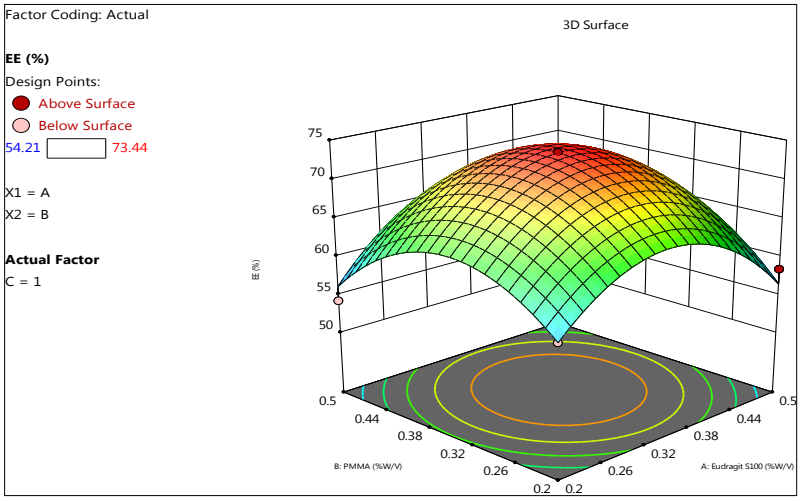

Fig. 6. 3D Surface Graph of entrapment efficiency (Eudragit S 100 and PMMA)

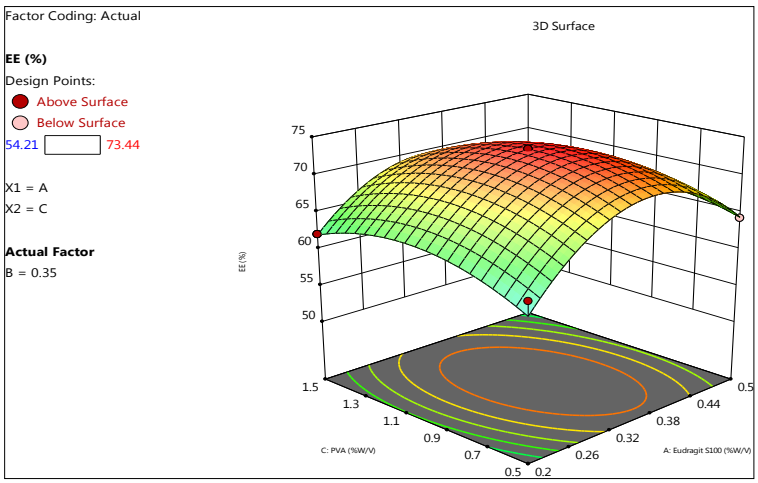

Fig. 7. 3D Surface Graph of entrapment efficiency (Eudragit S 100 and PVA)

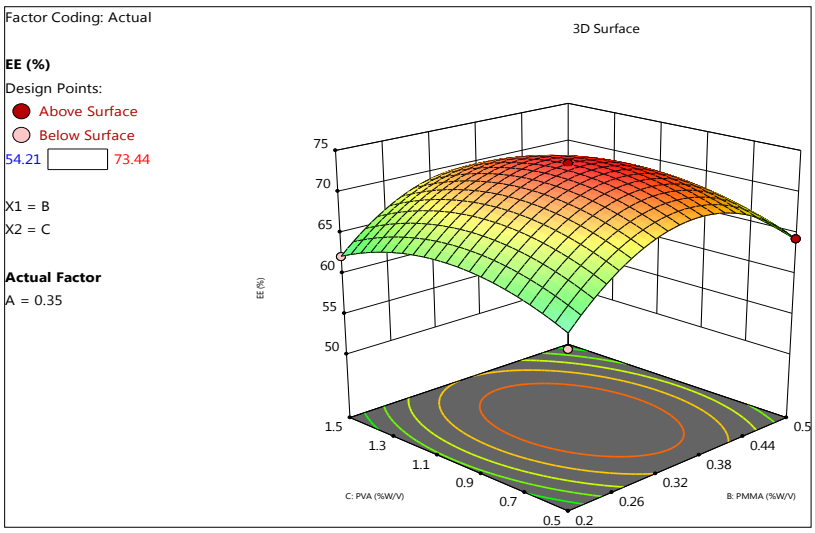

Fig. 8. 3D Surface Graph of entrapment efficiency (PMMA and PVA)

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

4. CONCLUSION

The optimization of a colon focused formulation is a difficult procedure that takes into account a vast number of factors and their interactions. The current study convincingly illustrates the efficacy of a Box-Behnken design in optimising colon targeting formulations. The generated polynomial equations and contour plots help in forecasting the values of chosen independent variables for the production of the best controlled release colon focused formulation of Deflazacort with the required features.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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