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Formulation and *in vitro* evaluation of polymeric metronidazole nanoparticles

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Abstract: The purpose of this research was to formulate metronidazole nanoparticles with the incorporation of acrylate methacrylate copolymer (Eudragit® RS 100) as the synthetic polymer using a nano spray-dryer. The formulated metronidazole nanoparticles were evaluated using scanning electron microscope, X-ray powder diffraction, differential scanning calorimetry and Fourier transform infra-red spectrophotometry. The results of the morphological analysis showed that the formulated nanoparticles were smooth, spherical with uniform distribution and polydispersity index (PDI) values between 0.31±0.12-0.39±0.14. The optimized formulation showed particle size in the range of 465.2±0.12-564.38±0.16 nm, zeta potential values ranged from 32.14±0.20-43.71±0.20 mV and entrapment efficiency had values between 34.19±0.51-39.28±1.10%. *In vitro* release analysis showed sustained release of metronidazole from the formulation with non-Fickian diffusion as the mechanism of drug release. The metronidazole nanoparticles formulated in this study was able to sustain the release of drug from the formulations which is a vital criterion for better patient adherence to therapy, improved therapeutic outcome and minimum incidence of adverse drug reaction and this can be exploited in the formulation of metronidazole nanoparticles in the management of amoebiasis, trichomoniasis and *Helicobacter pylori*-induced ulcer.

Keywords: Entrapment efficiency, metronidazole, nanoparticles, nanotechnology, zeta potential.

INTRODUCTION

Nanotechnology is an interdisciplinary field that combines knowledge from a variety of fields including chemistry, physics and biology. Nanotechnology is the science of controlling matter at the atomic or molecular level and it has the potential to significantly improve environmental technologies (Khan *et al.,* 2019). Nanoparticles are particles with a diameter of less than 100 nm. The physical properties of these materials change significantly as they are reduced in size compared to bulk materials. They can be made of metal, mineral, polymer or a combination of these materials. The majority of these changes are due to the development of quantum effects as the size reduces and are the cause of phenomena such as super paramagnetism, coulomb blockade and surface plasmon resonance (Ellin, 2006). The reduction in particle size also leads to a higher surface area to volume ratio. Nanoparticles exhibit various unique features such as thermal, optical, mechanical, structural and electromagnetic properties (Wickline *et al.,* 2006).

Nanoparticles can be formulated by dissolving a solution in a solvent such as dichloromethane followed by evaporation, freeze drying, sonification and filtration. Other methods of formulation of nanoparticles include crystallization, microbial synthesis and biomass reaction (Philips *et al.,* 2006; Ahmed *et al.,* 2016).

Nanoscience and nanotechnological approaches are accelerating the development of increasingly sophisticated tools for early detection and treatment of diseases such as cancer and atherosclerosis. Nanotechnology is also important in drug delivery to specific body sites and enhancing neurosurgery (Alexis *et al.,* 2008). Magnetic resonance imaging (MRI) can also be used to visualize brain cancers utilizing super paramagnetic iron oxide nanoparticles (Ali *et al.,* 2016). Some of the novel uses of nanotechnology under development include formulation of nanoparticles laden with paclitaxel that delivers the drug efficiently to its site of action in the treatment of ovarian and breast cancers, ribonucleic acid (RNA) nanoparticles carrying siRNA (small interfering RNA) and folate for the treatment of nasopharyngeal cancer by circumventing the blood-brain barrier and delivering drugs to combat brain tumors (Bahadar *et al.,* 2016).

Metronidazole is an anti-protozoal and anti-trichomonal agent that is used in the treatment of pelvic inflammatory disease, giardiasis, trichomoniasis and anaerobic infections. It has a biological half-life of 8 h and is commonly used along with other antibiotics to treat *Helicobacter pylori-induced* ulcer (Nagaraja *et al.,* 2020). It is normally administered at a dose of 200-400 mg eight (8) hourly for five (5) to seven (7) days, which is not convenient for some patients in terms of adherence to therapy as most patients prefer a once daily dosing (Sukhbir *et al.,* 2007; Gyanendra *et al.,* 2021). Formulation of sustained release polymeric metronidazole

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nanoparticles will further improve the physiological and clinical properties of the drug in the treatment of amebiasis and other protozoal infections as well as improved patient adherence to therapy and guarantee better therapeutic outcome. Elzatahry *et al.*, formulated chitosan nanoparticles as carriers for metronidazole and the result showed sustained release of the drug for a duration of 12h. Also, Sabbagh *et al.*, used the chitosan-alginate polymeric nanocomposite for the incorporation of metronidazole and investigated the drug release kinetics of metronidazole from the nanocomposite formulation which sustained the drug release over a duration of 63 h. Several studies have been done on the formulation of metronidazole into various acceptable sustained release dosage forms however, due to their relatively small particle size, nanoparticles have several advantages as a drug delivery mechanism compared to other dosage forms, including easy permeability, high drug loading, easy penetration of the mucus, low toxicity and resistance to degradation, as well as being rapidly taken up in inflammatory areas (Zhuang and Gentry, 2011; Hoang *et al.*, 2022).

The aim of this study was to formulate metronidazole polymeric nanoparticles using nano spray dryer and acrylate methacrylate copolymer (Eudragit RS 100) and to evaluate the morphology, particle size, drug loading, zeta potential and *in vitro* release of drugs from the formulated nanoparticles.

MATERIALS AND METHODS

Materials

Metronidazole pure sample (Aarti drugs, India) was used in this study as the active pharmaceutical ingredient, acrylate methacrylate copolymer (Eudragit RS 100) was obtained from Rhoma Pharma, Darmstadt (Germany), ethanol was purchased from Merck (Germany).

Table 1: Composition of the formulated nanoparticles

Formulations	Metronidazole (mg)	Eudragit RS 100 (%w/v)	Ethanol (mL)
MNP1	50	1	100
MNP1	100	1	100
MNP2	150	1	100

MNP1, MNP2 and MNP3 = Metronidazole nanoparticle formulations containing varying amount of metronidazole.

Methods

Formulation of metronidazole nanoparticles

Eudragit RS 100 was dissolved in ethanol using a magnetic stirrer at a revolution of 200rpm for 1h to obtain a clear polymeric solution to which varying amounts of metronidazole was added and stirred for another 10min. Prior to delivering the polymeric solution, the nano spray-dryer (B-90, Switzerland) was calibrated for 20min with ethanol to get the desired spraying, inlet and outlet temperature, gas flow, pump level and ambient temperature. During the application, a needle with a pore size of 5µm was used with an inlet and outlet temperature of 100°C and 45°C respectively (Nagarwal *et al.*, 2009; Li *et al.*, 2010). The dried metronidazole nanoparticles were stored in the collecting chamber (table 1).

Evaluation of nanoparticles

Morphology

The morphology of nanoparticles was determined using a scanning electron microscope (EVO50, Jeol, Japan). The sample was put on a sample holder and gold coated before the microscopy was done.

Zeta potential, polydispersity index (pdi) and particle size analysis

Zeta potential, PDI and particle size distribution were analyzed using a zeta sizer (zeta sizer nano ZS90, UK). Prior to estimation, samples were dissolved in distilled water and agitated continuously for 10min.

Thermal analysis

Pure metronidazole and the optimized nanoparticle formulation were thermally analyzed using Differential scanning calorimetry (DSC; Mettler Toledo-DSC-3, USA) against an aluminum reference and nitrogen gas at a flow rate of 40mL/min with a temperature increase of 10°C/min in the temperature range of 30-300°C.

X-ray diffraction (XRD)

The Rikagu generator (XRD Rikagu Rint 2000, Japan) was used to conduct XRD investigations of pure metronidazole and the optimized nanoparticle formulations at a speed of 30kV, 20mA current intensity, 3Θ angle and 3°min^{-1} in the range of 4-50°.

Fourier transform infrared spectrophotometry (FT-IR)

FT-IR spectra of pure metronidazole and the optimized nanoparticle formulations were determined at 4000-500 cm^{-1} wavelength using FT-IR (FTIR, Shimadzu, Model-RT-IR-8300, Japan).

Drug entrapment

The nanoparticles were digested in acetic acid (4%) solution for 30min with a probe sonicator (Misonix, USA) and then centrifuged for 4min at 1000rpm. Using a UV Spectrophotometer (UNICO 2150, UK), the concentration of metronidazole in the supernatant was determined. Drug entrapment (E) of the formulations was calculated using Equation 1 (Hoang *et al.*, 2022):

$$E(\%) = \frac{Ct - Cs}{Ct} \times 100 \quad (1)$$

Where Ct = total amount of drug in the formulation and Cs = amount of drug in the supernatant.

In vitro release study

In vitro release was performed in a Franz diffusion cell that was thermostatically controlled at 37±2°C in simulated gastric and colonic fluid using a dialysis membrane (Hi Media, Mumbai, India). The donor compartment contained a 2mL suspension of metronidazole that has been re-suspended in tween 80 (2%w/v) solution in simulated gastric/colonic fluid after being mixed for 4 sec (Nagarwal *et al.,* 2009). At different time intervals, 1mL samples were collected from the receptor compartment and replaced with fresh media of equal volume. Samples were diluted appropriately, filtered and spectrophotometrically examined at 277nm.

In vitro kinetics

The zero, first order, Higuchi and Korsemeyer-Peppas release models were used to analyze the data obtained from the *in vitro* drug release studies for the determination of the release kinetics.

STATISTICAL ANALYSIS

All studies were conducted in triplicate (i.e., n=3). The data obtained were statistically analyzed using GraphPad Instat 7.0 software (California, CA, USA) and the results were expressed as mean ±SEM.

RESULTS

Result of the morphology analysis

Fig. 1 shows the scanning electron microscopy (SEM) of the optimized metronidazole nanoparticles at 200x magnification and it revealed smooth, spherical and porous particles (Kim *et al.,* 2008).

Results of zeta potential, poly dispersity index (PDI) and particle size analysis

Table 2 shows the results of the zeta potential, PDI and particle size measurements. The particle sizes of the formulated nanoparticles ranged from 465.21±0.12-564.38±0.61nm. The polydispersity index (PDI) is a vital characteristic that describes the particle size distribution's width or spread. PDI values can range from 0 to 1, with values less than 0.1 indicating monodisperse particles and values greater than 0.1 indicating polydisperse particle size distributions (Ho *et al.,* 2020).

Differential scanning calorimetry (DSC) analysis

Fig. 2 shows the DSC thermograms of the optimized batch of metronidazole nanoparticles in comparison to pure metronidazole. The DSC thermograms revealed a melting point of between 162-165°C for the pure metronidazole sample and the optimized metronidazole nanoparticle formulation (Kumar *et al.,* 2019).

X-ray diffraction

Fig. 3 shows the XRD characteristics of optimized metronidazole nanoparticles in comparison to physical mixture of metronidazole. XRD elucidates the molecular structure of nanoparticles, studies the crystalline state as well as polymorphism investigations and offers important scientific information on the stability of nanoparticles (Al-Gaashani *et al.,* 2019).

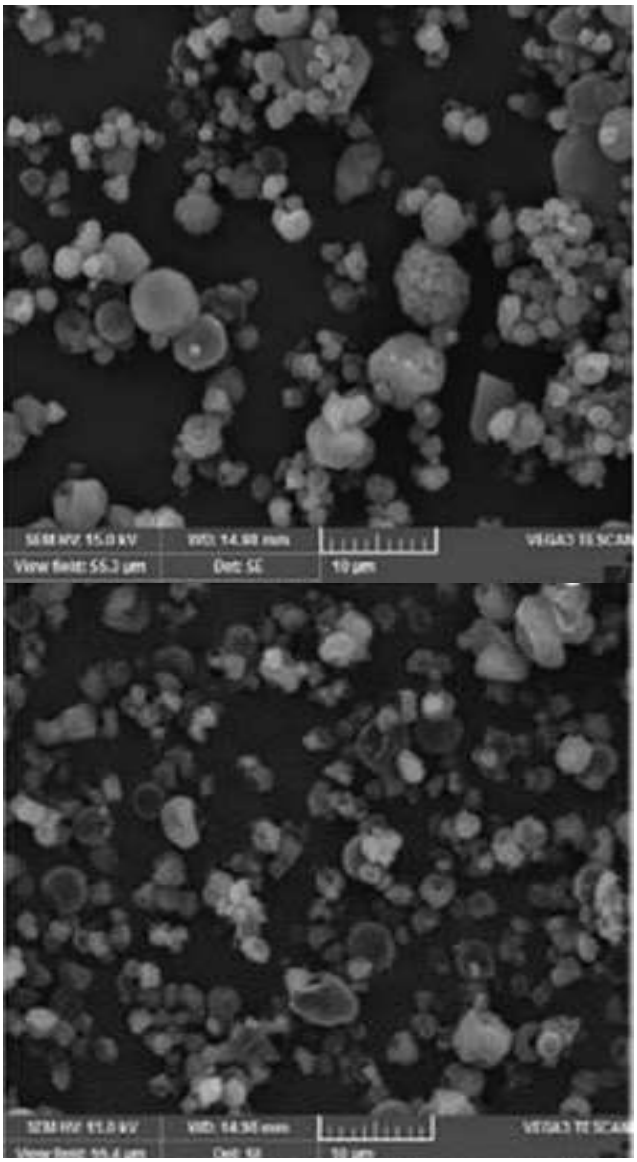

Fig. 1: SEM images of the formulated metronidazole nanoparticles

Fourier transform infra-red spectrophotometry (FTIR)

Fig. 4 compares the FT-IR spectra of the optimized metronidazole nanoparticles to pure metronidazole and it shows that both spectra were similar indicating that formulation of metronidazole into nanoparticles had no effect on the chemical structure of the final product (Chaudhary *et al.,* 2020).

Entrapment efficiency (EE)

Table 3 shows the results of the entrapment efficiency of the formulated nanoparticles and the values ranged from

34.19±0.51-39.28±1.10%. The drug may be integrated into the polymer matrix or adsorbed on the surface. Hence, while determining the total amount of drug in the nanoparticles, both the amount encapsulated and the amount adsorbed to the polymer surface was calculated. The loading capacity of MNP1 was discovered to be the highest among the other formulations in this study. EE of MNP3 was lowest among the prepared nanoparticles. This shows that as the amount of the drug increases, the amount of metronidazole in the polymer matrix decreases (Lopedota *et al.,* 2009).

Table 2: Results of the zeta potential, polydispersity index and particle size of the formulated metronidazole nanoparticles

Formulation	Zeta potential (mV) ± SEM	Polydispersity index ± SEM	Particle size (nm) ±SEM
MNP0	+32.14±0.20	0.31±0.12	465.21±0.12
MNP1	+41.06±0.14	0.36±0.11	521.16±0.14
MNP2	+43.71±0.12	0.39±0.14	564.38±0.16

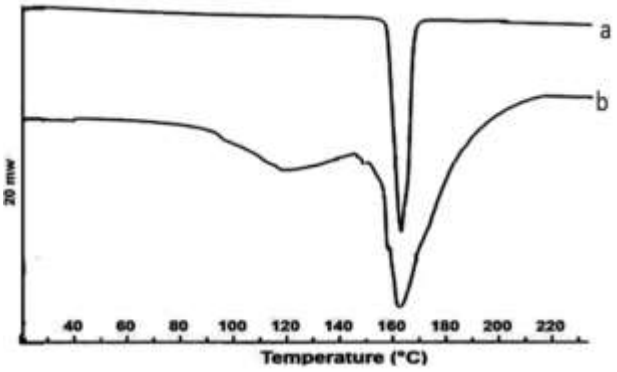

Fig. 2: DSC spectra of (a) metronidazole pure sample (b) optimized metronidazole nanoparticles.

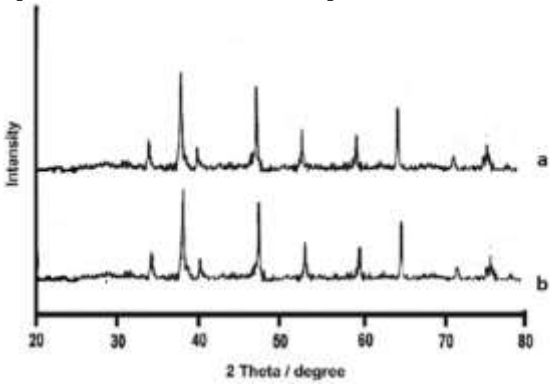

Fig. 3: XRD profile of (a) metronidazole and (b) optimized metronidazole nanoparticle formulation.

In vitro release kinetics

The results of the *in vitro* release profiles of the various formulated metronidazole nanoparticles are presented in fig. 5. From the results, 61%, 66% and 77% of drug was released from formulations MNP1, MNP2 and MNP 3 respectively within the first 2h followed by a sustained release of drug over a 48h duration (Jia and Kel, 2015).

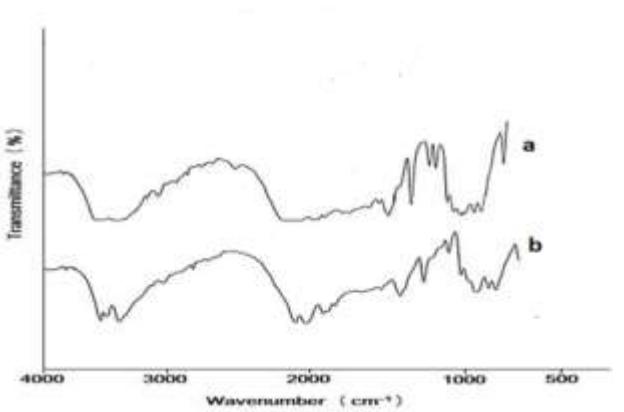

Fig. 4: FTIR spectra of (a) metronidazole nanoparticles (b) pure metronidazole Sample.

Table 3: Results of the entrapment efficiency of the formulated metronidazole nanoparticles

Formulation	EE (%) ± SEM
MNP1	39.28±1.10
MNP2	37.12±1.21
MNP3	34.19±0.51

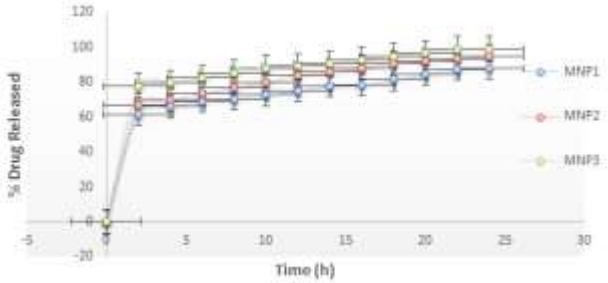

Fig. 5: *In vitro* drug release profile of the formulated nanoparticles.

Table 4: Correlation coefficient and release kinetics of different batches of metronidazole nanoparticle formulations.

Models	Zero		First		Higuchi		Korsmeyer and Peppas	
Formulations	r^2	K_0	r^2	K_1	r^2	K_H	r^2	n
MNP1	0.924	4.13	0.954	-0.054	0.992	19.03	0.576	0.59
MNP2	0.964	3.98	0.956	-0.027	0.995	17.55	0.635	0.61
MNP3	0.968	2.88	0.959	-0.036	0.997	16.84	0.657	0.63

DISCUSSION

The metronidazole particles were successfully loaded into the acrylate methacrylate copolymer (Li *et al.,* 2010). All metronidazole nanoparticles formulated in this study had a PDI value in the range of 0.31±0.12-0.39±0.14, indicating homogeneous particle distributions for all nanoparticles (Danao *et al.,* 2010). Eudragit RS 100 is a good cationic polymer in the formulation of pharmaceutical dispersions since it contains 4-8%

quaternary ammonium groups. Due to the cationic ammonium groups in its structure, all metronidazole nanoparticles formulated with Eudragit RS 100 had a positive zeta potential value (Li *et al.,* 2010). All of the formulated nanoparticles had zeta potentials between +32.14±0.20 to +43.71±0.12mV. Electrostatic and stearic stability determines the stability of nanoparticles dispersed in aqueous media and a high zeta potential value (+/-30mV) is associated with good colloidal dispersion stability. Hence, the formulated metronidazole nanoparticles were stable (Sukhbir *et al.,* 2009). Results from the DSC studies showed that there was no obvious change in the endothermic peak or melting point temperature as a result of the formulation of metronidazole into nanoparticles which implies compatibility between the active ingredients and the excipients used in the formulation (Wickline *et al.,* 2006). The result was also the same for the FTIR studies which revealed that there was no obvious change in the peaks of the optimized metronidazole nanoparticle spectrum, indicating that there was no chemical interaction between metronidazole and the excipients used in the formulation of the nanoparticles (Li *et al.,* 2010).

The XRD study was done to investigate metronidazole dispersion in the polymer matrix at the molecular level as well as the amorphous form of metronidazole nanoparticles. The even low-intensity metronidazole peaks were similar in the various XRD profiles which showed that metronidazole was molecularly distributed into the polymeric matrix of the synthetic polymer (Nagarwal *et al.,* 2009).

Results from the release kinetics studies showed that the initial rapid release observed from the formulated metronidazole nanoparticles was as a result of the rapid dissolution of superficially adsorbed metronidazole while the drug entrapped in the polymeric network of the nanoparticles was released in a sustained manner (Elzatahry *et al.,* 2015). MNP3 demonstrated highest amount of metronidazole release in 48h with a drug release of 98.86%. Determination of the release kinetics upon analysis, suggests a near zero order release profile for the formulations which can be attributed to diffusion mechanism of drug release from the core and partial erosion of the core polymer resulting in initial prompt release of the drug from the formulation which was thereafter followed by a cumulative sustained release over time as previous studies have shown that drug release mechanisms from nanoparticles are usually controlled by diffusion (Dash *et al.,* 2010; Isesele *et al.,* 2021). From the results obtained for the correlation coefficient and release kinetics of the various metronidazole nanoparticle formulations (table 4), it can be seen that the release mechanism of the nanoparticle formulations simulated the Higuchi release model (r^2=0.997) indicating that the drug was homogeneously dispersed within the polymer matrices and that the kinetics of release of the drug from the polymer matrices was diffusion controlled (Li and Jiang, 2016; Airemwen *et al.,* 2021). However, the results obtained for the Korsmeyer-Peppas diffusion model (n>0.5) indicate that the diffusion was non-Fickian (Higuchi, 1963; Korsemeyer *et al.,* 1983; Fu and Kao, 2010). The drug release profile from the metronidazole nanoparticle formulations was observed to be bi-phasic, starting with an initial burst release followed immediately by a slow release or sustained drug release rate (Aqel *et al.,* 2012; Bello *et al.,* 2015).

CONCLUSION

Metronidazole nanoparticles having uniform and spherical shapes were successfully formulated in this study using acrylate methacrylate copolymer. The sustained release of metronidazole from the formulated nanoparticles helps to meet the criteria for better patient adherence to therapy, improved therapeutic outcome and minimum incidence of adverse drug reaction and this can be exploited in the formulation of metronidazole nanoparticles in the management of amoebiasis, trichomoniasis and *Helicobacter pylori*-induced ulcer.

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