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Original Research Article

FORMULATION AND EVALUATION OF SELENIUM NANOPARTICLES OF METRONIDAZOLE FOR ORAL DELIVERY OF BIOACTIVES

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ABSTRACT

Nanoparticles have an enormous impact on society. Selenium nanoparticles are used in various oxidative stresses. Selenium nanoparticles (SeNPs) have attracted lots of attention recently owing to their excellent bioavailability and low toxicity. However, the stability of SeNPs needs to be improved. The 5-nitroimidazole drug metronidazole has remained the drug of choice in the treatment of anaerobic infections, parasitic as well as bacterial, ever since its development in 1959. In contrast to most other antimicrobials, it has a pleiotropic mode of action and reacts with a large number of molecules. The objective of the present study was to formulate and evaluate selenium nanoparticles of metronidazole for oral delivery of bioactives. The synthesized SeNPs were characterized using particle size, zeta potential, entrapment efficiency, scanning electron microscopic (SEM), *In-vitro* drug release. There was no interaction between the medication and the excipients in FT-IR experiments. The improved formulation's percent EE, particle size, and zeta potential were found to be 98.23%, 149.1 nm and -3.9 mV respectively. F4 was shown to be the most promising formulation among all created formulations in this investigation. Scanning electron micrograph of the prepared nanoparticle at 8.21 kx magnification showed that the nanoparticle were smooth surface morphology and spherical shape. Among all, the formulation F4 was found to be best formulation which releases 97.92% of the drug within 24hr. The data obtained from In-vitro release were fitted into the various kinetic models such as Zero Order, Higuchi, First Order and Korsmeyer–Peppas Model in order to determine the mechanism of drug release. When the regression coefficient values compared, it was observed that 'r' values of formulation F4 was maximum i.e 0.955 hence indicating drug release from formulations was found to follow Higuchi drug release kinetics. The current project also aims to improve the formulation's pharmacological acceptability.

Keywords: Nanoparticles, Selenium, Metronidazole, Scanning electron microscopic

INTRODUCTION

Metronidazole is one of the most preferred drug of choice for intestinal amoebiasis, giardiasis, trichomoniasis, bacterial vaginosis, surgical infections and duodenal ulcer associated with *Helicobacter pylori* infections, etc. (Busatti *et al.*, 2007; Graham *et al.*, 1991). Metronidazole is slightly soluble in water and is usually about 80% absorbed orally, although absorption in some patients can be much lower (Nasir and Naji, 2000). Conventional tablets of metronidazole provide

minimal amount of drug for the local action in colon, still resulting in the relief of amoebiasis, but with systemic side effects (Krishnaiah *et al.,* 2002). The minimum inhibitory concentration and bactericidal concentration of me-tronidazole is 3.1 and 6.3 mg/ml, respectively (Kuznetsova *et al.,* 2000). Metronidazole dosing regimens often include sets of daily doses extending over periods of several weeks. Since the elimination halftime of metronidazole is 6–8 h; the drug has to be taken 3–4 times per day in order to maintain the desired therapeutic level. Site specific and controlled release dosage forms will prolong the residence time and improve bioavailability (Skiba *et al.,* 2006). Hence, alternate dosage forms in which metronidazole is released slowly from a drug carrier have been suggested as a means to reduce dosing frequency and systemic toxicity (Baumgartner *et al.,* 2000; Iannuccelli *et al.,* 1998). Nanomedicines are emerging as a significant class of therapeutics. The most encouraging aspect of using nanoparticles in therapeutics is their capability to target or accumulate at the site of targeted tumour tissues with negligible toxicity. The nanomaterial, owing to its size, permeates through the kidney or liver due to surface decorations, and the cells diffuse through the blood vessels and vasculature of tumour tissues, thereby extending its presence in the blood circulation and this is explained through the phenomenon of EPR (enhanced permeation and retention). Moreover, nanoparticles have high surface area to volume proportions (Chowdhury *et al.,* 2016), yielding high adsorbing properties (Liu *et al.,* 2017). On this basis, nanoparticles are adsorbed with therapeutic drugs (Kumari *et al.,* 2018), phytochemicals with anticancer properties (Micova *et al.,* 2018), imaging agents (Raj, 2020), or target-specific genes or peptides as targeting ligands to the cancer receptor cells (Vairavel *et al.,* 2020). Selenium is an essential element as well as a micronutrient that has been frequently used in the treatment of diseases (Zonaro *et al.,* 2018). Selenium in nano form has safer and more costeffective antibacterial and antioxidant effects than in its other forms (Abbas *et al.,* 2021). Using different SeNPs concentrations (100, 200, and 250 µg/mL), the antimicrobial activity of SeNPs against various phytopathogenic bacteria and fungi was determined. However, 100 µg/mL showed the best results in controlling 99 percent of different bacteria, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Utilizing selenium nanoparticles (SeNPs), which are less toxic and have higher bioavailability and biological activity, is a novel method of fertilizing plants (Srivastava and Mukhopadhyay, 2015). SeNPs enhanced the phenolic compounds, total chlorophyll contents, antioxidant defense systems, and morphological and genetical attributes of the Vicia faba plant under biotic stress of Rhizoctonia solani (Hashem *et al.,* 2021). The antifungal potential of plant-based SeNPs at 100 ppm is the most effective to inhibit the growth of Alternaria alternata, which causes leaf blight in tomatoes (El-Gazzar and Ismail, 2020). SeNPs have been synthesized using a variety of techniques. However, reducing chemicals such as hydrazine and sodium ascorbate were employed chemically to synthesize SeNPs to create their multi-functional potential. The chemical processes are expensive, demand specialized equipment,

and are damaging to the environment. Synthesis of selenium nanoparticles is an eco-friendly, non-toxic, biocompatible, and cheap means of synthesizing NPs because plant extracts act as reducing and stabilizing agents (Liang *et al.,* 2021). In the present investigation, we attempted to develop and optimize selenium nanoparticles of metronidazole for oral delivery of bioactives.

MATERIALS & METHODS

Materials

Metronidazole was a gift sample from Shreya Life sciences (Aurangabad, Maharashtra, India). Sodium selenite was purchased from Himedia Laboratory, Mumbai. Ethanol, chloroform and Dichloromethane purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Demineralized and double distilled water was prepared freshly and used whenever required. All other reagents and chemicals used were of analytical grade.

Pre-formulation study of pure drug (Jain and Verma, 2020; Chowk, 2020; Kumbhar and Salunkhe, 2013; Behera *et al.,* 2012; Balla, and Goli, 2020)

Organoleptic properties

Organoleptic properties of metronidazole were observed by visual observation. The organoleptic studies of hydroquinone like general appearance like color, odor, state, etc. were performed.

Solubility study

Qualitative solubility of metronidazole in different solvents was determined according to USP NF, 2007. Approximately 1 mg of metronidazole was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, ethanol, DMSO, chloroform and water).

Melting Point

Melting point was analyzed by open Capillary method using Thiele's tube. Few quantity of the metronidazole was placed in a thin walled capillary tube 10-15 mm long, about 1mm inside diameter, and closed at one end. Liquid paraffin oil was filled in the thieles tube and placed in the contact of flame. The capillary was suspended into the thiele's tube and heat the sample slowly; thermometer was attached to check the temperature. The temperature at which the sample starts to melt wastaken as the melting point of the sample.

Partition coefficients

The partition coefficient of drug metronidazole was examined in, n-Octanol: water system. It was determined by taking 5mg of metronidazole (drug) in separating funnel containing, 20ml of n-Octanol and 20 ml water. The separating funnel was shaken for 2 hours in a wrist action shaker for equilibrium. Two phases were separated and the amount of drug in aqueous phase was analyzed spectro photo metrically at 294.0 nm.

$$K = \frac{Amount\ of\ drug\ in\ organic\ phase}{Amount\ of\ drug\ in\ aqueous\ phase}$$

Determinations of λ_{max} (maximum absorbance)

Preparation of metronidazole standard stock solution in methanol

Standard solution of metronidazole was prepared by dissolving accurately weighed 10 mg of metronidazole with 5 ml of methanol solvent, in a 10 ml volumetric flask. The volume was made up to 10 ml with methanol to obtain a stock solution of 1000 µg/ml. 1ml of this stock solution was taken and then

diluted up to 10 ml using respective solvent (methanol) to obtain a solution that has a concentration 100 µg/ml which is standard stock solution.

Lambda max

From the above stock solution 1 ml sample was transferred into a 10 ml volumetric flask and the volume was made up to mark with solvent to prepare a concentration of 10 µg /ml. The sample was scanned by UV-VIS Spectrophotometer in the range of 200- 800 nm, using respective solvent as a blank. The wavelength corresponding to the maximum absorbance (max) was found. This was further utilized to obtain a calibration curve.

Linearity or standard curve

Aliquots of 2, 4, 6, 8, 10 and 12µg/ml of 100µg ml drug working standard solution were accurately transferred into a series of 5 ml calibrated flask and made up to the mark with drug. The absorbance of the resulting solution was measured 327 nm against solvent blank. Calibration curve was prepared by plotting the absorbance vs concentration of drug.

Six points calibration curve were obtained in a concentration range from 2-12µg/ml for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.074x + 0.002 with correlation coefficient 0.990**.**

Fourier transmission infra-red spectroscopy

FT-IR spectrum of drug was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of Drug and 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region.

Formulation of Selenium nanoparticle

SeNPs were synthesized by mixing the metronidazole drug with a salt stock solution. The stock solutions of sodium selenite (10 mM) were prepared by adding 1.25 g of sodium selenite salt to 500 mL of distilled water and then allowed to heat at 80 ◦C along with magnetic stirring on a hot plate for 30 min. Then, Different concentration (100, 150, 200, 250, 300 mg) of drug (metronidazole) was added drop wise into the stock solution until its color changed from green to brick-red after 2 h of continuous heating and magnetic stirring. When the brick-red color formed, it was then allowed to cool. Centrifugation was performed using a centrifuge machine at 1000 rpm for 15 min at 25 ◦C. The supernatant was discarded, and the pellet was collected by adding methanol. After it was collected, the pellet was centrifuged thrice to remove remaining drug and salt. The resulting selenium nanoparticles were subjected to characterization and then were used for in vitro purposes (Shahbaz *et al.,* 2023).

Table 1: Composition of Selenium nanoparticle formulation

S. No.	Drug concentration (mg)	Sodium Selenite (10 mM) (ml)	Stirring (time)
1	100	10.0	15
2	150	10.0	15
3	200	10.0	15
4	250	10.0	15
5	300	10.0	15

Characterization of Selenium nanoparticle

(Balla and Goli, 2020; Penjuri, *et al.,* 2016)

Color change

Color change in the preparation of nanoparticle section will be monitored at different interval of 30 min, 60 min, 120 min and 180 min.

Particle size

The particle size is one of the most important parameter for the characterization of nanoparticle. The size of nanoparticle was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette.

Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanoparticle was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements.

Entrapment efficiency

%Entrapment efficiency was determined by indirect estimation. Drug -loaded nanoparticles were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug entrapment calculated by using below equation.

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc.*100

Scanning electron microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized nanoparticle were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography.

In-vitro drug release

The *in-vitro* drug release study of drug loaded nanoparticles formulations were studied by dialysis bag diffusion method. Drug loaded nanoparticles were dispersed into dialysis bag and the dialysis bag was then kept in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 1 °C throughout the experiment. During the experiment rpm was maintained at 100 rpm. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer at 327 nm. To analyze the *in vitro* drug release data various kinetic models were used to describe the release kinetics.

To analyze the in vitro release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release

rate is independent of its concentration. The first order Eq. (3) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. The results of *in- vitro* release profile obtained for all the formulations were plotted in modes of data treatment.

Zero - order kinetic model – Cumulative % drug released versus time. First – order kinetic model – Log cumulative percent drug remaining versus time. Higuchi's model – Cumulative percent drug released versus square root of time. Korsmeyer-Peppas model -- log cumulative % drug release vs log time (Korsmeyer–Peppas model)

RESULTS AND DISCUSSION

The solubility of metronidazole was determined in various non-volatile or volatile liquid vehicles such as Dimethyl sulfoxide, methanol, ethanol, water, and dichloromethane shown in Table 2. From the results, it was observed that the drug is freely soluble in methanol, ethanol and DMSO and soluble in water, sparingly soluble in Dichloromethane. The melting point of the metronidazole drug was found to be 162ºC, which is well within the limits of the drug specification. The partition coefficients of the metronidazole were found to be 0.31. λ_{max} of metronidazole was found to be 327.0 nm by using U.V. spectrophotometer (Shimadzu-1700) in linearity range 2-12µg/ml Figure1. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y = 0.074x + 0.002$ with correlation coefficient $R^2 = 0.990$**.** The IR spectrum of sample drug shows the peak values which are characteristics of the drug and the graph were shown in Figure 2. The particle size is one of the most important parameter for the characterization of

nanoparticles. The average particle sizes of the prepared selenium nanoparticle formulation

were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of nanoparticles was found to be range between 149.1 to 552.2 nm. These particle size values indicate that the all formulated nanoparticle is under the range (Below 1000 nm) of nanoparticle and F4 is the lowest particle size of all formulation shown in above Table 3 & Figure 3. Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range 0.1 to -3.9 mV with peak area of 100% intensity. These values indicate that the all formulated nanoparticle is stable Table 4 & Figure 4. This might be due to the fact that the variation in entrapment efficiency was due to the changes in the drug concentration. The prepared selenium nanoparticle possesses high drug entrapment efficiency and found to be in the range of 98.23 to 80.05% Table 5. Scanning electron micrograph of the prepared nanoparticle at 8.21 kx magnification showed that the nanoparticle were smooth surface morphology and spherical shape. The smooth surface morphology and spherical shape of nanoparticle was clearly observed in the SEM images Figure 5. The data of percentage drug release formulation were shown in Table 6. For kinetic study following plots were made: cumulative % drug release vs. time (zero

order kinetic models); log cumulative % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model); log cumulative % drug release vs log time (Korsmeyer–Peppas model). All Plots are shown in Figure 6-9 and results are summarized in Table 7. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of optimized formulation showed the constant drug release from the nanoparticle, the results of the zero order model was found to be $y = 4.256x + 12.22R^2 = 0.904$. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be $y = -0.171x + 2.372R^2 = 0.795$. The Higuchi model is used to describe the limits for transport and drug release. The Higuchi model of formulation was found to be, $y = 22.59x - 9.781R^2 = 0.955$. And the results of Korsmeyer peppas kinetic model was found to be $y = 1.314x + 0.442$ $R^2 = 0.860$s. *In-vitro* drug diffusion studies were carried out using dialysis bag method. In the above table R2 is correlation value. On the basis of best fit with the highest correlation (R2) value it is concluded that in the optimized formulation of nanoparticles follow the Highuchi kinetic model.

Table 2: Solubility study of metronidazole

Drug	Solvents	Observation/Inference
Metronidazole	Methanol	Freely soluble
	Ethanol	Freely soluble
	Water	Soluble
	Dichloromethane	Sparingly soluble
	DMSO	Freely soluble

Table 3: Particle size of Silver nanoparticle

S. No	Formulation	Particle size	PI value
1	SNPs (F1)	551.2 nm	3.449
2	SNPs (F2)	552.2 nm	4.206
3	SNPs (F3)	157.5nm	0.390
4	**SNPs (F4)**	**149.1 nm**	**0.374**
5	SNPs (F5)	169.6 nm	0.402

Table 4: Zeta potential

S. No	Formulation	Zeta potential
1	Nanoparticle (F1)	1.2 mV
2	Nanoparticle (F2)	0.1 mV
3	Nanoparticle (F3)	-1.2 mV
4	Nanoparticle (F4)	-3.9 mV
5	Nanoparticle (F5)	-2.1 mV

Table 5: Entrapment efficacy

S. No.	Formulations	Entrapment efficacy (%)
1.	**F1**	80.05
2.	**F2**	93.86
3.	**F3**	83.56
4.	**F4**	98.23
5.	**F5**	95.86

Table 6: Release kinetics study of optimized (F4) formulation

S. No	Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug released	log time
1.	0	0	100	0.000	0.000	0.000
2.	2	14.12	85.88	1.414	1.150	0.301
3.	4	25.01	74.99	2.000	1.398	0.602
4.	6	39.56	60.44	2.449	1.597	0.778
5.	8	56.19	43.81	2.828	1.750	0.903
6.	10	67.32	32.68	3.162	1.828	1.000
7.	12	72.08	27.92	3.464	1.858	1.079
8.	16	86.89	13.11	4.000	1.939	1.204
9.	24	97.92	2.08	4.899	1.991	1.380

Table 1: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Nanoparticle	Zero Order	$R^2 = 0.904$
	First Order	$R^2 = 0.795$
	Higuchi	$R^2 = 0.955$
	Korsmeyer peppas	$R^2 = 0.860$

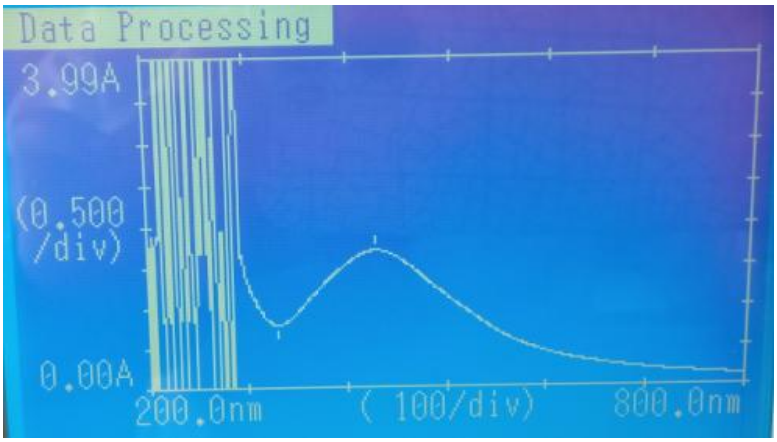

Figure 1: Lambda max of Metronidazole

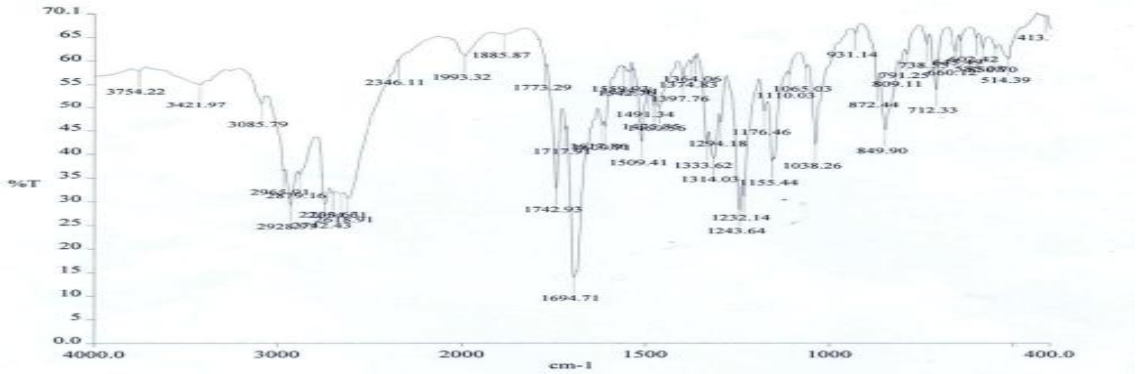

Figure 2: FTIR of metronidazole

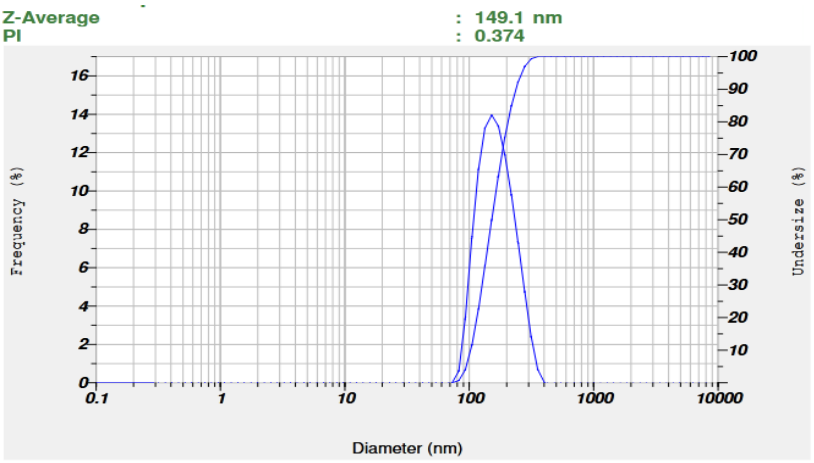

Figure 3: Particle size (F4)

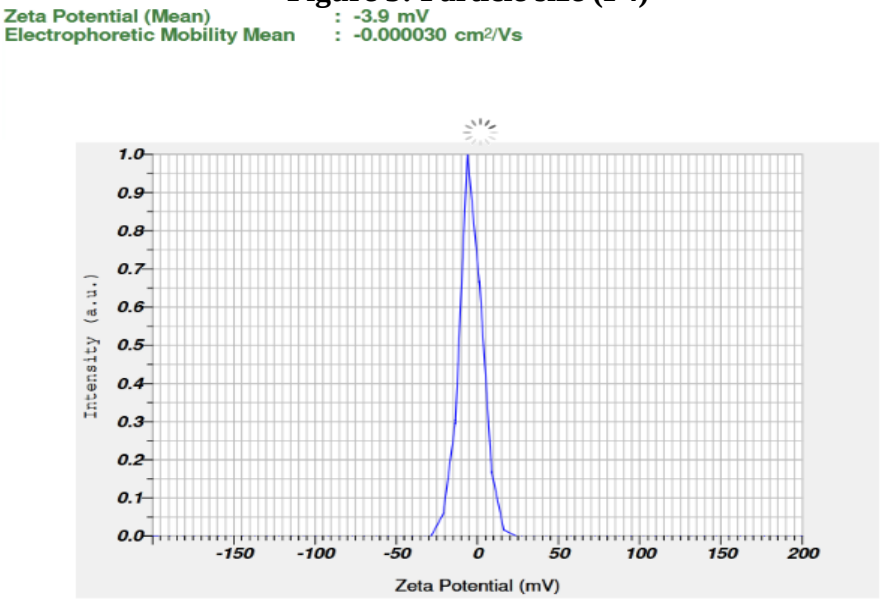

Figure 4: Zeta potential (F4)

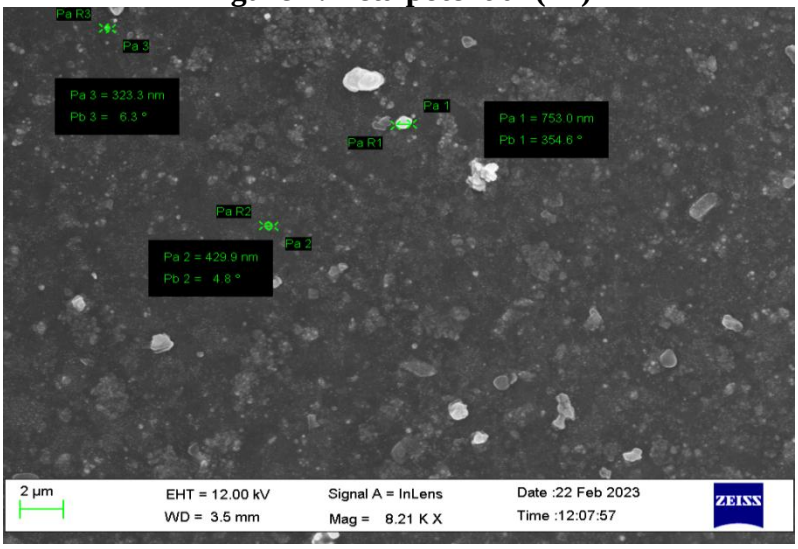

Figure 2: SEM (F4)

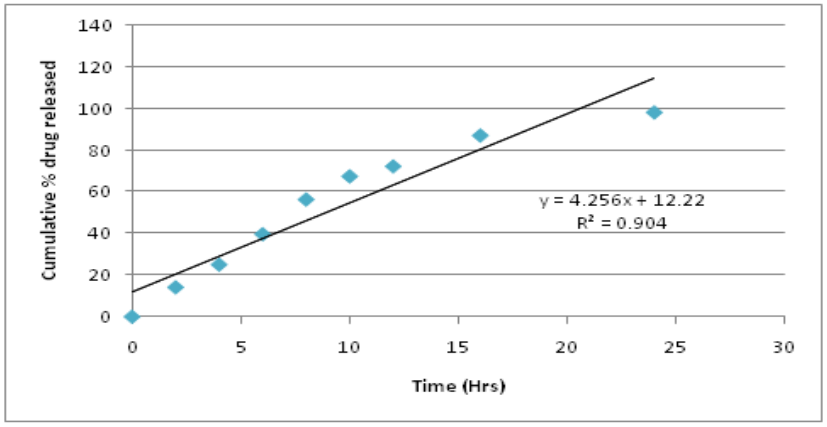

Figure 6: Zero order kinetic model

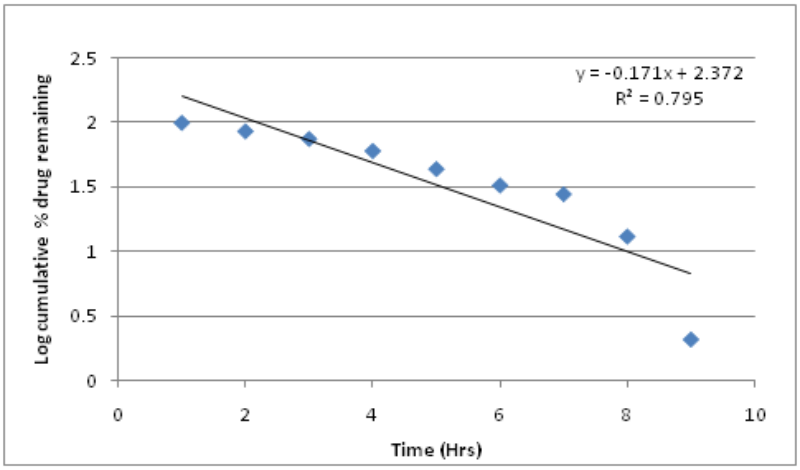

Figure 7: First Order kinetic model

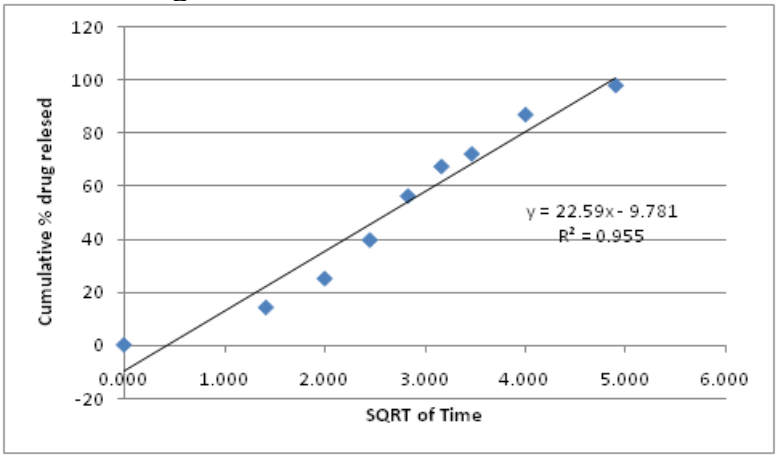

Figure 8: Higuchi model

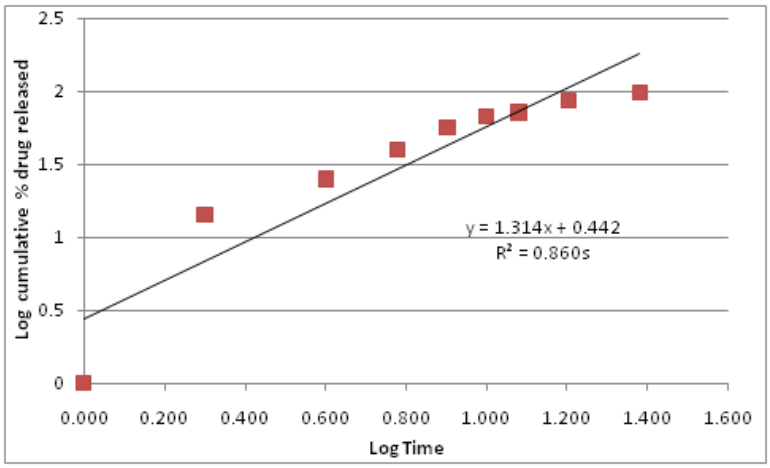

Figure 9: Korsmeyer peppas

CONCLUSION

Selenium is an essential micronutrient required for proper functioning of biological and metabolic mechanism within the human body. Deficiency of selenium leads to the generation of several harmful disorders such as cancer, neurological, muscular, immune, *etc*. Generally, selenium can be depicted within a very narrow concentration range due to its deficiency, physiological effect, and toxic doses. At optimal doses Se acts as an antioxidant, whereas at higher doses it shows pro-oxidant activity. Nanoparticle induced toxicity is still a major concern for researchers. The authors concluded from the literature that mostly SeNPs ranging from 50 to 200 nm were effective for their use as a therapeutic agent in cancer treatment and antioxidant and antimicrobial applications. Metronidazole is widely used to treat a variety of infections due to its high efficacy compared with others drugs; however, their side effects must be considered. Genotoxicity and neurotoxicity studies on humans should be done to clarify the role of metronidazole in human health. So this study focuses on the formulation and evaluation of selenium nanoparticle of metronidazole.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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