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FORMULATION AND EVALUATION OF MICROSPHHERES OF NITAZOXANIDE BY IONOTROPIC GELATIN METHOD

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Abstract:
In the present work, Microspheres of Nitazoxanide using PLGA and Chitosan as polymers were formulated to deliver Nitazoxanide via oral route. The results of this investigation indicate that Ionotropic gelation technique can be successfully employed to fabricate Nitazoxanide microspheres. In this work an effort was made to formulate microsphere of Nitazoxanide by using different polymers. Prepared formulations are evaluated for bulk density, tapped density, precent mucoadhesion, Percent compressibility, hausners ration, percentage yield, size and interaction study by FTIR and in vitro drug release. Formulation which passed all the evaluation parameters was considered as best formulation of Nitazoxanide. The present study conclusively that Nitazoxanide microsphere could be prepared successfully and formulation F3 was shows satisfactory result.
KEYWORDS: *Nitazoxanide, PLGA, Chitosan and Microspheres.*

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1. INTRODUCTION:

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site specific manner.

One of the most challenging areas of research in pharmaceuticals is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy of drugs[2,3]. The basic principle behind the controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamics properties of drug in such a way that its efficacy is maximized by reducing side effects, dose frequency and cure the disease in short time by using low amount of drug administered with the most suitable route [4,5, 6,7].

In 1997, first time microspheres were prepared for the sustained action of the drug. Since then, microparticles have proved to be good candidates for sustained and controlled release of drug and become an alternative of conventional or immediate release formulations. These particles are also a beneficial to deliver the active pharmaceutical ingredients which are pharmacologically active but are difficult to deliver due to limited solubility in water. In such type drugs, the attainment of required therapeutic concentrations of drug in the blood is problematic enabling to attain higher C_{max}, T_{max} and area under curve. Microsphere – based formulations can release a constant amount of drug in the blood or to target drugs to specific site in the body [8,9].

For many decades, medication of an acute disease or a chronic disease has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. This factor as well as other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems. The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major advance toward solving the problem associated with the existing drug delivery system.

The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while Temporal delivery refers to controlling the rate of drug delivery to the target tissue.

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is be dilled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.[10,11]

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e release from the dosage form should follow zero-order kinetics.[12]

1.1. DEFINITION AND GENERAL DESCRIPTION:

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes. The natural polymers include albumin and gelatin9-10 the synthetic polymers include polylactic acid and polyglycolic acid. Fig. 1.2 shows two types of microspheres: Microcapsules, where the entrapped substance is completely surrounded by a distinct capsule wall, and micromatrices, where the entrapped substance is dispersed throughout the microsphere matrix.

Microspheres are small and have large surface to volume ratios. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity.

Advantages of microspheres [18]

1. They provide protection before after administration for unstable drug.

2. They reduced concentration of drug at site other than the tissue or the target organ.
3. Decrease dose and toxicity.
4. Particle size reduction for enhancing solubility of poorly soluble drugs.
5. Provide constant and prolonged therapeutic effect.

Limitation:[19]

Some of the disadvantages were found to be as follows

1. The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
2. The fate of polymer matrix and its effect on the environment.
3. The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
4. Reproducibility is less.
5. Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to be encapsulated.
6. The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.

MATERIALS AND METHODS

Nitazoxanide Procured from Hetero Pharma limited Hyd, provided by SURA LABS, Dilsukhnagar, Hyderabad
PLGA Merk specialiities Pvt Limited
Chitosan Merk specialiities Pvt Limited
Sodium alginate Merk specialiities Pvt Limited
Calcium Chloride Merk specialiities Pvt Limited

INSTRUMENTS

1	UV-Visible spectrophotometer	Lab India, India
2	Electronic weighing balance	Sartorious
4	Magnetic stirrer	Remi Laboratories
5	Dissolution Apparatus	Lab India, Lab India
6	Ultrasonic cleaner	Remi Laboratories
7	FT – IR Spectrometer	Bruker Alpha
8	SEM	SEM (JEOL Ltd, Japan).

7. PREFORMULATION STUDIES

SPECTROSCOPIC STUDIES

PREPARATION OF 0.1N HCl (pH 1.2):

Take 8.5ml of HCl in a 1000ml volumetric flask and make up the volume with distilled water.[63]

DETERMINATION OF λ_{max}:

Weigh 10mg of Nitazoxanide and transferred into 10ml volumetric flask and dissolved in 10ml methanol (stock-I) to get concentration of 1000 µg/ml. From the stock-I take 1ml solution and make up 10ml with 0.1N HCL. From the second stock take 1ml solution and make up to 10ml with 0.1N HCL to get 10 µg/ml. Then scan from 200-400nm.[64-67]

Preparation of Standard Calibration Curve of Nitazoxanide:

1. 10 mg of Nitazoxanide was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 µg/ml.
2. From the stock solution- I, 1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100µg/ml.
3. From the stock solution- II, aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 2 to 10µg/ml. The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 246nm against reference solution 0.1N HCl (pH 1.2). The procedure repeated to pH 6.8 phosphate buffer and pH 7.4 phosphate buffer.[68-69]

METHOD OF PREPARATION

IONOTROPIC GELATION METHOD:

The microspheres were prepared by the Ionotropic gelation technique. The sodium alginate solution was prepared by dispersing the sodium alginate in de-ionized water under continuous stirring for 30 minutes. The weighed amount of the drug was thoroughly mixed with sodium alginate dispersion. By following the same procedure the alginate beads of different ratios of drug: polymer were prepared. The resulted homogeneous dispersion was extruded in to the 5% calcium chloride solution through hypodermic syringe with flat tip needle (20G) and stirred for 15 minutes at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for 30 minutes in the calcium chloride solution to complete the gelation reaction. The microspheres were then filtered and dried in hot air oven at 60ºC for 3 hr.[70-72]

CHARACTERIZATION OF MICROSPHERES:

Table 7.1: Prepared formulation of Microspheres

INGREDIENTS (mg)	FORMULATION CODES					
	F1	F2	F3	F4	F5	F6
Nitazoxanide	500	500	500	500	500	500
PLGA	150	300	450	-	-	-
Chitosan	-	-	-	150	300	450
Sodium alginate (w/v)	4%	4%	4%	4%	4%	4%
Calcium Chloride (w/v)	5%	5%	5%	5%	5%	5%

COMPATIBILITY STUDIES

A proper design and formulation of a dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. If the excipient(s) are new and if no previous literature regarding the use of that particular excipient with an active ingredient is available, then compatibility studies are of paramount importance. Hence, before producing the actual formulation, compatibility of Nitazoxanide with different polymers and other excipients was tested using the Fourier Transform Infrared Spectroscopy (FT-IR) technique.[98-100]

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR):

In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared using Shimadzu FT-IR 8400 spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers.[101-102]

Differential Scanning Calorimetry:

The possibility of any interaction between the drug and the Excipients during preparation of SLN was assessed by carrying out thermal analysis of optimised formulation using DSC. DSC analysis was performed using Hitachi DSC 7020, on 5 to 15 mg samples. Samples were heated in sealed aluminum pan at a rate of 10°C/min conducted over a temperature range of 30 to 350°C under a nitrogen flow of 50 mL/min.[103]

SEM (Scanning Electron microscope) studies

The surface morphology of the layered sample was examined by using SEM (Hitachi, Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs. These sample stubs were coated with a thin layer (30Å) of gold by employing POLARON-E 3000 sputter coater. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.[104]

Powder X-ray Diffraction (PXRD) Studies

The prepared mixtures were also analyzed using X-ray powder diffractometer (PXRD) which confirms the formation of the new solid phases. The difference in the 2 theta lines confirms the formation of the new solid phases as no two solids have same 2 theta lines, thus revealing the formation of new solid phases. It also reveals the information about the crystal structure, chemical composition, and physical properties of the material and also helps in structural characterization. This technique detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and co crystalline phases. Spectra of PXRD were taken on a sample stage Spinner PW3064. The samples were exposed to nickel filtrate Cukœ radiations (40 KV, 30 mA) and were scanned from 10° to 40°, 2Θ at a step size of 0.045° and step time of 0.5 s.[105]

Zeta Potential:

Zeta capability becomes anticipated on the premise of electrophoretic mobility under an electric powered field, the use of zeta Sizer Nano ZS (Malvern Instruments, UK). For the Zeta ability measurement, Samples have been diluted as 1:40 ratio with filtered water (v/v) before analysis. zeta potential have been then measured.[106]

8. RESULTS AND DISCUSSION

8.1. PREFORMULATION STUDIES

8.1.1. SPECTROSCOPIC STUDIES

Determination of λ_{max}

A solution of 10µg/ml of Nitazoxanide was scanned in the range of 200 to 400nm. The drug exhibited a λ_{max} at 346 nm in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer respectively.

Calibration curve of Nitazoxanide in simulated gastric fluid pH 1.2

Table 8.1 shows the calibration curve data of Nitazoxanide in simulated gastric fluid pH 1.2 at 346 nm Fig.8.1 shows the standard calibration curve with a regression value of 0.999, slope of 0.033 and intercept of 0.009 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml.

Table 8.1: Calibration curve data for Nitazoxanide in simulated gastric fluid pH 1.2

Concentration (µg /ml)	Absorbance
0	0
5	0.178
10	0.358
15	0.525
20	0.676
25	0.849

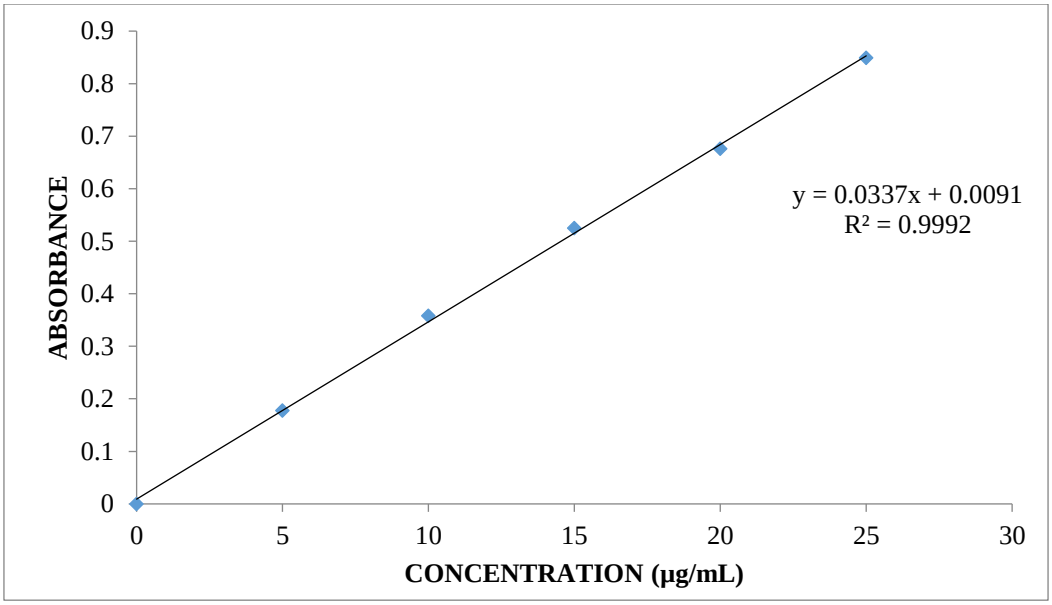


Figure 8.1: Standard graph Of Nitazoxanide in simulated gastric fluid pH 1.2

Calibration curve of Nitazoxanide in pH 7.4 phosphate buffer

Table 8.2 shows the calibration curve data of Nitazoxanide in pH 7.4 phosphate buffer at 346nm. Fig. 8.2 shows the standard calibration curve with a regression value of 0.997, slope of 0.027 and intercept of 0.020 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml.

Table 8.2: Calibration curve data for Nitazoxanide in pH 7.4 phosphate buffer

Concentration (µg /ml)	Absorbance
0	0
5	0.175
10	0.305
15	0.428
20	0.561
25	0.697

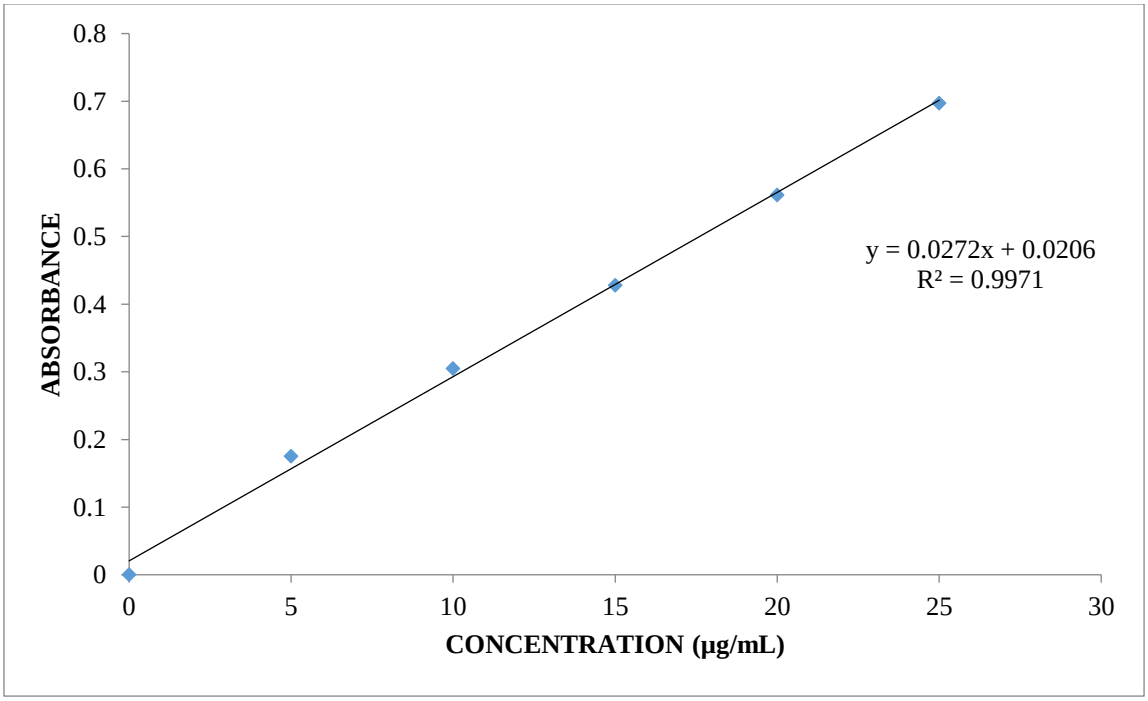


Figure 8.2: Standard graph Of Nitazoxanide in pH 7.4 phosphate buffer

Evaluation and characterization of microspheres

Micrometric Properties

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing PLGA as a polymer had a size range of 418.41µm to 452.14µm. Microspheres containing Chitosan as polymer exhibited a size range between 421.65µm to 460.15µm.

The particle size data is presented in Tables 8.3 and displayed in Figures. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

The bulk density of formulation F1 to F6 containing PLGA and Chitosan formulation was in the range of 0.277 ± 0.2 ± 0.2 to 0.625 ± 0.1 gm./cm^3 (as shown in table 8.3), tapped density 0.312 ± 0.2 to 0.833 ± 0.1 and Hausner's ratio 1.095 to 1.333.

The Carr's index of formulation F1 to F6 containing different grades of PLGA, and Chitosan 8.695 to 25.00 respectively. The angle of repose of formulation F1 to F6 containing PLGA, and Chitosan formulation was in the range <28.3 respectively (as shown in table 8.3) the values of Carr's index and angle of repose indicate good flow properties.

Table 8.3: Micromeritic property of microspheres of Nitazoxanide

Formulation code	Mean partical size	Bulk density ((gm./cm^3))	Tapped density (gm./cm^3)	Hausner's ratio	Carr's index	Angle of repose
F1	452.14	0.434 ± 0.2	0.476 ± 0.3	1.095	8.695	23.2 ± 0.2
F2	441.95	0.277 ± 0.2	0.312 ± 0.2	1.133	11.11	25.2 ± 0.1
F3	418.41	0.588 ± 0.3	0.666 ± 0.4	1.333	11.76	27.1 ± 0.1
F4	460.15	0.521 ± 0.3	0.631 ± 0.3	1.121	17.39	24.4 ± 0.4
F5	430.96	0.625 ± 0.1	0.833 ± 0.1	1.333	25.00	28.3 ± 0.4
F6	421.65	0.476 ± 0.3	0.526 ± 0.2	1.105	9.52	25.1 ± 0.1

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range.

DRUG ENTRAPMENT EFFICIENCY

Percentage Drug entrapment efficiency of Nitazoxanide ranged from 84.45 to 91.72 % for microspheres containing PLGA and Chitosan polymer, the drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 8.4, and displayed in Figures.

Table 8.4: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% Yield	Drug Content (mg)	% Drug entrapment efficiency
1	F1	89.31	96.14	86.14
2	F2	91.12	98.65	88.91
3	F3	96.08	99.76	91.72
4	F4	90.74	98.14	75.58
5	F5	96.91	96.52	84.45
6	F6	98.24	100.04	89.87

Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of polymer directly affects the swelling ratio. As the polymer to drug ratio increased, the percentage of swelling increased from 73.63 to 86.02% for microspheres containing PLGA as polymer, 67.29 to 80.32% for microspheres containing Chitosan as polymer. The percentage of swelling of the prepared microspheres is displayed in Figures. The percentage of swelling of the prepared microspheres is displayed in Figures. The effect of drug to polymer ratio on percentage swelling is displayed in Figure Table 8.5: Percentage swelling of the prepared microspheres.

Table 8.5: Swelling studies

S.NO.	FORMULATION CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	F1	10	12.45	80.32
2	F2	10	11.62	86.02
3	F3	10	13.58	73.63
4	F4	10	12.45	80.32
5	F5	10	13.95	71.68
6	F6	10	14.86	67.29

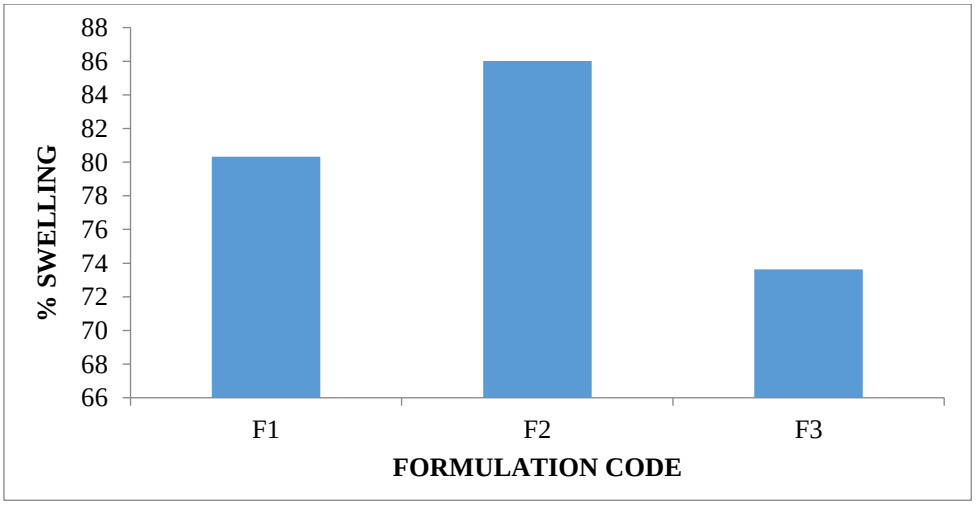


Figure 8.3 : Percentage swelling of microspheres containing PLGA

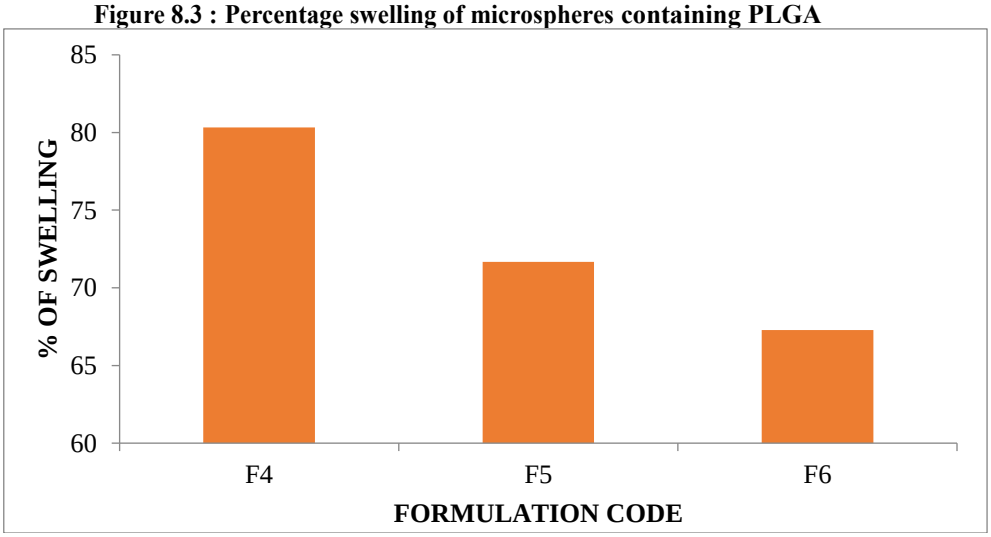


Figure 8.4 : Percentage swelling of microspheres containing Chitosan

***IN VITRO* MUCOADHESION TEST**

As the polymer to drug ratio increased, microspheres containing PLGA exhibited % mucoadhesion ranging from 61 to 70%, microspheres containing Chitosan exhibited % mucoadhesion ranging from 75 to 95%. The results of *in-vitro* mucoadhesion test are compiled in Table 8.6. Effect of polymer proportion on % mucoadhesion is depicted in Figures and comparative depiction of % mucoadhesion is depicted in Fig. Table Percentage mucoadhesion of the prepared microspheres.

Table 8.6: *In Vitro* Mucoadhesion Test of all Formulations

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	F1	20	15.48	61
2	F2	20	11.85	58
3	F3	20	15.14	70
4	F4	20	17.96	93
5	F5	20	20.71	95
6	F6	20	16.17	75

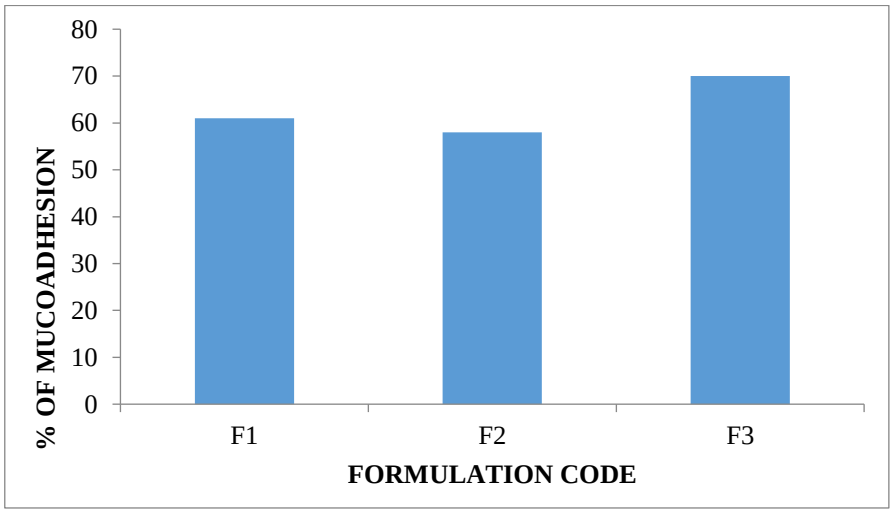


Figure 8.5: Percentage mucoadhesion of microspheres containing PLGA

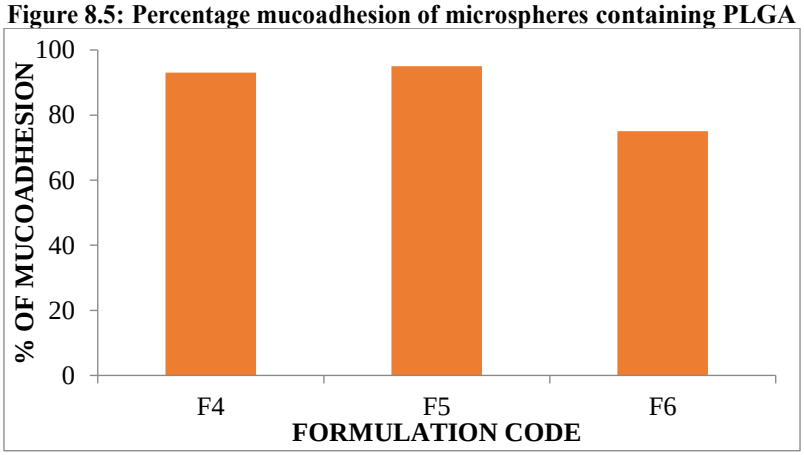


Figure 8.6: Percentage mucoadhesion of microspheres containing Chitosan

***IN-VITRO* DRUG RELEASE STUDIES**

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the *in-vitro* dissolution studies of formulations F1 to F6 are shown in table 8.7. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations F1 to F3, figure for formulations F4 to F6.

The formulations F1, F2, and F3 containing PLGA showed a maximum release of 97.58% at 10 hours, 98.12% 11 hours, 99.88% 12 hours respectively.

The formulations F4, F5 and F6 containing Chitosan polymer showed a maximum release of 97.14% 10

hours, 97.35% 12 hours, 91.17% 12 hours respectively.

This shows that more sustained release was observed with the increase in percentage of polymers. As the polymer to drug ratio was increased the extent of drug release increased. A significant increase in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling control release mechanism. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in slower release.

Table 8.7: *In-vitro* drug release data of Nitazoxanide microspheres

TIME (H)	Cumulative percentage of drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	21.89	16.87	16.18	17.82	13.91	15.67
2	28.96	25.50	27.92	24.31	18.68	21.75
3	35.75	31.89	36.27	34.93	24.90	26.90
4	48.18	45.23	49.96	47.72	36.53	33.83
5	55.09	52.19	58.19	53.15	47.95	40.76
6	62.10	60.97	65.76	64.91	52.18	47.92
7	78.67	68.57	72.51	68.75	63.87	53.76
8	85.79	74.21	78.93	73.81	68.56	62.81
9	90.14	78.92	82.74	82.94	78.97	70.47
10	97.58	87.28	87.94	97.14	84.28	78.38
11		98.12	90.75		91.84	84.10
12			99.88		97.35	91.17

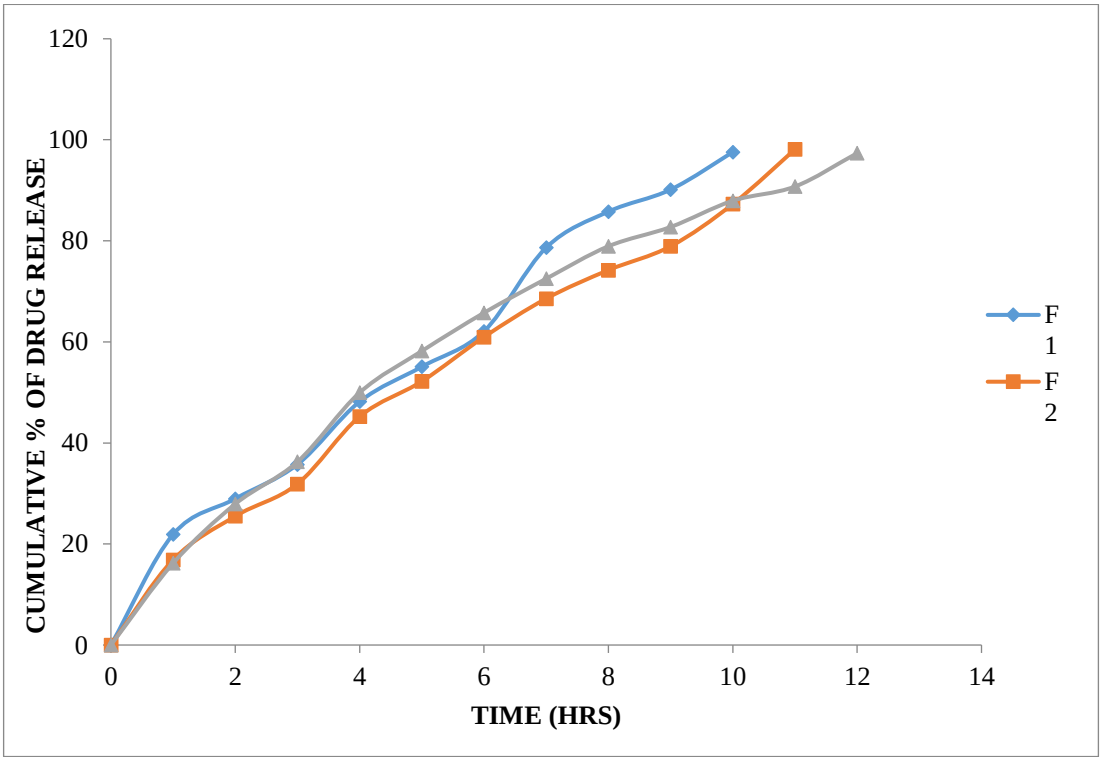


Figure 8.7: *In-Vitro* drug release profile of Nitazoxanide microspheres containing PLGA

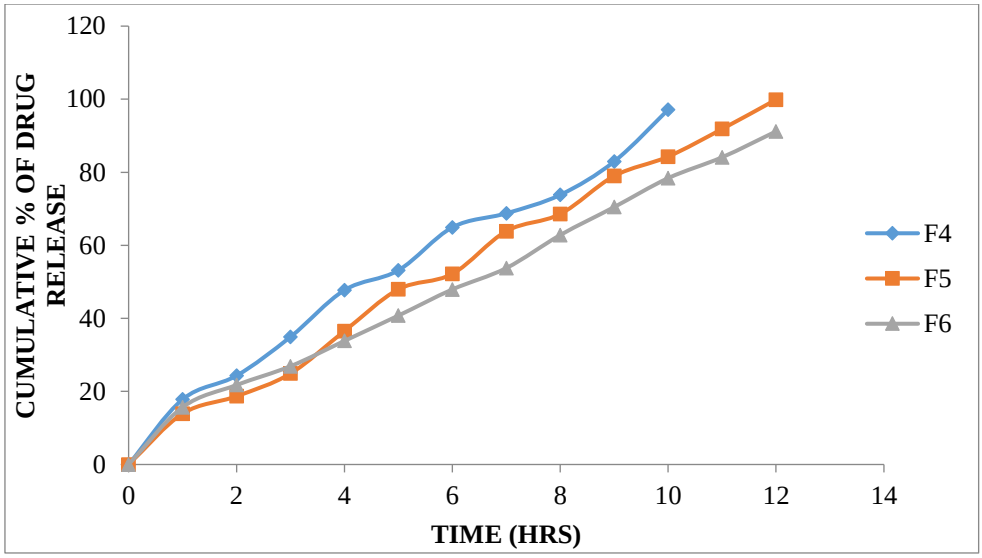


Figure 8.8: *In-Vitro* drug release profile of Nitazoxanide microspheres containing Chitosan

In vitro drug release from all the formulation was found to be slow and sustained over the period of 12 hours, among other formulation F3 showed better sustained release pattern and the cumulative percentage release at the end of 12 hours was found to be 99.88%.

***IN-VITRO* DRUG RELEASE KINETICS**

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the in-vitro drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeyer-Peppas model. The values are compiled in Table 8.10. The coefficient of determination (R2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the zero order release kinetics whereas release exponent value (n) ranged from 0.992. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows zero order release kinetics along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Table 8.8: Release kinetics studies of the optimized formulation (F3)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
13.91	1	1.000	1.143	0.000	1.935	13.910	0.0719	-0.857	86.09	4.642	4.416	0.226
18.68	2	1.414	1.271	0.301	1.910	9.340	0.0535	-0.729	81.32	4.642	4.332	0.309
24.9	3	1.732	1.396	0.477	1.876	8.300	0.0402	-0.604	75.1	4.642	4.219	0.423
36.53	4	2.000	1.563	0.602	1.803	9.133	0.0274	-0.437	63.47	4.642	3.989	0.653
47.95	5	2.236	1.681	0.699	1.716	9.590	0.0209	-0.319	52.05	4.642	3.734	0.908
52.18	6	2.449	1.718	0.778	1.680	8.697	0.0192	-0.282	47.82	4.642	3.630	1.012
63.87	7	2.646	1.805	0.845	1.558	9.124	0.0157	-0.195	36.13	4.642	3.306	1.336
68.56	8	2.828	1.836	0.903	1.497	8.570	0.0146	-0.164	31.44	4.642	3.156	1.485
78.97	9	3.000	1.897	0.954	1.323	8.774	0.0127	-0.103	21.03	4.642	2.760	1.881
84.28	10	3.162	1.926	1.000	1.196	8.428	0.0119	-0.074	15.72	4.642	2.505	2.137
91.84	11	3.317	1.963	1.041	0.912	8.349	0.0109	-0.037	8.16	4.642	2.013	2.628
99.88	12	3.464	1.999	1.079	-0.921	8.323	0.0100	-0.001	0.12	4.642	0.493	4.148

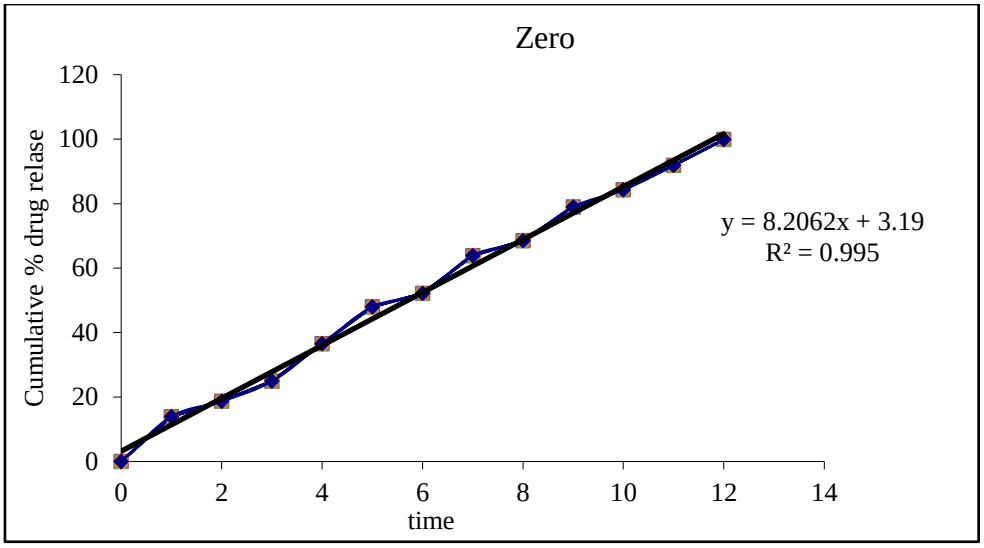


Figure:8.9: Graph of zero order release kinetics of optimized formula

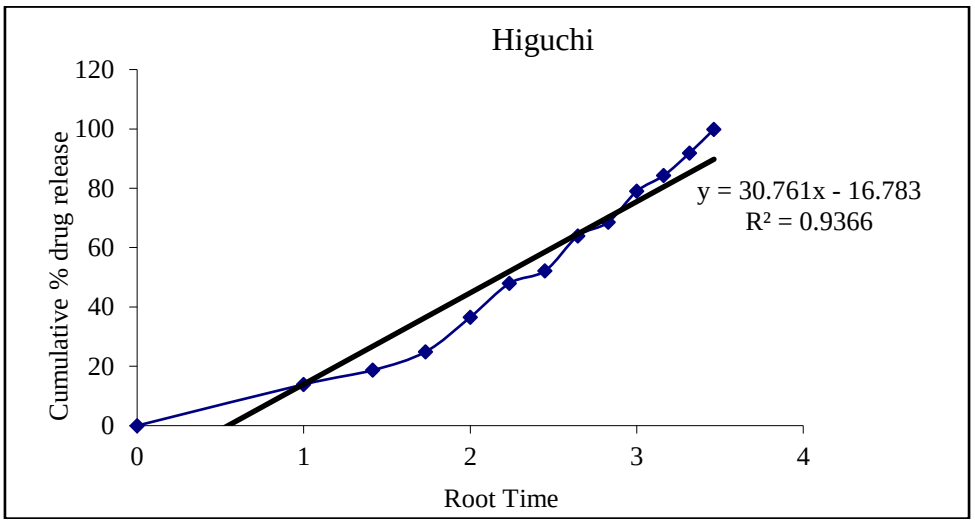


Figure:8.10: Graph of Higuchi release kinetics of optimized formula

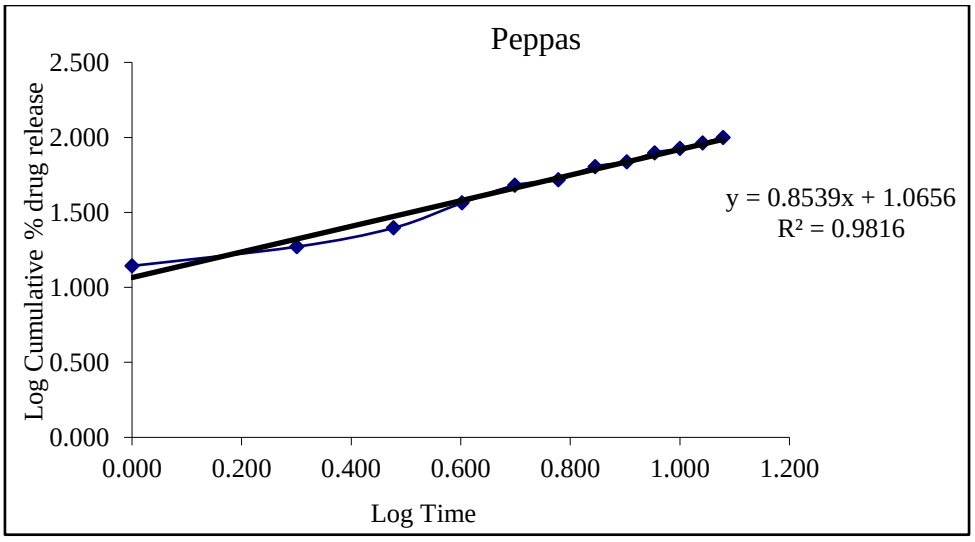


Figure :8.11: Graph of Peppas drug release kinetics of optimized formula

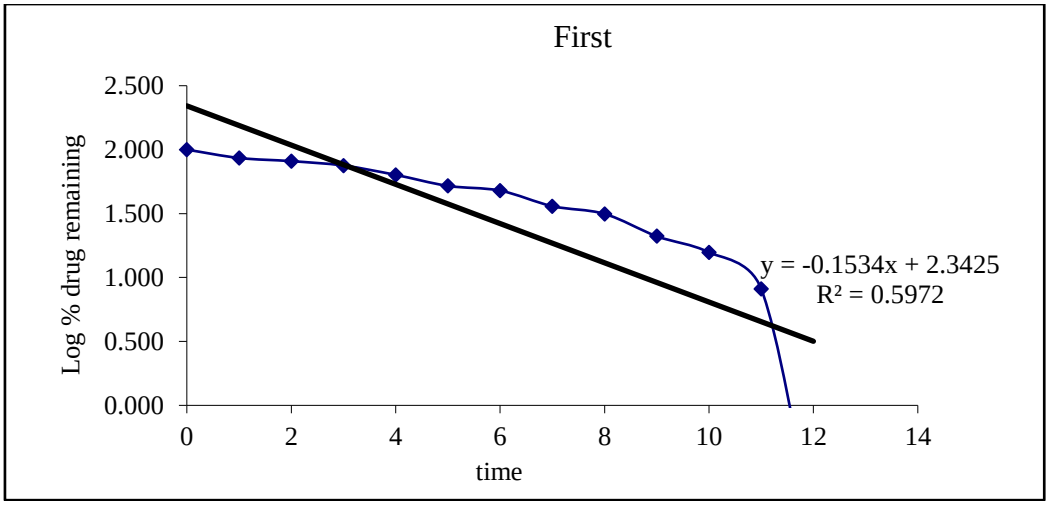


Figure:8.12: Graph of first order release kinetics of optimized formula

Optimised formulation F3 was kept for release kinetic studies. From the above graphs it was evident that the formulation F3 was followed zero order release kinetics.

COMPATIBILITY STUDIES

Drug polymer compatibility studies were carried out using Fourier Transform Infra Red spectroscopy to establish any possible interaction of Drug with the polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug.

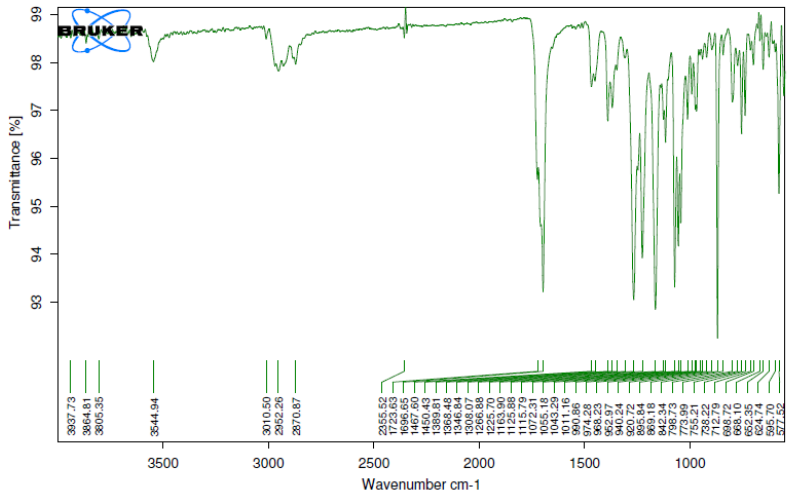


Figure 8.13: FT-IR spectra of Pure drug

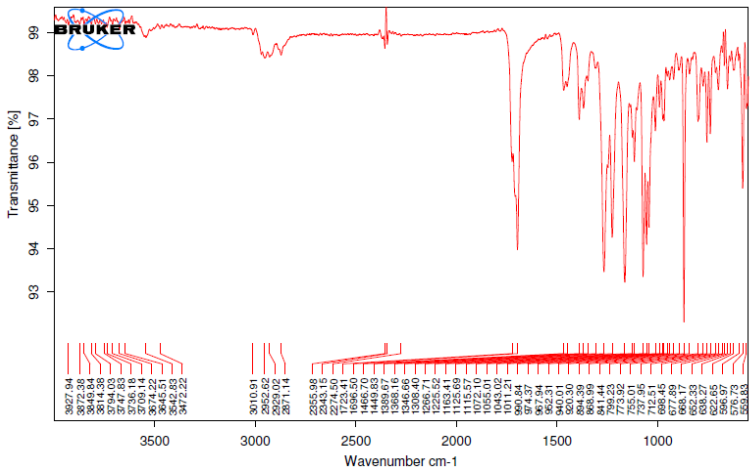


Figure 8.14: FT-IR spectra of Optimised formulation

SEM :

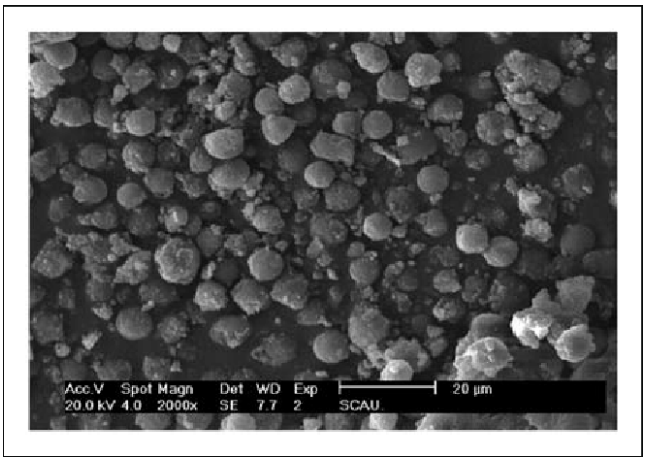


Figure 8.15: SEM of Optimised formulation

Table 8.9: Particle Sizes, PDI, and Zeta Potential of Microspheres formulations

FORMULATION	Particle Size(nm)	PDI	Zeta Potential (mV)
F1	1165.2	0.668	-26.12
F2	925.8	1.268	-24.81
F3	**314.3**	**0.168**	**-28.25**
F4	632.6	1.153	-23.52
F5	804.1	0.277	-16.55
F6	387.3	0.309	-20.83

Decrease in particle size shows increment in rate of dissolution. The PDI of optimized batch for solvent diffusion method is 314.3 while the PDI of batch for Ionotropic gelation technique is 0.168 (nm). The results indicate that the particles obtained by the solvent diffusion method shows narrow particle size distribution than the particles obtained by Ionotropic gelation technique.

The Zeta potential range from -10.80 mV to -28.25 mV to all the formulations. The negative charge on the surface of the nanoparticle is believed to facilitate uptake from the intestine by the Payers patch, leading to the lymphatic circulation, also it is believed to prevent entangling of the nanoparticles in the negatively charged mucous owing to the repulsion of like charges.

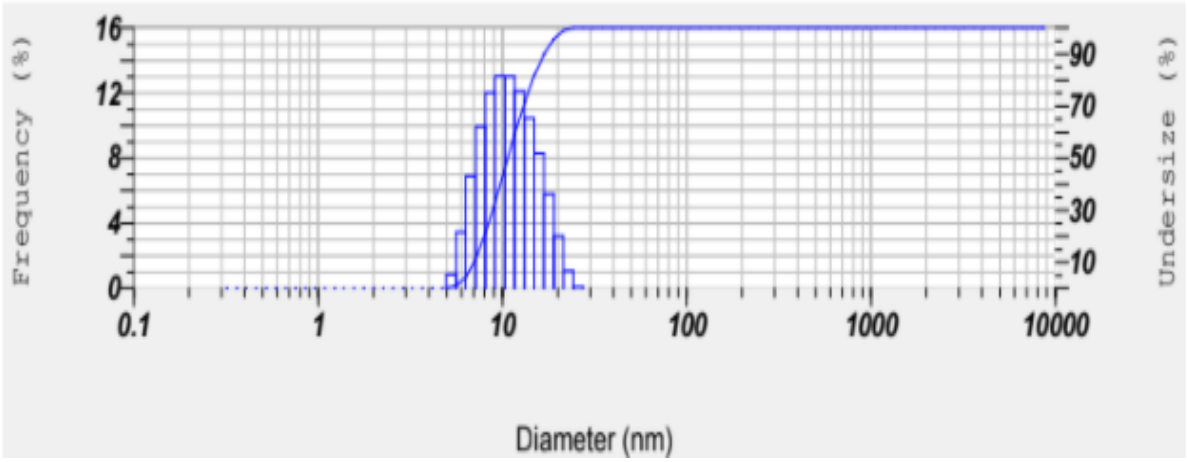


Fig 8.16: Particle size of F3 Formulation

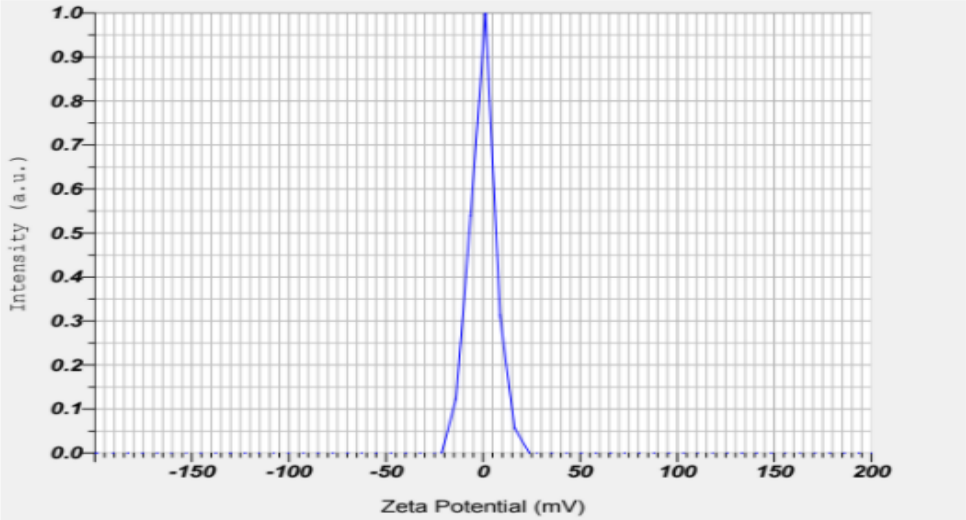
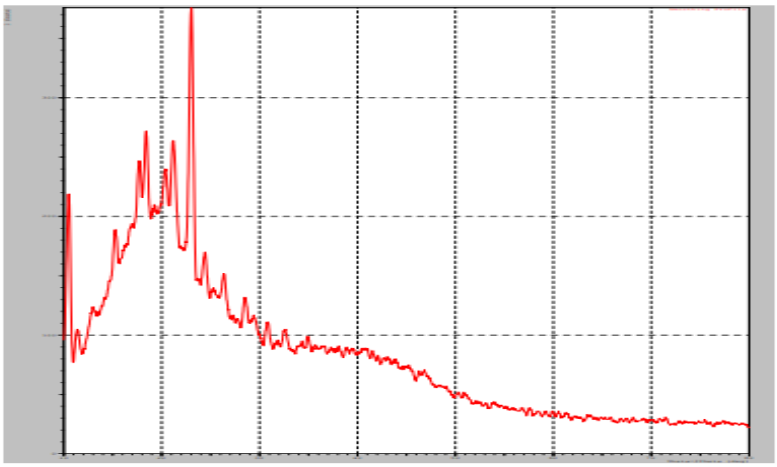


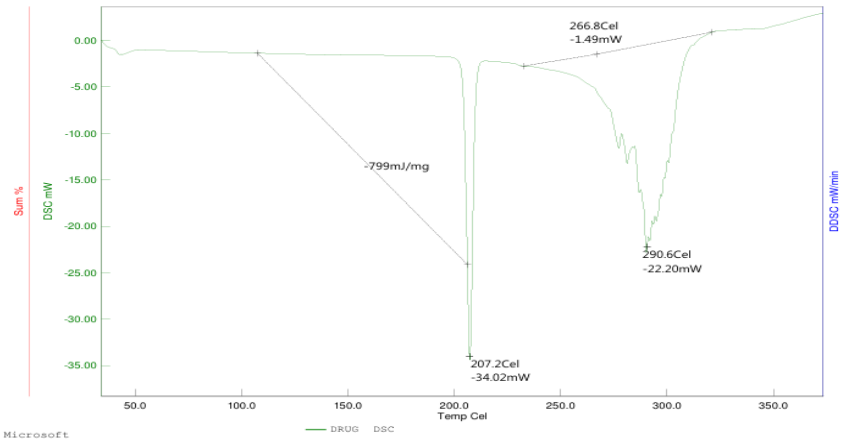
Fig 8.17: Zeta Potential of F3 Formulation

X-ray diffraction analysis



Graph 8.18: XRD graph of optimised formulations

X-RD patterns of pure Nitazoxanide formulated 1:3 ratio loaded PLGA prepared by Ionotropic gelation technique are displayed in Figure X-Ray diffractogram of pure Nitazoxanide produced peaks at different 2Θ angles show crystalline nature while in case of PLGA formulations, less intense peaks are observed than that of pure Entecavir. Along these lines, showing lack of diverse diffraction peaks of Entecavir. This result tells that majority of the drug was entrapped within the polymer and is dispersed homogeneously at molecular level.



Graph 8.19: DSC graph of pure drug

DSC was used to determine the melting point of Nitazoxanide. The DSC thermogram of Nitazoxanide shows a sharp endothermic at 207.2 °C confirming the melting point of Nitazoxanide.

9. CONCLUSION:

Microspheres are prepared with PLGA and Chitosan successfully by the Ionotropic gelation technique. Microspheres of Nitazoxanide showed excellent mucoadhesivity, % yield, Drug Content, % Drug entrapment efficiency and prolonged drug release up to 12 hours. Microspheres of different size and drug content could be obtained by varying the formulation variables. Thus the prepared microspheres may prove to be potential candidates for oral delivery devices. Formulation Batch F3 showed best appropriate balance between mucoadhesivity and drug release rate, which can be considered as a best fit for microspheres. The polymer ratio (PLGA) of 1:3 were selected as best formulation, The formulated system showed sustained release up to 12 h and the system is potentially useful to overcome poor bioavailability problems associated with Entecavir. Analysis of drug release mechanism showed that the drug release from the formulations the best fit model was found to be zero order release kinetics. Hence it can be concluded that Nitazoxanide loaded PLGA Microsphere may be useful to achieve sustained drug release profile suitable for oral administration.

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