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Formulation, Optimization and *In-vitro* Evaluation of Balsalazide Nanospheres

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Abstract: *Nanospheres are the particles having the size range between 10-200 nm in diameter. Nanospheres can be amorphous or crystalline in nature and also, they have the ability to protect the drug from enzymatic and chemical degradation. It has been shown that the hydrophobic surfaces of these particles are highly susceptible to opsonization and clearance by the reticulo endothelial system. The aim was present work is focused on formulation and In-vitro evaluation for balsalazide nanosphere. Nanosphare containing Balsalazide were prepared using nanoprecipitation method. 200 mg of polymer (Eudragit RS: Eudragit L) was dissolve in 50 ml water. Drug was dissolve in 20 ml of methanol. micrographs that nanosphare drug loaded nanosphare and enteric polymer coated nanosphare were discrete, spherical or oval Balsalazide slightly rough surface. From the result aspect ratio of the nanospheres are 1.041. Percentage Assay and content uniformity of all batches was acceptable and came in to the range, except batch F1. Drug entrapment efficiency of the formulations showed in the range of 90% to 96 %.*
Keywords: *Nanospheres, Balsalazide, Eudragite RS, Eudragite L, Nanosystem.*

INTRODUCTION

Nanoparticles can be divided into two main families: nanospheres, which have a homogeneous structure in the whole particle, and nanocapsules, which exhibit a typical core-shell structure. Nanospheres are the particles having the size range between 10-200 nm in diameter. Nanospheres can be amorphous or crystalline in nature and also they have the ability to protect the drug from enzymatic and chemical degradation[1]. It has been shown that the hydrophobic surfaces of these particles are highly susceptible to opsonization and clearance by the reticulo endothelial system. The tiny capsule of drug store house is called vesicles and the solid skeleton structure is called Nanospheres. Biodegradable Nanospheres include albumin Nanospheres, modified starch Nanospheres, gelatin Nanospheres, polypropylene dextran Nanospheres and polylactic acid Nanospheres. In addition there are two more types of Nanospheres, immune Nanospheres and magnetic Nanospheres [2, 3]. Immuno-magnetic nanospheres can be prepared by combining the above two kinds of nanospheres, which could significantly improve its targeting. There are various ways of targeting nanospheres on the tumor, as long circulation purpose and also for the drug delivery in the brain. Nanospheres can be prepared by various methods but the solvent displacement technique is the best method [4-6]. Administration of medication via such systems is advantageous because nanospheres can be ingested or injected and they can be tailored for desired release profiles and used site-specific delivery of drugs and in some cases can even provide organ-targeted release. According to biodegradability, it can be divided into biodegradable nanospheres and nonbiodegradable nanospheres. Biodegradable nanospheres include albumin nanospheres, modified starch nanospheres, gelatine nanospheres, polypropylene dextran nanospheres and polylactic acid nanospheres, etc. According to the current literature reports on nonbiodegradable nanospheres, polylactic acid is the only polymer approved to be used by people and used as a controlled-release agent In addition; reports on immune nanospheres and magnetic nanospheres are also common in recent years. Immune nanospheres possess the immune competence as a result of the antibody and antigen was coated or adsorbed on the polymer nanospheres. Nanospheres possess a unique magnetic feature, namely their reaction to a magnetic force [7]. These are generally coated with protective shells as magnetic polymer nanoparticles. Immuno magnetic nanospheres can be prepared by combining two kinds of nanospheres, which could significantly improve its targeting.

MATERIALS AND METHODS

Table 1: Chemical used for research work

Sl. No.	Name of Excipients	Manufacturer/supplier
1	Balsalazide	Akums Pharma LTD. Mumbai
2	Eudragit L	Evonik Industries
3	Eudrgit RS	FMC biopolymer, Mumbai
4	Magnesium stearate	FMC biopolymer, Mumbai
5	Talc	Homedicines
6	Lactose	Merck Corporation , Germany
6	Dextrose	Signet Corporation, Mumbai
7	Methanol	SD fine chemicals Mumbai
8	Sodium hydroxide	Amexin Pharma, Mumbai
9	Iso propyl alcohol	Ranchem , New Delhi
10	Hydrochloric acid	National Organic Chemicales,Taiwan

Standard Calibration Curve of Balsalazide:

Stock solution of Balsalazide was prepared by dissolving 50 mg of drug in 200 ml of 6.8 phosphate buffer. Aliquots of 1,2,3,4,5,6 ml (5 to 30 µg/ml) were transferred separately in to 50 ml volumetric flasks from the stock solution. Volume was adjusted up to the mark Balsalazide the same solvent. Absorbance of the above solutions was taken at 232 nm against the blank. Graph of absorbance *Vs* concentration was plotted [8].

Preparation of Nano sphere:

Nanosphare containing Balsalazide were prepared using nanoprecipitation method. 200 mg of polymer (Eudragit RS: Eudragit L) was dissolve in 50 ml water. Drug was dissolve in 20 ml of methanol. Both solution were mixed and add 50 ml of water and stirred for half an hour. Methanol and water was evaporated under reduced pressure using rotary flash evaporator until 10 Ml of solution was remaining. Than this suspension was centrifuge at 15000 rpm at 4^0C for half an hour [9]. The supernatant was discarded and remaining portion was washed with distilled water. The Nanosphares was dried over night at 60^0C and stored in desiccators.

Table 2: Formulation table of Nanosphare

Sr No	Material	F-1	F-2	F-3	F-4
1	Balsalazide (mg)	500	500	500	500
2	Eudragite RS (mg)	50	100	-	-
3	Eudragite L (mg)	-	-	50	100
4	Tween 80 (ml)	0.2	0.2	0.2	0.2
5	Methanol (ml)	20	20	20	20
6	Water (ml)	100	100	100	100

EVALUATION OF NANOSPHARE

Particle Size, Surface Morphology and Zeta Potential:

The surface morphology (roundness, smoothness, and formation of aggregates) and particle size were studied by scanning electron microscopy (SEM). Zeta potential of the best formulation was determined by zeta potential probe model DT- 300 [10, 11].

Drug Entrapment Efficiency:

Drug content was determined by centrifugation method. The Nanosphare were redisperse by centrifugation in Phosphate buffer pH 6.8 at 15,000 rpm for 40 min at 25^{0}C to separate the free drug in the supernatant. Concentration of Balsalazide in the supernatant was determined by UV-Visible Spectrophotometyrically at 232 nm after suitable dilution [12].

In -vitro Release Studies:

In-vitro release studies were carried out by using dialysis tubes with an artificial membrane. Initial 3 hour the nanosphare of Balsalazide drug release study was conducted using 0.1 HCl as dissolution medium then the prepared Balsalazide Nanosphare and 10 ml of Phosphate buffer pH 6.8 was added to the dialysis tube and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8.The medium in the receptor was agitated continuously using a magnetic stirrer a temperature was maintained at 37±1^{0}C. 5ml of sample of receptor compartment were taken at various intervals of time over a period of 24 h and each time fresh buffer was replaced. The amount of drug released was determined spectrometrically at 304 nm [13].

OPTIMIZATION OF NANOSPHERE TABLET FORMULATION

Optimization of Eudragite RS and Eudragite L as Polymer for Nnaosphere Tablet:

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The Content of Eudragite RS) and Content of Eudragite RS (X2) were selected as independent variables [14-16]. The time required for 50% drug release ($t_{50\%}$), release at 4hrs (Q 4hrs) and similarity factor f2 were selected as dependent variables.

Table 3: Optimization Parameter for Eudragite RS and Eudragite L

			Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	ID	Run	A: Eudragite RS	B: Eudragite L	*Drug Entrapment Efficiency*	*Drug loading*	Drug Release
			mg	mg	%	%	%
5	5	1	100	150	49.65	23.75	86.32
9	9	2	150	200	43.98	29.12	90.14
3	3	3	150	100	41.06	18.45	76.86
8	8	4	100	200	51.45	31.94	92.47
4	4	5	50	150	58.14	24.62	88.74
2	2	6	100	100	42.84	17.85	78.65
6	6	7	150	150	48.45	21.89	85.47
1	1	8	50	100	54.78	19.24	82.29
7	7	9	50	200	62.58	33.12	94.24

FORMULATION OF BALSALAZIDE NANOSPHARE TABLET

Balsalazide prepared Nanosphere was passed through #40 sieves, then lubricant magnesium stearate and talc was added then compressed into tablets by rotary tablet punching machine. Then film coating is done by 6% w/v solution of Cellulose acetate Phthalate in isopropyl alcohol using 2% tween-80 as plasticizer in coating pan [17-19]. The weight of tablet was kept constant for all formulations. A minimum of 100 tablets were prepared for each batch.

Table 4: Polymer Concentration of Tablet Formulation

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7*	F-8	F-9
Balsalazide									
Eudragite RS	50	100	150	50	100	150	50	100	150
Eudragite L	100	100	100	150	150	150	200	200	200

Lactose	75	50	25	75	50	12.5	50	12.5	12.5
Dextrose	100	75	50	50	25	12.5	25	12.5	12.5
Talc	5	5	5	5	5	5	5	5	5
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Starch past	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Stability Study

The stability study was carried out for optimized formulation as per ICH guidelines. The nanoparticle of the best formulation were placed in screw capped glass container and stored at ICH storage ($40^0C\pm2^0C/75\%RH\pm5\%RH$) condition for a period of 60 days. The samples were analyzed for physical appearance and for the drug content [20].

Table 5: ICH guidelines for stability study

Study	Storage condition	Time period
Long term	$25^0C\pm2^0C/60\%RH\pm5RH$	12 month
Intermediate	$30^0C\pm2^0C/65\%RH\pm5\%RH$	6 month
Accelerated	$40^0C\pm2^0C/75\%RH\pm5\%RH$	6 month

RESULT AND DISCUSSION

Determination of Analytical Wave length

A standard stock solution of Balsalazide in 6.8 phosphate buffer was prepared having a concentration 600 µg/mL. A 5.0 mL portion of stock solution was further diluted Balsalazide water in a 100.0 mL. volumetric flask up to mark to get final concentration 30 µg/mL. The standard solution of Balsalazide (30 µg/mL) was scanned in the range of 400-200 nm. in 1.0 cm cell against solvent blank and spectra was recorded , the absorbance maxima was observed at 232.0 nm.

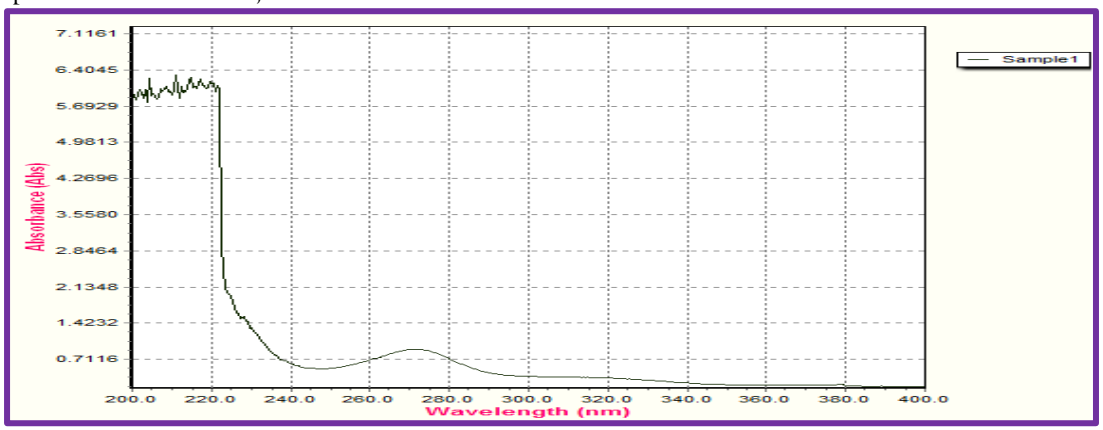

Figure 1: UV spectrum of Balsalazide in 6.8 phosphate buffer

X-ray Diffraction of Balsalazide Pattern:

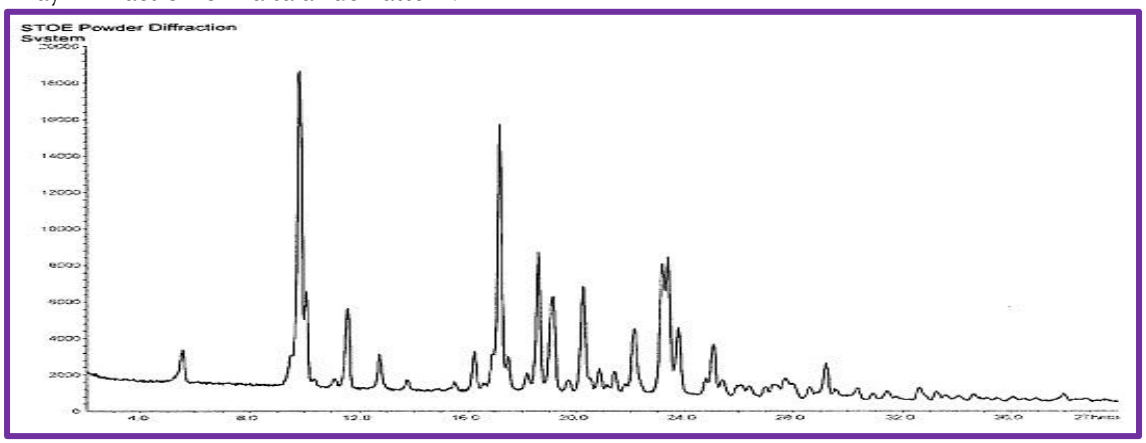

Figure 2: X-ray powder diffraction pattern of Balsalazide in crystalline form

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Calibration curve for Balsalazide in 6.8 Phosphate buffer:
Phosphate buffer pH 6.8 was used for the preparation of Balsalazide concentration and absorption was measure by Shimadzu UV-1601 UV/Vis double beam spectrophotometer. The λ max of Balsalazide was found to be 232 nm.

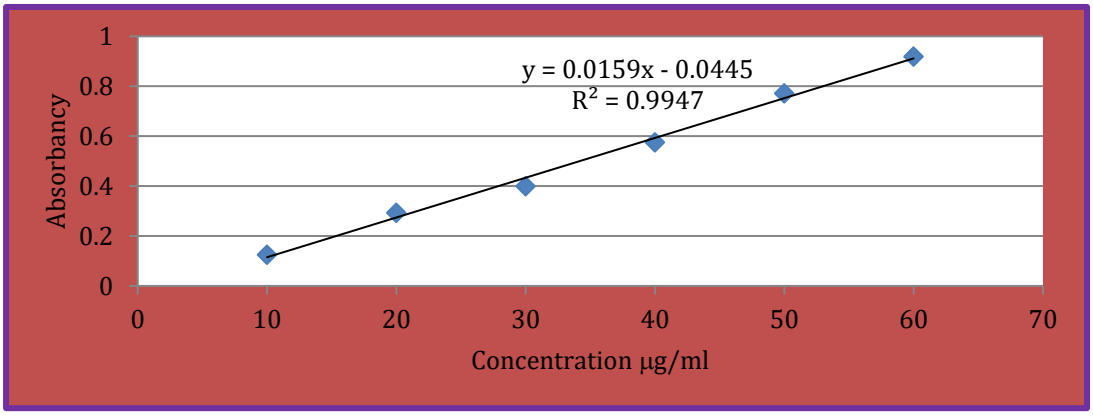


Figure 3: Drug calibration curve in Phosphate buffer pH6.8

Calibration curve of Balsalazide in 0.1 N HCl:

Balsalazide concentration was prepared in 0.1 N HCl and absorption were measure by Shimadzu-1601 UV/Vis double beam spectrophotometer. The λ max of Balsalazide was found to be 232 nm.

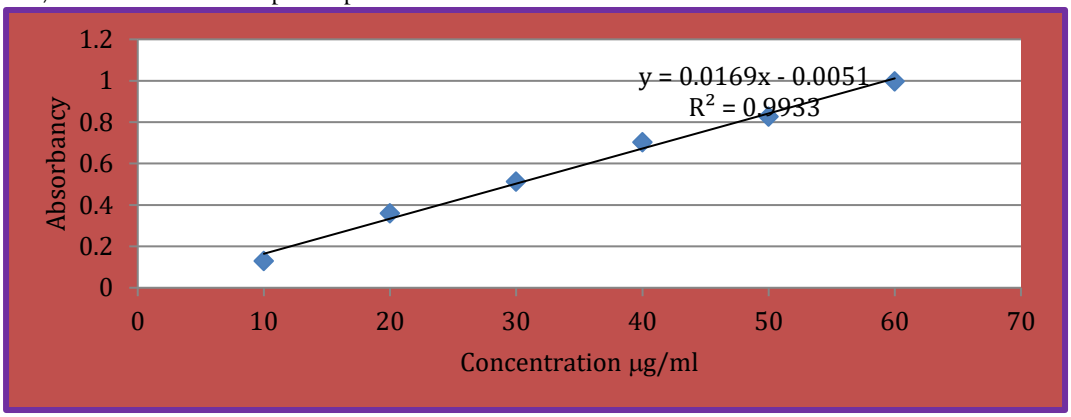
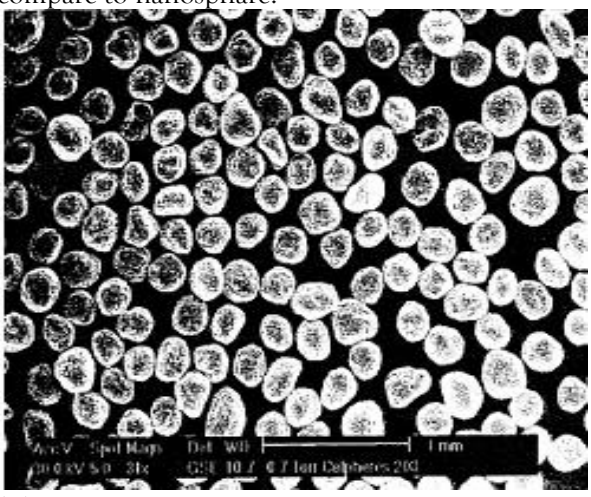


Figure 4: Drug calibration curve in Phosphate buffer 0.1 N HCl

Shape Analysis

The shape analysis of the nanosphare, drug loaded nanosphare and polymer coated nanosphare obtained is shown in figure. It was evident from SEM photo micrographs that nanosphare drug loaded nanosphare and enteric polymer coated nanosphare were discrete, spherical or oval Balsalazide slightly rough surface. No significant change of shape was found in drug loaded nanosphare and polymer coated nanosphare as compare to nanosphare.



(A)

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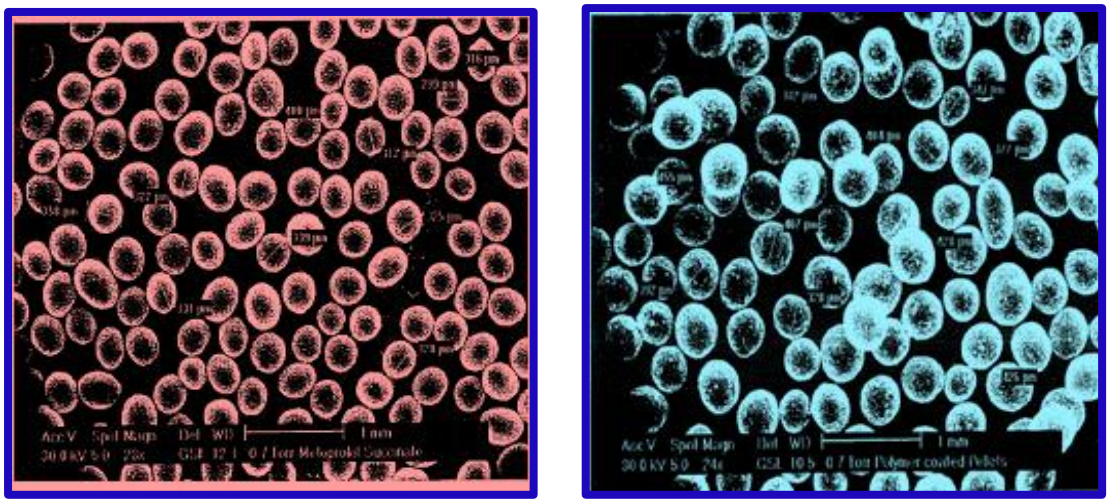


(B) (C)

Figure 5- Scanning electron micrographs of (A) Nanosphare (B) Drug loaded nanosphare and (C) Polymer coated nanosphare

Aspect Ratio:

Aspect ratio was done for the nanosphare's spherocity, for the flow property. Hot stage microscope was used for the measurement of the height and width of the nanosphare. Aspect ratio was calculated from following formula:

$$\text{Aspect ratio} = \frac{\text{Length of nanosphare}}{\text{Width of nanosphare}}$$

Aspect ratio should be very near to 1 for the best spherical shape.

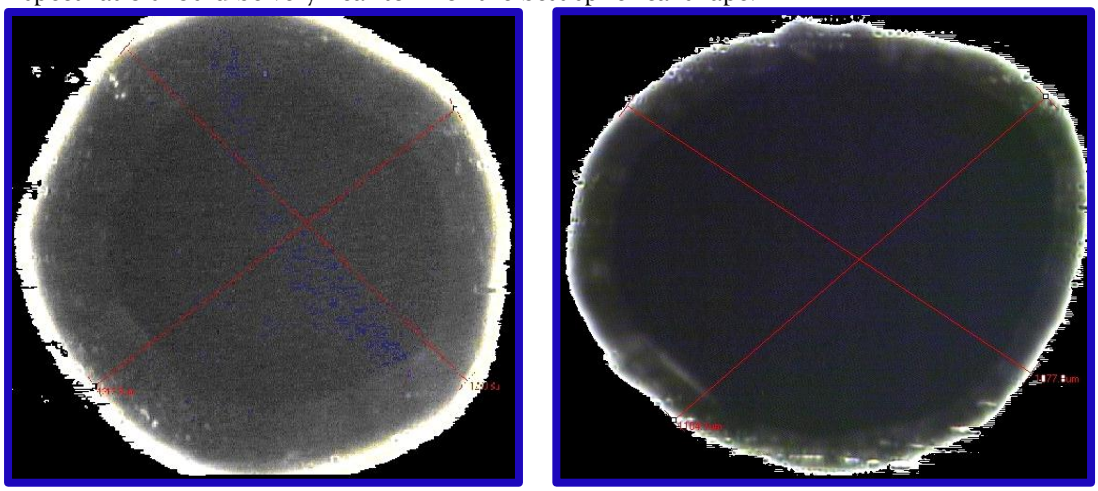


Figure 6: Aspect ratio of different batches of nanosphare

Table 6: Aspect ratio of different batches

SR.NO.	LENGTH (nm)	WIDTH (nm)	ASPECT RATIO
F1	1422.0	1369.0	1.038
F2	1260.8	1212.3	1.040
F3	1188	1103.2	1.076
F4	1104.1	1077.8	1.024

RESULT

From the above result aspect ratio of the nano-spheres 1.042

Scanning Electron Microscopy:

These studies were done for the nanosphare's shape and surface study. Following study gives perfect idea about shape and surface of the nanospheres prepared.

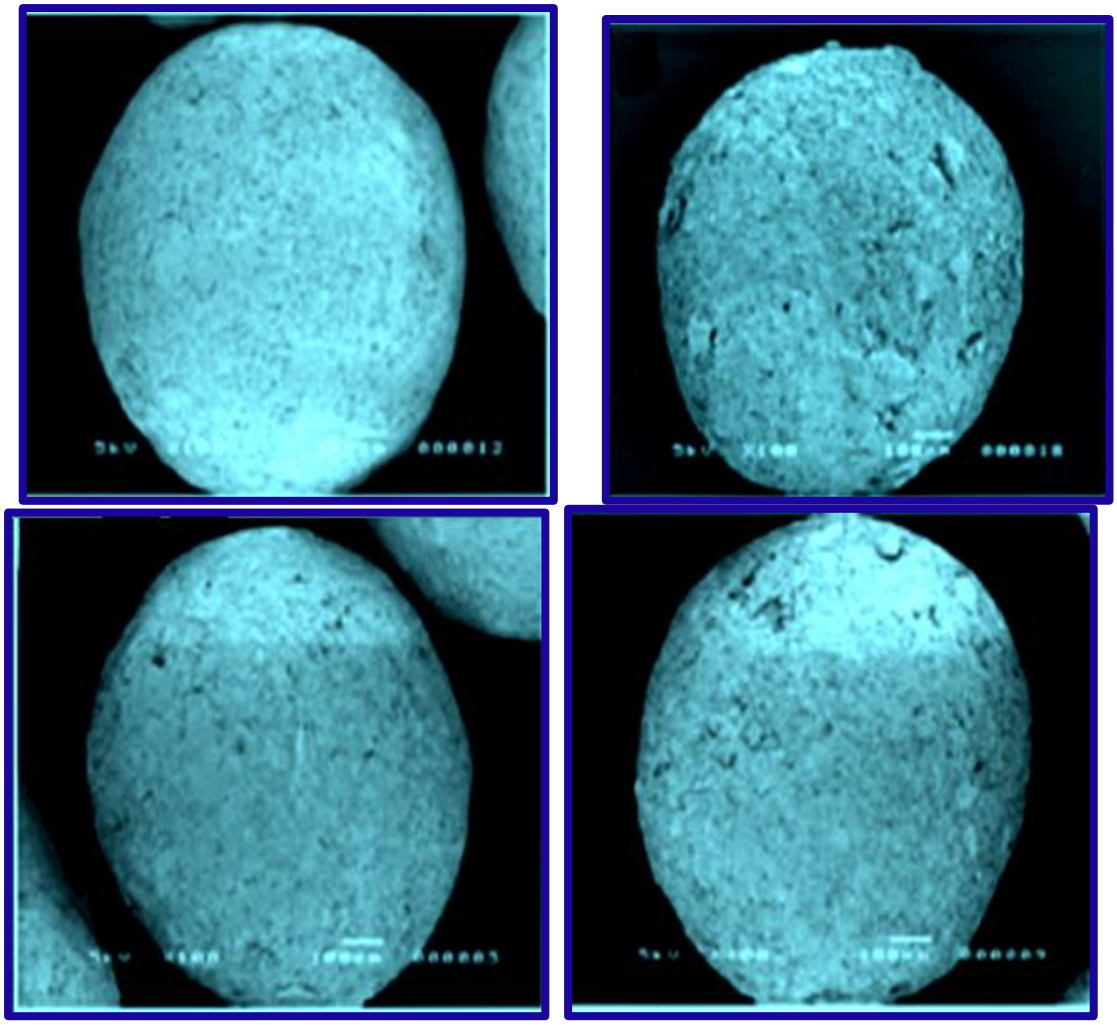


Figure 7: Scanning electron micrographs of shape and surface size of F1, F2, F3 and F4 polymer coated nanosphare

Zeta potential:

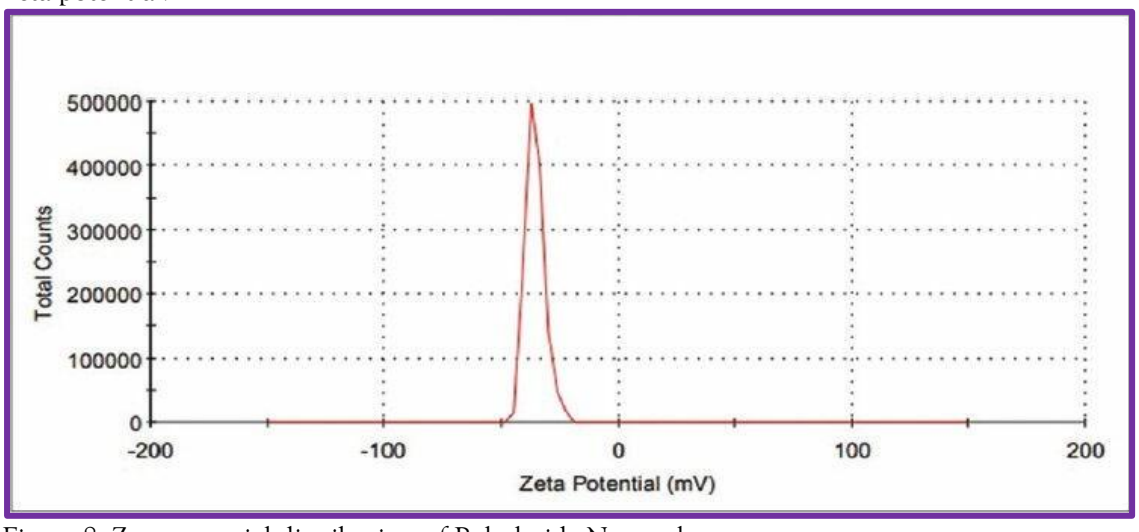


Figure 8: Zeta potential distribution of Balsalazide Nanosphere

Percentage Assay, Percentage yield and Content Uniformity

Percentage assay, Percentage yield and Percentage CU of all batches were done as described in following table.

Percentage Assay and CU of all batches was acceptable and came in to the range, except batch F1. Percentage assay & Percentage CU results of all batches shown that all batch contain equivalent amount of Balsalazide in range. But, Batch F2 shows the comparable and exact result of Percentage assay & Percentage CU. So, from the results it was concluded that Batch F2 was good for sustained coating. Assay results of all batches are shown in table below.

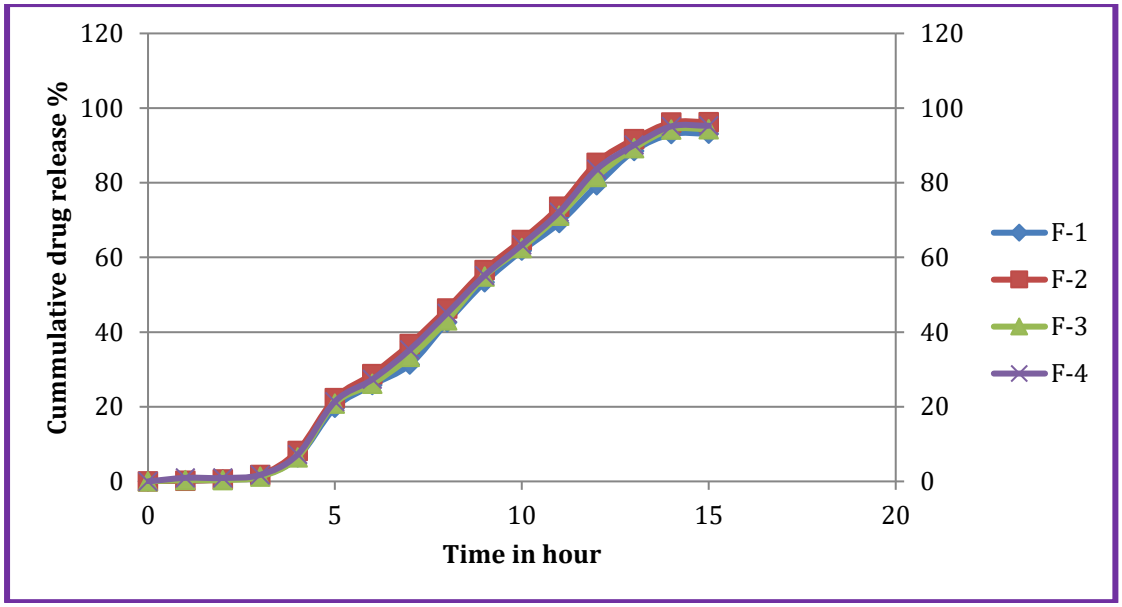
Table 7: % Assay& CU of F1- F4

B. NO.	Avg. % Assay	Avg. % CU	Avg. % Yield
F1	93.1	95.8	97.96
F2	99.8	100.2	98.75
F3	101.8	102.8	98.43
F4	100.2	102.2	98.13

Fig 9: Coated nanosphare of F_2

In-vitro Drug Release Studies of Balsalazide Nanosphare:

Time (hrs.)	F-1	F-2	F-3	F-4
0	0.0	0.0	0.0	0.0
1	0.21	0.15	0.31	0.95
2	0.53	0.49	0.36	0.92
3	1.49	1.72	1.26	1.76
4	6.42	8.17	6.46	7.18
5	19.72	22.26	20.85	21.39
6	25.82	28.74	26.12	27.33
7	31.29	36.76	33.32	35.27
8	42.64	46.29	43.16	45.04
9	53.32	56.61	54.88	55.16
10	61.79	64.69	62.55	63.38
11	69.21	73.57	71.21	72.15
12	79.32	85.35	81.53	83.59
13	88.52	91.67	89.23	90.09
14	93.01	96.12	94.24	95.11
15	93.13	96.24	94.35	95.26



Drug Entrapment Efficiency of Nanosphare:

Drug entrapment efficiency of the formulations showed in the range of 90% to 96 %. The results have been shown in table.

Table 8: Drug Entrapment of nanosphare

Sr. no.	Batch no.	% Entrapment
1	F-1	93
2	F-2	95
3	F-3	91
4	F-4	92

OPTIMIZATION PARAMETER OF EUDRAGITE RS AND EUDRAGITE L AS POLYMER FOR NANOSPHERE TABLET FORMULATION:

Drug Entrapment Efficiency:

Table 9: Optimization Parameter for Eudragite RS and Eudragite L with response

			Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**
Std	ID	Run	A: Eudragite RS	B: Eudragite L	*Drug Entrapment Efficiency (DE)*	*Drug loading (DL)*	Drug Release
			mg	mg	%	%	%
5	5	1	100	150	49.65	23.75	86.32
9	9	2	150	200	43.98	29.12	90.14
3	3	3	150	100	41.06	18.45	76.86
8	8	4	100	200	51.45	31.94	92.47
4	4	5	50	150	58.14	24.62	88.74
2	2	6	100	100	42.84	17.85	78.65
6	6	7	150	150	48.45	21.89	85.47
1	1	8	50	100	54.78	19.24	82.29
7	7	9	50	200	62.58	33.12	94.24

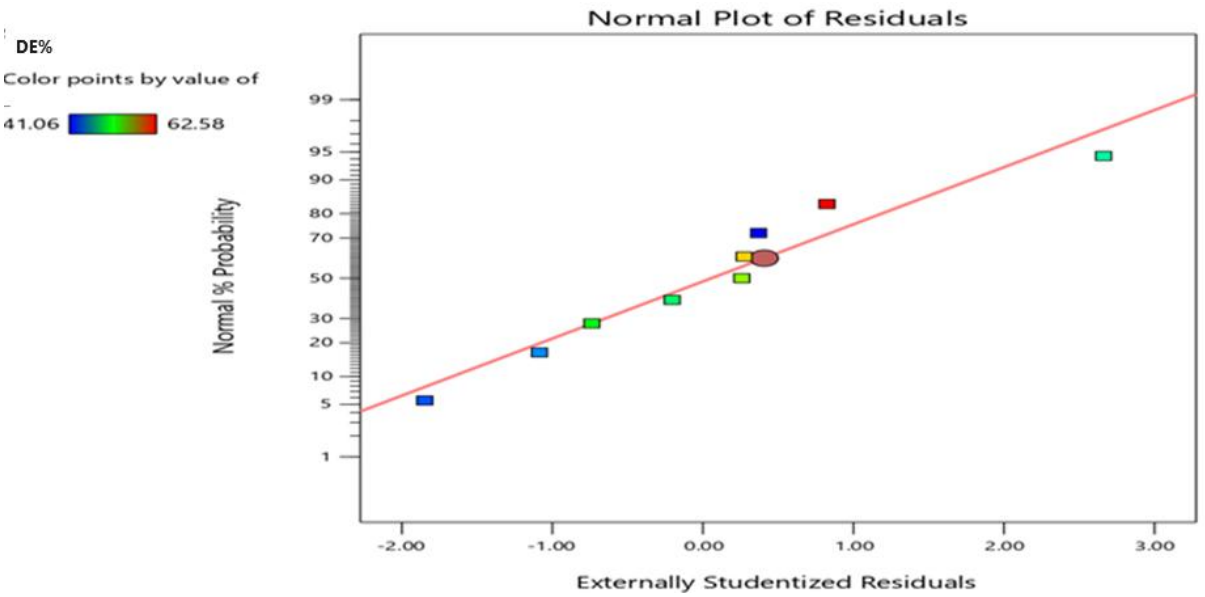


Fig 10: Residual Plot for Drug entrapment efficiency (DE %)

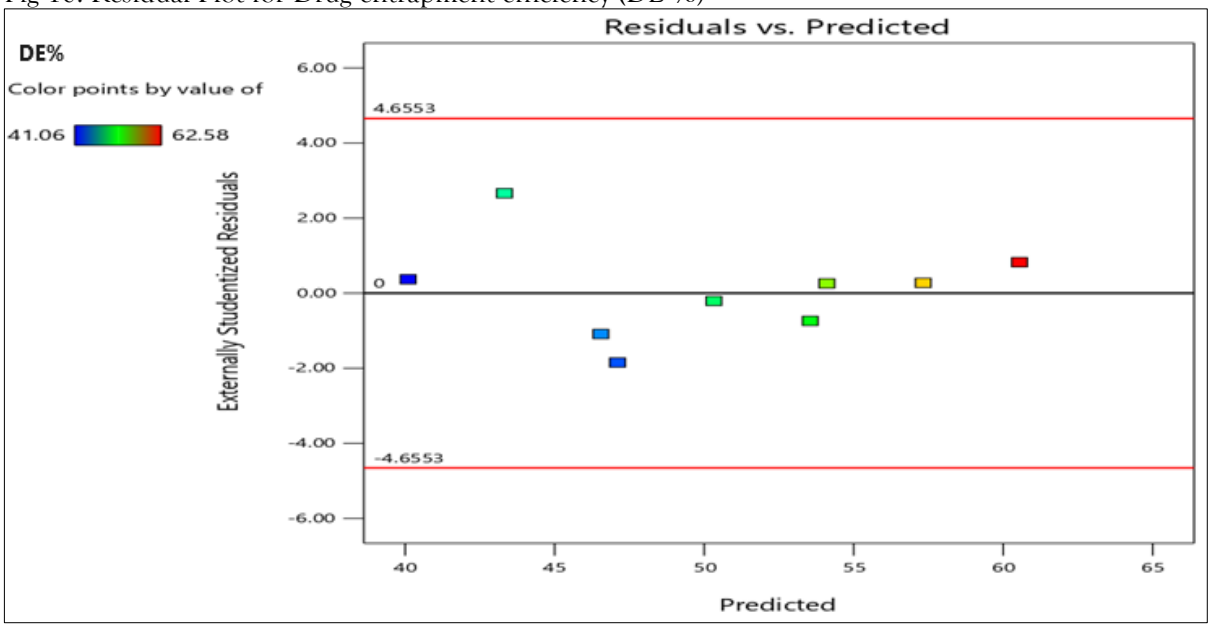


Fig 11: Residual *vs* Predicted for Drug entrapment efficiency (DE %)

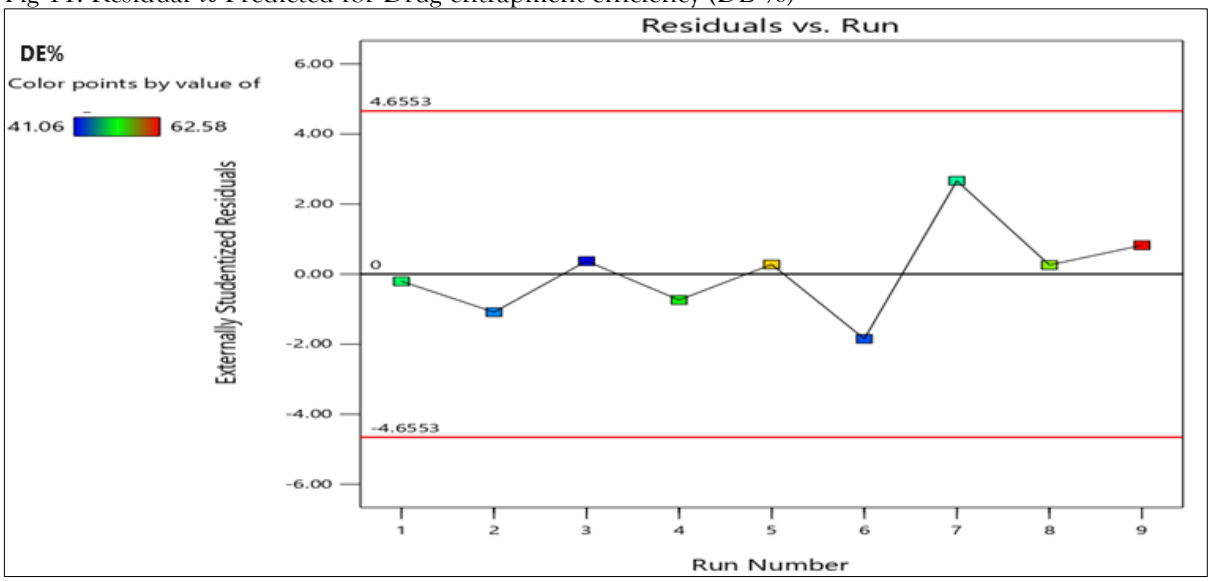


Fig 12: Residual *vs* Run for Drug entrapment efficiency (DE %)

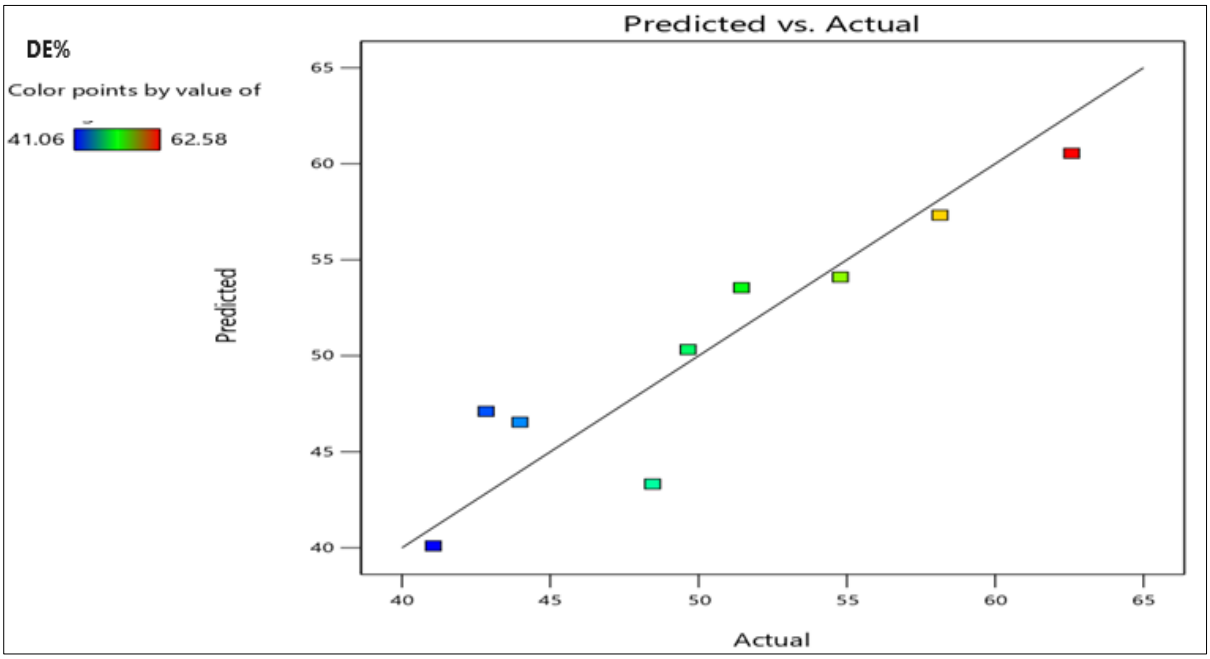


Fig 13: Predicted *vs* Actual for Drug entrapment efficiency (DE %)

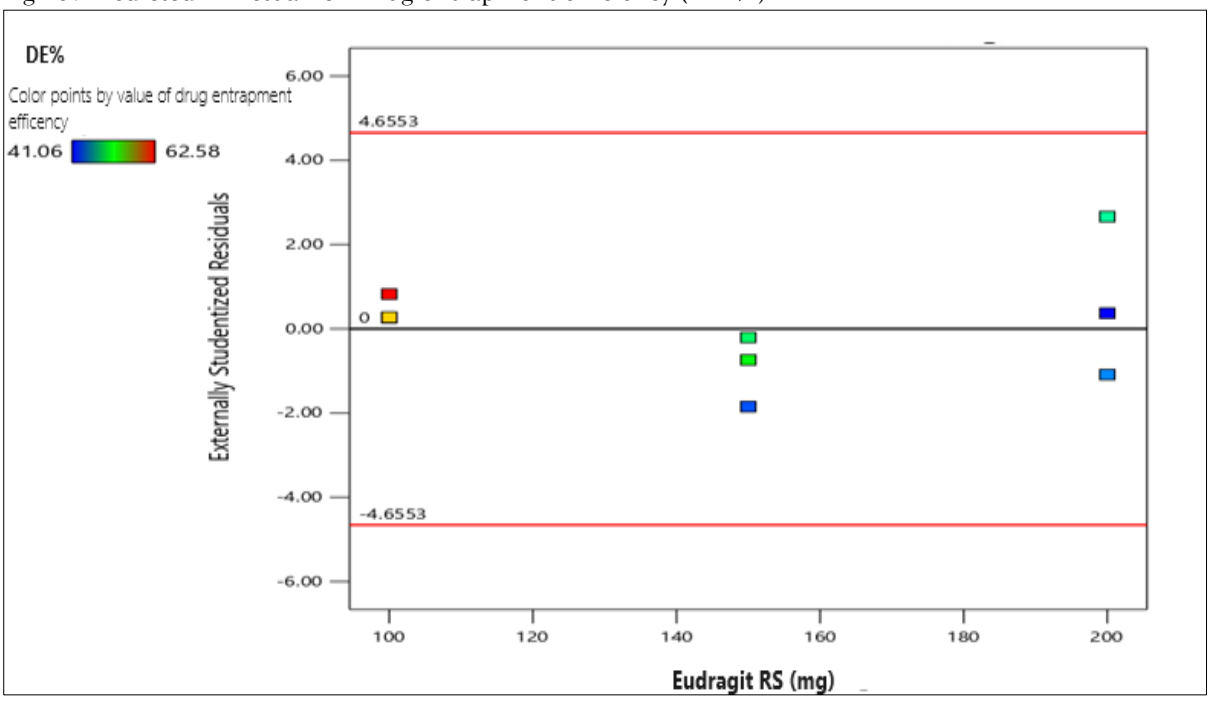


Fig 14: Residual *vs* Eudragit RS for Drug entrapment efficiency (DE %)

The Model F-value of 17.23 implies the model is significant. There is only a 0.33% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Predicted R^2 of 0.6782 is in reasonable agreement with the Adjusted R^2 of 0.8023; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 11.012 indicates an adequate signal. This model can be used to navigate the design space.

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Table 10: Final Report of Drug entrapment efficiency (DE %) for Eudragit RS

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value dffits	Standard Order
1	49.65	50.33	-0.6756	0.111	-0.223	-0.204	0.002	-0.072	5
2	43.98	46.55	-2.57	0.444	-1.070	-1.086	0.305	-0.972	9
3	41.06	40.10	0.9578	0.444	0.400	0.370	0.043	0.331	3
4	51.45	53.55	-2.10	0.278	-0.767	-0.738	0.075	-0.457	8
5	58.14	57.33	0.8128	0.278	0.297	0.274	0.011	0.170	4
6	42.84	47.10	-4.26	0.278	-1.560	-1.847	0.312	-1.146	2
7	48.45	43.32	5.13	0.278	1.876	2.662	0.451	1.651	6
8	54.78	54.11	0.6744	0.444	0.281	0.259	0.021	0.231	1
9	62.58	60.55	2.03	0.444	0.847	0.824	0.191	0.737	7

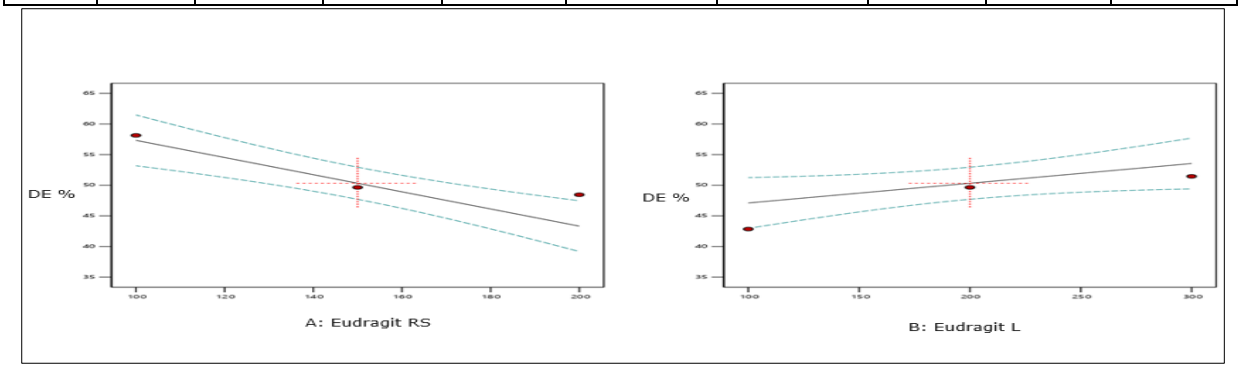

Fig 15: Drug entrapment efficiency (DE %) for Eudragite RS and Eudragite L

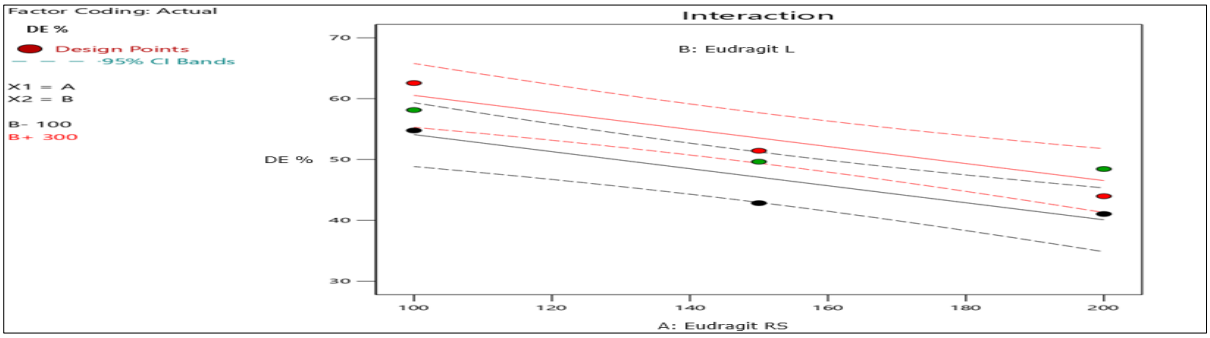

Fig 16: Interaction of Eudragite RS and Eudragite L

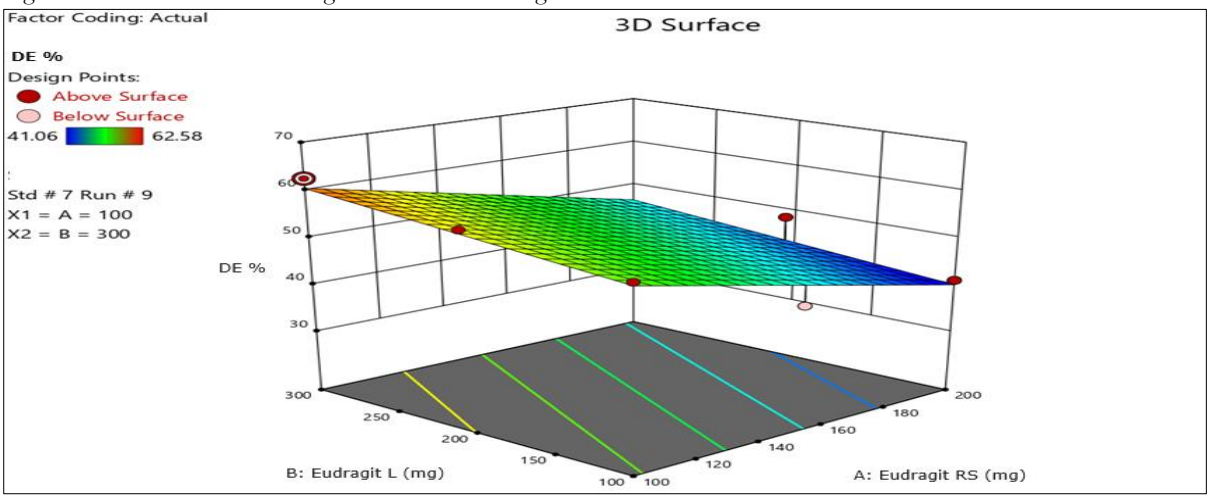

Fig 17: 3D-View of drug entrapment efficiency (DE %) for Eudragite RS and Eudragite L

CONCLUSION

On the basis of drug entrapment efficiency we concluded that method used for formulation of Nanosphare were optimized method. Formulation F-2 Showed 96.02% drug was entrapped which is best formulation among all other nanosphare formulation. Formulation F-3 showed least drug entrapment. *In-vitro* Drug release studies of Balsalazide nanosphare showed that Formulation F-2 release maximum drug (96.16%) after 15 hour. The FTIR spectra showed that drug and polymer used in formulation of Nanosphare are compatible with each other, Polymer concentration was optimized for the nanosphere tablet formulation.

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