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FORMULATION CHARECTERIZATION AND OPTIMIZATION OF PROCESS VARIABLES OF CHITOSAN NANOPARTICLES CONTAINING SULFASALAZINE

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ABSTRACT

Polymeric nanoparticles are solid particles prepared from polymers in which drug is dissolved, entrapped, encapsulated or attached. Chitosan Nanoparticles gained a lot of attention as drug delivery carriers because of their better stability, safe, non toxic and providing versatile routes of administration. The objective of the present study is to prepare, optimized formulations of chitosan nanoparticles containing sulfasalazine using Ionotropic gelation method, and to study the effect of formulation variables like polymer, cross linking agent, and drug concentration and process variables like stirring speed on product yield, drug content, entrapment efficiency, loading capacity, particle size, and zeta potential. Sodium tripolyphosphate (STPP) is used as cross linking agent and sulfasalazine is choosed as model drug. Nanoparticles were obtained in the average particle size range from 262nm to 853nm for different formulations. All the nanoparticles contain maximum positive surface charges in the range of 33.21 to 8.51mV. Entrapment efficiency of Nanoparticles ranged between 66 to 85 % and drug content ranged from 69.23 to 82.0%. The prepared particles showed good drug-loading capacity. *In vitro* release of sulfasalazine loaded Nanoparticles showed a rapid initial burst, followed by a slow drug release and followed zero order kinetics with non fickian diffusion model. Formulation IV showed good sustained release & maximum drug release (85%) within 9 hours interval, as compared to other samples. Nanoparticles got good stability at refrigeration temperature (4^0C). Good results are observed in FIV-SS3 sample i.e., at 900rpm.

KEYWORDS: Chitosan, Sulfasalazine, Stirring Speed, STPP, Ionic Gelation, Nanoparticles.

1. INTRODUCTION

Nanotechnology involves creation and utilization of materials, devices or systems on nanometer scale. The pharmaceuticals designed using Nanotechnology is called Nanopharmaceuticals. Nanoparticles are defined as particulate dispersions or solid particles with size in the range of 1-1000nm. Nanoscale devices smaller than 50 nanometres can easily enter most cells, while those smaller than 20 nanometres can move out of blood vessels as they circulate through the body. Because of their small size, nanoscale devices can readily interact with bio molecules on both the surface and inside cells. Polymeric Nanoparticles are solid particles prepared from polymers in which drug is dissolved, entrapped, encapsulated or attached.

Chitosan Nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration. Sulfasalazine is a sulfa drug, a derivative of mesalazine. It is used in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease. It is commonly used drug but, till today a less research work has been done on this drug. Ionotropic gelation method is selected because of its simple handling, economical, less consuming time, and availability of different modifications. Sustained release system is designed to release a drug at a predetermined rate in order to maintain a constant drug concentration for a specific period of time with minimum side effects.

In this work, the influence of optimized conditions is systematically investigated. The effects of formulation variables and process variables on entrapment efficiency, loading capacity, particle size, and zeta potential were studied. Stability studies and release of model drug sulfasalazine, is also performed, for best samples selected from optimized formulations based on particle size to testify the potential application of these Nanoparticles. Yet So far less attention has been given to the Nanoparticle carriers and optimization studies, in particular sulfasalazine loaded chitosan Nanoparticles.

2. MATERIALS AND METHODS

2.1. Materials: Sulfasalazine was purchased from Posh Chemicals, Hyderabad. Chitosan (LMW) is bought from Sigma Aldrich-Bangalore; Sodium Tripolyphosphate (TPP) is purchased from Finar Chemicals, Hyderabad. Acetic acid, Ethanol, Methanol, Buffer Solution (pH-7.0), & Milli-Q water purchased from Orbital Scientific Products- Hyderabad and all are analytical grade.

2.2. Formulation of Sulfasalazine loaded chitosan Nanoparticles: Chitosan Nanoparticles were prepared by using Ionotropic gelation method. Nanoparticles were formed with the electrostatic interactions occurring between the negatively charged sodium tripolyphosphate (TPP) and positively charged amino groups of chitosan polymer. Sodium tripolyphosphate is used as a cross linking agent because it is nontoxic, multivalent and able to form colloidal particulate solution with chitosan polymer. Chitosan were weighed in different amounts and dissolved in 1%v/v acetic acid. STPP were weighed and dissolved in milli-Q water. Sulfasalazine were dissolved in 3% Ethanol. Polymer and drug solution were added in appropriate ratios and stirred under magnetic stirring at 600rpm for 5-6hrs. And STPP solutions were added drop wise in the time interval of 5 min. Four formulations were prepared by optimizing the formulation and process variables like polymer, STPP, Drug and Stirring speed. First formulation contains different polymer concentrations (0.2 to 0.4%w/v). Second formulation contains different cross linking agent concentrations (0.4 to 0.8%w/v). Third formulation contains different drug concentrations (0.06 to 0.12%). Fourth formulation contains different stirring speed (400, 600, 800 rpm).

3. CHARACTERZATION OF DRUG LOADED CHITOSA NANOPARTICLES

3.1 Average Particle Size, Particle Size Distribution and Zeta Potential: The average Particle Size of the optimized formulations was determined by photo correlation spectroscopy (a technique used to determine the diffusion coefficient of small particles in a liquid) with a particle size analyzer (Delsa Nano C- Beckman Coulter) equipped with the SOP software. Samples were prepared by dispersing the suitable amount of Nanoparticles in 5ml of distilled water and ultra sonicated for 1 hr. The surface charge (zeta potential) was determined by measuring the velocity of the particles suspended in a liquid medium under an applied electric field i.e., Electrophoretic Mobility. (Delsa nano-C Beckman coulter) Zeta potential analyzer is used. Samples were prepared by dispersing the suitable amount of Nanoparticles in 5ml of distilled water and ultra sonicated for 1 hr.

3.2 Drug Content: Drug content in the preparation was determined by crushing and dissolved in buffer solution of pH 7.0 and stirred for 30 minutes. In this method, the Nanoparticles (20mg) were crushed and stirred in 20ml buffer solution pH 7.0 until dissolved; it was filtered through a Millipore filter and drug content was determined after suitable dilution, at 352nm by UV spectrophotometer (Shimadzu 1700). A standard calibration curve of sulfasalazine was plotted by UV Spectrophotometrical method for this purpose. The Drug content of Nanoparticles was calculated according to equation (1).

$$\mathrm{DC}\% = \frac{(\text{Amount of drug present in the sample})}{(\text{Total amount of drug loaded initially})} \times 100 \quad \ldots\ldots(1)$$

3.3 Entrapment Efficiency: For determination of entrapment efficiency, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV-spectrophotometer at 352nm. In this method the Nanoparticles 20mg were dispersed in Centrifuge tubes which consist of 20ml of buffer solution (pH-7.0). These tubes are centrifuged at 15000 rpm for 60 minutes at 5^0C by Remi C-25 cooling centrifuge. The %EE and %LC is calculated by using the equation.(2)

$$\%\ \text{Entrapment efficiency} = \frac{(\text{Total drug content} - \text{free drug in supernatant})}{(\text{Totaldrug content})} \times 100 \ldots\ldots(2)$$

$$\%\ \text{Loading capacity} = \frac{(\text{Total drug content} - \text{Free drug in supernatant})}{(\text{nanoparticles weight})} \times 100$$

3.4 *In vitro* drug release studies: Based on the particle size results four best samples were chosen form optimized formulations to conduct Invitro experiments. A known amount of Nanoparticles was transferred to a conical flask and 20 mL of the Phosphate buffer pH 7.0 was added to the flask. Experiment is conducted in an incubator shaker in which temperature and rotation were adjusted to 37°C and 90 rpm, respectively. At definite time interval of 1 hrs, 3ml of the dispersion was withdrawn and replaced with equivalent volume of dissolution

medium to maintain the sink conditions. The amount of drug released from the nanoparticles was then analyzed by using UV Spectrophotometrical method. The mechanism of drug release was analysed by fitting the drug release data into various release models like zero order, first order, Higuchis model and Korsmeyer peppas model.

3.5 Stability Studies: The stability of sulfasalazine loaded Nanoparticles was evaluated in terms of its drug content and entrapment efficiency. sample is taken in to three different bottles and were stored at three different conditions like Refrigeration temperature (4°C), at room temperature (25°C)and at >40°C for a period of 3 months. The samples were analyzed for drug content after 3 months by UV-Spectrophotometrical method at 352nm. The results were compared with actual results of before storage.

4. RESULTS AND DISCUSSIONS

Optimized formulations of sulfasalazine loaded chitosan Nanoparticles were prepared (FI-FIV) by Ionotropic gelation method. A colloidal particulate solution is formed by adding sodium tripolyphosphate in to aqueous solution containing chitosan and sulfasalazine due to the electrostatic interactions occurring between the negatively charged sodium tripolyphosphate (TPP) and positively charged amino groups of chitosan polymer.

Drug content: From the results it was observed that, as polymer concentration increases, drug content increasing (69 to 79 %). Whereas decreasing with increasing drug concentration from 82 to 62 %. As cross linking agent and stirring speed increases, drug content is increasing from70 to 81%. Maximum drug content is obtained by increasing stirring sped from 400 to 800rpm.

Entrapment Efficiency: As polymer, cross linking agent concentrations and stirring speed increases entrapment efficiency increasing from (66 to 88 %). %EE decreasing with increasing drug concentration. Maximum %EE is observed in 900 rpm stirring speed (88%).

Particle size and zeta potential: In the study of optimized formulations, chitosan concentration was effected the particle size from 262nm to 589nm and zeta potential as +27.23mV to + 33.01mV. The cross linking agent and drug concentration also affects the particle size and zeta potential with respect to the concentration of chitosan from1281nm to 512nm and +20.14mV to +8.51mV respectively. The same parameters were affected with using the different stirring speed from 853nm to 284nm and +6.31mV to +22.61mV.

Invitro studies: the Invitro studies of selected samples were performed and the cumulative % drug release was observed. The samples P2, T3, D2, SS3, showed a release of 82.56%, 76.01%, 82.12%, 84.62% after 9 hours. More sustained and maximum drug release was observed in SS3 sample i.e for 900rpm. The cumulative % drug release after 9 hours is reported in Fig 2. The release kinetics shows that all the samples were follows Zero Order Release model and the mechanism of drug release from the chitosan Nanoparticles was Non-Fickian or Anomalous Diffusion which signifies that the drug release is both diffusion –controlled and swelling controlled.

Stability Studies: Stability Studies of P2 sample were shown in fig. In refrigeration condition at 4^0C and RT 25^0C there was no remarkable change in the drug content and %EE compared to $>40^0$C. The particles kept at higher temperature get degradation when compared to other conditions. This indicates that particles are stable in refrigeration temperature and room temperature.

Table.1.Optimized formulations of Drug loaded chitosan Nanoparticles

Formulation code	Samples code	Chitosan Con.(%w/v)	Tripoly phosphate Con. (%w/v)	Drug Con.(%w/v)	Stirring Speed (rpm)	Time (hrs)
FI	P1	**0.2**				5-6 hrs
	P2	**0.3**	1	0.1	600	
	P3	**0.4**				
FII	T1		**0.4**			5-6 hrs
	T2	0.3	**0.6**	0.1	600	
	T3		**0.8**			
FIII	D1			**0.06**		5-6 hrs
	D2	0.3	1	**0.08**	600	
	D3			**0.12**		
FIV	SS1				**400**	5-6 hrs
	SS2	0.3	1	0.1	**600**	
	SS3				**800**	

***(P-Polymer, T-Tripolyphosphate, D- Drug, SS- Stirring Speed).**

Table.2.Effect of Formulation and Process variables on characterization studies

Formulations	Sample code	Product yield (%)	Drug content (%)	Entrapment efficiency (%)	Average particle size (nm)	Zeta potential (mV)
I	P1	87.50	69.23	78.68	262.14	+33.21
	P2	86.08	73.23	82.12	379.62	+22.61
	P3	85.50	79.50	83.10	589.34	+18.13
II	T1	86.12	70.10	66.17	1281.1	+17.96
	T2	87.56	62.30	71.04	796.8	+2.13
	T3	83.23	75.14	75.67	576.92	+8.51
III	D1	84.30	82.03	72.13	2168.6	+20.14
	D2	83.12	78.09	76.37	512.90	-
	D3	88.13	62.00	68.90	1367.1	+4.95
IV	SS1	84.08	71.32	71.32	853.79	+6.31
	SS2	86.01	73.23	82.12	379.62	+22.61
	SS3	92.21	81.16	87.99	284.31	+20.98

Table.3.In-vitro drug release kinetics data

Formulation code	Zero order R^2	First order R^2	Higuchi's R^2	Korsmeyer Peppas	
				R^2	N
P2	0.967	0.930	0.947	0.954	0.568
T3	0.971	0.963	0.957	0.974	0.591
D2	0.985	0.930	0.914	0.960	0.673
SS3	0.976	0.910	0.943	0.968	0.617

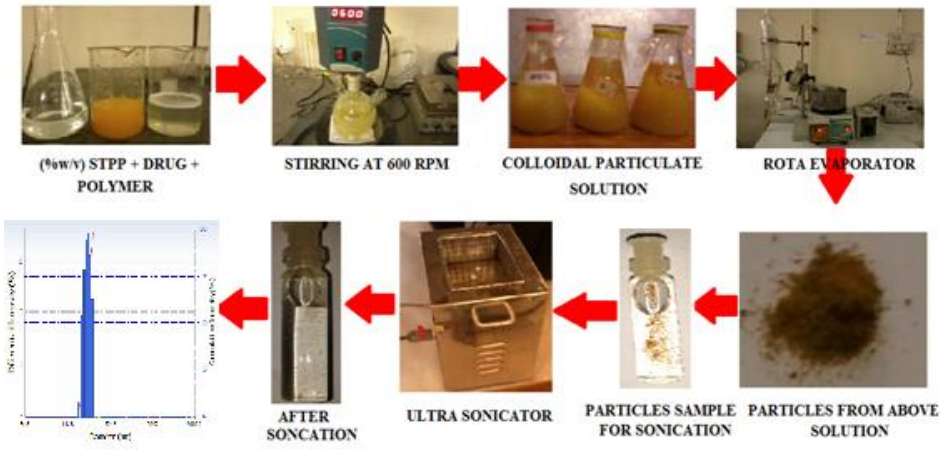


Figure.1.Experimental work

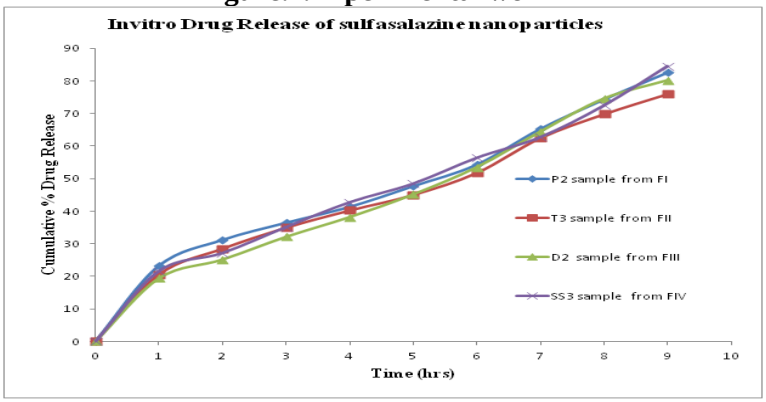


Figure.2.Invitro release of sulfasalazine loaded chitosan Nanoparticles from the optimized formulations

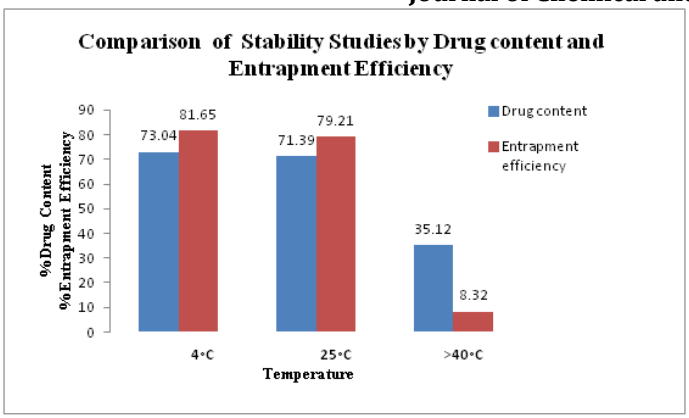


Figure.4.Stability studies of P2 sample at different temperatures stored for 2 months

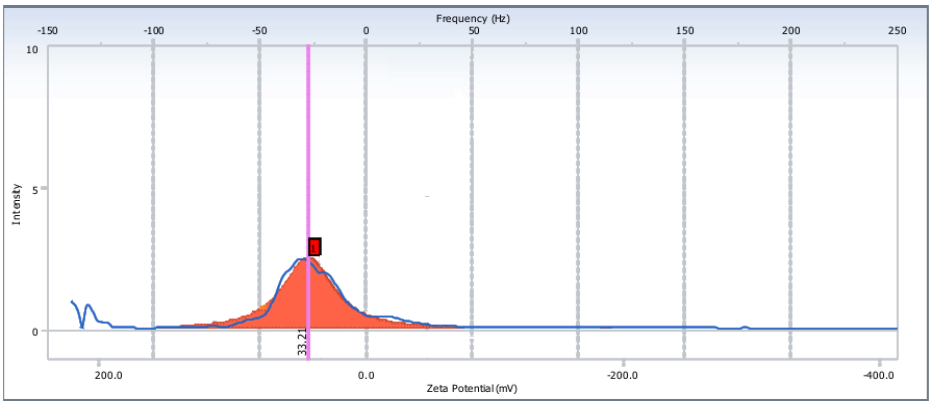
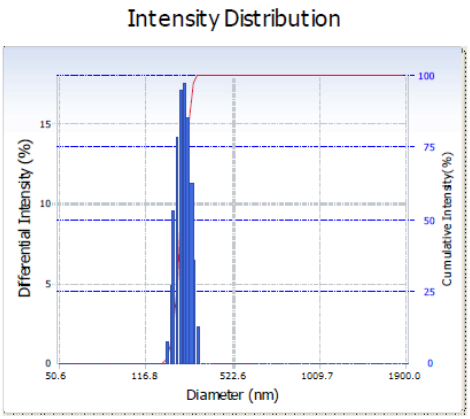
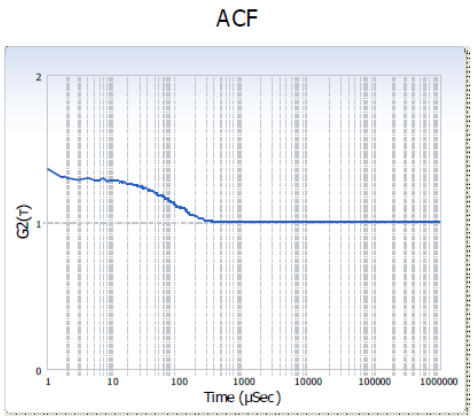


Fig 5: Zeta potential analysis of P1 sample (+33.21 mV)





Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	262.1	63.77
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	262.1	63.77
Residual :	1.076e-002	(O.K)

Cumulants Results

Diameter	(d)	: 681.6	(nm)
Polydispersity Index	(P.I.)	: 0.298	
Diffusion Const.	(D)	: 2.216e-009	(cm^2/sec)

Measurement Condition

Temperature	: 28.5	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3324	
Viscosity	: 0.8216	(cP)
Scattering Intensity	: 8157	(cps)

Figure.6.Particle size analysis of P1 sample (262.1nm)

5. CONCLUSION

In conclusion, Ionotropic gelation method can produce sulfasalazine loaded chitosan Nanoparticles with optimum particle size and zeta potential and maximum drug entrapment. The physical properties or parameters of sulfasalazine loaded chitosan Nanoparticles can be varied by changing a Formulation and process variables. Invitro studies showed a sustained release and follow zero order release with non-fickian diffusion model. Among all the variables stirring speed has showed maximum effect.

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