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FORMULATION AND EVALUATION OF NORFLOXACIN MUCOADHESIVE MICROSPHERES USING OKRA GUM

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ABSTARCT
The immobilisation of drug in the hydrogels has proven to be of great utility in pharmaceutical applications. Oral controlled drug delivery system represents the most popular form of sustained drug delivery system as microspheres using Alginate- okra gum, calcium gel. The present study is to formulate and evaluate alginate and okra gum microspheres of Norfloxacin in different concentrations (ratios) adopting ionotropic gelation technique. The microspheres were characterised for morphology, size, drug content, loading efficiency, drug-polymer interactions, swelling ratio (index) and in-vitro release studies. In present study Microencapsulation of water insoluble drugs using hydrophilic polymer like sodium alginate, natural gum (okra gum) which moisturise and swell in contact with aqueous media, are becoming extremely popular in controlling the release of water insoluble drug. Norfloxacin is a synthetic broad-spectrum antibacterial drug used in the treatment of respiratory, biliary and urinary tract infections. Norfloxacin has short half-life of 3 to 4 hrs requires multiple administration of drug leads to fluctuations in plasma concentration. Based on dose related, renal toxicity, seizures, nausea and vomiting with Norfloxacin make it an appropriate candidate for the sustained release. Obtained microspheres showed good flow properties, spherical in shape with uniform surface morphology with their size range of 761.2 ± 1.30 to 971.4 ± 1.06 µm. The drug content was uniform and reproducible in each batch of microspheres. Overall encapsulation efficiencies were in the range of 82.0 ± 0.11 to 90.1 ± 0.08 %. FTIR studies revealed the absence of drug polymer interactions. Swelling index of microspheres was enhanced with the polymer concentration. The microspheres prepared with alginate and okra gum (formulation) have exhibited higher drug release 98.68% as compared to other formulations. In-vitro release profiles revealed that high concentration of hydrophilic natural gum i.e., okra gum and alginate has resulted retard in drug release. The mechanism of drug release from alginate and okra gum was zero order non fickian kinetics for a period of 12hrs. In conclusion, alginate-okra gum mucoadhesive microspheres could be promising vehicle for oral sustained release of Norfloxacin.

KEYWORDS: Microspheres, Norfloxacin, alginate, okra gum, ionotropic gelation method, swelling index, in-vitro release.

INTRODUCTION

Novel drug delivery system delivers a therapeutic substance to the target site in a well-controlled and sustained model.[1] Microspheres or microparticles are defined as a free-flowing spherical particles consisting of polymer matrix and drug. They consist of proteins or synthetic polymers which are biodegradable in nature having a particle size less than 200µm.[2]

Microspheres can be referred as small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres can also be called as microparticles. Microspheres had been explored significantly for their use in the subject of drug transport and various polymers had been utilized for the formulation of the microspheres, which in turn have been assessed for distinctive purposes. Eventually the whole dose and few adverse reactions can be decreased due to the fact that a steady plasma concentration is maintained.[3]

Microspheres are of two types; Microcapsules and Micrometrics. Microcapsules are those in which entrapped substance is surrounded by distinct capsule wall and Micrometrics in which entrapped substance is dispersed throughout the microsphere matrix. The objective develop, characterize, and evaluate mucoadhesive microspheres of Norfloxacin using various mucoadhesive polymers. Norfloxacin is a synthetic antibacterial agent belonging to the

fluoroquinolone class of antibiotics. The microspheres are effective carriers to maintain prolonged effective concentration of a drug Norfloxacin (biological half-life 3-4.00±0.49 h). In the treatment of Gastrointestinal Infections, the equivalent of the 400 mg of norfloxacin is used in daily dose. In the present work, we prepared sustained release of mucoadhesive microspheres of Norfloxacin by ionotropic gelation technique, and their morphological characters, drug content, mucoadhesive property, and drug release properties were evaluated.

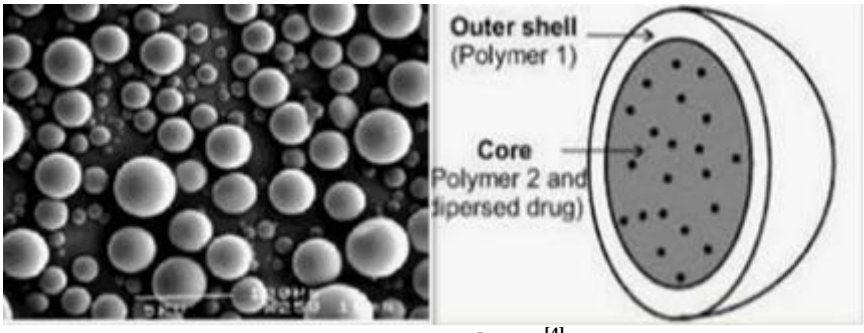

Fig. 1: Microspheres.[4]

MATERIALS AND METHODS

Norfloxacin I.P was procured from Anant pharmaceuticals Pvt Ltd., Ambernath, Sodium alginate from Rolex laboratory Pvt Ltd., Mumbai, Calcium chloride fused, purchased from Finer chemical Pvt Ltd., Ahmedabad, Dimethyl sulfoxide from Meru chem Pvt Ltd.

Preparation of Okra Gum (Abelmoschus esculentus)

Okra gum is a sticky, mucilaginous, and pale yellowish coloured gum. The preparation of the gum is consisting of the four steps. The final step is quite lengthy and it takes up to weeks to complete.

Washing – For the extraction of the mucilaginous gum from the okra, the 1Kg okra was obtained from the local market and it was washed completely to remove the soil and dirt particles. Then it was dried to remove water. The okra was thinly sliced or it was chopped into the small pieces. The seeds were removed from the okra because consists it does not consist of mucilage.

Filtration – After the cutting of the okra, the smaller pieces were soaked into the water overnight to extract out the mucilage. After thickening of the mucilage, the gummy material was filter out from rest part of okra by using muslin cloth.

Precipitation – In the third step the precipitation of the gum was done. For the precipitation of the gum, the acetone was added at a ratio of 3 parts of acetone to one part of gum extract. This results in the precipitation of the gummy material.

Drying – After the complete precipitation of the gum it was separated and then it was dried in a hot air oven at 60℃, The gum was stored in the air tight container.

Physiological properties of okra gum

From okra pods 0.46% w/w of mucilage was collected as shown in Table 2

The presence of mucilage in okra was confirmed by the development of purple to violet colour ring and pink colour (positive) upon the treatment of Molisch reagent and ruthenium red test respectively as shown in Table The prepared okra gum was sticky and yellowish in colour. After drying colour turns to dark brown. The gum was studied for stability and no unusual changes were found in the gum. The swelling index of the modified gum was found as 205, which indicates that the gum has good swelling capacity. This result are shown in Table 4.

Preparation of Norfloxacin microspheres

The microspheres containing Norfloxacin were prepared by employing ionic gelation method using sodium alginate and okra gum as natural polymers. The required amount of sodium alginate and okra gum was soaked in distilled water in a beaker (100ml) for 12hrs. The active substance Norfloxacin (100mg) was dispersed in dimethyl sulfoxide and mixed uniformly with sodium alginate and okra gum.

Aqueous polymer dispersion with drug solution was added in a drop wise at the rate of 1ml/min in a 100 ml of 5% w/v of calcium chloride solution through a syringe with needle no-23(0.63×25mm). Further the medium was stirred for 20 mins at 800rpm to complete the curing reaction and to produce spherical rigid microspheres.

The microspheres were collected by decantation and washed twice with distilled water. The product was dried at 40ºC for 12 hours in an oven and stored in desiccator. The dried microspheres formulation.

Evaluation of Norfloxacin Microspheres

The microspheres prepared were evaluated for for particle size analysis, drug content, drug entrapment efficiency, swelling index, *in vitro* release, morphological characters and drug-polymer interactions.

The microspheres were analyzed for particle size by optical microscopy. The instrument was calibrated and 100 microspheres sizes were calculated under

magnification. For determination of drug content, 100mg of norfloxacin microspheres were powdered, and 50mg of the powder was transferred to 100 ml volumetric flask, dissolved in water and made the volume to 100 ml. The solution was kept for 1hour with occasional shaking and filtered through whatmen filter paper. The filtrate was collected and diluted with sufficient amoun of distilled water, maintaining the concentration of the drug within the standard plot range. The diluted solution was analyzed for the norfloxacin content by UV-spectrophotometer at 274nm.

Drug entrapment efficiency was studied on drug content obtain, by using a UV–Visible spectrophotometer (Shimadzu, Japan) at 274nm. The amount of drug entrapped in the microspheres was calculated by the following formula.

$$\%\text{Drug Entrapment Efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug concentration}} \times 100 \ldots\ldots (1)$$

In Swelling studies, a know weight (100mg) of microspheres was soaked in 20 mL 0.1N HCL for 30 min. After 30 min, the microspheres were then removed and excess 0.1N HCL was wiped using a dry filter paper and their final weights were determined. Then the swelling ratio was calculated as per the following formula

$$\%\text{Swelling index} = \frac{\textit{Weight of wet}\ \text{microspheres} - \textit{Weight of dried}\ \text{microspheres}}{\textit{Weight of dried}\ \text{microspheres}} \times 100 \ldots (2)$$

In-vitro dissolution studies were conducted to determine the release pattern of the drug from Norfloxacin microspheres. Dissolution test for Norfloxacin microspheres was carried out using 8 station USP Type II dissolution test apparatus (Electro Lab, TDT-O8L, Mumbai). The dissolution studies were carried out in 900 ml 0.1 N HCl at 37 ± 0.5oC. The speed of the paddle was set at 100 rpm. Sampling was done every 1 h interval. An aliquot of 5 ml sample was withdrawn at each time interval and replaced with equal volume of fresh medium. The samples withdrawn after suitable dilution were analyzed in the UV spectrophotometer at 274 nm. The mean of three determinations was used to calculate the drug release from each formulation.

Table 1: Formulation of different microspheres of Norfloxacin.

Ingredient (mg)	F1	F2	F3	F4	F5	F6
Norfloxacin (mg)	100	100	100	100	100	100
Sodium alginate(mg)	4	6	4	6	4	6
Okra gum(mg)	50	100	150	50	100	150
Calcium chloride (%)	5	5	5	5	5	5
RPM	800	800	800	800	800	800

CHARACTERIZATION OF MICROSPHERES

Fourier transformer infrared spectroscopy (FTIR) study

The compatibility between drug, polymer and other excipients was detected by FTIR spectra. The pellets were prepared on KBr-press. The spectra were recorded over the wave number range of 4000 to 400 cm^{-1}. The FTIR spectra support the identification of the functional groups present in the compound. The FTIR spectra are also used in comparing with a standard FTIR spectrum of the pure drug to detect any physicochemical incompatibility between the drug and different excipients. FTIR of pure drug, okra gum and sodium alginate was carried out.

Scanning electron microscopy (SEM)

The surfaces and cross-section morphologies of the microspheres were observed using a scanning electron microscope (SEM) operated at an acceleration voltage of 25 kV. The microspheres were made conductive by sputtering thin coat of platinum under vacuum using Jeol JFC-1600 autofine coater and then the images were recorded at different magnifications. The formulation F4 was subjected to SEM studies.

RESULTS AND DISCUSSION

Micropsheres of norfloxacin with natural polymer namely sodium Alginate and okra gum could be prepared by inotropic gelation process. Micropsheres were found to be discrete, large, spherical, and free flowing. The size could be separated, more uniform size range of micropsheres could readily be obtained. The sizes analysis of different micropsheres should be about 761.2 ± 1.30 - 918 ± 0.98. The size of micropsheres was decreased with the increase in the polymer. The size was also reduced with higher levels of calcium chloride and reaction time.

The drug content and drug entrapment efficiency are shown in table 5. The overall drug content was uniform and reproducible in each batch of micropsheres prepared. The drug entrapment efficiency was in the range of 82.0 ± 0.11 - 90.1 ± 0.08 %. The drug entrapment efficiency was lower in increase in polymer. It was also observed that the entrapment efficiency has Enhanced slightly with an increase in cross-linking time, whereas an increase in cross-linking concentration did not influence drug-loading process.

The swelling index of all formulations of Norfloxacin microspheres was between 20.54 ± 0.09% to 33.13 ± 0.22% (Table 5). This indicated that the swelling index of Norfloxacin microspheres increases with increase in the concentration of polymer. Sodium alginate is a hydrophilic polymer and along with okra gum forms mucilage. When the concentration of sodium alginate and okra gum increases, there are more hydrophilic sites available to attract and hold water molecules, leading to greater swelling.

The cumulative percentage drug released from all the prepared Norfloxacin microspheres was found between 74.01% to 98.68% (Table 6). In-vitro release profile showed initial burst effect due to entrapment of drug over the surface of microspheres and later drug released in slow manner over a sustained period of time upto 12hrs. It indicated that increasing the concentration of polymer has resulted decrease in release rate. The retardation in the release rate was due to thicker water insoluble calcium alginate polymer coating along with mucilage non coating of okra gum of microspheres. This can be attributed to increasing diffusional path length through the polymer when polymer concentration increases.

The order of drug release was as follows F1>F4>F5>F2>F3>F6. Formulation containing Norfloxacin F1 was selected as optimised formulation based on percentage release of drug. The release of drug from microsphere depends on particle size. The higher drug release was obtained with smaller particle size. Further the concentration of calcium chloride seems to have little influence on drug release. Also, the curing time seems to have minor influence on drug release.

The IR spectra of pure drug, okra gum, sodium alginate and formulations F3 were studied in detail in order to ascertain whether there is any interaction of drug with excipients (Fig.3,4 and 5). The drug excipient interaction study provides stability data of the drug and self-life of drug. The FTIR spectroscopy is the best method to evaluate the drug excipient incompatibility study. The study of FTIR spectroscopy indicates that the characteristic peaks due to pure Norfloxacin have appeared without any change in their position after successful encapsulation indicating no chemical interaction between Norfloxacin and excipients and the stability of drug microencapsulation process, hence the drug is compatible with excipient used.

The morphological characterisation of microspheres was done by SEM analysis. The SEM pictures of the optimized microspheres (F4) at different magnification (whole microspheres) are shown in Fig.6. The surface of microspheres was rough and irregular (Fig.6A). The microspheres were found spherical in shape and were distinct in nature (Fig.6B).

During the formulation process, particles may interact and agglomerate, leading to a rougher surface. (Fig.6C and 6D)

Table 2: Percentage yield of okra gum.

Sl no.	Batches	Quantity of okra pods (gm)	Weight of mucilage after drying (gm)	Percentage yield (%)
1	Batch 1	5000	26	0.46
2	Batch 2	5000	23	0.44

Table 3: Results of identification test for mucilaginous substance.

Sl no.	Description	Observation	Results
1	Molisch's test	Purple to violet colour ring appears	Presence of carbohydrates
2	Ruthenium test	Pink colour	Presence of mucilage

Table 4: Evaluation of okra gum.

Sl no.	Appearance	λ_{max}	p^H	Swelling index
1	Dark	201nm	6.5	205

Table 5: Characterisation of Norfloxacin microspheres F1 – F6.

Formulation code	Particle size (µm)	Percent yield (%)	Entrapment efficiency (%)	Drug content (%)	Swelling index (%)
F1	761.2 ± 1.30	87.46 ± 0.4	82.0 ± 0.11	82.1 ± 0.08	33.13 ± 0.22
F2	772.06 ± 0.98	89.75 ± 0.16	82.4 ± 0.07	82.3 ± 0.06	26.18 ± 0.26
F3	971.4 ± 1.06	93.9 ± 0.03	86.0 ± 0.09	86.25 ± 0.04	22.83 ± 0.19
F4	815.58 ± 0.95	96.29 ± 0.13	83.9 ± 0.06	83.66 ± 0.06	32.12 ± 0.18
F5	896.2 ± 1.28	91.32 ± 0.77	84.3 ± 0.04	84.55 ± 0.07	28.76 ± 0.10
F6	918.6 ± 0.98	95.75 ± 0.03	90.1 ± 0.08	90.22 ± 0.03	20.54 ± 0.09

***Average of 3 determinations (± SD)**

Table 6: Comparison of *In-vitro* release profile of Norfloxacin microspheres(F1,F2,F3,F4,F5 and F6).

Time (h)	% Drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	30.00	30.00	30.00	30.00	30.00	30.00
2	30.98	30.48	30.83	32.45	30.76	30.84
3	37.02	32.27	32.33	38.48	32.96	32.06
4	40.28	34.90	34.95	43.15	34.54	34.09
5	48.42	37.00	39.18	48.72	39.58	38.39
6	53.50	41.59	44.13	56.62	43.11	41.31
7	68.84	46.60	49.63	61.46	48.05	45.93
8	77.62	54.13	53.95	67.04	52.41	49.93
9	81.19	60.53	57.86	72.04	68.41	54.43
10	88.01	66.49	64.40	78.09	76.82	60.56
11	94.99	74.83	70.38	86.85	86.53	66.05
12	98.68	82.57	80.71	94.03	93.36	74.01

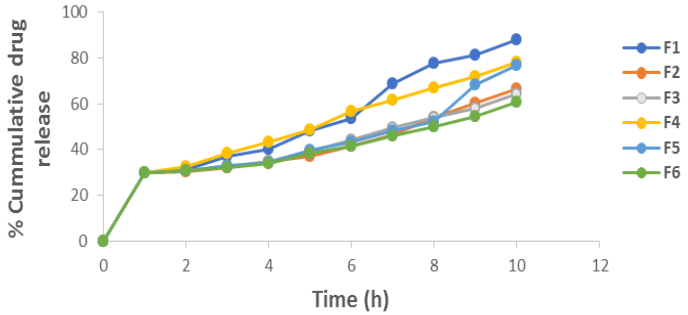

Fig. 2: Comparison of *In-vitro* release profile Atenolol floating tablets (F1,F2,F3,F4,F5 and F6).

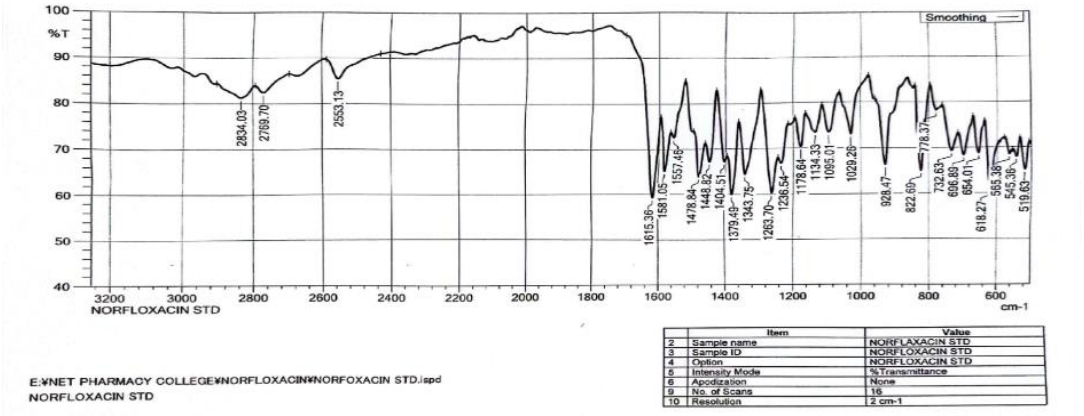

Fig. 3: FTIR Spectra of pure drug Norfloxacin.

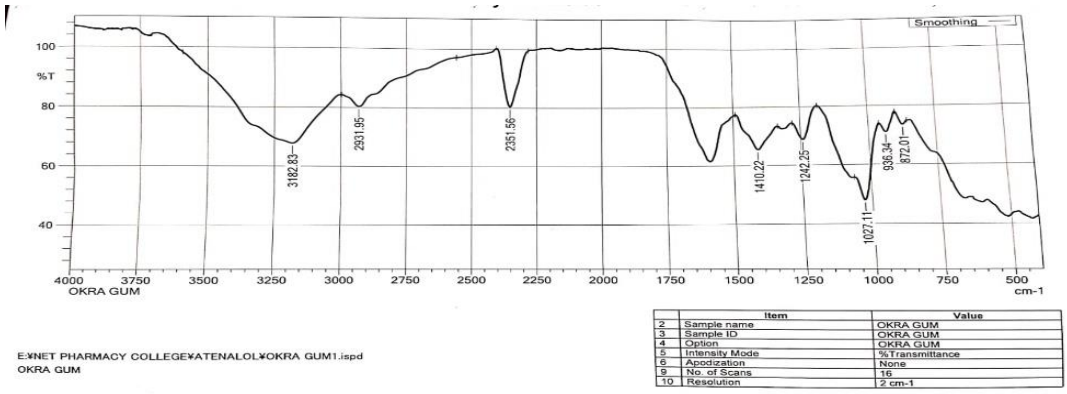

Fig. 4: FTIR Spectra of Okra gum.

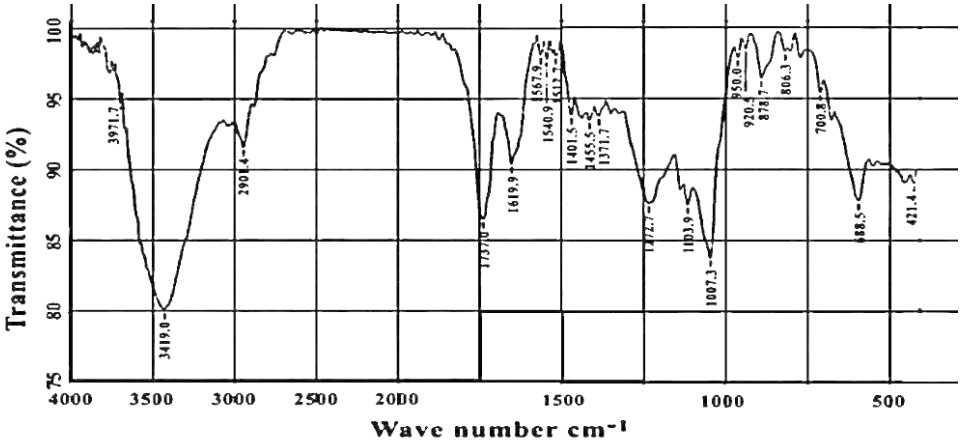

Fig.5: FTIR Spectra of Sodium alginate.

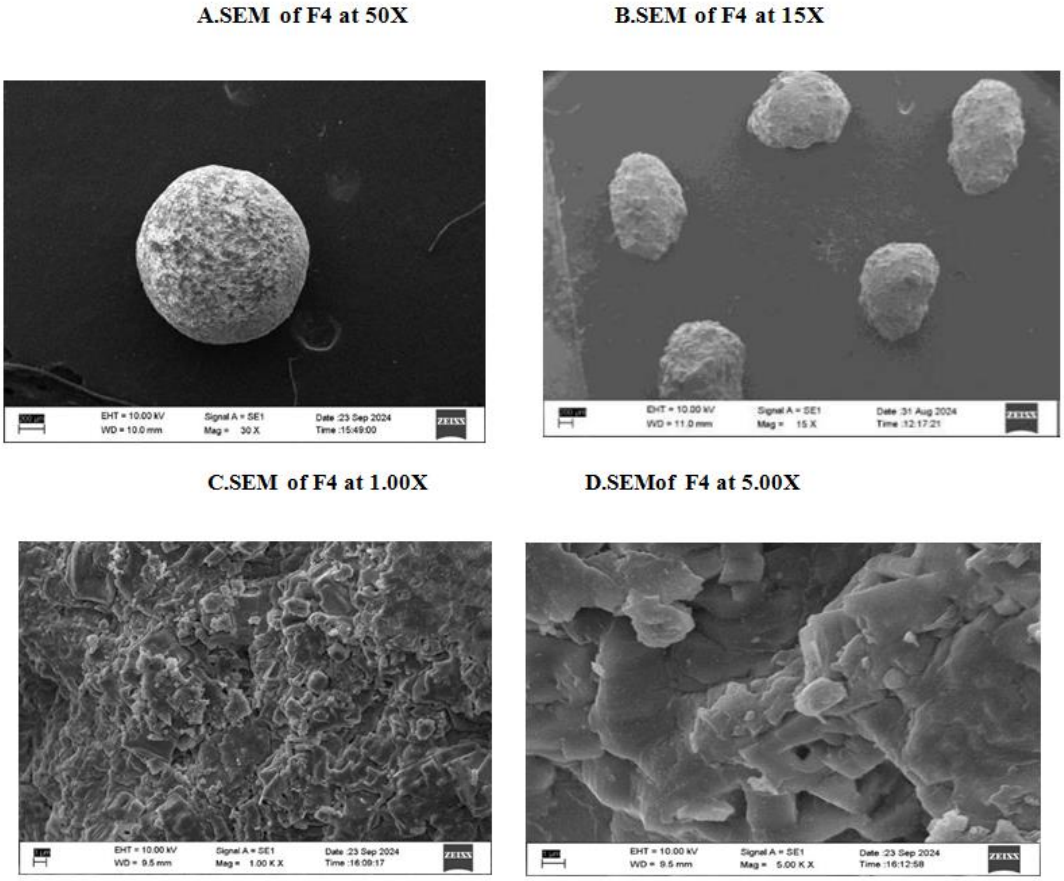

Fig. 6: SEM Photograph of optimised Norfloxacin microspheres F4 at different magnification (Whole microspheres).

CONCLUSION

The study successfully developed and optimized Norfloxacin microspheres using sodium alginate, okra gum, and calcium chloride through the ionic gelation method. Preformulation studies confirmed the compatibility of Norfloxacin with the excipients. Okra gum proved to be an effective rate-controlling polymer, and the microspheres exhibited excellent flow properties and a high percentage yield, ranging from 87.46% to 96.29%. The particle size and swelling index of the microspheres increased with higher polymer concentrations, while drug entrapment efficiency and drug content were observed within acceptable ranges, showcasing effective drug encapsulation. In-vitro release profiles demonstrated controlled and sustained drug release, with formulation F1 showing optimal release (98.68% in 12 hours) and stability confirmed by FTIR studies. SEM analysis revealed spherical microspheres with rough surfaces. Overall, these microspheres demonstrated promising in-vitro results, offering

significant potential for further pharmacokinetic studies and the development of effective drug delivery systems.

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