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Research Article

Formulation and Evaluation of Metronidazole Loaded Nanosponges for Topical Delivery

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Abstract

Formulations of metronidazole M1, M2, M3, M4, and M5 with the polymer ethyl cellulose and M1*, M2*, M3*, M4*, and M5* with the polymer Eudragit RS100 at varying concentrations were prepared and characterized by Zeta sizer, scanning electron microscope (SEM), differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), and X-ray diffraction (XRD). The resulting gels were evaluated for their spreadability, skin irritancy, entrapment efficiency (EE), and drug release. Size in M5* (the most promising nanosponge) was 292.2 nm with a polydispersity index (PDI) of 1.00, and in M2, 371.8 nm size with a PDI of 0.809 was observed. The EE and production yield (PY) with M5* were observed to be 68.40 % and 66.9 %, respectively, while the EE and PY with M2 were 66.70 % and 58 %, respectively. FTIR did not reveal any incompatibility between the polymer and the drugs. SEM results revealed that the shape of nanosponges appeared to be spherical and porous. 10% of the drug was released from nanosponges in Ist hour and almost 70 % in 8 hours.

Keywords: Entrapment efficiency, Franz cell diffusion, nanosponges, polydispersibility index, production yield.

1. Introduction

Nanosponges are interlinked complex structures made up of polymers, are colloidal in nature, and possess nano-size cavities. They enhance the bioavailability, lessen undesired properties and alter the drug discharge profile (Abbas et al. 2017). Their external surface is normally permeable in nature and allows continuous release of the drug from its dosage form. They are mainly employed for topically administered dosage forms. They can be utilized to deliver drugs at the specified and intended target sites, hence avoiding and preventing drug loss for various reasons, one of which is plasma protein binding. By enhancing certain parameters such as the ratio of the drug in comparison with polymer and the speed at which stirring is being carried out, it is certainly possible to synthesize nanosponges of 25μ in size bearing up to 250 thousand superficial pores and an average pore structure of about ten feet along with 1 ml/g average pore volume (Kaura et al. 2022).

Metronidazole, a nitroimidazole derivative, is an antimicrobial agent with strong activity against bacterial and parasitic infestations. It has been reported to be utilized for trichomoniasis for around two decades and furthermore possesses higher activity against amoebiasis and giardiasis (Salunkhe et al. 2018). Normal undesirable effects

associated with metronidazole include the gastrointestinal tract and neurological disorders at higher doses (Ozyazici, Gökçe, and Ertan 2006).

Various methods of nanosponges synthesis have been reported in the literature, including solvent emulsion method, ultrasound-assisted synthesis, and emulsion polymerization. This study adopts the emulsion polymerization technique to prepare metronidazole-loaded sponges. This technique utilizes ethyl cellulose (EC) and polyvinyl alcohol (PVA) to synthesize nanosponges (Patel and Oswal 2012).

2. Materials & Methods

All the chemicals used were of the highest grade of purity. Dichloromethane (methylene chloride) (DCM) was from BDH Laboratory supplies, England. Metronidazole (MET) was a kind gift from Shaigan Pharmaceuticals Ltd; Rawalpindi, Pakistan. EC, Eudragit RS100, and PVA were purchased from Sigma Aldrich.

2.1. Preparation of Metronidazole Nanosponges

0.5% of PVA solution was prepared by weighing 0.5g of PVA using a high precision weighing balance (hi-tech. electronic compact scale) (SF-400A) and transferred to the 250 ml beaker containing 50 ml of distilled water. The beaker was placed in the water bath, having the temperature set at 65°C. The mixture was stirred continuously using a glass rod until PVA was completely dissolved. It was then cooled down to room temperature and transferred to a volumetric flask for the volume makeup. After making up the volume, the solution was transferred to the beaker and labeled as the solution (A). This PVA solution was used for further process (Bhowmik et al. 2018).

100mg of MET was weighed on an analytical balance (Sartorius TE214S) and then crushed using pestle mortar up to the desired size. A relative amount of ethyl cellulose was also weighed in a separate petri plate using the same weighing balance. Both of these materials were transferred to a 100ml beaker. A 20ml of DCM measured through a 20ml pipette was added to the beaker. Continuous stirring was carried out to ensure that both materials were completely dissolved in the DCM.

After the complete solubilization of the materials in DCM, this beaker was labeled as the solution (B). Then 100ml of PVA solution, previously named solution A was placed on a magnetic stirrer set at 1000 RPM. With the help of a syringe (10cc), solution B was slowly added into PVA solution (solution A) and stirred for 2 hours at 1000 rpm. The sample was allowed to stay for 24 hours at room temperature. It was then filtered using the Whatman filter paper. After filtration, the filtrate was dried in an oven and placed in a desiccator till further used for testing (Sreeharsha et al. 2020). The above-explained method was repeated for various concentrations of Ethyl cellulose and Eudragit RS100. Following tables contain the values of drugs and polymers used to prepare various formulations (Table 1 and 2).

2.2. Preparation of Gels

Weighed amount of poloxamer P (407) was dissolved in phosphate buffer and stored in the refrigerator at 4 ^{0}C for 24 hours until the solution turned clear. Carbopol was separately dissolved in phosphate buffer and stored at room temperature for 24 hours for complete hydration. The same was repeated for hydroxypropyl methylcellulose (HPMC) and polyvinyl alcohol (PVA). All the polymers were then mixed with continuous stirring, and a few drops of benzyl alcohol were added as a preservative. Nanosponge-loaded metronidazole was added with continuous stirring into the polymer solution. A few drops of lactic acid and sodium bicarbonate were added to decrease gelling temperature (Sreeharsha et al. 2020).

2.3. Evaluation and Characterization Studies of Prepared Nanosponges

Prepared nanosponges and the loaded gel was evaluated using following analytical techniques.

Table 1. Formulaiton of metronidazole-ethylcellulose.

MATERIAL	M1	M2	M3	M4	M5	M6
Metronidazole	100mg	100mg	100mg	100mg	100mg	100mg
Ethylcellulose	30 mg	50mg	70mg	100mg	130mg	150mg
Drug: Polymer: PVA	1:0.3:0.5	1:0.5:0.5	1:0.7:0.5	1:1:0.5	1:1.3:0.5	1:1.5:0.5
Dichloromethane	20ml	20ml	20ml	20ml	20ml	20ml
PVA(W/V)	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Distilled water	100ml	100ml	100ml	100ml	100ml	100ml

Table 2. Formulaiton of metronidazole (eudraget RS 100).

MATERIAL	T1	T2	T3	T4	T5	T6
Metronidalzole	100mg	100mg	100mg	100mg	100mg	100mg
Eudragit RS100	30mg	50mg	70mg	100mg	130mg	150mg
Drug:Polymer:PVA	1:0.3:0.5	1:0.5:0.5	1:0.7:0.5	1:1:0.5	1:1.3:0.5	1:1.5:0.5
Dichloromethane	20ml	20ml	20ml	20ml	20ml	20ml
PVA(W/V)	0.5%	0.5%	o.5%	0.5%	0.5%	0.5%
Distilled water	100ml	100ml	100ml	100ml	100ml	100ml

2.3.1. X-ray diffraction (XRD) Spectroscopy

XRD discovers the physicochemical way of a substance, regardless of whether it is in crystalline or imprecise shape. In the case of additional sharp peaks detected in the range, the substance is transcendently crystalline. Thus, XRD additionally decides the arrangement of solid dispersion formulations. In the case of solid dispersion, the sharpness of peaks of the medication is diminished along with the enhancement of its dissolvability characteristics (Estelle et al. 1993).

2.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

The majority of analytical applications of FTIR spectroscopy are confined to the mid-infrared region extending from 4000 to 400 cm^{-1} (2.5 to 25 μm) (Aremu and Oduyela 2015). Possible drug-to-excipient interactions can be determined using FTIR. This tool is also used to determine the probable drug-excipients compatibility profile.

2.3.3. Differential Scanning Calorimetry (DSC)

In differential scanning calorimetry (DSC) both the test and the reference substance are exposed to controlled temperature conditions which is appropriately regulated. The difference in heating pattern is recorded for both the sample and reference material (Codispoti et al. 2001).

2.3.4. Particle Size Analysis

Malvern Zeta sizer or laser light diffractometer was used to determine the particle size of metronidazole nanosponges. All samples were analyzed at a fixed angle of 90ºC. For measurement, each sample was diluted with distilled water (Aremu and Oduyela 2015).

2.3.5. Scanning Electron Microscopy (SEM)

The surface morphology of nanosponges was depicted by SEM. Preparation of the sample was carried out by

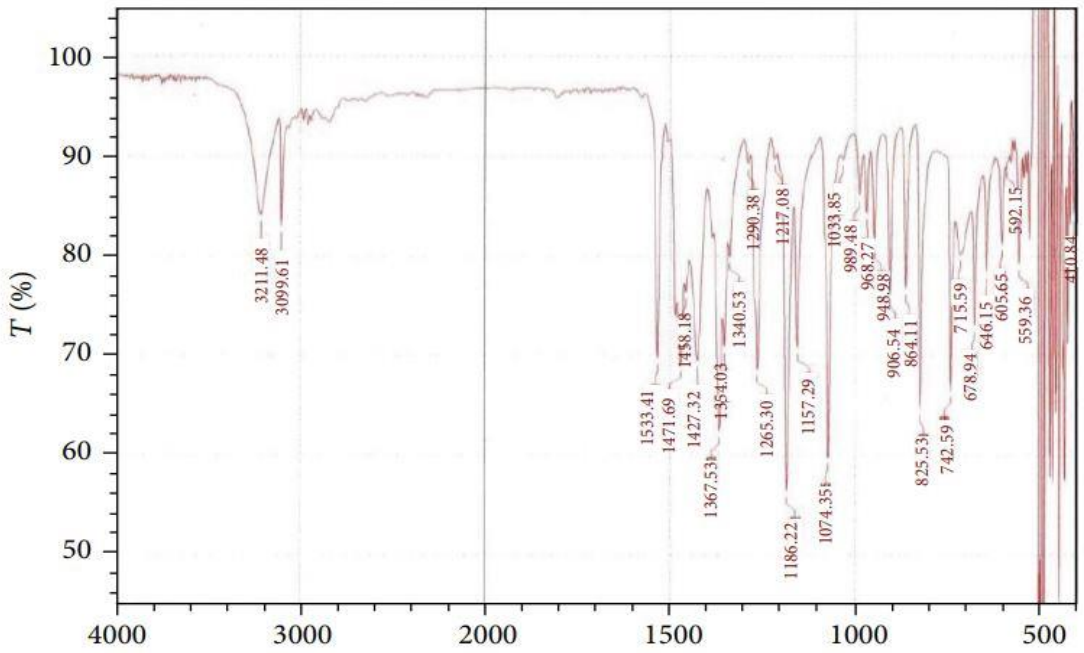


Figure 1. FTIR spectra pure metronidazole.

spraying it on the adhesive tape previously stuck to an aluminum stub coated with platinum. The prepared sample stub was positioned in SEM. The photomicrographs of the sample were taken at a scanning voltage of 20 KV. The average particle size was determined from SEM images.

2.3.6. Production Yield (%)

The weight of prepared lyophilized nanosponges was recorded by weighing them on a digital weighing balance. The production yield was determined using the following formula.

$$\text{Percent production yield} = \frac{\text{Nanosponges final weight}}{\text{Weight of solid mass (polymer + drug)}} \times 100$$

2.3.7. Drug Entrapment Efficiency (DEE)

Percent DEE was determined using the following formula.

$$\text{DEE (Drug entrapment efficiency) (\%)} = \frac{\text{Drug loaded experimentally}}{\textit{Theoretical values}} \times 100$$

2.3.8. *In vitro* Drug Release Studies (Franz Cell Diffusion Method)

Franz cell diffusion was used to carry out the *in vitro* release study of metronidazole nanosponges and the nanosponges-loaded hydrogel(Ng et al. 2010).

2.4. Kinetics of *in vitro* Drug Release

The following mathematical models were applied to the obtained *in vitro* release data

- Cumulative % drug released Vs. T (Zero order kinetics)
- Log cumulative % drug retained Vs. T (First order kinetics)
- Cumulative % released Vs. The square root of T (Higuchi model)
- Log cumulative% drug released Vs. Log T (Korsmeyer-Peppas model)
- Peppas model, the value of 'n' characterizes the release mechanism of the drug.

3. Results

3.1. FTIR

FTIR analysis was conducted using Bruker FTIR at a range of 4000^{-1}cm – 400^{-1}cm of chemical compounds and as a primary tool for the analysis of nanosponges. The FTIR spectrum of the sample

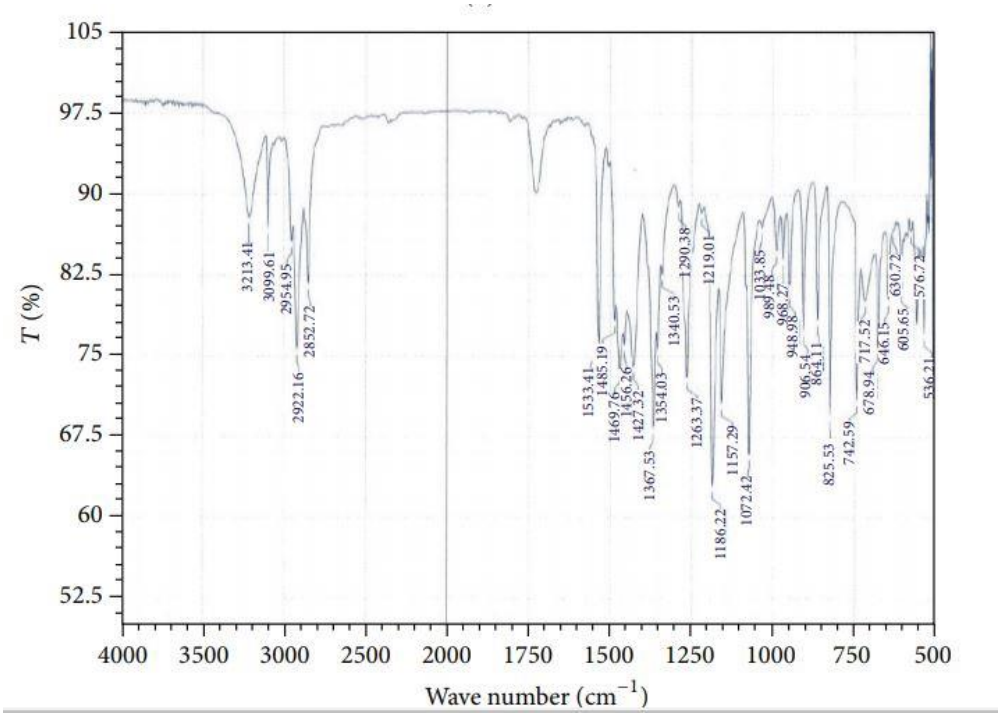


Figure 2. FTIR spectra metronidazole-Eudragit RS100.

(Figure 2 and 3) shows the observed peaks for hydroxyl O–H stretch at (3213.418 cm^{-1}) in the sample (3211.48) for pure drug, carbon-hydrogen (sp^3) C–H stretch (3099.61 cm^{-1}) for both the pure and the sample compounds. The NO_2, N–O stretch (1533.41 cm^{-1}) was observed to be the same as that of the pure drug in a metronidazole-Eudragit combination. There was a slight deviation from that of the pure drug in the sample of metronidazole-ethyl cellulose, C–OH, C–O bend (1074.35 cm^{-1}) pure drug while values obtained from peaks of the sample remained 1072.42 for metronidazole-Eudragit, 1070.49 for metronidazole-ethyl cellulose and C–NO_2, C–N stretch (825.53 cm^{-1}) for the pure drug was recorded to be same in both the sample product as of pure drug compound. These are the characteristic peaks of the drug, and there was no major change in the peak pattern noted from the FTIR spectra of both samples, which indicates that the integrity of the drug in the nanosponge is still preserved. Figure 1 shows the FTIR spectrum of a pure drug, i.e., metronidazole.

3.2. X-Ray Diffraction Studies

A notable difference exists in the diffraction pattern of a newly produced substance from that of an uncomplexed nanosponge when the drug is in liquid form because liquids are considered to have no specific diffraction pattern of their own. This difference indicates complex formation. A comparison among the diffractogram of supposed complex and combination of drug and polymer has to be studied in the case of the solid drug substance. Figure 4 shows the complex formation between drug metronidazole and the polymer Eudragit RS 100 obtained from the formulations M2*, while Figure 6 represents the complexation between the drug metronidazole and polymer ethyl cellulose obtained from formulation M3.

3.3. DSC Studies

The recording of DSC thermograms was carried out using TA Instruments, (Auto Q20 DSC, United State) which had been previously calibrated using indium. The temperature range for heating of the

sample was kept between 30-350°C and the samples were at the rate of 30 °C/min in closed aluminum pans under an argon purge. Triplicate observations were used to determine mean and standard deviation values. The results of the sample showed a peak at 78.32, which indicated the dehydration process. No interaction or incompatibility was seen among the polymers and with the drug, as there exists no change in the peak as compared to the literature. DSC of metronidazole-loaded nanosponges is shown in figures 6 and 7. Figure 6 shows the DSC spectra of metronidazole-loaded nanosponges with ethyl cellulose as polymer obtained from the formulation M5 while figure 7 shows the DSC spectra of metronidazole loaded nanosponges having Eudragit RS 100 as polymer and are obtained from formulation M6.

Table 3 Tabulated studies of zeta size and zeta potential.

Formulation code	Zeta size Avg. (nm)	Zeta Potential Avg. (mV)
M2	371.8 nm	-11.60
M5*	292.2 nm	-8.72

Table 4 Tabulated values of polydispersity index

Formulation Code	**PDI**
M2	0.809
M5*	1.000

3.4. Surface Morphology or Topography of Nanosponges

The surface morphology of metronidazole nanosponges formulation M6* prepared by solvent emulsion diffusion technique was studied and evaluated using SEM. The operational voltage was kept at 10 and 20KV. The sample preparation was spread uniformly on the slab in the form of a concentrated suspension and later dried using a vacuum. A cathode evaporator fitted with 20 (nm) thick gold layer was used to observe the sample. Image processing software was used to capture and enhance the quality of photographs. Mean particle size was obtained by measuring the individual nanosponge diameters. The difference in the crystallization state of the raw materials and the product seen under an electron microscope indicates the formation of the inclusion complexes. The results reveal that the metronidazole-loaded nanosponges were spherical in shape, porous, and spongy in nature. SEM of formulation M6* is shown in Figure 8 (a & b).

3.5. Particle Size Distribution

The nanosponge particle size distribution was found by means of PDI. PDI is considered to be a measure of the size distribution (Shoaib et al. 2018). It was found to be within the range of 0.809 to 1.00. The values less than 1 show that narrow size distribution of particles exists in nanosponges formulated. Values of PDI are shown in table 3.

3.6. Particle Size Determination

The mean values for particle size of nanosponges were calculated with the help of Zeta sizer. The mean size was found to be within the limit of 292-379 nm. The particle size increases with a decrease in polymer quantity. Particle sizes are tabulated in table 3, containing particle size for the formulation metronidazole-EC (M2) and metronidazole-ERS100 (Eudragit RS 100) formulation M5*. Figures 9 and 11 are the representation of particle size distribution of metronidazole formulations prepared using ethyl cellulose and Eudragit RS 100, respectively. The formulations M2 and M5* showed the best results. Figures 10 and 12 show the Zeta potential.

3.7. Production Yield

It was observed that the values of production yield obtained from all the nanosponge formulations were recorded to be between 48.3-68 %, as shown in table **4**. As the polymer ratio increases, ultimately, the production yield increases but to certain optimum limits of the polymer concentration. Because further increasing the ratio of polymer, drug is not filtered properly.

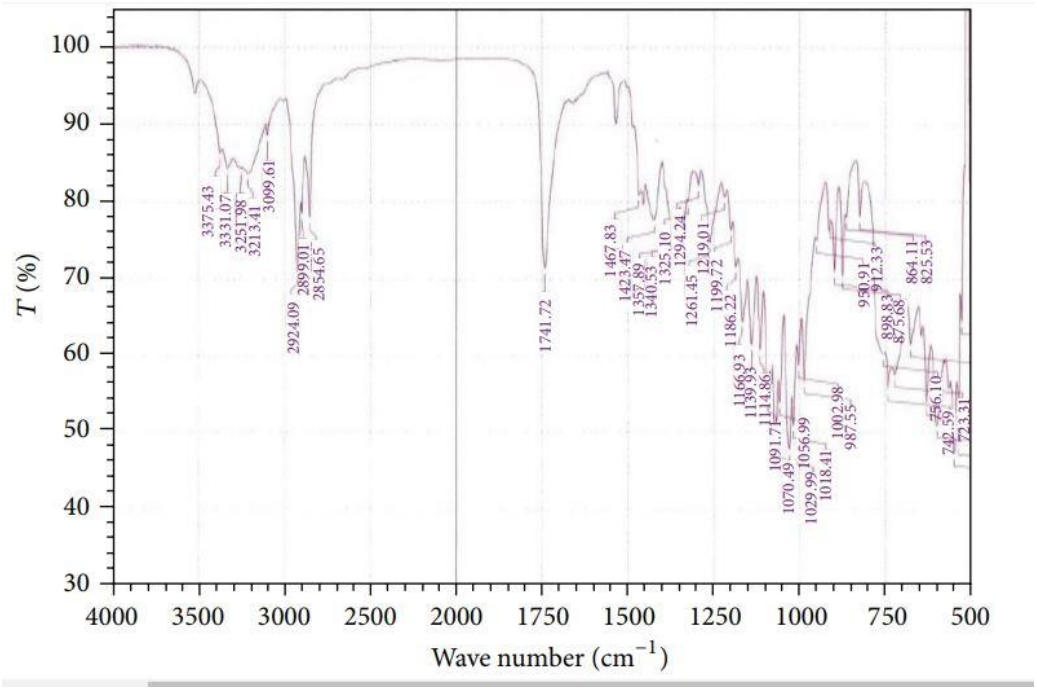


Figure 3. FTIR spectra metronidazole-ethyl-cellulose.

3.8. In-Vitro Diffusion Studies on Nanosponges

Based on the polydispersity index, Zeta potential, and SEM analysis, the M6 formulation of nanosponges was subjected to further studies. The formulation was analyzed using a Franz diffusion cell at an rpm of 100 and about 37.0 (±0.5) ◦C temperature. Absorbance was calculated at 235 nm peak against phosphate buffer as blank. The obtained results were plotted for cumulative drug release (CDR) vs time parameters. Table **6** shows the release data from the metronidazole-loaded nanosponges. Duplicate readings were noted. It clearly shows that almost 10% of the drug is released from nanosponges in the first hour and almost 70 % in 8 hours. Readings were plotted in different models to note the cumulative controlled release of the drug with time.

3.9. Pharmacokinetic Models

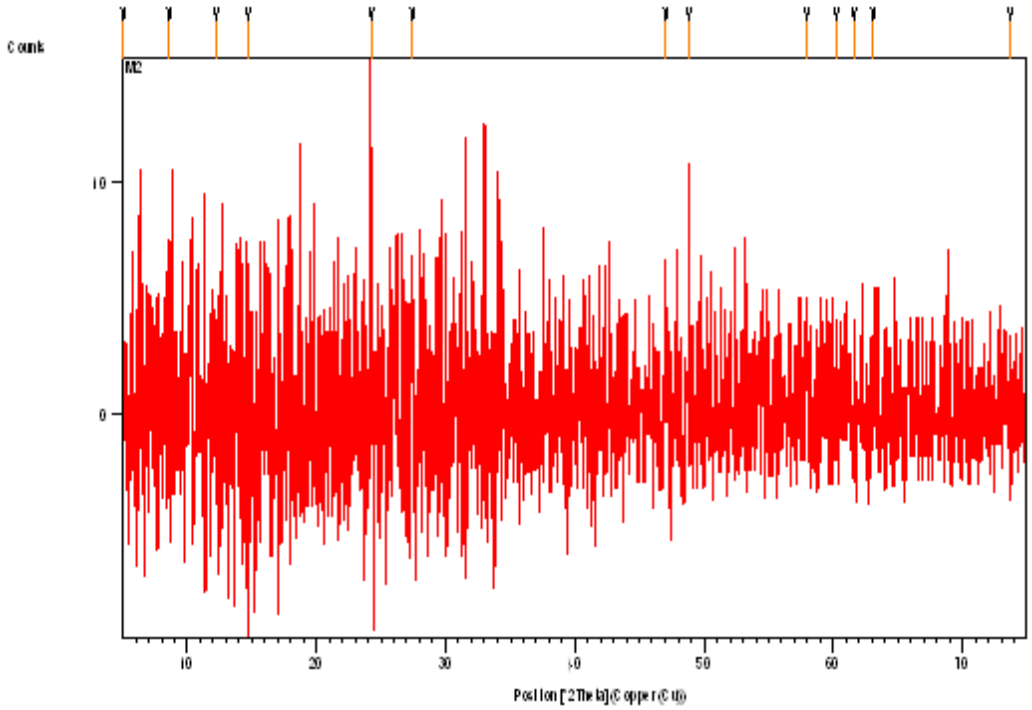
Readings noted from *in- vitro* Franz diffusion test were fitted in different models, and graphs that were obtained are shown in various figures. In figure 13, the cumulative log %age amount of drug release is plotted against time, which is representative of first-order kinetics. The plot of percentage release against the square root of time represents the Higuchi model, as shown in figure 14. The cube root of percent drug retained vs time represents the Hixon Crowell model as given by figure 15, while figure 16 represents the log of cumulative % release vs log time plot. These results showed that the formulation released the drug constantly over a period of time, as shown by first-order kinetics and the Higuchi model.

4. Discussion

In this study, metronidazole-loaded nanosponges were designed using polymers such as ethyl cellulose and Eudragit with grade RS. One of the major advantages of Eudragit is that it has pH-independent swelling properties, no toxicity, and a well-controlled release profile due to the presence of an ammonium group. Keeping in view all the characteristic features of the polymer and its compatibility with the metronidazole, formulation was proceeded for designing.

When the ratio between drug and polymer is increased up to certain limit the particle sizes is decreased. In this study, only polymer ratio was

Graphics



Peak List

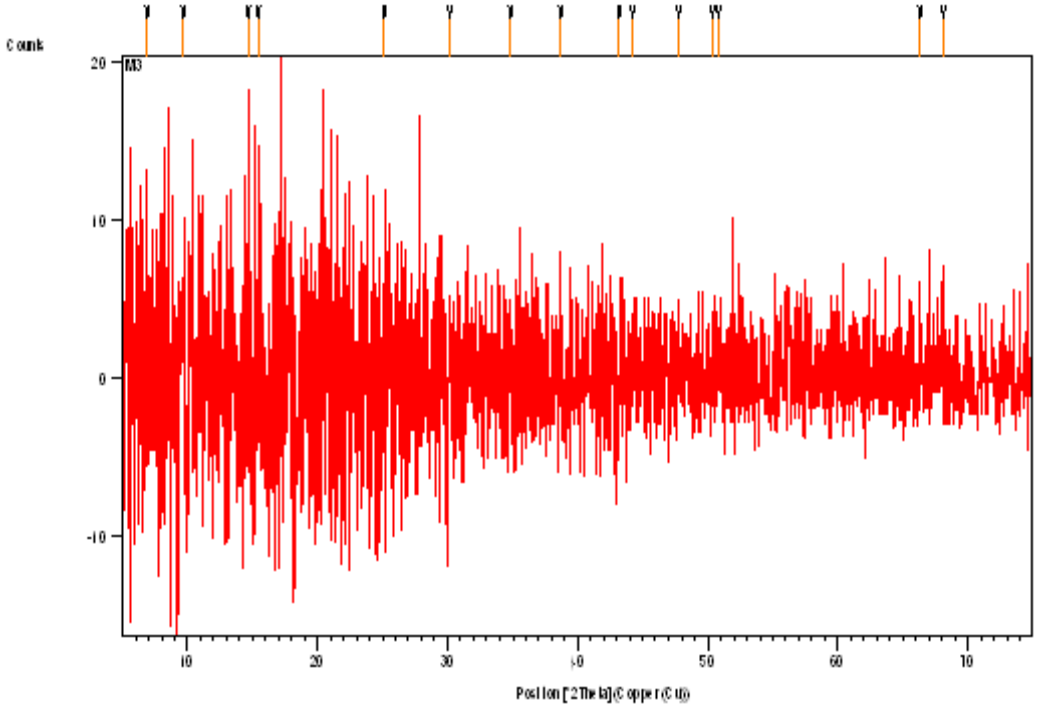
Pos.[°2Th.]	Height[cts]	FWHM[°2Th.]	d-spacing[Å]	Rel.Int.[%]
5.0809	5.22	0.2952	17.39293	61.82
8.6256	7.86	0.0886	10.25159	93.18
12.2372	3.03	0.7085	7.23293	35.96
14.7267	7.74	0.0590	6.01536	91.72
24.2551	8.44	0.1771	3.66958	100.00
27.4468	2.18	0.2362	3.24968	25.82
47.0108	3.65	0.2362	1.93297	43.28
48.8252	2.21	0.2362	1.86530	26.19
57.9467	1.93	0.2362	1.59152	22.87
60.2210	2.48	0.1181	1.53674	29.34
61.6167	5.31	0.0720	1.50400	62.92
63.1113	2.10	0.4723	1.47315	24.95
73.6728	3.85	0.1440	1.28483	45.67

Figure 4.0 XRD-Spectra metronidazole: eudragit RS 100.

changed while all other variables, i.e., the quantity of the drug, cross-linker, internal phase, stirring speed, and stirring time, were kept constant. The potential charge is helpful in confirming the stability of the formulation (Kaur, Aggarwal, and Harikumar 2015).

From Zeta sizer results, it was evident that the M5 formulation has the best particle size, i.e., 292.2nm.

Graphics



Peak List

Pos.[°2Th.]	Height[cts]	FWHM[°2Th.]	d-spacing[Å]	Rel.Int.[%]
6.9091	8.07	0.9446	12.79427	44.92
9.7024	10.44	0.2952	9.11610	58.08
14.6718	17.97	0.0886	6.03774	100.00
15.4099	3.82	0.4723	5.75020	21.28
25.1391	1.01	0.1476	3.54251	5.60
30.1789	4.11	0.1771	2.96142	22.86
34.8262	0.12	0.2952	2.57616	0.65
38.7065	3.86	0.2362	2.32636	21.48
43.2165	2.18	0.1771	2.09347	12.11
44.1792	2.35	0.5904	2.05006	13.08
47.7635	2.86	0.9446	1.90425	15.92
50.3680	3.62	0.0720	1.81023	20.14
50.8309	1.21	0.4723	1.79631	6.71
66.3562	1.96	0.2362	1.40876	10.92
68.1323	7.69	0.0720	1.37517	42.80

Figure 5. XRD-Spectra metronidazole: ethyl cellulose.

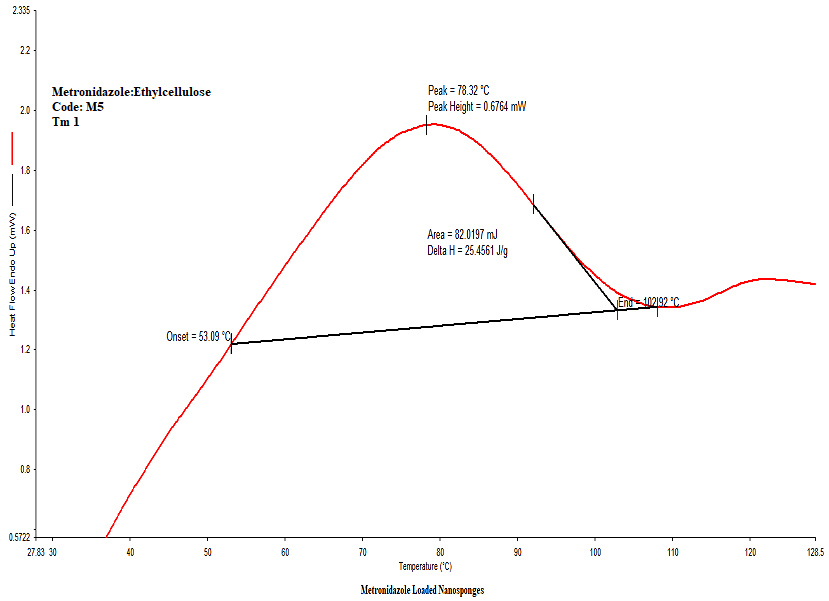


Figure 6. DSC spectra metronidazole-ethylcellulose.

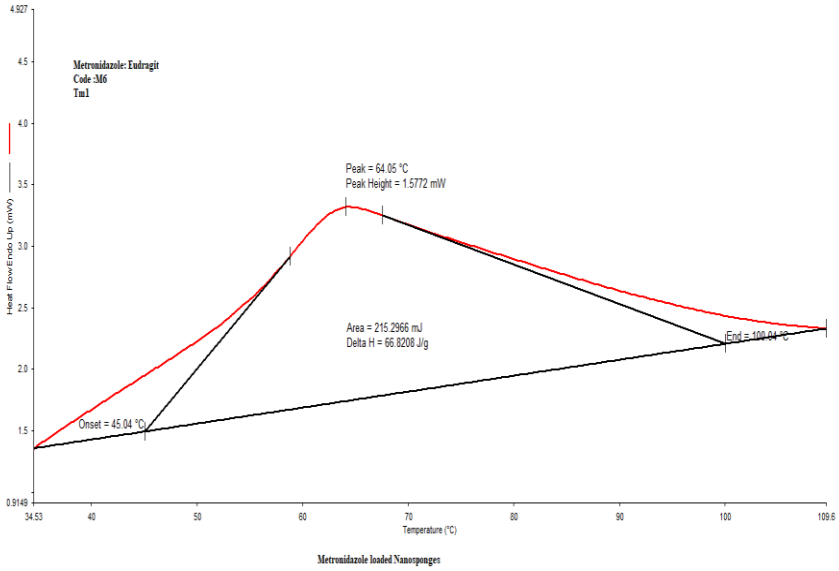


Figure 7. DSC spectra metronidazole-Eudragit RS 100.

Production yield is indirectly linked to the pace at which it is being stirred. By increasing the speed, a reduction in nanosponge size is observed, but a decrease in production yield is inevitable due to the fact that nanosponges stick with the stirrer. The research has suggested that the quantity of internal phase also shows its significant impact on the formation of nanosponges. From our result, it has been shown that better entrapment efficiency was found for the M5 formulation, which shows that almost 65.9 % of metronidazole is entrapped in the nanosponges.

From the results of DSC, it was demonstrated that there was no formation of new peaks, which confirms that there was no interaction between the molecules of the drug and the polymer used.

Morphological studies of metronidazole-loaded nanosponges were further studied with the help of

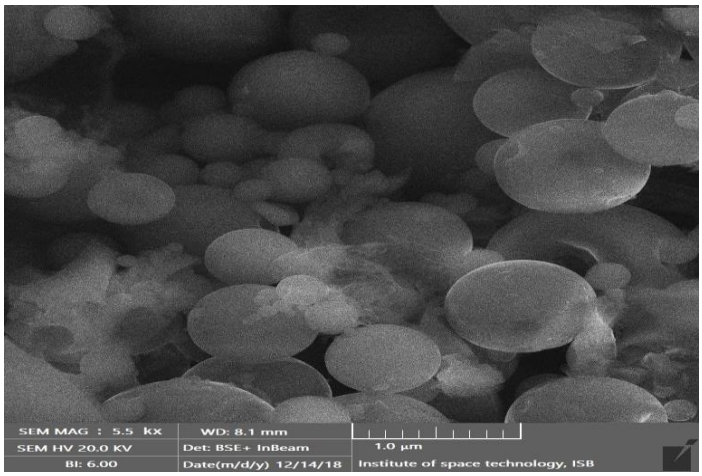


Figure 8. (a) SEM of Formulation M6*.

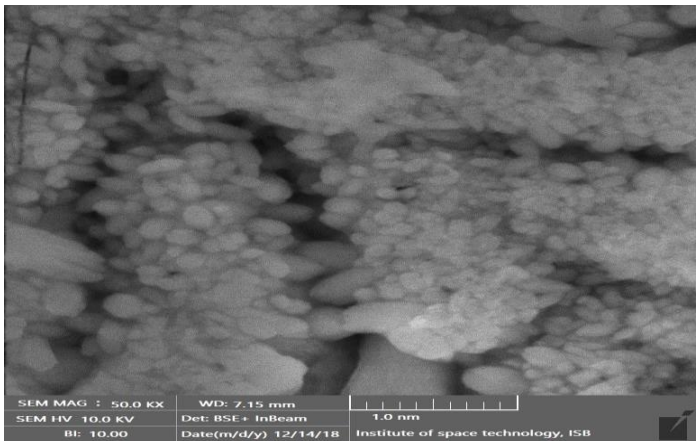


Figure 8. (b) SEM of Formulation M6*.

SEM. It was revealed from SEM results that the nanosponges are spherical in shape with a porous structure. On the basis of particle size, Zeta charge, SEM morphology, and % entrapment efficiency, M5* formulation was selected and was studied for *in-vitro* studies. In this current study, metronidazole-loaded nanosponges were evaluated for diffusion studies by using the Franz diffusion cell. Franz diffusion cell measures the amount of drug diffused from the said formulation and the amount of drug washed away. It is a helpful technique for studying the drug release process as well as the drug permeation across the skin membrane.

Our study used a Franz diffusion cell with a dialysis membrane of about 0.22-micrometer pore size. Results revealed that *in-vitro* kinetics of metronidazole provides a constant release that follows first order over a period of 8 hours. Data obtained depicts that the nanosponges formulated from Eudragit RS 100 showed no burst effect and were released in a controlled manner, and it has hence shown that the drug is not weakly adsorbed at the surface of nanosponges.

The ease of penetration through skin layers for nanosponges is attributed to their nanosized and colloidal nature. The predictability of drug release pattern at the intended site from the dosage form is reported to be highly evident in the case of

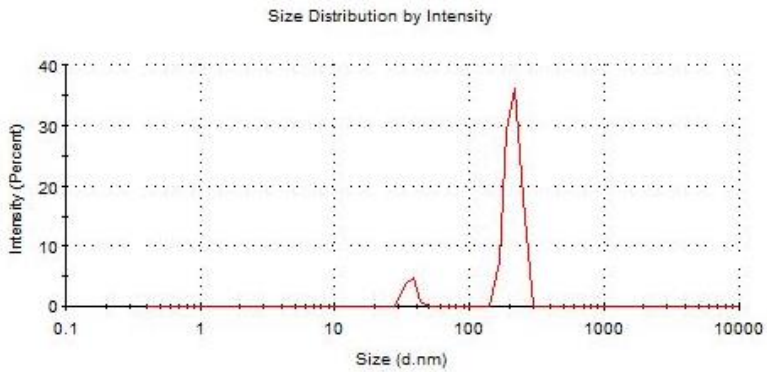


Figure 9. PDI metronidazole-ethyl-cellulose nanosponges

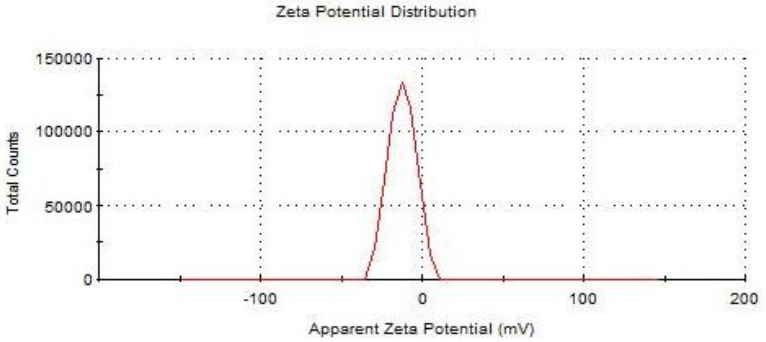


Figure 100 ZPD metronidazole-ethyl-cellulose nanosponges

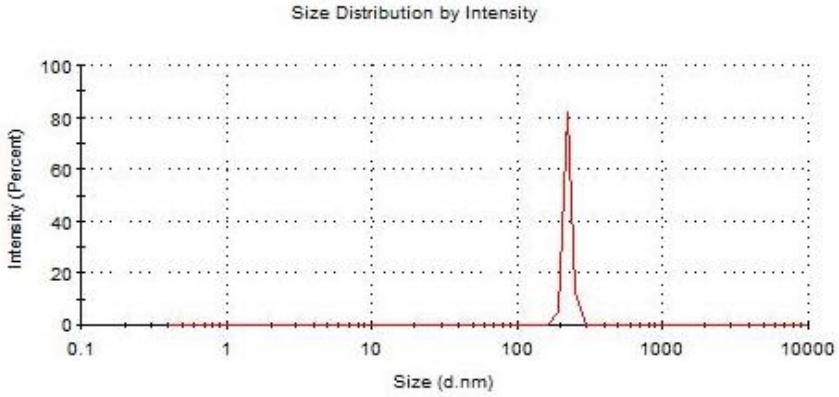


Figure 11. PDI metronidazole-Eudragit RS 100 nanosponges.

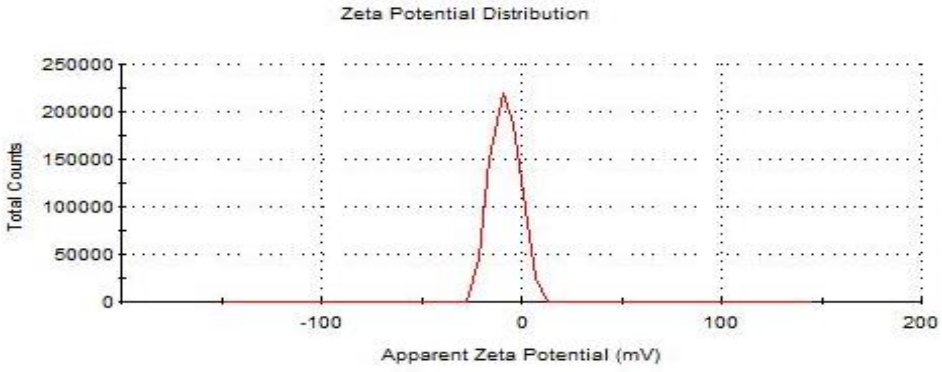


Figure 12. ZPD metronidazole-Eudragit RS 100 nanosponges

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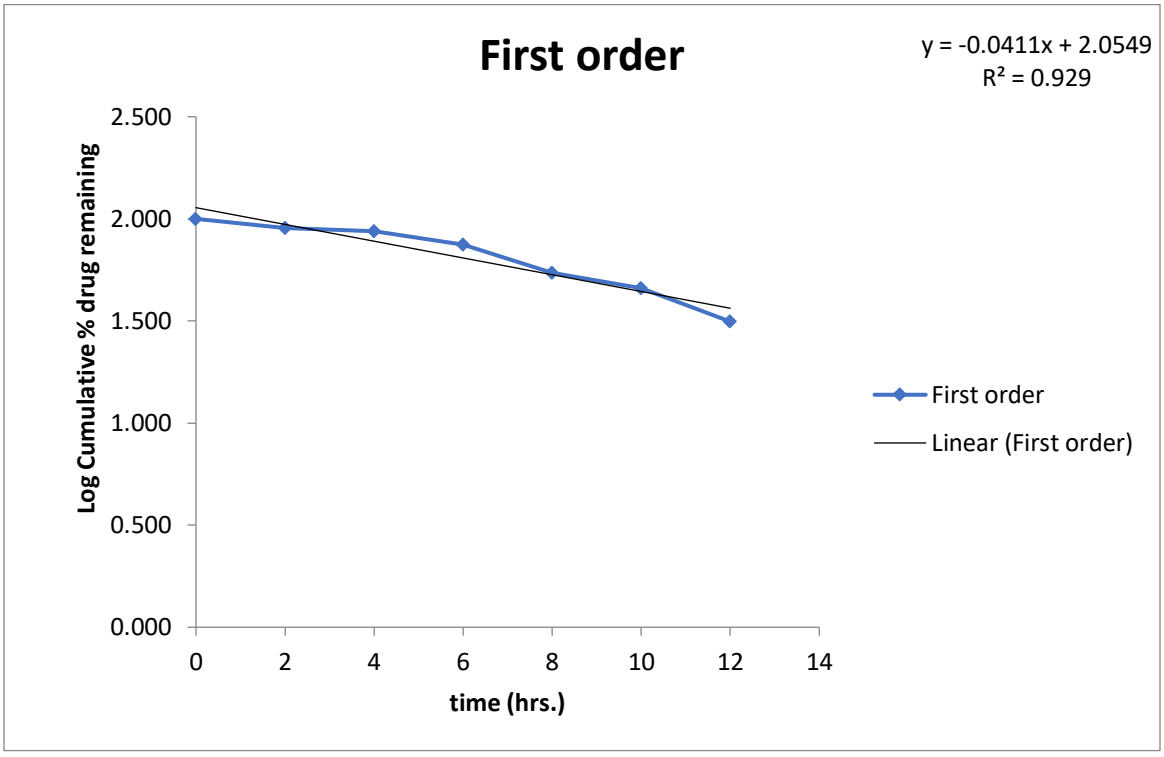

Figure 13. First order release pattern of metronidazole.

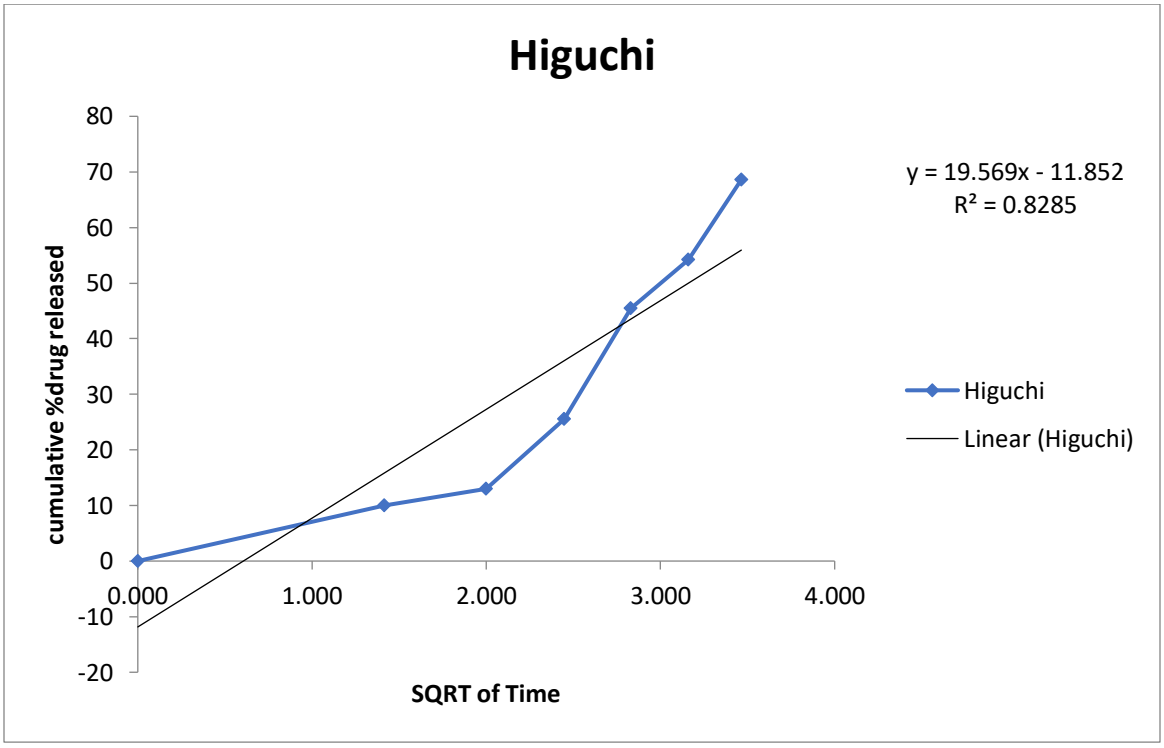

Figure 14. Higuchi model for the release pattern for metronidazole.

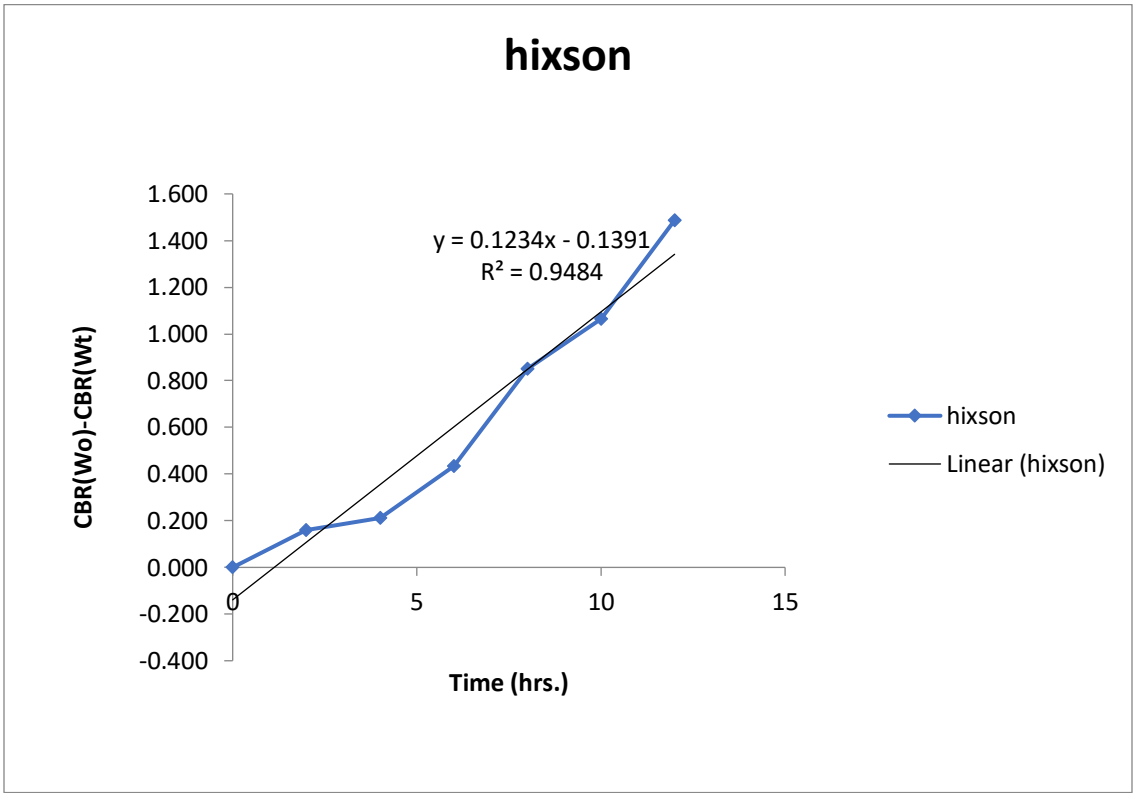

Figure 15. Hixon Crowell model for release pattern for metronidazole.

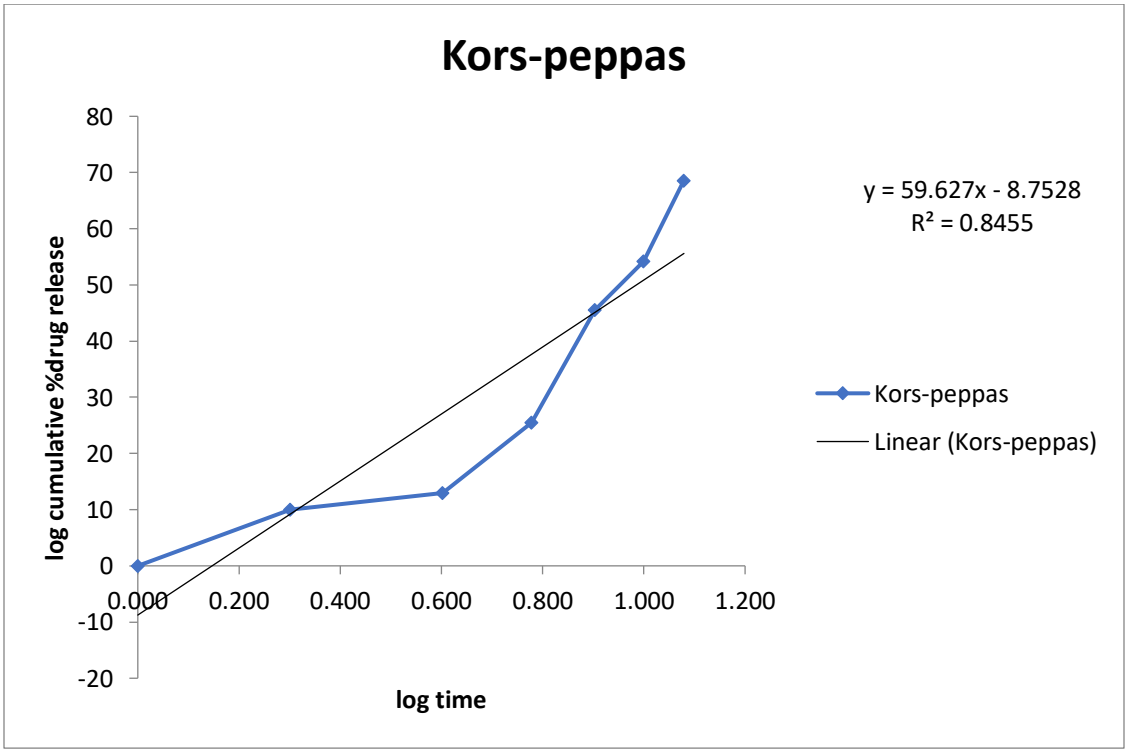

Figure 16. Korsmeyer peppas model for release pattern for metronidazole.

nanosponges. Reduction in frequent application in the case of the topically applied drug has resulted in patient compliance and ease when designed from nanosponges. Their utilization as topically administered drugs has also resulted in a prolonged stay of the drug at the site of action on the skin.

5. Conclusions

In the context of obtained facts and figures, it can be deduced that metronidazole nanosponges possess novelty in their biocompatibility, versatility, and polymeric cross-linking, resulting in the enhancement of aqueous solubilization of the metronidazole. These nanosponges possess the capability to discharge the drug from its dosage form in the expected and designed pattern at the intended site. This innovative technology not only provides a safe delivery method for the active drug by its encapsulation but also results in a reduction in its undesired effects and an improvement in stability. Furthermore, a greater elegance in this dosage form can be attained for metronidazole.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Study Approval

Yes. The study was approved by the Institutional Review Board of the Riphah International University Islamabad, Pakistan.

Consent Forms

NA.

Authors Contribution

RAK, AS1, AS2; conceptualized the study, RAK; wrote the final manuscript, AS2, MAS, MNA helped in the formal analysis, RAK, and AS1; did the experimental analysis, and RAK and AS2; supervised the whole project.

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