Original Article

Synthesis & Evaluation of Drug Release from Amoxicillin Loaded Chitosan Nanoparticles Incorporated in the Periodontal Membranes

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ABSTRACT

Objective: (1) To fabricate Amoxicillin loaded chitosan nanoparticles and evaluate them for their morphology, size and drug releasing profile. (2) To incorporate these nanoparticles in the fabricated periodontal membranes and evaluate their pattern of drug release.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Study was conducted at RIPS (Riphah Institute of Pharmaceutical Sciences) Islamabad in collaboration with IST (Institute of Space and Technology) and Quaid e Azam University Islamabad from April 2020 to February 2021 for a duration of ten months.

Materials and Methods: Amoxicillin loaded chitosan nanoparticles were fabricated by ionic gelation method and were characterized by using SEM and Zeta sizer. The pattern of the drug release was observed over a period of 24 hours by using dissolution apparatus. These nanoparticles were incorporated in the fabricated membranes and the pattern of drug release from the membranes was also observed.

Results: Amoxicillin loaded nanoparticles displayed spherical shape with a uniform size of 150.6±2.5 nm and depicted an initial burst followed by a sustained drug release pattern. An initial burst release of the drug followed by a slow and sustained pattern of amoxicillin was also observed from the membranes until 24 hours.

Conclusion: It can be suggested from the results that the fabricated Amoxicillin nanoparticles can serve as effective carriers for the drug. Also, the fabricated membranes incorporated with drug loaded nanoparticles show their potential to be used in the periodontal therapy possessing controlled drug release feature.

Key Words: Amoxicillin loaded nanoparticles, Chitosan, Dissolution apparatus, Lyophilization, Sustained drug release

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INTRODUCTION

Periodontal diseases are the most widespread chronic diseases affecting >743 million people globally[1]. In periodontitis there is severe infection resulting in the formation of periodontal pocket which in severe cases leads to periodontal tissue destruction and formation of infrabony defects resulting in early tooth loss[2].

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The treatment focuses on eradication of pathogenic microbes and tissue regeneration.

Traditional treatment is the mechanical removal of the dental plaque along with the administration of antibiotics or antiseptics[3]. The most commonly prescribed drugs for periodontal infections are tetracycline and amoxicillin. Amoxicillin has broad spectrum antibacterial potency but its therapeutic potency is limited due to its half-life of 60 minutes which necessitates its repeated administration to maintain adequate plasma levels. The repeated use leads to unwanted side effects along with developing resistance to the drug[4]. To overcome the drawbacks of systemic administration, there is strong emphasis on the generation of controlled and sustained drug dispensing structures[5].

The most approved and established GBR (Guided bone regeneration)/GTR (Guided tissue regeneration) therapy for regeneration of the periodontal tissue is the use of membranes. Various antibiotics have been directly incorporated in the membranes to control the bacterial contamination both at the non-surgical and surgical stage of the GTR/GBR procedures and has been studied

in the past. However, the direct incorporation of the drugs exhibited drawbacks such as the burst release of the drugs, inadequate stability, and inability to achieve specific targeting[6].

Controlled drug release through nanoparticulate system not only maximizes the drug's bioavailability, pharmacokinetics, efficacy of the drugs but also minimizes the side effects related with the free drug[6]. Chitosan has shown to be effective for drug delivery especially when formulated as nanoparticles. Nanoparticles of chitosan provide excellent properties because of their nanosize, high zeta potential and high surface area[7].

Hence, the aim of the current study was to fabricate chitosan nanoparticles loaded with amoxicillin and incorporate them in the fabricated membranes to overcome the limitations of systemic administration of the antibiotic.

MATERIALS AND METHODS

Materials: The following materials were used in the current study:

Acetic acid, Chitosan (LMW),1-ethyl-3(3dimethy-laminopropylcarbodiimide(EDC), N-Hydrosuccinimide (NHS), Amoxicillin, Sodium Hydroxide(NaOH), De-ionized water was purchased from Sigma-Aldrich. Sodium Tripolyphosphate(TPP) was purchased from A-Aesar and Hydrochloric acid. (HCL) from Reidel-de-haen.

Preparation of ACNs (Amoxicillin chitosan nanoparticles): Amoxicillin chitosan nanoparticles were prepared using ionic gelation method (8). Chitosan (45mg) (0.1% w/v) was thoroughly mixed in acetic acid solution (45ml) (1%) on a magnetic stirrer (Eisco Scientific USA MGST2-V2) maintaining pH of the solution at 5 with sodium hydroxide. Then 100mg of Amoxicillin was dissolved in the prepared mixture of chitosan and acetic acid for ten minutes. Sodium Tripolyphosphate (TPP) (22.5 mg) (0.15% w/v) was dissolved in de-ionized water (15ml) with pH set to 4 with HCL separately. With continuous mixing, at 950 rpm for 20 minutes, TPP was added in drops to the chitosan mixture and was sonicated in the sonicator for half an hour. This whole mixture was poured in test tubes of the centrifuge machine and centrifuged for 25 minutes at a speed of 4000 rpm. Finally, the centrifuged solution was lyophilized below -50◦C for 24 hours at a pressure around 6mTorr using TFD5530 Bench top freeze dryer (IIShinBioBase, Republic of Korea) to obtain amoxicillin incorporated nanoparticles which were kept in the refrigerator at 4◦C. These nanoparticles were then incorporated in the Nanohydroxyapatite/ Chitosan periodontal membranes fabricated by using solvent casting and lyophilization methods.

Characterization of ACNs:

Polydispersity index, particle size and zeta potential: Polydispersity index, particle size, zeta potential of the prepared nanoparticles was estimated using Zetasizer Zs 90 (Malvern Instrument, Malvern, Worcestershire, UK)(8). The procedure was performed in triplicate and the obtained results were presented as mean ± standard deviation.

Surface morphology: The morphology of amoxicillin chitosan nanoparticles was observed by a scanning electron microscope (Mira 3 Tescan) (8). The particles were placed on a glass plate which was coated with gold using a gold sputter coating machine. The SEM images were obtained at magnifications of 50kx using secondary electron at an accelerating voltage of 20k.

Drug Release Study of ACNs

The first step to study invitro drug release is to obtain the standard calibration curve to get the value of R^2 (coefficient of determination). The calibration curve of amoxicillin was obtained by plotting the absorbance versus the known drug concentration.

To study the drug release of ACNs, dialysis bag method[8] was used to estimate the liberation of amoxicillin from ACNs in phosphate buffer saline (PBS) at a pH of 7.4. ACNs (equivalent to 100mg) were put in the dialysis bags having molecular weight cut off 12000-14000(Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA) and sealed at both ends. In addition, for comparison free Amoxicillin dispersion (equivalent to 100 mg) was also taken in a separate dialysis bag and sealed at both ends. Maintaining the temperature at 37 ± 0.5◦C and stirring at 100 rpm, the bags were immersed in the vessels containing 500ml PBS of the dissolution apparatus (Galvano Scientific). At predetermined intervals of 0,1,2,4,6,8,10,12,18 and 24 hours,3 ml aliquots of supernatant were withdrawn and the percent drug release was estimated by obtaining the absorbance through UV-visible spectrophotometer at 227nm.Following the withdrawal of aliquots, each vessel was replenished by the same volume of fresh media. Procedure was performed thrice. The concentration of amoxicillin was calculated through comparison with a standard curve and the obtained drug release percent was presented in a graph.

Drug Release Study of the Membranes: To study the drug release of the membranes, basket rack assembly was used to estimate the release of amoxicillin from the membranes in phosphate buffer saline (PBS) at a pH of 7.4. Nanohydroxyapatite chitosan membranes having equal weight and same size were put in the baskets and secured. Maintaining the temperature at 37 ±0.5◦C and stirring at 100 rpm, the baskets were immersed in the vessels containing 500ml (PBS) of the dissolution apparatus (Galvano Scientific). At predetermined intervals at 0,1,2,4,6,8,10,12,18 and 24 hours,3 ml of supernatant was withdrawn and the percent drug release was estimated by obtaining the absorbance through UV-visible spectrophotometer at 227nm.Following the withdrawal of supernatant, each vessel was replenished by the same amount of fresh media. The whole

procedure was repeated thrice. The concentration of amoxicillin was calculated through comparison with the standard curve and the percent of drug release was represented through graph.

RESULTS

Polydispersity index, Particle size and Zeta potential: The Prepared Amoxicillin Chitosan nanoparticles depicted a particle size of 150.6 ± 2.5 nm with a PDI value of 0.164± 0.014(normal value 0.2 or below 0.2) showing a narrow size distribution revealing homogeneity of the particles. A zeta potential of 18.5 ± 1.4 mV (normal value ranges between ±15 to ±45 mV) represents a stable formulation. A unimodal graph below (Fig.3.1) depicts a uniform particle size with similar morphology and equal size distribution.

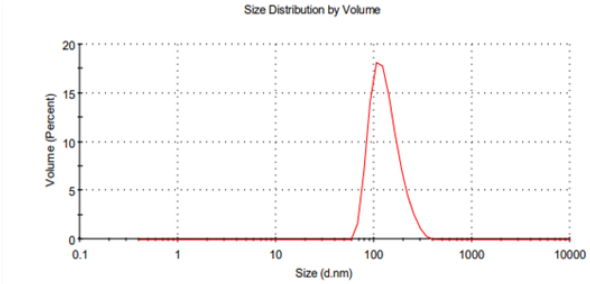

Figure No.3: Showing unimodal graph representing size of the nanoparticles by volume.

SEM Analysis: The morphological characteristics of the prepared ACNs examined through SEM showed spherical particles having a smooth and uniform surface as shown in Fig 3.2 at magnification of 50 kx and a voltage of 20 kv.

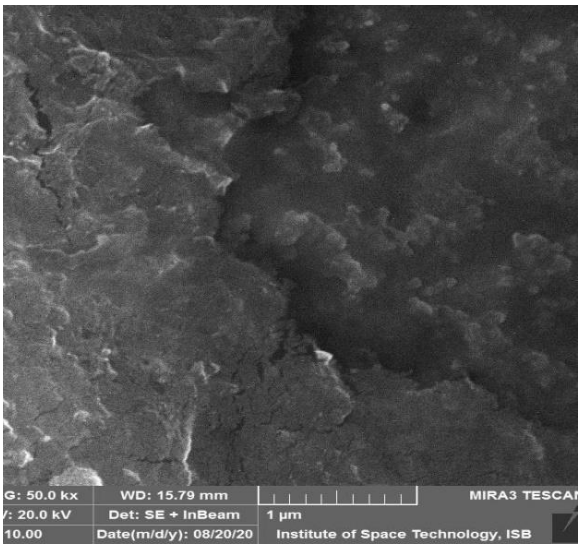

Figure No.2: SEM images showing spherical nanoparticles with a smooth and uniform surface.

Drug Release Profile of ACNs & Membranes: The callibration curve of Amoxicillin at 227nm was obtained showing value of R^2 0.9997 as presented in (Fig 3.3 a).The value of R^2 indicates that the unknown calculated values in the study would be accurate across the entire callibration range.

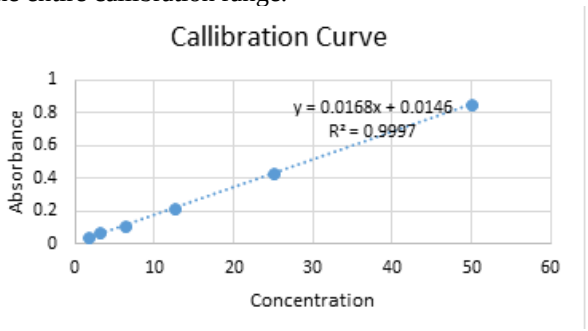

Figure No. 3.3(a): The callibration curve for Amoxicillin.

Amoxicillin released from the ACNs depicted a biphasic extended drug release pattern as presented in the (Fig. 3.3b). A burst release of 17% of Amoxicillin from the nanoparticles was observed initially followed by a gradual release of 43% untill 24 hrs.The fabricated membranes also depicted an initial burst release of 17% Amoxicillin followed by a gradual release of 25%.The free Amoxicillin in comparison depicted an immediate and burst release pattern of the drug starting from fast release of 30 % within first two hours and continuing upto 90% indicating that the fabricated ACNs performed well in controlling the drug release both alone and when incorporated in the fabricated membrane.

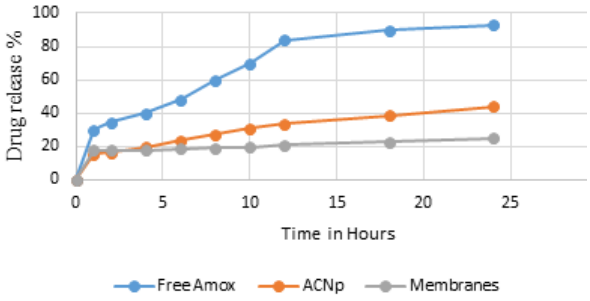

Figure No.3.3(b): Drug release profile of Amoxicillin from ACNs & membranes in comparison with free amoxicillin.

DISCUSSION

To evaluate the physical parameters of the prepared formulations three factors namely, particle size, PDI and zeta potential are considered significant. It is proposed that the magnitude of nanoparticles must be small (<200 nm) in order to impede their elimination by the phagocytic cells and to extend the duration required for their retention[9].

The particle size of the prepared nanoparticles was 150.6±2.5nm which is in accordance with the previous studies[8]. However, the particle size of the chitosan

nanoparticles in the previous studies varied between 91 to 121nm. This difference in the results is attributed to the difference in the speed of the centrifuge machine used (4000rpm in current study and 6000 to 10000rpm in previous), the greater the speed the more is the breakage of the glycosidic bonds in the chitosan, resulting in the formation of smaller nanoparticles[10]. On the contrary it is also a well-known fact that the lower concentrations of polymer and TPP result in the formation of nanoparticles with a size >100nm[8] which is in accordance with the results of the current study.

The PDI value represents the particle uniformity in terms of their size. It depicts the distribution of size population with in a given sample. Value ranges from 0.0 to 1.0. The acceptable PDI values for polymer based nanoparticle materials ranges from 0.2 and below[11]. According to Zeb et.all, the uniform particle size having similar morphologies and equal size distribution can also be depicted with a unimodal graph[12] which is shown in the current study (Fig.3.1).

To avoid aggregation within the formulation, a charge called zeta potential is imparted on the surface of the nanocarrier, which adds to the stability of the formulation. Sufficient stability of the nano formulations is achieved when the range of zeta potential is within ±15 to ±45 avoiding aggregation[13]**.** Hence, the results indicate the formation of stable nanoparticles.

The morphology of the ACNs observed through SEM imaging, show spherical nanoparticles having a uniform surface. These findings are similar with the previous studies showing spherical and smooth surface of the prepared amoxicillin chitosan nanoparticles[8]. According to literature, this particular shape and uniform surface of the particles provides a lesser surface for erosion and facilitates the release of the drug as compared to non-homogenous irregular shaped particles[14].

The drug release profile of the ACNs, membranes with ACNs and Amoxicillin Dispersion were compared. The initial fast and rapid liberation of the antibiotic is ascribed to the dispersion of the drug particles which are poorly bound or absorbed on the exterior of the nanoparticles[15].This early rapid and fast liberation can also take place due to swelling or expansion of the polymer[16]. This phenomenon occurs because of the ability of the polymer to consume water or the adjoining body fluid. The absorption of the fluid untangles the chains of the polymer and advances till the entire polymer breaks down and in turn releases the drug. Secondly the dispersion of the drug from the inside of the polymer network to the exterior is difficult as the chains of the polymer impede the movement, hence slowing the release[17]**.**

The release of the drug is also dependent on the shape and size of the nanoparticles. A uniform surface provides lesser surface for erosion which facilitates slow release of the drug as compared to an irregular non homogenous surface[18].The drug release from the membranes is attributed to the ability of chitosan to absorb water or the fluid which entangles the polymer chains and results in breakdown of the entire polymer releasing the drug[15].

Therefore, through this sustained release not only the bacterial proliferation can be prevented for a longer duration but the antibiotic related side effects can also be reduced.

CONCLUSION

It can be concluded from the study that the fabricated chitosan nanoparticles containing amoxicillin can serve as beneficial drug carriers. Moreover, the fabricated membranes showing sustained drug release can be used in severe periodontal conditions providing protection against pathogens until the tissue regenerates.

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Author's Contribution:

Concept & Design of Study:	Nausheen Ashraf
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Final Approval of version:	Nausheen Ashraf

Conflict of Interest: The study has no conflict of interest to declare by any author.

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