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RESEARCH ARTICLE

DESIGN AND EVALUATION OF MINOCYCLINE HYDROCHLORIDE NANOPARTICLES AS LOCAL DELIVERY IN TREATMENT OF PERIODONTITIS

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ABSTRACT

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms. Local delivery of antibiotics has been investigated for the possibility of overcoming the limitations of conventional therapy. The goal of this research work is to prepare nanoparticles of minocycline hydrochloride,then these tiny particles are formulated into *in situ* gels as a local drug delivery system within the periodontal pockets for the effective treatment of periodontitis. FTIR studies indicated that there was no chemical interaction between drug and polymer and stability of the drug. Nanoparticles containing Minocycline hydrochloride were developed by using eudragit RL 100 as polymer andthey were evaluated for drug content, particle size analysis and stability studies. The average particle size of minocycline nanoparticles were within the range. On the other hand optimized formulations of *in situ* gels containing nanoparticles and pure drug were prepared by using gellan gum (0.6 % w/v) and comparative *in vitro* diffusion study have done for these *in situ* gel formulations. No appreciable difference was observed in the extent of degradation of product during 60 days in which nanoparticles were stored at 40°C/ 75% RH.

Key words: periodontitis**,** minocycline hydrochloride, nanoparticles and*in situ* gels.

INTRODUCTION

Dental diseases are recognized as the major public problem throughout the world and these are amongst the most widespread chronic disorders affecting the mankind[1]. These are among the most common diseases in humans and include dental caries, gingivitis, periodontitis and many more oralconditions.Periodontitis is very common and is widely regarded as the second most common disease worldwide, after dental decay and in the United States has a prevalence of 30-50% of the population but only about 10% have severe forms. Periodontitis or pyorrhea is a set of inflammatory diseases affecting the periodontium, i.e., the tissues that surround and support the teeth. Periodontitis involves progressive loss of the alveolar bone around the teeth and if left untreated, can lead to the loosening and subsequent loss of teeth[2].

Periodontitis is caused by anaerobic bacteria, fungi and other microorganisms that adhere to and grow on the tooth's surfaces. *Treponamadenticola, Porphyromonasgingivalis, Prevotellaintermedia,Actinobacillusactinomycetemcomitans,Treponamasocranskiiporphyromonasintermedia*are few known examples[3].

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Periodontal disease has many states or stages, ranging from easily treatable gingivitis to irreversible severe periodontitis. The most prevalent form of periodontal disease in a mild form called gingivitis it is characterized by inflammation of the gums, redness, swelling and frequent bleeding. The symptoms of periodontitis are similar to those of gingivitis but are more severe due to higher accumulations of bacteria and stronger inflammatory responses[4].

In conventional mode of drug administration, many drugs do not reach target areas in the body in sufficient concentration because of premature inactivation and excretion. The systemic drug administration has been useful in treating periodontitis but the disadvantage is that, drug is diluted several thousand folds before it reaches the site and exposes the rest of the body to potential side effects. This problem can be overcome by administering the drug directly to the intended site of action with lesser dose. Sustained drug delivery systems are able to provide very precise control over drug release for a prolonged period of time eliminating the need for frequent dosing and minimizing side effects, thereby increasing patient compliance and comfort. A site-specific system aims at delivering the therapeutic agent at sufficient levels inside the pocket and at the same time minimizing the side effects[5]. Modern drug delivery systems like nanoparticles are designed for targeted controlled and sustained drug releasewhich is safe and capable of producing consistent blood levels of drug in the body for required period of time. It also improves keeping and handling properties of the drug[6]. Different drugs used for local delivery are tetracyclines including doxycycline, minocycline, metronidazole and chlorhexidine. However, local delivery of these agents provides high concentrations that are bacteriocidal. Local application of tetracyclines has been associated with minimal side effects[7]. Furthermore, there are limited data to suggest that local delivery of antibiotics may also be beneficial in preventing recurrent attachment loss in the absence of maintenance therapy[8]. Tetracycline and its congeners such as minocycline and doxycycline are generally used to treat periodontitis[9].Minocycline hydrochloride is a broad-spectrum tetracycline antibiotic and has a broader spectrum than the other members of the group and it is the most lipid-soluble of the tetracycline class antibiotics[10]. This project is undertaken to improve the drug delivery to the periodontal pockets for the treatment of periodontitis by formulating and evaluating the nanoparticles of minocycline hydrochloride. The developed minocycline hydrochloride nanoparticles are formulated into *in situ* gels as a local drug delivery system within the periodontal pockets for the effective treatment of periodontitis.

MATERIALS AND METHODS

Chemicals and Reagents

MinocyclineHydrochloride, Gellan gum (HI media laboratories Ltd, Mumbai), Chitosan (Central Institute of Fisheries Technology, Cochin, India), Eudragit RL 100 (Evonik Degussa India Pvt. Ltd, Mumbai).Glacial acetic acid, Liquid paraffin, Petroleum ether, Gluteraldehyde 25%, Acetone, Span 80,Poly Vinyl Alcohol, Dichloromethane from SD Fine Chemicals Limited, Mumbai, India.Sodium citrate (BML Industries, Banglore).

Preparation of minocycline hydrochloride (MH) nanoparticles:

MH nanoparticles were prepared by high speed homogenization technique using Eudragit RL 100 as polymer. Drug to polymer ratio was maintained at 1:10. Then 100mg drug was dissolved in 5ml of 1% PVA solution. Surfactant used to stabilise the drug & to dissolve.Eudragit RL 100 was dissolved in 8 ml of dichloromethane and homogenized under high speed homogenizer at 10,000 rpm. Drug solution was added drop wise to the polymer solution for 15 min at 10,000 rpm. Added above drug-polymer solution dropwise to the 1% PVA 10 ml solution which is

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maintained under homogenization for 15 min. at 10,000 rpm. Then sonicated the solution at 80/8/8in ice-bath toreduce the heat produced by the sonicator and evaporate the organic solution for 4 hrs.Further the resultant solution is centrifuged at 22,000 rpm for 30 min. Nanoparticles were collected by decanting the supernatant[11].

Evaluation of Minocycline Hydrochloride Nanoparticles

Average particle size determination by zeta sizer:

Average particle size (in nanometers) and size distribution of the minocycline hydrochloride nanoparticle suspension was measured using a Malvern nano zeta sizer instrument[11].

Drug content:

Nanoparticle suspension of 0.1 ml was taken and appropriate dilutions were done with water and drug content was determined spectrometrically[12].

Formulation of *in situ* gels

Polymer solution (gellan gum) of 60 mg concentration was prepared by adding to deionised water containing 0.17% w/v sodium citrate and heated to 90°C while stirring. After cooling to below 40°C, minocycline hydrochloride nanoparticle solution was added to the polymer solution. Make up the volume to 10 ml. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing. Using this same procedure gel containing minocycline hydrochloride pure drug were also prepared[13].

Release studies nanoparticles

Drug release studies from the *in situ* gel were carried out by using a cellophane membrane. Apparatus was designed as it is a glass tube had a length of 10.5 cm and a diameter of 2.1 cm. The lower base was tied with cellophane membrane containing *in situ* gel and this was placed in a beaker containing 100 ml of phosphate buffer pH 6.8 (simulated salivary pH) as diffusion medium which is maintained at 37^{o}C with 50 RPM. Samples (5 ml) were withdrawn at different time intervals from the reservoir till the gel was completely eroded (3 hrs). The cumulative percent drug release was determined by measuring the absorbance at 265 nm by using UV Spectrophotometer (Shimadzu – 1700)[14].

Stability studies of minocycline hydrochloridenanoparticles

The nanoparticles and microspheres were packed in amber-colored bottles, tightly plugged with cotton and capped. They were then stored at 40°C/ 75% RH for two months and evaluated for their physical appearance, drug content[15].

RESULTS AND DISCUSSION

Evaluation ofMinocycline Hydrochloride Nanoparticles

Particle size analysis:

Particle size analysis of the minocycline hydrochloride nanoparticles was determined by using a malvern zeta sizer instrument. It was found that the average particle size of nanoparticles was found to be 712.6 nm, the results were shown in figure-1.

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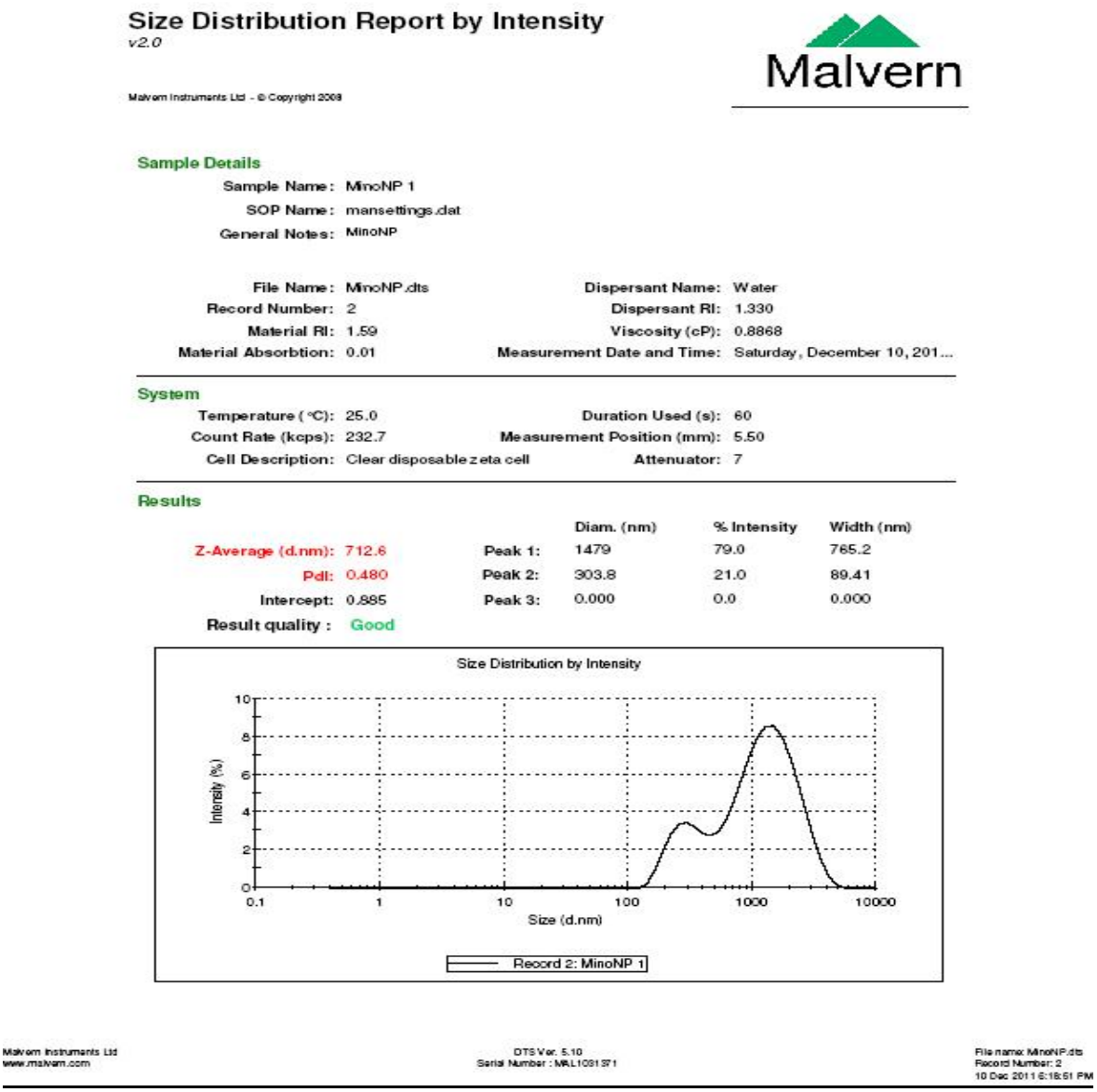

Fig 1: Particle size & distribution of MN by zeta sizer.

Drug content:

The average drug content in 1 ml of nano suspension was found to be 5.464 mg/ml which is equal to 98.35% of drug.

***In vitro* release of minocycline hydrochloride nanoparticles:**

The *in vitro* diffusion profile of drug loaded gels containing nanoparticles and pure drug is shown in figure-2. Minocycline pure drug (MP) has shown least drug release (65.92 %) compared to formulation minocycline nanoparticles(MN) maximum drug release 84.53%within three hours of study (Table 1).

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Table 1: Drug release data of Pure drug and Nanoparticles

TIME (min)	% CUMULATIVE DRUG RELEASE	
	MP	MN
0	0± 0.000	0±0.000
15	7.09±0.124	6.54±0.112
30	16.57±0.214	20.32±0.114
45	21.87±0.321	29.13±0.321
60	32.77±0.145	36.21±0.223
90	39.95±0.231	44.09±0.124
120	44.42±0.312	49.17±0.331
150	55.78±0.341	69.89±0.121
180	65.92±0.123	84.53±0.199

Stability studies:

The prepared nanoparticles were collectedin tightly closed amber-colored bottles. They were then stored at 40°C/ 75% RH for two months and evaluated for their physical appearance, drug content.The results of the stability studies are given in table 5 and found to be satisfactory.

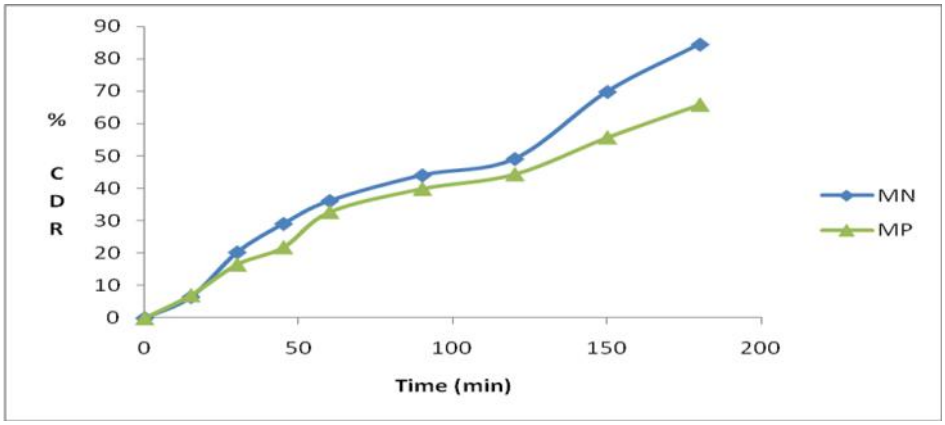

Fig 2: Comparative *in vitro* drug release profile for the formulations MN (Drug loaded naoparticles) and MP (Pure drug).

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Table 5: Stability data of minocycline hydrochloride nanoparticles 40ºC / 75% RH.

TIME IN DAYS	NANOPARTICLES	
	Physical appearance	Drug content
0	+++	98.35 %
30	+++	98.02 %
60	+++	97.87 %

CONCLUSION

The present study showed that the nanoparticles of minocycline hydrochloride can be prepared successfully in laboratory scale. The various evaluation parameters of nanoparticles like particle size, drug content and size distribution the results were satisfactory. Further these tiny particles can be incorporated into *in situ* gel. *In vitro* release studies revealed that nanoparticles are more efficient than pure drug and the data was found satisfactory. However, more clinical studies are essential to prove therapeutic significance and effectiveness for the intended treatment.

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