DEVELOPMENT OF PARTICULATE DRUG DELIVERY SYSTEM FOR SULFASALAZINE FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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Abstract

The aim of the present investigation is to prepare and evaluate Sulfasalazine nanoparticles and micro particles by solvent evaporation technique.

Six formulations of microspheres and nanoparticles were prepared by varying the concentrations of drug to polymer ratio & by using ethyl cellulose and eudragits100 as polymers. The obtained microspheres, and nanoparticles were characterized for surface morphology, FTIR, particle size, zeta potential, drug content, entrapment efficiency and In-vitro dissolution/diffusion studies.

Among the six formulations of microspheres M4 was found to be best formulation with entrapment efficiency of 95.8%, zeta potential value of -37.6mV. Among the sixformulations of nanoparticles N2 was found to be best formulation with entrapment efficiency of95.6% and zeta potential value of -35.8mV. Microspheres and nanoparticles were follows zero order kinetics with non-fickian diffusion mechanism.

Key words: -entrapment efficiency, Loading capacity, Particle size, Zeta potential, Drug release

Introduction: - Nanoparticles are sub-Nano sized colloidal structures ranging from 10 to 1000 nm and are composed of synthetic or semi synthetic polymers. Nanoparticles have been actively explored at delivery system for small drug molecules as well as macromolecules such as nucleic acids, peptides, proteins and hormones[1,2].

Polymeric nanoparticles offer few specific advantages of enhancing the stability of drugs/proteins and possess useful controlled release properties[3,4].

Microspheres are solid spherical particles ranging from 1-1000µm. They are spherical free flowing particles comprising of proteins or synthetic polymers, which are biodegradable in nature. Microspheres are sometimes referred to as micro particles[5,6].

An effectively-designed controlled drug delivery system can control few of the problems of the conventional therapy and enhance the therapeutic efficacy of a given drug. There are numerous approaches in delivering a therapeutic substance to the specific site in a sustained controlled release fashion. One such approach is utilizing microspheres as carriers for drugs.

Microspheres can be manufactured from several natural and synthetic materials. Glass microspheres, polymeric microspheres and ceramic microspheres are generally available. Solid and hollow microspheres vary broadly in density and, hence, they are used for different applications. Hollow microspheres are generally used as additives to lower the density of a material[7,8].

Sulfasalazine is an anti-inflammatory agent used in the treatment of inflammatory bowel diseases like ulcerative colitis and crohns disease. It is a disease altering anti-rheumatoid drug used in the second line treatment of rheumatoid arthritis when patients do not 2 respond to the NSAID's treatment. DMARDs not only reduces the pain and swelling of arthritis, but also prevent damage to joints, reducing risk of long-term loss of function. Available marketed formulations of sulfasalazine are salozopyrin enteric coated tablets500mg, Azulfidine 500mg tablets, enteric coated tablets. The usual dose of sulfasalazine at initial treatment is 1gm per day and increased up to 2-3 grams per day. It has side effects such as thrombocytopenia, megaloblastic anemia, Bone marrow depression, Folic acid deficiency, Impairment of male fertility (Oligospermia), Intestinal nephritis due to 5-ASA, Diarrhea, The biological half-life is 5-10hr; The bioavailability of sulfasalazine is 15% and as the conventional dosage forms has drawback of missing the doses, there is a need to develop a novel drug delivery system such as microspheres, nanoparticles and liposomes to increase the sustained release action thereby reducing the dose, dosing frequency and colonic side effects of the drug. As So in order to reduce the dose, dosing frequency and colonic side effects of the drug micro particulate and Nano particulate drug delivery system was designed for sulfasalazine.[9,10].

MATERIALS AND METHODOLOGY

PREPARATION OF SULFASALAZINE LOADED EC & EUDRAGIT S100 BY SOLVENT EVAPORATION METHOD

Materials Sulfasalazine (gift sample from Posh chemicals) Ethyl cellulose (SD fine-chem Limited, Mumbai.), Dichloromethane (SD fine-chem Limited, Mumbai.), Sodium CMC (SD fine-chem Limited, Mumbai), Tween 80 (SD fine-chem Limited, Mumbai

OPTIMISATION PARAMETERS: **OPTIMISATION PARAMETERS:**

Various parameters have been optimized in order to obtain sulfasalazine microspheres

Optimization different organic solvents:

The formulations were prepared by varying different organic solvents such as DMSO, DCM, and methanol.

Optimization of organic-aqueous phase ratio:

Three formulations were prepared by varying organic-aqueous phase ratio i.e. 1:2, 1:5 and 1:10 respectively.

Optimization of stirring speed (rpm)

Four formulations were prepared by varying stirring speed i.e. 400, 600, 800 and 1000 rpm respectively. Various parameters were optimized for the preparation of microspheres[11,12].

Preparation of sulfasalazine loaded EC microspheres by solvent evaporation method:

ECwas weighed and dissolved in dichloromethane to form homogenous solution. Sulfasalazine was weighed accurately and added to the above solution. The above organic solution was added dropwise to aqueous phase containing 50ml distilled water, sodium CMCand tween 80 as an emulsifying agent being stirred at 800rpm to emulsify the added dispersion as a fine droplet. The solvent removal was achieved by continuous stirring at room temperature for 3hrs to produce spherical microspheres. The formed microspheres were collected by filtration and washed repeatedly with distilled waterand air dried[13,14]. Threeformulations of microspheres were prepared by varying the concentration of drug: polymer ratios. Refer table 1 and 2.

Table 1: Composition of Sulfasalazine loaded EC microsphere formulations prepared by solvent evaporation method

S.No	Formulation code	Sulfasalazine	Ethyl cellulose	Dichloro methane	Sodium CMC	Tween 80
1	M1	50mg	150mg	10ml	50mg	1ml
2	M2	50mg	100mg	10ml	50mg	1ml
3	M3	50mg	50mg	10ml	50mg	1ml

Table 2: Composition of Sulfasalazine loaded EudragitS100 microsphere formulations prepared by solvent evaporation method

S.No.	Formulation code	Sulfasalazine	Eudragit S100	Acetone	Ethanol	Heavy liquid paraffin
1	M4	50mg	150mg	5ml	5ml	50ml
2	M5	50mg	100mg	5ml	5ml	50ml
3	M6	50mg	50mg	5ml	5ml	50ml

PREPARATION OF SULFASALAZINE LOADED EC & EUDRAGIT S100 NANOPARTICLES BY EMULSION SOLVENT EVAPORATION METHOD

Experimental methodology:

Sulfasalazine loaded ethyl cellulose / Eudragit S 100nanoparticles were formulated byemploying Emulsion–Solvent evaporation technique[15,16].Sulfasalazineand ethyl cellulose /EudragitS100 were dissolved in ethanol at various drug-polymer ratios.Then this organic dispersion was emulsified by mixing at a speed of 700rpm, with a REMI LAB overhead stirrer provided with a three labelled paddle rotor, into an aqueous external phase containing tween- 80 (0.25%) at

roomtemperature.The organic phase was added drop wise to the aqueous phase at a continuous rate. Stirring of the O/W emulsion was continued until the ethanol was evaporated. Then the resultant dispersion was collected by rotary vacuum evaporator and kept for dryingfor further studies. Six formulations of sulfasalazine loaded EC & ED100 nanoparticleswere prepared by changing the concentration of drug: polymer ratios. Refer table 3.

Table3: Composition of sulfasalazine loaded EC & ED100 nanoparticles prepared by Emulsion solvent evaporation method

S.No.	Formulation code	Sulfasalazine	Eudragit S100	Ethyl cellulose	Ethanol	Tween 80
1	N1	100mg	-	100mg	10ml	0.25ml
2	N2	100mg	-	150mg	10ml	0.25ml
3	N3	100mg	-	200mg	10ml	0.25ml
4	N4	100mg	100mg	-	10ml	0.25ml
5	N5	100mg	150mg	-	10ml	0.25ml
6	N6	100mg	200mg	-	10ml	0.25ml

CHARACTERIZATION AND EVALUATION PARAMETERS:

Scanning electron microscopy

Surface morphology was determined for all six formulations using Scanning electron microscopy (S -3700N, Hitachi, Japan) [17,18]

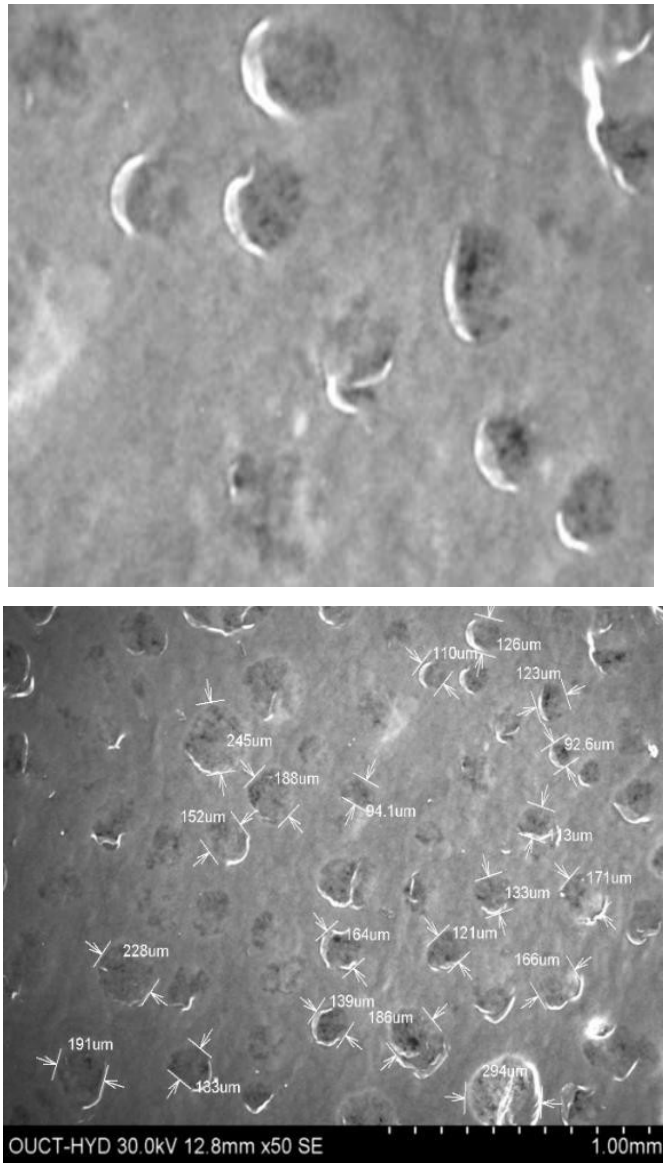

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Figure 1: SEM images of best formulation of microspheres

The SEM images were found to be spherical with size was in micro range as shown in figure 1.

Particle Size Distribution: -The prepared best microsphere formulations were characterized for particle size using Zetasizer (Malvern Instruments Ltd). The analysis was done at a temperature of 25ºC with double distilled water as dispersion medium [19,20].

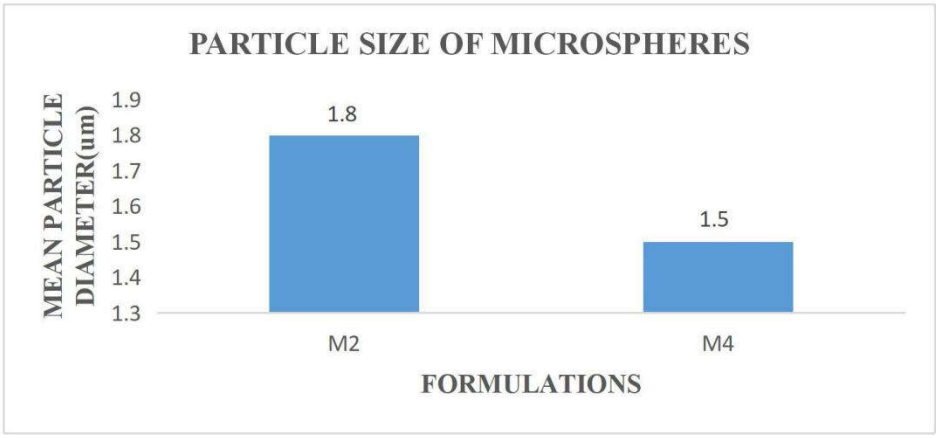


Figure 2: comparison of mean particle diameter of best formulations of Sulfasalazine microspheres prepared by solvent evaporation technique.

The formulations were in micro range. The mean particle diameter of best formulations was found to be1.8µm,1.5µm respectively as shown in figure 2.

Zeta potential: - The prepared best microspheres formulations were characterized for zeta potential value in order to know the stability of the formulations[21]. The analysis was done at a temperature of 25oC with double distilled water as dispersion medium as shown in figure 3 and 4.

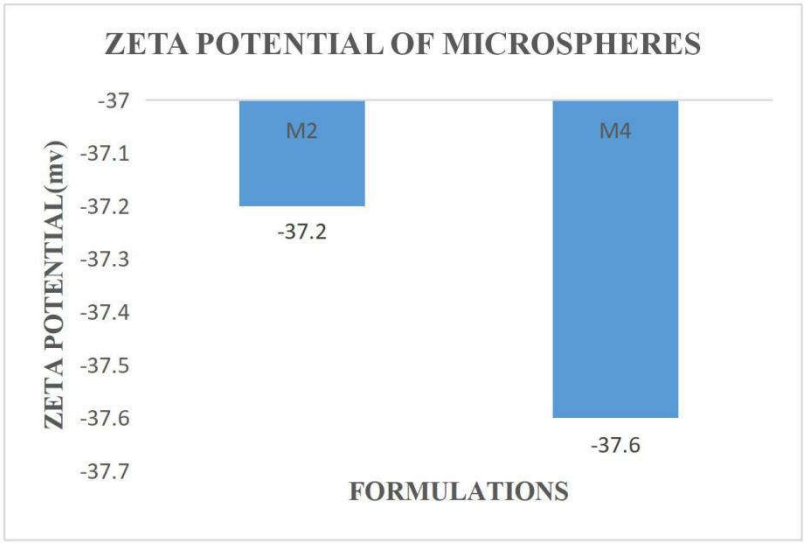
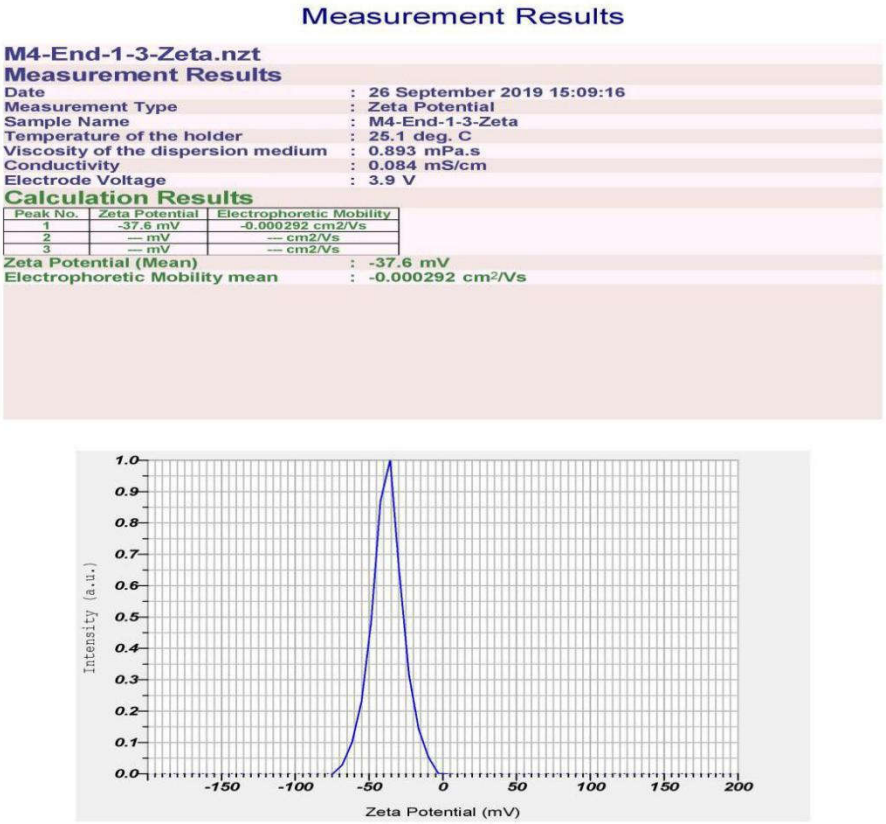


Figure 3: comparison of zeta potential values of best formulations of Sulfasalazine microspheres prepared by solvent evaporation technique.

From the results of the formulations were found to be stable. The zeta potential values of best formulations were found to be -37.2 mV, -37.6mV respectively.



Measurement Results

M4-End-1-3-Zeta.nzt

Measurement Results

Date	:	26 September 2019 15:09:16
Measurement Type	:	Zeta Potential
Sample Name	:	M4-End-1-3-Zeta
Temperature of the holder	:	25.1 deg. C
Viscosity of the dispersion medium	:	0.893 mPa.s
Conductivity	:	0.084 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-37.6 mV	-0.000292 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-37.6 mV
Electrophoretic Mobility mean	:	-0.000292 cm²/Vs

Figure 4: Zeta potential report of optimized formulation of Sulfasalazine loaded microspheres(M4) prepared by solvent evaporation technique.

FTIR Spectrum

The prepared six formulations were characterized for drug- excipient interactions using FT-IR (Horiba scientific, Ltd).

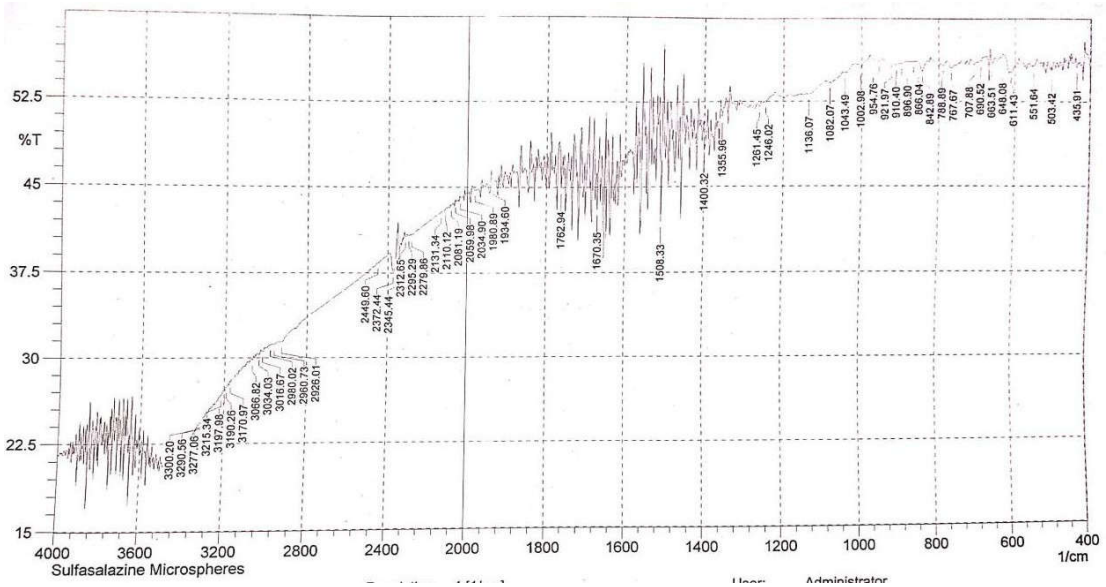


Figure 5: FTIR spectrum of optimized formulation of Sulfasalazine loadedmicrospheres(M4) by solvent evaporation technique

IR spectra developed for microsphers. The prominent peaks of sulfasalazine was observed at the region of 3197 due to O-H stretching, a peak at 3016 due to peak of amine N-H, a peak at 1670 due to C=O stretching, a peak at 1400 due to SO2 group and a peak at 1261 due to C-O stretching was observed. From the FTIR spectra of microspheres it was observed that all the characteristic peaks of sulfasalazine were present in microspheres. Fig 5 indicating compatibility of drug with excipients used in microspheres.

Drug content: - The prepared six formulations were evaluated for drug content as shown in figure 6.

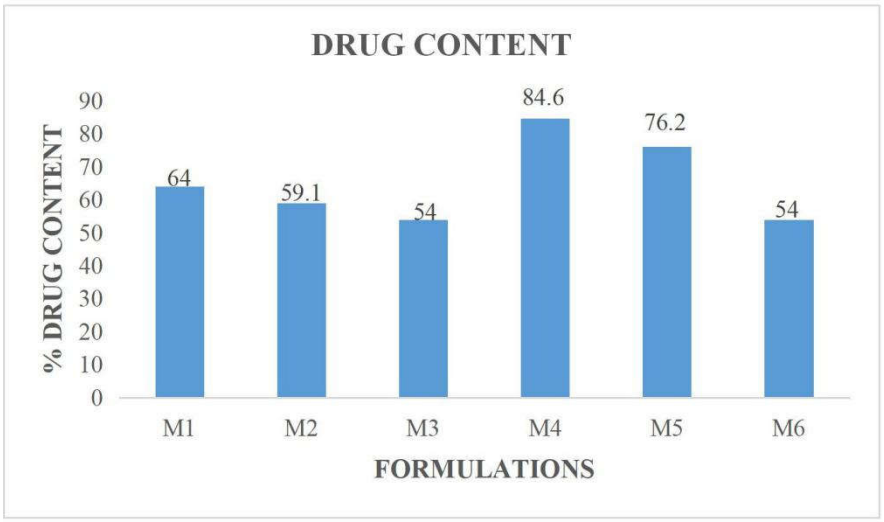


Figure 6: Comparison of drug content among the six formulations of sulfasalazine loaded EC & ES100 microspheres.

Drug content of M1,M2,M3,M4,M5 and M6 was found to be 64%,59.1%,54%,84.6%,76.2% and 54% respectively. From all the formulations M4 formulation found to be the highest one.

Encapsulation efficiency: - All the six formulations were evaluated for drug entrapment efficiency using cooling ultracentrifuge (Eltek, Mumbai). The result was shown in figure 7

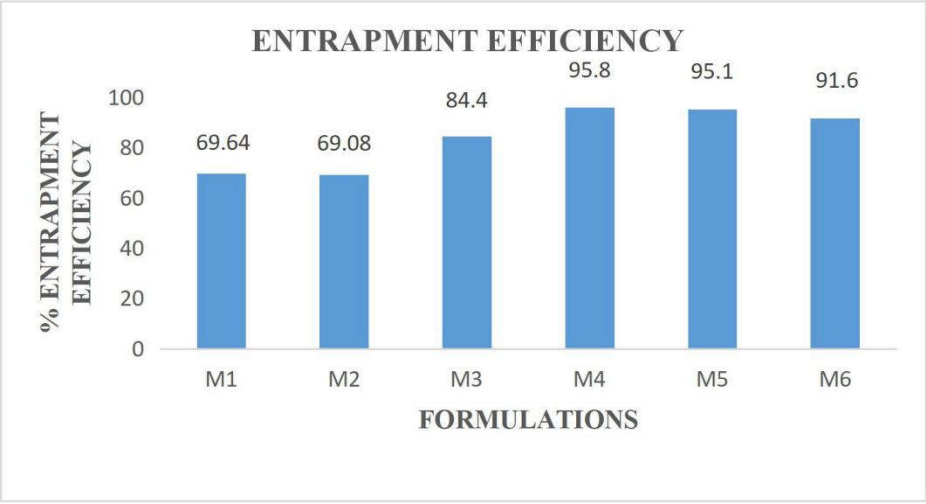


Figure 7: Comparison of drug entrapment efficiency among the six formulations of sulfasalazine loadedEC & ES100 microspheres.

Entrapment efficiency of M1, M2,M3,M4,M5 and M6 was found to be 69.64%, 69.08% 84.4%, 95.8%, 95.1% and 91.6% respectively. Out of all formulations M4 formulation found to be showing highest entrapment efficiency.

Loading capacity: - All the prepared formulations are evaluated for loading capacity

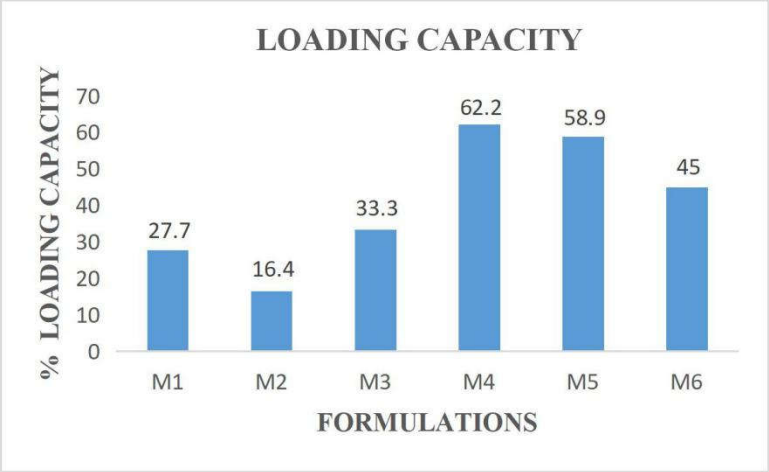


Figure 8: Comparison of loading capacity among the six formulations of sulfasalazine loaded EC& ES100 microspheres.

From figure 8 the loading capacity of all the formulationswere found to be 27.7%, 16.4%, 33.3%, 62.2%, 58.9% and 45% respectively. Among them M4 has greater loading capacity than other formulations.

Invitro release studies

Invitro drug release studies were performed by using orbital shaker and thestudy was conducted for period of 12 hours as shown in figure 9.

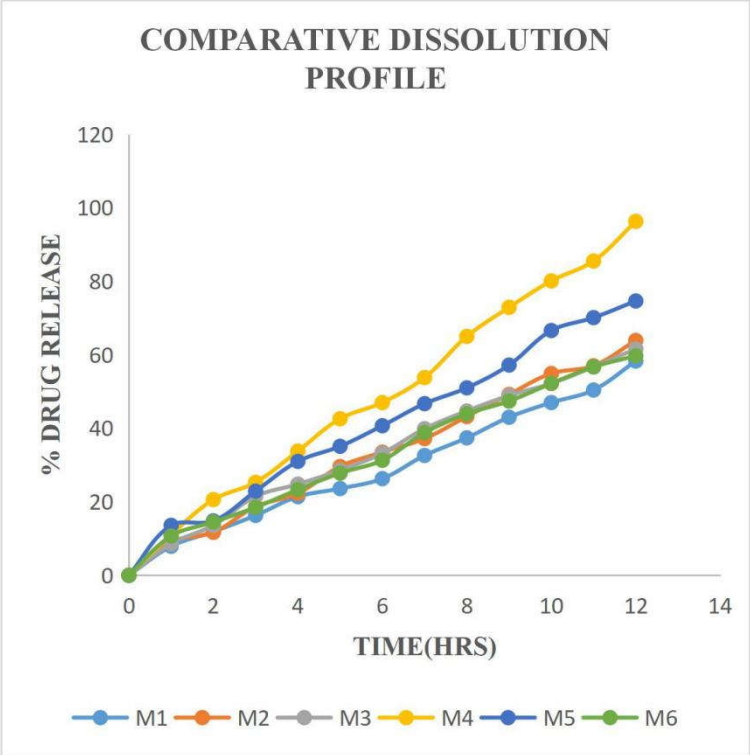


Figure 9: Comparative invitro drug release of sulfasalazine microspheres.

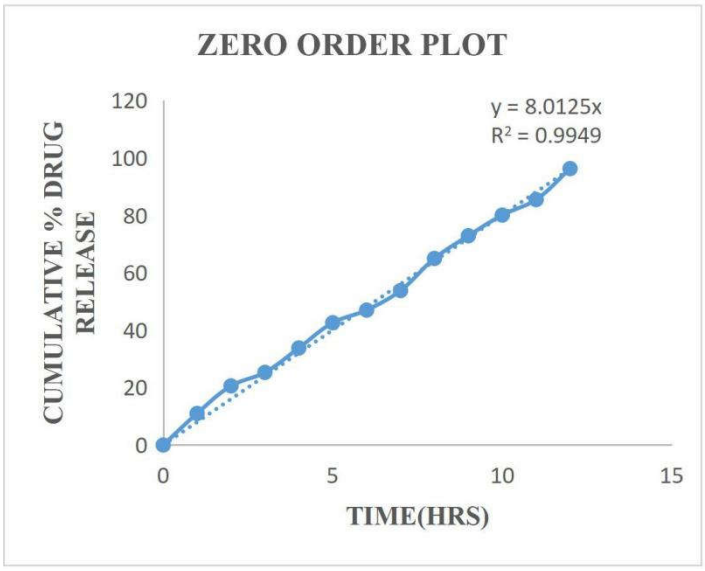
Invitro drug release ofM1,M2,M3,M4,M5 and M6 was found to be 58.3%,63.9%, 61.6%, 96.3%, 74.63% and 59.7% sustained for 12hrs respectively. From all formulations M4 formulation found to be sustained with 96.3% drug release rate. Polymer concentration has an effect on formulation degradation and drug release rate. Withenhance in polymer concentration the sustain release profile of the formulation was found to be enhanced. This is because increase in polymer concentration reduces the diffusivity of solvent through the formulation resulting in decreased drug release rate and thereby attain 14 sustained release.

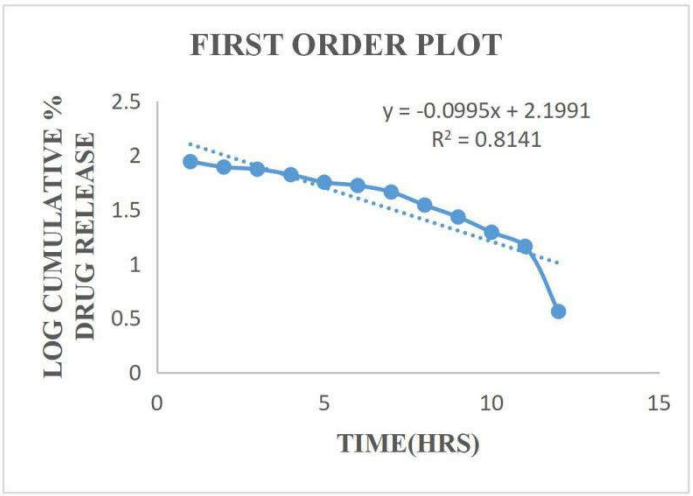
Comparison of best formulation with various kineticmodels

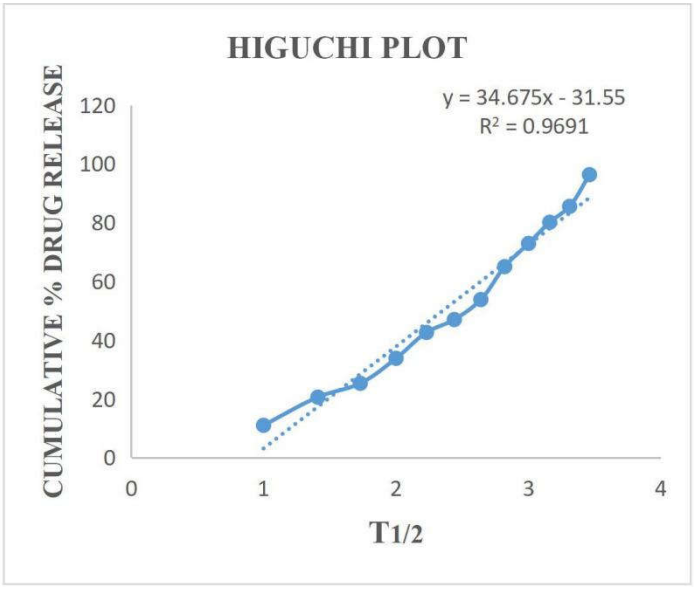
Comparison of better formulation with various kinetic models. Several plots (Zero order plot, first order plot, higuchi plot and peppas plots) were drawn in order to know the release kinetics and drug release mechanism. The data was shown in Figure 10 and table 4 and 5

Table 4: In-vitro drug release data of best formulation of sulfasalazine microspheres(M4)

S.No	%drug release (X±S.d) (n=3)	%drug remaining	log% drug release	TIME (hrs)	T1/2	log T	log% drug remaining
1	11±0.88	89	1.04	1	1	0	1.86
2	20.6±0.68	79.4	1.31	2	1.41	0.301	1.79
3	25.3±0.59	74.7	1.40	3	1.73	0.477	1.78
4	33.8±0.32	66.2	1.52	4	2	0.602	1.73
5	42.6±0.05	57.4	1.62	5	2.23	0.698	1.66
6	47±0.80	53	1.67	6	2.44	0.778	1.56
7	53.8±0.41	46.2	1.73	7	2.64	0.845	1.48
8	65±0.76	35	1.81	8	2.82	0.903	1.4
9	72.9±0.94	27.1	1.86	9	3	0.95	1.30
10	80.1±0.09	19.9	1.90	10	3.16	1	1.13
11	85.5±0.18	14.5	1.93	11	3.31	1.04	0.95
12	96.3±0.21	3.7	1.98	12	3.46	1.07	0.73


ZERO ORDER PLOT
y = 8.0125x
R² = 0.9949
CUMULATIVE % DRUG RELEASE
120
100
80
60
40
20
0
0
5
10
15
TIME(HRS)


FIRST ORDER PLOT
y = -0.0995x + 2.1991
R² = 0.8141
LOG CUMULATIVE % DRUG RELEASE
2.5
2
1.5
1
0.5
0
0
5
10
15
TIME(HRS)


HIGUCHI PLOT
y = 34.675x - 31.55
R² = 0.9691
CUMULATIVE % DRUG RELEASE
120
100
80
60
40
20
0
0
1
2
3
4
T1/2

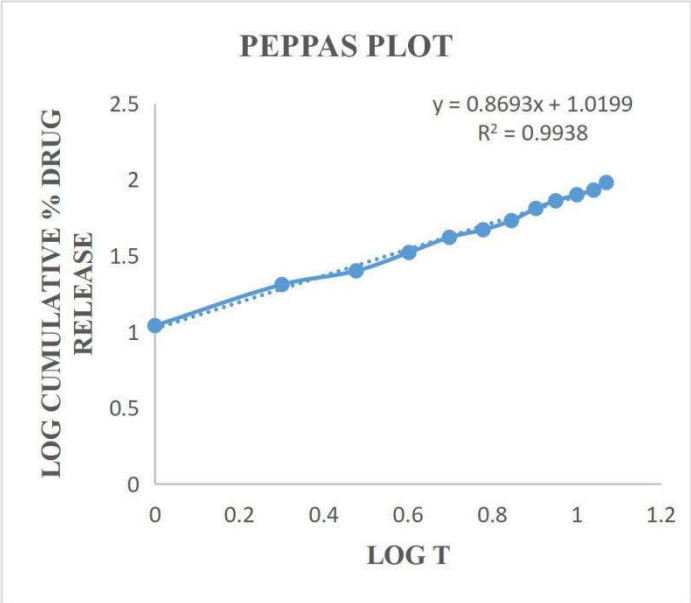


Figure 10: Drug release kinetic plots of best formulation of sulfasalazine microspheres

Table 5: Data for in-vitro plots of sulfasalazine microspheres using Eudragit S100polymer

Formulation	Zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppas plot (n)
M4	**0.994**	0.814	0.969	**0.869**

From the results it was concluded that the drug release was following zero order kinetics with non-fickian diffusional pathway.

RESULTS AND DISCUSSION OF SULFASALAZINE NANOPARTICLES

Based on the literature review a general procedurewas followed for the preparation of sulfasalazine nanoparticles. The process variables were optimized to yield sulfasalazine nanoparticles withhigh drug content, entrapment efficiency and sustained release.

CHARACTERIZATION AND EVALUATION PARAMETERS:

Scanning electron microscopy Surfacemorphology was determined for all six formulations using Scanning electron microscopy (S-3700N, Hitachi, Japan).

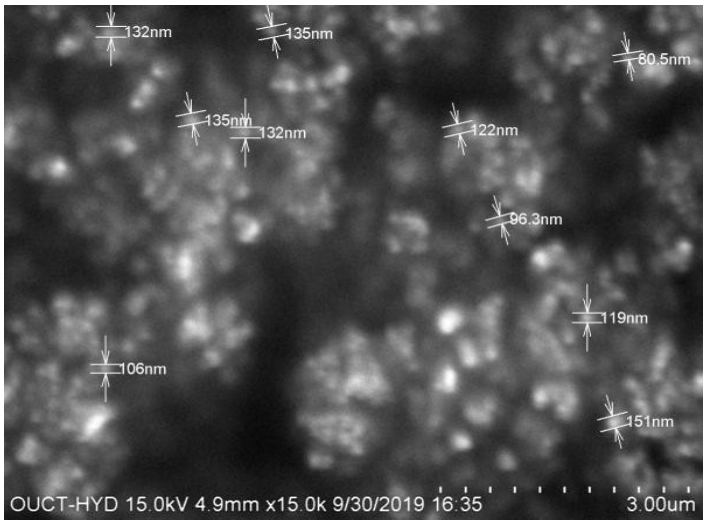


Figure 11: SEM images of best formulation of nanoparticles.

The SEM images were found to be spherical and the size was in nano range as shown in figure 11

Particle Size Distribution

The prepared best nanoparticleformulations were characterized for particle size using Zetasizer (Malvern Instruments Ltd). The analysis was done at a temperature of 25oC with double distilled water as dispersion medium[26].

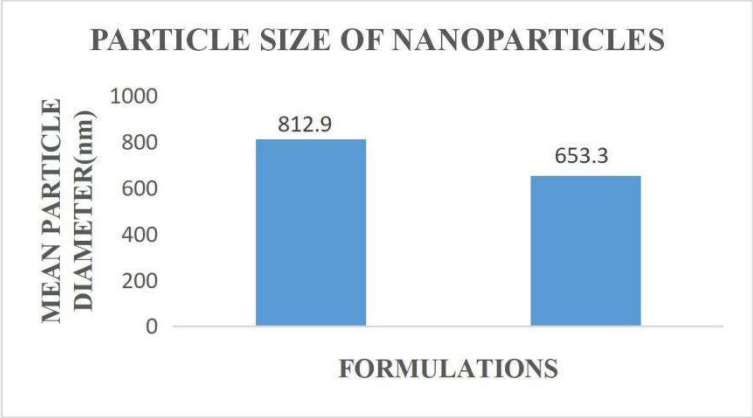


Figure 12: comparison of mean particle diameter of best formulations of Sulfasalazine nanoparticles prepared by emulsion solvent evaporation technique.

The formulations were in Nano size range.The mean particle diameter of best formulations was found to be 812.9 & 653 .3nmrespectively as shown in figure 12.

Zeta potential

The prepared best nanoparticles formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25oC with double distilled water as dispersion medium. Figure 13: comparison of zeta potential values of best formulations of Sulfasalazine nanoparticles(N2) prepared byemulsion solvent evaporation technique. The result was shown in figure 13.

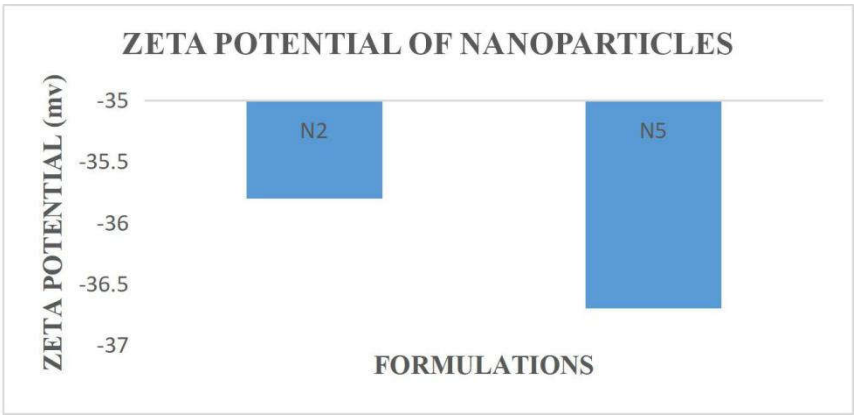


Figure 13: comparison of zeta potential values of best formulations of Sulfasalazine

nanoparticles(N2) prepared by emulsion solvent evaporationtechnique.

From the results of the formulations were found to be stable. The zeta potential values of best formulation was found to be -35.8 mV & -36.7mV respectively.

FTIR Spectrum

The prepared six formulations were characterized for drug- excipient interactions using FT-IR (Horiba scientific, Ltd).

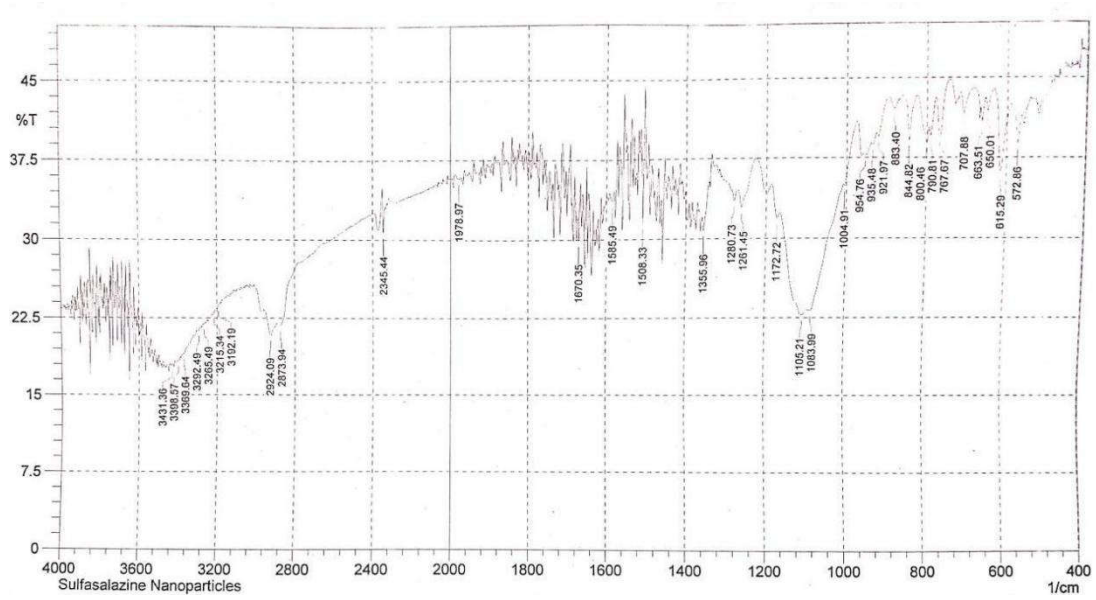


Figure 14: FTIR spectrum of optimized formulation of Sulfasalazine loaded nanoparticles by emulsion solvent evaporation technique.

IR spectra developed for Nanoparticles. As per the figure 14 the prominent peaks of sulfasalazine was observed at the region of 3192 due to O-H stretching, a peak at 2873 due to peak of amine N-H, a peak at 1670 due to C=O stretching, a peak at 1355 due to SO2 group and a peak at 1261 due to C-O stretching was observed. From the FTIR spectra of nanoparticles. It was observed that all the characteristic peaks of sulfasalazine were present in nanoparticles. fig indicating compatibility of drug with excipients used in nanoparticles.

Drug content:- The prepared six formulations were evaluated for drug content.

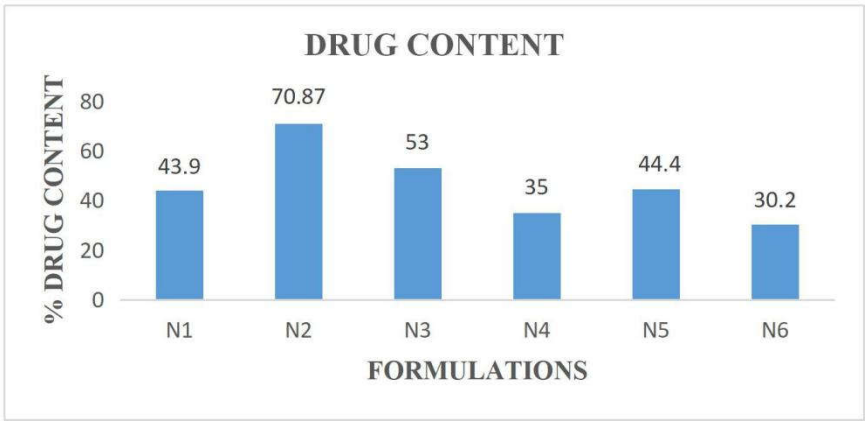


Figure 15: Comparison of drug content among the six formulations of sulfasalazine loaded EC & ES100 Nanoparticles.

The drug content of N1, N2, N3, N4, N5 and N6 formulations were found to be 43.9%, 70.87%, 53%, 35%, 44.4% and 30.2%. N2 has high drug content when compared to other formulations as shown in figure 15.

Encapsulation efficiency

Encapsulation efficiency All the six formulations were evaluated for drug entrapment efficiency using cooling ultra centrifuge (Eltek, Mumbai).

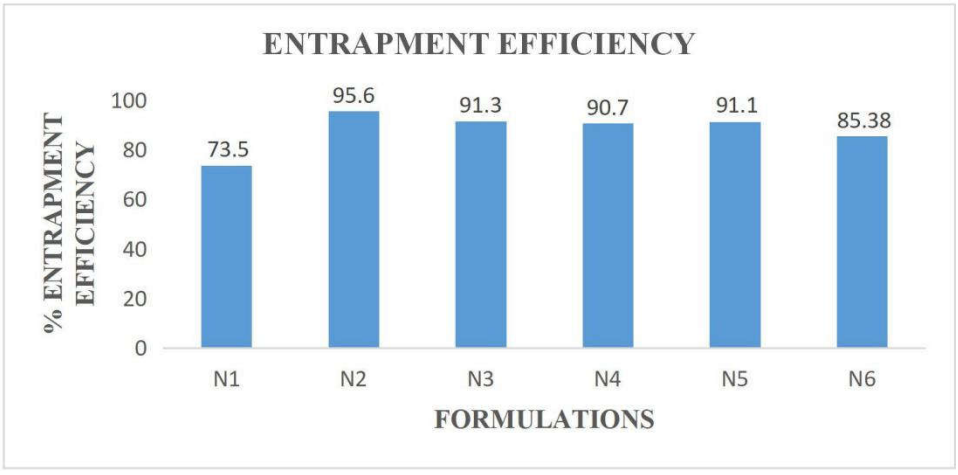


Figure 16: Comparison of drug entrapment efficiency among the six formulations of sulfasalazine loaded

EC & ES100 Nanoparticles. The entrapment efficiencies of N1, N2, N3, N4, N5 and N6 formulations were found to be 73.5%, 95.6%, 91.3%, 90.7, 91.1% and 85.38%. Among the formulations, N2 has greater entrapment efficiency. As N2 formulation has the propotion of 1:2 for drug and polymer, maximum amount of the drug was entrapped in the polymer. The polymer concentration was found to be sufficient to entrap the drug into it as shown in figure 16.

Loading capacity

The prepared formulations were evaluated for loading capacity.

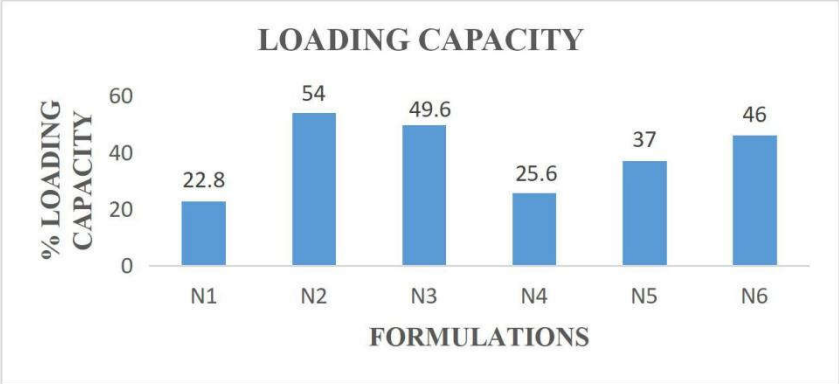


Figure 17: Comparison of loading capacity among the six formulations of sulfasalazine loaded EC & ES100 Nanoparticles.

The loading capacities of all the formulations were found to be 22.8%, 54%, 49.6%, 25.6%, 37% and 46% respectively. Among them N5 has greater loading capacity than other formulations as shown in figure 17.

Invitro release studies

Invitro drug release studies were performed by using orbital shaker and thestudy was conducted for period of 12 hours. The result was shown in figure 18.

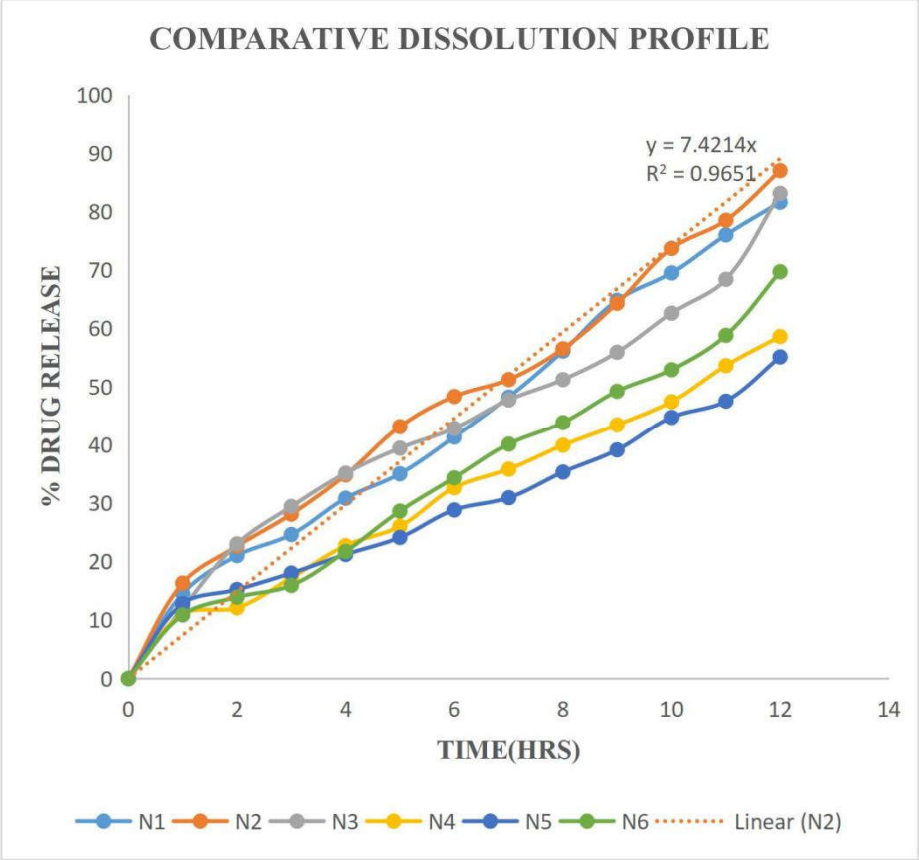


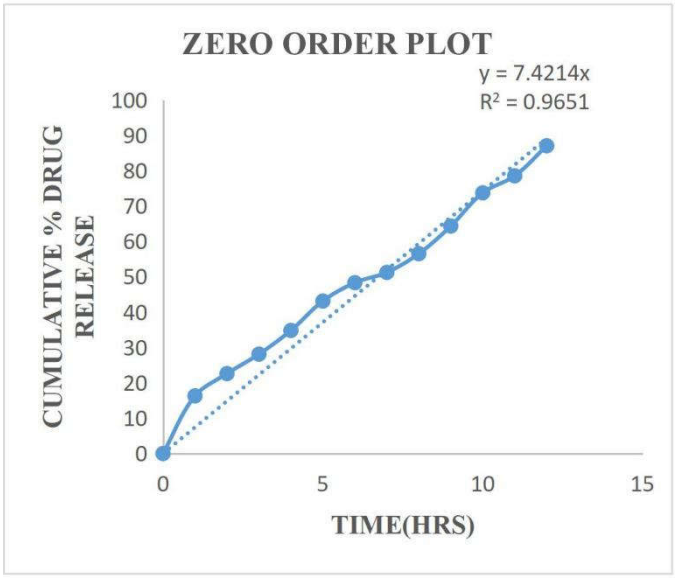
Figure 18: Comparative invitro drug release of sulfasalazine nanoparticles.

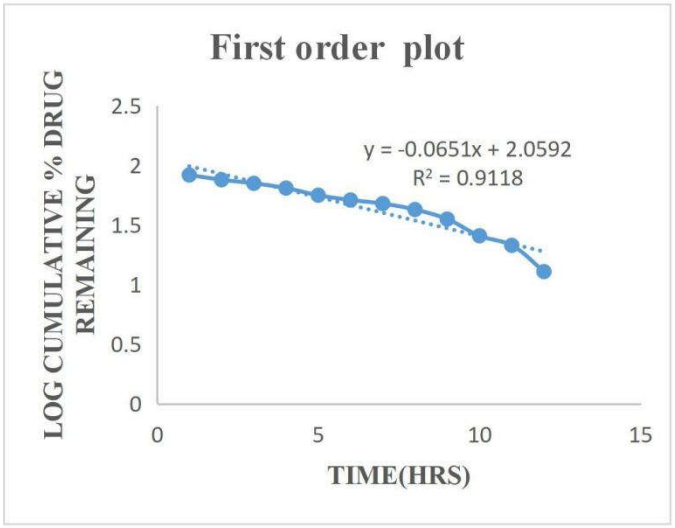
Invitro drug release of N1,N2,N3,N4,N5 and N6 was found to be 81.2%, 87%, 83.1%, 58.6%, 55.1% and 69.7% in 12hrs respectively. From all formulations N2 formulation found to be sustained with 87% drug release rate.

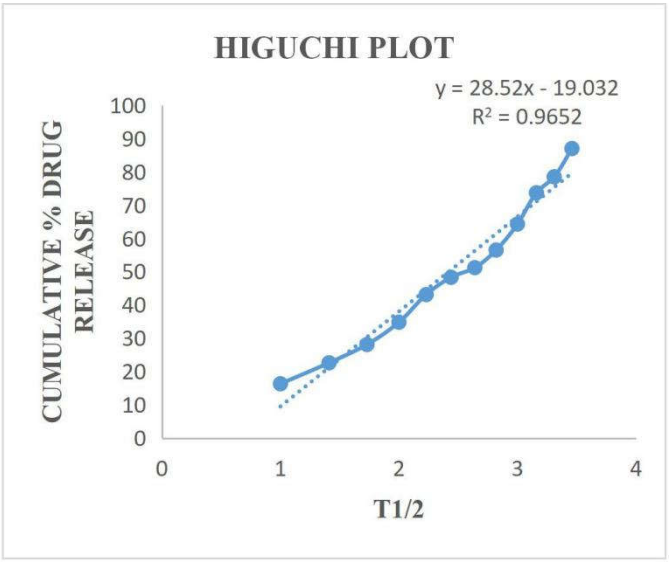
Comparison of better formulation with various kinetic models Several plots (Zero order plot, first order plot, higuchi plot and peppas plots) were drawn in order to know the release kinetics and drug release mechanism. The data was shown in figure 19 and table 6 and 7.

Table 6: Invitro drug release data of best formulation of sulfasalazine nanoparticles(N2).

S.No	%drug release (X+S.d) (n=3)	%drug remaining	log% drug release	TIME (hrs)	T1/2	log T	log% drug remaining
1	16.3±0.86	83.7	1.21	1	1	0	1.92
2	22.6±0.99	77.4	1.35	2	1.41	0.301	1.88
3	28.1±0.44	71.9	1.44	3	1.73	0.477	1.85
4	34.8±0.61	65.2	1.54	4	2	0.602	1.81
5	43.1±0.25	56.9	1.63	5	2.23	0.698	1.75
6	48.3±0.73	51.7	1.68	6	2.44	0.778	1.71
7	51.2±0.07	48.8	1.70	7	2.64	0.845	1.68
8	56.5±0.94	43.5	1.75	8	2.82	0.903	1.63
9	64.3±0.19	35.7	1.80	9	3	0.95	1.55
10	73.7±0.38	26.3	1.86	10	3.16	1	1.41
11	78.5±0.52	21.5	1.89	11	3.31	1.04	1.33
12	87±0.73	13	1.93	12	3.46	1.07	1.11


ZERO ORDER PLOT
y = 7.4214x
R² = 0.9651
CUMULATIVE % DRUG RELEASE
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
TIME(HRS)


First order plot
y = -0.0651x + 2.0592
R² = 0.9118
LOG CUMULATIVE % DRUG REMAINING
2.5
2
1.5
1
0.5
0
0
5
10
15
TIME(HRS)



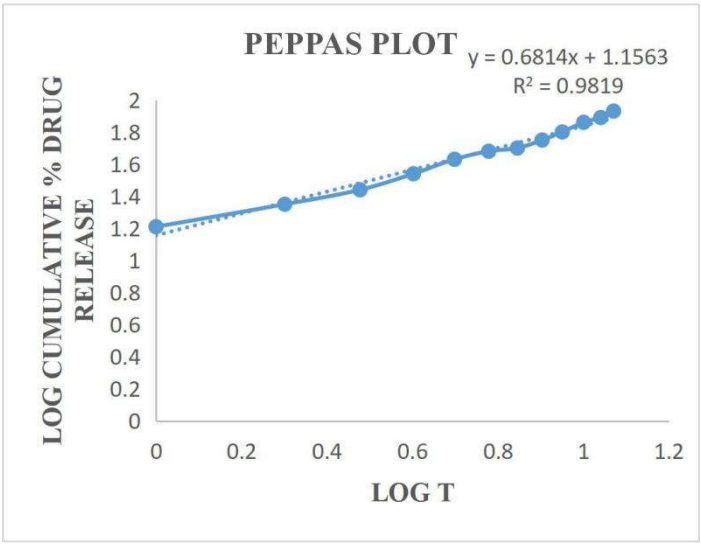


Figure 19: Drug release kinetic plots for best formulation of sulfasalazine nanoparticles

Table 7: Data for invitro plots of best formulation of sulfasalazinenanoparticles

Formulation	Zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppas plot (n)
M4	**0.965**	0.911	0.965	**0.681**

Conclusion: Using ethyl cellulose and Eudragit S100 as polymers, sulfasalazine nanoparticleswere prepared by emulsion solvent evaporation method. Six formulations were prepared by varying drug to polymer ratio. Out ofthe six formulations the N2 formulation containing ethyl cellulose as polymerwas found to be the best formulation with high drug content of 70.87%, entrapment efficiency of 95 .6% andinvitro drug release data of 87% and drug release was sustained upto 12hrs.

DISCUSSION:

Sulfasalazine is an anti-inflammatory drug and used in the treatment of inflammatory bowel diseases including ulcerative colitis and chron's disease. As the drug ishaving the low bioavailability and having side effects,this research is aimed to develop novel drug delivery system for sulfasalazine like microspheres and nanoparticles to sustain the release of drug in colon region. FTIR studies were conducted for sulfasalazine, microspheres and nanoparticles formulations inorderto check the compatibility of drug with excipients. From IR spectrait was observed that all the characteristics peaks of sulfasalazine were present in combination spectra. Hence its was concluded that there are no interactions between drug and excipients indicating compatibility of drug excipients.Microspheres were prepared by solvent evaporation technique. Six microsphere formulations were prepared. ThePreparedmicrospheres were evaluated for drug content, entrapment efficiency, invitro drug release andfor zeta potential. Three formulationsM1(1:3), M2(1:2) and M3(1:1) were prepared by using EC as a polymer. Among them M2 formulation is showing better result with entrapment efficiency of 69.08%, particle size of 1.8 µm and zeta potential of -37.2mV indicating good stability. Three formulation M4(1:3), M5(1:2) and M6(1:1) were prepared by using ED100 as a polymer. Among them M4 formulation is showing better result with entrapment efficiency of 95.8%, particle size of 1.5µm and zeta potential of -37.6mV indicating good stability. From all the evaluation and characterization parameters it was concluded that M4 formulation containing ED 100 (1:3) as a polymer is showing highest entrapment efficiency of 95.8% and zeta potential of -37.6mV indicating good stability when

compared to other microsphere formulations. With the result it is concluded that polymer concentration has an effect on formulation degradation and drug release rate. Withincrease in polymer concentration the sustain release profile of formulation was found to be increased. This is because increase in polymer concentration reduces the diffusivity of solvent through the formulation resulting in decreased drug release rateand thereby attained sustained release. Nanoparticleswere prepared by emulsion solvent evaporation technique. Six nanoparticle formulations were prepared. Theprepared nanoparticleswere evaluated for drug content, entrapment efficiency, invitro drug release andfor zeta potential. Three formulationsN1(1:1), N2(1:2) and N3(1:3)were prepared by using EC as a polymer. Out of them N2 is showing better result with high entrapment efficiency of 95.6%, invitro drug release of 87% and Zeta potential of -35.8mV indicating good stability. Three formulationN4(1:1), N5(1:2) and N6(1:3) were prepared by using ED100 as a polymer. Out of them N5 is showing better result with high entrapment efficiency of 91.1%, invitro drug release of 55.1% and zeta potential of -36.7mV respectively. Comparison was made between the best formulations of EC and ED100 microspheres, N2 formulation having EC(1:2) as a polymer was considered as best formulation.

Jyothika mattam 2015 prepared sulfasalazine microspheres by solvent evaporation method using tween 80 1% as an emulsifying agent. Solvent evaporation method was used to entrap hydrophobic drugs. Five formulations were prepared by varying the drug: polymer ratio using ethyl cellulose as polymer. The best formulation was showing product yield of 86.5%, drug content of 94.2%, entrapment efficiency of 84.6% and invitro drug release of 90.7% for 12 hours. Where as in the present work the best formulation of microspheres M4 containing ED 100 (1:3) as a polymer is showing highest entrapment efficiency of 95.8% and zeta potential of -37.6mV indicating good stability.

CONCLUSION: - In the present study Sulfasalazine loaded microspheres and nanoparticles were successfully prepared.Microspheres and nanoparticleswere prepared by solvent evaporation technique.For the preparation of microspheres in solventevaporation technique acetone, ethanol, dichloromethane were used as organic solvents and various process parameters like organic-aqueous ratio, stirring speed, various organic solvents were optimized. Sixformulations were prepared by varying the concentrations of drug and polymer& by using EC and ED100 as a polymer. Out of the six formulations, M4 formulation containing ED100 as a polymerwas found to be the best formulation with drug content of 84.6%, entrapment efficiency of 95.8%, loading capacity of62.2%. Invitrodrug release data showed 96.3%% of drug release sustainedupto 12hrs. Theformulation followed Zero order kinetics with non fickian diffusion pathway. For thepreparation of nanoparticles inemulsion solvent evaporation technique tween-80 was used as an emulsifying agent and ethanol was used as organic solventand various process parameters likestirring speed and stirring ratios were optimized. Six formulations were prepared by varying the concentrations of drug and polymer

& by using EC and ED100 as a polymer. Out of the six formulations, N2 formulation containing EC as a polymerwas found to be the best formulation with drug content of 70.87%, entrapment efficiency of 95.6%, loading capacity of49.6%. Invitrodrug release data showed 87% of drug release sustainedupto 12hrs.The formulation followed Zero order kinetics with non fickian diffusionpathway. Both micro and nano formulations are considered as good drug delivery systems for sulfasalazine because of their highest entrapment efficiency and sustain drug release property.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No animals/humans were used in this study.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
The data supporting the findings of this study are available within the article.

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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

1. SreeGiri Prasad B., Gupta V. R. M., Devanna N., Jayasurya K.,(2014) Microspheres as drug delivery system – A review, *JGTPS*. 2014;5(3): 1961-72.

2. Mohan M., Sujitha H., Dr. Rao V. U. M., Ashok M., Arun kumarB. A brief review on mucoadhesive microspheres, *IJRRPAS*. 2014;4(1):975-86.

3. Ramteke K.H, Jadhav V.B, Dhole S.N.Microspheres as carriers used for novel drug delivery system *IOSR Journal of Pharmacy 2012;*Vol. 2(4):PP44-48.

4. Y.M.Chein, 1992 Novel Drug Delivery System, second edition, revised & expanded, Marcel Dekker, Inc, New York.

5. Agusundaram M, Madhu Sudana Chetty et al. 2009 Microsphere as A Novel Drug Delivery System A Review. *International Journal of ChemTech Research.2009:* 1(3):526-534.

6. Kavita Kunchu, Raje Veera Ashwani et al. 2010 Albumin Microspheres: A Unique system as drug delivery carriers for non steroidalanti-inflammatory drugs 2010;5(2):12.

7. Singh prashant, prakash Dev, Ramesh B, Singh Neha, Mani Tamizh T 2011 Biodegradable polymeric microspheres As Drug Carriers; *A review Indian Journal of Novel Drug Delivery*.2011;3(2):70-82.

8. Patel N.R. , Patel D.A., Bharadia P.D.,PandyaV., Modi V. Microsphere as a novel drugdeliver, *Int Journal of Pharmacy & Life Sciences*.

2011;2(8):992-997.

9. Prasanth V.V., Moy A.C., Mathew S.T.,MathapanR. Microspheres: an overview. Int J ofPharm& BiomedicalSci..2011;2(2):332-338

10. JainD.,PandaA.K.,Majumdar D.K., EudragitS100 Entrapped Insulin MicrosphereforOralDelivery, *AAPS Pharm Sci Tech*.2005;6(1):101-107.

11. Chinna G.B., Shyam S.R., Vimal K.M., SleevaR.M., SaiK.M. Formulation and Evaluation ofIndomethacin Microspheres using natural andsynthetic polymers as Controlled Release DosageForms, *Int J of Drug Discovery*.2010 ;2(1):8-16.

12. ZhouW.Q., Gu T.Y., Su Z.G., Ma G.H., Syntheses of macroporous poly (styrene-divinyl benzene) microspheres by surfactant reverse micellesswelling method, Science DirectPolymer. 2007;48:1981-1988.

13. Ramteke K.H., Jadhav V.B., Dhole S.N., Microspheres: As carriers used for novel drug delivery system, *IOSRPHR*. 2012;2(4):44-48.

14. Kataria Sahil1, Middha Akanksha1, Sandhu Premjeet1, Ajay Bilandi and Bhawana Kapoor. Microsphere: a review*, International journal of research in pharmacy and chemistry*.2011;1(4): pg1184-1198.

15. S.Tamizhrasi, A.Shukla, T.Shivkumar, V.Rathi, J.C.Rathi. Formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles. International Journal of Pharm Tech Research. 2009;1(3):411-415.

16. C.E. Mora-Huertas, H. Fessi, A. Elaissari. Polymer-based nanocapsules for drug delivery. *International Journal of Pharmaceutics*. 2010; 385:113–142.

17. Abhishek Garg et al. Formulation, Characterization and Application on Nanoparticle: A Review. Der Pharmacia Sinica. 2011 ; 2(2):17-26.

18. Calvo P., Remunan-Lopez C., Vila-Jato JL. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. Journal of Applied Polymer Science.1997; 63:125-132.

19. Betancor, L., Luckarift HR. Bioinspired enzyme encapsulation for

biocatalysis. Trends in Biotechnology. 2008; 26:566-572.

20. Chiranjib Chakraborty, Soumen Pal, George Priya Doss.C, Zhi-Hong Wen, Chan-Shing Lin. Nanoparticles as smart pharmaceutical delivery. *Frontiers in Bioscience*. June 2013; 18:1030-1050.

21. Abhishek Garg, Sharad Visht, Pramod Kumar Sharma and Nitin Kumar.2011, Formulation, Characterisation and Application on Nanoparticle: A Review. *Der Pharmacia Sinica*. 2011 ; 2(2):17-26.