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CONTROLLED RELEASE BIOADHESIVE NANOPARTICULATE SYSTEM OF CIPROFLOXACIN: FABRICATION AND EVALUATION FOR PERIODONTITIS TREATMENT

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ABSTRACT

Periodontitis is a gum infection with symptoms like gum inflammation, tooth loss etc. caused due to bacteria residing in biofilms at or below the gingival margin. Control remedies like tablets and surgical methods are used but have their own side effects like liver toxicities and postoperative complications. Widespread number of local drug delivery systems have been introduced one of them being nanoparticles having significant surface area and sustained release properties at affected site. In our experiment we have formulated ciprofloxacin (CF) nanoparticles using PLGA as polymer further coated with chitosan (CH) by single emulsification method and carried out their evaluation and optimization. Evaluation parameters like size & morphology, in-vitro drug release, mucoadhesion studies, DSC, irritation testing and stability studies were carried out to achieve best of the conditions for nanoparticle formulation. Since, ciprofloxacin is a second generation fluoroquinolone antibiotic with 3-4h shelf life, 1µg/ml or less minimum inhibitory concentration (MIC), effective against both gram positive and gram negative bacteria act as a potent drug for nanoparticle formulation for periodontitis. Single Emulsification method resulted in smaller sized ciprofloxacin nanoparticles (NPs). Moreover, results of in-vitro drug release showed that chitosan coated ciprofloxacin PLGA NPs (CH-CF-PLGA-NPs) have sustained drug release profile. CH coated nanoparticles were found to be non irritant and show good mucoadhesion. Hence prepared CH-CF-PLGA-NPs found to be suitable for dental carries like periodontitis.

Keywords: Chitosan, Ciprofloxacin, Mucoadhesion, Periodontitis, PLGA Nanopartciles, Single Emulsification

ABBREVIATIONS

AC	:	Acetone
ACN	:	Acetonitrile
CF	:	Ciprofloxacin
CF-PLGA-NPs	:	Ciprofloxacin Loaded PLGA Nanoparticles
CH	:	Chitosan
CH-CF-PLGA-NPs	:	Chitosan Coated Ciprofloxacin Loaded PLGA Nanoparticles
DE	:	Double Emulsification
DSC	:	Differential Scanning Calorimetry
EE	:	Entrapment Efficiency
HET-CAM	:	Hen’s Egg-Chorioallantoic Membrane
MPS	:	Mean Particle Size
MWCO	:	Molecular Weight Cut-Off
NPs	:	Nanoparticles
PI	:	Poly dispersity Index
PLGA	:	Poly Lactic Glycolic Acid
PTA	:	Phospho Tungstic Acid
PVA	:	Poly Vinyl Alcohol
SE	:	Single Emulsification
TEM	:	Transmission Electron Microscopy
ZP	:	Zeta Potential

1. INTRODUCTION

Periodontitis is a critical gum infection caused by bacteria residing in biofilms at or below the gingival margin. Symptoms include gum inflammation, tooth loss, gentle tooth, bad breath, painful chewing, alveolar bone loss and other significant intricacies **[1]**. Plaque formation on teeth, ongoing gum inflammations, gingivitis, poor oral health habits, hormonal changes, obesity, vitamin C deficiency, genetics, are some of the causes **[2]**.

Conventional control remedies for periodontitis treatment include suppression or elimination of subgingival pathogens by non- surgical procedures like, scaling and administration of antibiotics (tetracycline, metronidazole, amoxicillin, clindamycin, cephalexin, ciprofloxacin, azithromycin, spiramycin etc.) **[3]**. Application of chewable substances like tablets and lozenges, use of ointments, mouthwashes and dental irrigation are some other convenient methods. Pocket reduction to reduce space between tooth and gums, bone graft surgery for dental implants, ridge augmentation to restore the natural contour of the jawbone, surgical methods areapplied **[4]**. Sporadically, antibiotics may exert antagonisation interactions causing impaired liver toxicities. On the other hand, surgical procedures may accompany post operative complications like reduced mouth opening, pain, difficulty in masticatory compatibility and swelling. Moreover, these methods are not efficient in delivering drug at desired site for sufficient duration **[5]**.

A thrive in understanding of etiology, epidemiology and microbiology of periodontal affected tooth have reformed the clinical schedule for periodontitis **[6]**. A widespread number of local drug delivery system have been propounded including fibres, strips, films, gels, sponges, microparticles etc to carry forms of liposomes, niosomes, nanoparticles, and microemulsions **[5]**.

Since nanoparticles have significant surface, size and quantum effects, improved transparency, heat resistance and decreased gas permeability, they usually exhibit preferable performance than traditional materials. Moreover nanoparticle preparations manifests sustained release of the drug at sublingual spaces, adverse effects and toxicities are minimized through the use of these colloidal vehicles. Alternatively, nano-particles have increased stability in biological fluids and during storage. Nanoparticles can be manufactured by both biodegradable and non-biodegradable polymers. Poly (lactic acid) (PLA), poly (glycolic acid) (PGA), PLGA, poly (methyl acrylate) NP's are majorly used **[7]**. These systems with bioadhesive and mucoadhesive polymers hold the drug in the periodontal pocket for a longer duration allowing a deep contact between the drug and the affected tissues which forms the basic characteristic property for the generation of nanoparticles **[8]**. In comparison, to the work published so far, an attempt has been made to develop ciprofloxacin nanoparticulate system which is a broad spectrum antibiotic.

Ciprofloxacin belongs to the second generation of fluoroquinolone antibiotics with a plasma half life of 3-5h. It can act against both gram-positive and gram-negative bacteria's *(Staphylococcus aureus and Eescheria coli respectively)* **[9]**. Moreover, it acts as a potent drug in inhibition of bacteria causing periodontitis **[10]**. Ciprofloxacin acts as DNA gyrase inhibitor which is an essential enzyme for DNA replication at the time of bacterial cell division thereby inhibiting bacterial cell growth **[11]**.

Thus, the aim of this study was to develop ciprofloxacin nanoparticles by emulsifycation method with great therapeutic efficacy and minimum side effects in periodontitis treatment and also carry out their characterization on various parameters like morphology, particle size, entrapment efficiency, DSC, in-vitro drug release, mucoadhesion, irritation and stability studies.

2. MATERIALS AND METHODS

2.1 Materials

Ciprofloxacin was received from Sun Pharmaceutical Industries Ltd. (Gurugram, India) as a gift sample. Chitosan (Chitoclear, degree of deacetylation 96%, viscosity 15cp) was received from Primex EHF (Siglufjordur, Iceland), Poloxamer from Sigma Aldrich (Mumbai, India) and other solvents of analytical grade were used.

2.2 Preparation of Nanoparticles (NPs)

Preparation of NPs depends on physicochemical character of polymer and nature of drug to be loaded. In our case both single emulsification and double emulsification methods were used for the preparation of ciprofloxacin loaded NPs. These two different emulsification methods were carried out to compare effects of different variables such as stabilizer (poloxamer, PVA, carbopol), organic phases (acetonitrile, acetone) & concentration of PLGA on Poly Dispersity Index (PI), Mean Particle Size (MPS) & Encapsulation Efficiency (EE) of NPs.

2.2.1 By Single emulsification method

It is one of the frequently used methods carried out in two steps. Step 1 involves emulsification of the polymer solution into an aqueous phase **[12]**. PLGA was precisely weighed and dissolved in 5ml of organic solvent containing drug. This organic phase was poured into the aqueous phase containing a stabilizer which was stirred magnetically (REMI, Mumbai, India) (1800rpm) at room temperature to obtain an o/w emulsion. Step 2 involved break down of emulsion leading to nanospheres precipitation when subjected to high pressure homogenization at 500 bar pressure for 3 cycles to obtained drug loaded PLGA NPs.

2.2.2 By Double emulsification method

PVA (small volume) was added into 5ml of organic polymer solution of PLGA and ciprofloxacin drug under vigorous stirring in a magnetic stirrer (REMI, Mumbai, India) at 1800rpm to form a w/o emulsion. This emulsion was added in outer aqueous phase (large volume) and further subjected to homogenization to form w/o/w emulsion. Then next the resulting emulsion was subjected to dilution and left agitated (700rpm) for next 2 to 4 h for the removal of organic solvent in a magnetic stirrer **[13, 14]**.

2.3 Coating of Nanoparticles

For the development of a potent formulation in dental application which has a prolong residing time with control release, suitable mucoadhesive polymer was used of the coating of nanoparticles. Three coating polymers (chitosan, sodium alginate and poloxamer) were compared with their influence on PLGA nanoparticles.

2.4 Evaluation of Nanoparticles

2.4.1 Morphology

Size of the NPs sample and its surface morphology was investigated by a microscopic technique using Transmission Electron Microscope. TEM was carried out with negative staining of Phosphotungstic acid (PTA) solution (2%w/v). One drop of this aqueous dispersion was placed on a 400 mesh copper grid with carbon film. The excess of nanoparticles were eliminated by the reduction of droplet after 5min using a filter paper. At last, this sample was placed in the TEM after being air dried.

2.4.2 Particle size and Zeta potential

Poly Dispersity Index i.e. measurement of nanoparticles distributed in the given polymer sample and Mean Particle Size (MPS) were determined by using Diffraction Light Scattering (Zetasizernano ZZS, Malvern instruments Inc. Southborough, MA). All the readings were carried out in the series of triplicate.

2.4.3 Drug loading and Entrapment Efficiency

The suspension of nanoparticles was ultra-centrifuged (REMI, Mumbai, India) at 18000rpm for nearly 1hour for the separation of supernatant free drug.

Sediments of NPs were washed two times with distilled water after the removal of supernatant for separating the absorbed drugs. Separated nanoparticles were freeze dried (Meta DRYWINNER, Germany), weighed and their yield was calculated as:

$$\textbf{Nanoparticles Recovery } (\%) = \frac{\textbf{Amount of NPs Recovered}}{\textbf{Amount of } (\textbf{Polymer} + \textbf{Drug} + \textbf{Excipients})} \textbf{ X 100}$$

2.4.4 In vitro drug release study

This study is important to as it affects the bioavailability of the drug thereby affecting its therapeutic efficacy. To study the release of drug, 1ml of sample of nanoparticles was induced in a dialysis membrane bag (MWCO: 12000 g/mol). Further this bag was placed in a glass bottle of 200ml containing 100 ml of 0.1N HCl as dissolution medium agitated in a water bath at 37°c. To maintain sink condition, the exchange of release medium was done and analysis was performed using RP-HPLC method adopted after re-validation to determine the concentration of drug released **[15]**. All readings were carried out in the series of triplicate.

2.4.5 Mucoadhesion test

PLGA nanoparticles were placed in a glass tube of 10ml at 37°C containing phosphate buffer solution almost (pH 7.4) for a period of 5min so that the solution almost covers the NPs. A goat intestinal membrane of diameter 2cm² same as that of glass tube was placed on the surface of wet NPs so that it got covered by them and was allowed to remain there for 5min. Next, the membrane was removed with the attached NPs and the glass tube with remaining NPs was dried at 60°c with constant weight.

The percentage of nanoparticles adhered was calculated using the following formula:

$$\textbf{Adhered Nanoparticles} = \frac{\textbf{Initial weight of NPs} - \textbf{Weight of NPs unattached}}{\textbf{Initial weight of NPs}} \textbf{ X 100}$$

2.4.6 Differential scanning calorimetry (DSC)

DSC (Pyris – 6, Perkin Elmer, Massachuetts, Neitherlands) was used to assess the thermal behavior of individual excipients and to determine the physical state of ciprofloxacin entrapped in the formulation. Sample of individual substance as well as physical mixture and drug loaded NPs was analyzed by DSC. Weighing of dried samples was carried out directly in pans (3-5mg) made of aluminium and scanning was done between 40-300°C with heating rate of 10°C/min in an atmosphere of nitrogen **[16]**.

2.4.7 HET-CAM method for irritation testing

Chorioallantoic membrane is a highly vascularized membrane which surrounds a developed embryo of a chick. It is used for the evaluation of in-vitro toxicity of mucoadhesive coated NPs. The HET-CAM assay is used to determine the reaction of blood vessels to the drug and the damage caused to analyze toxicity levels of chemicals used and that of the formulation **[17]**.

2.4.8 Stability studies

For stability studies, coated and uncoated nanoparticles were stored in either dried form or in suspension form in a place protected from light at room temperature for 90 days. Assessment of particle size was carried out to see the effect of storage time **[18]**.

3. RESULT AND DISCUSSION

3.1 Preparation and Optimization of Placebo and Drug Loaded PLGA Nanoparticles

The prepared empty nanoparticles size was determined in the range of 405.4±8.1nm for all the samples prepared by SE method and 683.2±10.2nm for DE method. NPs loaded with drug showed MPS as 477.6±9.6nm for SE and 454.7±9.5nm for DE as seen in **Table 1(A)**. Drug loaded NPs showed larger dimensions as compared to empty nanoparticles but showed insignificant effect as one tail $p>0.05$. It was clearly demonstrated by the data that larger particles ($p<0.01$) were produced by DE method as compared to particles produced by SE method. PI depicted relatively narrow distribution due to their value less than 1. Zeta potential values observed for both drug loaded and empty nanoparticles were negative ranging from -44.2±0.8mV to -38.8±0.5mV, typically observed in these systems prepared with PLGA **[19]**.

Table 1(B) showed MPS of nanoparticles produced in Acetonitrile with SE 497.2±10.6nm and with DE 740.4±12.1nm was larger than that produced in Acetone solution with MPS of 477.6±10.2nm with SE and 705.2±11.3nm with DE. But DE and SE method did not depict statistically significant effect of organic phase on MPS. As there was efficient extraction of ciprofloxacin to acetone and both the solvents showed similar particle size, acetone was used as an organic phase for further experiments.

High concentrations of stabilizer showed smaller MPS with slow reduction in PI for NPs prepared by both SE and DE method **(Table 2)**. In SE method PVA concentration increment from 0.5% to 1.5% ($p<0.01$) showed a reduction in mean diameter from 532.7 ± 8.5nm to 384.1 ± 11.7nm but a little increment was observed in MPS at 2% concentration. In DE method there was significant reduction in MPS from 777.6 ± 9.3nm to 651.7 ± 9.6nm as the concentration of PVA was increased from 0.5% to 2%. It was observed from the difference of two results that the dominant factor for controlling MPS of the NPs was PVA concentration for both the preparation methods **[20]**. Since higher EE and smaller MPS were shown by nanoparticles using PVA, it was therefore chosen as a stabilizer for final formulation.

A significant decrease in the MPS was observed as the PLGA: drug ratio was increased **(Figure 1)**. Whereas, no NPs were observed at 5:1, PLGA: drug concentration. Instead polymer aggregates were formed for both the methods. Decrease in MPS was observed from 584.7nm to 378.6nm in SE and 785.6nm to 650.7nm in DE when PLGA: drug ratio increased from 7.5:1 to 20:1 **(Figure 1)**. A constant value of PLGA: drug ratio, 15:1 was kept with varying PLGA concentrations from 200mg to 600mg to observe its influence on MPS and EE with a fixed organic phase volume. With increase of PLGA concentration ($p<0.05$) an increase in mean diameter of NPs was observed using both methods **(Figure 2)**. Since the concentration of PVA was not enough to cover the PLGAs increasing concentration, an increase in viscosity of the organic phase was observed with increasing PLGA concentration **[21]**. Further it was observed that EE of NPs was independent of the PLGA concentration.

It was also observed that EE of ciprofloxacin NPs showed an increase with PLGA: drug ratio till a certain limit and then again showed a negative relationship with concentration of PVA **(Figure 3)** and a null relationship with concentration of PLGA. PVA concentration proved to be the major variable for forming ciprofloxacin loaded NPs from the results of multiple linear regression analysis.

After various factors for nano-formulations were optimized, it was determined that MPS, PI and zeta potential results were better yielded by SE method. Acetone was chosen as a reasonable dissolvable frame-work and PVA was utilized as powerful stabilizer. On similar examination of different parameters, SE technique was found to be suitable for the preparation of ciprofloxacin loaded PLGA Nanoparticles and coded as CF-PLGA-NPs **(Table 3)**.

After that nanoparticles prepared by single emulsification technique undergone into homogenization at 500 bars for 3 cycles, the drastic decrease in MPS of 231.5 ± 9.8nm with poly Dispersity index of 0.27 ± 0.03 and zeta potential of -42.5Mv was observed. The EE of these CF-PLGA-NPs was 68.6%.Optimum formulation conditions and their results were mentioned in **Table 4**.

3.2 Coating of PLGA Nanoparticles

Three different coating polymers were characterized for their impact on mean size, zeta potential, entrapment efficiency and nanoparticles recovery. As can be seen from **Table 5**, the nanoparticle recovery was 0%when sodium alginate was utilized as a coating agent. The anionic sodium alginate doesn't communicate with anionic PLGA nanoparticles. In addition, sodium alginate presence increases the viscosity and recovery of nanoparticles in such viscous solution is not possible. The molecule size of the nanoparticles recovered after adsorption with non-ionic poloxamer had a molecule size (238.7 ± 9.5 nm) almost like that of uncoated PLGA-NPs. As there was no shift in zeta potential also, confirmed that these nanoparticles were uncoated indicating the absence of PLGA nanoparticles and poloxamer interaction. Recovery was decreased in the presence of poloxamer may be due to an increase in viscosity.

Rather than sodium alginate and poloxamer, cationic chitosan (CH) was seen as adequately interact or adsorbed on PLGA nanoparticles.CH is a cationic polysaccharide having various – OH and – NH2 groups that makes intra-molecular hydrogen bonds with anionic PLGA. Diffusion of water is restricted due to the formation of CH entangled network like structure. PLGA nanoparticles coated with CH could be made by adding chitosan solution into the external phase with PVA solution.CH coated nanoparticles zeta potential moved to positive side, presumably because of strong interaction between positively charged CH and negatively charged PLGA core **[22]**. After adsorption of CH, an increment in the size of PLGA nanoparticles coated with CH was observed because of the atomic layers of chitosan covered on the outside of PLGA nanoparticles. From our outcomes it appeared that CH was effectively adsorbed on PLGA charged negatively.

3.3 Evaluation of Optimized Nanoparticles

Particle Size and Morphology

A good distribution was indicated by the TEM photos of the PLGA-NPs **(Figure 4)** and a small size of the nanoparticles compared with CH-PLGA-NPs (Fig. 4A). Mean diameter of 220 nm and 278nm was shown for CF-PLGA-NPs and CH-CF-PLGA-NPs respectively by TEM. The lump regions showed the presence of CH coated PLGA nanoparticles **(Figure 4B)**.

In-Vitro Drug Release

A biphasic pattern was observed from the drug release profile of the prepared nanoparticles. The ciprofloxacin release profile from PLGA-NPs is portrayed by an initial rapid release (burst) phase to a steady release phase. The initial burst impact can be ascribed to the spread of drug close to the nanoparticles surface and the steady discharge appeared in the second part due to the presence of ciprofloxacin encapsulated inside nanoparticles. The % drug discharged in beginning of 4 h from CF-PLGA-NPs and from CH-CF-PLGA-NPs was 26.77% and 15.03% respectively, drug release rate after initial burst phase was lower in case of CF-PLGA-NPs compared to CH-CF-PLGA-NPs. Cumulative drug release rate after 28 days was 71.65% for CF-PLGA-NPs and 84.97% for CH-CF-PLGA-NPs **(Figure 5)**. Presence of hydrophilic chitosan at the outside of the PLGA NPs is the reason for cumulative higher release of ciprofloxacin from chitosan coated nanoparticles. Drug diffusion is increased with degradation of PLGA.CF-PLGA-NPs and CH-CF-PLGA-NPs followed the zero-order model (R^2 =0.9961 & 0.9818 respectively) for burst discharge stage during initial 4 hrs as showed by the overall curve fitting of drug release. Korsmeyer–Peppas model best (R^2 =0.9912 & 0.9982 respectively) depicted the sustained release during later 24 hrs with the basic estimation of n being 0.1742-0.1895 recommending Fickian diffusion process. This was further upheld by the fact that the sequential procedure of polymer hydration, solvent penetration, drug dissolution and polymer erosion decide the drug release from the hydrophilic matrices.

Mucoadhesion Studies

CF-PLGA-NPs were seen as non-adhesive and there mucoadhesive quality is zero, this demonstrated their poor pocket retention. Rather than this CH-CF-PLGA-NPs were seen as mucoadhesive (connected NPs wt %,~ 85 %). The mucoadhesive quality of NPs was analyzed for 1 day and it was discovered that CH-CF-PLGA-NPs hold their mucoadhesion for this period. It was seen that mucoadhesive quality declined after 24 hrs and was around 60 % for CH-CF-PLGANPs **(Figure 6)**.

Differential Scanning Calorimetry (DSC)

Two exothermic peaks of melting by pure ciprofloxacin were shown in curve A **(Figure 7)** at approx 154°c and 366°c. On the other hand chitosan shown melting exotherm at 82°c **(Curve B, Figure 7)**. Glass transition state was exhibited by PLGA at 44°c **(Curve C Figure 7)** but not melting points. Superimposition of DSC curves of isolated compounds represented the peaks in DSC curve of the physical mixture **(Curve D Figure 7)**. There was no crystalline material detected in the DSC study of CF loaded CH-CF-PLGA-NPs **(Curve E Figure 7)**. Therefore it was that the formulated nanoparticles of given drug was in amorphous form or in disordered crystalline phase of a molecular dispersion or a solid solution state in the PLGA matrix after fabrication. An interaction between

PLGA and chitosan was observed **(Curve E, Figure 7)** due a glass transition in them. This interaction between chitosan and PLGA confirms their entanglement resulting in efficient and stable coating of chitosan over PLGA.

Irritation Testing

HET CAM studies were used to determine the irritation studies of both CF-PLGA-NPs and CH-CF-PLGA-NPs. HET CAM was chosen because of the sensitive nature of its membrane. The scores acquired for CF-PLGA-NPs and CH-CF-PLGA-NPs were 0.75 and 0.48 respectively. The outcome demonstrates that both the NPs prepared were appropriate for use and non-irritation to mucous membrane. Scores for the isotonic sodium chloride and 0.1N NaOH solution were 0.32 and 10.9 respectively.

Stability Studies

The result from stability studies **(Table 6)** indicates that both coated and uncoated nanoparticles remain stable if stored in form of dried powder. The suspension form shows larger particle size in both coated and uncoated nanoparticles after 3 month which might be due to aggregation of particles in suspension. Hence, it is recommended to store prepared NPs in freeze dried form for long term storage.

Table 1

(A) MPS, PI and zeta potential of PLGA empty and drug loaded nanoparticles prepared by SE and DE at 1% PVA, 2% PLGA (50:50) and PLGA: drug ratio 15:1

Parameter	Single Emulsification		Double Emulsification	
	Empty-NPs	Drug loaded -NPs	Empty-NPs	Drug loaded-NPs
MPS (nm)	405.4± 8.1	477.6±9.6**	683.2±10.2	454.7±9.5
PI	0.69±0.04	0.70±0.07	0.74±0.02	0.76±0.03
Zeta potential (mV)	-38.8±0.5	-42.5±0.2	-41.7±0.5	-44.2±0.8

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

(B) Influence of type of organic solvent on MPS and EE using SE and DE at 1% PVA, 2% PLGA, 15:1 PLGA: drug ratio

Organic solvent	Single Emulsification			Double Emulsification		
	MPS (nm)	PI	EE (%)	MPS (nm)	PI	EE (%)
Acetone	477.6±10.2	0.87±0.08	73.3±0.7**	705.2±11.3	0.93±0.04	62.1±1.2 ##
Acetonitrile	497.2±10.6	0.91±0.09	62.5±0.5	740.4±12.1	1.2±0.08	34.5±0.9

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

Table 2: Effect of different stabilizers on MPS, PI and EE prepared by SE and DE at 2% PLGA, 15:1 PLGA: drug ratio

Stabilizer	Concentration	SE			DE		
		MPS	PI	EE	MPS	PI	EE
PVA	0.5 %	532.7±8.5	0.82±0.04	73.7±0.9	777.6±9.3	0.98±0.05	36.5±0.3
	1.0 %	477.6±9.4 384.1±11.7**	0.76±0.09 0.70±0.08	70.3±0.7 61.2±0.4	725.2±10.1$^{\#}$ 686.4±11.4$^{\#\#}$	0.83±0.03	38.4±0.5
	1.5 %	408.6±10.6*	0.65±0.07	56.4±0.2**	651.7±9.6$^{\#\#}$	0.78±0.02	42.8±0.8
	2.0 %					0.72±0.08	48.5±0.2$^{\#\#}$
Carbopol	0.5 %	1329.2±20.3	1.4±0.07	70.1±0.6	1635.1±24.3	1.9±0.08	52.5±0.4
	1.0 %	1497.9±27.2	1.8±0.06	74.5±0.8	NP	-	-
	1.5 %	NP	-	-	NP	-	-
	2.0 %	NP	-	-	NP	-	-
Poloxamer	0.5 %	602.4±10.1	1.92±0.03	54.5±0.3	998±15.1	2.1±0.04	45.6±0.7
	1.0 %	578.1±10.8	0.83±0.05	43.3±0.2	867±13.5	1.9±0.02	36.7±0.9
	1.5 %	487.5±9.2*	0.76±0.09	38.2±0.7	821±10.6$^{\#}$	1.4±0.06	31.2±0,4$^{\#}$
	2.0 %	267.2±8.61*	0.79±0.04	33.5±0.1**	793±9.3$^{\#}$	1.5±0.01	27.1±0.3$^{\#\#}$

NP- Not prepared. Data are shown as the means±SD. (n=3); **p<0.01, *p<0.05 compared with SE method using 0.5% stabilizer; $^{\#\#\#}$p<0.001, $^{\#\#}$p<0.01, $^{\#}$p<0.05 compared with DE method using 0.5% stabilizer

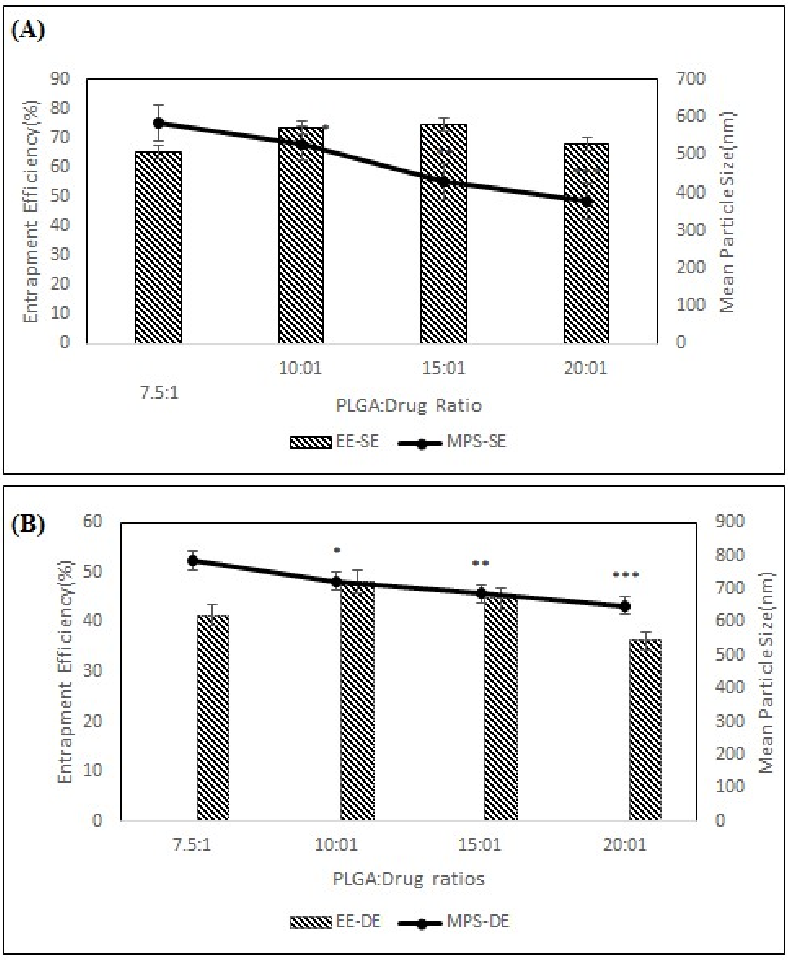

Figure 1: Effect of PLGA: drug ratio on MPS and EE of NPs prepared at 1% PVA, 2% PLGA. Data are shown as the mean±SD. (n=3), (A) by Single emulsification; (B) by Double emulsification method ***p<0.001, **p<0.01, *p<0.05 compare to values of MPS and EE at 7.5:1

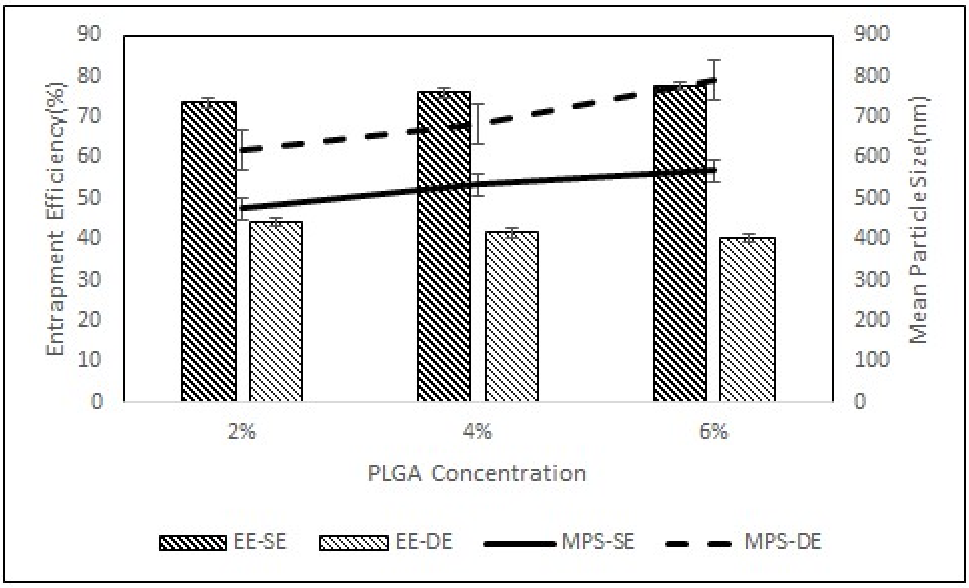

Figure 2: Effect of PLGA concentration on MPS and EE of NPs prepared by SE and DE at 1% PVA and PLGA: drug ratio 15:1. *p<0.05 compare to MPS at 2% using SE, †p<0.05 compared with MPS at 2% using DE

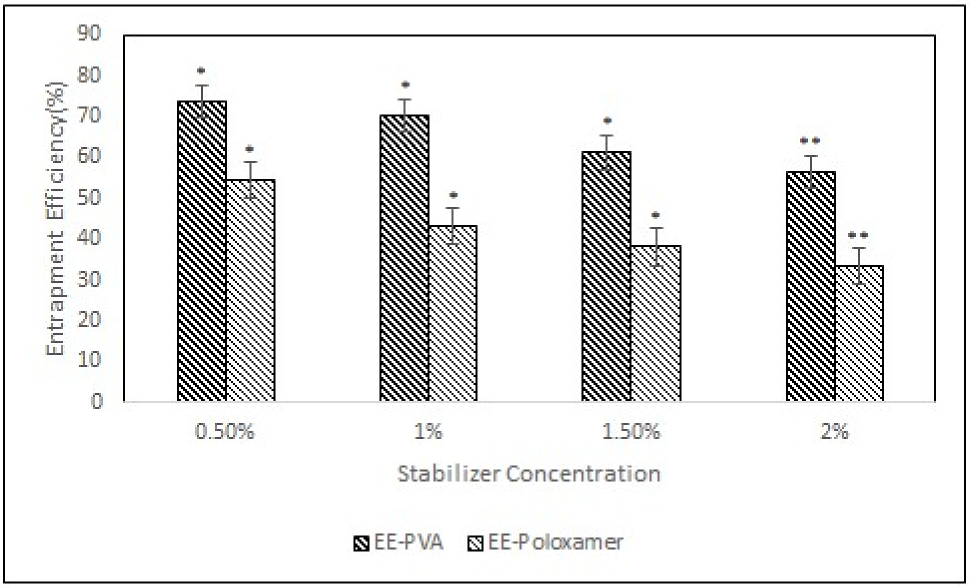

Figure 3: Effect of type of stabilizer and its concentration on EE of PLGA NPs using SE method at 2% PLGA, 15:1 PLGA: drug ratio. Data are shown as the mean±SD. (n=3), significantly different, **p<0.01, *p<0.05

Table 3: Comparison of effect of formulation variables on MPS and EE of NPs prepared by SE and DE methods

Variable	Single		Double	
	MPS (nm)	EE (%)	MPS (nm)	EE (%)
PLGA : Drug concentration				
5:1	X	X	X	X
7.5:1	584.7	65.4	785.6	41.4
10:1	531.6	73.5	724.1	48.1
15:1	430.1	74.7	685.4	44.7
20:1	378.6	68.1	650.7	36.3
PLGA concentration				
2%	476.6	73.5	621.1	44.3
4%	534.3	75.9	684.8	41.7
6%	568.7	77.6	788.4	40.3
8%	X	X	X	X
PVA concentration				
0.05%	531.7	72.9	831.6	41.7
1%	476.6	73.5	724.2	44.2
1.5%	383.1	62.5	740.4	49.5
2%	407.9	66.2	705.7	53.5

Table 4: Optimized formula for preparation of CF loaded PLGA nanoparticles by single emulsification method

Optimization Parameters	Optimized value	Result
Organic solvent	Acetone	Drug loaded CF-PLGA NPs were obtained with drug encapsulation app. 68 %, and mean particle size app. 231 nm
Stabilizer	PVA	
Stabilizer concentration	1%	
PLGA:Drug ratio	15:1	
PLGA concentration	2%	
Homogenisation	500 bars for 3 cycles	

Table 5: Influence of coating polymer on physicochemical properties of PLGA-NPs

Coating polymer	Nanoparticle recovery (%)	Particle size (nm)	Zeta potential(mV)	Entrapment efficiency (%)
No polymer	**68.2**	**231.5±9.8**	**-42.5**	**68.6**
Chitosan	**80.4**	**294.2±10.1**	**+35.2**	**73.4**
Sodium alginate	**0**	**-**	**-**	**-**
Poloxamer	**20.3**	**238.7±9.5**	**-40.1**	**-**

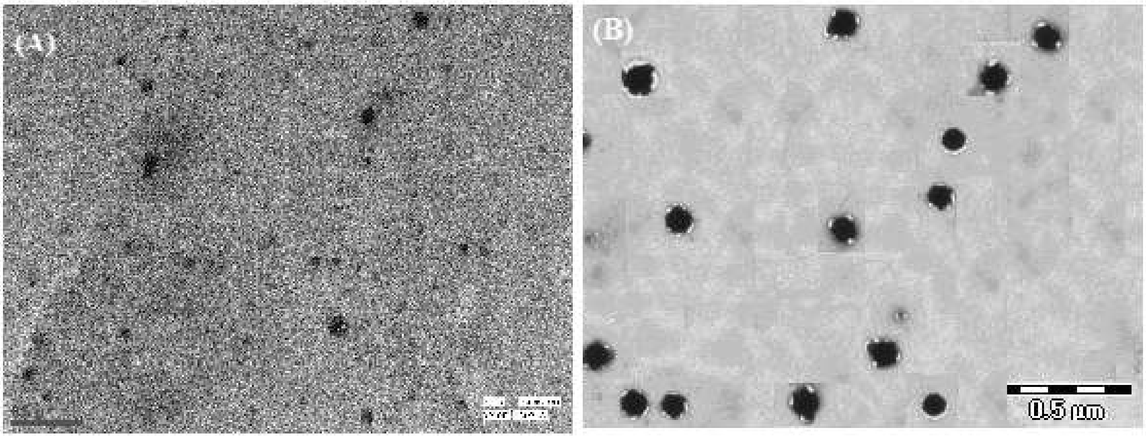

Figure 4: Transmission electron micrograph of (A) CF-PLGA-NPs; (B) CH-CF-PLGA-NPs

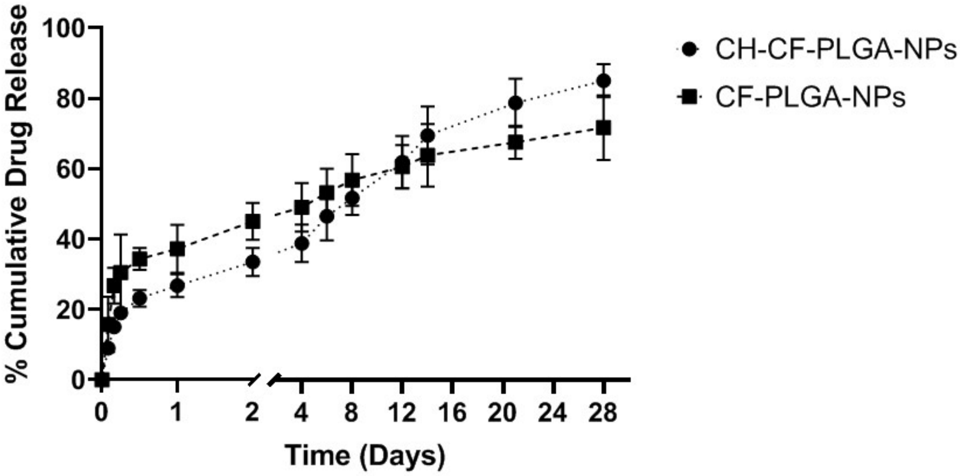

Figure 5: % Cumulative CF release profiles of CF-PLGA-NPs and CH-CF-PLGA-NPs

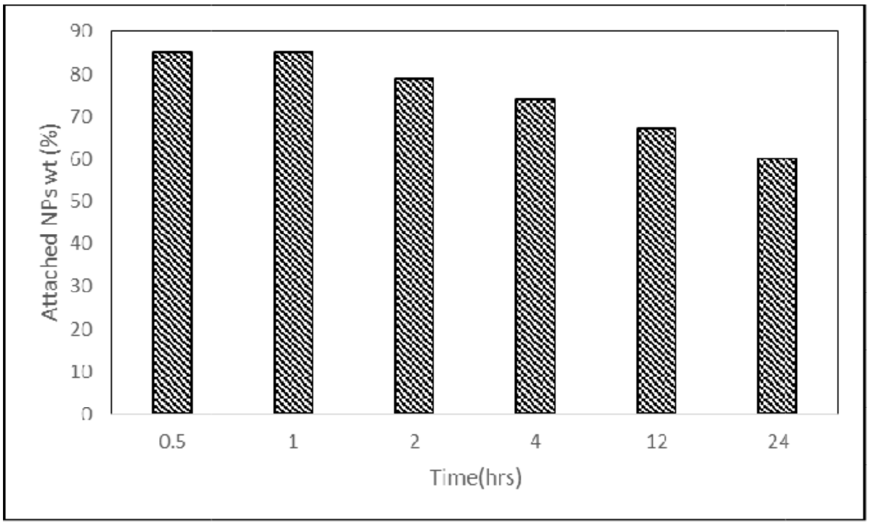

Figure 6: Bioadhesive strength of CH-CF-PLGA-NPs for over 24 hrs

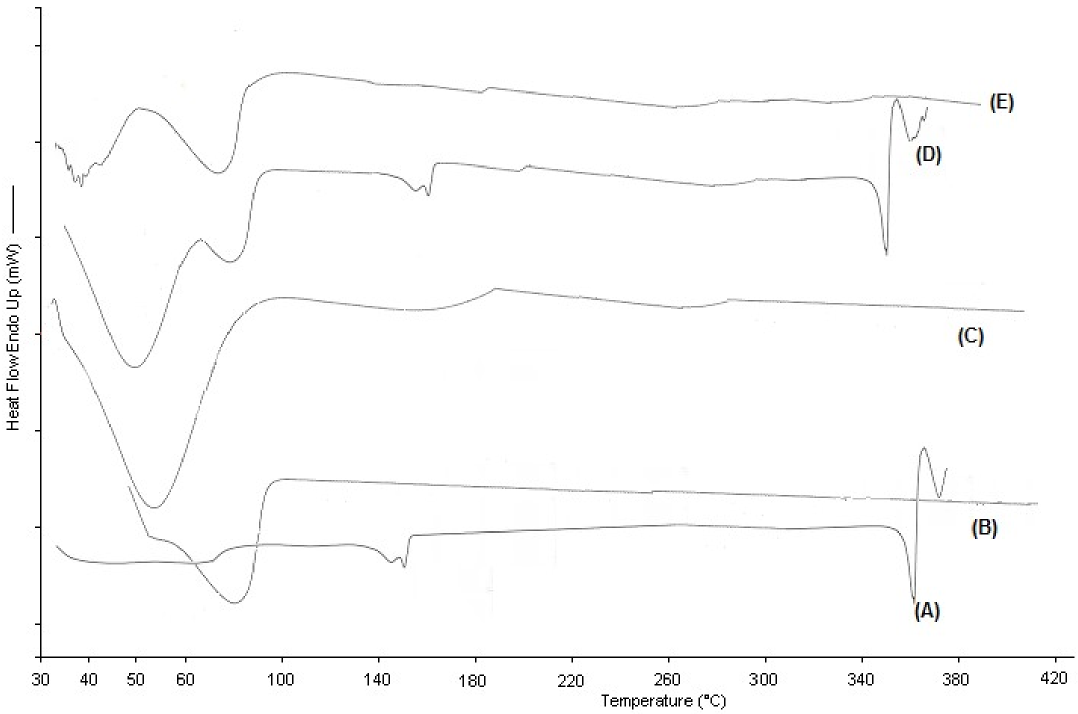

Figure 7: DSC curves of (A) CF (B) CH (C) PLGA (D) Physical mixture, and (E) CH-CF-PLGA-NPs

Table 6: Stability of drug loaded NPs and chitosan coated NPs formulations at room temperature

Storage Form	Particle size (nm)	
	Initial	After 3 months
Powder form		
CF-PLGA-NPs	231.5±9.8	239.7±9.5
CH-CF-PLGA-NPs	294.2±10.1	298.3±9.6
Suspension form		
CF-PLGA-NPs	231.5±9.8	282.8±7.5
CH-CF-PLGA-NPs	294.2±10.1	386.1±9.2

4. CONCLUSION

For successful treatment of periodontitis, a formulation is required with a prolonged stay at required site, and for this the formulation must stay intact in the periodontal pocket. Results from the tests uncover that chitosan covered ciprofloxacin PLGA nanoparticles given more noteworthy mucoadhesion and had much better drug release design when compared with uncoated nanoparticles. The lower in-vitro toxicity of the created CH-CF-PLGA-NPs when contrasted with the positive control recommends the appropriateness of these nanoparticles for intra-pocket delivery. The developed surface improved nanoparticulate framework acted as a good antimicrobial carrier in place of scaling and other methods for treating periodontitis.

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6. AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist in publication of this manuscript.

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