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Original Article

DEVELOPMENT OF SODIUM ALGINATE COATED NANOPARTICLES OF METRONIDAZOLE FOR THE TREATMENT OF PERIODONTITIS

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ABSTRACT

Objective: The present study was aimed to develop metronidazole (MNZ) loaded poly lactic-co-glycolic acid (PLGA) based mucoadhesive nanoparticles (NPs) in prolonged treatment in periodontitis.

Methods: Nanoparticles were prepared by using single (SE) and double (DE) emulsion method to determine the suitability of methods. Prepared NPs were evaluated for surface morpholgy, mean particle size, polydispersity index, zeta potential, mucoadhesion ability and invitro-drug release,

Results: SEM images confirmed that NPs were of spherical shape and smooth surface. Mean particle size, of MNZ loaded NPs were found 583.28±18.22 and 872.72±63.18 prepared by SE and DE method. Similarly, polidispersity index (0.68±0.1 and 0.83±0.06) and zeta potention (-33.29±0.7 and-31.28.0.6) was found in acceptable range. Prepared NPs were surface treated with Sodium alginate (SA) to increase mucoadhesive property. It was observe that particles remain adhere till 24 hr with biological membrane. Prepared NPs allow release of MNZ upto 24 h in sustained manner.

Conclusion: This study confirms that the prepared MNZ loaded NPs may be used as an better alternate with addition application such as prolonged action thus improved patient compliance.

Keywords: Metronidazole, Nanoparticles, Periodontitis

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INTRODUCTION

Natural or synthetic polymers have wide applications by making system bioadhesive or mucoadhesive thereby extending the retention time. This concept of prolonged retention can be utilized for drug delivery systems for localized periodontal therapy by retention on the mucosal surface and controlled drug release at the site of action. The prospects of bioadhesion encompass prolonged drug delivery, localized therapy, targeting of specific tissues and a close contact with the substrate [1, 2].

Oral Cavity is an assessable site for deploying devices. Buccal tissues and periodontium are the potential sites for localized and topical drug delivery. The common oral lesions such as aphthous ulceration can be treated via buccal cavity and the periodontium, where destructive periodontal diseases might benefit from improved therapeutics. So, several studies have recently suggested that mucoadhesive polymers might be useful for periodontal pocket therapy for longer retention of formulation at its desire site [3, 4].

As discussed above, bioadhesive or mucoadhesive polymers can prolong the retention at the mucosal surface by providing intimate contact between the dosage form and absorbing tissues in the periodontal pocket. Therefore, bioadhesive and biodegradable nanoparticles with controlled release characteristics, withdraws the attention of various researchers in the developmental phase of long-retentive drug delivery systems.

In addition to bioadhesivity, controlling the release of a drug from the dosage form is also desirable. Controlled rate of drug delivery provides a persistent delivery of drugs at anticipating and reproducible kinetics for a predetermined period. The prospectives of this concept is the minimization of drug related side effects (due to controlled therapeutic blood levels instead of oscillating blood levels) and improved patient compliance (due to reduced frequency of dosing). Furthermore, if system is biodegradable, sustained-release, then it can be placed into the periodontal pocket to maintain therapeutic concentration for prolonged periods and patients will not required to revisit the dentist to have these devices removed at the completion of therapy thereby making them cost effective.

Among the imidazole derivatives, MNZ is one compound with potent antiprotozoal activity against *E. Hystolytica, T. vaginalis* and *giardia* including periodontal pathogens [5]. Due to its short half-life, it requires frequent dosing. Therefore, objective of the present study was to develop biodegrable mucoadhesive nanocarrier system containing MTZ for sustained release of the drug in periodontal pocket and cover the most of the microorganisms present in deep pocket.

MATERIALS AND METHODS

Materials

MNZ was kindly gifted as sample from Plethico Pharmaceutical Pvt. Ltd, Indore, India. Poly Lactic Glycolic Acid (PLGA, 50:50) and sodium alginate (SA) was purchased from Sigma Aldrich (St. Louis, MO). The other ingredients used were of analytical grade and purchased from Central Drug Laboratory, New Delhi, India and Sigma Aldrich (St. Louis, MO).

Fourier transform infrared spectroscopy (FT-IR)

In order to determine chemical incompatibility between drug and polymer, FTIR absorption spectra were taken using FT-IT spectrophotometer (Jasco, Eastern, Maryland, Japan). Samples were mixed with potassium bromide (KBr) in equimolar quantity, punched and then spectra were obtained using the analyzer. FTIR spectra of MNZ, SA, PLGA and physical mixture were scanned in the range of 4000-400 cm^{-1}.

Preparation of nanoparticles (NPs)

The MNZ loaded NPs were prepared by emulsion-solvent evaporation method. In order to determined the suitability of method two different processes was used. These methods are discussed here

Single emulsion (SE) method

This method was used as per previous literature by with slight modification [6]. Briefly, PLGA (2% w/v) was accurately weighed

and dissolved in 5 ml of acetone as organic solvent containing MNZ (drug and polymer ratio 1:10). Similarly aqueous phase was prepared by dissolving polyvinyl alcohol (PVA, 1% w/v) as stabilizer in distilled water. Organic phase was slowly dispersed into the aqueous phase and stirred magnetically (REMI, Mumbai, India) (1800 rpm) to form o/w emulsion. The prepared emulsion was converted into nano-droplets by subjecting to high pressure homogenization. The resulting nano-emulsion was left agitated at 700 rpm with a magnetic stirrer for next 2 to 4 h to remove organic solvent. The turbid nanosuspension thus obtained is centrifuged (REMI, Mumbai, India) at 18000 rpm for 30 min and washed twice with water and then freeze dried (Heto DRYWINNER, Germany) to obtain MNZ loaded PLGA NPs.

Double emulsification (DE) Method

This method was used as per the previous reports of Vandervoort and Ludwig, 2002; Song *et al.*, 2008 with slight modifications [7,8]. Aqueous PVA solution was dispersed into organic phase (5 ml) contains PLGA and drug (10:1) dissolved in acetone under magnetic stirring (REMI, Mumbai, India) at 1800 rpm. This primary emulsion was dispersed in outer aqueous stabilizer solution (10 ml) and subjecting to homogenization. The resulting double emulsion (w/o/w) was further diluted and left agitated (700 rpm) with a magnetic stirrer for next 2 to 4 h to remove organic solvent. Consequently, the MNZ loaded NPs were isolated by cooling centrifugation at 18000 rpm for 30 min and washed twice before lyophilization.

NPs characterization

Morphology

The surface morphology and geometrical size of prepared MNZ loaded NPs were determined using scanning electron microscope EVO18 research, ZEISS). Drug loaded NPs (prepared by both methods) were dusted on double sided tape on an aluminium stub and coated with gold using a cold sputter coater to a thickness of 300 Å. Images of NPs were captured using 20 kv electron beam.

Mean particle size (MPs), polydispersity index (PI) and zeta potential

Mean particle size (MPS) and polydispersity index (PI) was measured using light scattering diffraction method (Zetasizernano ZS, Malvern instruments Inc, Southborough, MA). Typically, a sample was placed in a cuvette and the measurements were done at 25 °C after dispersing the NPs into suitable media. Similarly, Zeta potential was measured with a disposable capillary cell with a volume of 1 ml. The mean value was determined using a mono-modal distribution. Each analysis was performed in triplicate.

Drug loading and entrapment efficiency

Drug loading and percentage entrapment efficiency of prepared MNZ loaded NPs was determined using method reported by Stolnik *et al.* 1998 [9]. Free drug in the supernatant of NPz suspension was separated by centrifugation of NPs suspension at 18000 rpm for 1 h. The supernatant was removed and nanoparticles sediments were washed twice with distilled water in order to remove the MNZ from the surface of NPs. Drug content in the supernatant was calculating by using UV-visible spectrophotometer (UV-1601, Shamdzu Co. Ltd. Japan)**.** The nanoparticles obtained were dried using freeze dryer.

Drug loading (DL) was then determined using the following equation:

$$\text{Drug loading (\%)} = \frac{\text{Amount of drug in nanoparticles x 100}}{\text{Amount of nanoparticles recovered}}$$

Similarly, the entrapment efficiencies (EE) of NPs were determined with the percent ratio of the actual amount of drug incorporated into NPs to the total amounts of drug used. The equation is as follow:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug in nanoparticles x 100}}{\text{Amount of drug used}}$$

Coating of drug loaded PLGA NPs

The objective of the present study is to prepared mucoadhesive NPs to improve the retention of delivery system into dental cavity for longer duration of time. The presence of mucoadhesive polymer on the surface of PLGA NPs can modulate the interfacial property of the carrier and hence can positively influence the application potentialities both in terms of mucoadhesion and improved drug permeation. For this reason we investigated the possibility of coating the surface of MNZ loaded NPs with sodium alginate as mucoadhesive polymers by simple adsorption process [6]. The freeze dried PLGA nanoparticles were suspended in sodium alginate solution (1% w/v) for 24 h. The coated particles were then collected after centrifugation, washing and freeze drying.

Mucoadhesion study

To determine the mucoadhesion ability of prepared NPs the method was used described in the literature of Sharma *et al.*, 2013 with slight modification [10]. The MNZ loaded NPs (both batch) were dispersed in a 10 ml glass tube at containing phosphate buffer solution (pH 7.4) at 37±0.5°C for 5 min in such a way that surface of all the NPs covered by the media. NPs (2.5 mg) were transferred on the glass slide which was previously mounted by freshly excised pieces of goat intestinal mucosa (2×2 cm) (collected from slaughter house) and left for 30 min in desiccators to facilitate the interaction between NPs and mucosa. The membrane with the attached NPs was removed after 12 and 24 h. Remaining NPs on the glass beaker were dried at 60C till constant weight. The percent of adhered nanoparticles (AN) was estimated using the following equation:

$$\text{AN \%} = \frac{\text{Initial weight of NPs - Weight of unattached NPs}}{\text{Initial weight of NPs}} \text{ X 100}$$

***In vitro* drug release studies of NPs**

Release of MNZ from the prepared NPs (coated and uncoated NPs from SE and DE) was estimated using dialysis method [10]. Briefly, required quantity of NPs (equivalent to single dose of MNZ)) was transferred into phosphate buffer (pH 6.8) as dissolution media (containing 0.5% (w/w) Tween 80 as solubilizer). The rotational speed was set to 100 rpm, and the temperature was set to 37±0.05 ◦C. At predetermined time schedule, release medium (1 ml) was replaced with the equal quantity of fresh media. Concentration of released MNZ from the NPs was measured with UV spectrophotometer (UV-1601, Shamdzu Co. Ltd. Japan) at 288 nm. All experiments were in triplicate and the results were represented in±SD.

RESULTS AND DISCUSSION

FT-IR spectra

For the determination of possibility of chemical integration, FT-IR spectra of pure MNZ, SA, PLGA and physical mixture was obtained (fig. 1). Spectra of MNZ (fig. 1a) showed O-H stretching at 3239.47 cm-1 and C-H stretching at 3102.38 cm-1. Peak at 3017.29 and 1542.39 cm-1was due to asymmetric–CH2 and asymmetric NO2 group [11]. FT-IR spectra of PLGA showed presence of–CH,–CH2,–CH3 stretching (2850-3000 cm-1), carbonyl stretching (1700-1800 cm-1), and C-O stretching (1050-1250 cm-1). The obtained FT-IR spectrum indicates that all characteristic peaks of MNZ were present in the spectrum of physical mixture, which indicates no significant physical or chemical interaction between MNZ and polymers.

Preparation of NPs

MNZ loaded NPs were prepared using single and double emulsification solvent evaporation methods. During emulsification method a native emulsion is formed due to dispersion of an organic phase into aqueous phase. This emulsion was stabilized using surfactant and stabilizers. Prepared NPs were characterized for various parameters

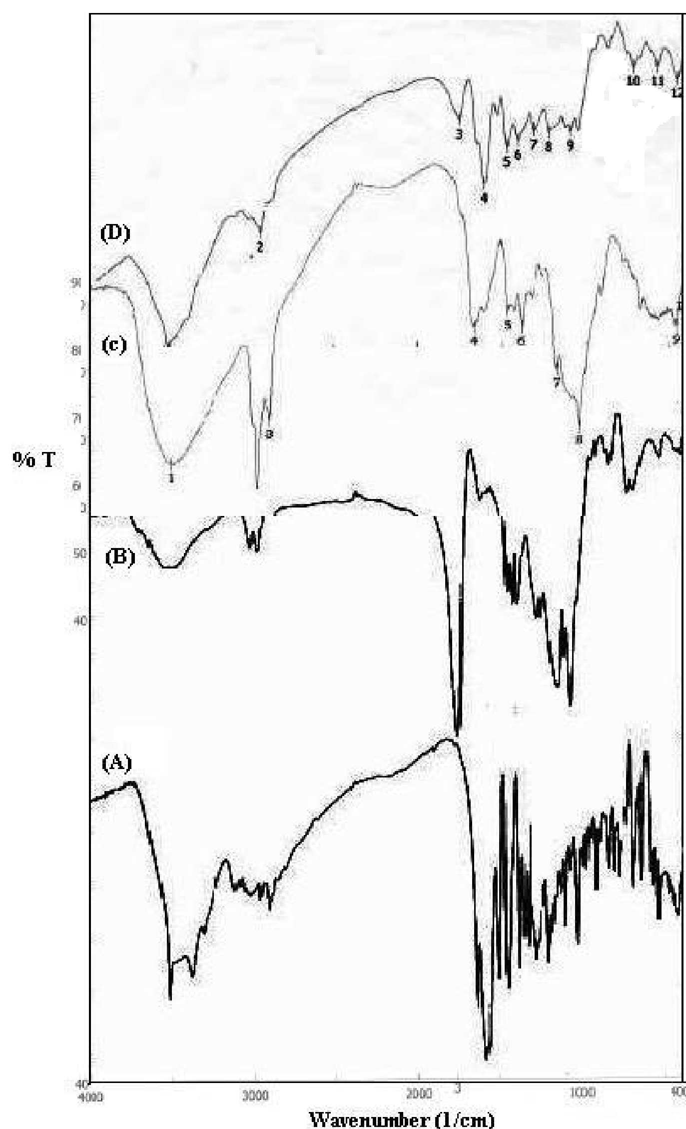

Fig. 1: FTIR spectra of (A) MNZ (B) SA (C) PLGA and (D) physical mixture

Characterization of NPs

Particle size and zeta potential

Particle size of prepared MNZ loaded NPs and empty NPs were determined using light scattering method and obtained results are summerized in table 1. Results indicates that empty NPs were in the nanometric range showing mean diameter 385±13.94 nm with SE and 792.72±55.92 nm with DE and exhibited a narrow size distribution. MNZ loaded particles exhibited mean particle size 583.28±18.22 and 872.72±63 nm prepared by SE and DE, respectively. Result indicates that drug loaded NPs was bigger than empty NPs. Results also demonstrated that NPs prepared by DE method were significantly large ($p<0.01$) compare to those produced by SE method. This could be explained on the basis of the fact that in single emulsification method internal aqueous compartment is absent, only two immiscible liquid phases (AC phase in aqueous buffer external phase) are present. As we have mentioned earlier, the AC organic phase has low viscosity, so shear (mechanical work) required for emulsification process was not as much of DE. In DE method, primary emulsion need to be dispersed in external buffer phase and the viscosity of primary emulsion is enough high and required high shears for emulsification. So in identical formulation and process parameters, NPs produced by DE are bigger ($p<0.01$) compare to SE [12, 13].

Particle size distribution of prepared NPs was determined using PI determination. Obtained data of PI are also given in table 1 and fig. 2. Vales are showing relatively narrow size distribution in NPs prepared by SE and DE method.

Result of ZP are given in table 1 indicate that both empty and drug-loaded NPs prepared by SE and DE exhibited negative charge with values ranging from-27.19±0.4 to-33.29±0.7 Mv. The negative surface charge of PLGA NPs can be attributed due to the presence of carboxyl groups of the polymer on the nanoparticles surface, as reported previously for drug free PLGA-NPs [8]. Results of ZP also indicate slight increments in negative surface value with drug loading.

Surface morphology

Surface morphology of prepared NPs were observed using SEM. Obtained SEM images are given in fig. 3. Images indicate that the prepared NPs were of spherical shape with a smooth surface. No difference was observed in the morphological properties of nanoparticles due to presence of the drug.

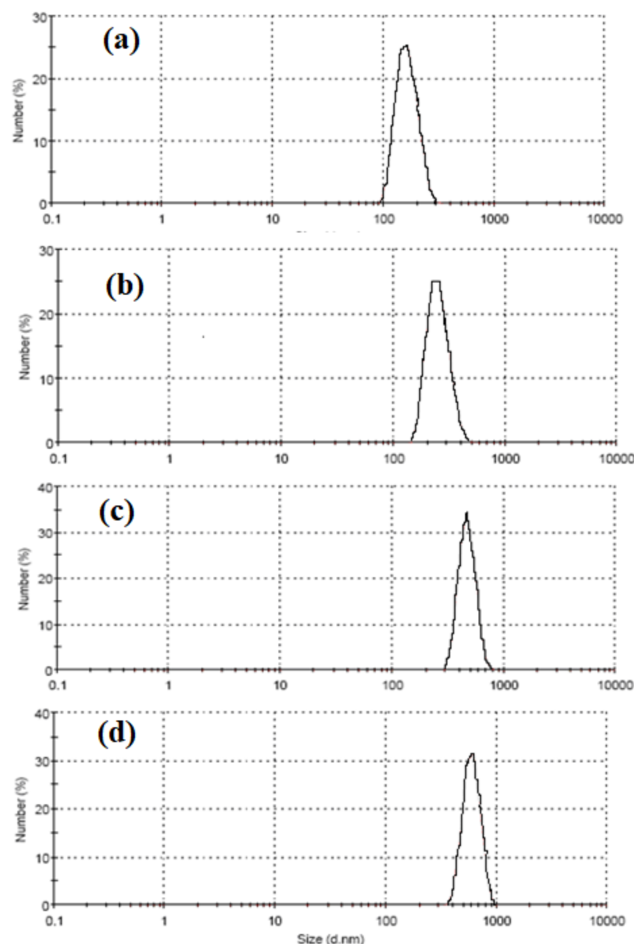

Fig. 2: Particle size distribution graph for empty PLGA-NPs prepared by SE (a) MNZ loaded PLGA-NPs prepared by SE (b) empty PLGA-NPs prepared by DE (c) MNZ loaded PLGA-NPs prepared by DE (d)

Table 1: MPS, PI and zeta potential of PLGA empty and drug loaded nanoparticles prepared by SE and DE

Parameter	Single emulsification		Double emulsification	
	Empty-NPs	MNZ loaded-NPs	Empty-NPs	MNZ loaded-NPs
MPS (nm)	385±13.94	583.28±18.22**	792.72±55.92	872.72±63.18
PI	0.62±0.05	0.88±0.1	0.74±0.007	0.83±0.06
Zeta potential (mV)	-27.19±0.4	-33.29±0.7	-28.39±0.5	-31.28±0.6

Data are shown as the mean±SD. (n=3); **p<0.01 compared with DE method

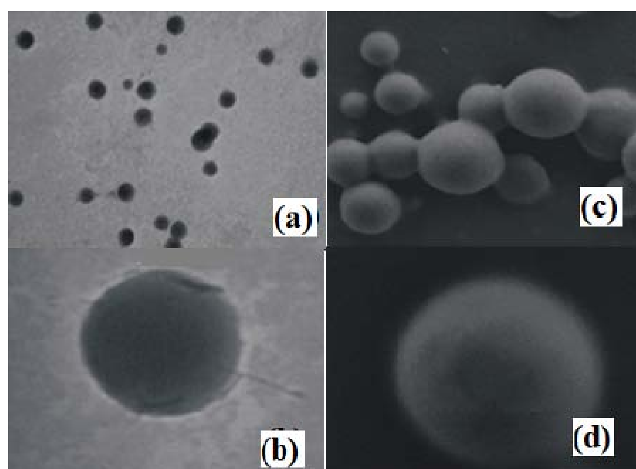

Fig. 3: Microphotographs of empty PLGA-NPs prepared by SE (a) MNZ loaded PLGA-NPs prepared by SE (b) empty PLGA-NPs prepared by DE (c) MNZ loaded PLGA-NPs prepared by DE (d)

Drug loading and entrapment efficiency

Drug loading and entrapment efficiency of NPs prepared by SE and DE methods was determined and obtained data are depicted in fig. 4. Data showed that drug loading and entrapment efficiency was reduced significantly (p<0.001) from 7.3±0.07 % to 6.1±0.02 and 73.29±0.37 to 61.28±2.18 when NPs were prepared using SE and DE method respectively which may be due to highly viscous droplets during the preparation of single emulsion method. Highly viscous droplets prevent the migration of drug to the surrounding media [14, 15]. Data suggest that single emulsification method was found suitable for the preparation of drug loaded PLGA Nanoparticles.

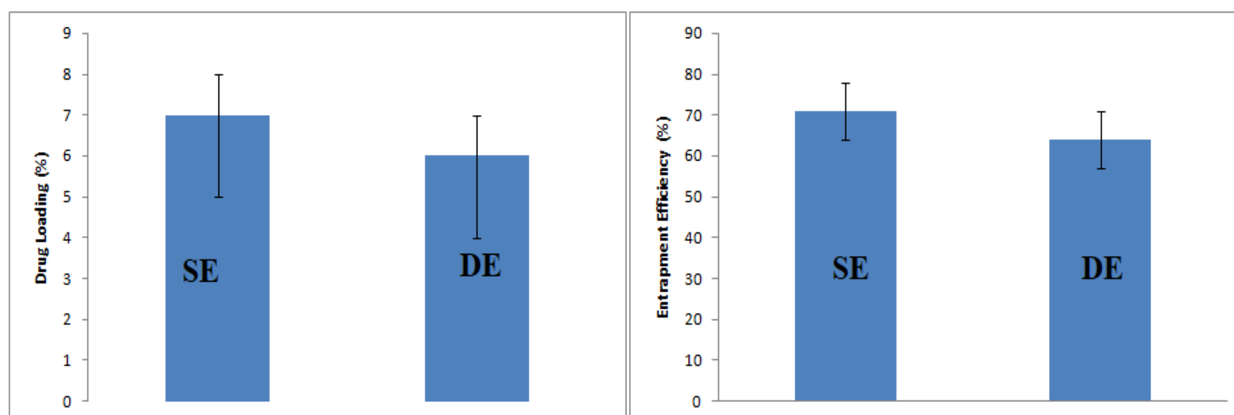

Fig. 4: Drug loading (%) and entrapment efficiency (%) of MNZ loaded NPs prepared by SE and DE method. Data are presented as mean±SD (n=3)

Mucoadhesion

Mucoadhesion ability of SA coated NPs prepared by SE and DE method was determined and obtained data are depicted in fig. 5. Data indicates that NPs prepared by SE method possess better adhesion (57.82±8.48 %) with biological membrane as compared to NPs prepared by DE (32.46±9.37 %). Better bioadhesion of NPs prepared by SE can be attributed by the smaller particle size compare to the NPs prepared by DE. Smaller particles of NPs (SE) exhibit comparative higher available surface area for mucoadhesion. The mucoadhesive strength of NPs was determined for 1 d and it was found that as the duration increase the mucoadhesive strength decreases and after 24 h it was found to be only 29.04±8.64 and 18.27±3.88 % for NPs prepared by SE and DE.

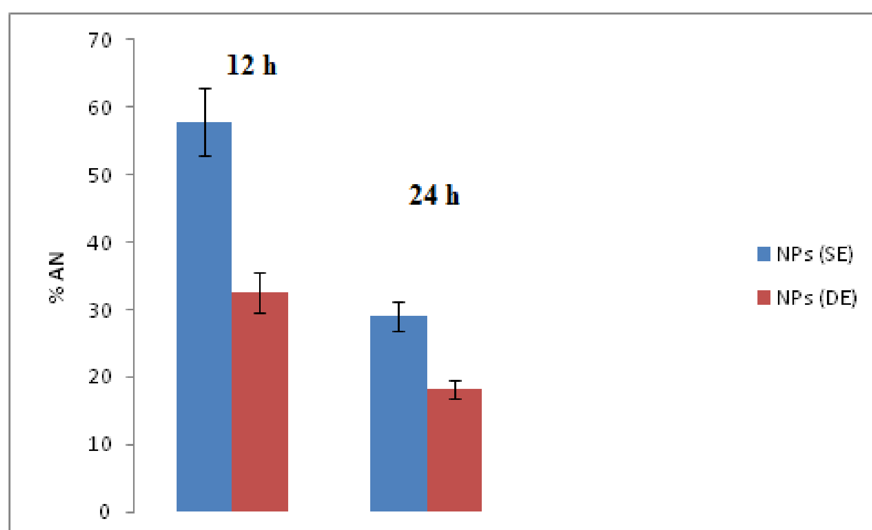

Fig. 5: Percentage adherent nanoparticles (prepared by SE and DE method) at 12 and 24 h, data are presented as mean±SD (n=3)

Drug release

Percentage release of MNZ from coated and uncoated NPs was estimated by dialysis method. Obtained data are depicted in fig. 6. Data revealed that all NPs except SA coated NPs prepared by DE method allowed MNZ release within 24 h time course study. Only, 95.38±8.37 percentage MNZ was release from the SA uncoated NPs prepared by DE method. This was observed NPs prepared by SE method allow rapid release of MNZ compared to NPs prepared by DE method. This result may be correlated with the size of prepared NPs. Since Lower size NPs prepared by SE method possess larger surface are for drug release compared to the NPs prepared by DE method [16]. It was also observed that coating of SA on any type of NPS retard the drug release. This may be due to the formation of three dimensional structure of SA after hydration. Now the released drug from the NPs has to be crossed this barrier of SA for its complete migration into dissolution media.

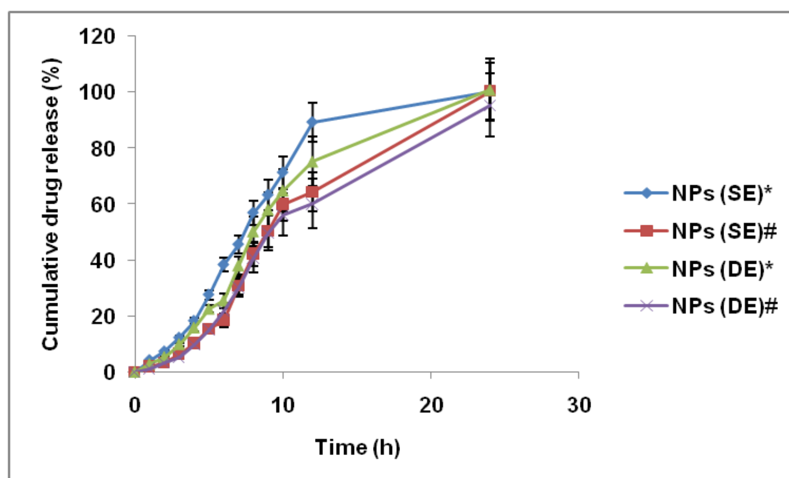

Fig. 6: Percentage release of MNZ upto 24 h from SA-coated* and SA-uncoated# NPs prepared by SE and DE method. Data are presented as mean±SD (n=3)

CONCLUSION

The study was successful in order to develop a MNZ loaded NPs for sustain delivery upto 24 h. Single emulsification solvent evaporation method was found suitable for the preparation of MNZ loaded NPs. Obtained data of particle size, zeta potential, drug loading, mucoadhsion and *in vitro* drug release confirmed the suitability of prepared NPs for successful delivery of MNZ in periodontitis upto 24 h. Further, few study such safety and efficacy study of NPs in *in vivo* animal or human model may also be conduct for complete establishment of delivery system.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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