COMPARATIVE STUDIES OF NANOPARTICLES AND MICROSPHERES OF MINOCYCLINE HYDROCHLORIDE AS LOCAL DELIVERY IN TREATMENT OF PERIODONTITIS

Mohammed Gulzar Ahmed*, Vinay C. H, Sanjana. A

Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Karnataka -571448

Abstract:
Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms. Local delivery of tetracycline antibiotics has been investigated for the possibility of overcoming the limitations of conventional therapy. The goal of this research work is to prepare nanoparticles and microspheres containing minocycline hydrochloride, 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. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of the drug. The microspheres were prepared by emulsion cross linking method using chitosan as a polymer. Nanoparticles containing Minocycline hydrochloride were developed by using eudragit RL 100 as polymer. Microspheres and nanoparticles were evaluated for drug content, particle size analysis and stability studies. The physico-chemical parameters reveal that microspheres have a discrete spherical structure without aggregation. The average particle size of minocycline nanoparticles and microspheres were within the range. On the other hand optimized formulations ofin-situ gels containing nanoparticles, microspheres 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 and microspheres were stored at 40°C/ 75% RH.
Key words: *periodontitis,minocycline hydrochloride, nanoparticles, microspheres and In-situ gels.*

*Corresponding Author:
Dr. Mohammed Gulzar Ahmed,
Professor and Head, Department of Pharmaceutics
Sri Adichunchanagiri College of Pharmacy,
B.G. Nagar, Karnataka, India.
Tel: +91-8234-287870.   9448401238 (m).
E.mail: mohammedgulzar1@gmail.com

QR code

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]. Dental diseases can cause much more serious problems than a toothache; they can affect our ability to chew, smile, or speak properly. 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].

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 targetareas 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 associated with systemic drug administration [5].Recently, intensive research is being performed all over the world to improve the effectiveness of delivery systems. Modern drug delivery systems like microspheres and nanoparticles are designed for targeted controlled and sustained drug release. There is a need of making any drug nanoparticles or microspheres is to produce a drug delivery system which 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.Minocycline 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 and microspheres of minocycline hydrochloride.The developed minocycline hydrochloride nanoparticles and microsphere 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

Materials:

Minocycline Hydrochloride, 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, Dichloromethanefrom SD Fine Chemicals Limited, Mumbai, India.Sodium citrate (BML Industries, Banglore).

Methods:

The compatibility studies of drug and excipients were determined by FT-IR studies. Both pure drug and excipients were individually analysed and further the physical mixture and formulations were also studied.

Preparation of Minocycline Hydrochloride Nanoparticles [11]:

Minocycline hydrochloride nanoparticles were prepared by high speed homogenization technique using Eudragit RL 100 as polymer. Drug to

polymer ratio was maintained at 1:10. 100mg drug was dissolved in 5ml of 1% PVA solution. Surfactant used to stabilise the drug and 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. Add above drug-polymer solution dropwise to the 1% PVA 10 ml solution which is maintained under homogenization for 15 min. at 10,000 rpm. Then sonicate the solution at 80/8/8in ice-bath toreduce the heat produced by the sonicator and evaporate the organic solutionfor 4 hrs.Further the resultant solution is centrifuged at 22,000 rpm for 30 min. Nanoparticles were collected by decanting the supernatant.

Preparation of Minocycline Hydrochloride Microspheres [12]:

Chitosan microspheres were prepared by emulsion cross linking method. 2 g of chitosan was dissolved in 4 % acetic acidby overnight stirring in a magnetic stirrer. Accurately 500 mg of minocycline hydrochloride was weighed and dispersed in chitosan solution and mixed well. Drug-polymer mixture of 6 ml was added to 40ml liquid paraffin containing 3ml of span 80 and stirring was performed in a magnetic stirrer to form w/o emulsion.After 30 minutes homogenization, 2 ml of glutaraldehyde 25% was added. It was left for stabilization and cross linking for a period of 3 hrs. Microspheres thus obtained were centrifuged at 2000 Rpm and sediment was then collected. Washed with petroleum ether for three times and acetone for three times and then dried in hot air oven.

Micromeritic Properties [13, 14]:

Angle of Repose:

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel standing method. Improper flow of microspheres is due to frictional forces between the microspheres. These frictional forces are quantified by angle of repose.A glass filling funnel is held in place with a clamp on the ring support over a glass plate. Microspheres were weighted passed through the funnel, which was kept at a height 'h' from the horizontal surface. The passed microspheres formed a pile of a height 'H' above the horizontal surface and the pile was measured and the angle of repose was determined for all the batches by using the formula.

$$\text{Angle of repose } (\theta) = \tan^{-1}(H/R)$$

H = Height of the pile

R= Radius of the pile.

Bulk Density and Tapped Density:

Bulk and tapped densities were measured by using 10ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated. Each experiment for micromeritic properties was performed in triplicate manner.

Carr's index:

Carr's index=$\dfrac{(\text{Tapped density} - \text{Bulk density}) \times 100}{\text{Tapped density}a}$Compressibility index or Carr's index value of microspheres was computed according to the following equation:

Evaluation of Minocycline Hydrochloride Nanoparticles

Average particle sizedetermination by zeta sizer [7]:

Average particle size (in nanometers) and size distribution of the minocycline hydrochloride nanoparticle suspension was measured using a Malvern nano zeta sizerinstrument.

Drug Content [12]:

0.1 ml of nanoparticle suspension was taken and appropriate dilutions were done with water. Absorbances were measured by UV spectrophotometer (Shimadzu UV"1700), drug content was calculated by using standard graph. Readings were taken for average of 3 samples.

Evaluation of Minocycline Hydrochloride Microspheres

Analysis of Particle size [15]:

The particle size and particle size distribution of microspheres was evaluated using optical microscope. The freshly prepared microspheres were spread on a clean and dried glass slide and examined on an optical microscope and size of the microspheres was measured by using the pre-calibrated ocular micrometer and stage micrometer. About 100 particles of each formulation were observed and counted.

Drug Content [12]:

50 mg of chitosan microspheres loaded with minocycline hydrochloride were transferred into glass mortar and crushed and further digested in methanol for 10min to dissolve the drug. The solution was filtered and an aliquot was assayed sphectrophotometrically to quantify for drug content.

Formulation of *in-situ* gel [16]

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 10ml. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing. Using this same procedure gel containing minocycline hydrochloride microspheres, nanoparticles and pure drug were prepared.

Comparative Studies of *In-Vitro* Release [17]

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 37ºC with 50 RPM. Samples (5ml) 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.

Stability Studies [18]

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 three months and evaluated for their physical appearance, drug content.

RESULTS AND DISCUSSION:

The compatibility studies revealed both drugs and excipients were compatible after FT- IR studies, the results shown in Figure 1.

Micromeritic Properties:

Minocycline hydrochloride microspheres shown angle of repose value 22º 55"± 0.176, i.e., less than 25, which shows free flowing nature of the formed microspheres.Bulk and tapped densities showed good packability of the microspheres. Carr's index of minocycline hydrochloride microspheres was14.86± 0.173 (Table 1).

Evaluation of Minocycline 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 (Figure2).

Drug Content:

The average drug content in 1 ml of nano suspension was found to be 5.464 mg/ml (Table 2).

Evaluation of Minocycline Hydrochloride Microspheres

Particle Size Determination:

Microsphere size was determined by using an optical microscope under regular polarized light and the mean microsphere size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer. Ocular and stage microscopy shows that the microspheres obtained were spherical and free flowing. The particle size of minocycline hydrochloride microspheres was in the range of 94-126 µm.

Drug Content:

The result shows minocycline hydrochloride microspheres contains drug content 884.8µg/ml (Table 3).

Comparative Studies of *In-Vitro* Release of Minocycline Hydrochloride Nanoparticles and Microspheres:

The *in-vitro* diffusion profile of drug loaded gels containing nanoparticles, microspheres and pure drugis shown in figure 3. Formulation MP (pure drug) have shown least drug release (65.92 %) compared to formulation MM (minocycline hydrochloride microspheres) that is 73.68 % within 3 hrs and formulation MN(minocycline nanoparticles) have shown maximum drug release 84.53% (Table 4).

Stability Studies of Nanoparticles and Microspheres:

The nanoparticles and microspheres were stored tightly closed amber-colored bottles at 40°C/ 75% RH for three months and evaluated for their physical appearance, drug content.The results of the stability were found to besatisfactory (table 5).

CONCLUSION:

The above study showed that the nanoparticles and microspheres of minocycline hydrochloride can be prepared successfully in laboratory scale. Further these tiny particles can be incorporated into *in-situ* gel. *In-vitro* release studies revealed that nanoparticles are more efficient than microspheres and pure drug. However, more clinical studies are essential to prove therapeutic significance.

ACKNOWLEDGEMENT:

REFERENCES:

1. Jain NK. Controlled and novel drug delivery. New Delhi: CBS publishers & distributors; 1997. p. 132-42.
2. http://en.wikipedia.org/wiki/Periodontitis.
3. Samina R, Alka A, Ali J, Khar, RK. Site specific Delivery systems for Treatment of Periodontitis. Indian Journal of pharmaceutical sciences 2003; 65(2):106-12.
4. Jemin K, Salomon A. Periodontal disease and systemic conditions: a bidirectional relationship. 2006:94(1);10–21.
5. Mohammed GA, Narayana CR, Harish NM, Prabhakar P. Formulation and *in-vitro* evaluation of Chitosan films containing tetracycline for the treatment

of periodontitis. Asian Journal of Pharmaceutics 2009;113-19.

6. Chiao Charles SL and Robinson JR. Sustained Release Drug Delivery System. Remington's Pharmaceutical Sciences, 19th ed. Mac Publishing Company 1999; 1660.

7. Jayvadan KP, Dhaval JP, Vikram MP. An Overview: Nanoparticles. International Journal of Pharmaceutical Sciences and Nanotechnology. 2008;1(3):215-20.

8. Pragati S, Ashok S, Kuldeep S. Recent advances in periodontal drug delivery systems. International Journal of Drug Delivery. 2009:1-14.

9. Divya PV, Nandakumar K. "Local Drug Delivery-Periocol" In Periodontics. Trends BiomaterArtif Organs. 2006;19(2):74-80.

10. http://en.wikipedia.org/wiki/Minocycline.

11. Yesim A, karine A, Maria JA, Pilar C, Neslihan GR, Patrick C, Yilmaz C. Preparation and *in-vitro*evaluation of chitosan nanoparticles containing a caspase inhibitor. International Journal of Pharmaceutical Sciences 2005;298:378-83.

12. Jain S K, Rai G, Saraf D K, Agrawal G P; The preparation and evaluation of albendazole microspheres for colonic delivery. Pharmaceutical Technology. 2004; 28(12): 66-71.

13. CVS Subrahmanyam. Textbook of Physical Pharmaceutics, Micromeritics 2nd ed., VallabhPrakashan 2002; 222-24.

14. ParulTrivedi, AML Verma, N Garud. Preparation and characterization of aceclofenac microspheres. Asian J pharm 2008 ;(2): 110-15.

15. Anand G, Chirag N, Krunal P, Panchaxari D, Vinayak, Mastiholimath. Formulation and Evaluation of Floating Microspheres of Captopril for Prolonged Gastric Residence Time. Indian Journal of Novel Drug Delivery. 2007;3(1):17-23.

16. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EVS. Formulation and Evaluation of *in-situ* Gels Containing Clotrimazole for Oral Candidiasis. Indian J Pharm Sci. 2009;71(4):421–27.

17. Shastri DH, Patel LD, ParikhRK. Studies on *In-situ* hydrogel: A smart way for safe and sustained ocular drug delivery. Journal of young pharmacist. 2010;2(2):116-120.

18. ICH Q1A (R2) Stability testing guidelines: Stability Testing of new drug substances and products.[online]. [Cited 2008 Nov 10]; Available from: URL: http://www.tga.health.gov.au/docs/pdf/eug uide/inch/273699r2en.pdf.

List of Tables and Figures

Table 1: Micromeritic Properties of MH Microspheres (n = 3)

S.No	Angle of Repose(θ) Mean ± SD*	Bulk Density (g/ml) Mean ± SD*	Tapped Density (g/ml) Mean ± SD*	Carr's Index (%) Mean ± SD*
1	22º 55"± 0.176	0.441± 0.0053	0.518± 0.0068	14.86± 0.173

Table 2: Drug Content of Minocycline Hydrochloride Nanoparticles

S.No	Volume of nano suspension	Absorbance	Dilution factor	Conc of drug mg/ml	% of drug
1	0.1 ml	0.1366	1000	5.464	98.35 %

Table 3: Drug Content of Minocycline Hydrochloride Microspheres.

S.No	Amount of microspheres	Absorbance	Dilution factor	Conc of drug µg/ml	% of drug
1	100 mg	0.2212	100	884.8	88.4 %

Table 4: Drug Release Data of Formulation MN, MM and MP.

TIME (min)	% cumulative drug release		
	MN	MM	MP
0	0	0	0
15	6.54	4.72	7.09
30	20.32	18.41	16.57
45	29.13	24.67	21.87
60	36.21	32.33	32.77
90	44.09	40.87	39.95
120	49.17	48.39	44.42
150	69.89	63.06	55.78
180	84.53	73.68	65.92

A. Pure Drug of Minocycline Hydrochloride.

B. Drug with Chitosan.

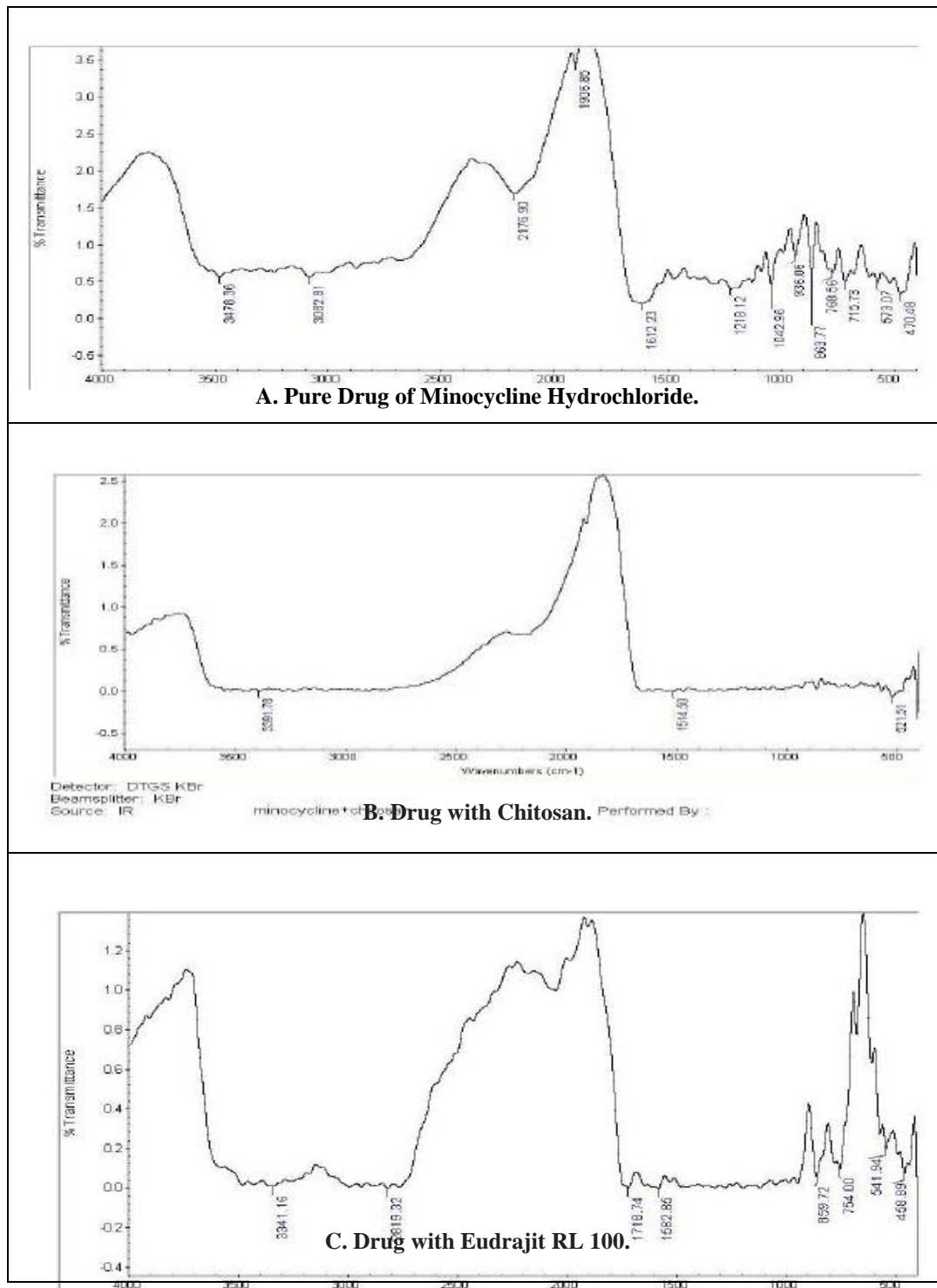**C. Drug with Eudrajit RL 100.**
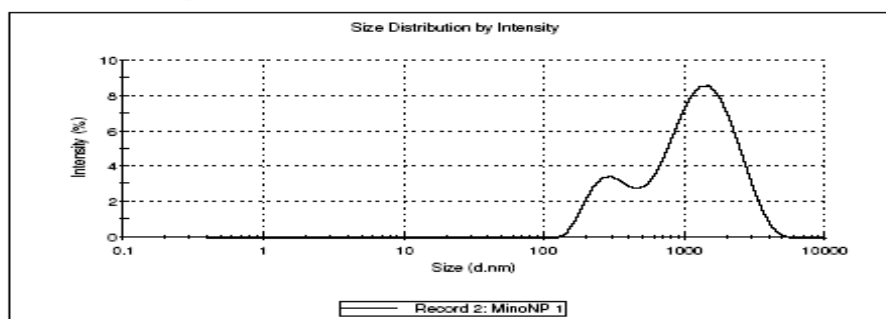
Fig 1: FTIR Studies of Pure Drug, with Excipients.

Table 5: Stability Data of Minocycline Hydrochloride Nanoparticles and Micro Particles Stored at 40ºC / 75% RH.

Time In Days	Nanoparticles		Microspheres	
	Physical appearance	Drug content	Physical appearance	Drug content
0	+++	98.35 %	+++	88.4 %
30	+++	98.02 %	+++	88.29 %
60	+++	97.87 %	+++	88.11 %

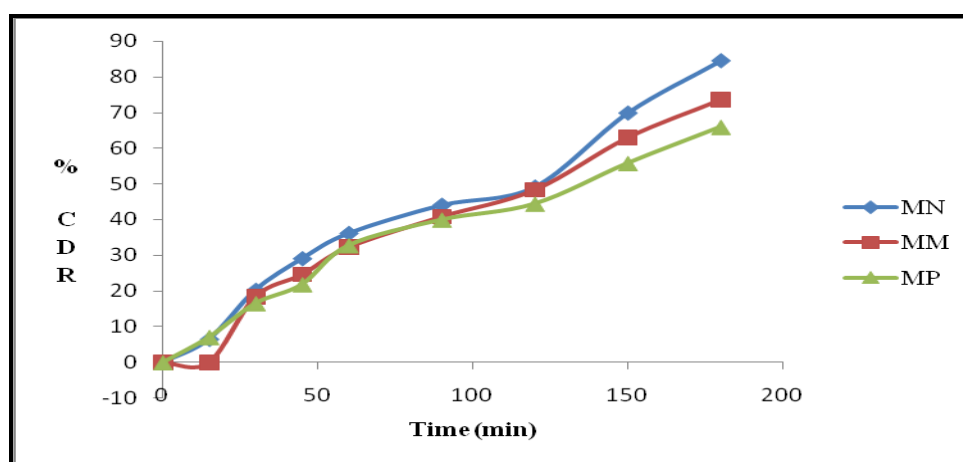**Fig 2: Particle size of Minocycline Hydrochloride Nanoparticles by Zeta Sizer.**

Fig 3: Comparative *In-Vitro* Drug Release Profile For The Formulations MN (Drug Loaded Naoparticles), MM (Drug Loaded Microparticles) and MP (Pure Drug).