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Formulation and in-Vitro Evaluation of Sunitinib Loaded Nanoparticles for Treatment of Breast Cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited treatment options due to the lack of estrogen, progesterone, and HER2 receptors. Sunitinib, a multi-kinase inhibitor, has shown potential in cancer therapy but suffers from low bioavailability and severe side effects. This study aimed to develop and characterize sunitinib-loaded hyaluronic acid-chitosan polyelectrolyte complex nanoparticles (SPEC) for targeted delivery to TNBC. The nanoparticles were prepared using the polyelectrolyte complexation method and optimized using a Central Composite Design (CCD). The optimized formulation exhibited a particle size of 193.8 ± 4 nm, zeta potential of 24.12 ± 0.8 mV, polydispersity index of 0.3 ± 0.01, and entrapment efficiency of 73.2 ± 1.2%. In vitro cytotoxicity studies demonstrated enhanced efficacy of SPEC against MDA-MB-231 and 4T1 cancer cells compared to free sunitinib. The results suggest that SPEC could be a promising nanocarrier for targeted delivery of sunitinib in TNBC therapy.

INTRODUCTION

Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer characterized by the absence of estrogen, progesterone, and HER2 receptors, making it resistant to conventional hormone therapies. Sunitinib, a multi-kinase inhibitor, has shown potential in inhibiting tumor angiogenesis and proliferation. However, its clinical application is limited by poor solubility, low bioavailability, and severe side effects. To address these challenges, this study focused on developing a targeted drug delivery system using hyaluronic acid (HA) and chitosan (CS) to form polyelectrolyte complex nanoparticles (PECNPs). HA, a natural polysaccharide, targets CD44 receptors overexpressed in TNBC cells, while CS enhances the stability and biocompatibility of the nanoparticles. The study aimed to optimize the formulation using CCD and evaluate its physicochemical properties, in vitro cytotoxicity, and drug release profile.

MATERIALS AND METHOD

Sunitinib (Cipla, Mumbai, India)
Hyaluronic acid (Meteoric Biopharmaceuticals Pvt. Ltd., Ahmedabad, India)
Chitosan (Loba Chemicals, Mumbai, India)

Glacial acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, potassium chloride, potassium phosphate, disodium hydrogen phosphate, potassium dihydrogen orthophosphate, acetonitrile (HPLC grade), methanol (HPLC grade), and sodium phosphate tribasic dodecahydrate were purchased from Merck Specialities, Mumbai, India.

Method:
Preparation of Blank PECNPs: HA and CS solutions were prepared in deionized water and acetic acid, respectively. The HA solution was added dropwise to the CS solution under magnetic stirring to form PECNPs.

Drug Loading: Sunitinib was dissolved in ethanol and added to the HA solution before mixing with CS to form SPEC.
Optimization Using CCD: A Central Composite Design was employed to optimize the formulation. Independent variables included HA concentration (0.5–2 mg/mL), CS concentration (0.5–2 mg/mL), and sunitinib concentration (0.1–2.5 mg/mL). Dependent variables were particle size, zeta potential, polydispersity index, and entrapment efficiency.

Characterization: Particle size, zeta potential, and polydispersity index were measured using a Horiba particle size analyzer. Entrapment efficiency was determined by UV-Vis spectrophotometry. Morphology was analyzed using scanning electron microscopy (SEM).

In Vitro Drug Release: The release profile of sunitinib from SPEC was studied using a dialysis bag method in phosphate buffer (pH 5.5).

RESULTS AND DISCUSSION

Organoleptic properties

Table 1: Organoleptic properties of drug and polysaccharides.

Organoleptic Properties	Observations		
	Sunitinib	**Chitosan**	**Hyaluronic acid**
Colour	white	White	white
Odour	Odourless	Odourless	Odourless
Taste	Tasteless	Tasteless	Tasteless
Description	Solid white powder	White/ivory powder	White powder

Table : Melting point of dug and polysaccharides.

Parameters	Melting Point	
	Observed	Reported
Sunitinib	241°C	240-243°C
Chitosan	87°C	88°C
Hyaluronic acid	243°C	241-247°C

Table : Drug-Excipients compatibility studies.

S. No.	Ingredients	Quantity (mg)	Initial	Room temperature (4 weeks)	40±2°C /75±5 % RH (4 weeks)
1	SUN: HA	1:1	Off white	No change	No change
2	SUN: CS	1:1	Off white	No change	No change

Solubility studies:
SUR is BCS class II drug; it is less soluble in water. The solubility of SUR in water was found to be 0.0506 µg/ml.

FTIR Analysis of Sunitinib:

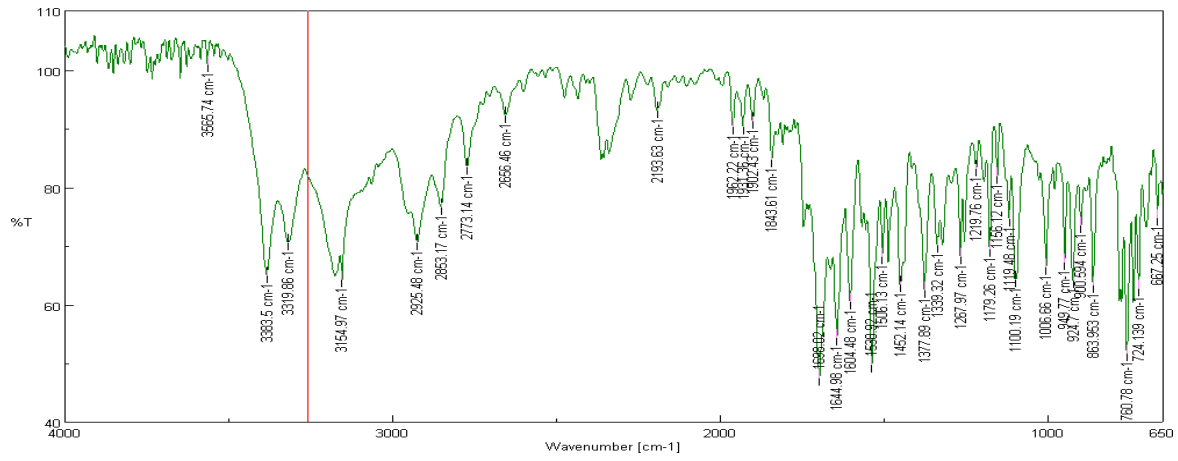

Fig. No. : FTIR spectrum of Sunitinib.

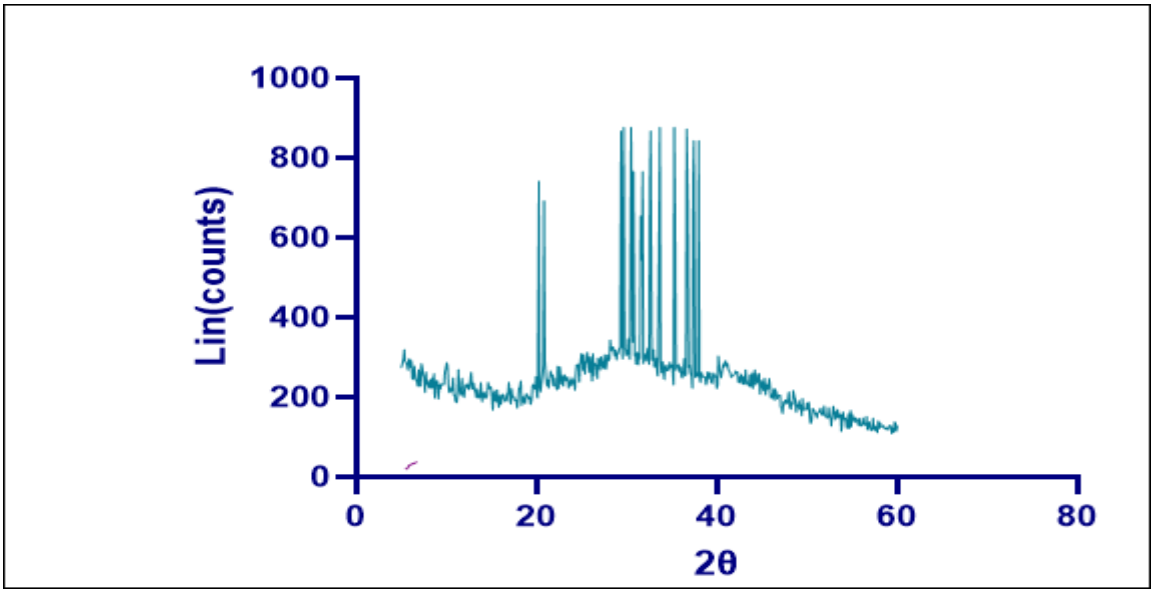

Fig. No. : XRD diffractogram of Sunitinib.

Differential scanning calorimetric analysis of Sunitinib:

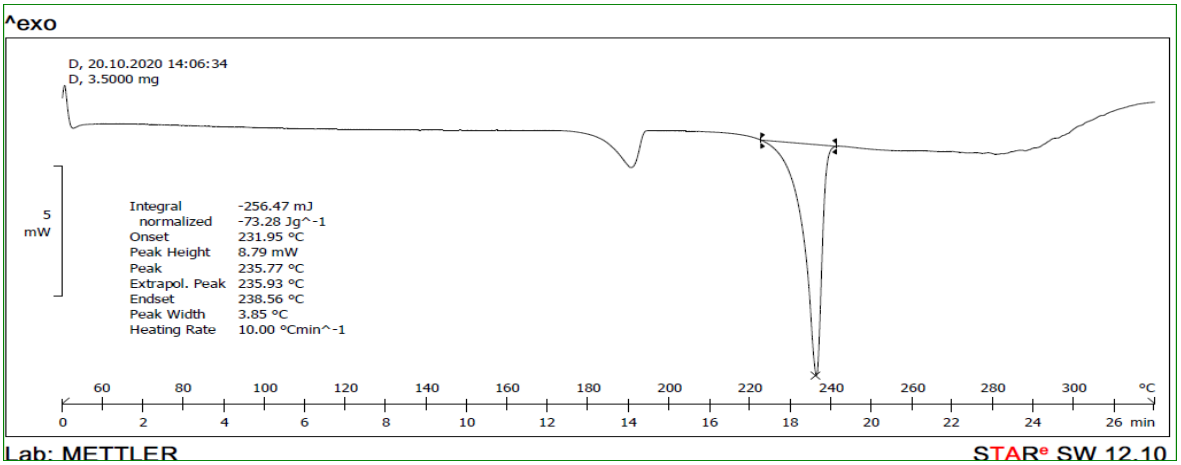

Fig. No. : DSC thermogram of Sunitinib.

Selection Of Method For Preparation Of Nanoparticles:

Table . Different method of preparation of nanoparticles.

Method	particle size (nm)	Polydispersity index	Zeta potential (Mv)	Stability at r
Ionotropic gelation	2900	0.88	19.1	Precipitationof micron-sized particles was seen.
Inverse Ionotropic gelation	1636	1.3	21.2	Precipitation particles settle downat the bottom.
Polyelectrolyte complex 1.Adsorption	496	0.43	16.3	Stable butturbid solution
2.mixing	90	0.2	23.03	STabl whitish solution

Preparation Of Blank Polyelectrolyte Complex Nanoparticles (PECNPs):

Effect of pH on particle size and zeta of blank PECNPs:

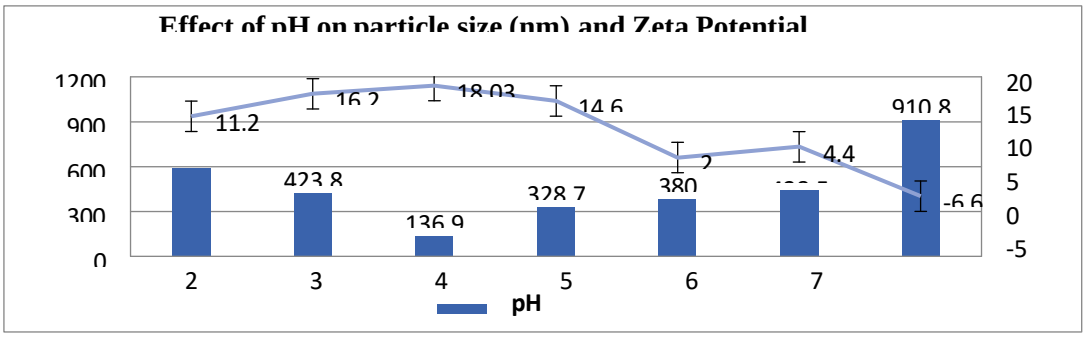

Fig. No. : Effect of pH on particle size and zeta potential of blank PECNPs

Effect of Ionic Strength on PECNPs particle size and polydispersity index:

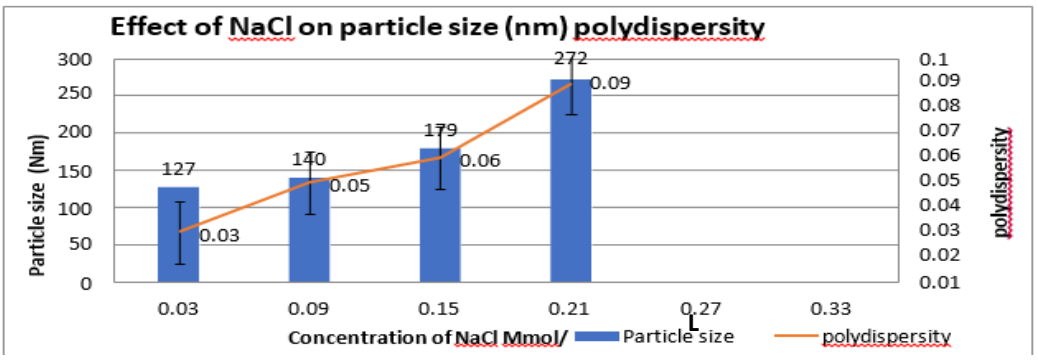

Fig. No. : Effect of concentration of NaCl on particle size and polydispersity index.

Effect of drug loading on particle size, zeta potential, polydispersity index and entrapment efficiency:

Table : Analysis of result of the B1 to B18 batches

Batch No.	HA mg/m l	CS mg/ ml	SUR mg/ ml	PS (nm)	ZP(mV)	PI	EE (%)
B1	**0.5**	**0.5**	**0.1**	**125**	**23**	**0.2**	**81.7**
B2	0.5	0.5	0.5	172.3	23	0.4	69
B3	0.5	0.5	1	279.5	23.9	0.3	57.3
B4	0.5	0.5	1.5	309.9	23.18	0.2	53.4
B5	0.5	0.5	2	575	22.7	0.4	41.2
B6	**0.5**	**0.5**	**2.5**	**1326**	**23.2**	**0.5**	**26.2**
B7	0.5	1	0.1	144	25.09	0.2	78.5
B8	0.5	1	0.5	159	25.23	0.2	72.5
B9	0.5	1	1	274.4	25.09	0.2	58.3
B10	0.5	1	1.5	325.4	25.4	0.3	50.5
B11	0.5	1	2	358.9	25.2	0.4	49.9
B12	0.5	1	2.5	519.4	25.04	0.4	43.1
B13	**2**	**0.5**	**0.1**	**127.3**	**-13.22**	**0.2**	**83**
B14	2	0.5	0.5	201.6	-13.4	0.5	63
B15	2	0.5	1	239.7	-13.46	0.6	65.1
B16	2	0.5	1.5	494.5	-13.4	0.6	46.5
B17	2	0.5	2	576.5	-13.7	0.2	41.8
B18	**2**	**0.5**	**2.5**	**606.3**	**-13.2**	**0.2**	**37.2**

Table : Variables and their levels studied in CCD.

Independent Variables	Low(-1)	High(+1)
A: Amount of hyaluronic acid	0.5 mg/ml	2 mg/ml
B: Amount of chitosan	0.5 mg/ml	2 mg/ml
C: Amount of drug	0.1 mg/ml	2.5 mg/ml
Dependent Variables	**Constraints**	**AccepTable range**
Y1: Particle size	Minimize	<100 nm
Y2: Zeta potential	In range	-30 to +30
Y3: polydispersity index	In range	0.2-0.7
Y4: Entrapment efficiency	Maximize	100 %

Table : Different combinations used to prepare SPEC using CCD along with the responses studied

Independent variables				Dependent variables			
Batch code	**Conc. Of HA(A)**	**Conc. of CS(B)**	**Conc. of SOR (C)**	**Particle Size (nm)**	**Zeta Potential (mV)**	**Polydispersity Index**	**Entrapment Efficiency (%)**
N1	-1	1	-1	239.7	-13.46	0.6	67.1
N2	0	0	-1	173.5	23.9	0.4	70
N3	0	0	0	193.8	24.12	0.3	73.2
N4	-1	1	1	575	27.7	0.4	50.7
N5	-1	-1	-1	124.3	23.26	0.2	82
N6	1	1	-1	265.1	24.1	0.23	68.5
N7	0	1	0	325.4	25.4	0.35	58.8
N8	0	0	0	191.5	24.01	0.28	75.8
N9	0	-1	0	158.6	25.09	0.2	78.5
N10	1	1	1	298.4	25	0.4	51.5
N11	1	-1	-1	125.3	-13.2	0.2	82.1
N12	0	0	0	198	24.1	0.3	71.4
N13	0	0	1	195.7	24.6	0.26	72.2
N14	0	0	0	194.3	24.2	0.29	74.5
N15	0	0	0	196	24.3	0.3	73.9
N16	-1	0	0	151	25.23	0.2	79.5
N17	1	0	0	345.7	25.1	0.3	54.9
N18	1	-1	1	611	-13.2	0.2	38
N19	0	0	0	195.2	24.57	0.29	71.8
N20	-1	-1	1	1046	23.4	0.5	37.2

Effect of factors on Particle size:

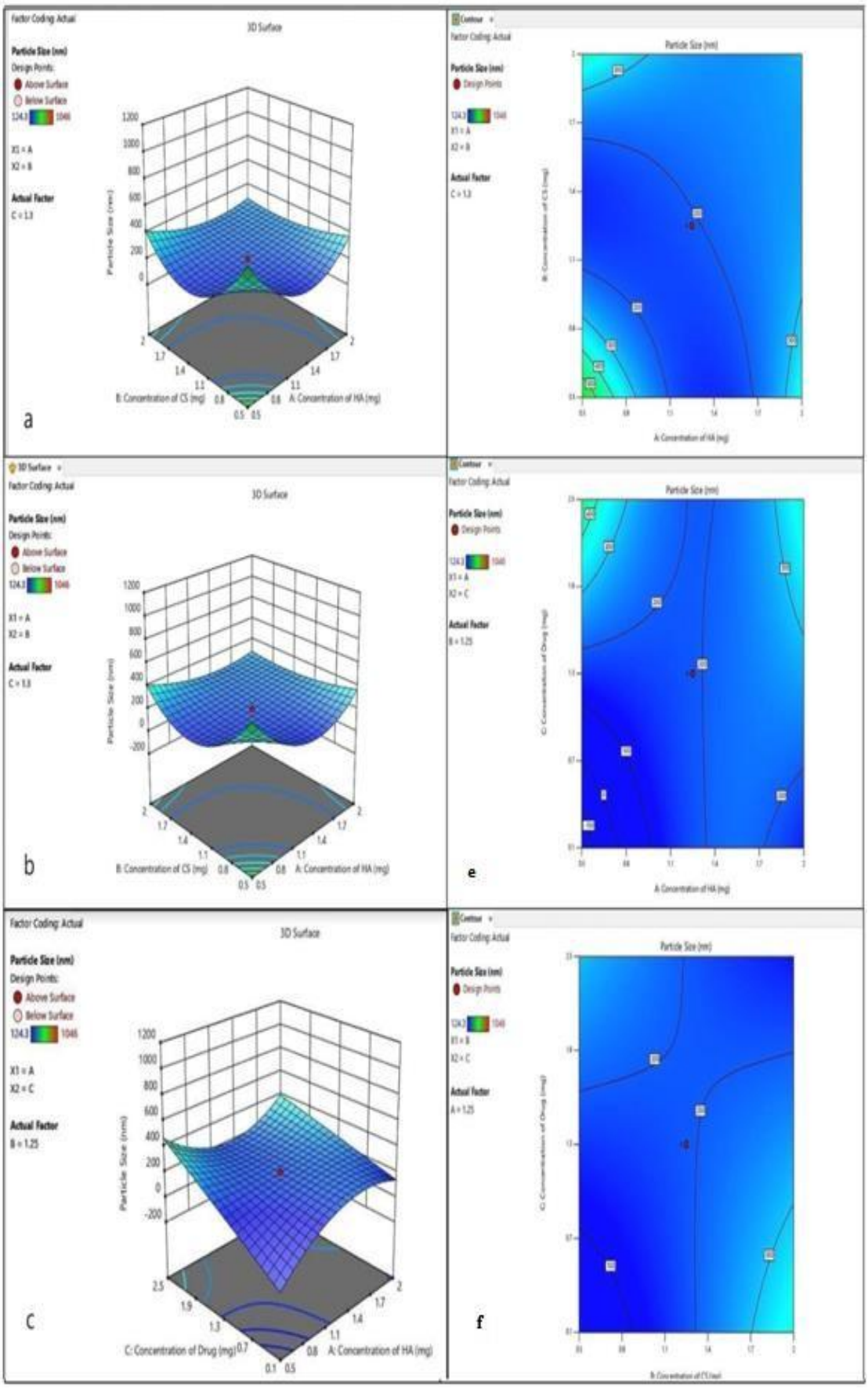

Fig.No.. Three D-response surface plots and Contour plots showing the effect of different variables on the particle size of SPEC (a & d) AB, (b & e) AC, and (c & f) BC Where, A:HA, B:CS, C:SUR

Effect of factors on zeta potential:

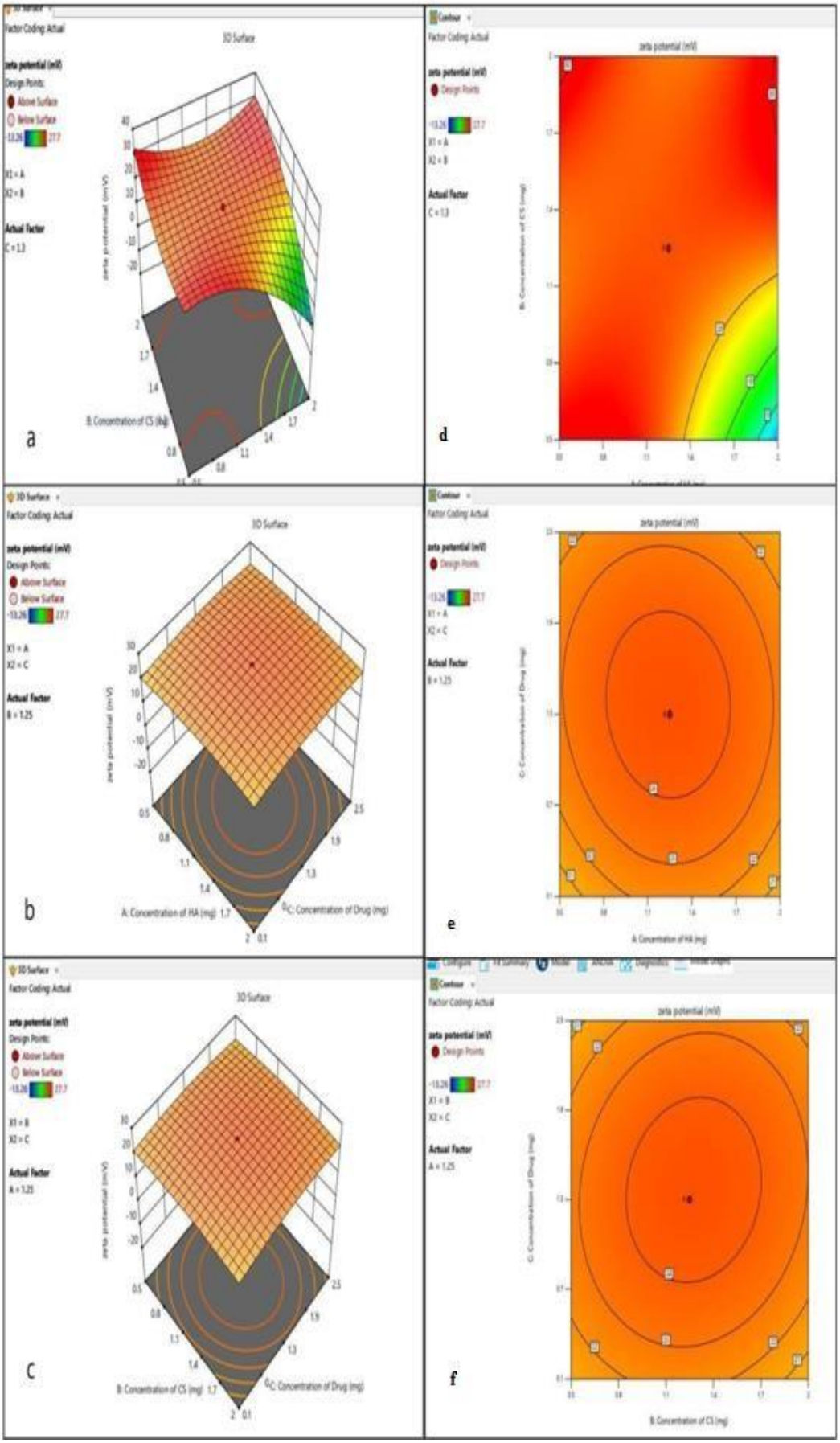

Fig.No.. Three D-response surface plots and Contour plots showing the effect of different variables on the zeta potential of SPEC: (a & d) AB, (b & e) AC, and (c & f) BC on Polydispersity index:

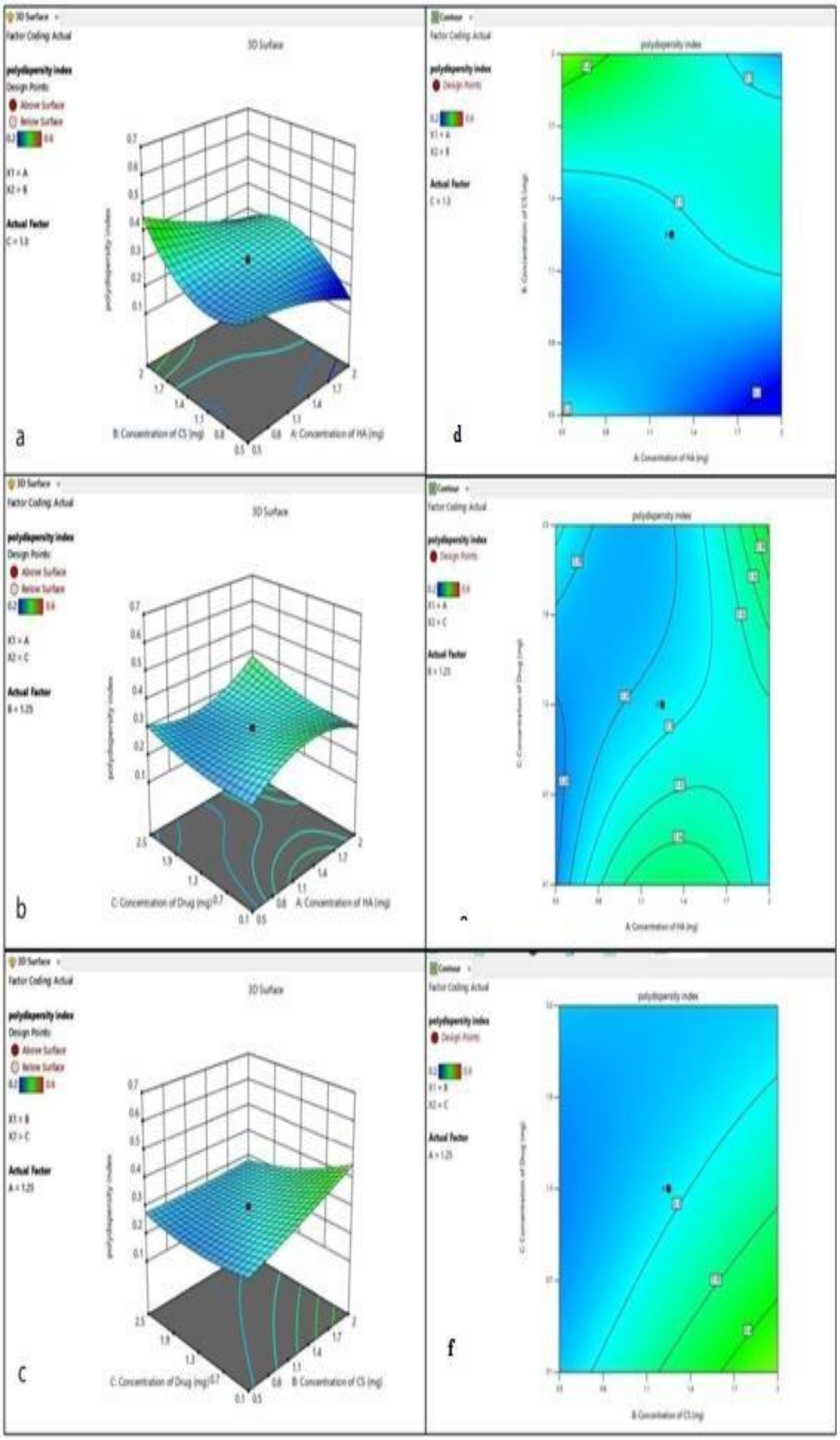

Fig.No.. Three D-response surface plots and Contour plots showing the effect of different variables on the polydispersity index of SPEC SPEC : (a & d) AB, (b & e) AC, and (c & f) BC Where, A: HA, B: CS, C: SUN

Effect of factors on Entrapment efficiency

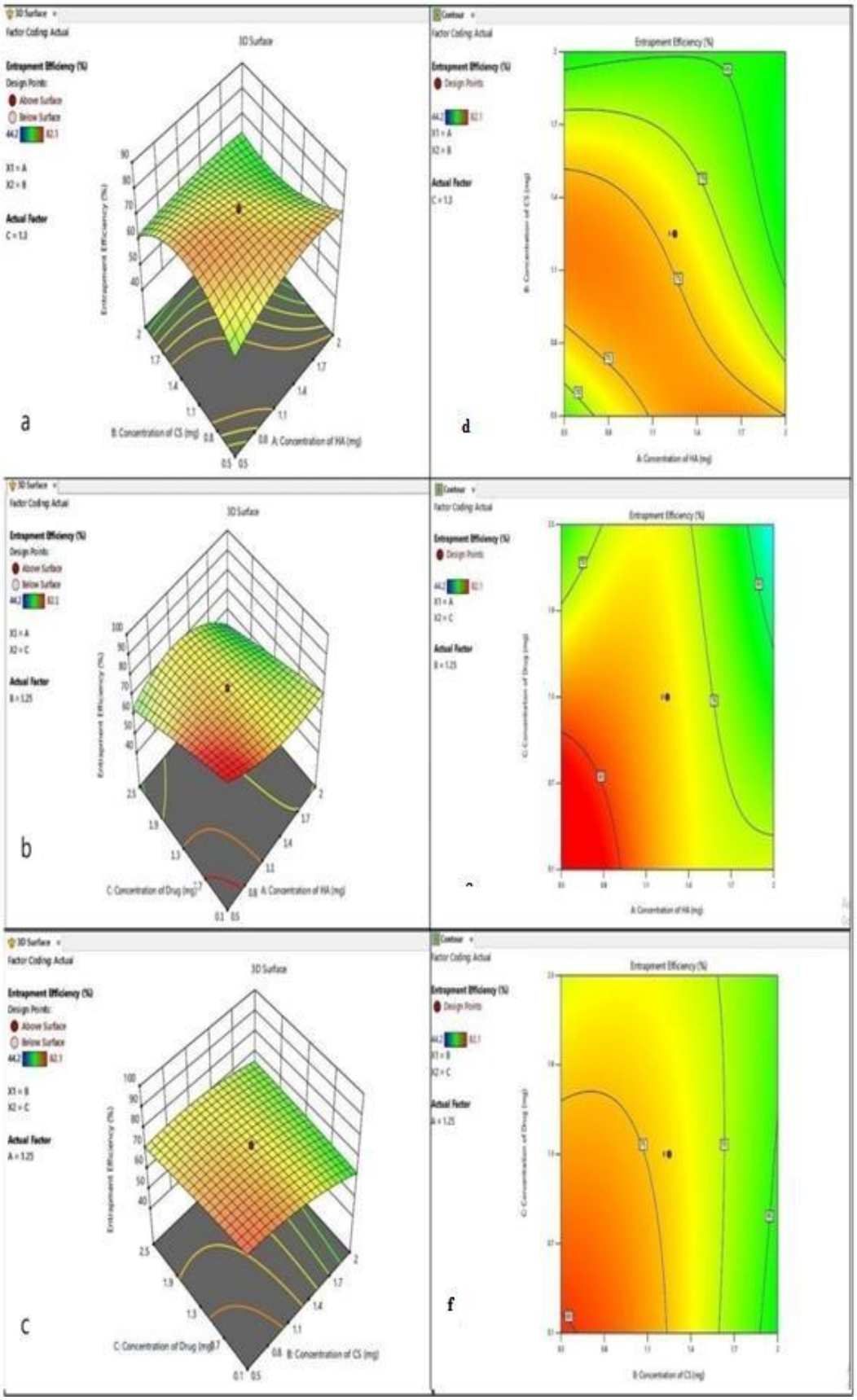

Fig.No.. Three D-response surface plots and Contour plots showing the effect of different variables on the entrapment efficiency of SPEC: SPEC (a & d) AB,b & e) AC, and (c & f) BC Where, A: HA, B: CS, C: SUN

Desirability and validation of the model:

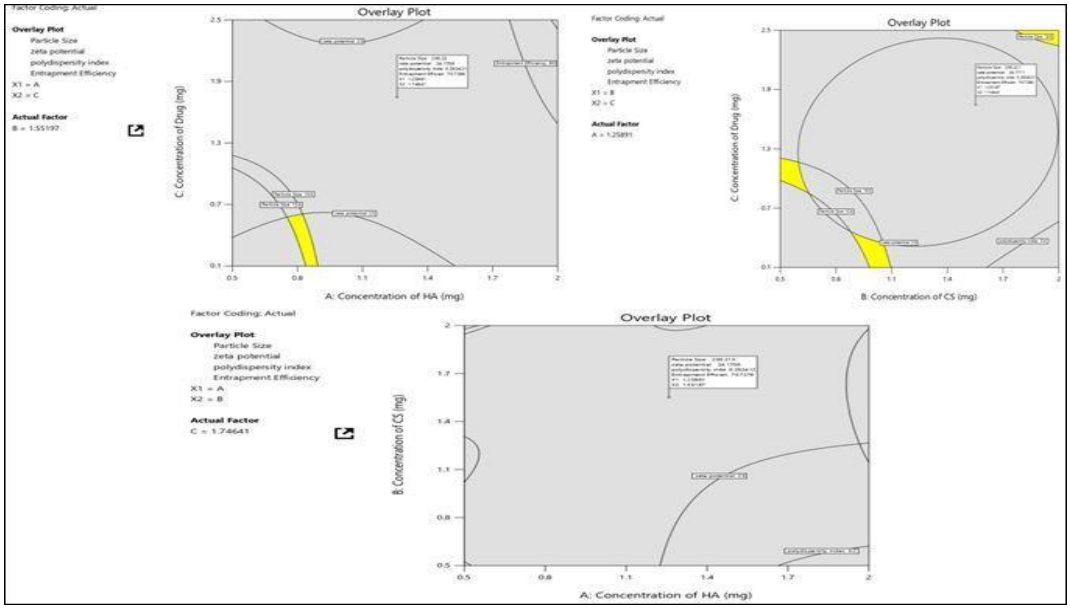

Fig.no. .Overlay plot indicating yellow color region as the optimized region and flagged point as the composition of optimized SPEC.

Table : Comparison of the predicted and experimental values of the response variables of validated SPEC (N11)

Responses	Predicted values	Experimental values
Particle size[nm]	121.272	125
Zeta potential [mV]	-12.49	-13.7
Polydispersity index	0.2	0.21
Entrapment efficiency [%]	82.1	82.07

*Note: Data represent the mean value ± standard deviation (n=6).

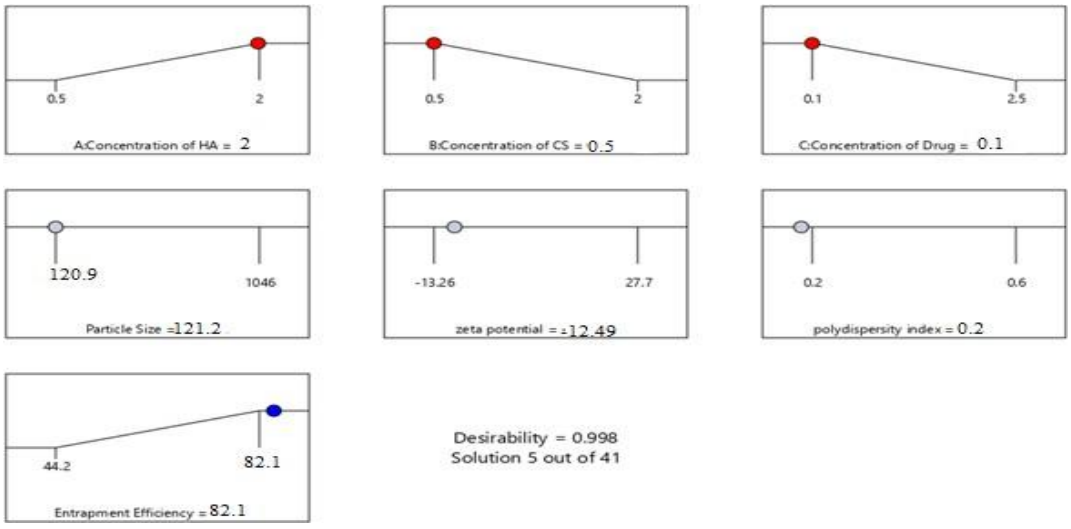

Fig. No. : Solution given by DOE with highest desirability.

Characterization Of blank PECNPs and Optimized Batch:

Scanning electron microscopy:

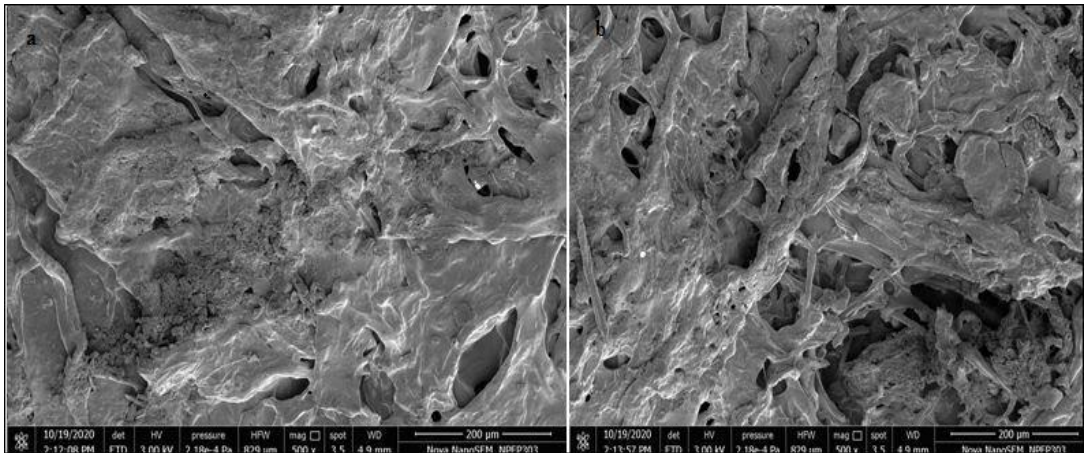

Fig. No. : SEM images of blank PECNPs (fig. a) and SPEC fig. b)

Particle size and polydispersity index of blank PECNPs and SPEC:

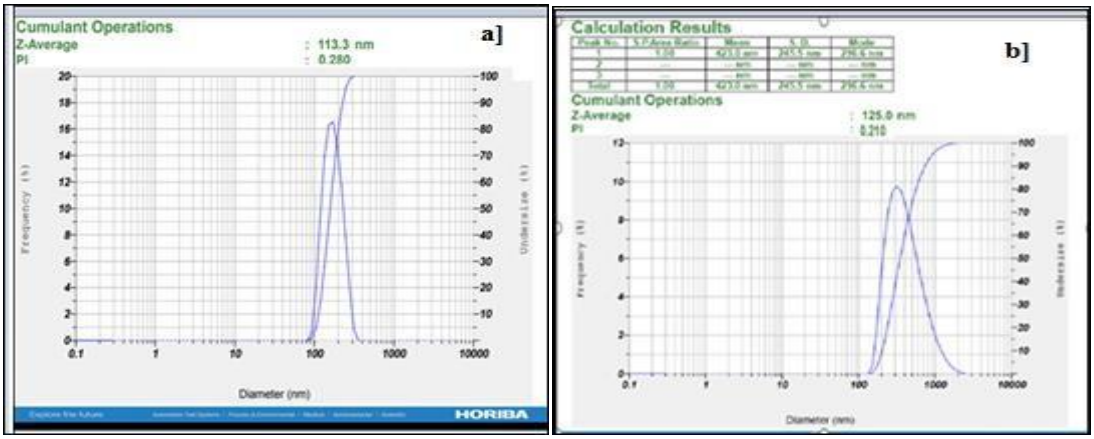

Fig. No. : Particle size of a] blank PECNPs and b] SPEC

Zeta potential of blank PECNPs and SPEC:

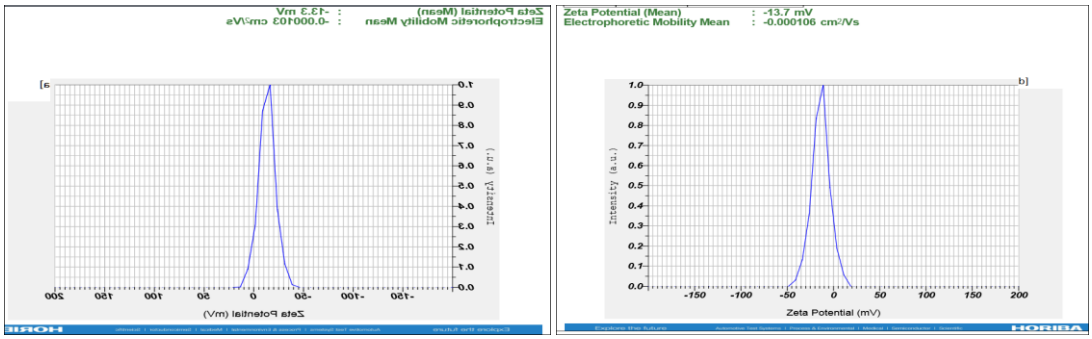

Fig. No. : Zeta potential of a] blank PECNPs (-13.3) b] SPEC -13.7)

FTIR Analysis:

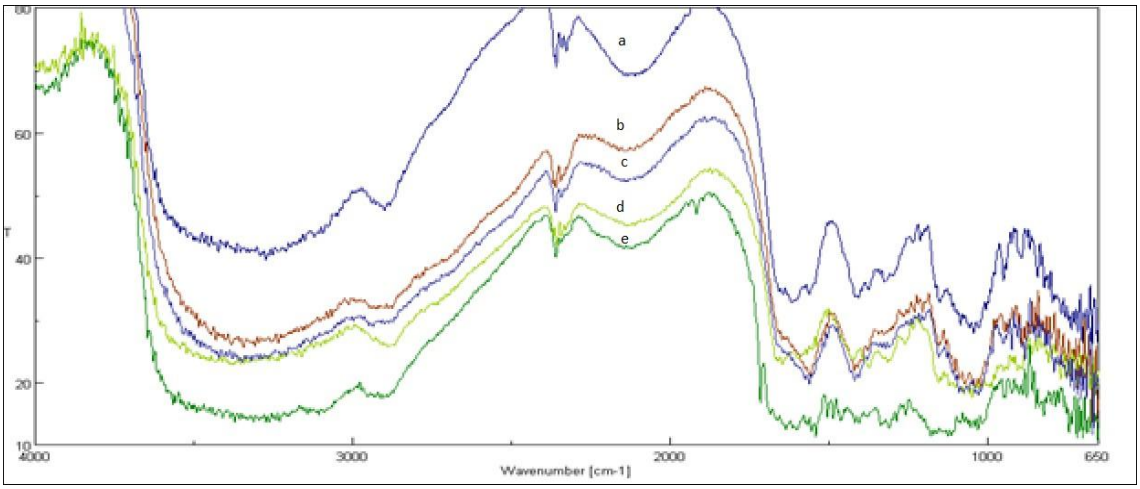

Fig. No. : FTIR spectrum of (a) HA, (b) CS, (c) pure drug, (d) blank PEC- NP, and (e) SPEC

Differential Scanning Calorimetry:

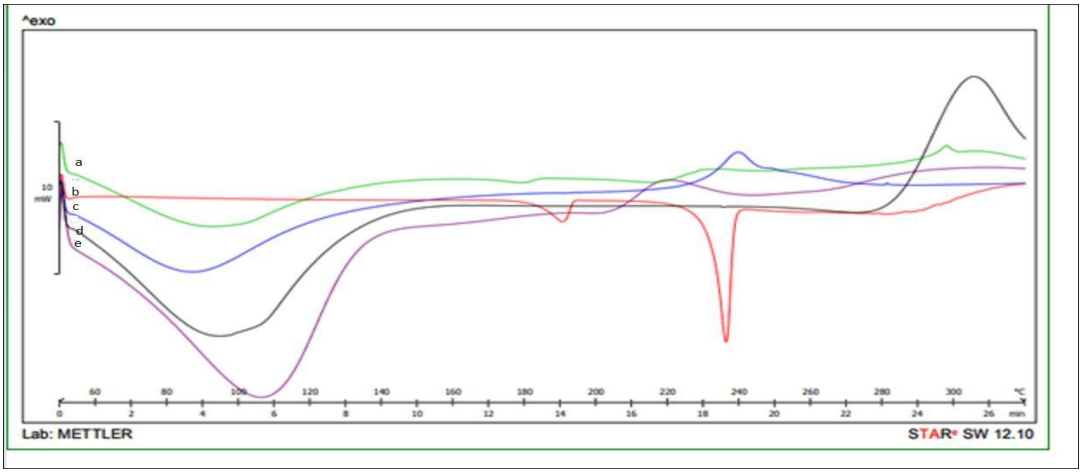

Fig. No. DSC thermograms of (a) HA (b) pure drug (c) CS (d) blank PEC- NPs and (e) SPEC

In Vitro Drug Release Study:

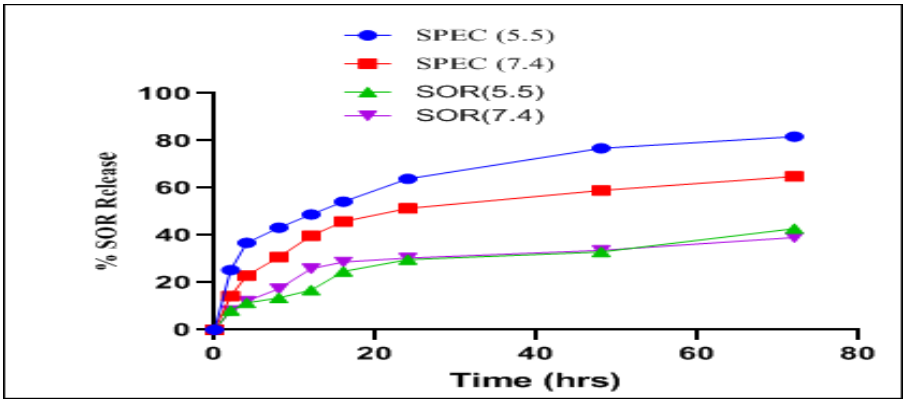

Fig. No. : In vitro drug release study of optimized batch and pure sunitinib in (5.5 pH buffer and 7.4 buffers).

Determination of aqueous Solubility of SUN in the form of SPEC:
The solubility of SUR in water and in the form of SPEC was found to be 0.0506 µg/ml and 0.83 µg/ml respectively.

Stability Studies Of SPEC:

Table : Stability study data of particle size, zeta potential, and encapsulation efficiency.

At 5°C±3°C	Particle size[nm]	Zeta potential [mV]	Polydispersity index	Entrapment efficiency [%]
Initial	125.3	-13.7	0.21	82.07
15 days	129.8	-13.6	0.2	81.9
30 days	132	-13.4	0.21	82.1
45 days	136.5	-13.6	0.2	81.8
60 days	127	-13.5	0.2	82.2
75 days	126.4	-13.5	0.2	81.6
90 days	126	-13.5	0.2	81.6

*Note: Data represent the mean value ± standard deviation (n=6).

CONCLUSION

The study successfully developed and characterized sunitinib-loaded hyaluronic acid-chitosan polyelectrolyte complex nanoparticles (SPEC) for targeted delivery in TNBC. The optimized formulation exhibited desirable physicochemical properties, enhanced cytotoxicity, and sustained drug release. These findings suggest that SPEC could be a promising nanocarrier for improving the therapeutic efficacy of sunitinib in TNBC treatment. Further in vivo studies are warranted to validate the potential of this formulation in clinical settings.

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