ORIGINAL ARTICLE

Development and evaluation of biodegradable microspheres embedded in *in situ* gel for controlled delivery of hydrophilic drug for treating oral infections: *In vitro* and *in vivo* studies

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Present investigation was aimed at developing biodegradable polymeric microspheres of Tetracycline hydrochloride to treat oral infections by using Poly (D, L lactic-co-glycolic) acid (50:50) as polymer. Microspheres were prepared using oil-in-oil (O/O) and water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method. Microspheres prepared by W/O/W were spherical in shape compared those prepared with O/O method. Thus, the microspheres formulated by W/O/W method were further evaluated for particle size, morphology, entrapment efficiency, and percent drug release. Effects of salt addition, polymer concentration on the characteristics of microspheres and tetracycline release profile were investigated. An increase in polymer concentration decreased drug release and increased entrapment efficiency of drug. *In vitro* studies indicated that release of drug from microspheres could be controlled for 10-15 days depending on drug: Polymer concentration. Formulation E released 99.10% of drug from microspheres in 10 days. Addition of sodium chloride to outer aqueous phase produced spherical microspheres with smooth surface and also increased entrapment efficiency. Microspheres were further dispersed in optimized formulation of mucoadhesive *in situ* gel of Pluronic F127, which acts as carrier for microspheres. *In vivo* studies were conducted on patients who underwent molar tooth extraction to check efficacy of designed formulation.

Key words: *Double emulsion, in situ gel, microspheres, molar tooth extraction, poly (D, L lactic-co-glycolic) acid, tetracycline hydrochloride*

INTRODUCTION

Oral diseases are major health problem in all parts of the world. Oral hygiene is defined as scientific care of teeth and mouth There are more than 250 different types of diseases that affect oral cavity.[1] Effectiveness of dental products is limited due to lack of site specificity, high drug dose leading to increased side-effects, drug resistance, drug dilution through systemic route, patient noncompliance since increase in frequency of dose administration. Thus, local drug delivery using biodegradable polymers help to maintain a therapeutic concentration of drug at the affected site for prolong period with reduced dose and thus reduced side-effects, improved patient compliance and can be administered in unit dose.[2] Tetracycline is widely used for treatment of oral infections to control microbial growth. Tetracycline hydrochloride (HCl) is a broad spectrum antibiotic. It is bacteriostatic and inhibits bacterial protein synthesis by binding to the 30S bacterial ribosome and preventing access of aminoacyl transfer ribonucleic acid (RNA) to the acceptor site on the messenger RNA-ribosome complex. It is active against oral pathogens such as

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Streptococcus mutans, Treponema denticola, Actinomyces viscosus, Porphyromonas gingivalis.[3]

Aliphatic polyesters like poly (D, L lactic-co-glycolic) acid (PLGA) are widely used to formulate controlled release devices. PLGA provides a controlled drug release from months to years depending on the type of polymer grade used.[4] Furthermore, PLGA supports in cell attachment and cell proliferation in case of wound healing mainly after tooth extraction and thus reduce duration of wound healing.[5]

For hydrophilic drugs double emulsion solvent evaporation method is commonly used in which drug is dissolved in aqueous phase and entrapped into organic phase consisting of polymer.[6] Tetracycline HCl is a hydrophilic drug hence various formulations of microspheres were formulated using water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method. Similar methods have been reported for encapsulating water soluble drugs.[7] The effect of polymer concentration and salt addition have been studied. *In vitro* drug release was studied using thermostatic incubator shaker for tetracycline loaded microspheres, pure tetracycline HCl dispersed in *in situ* gel and tetracycline HCl loaded microspheres dispersed in *in situ* gel using distilled water as media. Different drug: Polymer ratios were designed and formulation which provided drug release for 10 days was optimized and efficacy was checked on patients who underwent mandibular molar tooth extraction. Currently, only conventional methods like systemic antibiotics, local antibiotic gels are being widely used to prevent post extraction microbial infection. The optimized formulation of tetracycline HCl loaded microspheres dispersed in *in situ* gel were delivered in patients who underwent mandibular molar tooth extraction and parameters such as wound healing, irritation and inflammation at extracted tooth site, patient compliance were studied for 7 days.

MATERIALS AND METHODS

Materials

Tetracycline HCl was kindly received as a gift sample from Hindustan Antibiotics, Pune, India. PLGA 50:50 (RESOMER RG 504) (M_w = 45,000) was received as a gift sample from Evonik Degussa India Pvt. Limited, India. Poloxamer 408 was received as a gift sample from BASF, Navi Mumbai, India. Hydroxyl propyl methyl cellulose (HPMC) K4M and Carbopol 974P were received as gift sample from Colorcon Asia Pvt. Limited, Goa, India and Lubrizol, India respectively. Analytical reagent grade samples of Acetone, light liquid paraffin, magnesium stearate, hexane, dichloromethane (DCM), sodium chloride (NaCl), poly (vinyl alcohol) (M_w = 40,000), triethanolamine, methylparaben sodium, propylparaben sodium were purchased from Research Lab Fine Chem Industries, Mumbai. Dialysis membrane 110 was purchased from Hi-media laboratories Pvt. Limited Mumbai. Distilled water was used throughout the process.

Methods

Preformulation studies

Compatibility studies of tertracycline hydrochloride with all the excipients used in the study was carried out using Fourier transform infrared spectral studies and Differential calorimetric analysis of plain drug and physical mixture of drug and excipients.

Preparation and characterization of microspheres loaded with tetracycline hydrochloride preparation of microspheres

Tetracycline HCl is a hydrophilic drug, hence microspheres were prepared using oil-in-oil (O/O) and W/O/W emulsion solvent evaporation method with few alterations in both the methods.[8] Single emulsion solvent evaporation method (O/O)[9,10] was carried out as follows:

Weighed amount of PLGA was dissolved in 3 ml Acetone. Further 20 mg of tetracycline HCl and 20 mg of magnesium stearate were dispersed in the polymer solution under stirring. Magnesium stearate helps to prevent the aggregation of microspheres. This drug containing polymer dispersion (internal phase) was then added 25 ml of light liquid paraffin containing 6 ml of Hexane under stirring. Stirring was continued at 800 rpm under mechanical stirrer. On hardening of microspheres the microspheres were washed with hexane to remove the surface adhered paraffin. Further microspheres were filtered and air dried.

For W/O/W double emulsion solvent evaporation method[11] tetracycline HCl and PLGA were used in different ratios. Tetracycline (100mg) was dissolved in 2 ml of distilled water. PLGA was dissolved in DCM to form oil phase. Aqueous solution of tetracycline was then emulsified with oil phase using probe sonicator (Oscar Ultrasonics, SONAPROS PR-250 MP) for 5 min. to form W/O phase. This W/O phase was then added to 100 ml of 1% aqueous solution containing 2% NaCl and homogenized at 4500-5000 rpm using high speed homogenizer (Remi motors) for 5 min. to form a stable emulsion. To this emulsion 50 ml of distilled water was added and further stirring was continued for 3 h at 600 rpm using mechanical stirrer (Remi motors). Stirring was continued till DCM evaporated. Later microspheres were separated by filtration. Further the microspheres were washed with distilled water to remove unentrapped drug. Microspheres were dried at room temperature. Various formulations of microspheres are shown in Table 1 with their respective formulation codes.

Characterization of tetracycline loaded microspheres

Encapsulation efficiency

To determine entrapment of tetracycline in microspheres, 25 mg of microspheres were dissolved in 5 ml of DCM. Appropriate aliquots were removed from this solution and transferred to 10 ml volumetric flask and volume was made up to mark with DCM. This solution was analyzed for tetracycline content using ultraviolet (UV) visible

spectrophotometer (Shimadzu V-630) at λ_{max} value of 367 nm. The percent encapsulation efficiency was calculated as:

Percentage encapsulation efficiency = Actual drug loading/ theoretical drug loading × 100.

Percentage yield

The percentage yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres.Percentage yield was calculated as:

Percentage yield = [Weight of dried microspheres/weight of drug used + weight of polymer] ×100

Particle size measurement

Particle size was measured using optical microscope (Motic BA210 Digital). Microspheres were dispersed in distilled water and then placed on a glass slide and covered with cover slip. Microspheres were observed under ×40. Particle size was determined for 100 particles for each batch.

Shape and surface morphology

The shape and surface morphology of tetracycline HCl microspheres were examined by Scanning Electron Microscope (SEM) (JEOL 5400, Japan) and optical microscope (Motic BA210, Digital). SEM images of microspheres were taken by coating the microspheres with gold to make them conductive and placed on a copper stub. For optical microscopy, the microspheres were dispersed in water by sonication and placed on glass slide and observed under magnification of ×40.

Table 1: Formulation of microspheres

Trial	Drug: Polymer	Water (ml)	DCM (ml)	PVA (%)	NaCl (%)
A	1:1	2	4	1	-
B	1:3	1	4	1	-
C	1:4	1	4	1	-
D	1:5	2	8	1	-
E	1:5	2	8	1	2
F	1:7	2	8	1	2
G	1:10	2	8	1	2
H	1:15	2	8	1	2
I	1:20	2	8	1	2

DCM: Dichloromethane, PVA: Poly (vinyl alcohol), NaCl: Sodium chloride

In vitro drug release

Tetracycline loaded microspheres were suspended in 5 ml distilled water and placed in a dialysis bag (previously soaked for 60 min. in distilled water). The dialysis bag was kept in conical flask containing 60 ml of distilled water as dissolution media. The flask was kept in thermostatic incubator shaker (Chromous Biotech, India) and was shaken at 100 rpm and temperature was maintained at 37° ± 0.5°C. The amount of drug released was determined by withdrawing each time 10 ml aliquots at the selected specific time intervals. The volume withdrawn was replenished with an equal volume of distilled water. Samples were analyzed by UV visible spectrophotometer (Shimadzu V-630) at the λ_{max} value of 275 nm using distilled water as blank.

Preparation and characterization of in situ gel

Preparation of *in situ* gel

Weighed amount of Pluronic F-127 was slowly added under continuous stirring to distilled water whose temperature was maintained at 4 ± 1°C throughout the preparation. Mucoadhesive polymers like HPMC K4M or Carbopol 934P and preservatives methylparaben sodium and propylparaben sodium were added to Pluronic dispersion with stirring. The dispersion was stored in the refrigerator overnight until a clear solution was obtained.[12,13] Various formulations of *in situ* gel are shown in Table 2 with their respective formulation code.

*Each trial contain 0.1% Methyl paraben and 0.01% Propyl paraben as preservatives

pH of gel

The pH of gel should be such that there should be no irritation to the buccal mucosa. The pH of gel was measured with digital pH meter (systronics). Twenty percent gel was prepared and tip of pH meter was dipped in the gel and pH was read on the indicator screen.

Thermogelling properties of gel

Gelation temperature was determined by tube inversion method.[14] Aqueous solution of gel (18% and 20%) was prepared in distilled water. One l of solution was transferred to glass test tube and immersed in water bath whose temperature was maintained at 4C and test tube was sealed with aluminum foil. The temperature of water bath was increased in increments of 1°C. Conversion of solution to

Table 2: Formulations of *in situ* gel

Trial	Pluronic F127 (%)	HPMC K4M (%)	Carbopol 974P (%)	Methylparaben sodium (%)	Propylparaben sodium (%)
G1	18	-	-	0.1	0.01
G2	20	-	-	0.1	0.01
G3	18	-	0.25	0.1	0.01
G4	18	-	0.1	0.1	0.01
G5	18	0.25	-	0.1	0.01
G6	18	0.1	-	0.1	0.01

HPMC: Hydroxypropyl methycellulose

gel was confirmed when there was no flow of solution on inversion of test tube.

Gelation time

The gelation was checked visually by noting the time required for gelation of liquid at 37 ± 1°C.

Viscosity

The viscosity of *in situ* gel was measured by using Brookfield digital viscometer (Brookfield DV II + Pro). 18% and 20% of gel was prepared and its viscosity was measured at 37 ± 1°C with spindle number 64 at 50 rpm.

Mucoadhesive strength

An important feature of formulations, designed for implantation into the oral cavity, is the ability to exhibit retention within the oral cavity. Assessment of the mucoadhesive strength is determined in terms of detachment stress that is stress required to detach the gel from the mucosal membrane. Porcine buccal mucosa was used as biological membrane to determine mucoadhesive strength of gel. The apparatus consisted of a rubber cork to hold the gel, a glass container in which glass block was placed to which porcine buccal mucosa was attached, a container for addition of water. The rubber cork and container for addition of water was tarred. Gel was attached to the cork with help of double sided tape. Porcine buccal mucosa was attached to the glass block using double sided tape. The glass block was lowered in the glass container, which was filled with Krebs physiological solution just to keep the tissue wet during the study. Pressure was applied to rubber cork for specified time for attachment of gel to mucosa. To the rubber cork a thread was tied and was passed through a pulley whose other end was attached to a container. Water was added drop wise to the container until gel got detached from the mucosa. Weight of water was taken as mucoadhesive strength of formulation. Mucoadhesive strength was calculated as:

Mucoadhesive strength (Newton/m^2) = Mass applied (kg) × acceleration due to gravity/surface area

In vitro drug release studies

The release rate of plain tetracycline HCl from *in situ* gel was determined using USP dissolution testing apparatus II (Paddle type). Pure tetracycline HCl was dispersed in *in situ* gel and placed in dialysis bag (previously soaked for 60 min in distilled water) and was tied to the paddle. The dissolution test was performed using 150 ml of distilled water at 37 ± 0.5°C and 100 rpm. The amount of drug released was determined by withdrawing each time 10 ml aliquots at the selected specific time intervals. The volume withdrawn was replenished with an equal volume of distilled water. Samples were analyzed by UV visible spectrophotometer (Shimadzu V-630) at the λ_{max} value of 275 nm using distilled water as blank.

Preparation and characterization of combination delivery system of tetracycline hydrochloride loaded microspheres dispersed in Pluronic F127 in situ gel

Preparation of combination delivery system

Weighed amount of microspheres were taken. In a beaker *in situ* gel was prepared and temperature was maintained at 5 ± 0.5°C. Further, the microspheres/gel combination delivery system was obtained by adding microspheres under stirring to the *in situ* gel solution. The combination delivery system prepared in this way was a solution containing suspended microspheres. As the temperature reached 37 ± 0.5°C the solution containing microspheres turned into gel.

In vitro drug release studies

Tetracycline microspheres dispersed in *in situ* gel were placed in a dialysis bag (previously soaked for 60 min in distilled water). The dialysis bag was kept in conical flask containing 60 ml of distilled water as dissolution media. The flask was placed in thermostatic incubator shaker (Chromous Biotech) and shaken at 100 rpm with temperature maintained at 37 ± 0.5°C. The amount of drug released was determined by withdrawing each time 10 ml aliquots at the selected specific time intervals. The volume withdrawn was replenished with an equal volume of distilled water. Samples were analyzed by UV visible (Shimadzu V-630) spectrophotometer at the λ_{max} value of 275 nm using distilled water as blank.

In vivo study

In vivo study was carried out to evaluate the efficacy of *in situ* gel containing tetracycline HCl loaded microspheres on patients who underwent mandibular molar tooth extraction. The study was carried out at Sinhgad Dental College and Hospital, Vadgaon, Pune with prior approval of Institutional Human Ethical Committee. The principles of the Helsinki Declaration were followed and informed consent was obtained from patients before conducting the trial. Patients who underwent mandibular molar tooth extraction in the Department of Oral and Maxillofacial Surgery of Sinhgad Dental College and Hospital, Pune were enrolled in the study. Male and female patients with age group from 18 to 50 years who underwent mandibular molar tooth extraction were selected for the study. Patients with any systemic disease or disorder, patients having any allergic reaction or sensitivity to tetracycline HCl, pregnant and lactating women were excluded from the study.

After obtaining approval from Human Ethical Committee and as per the protocol the patients were divided in two groups with four patients in each group. Group I acted as control group which received standard treatment of analgesics and antibiotics systemically and group II acted as test group which received systemic treatment of analgesics along with local antibiotic that is *in situ* gel containing tetracycline HCl microspheres.

Clinical procedure

Following tooth extraction bleeding was controlled and then with the help of a sterile prefilled syringe, which contained

0.5 ml of *in situ* gel containing tetracycline HCl microspheres was placed in the tooth socket, which would deliver a dose of 12.7 mg of tetracycline HCl locally in patients of test group [Figures 1 and 2]. The socket was sutured and then a sterile gauze swab was placed on the sutured tooth socket. Patients were given postoperative instructions. The patients were recalled again on the day after tooth extraction to check complications if any and again after 7 days for suture removal. The study duration was for 7 days. The various parameters, which were evaluated during the study by investigators were local irritation, feel of the gel, ease of application, dose administration, inflammation, and wound healing. All parameters were assessed and reported on the day after tooth extraction and on the 7th day during suture removal.

RESULTS AND DISCUSSION

Preformulation studies

Fourier transform infrared study

Fourier transform infrared spectra of pure tetracycline HCl and drug-excipients physical mixture of tetracycline HCl with PLGA, Pluronic F127, and HPMC K4M which were used together in formulation of combination delivery system are displayed in Figures 3a and b. There is no characteristic difference in the wavelength of peaks obtained for pure drug and that for drug-excipients physical mixture. This indicated that there is no strong interaction between drug and different polymers used for formation of combination delivery system.

Differential scanning calorimetry studies

The DSC thermogram of plain tetracycline HCl and drug-excipients physical mixture of tetracycline with PLGA, Pluronic F127, and HPMC K4M which were used together in formulation of combination delivery system are displayed in Figures 4a and b. Tetracycline HCl exhibited a single sharp exothermic peak at 251.26°C. This peak was also observed in the thermogram of the drug-excipients physical mixture, but slightly shifted by 2-3C towards higher temperature. This indicated that there is no strong interaction between drug and different polymers used for formation of combination delivery system.

Figure 1: Prefilled syringe containing tetracycline microspheres dispersed in *in situ* gel

Microspheres prepared by oil-in-oil (o/o) method

The microspheres were irregular in shape. Drug remained in dispersed state in polymer solution as tetracycline HCl is insoluble in acetone, this can lead to decreased entrapment efficiency. Thus, based on this observation double emulsion solvent evaporation method was further tried.

Microspheres prepared by (w/o/w) double emulsion - solvent evaporation method

Percentage yield and particle size

Table 3 shows percent yield and particle size for formulations A to I.

Formulation F showed highest yield. The percent yield was good for all formulations. Loss occurred mainly during addition of primary emulsion to external aqueous PVA solution for formation of secondary emulsion. The particle size of formulations from A to I ranged from 15 µm to 22 µm when measured using optical microscope (Motic BA210, Digital). Particle size was small enough to place the microspheres in the oral cavity. Also, within each batch range of sizes were observed.

Effect of salt addition on surface morphology

Figures 5a and b and Figures 6a and b shows surface morphology of microspheres formulated without salt addition and with salt addition, respectively. Microspheres formulated without salt addition produced microspheres with pores on surface (formulation D). On the contrary, addition of 2% NaCl showed spherical microspheres with smooth surface (formulation E). Addition of NaCl produced less pores on surface of microspheres. NaCl acts as an osmogen which helps to maintain the osmotic pressure between the two phases thus decreasing the influx of water into internal phase (dispersed phase) and thus reducing surface pores. Similar results were reported in literature.[7,15]

Effects of polymer concentration and salt addition on entrapment efficiency

The influence of polymer concentration and salt addition on entrapment efficiency is shown in Table 4. At low

Figure 2: Placement of microspheres into extracted molar tooth socket with help of sterile syringe

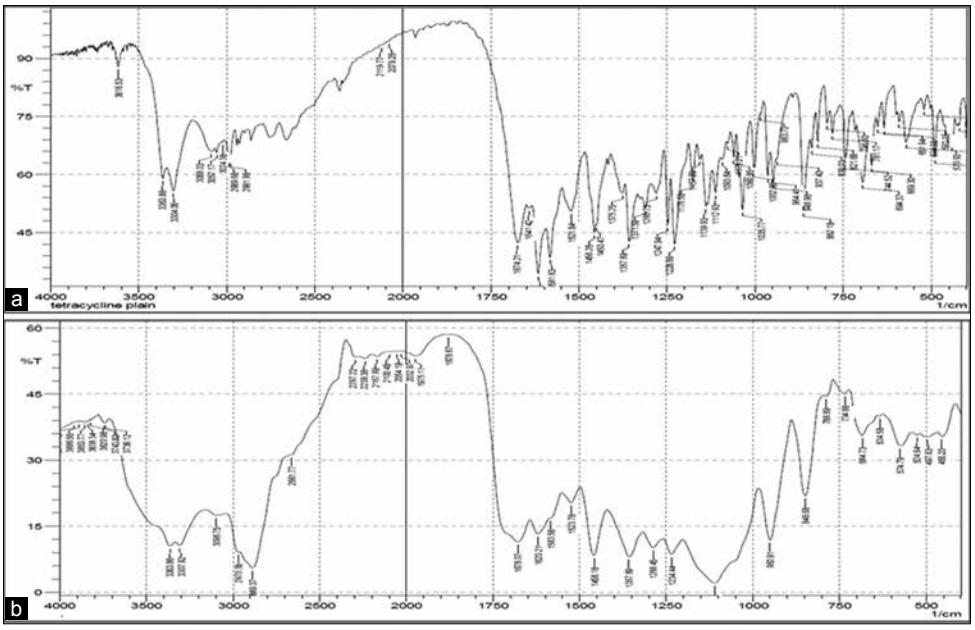

Figure 3: (a) Fourier transform infrared spectra of pure tetracycline hydrochloride (b) Fourier transform infrared spectra of drug-excipients physical mixture

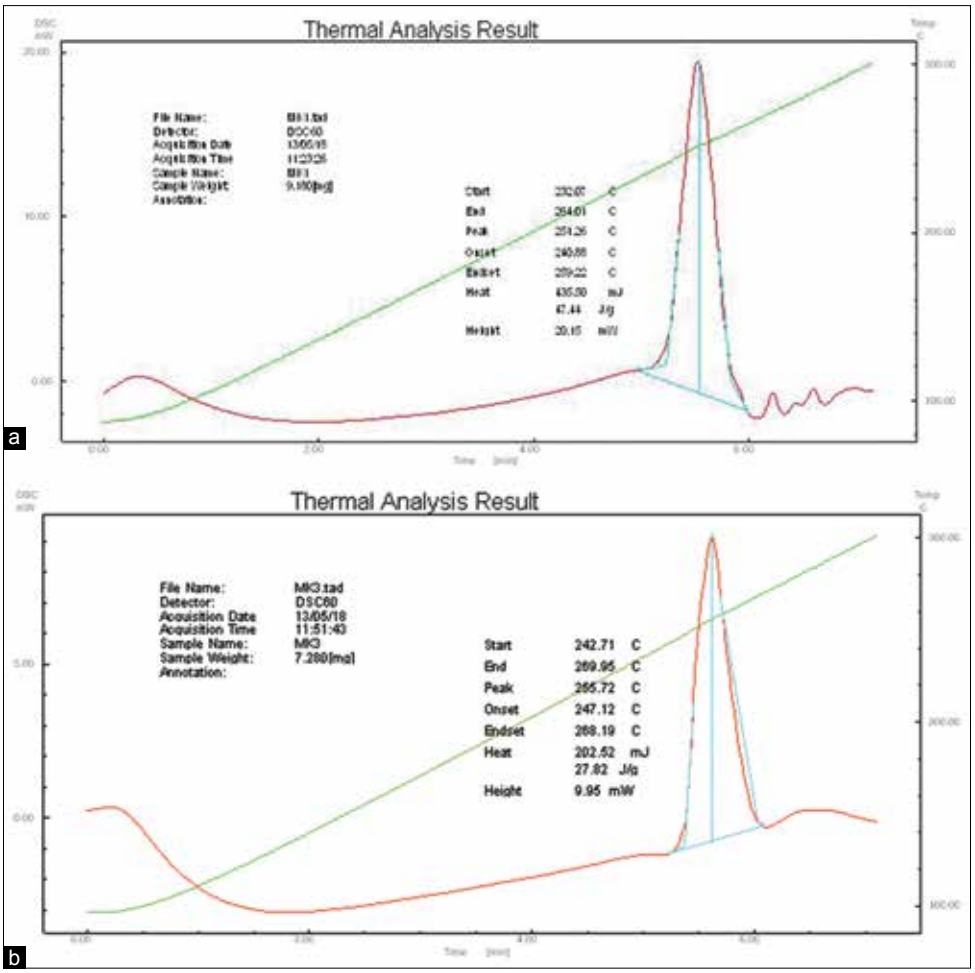

Figure 4: (a) Differential scanning calorimeter thermogram of plain tetracycline hydrochloride, (b) Differential scanning calorimeter thermogram of drug-excipients physical mixture

Table 3: Results for percentage yield and particle size of microspheres

Trial	A	B	C	D	E	F	G	H	I
Drug:Polymer	1:1	1:3	1:4	1:5	1:5	1:7	1:10	1:15	1:20
% yield	42.00	44.70	52.80	71.27	84.54	70.35	70.67	68.66	72.21
Particle size (μm)	12.85±3.2	21.51±2.01	23.25±3.21	20.56±4.04	15.46±2.35	19.25±2.33	20.56±4.04	22.70±3.51	25.51±4.04

Table 4: Effects of polymer concentration and salt addition on entrapment efficiency of microspheres

Trial	A	B	C	D	E	F	G	H	I
Drug:Polymer	1:1	1:3	1:4	1:5	1:5	1:7	1:10	1:15	1:20
NaCl (%)	-	-	-	-	2	2	2	2	2
EE (%)	6.85	11.86	13.80	23.50	31.36	34.33	43.65	52.69	62.66

NaCl: Sodium chloride, EE: Encapsulation efficiency

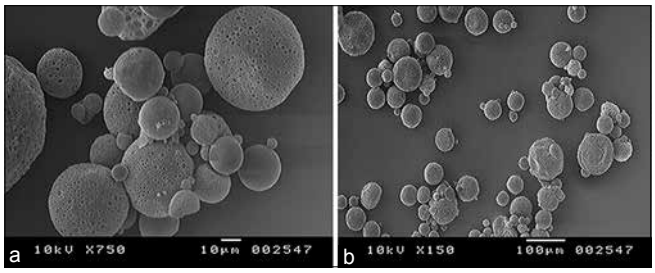

Figure 5: (a) Scanning electron microscope of formulation D, (b) Scanning electron microscope of formulation D

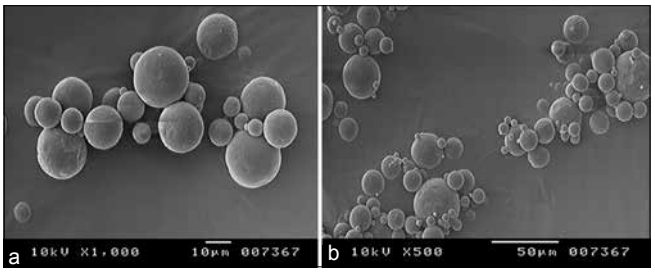

Figure 6: (a) Scanning electron microscope of formulation E, (b) Scanning electron microscope of formulation E

polymer concentration entrapment efficiency was found to be low (formulation A-C). Entrapment efficiency of formulation I was found to be highest due to high polymer concentration, which forms a barrier against leaching of drug from internal aqueous phase to external aqueous phase. Entrapment efficiency also increased on addition of NaCl which mainly acts as osmogen. It maintains the osmolarity between internal aqueous phase and external aqueous PVA phase to prevent migration of drug from internal aqueous phase to external aqueous phase thus leading to increased entrapment efficiency. Similar results have been reported earlier in literatures that increase in polymer concentration increases entrapment efficiency.[16,17] Based on entrapment efficiency and surface morphology trial E-I were further optimized for *in vitro* drug release studies.

The influence of polymer concentration on drug release

As shown in Table 5, increased polymer concentration leads to formation of dense polymer matrix in microspheres and hence takes more time for erosion of polymer matrix leading to delayed drug release (formulation I). No significant difference in drug release rates from trial E and F were observed. Based on release obtained formulation E was selected as optimized formulation, which released 99.10% drug in 10 days and was selected for further study.

Hence, depending on type of oral infection and treatment regimen different trials of microspheres formulated in this study can be used. For instance, if a person is suffering from periodontal infection and is in chronic stage where the treatment regimen is for 1 month microspheres formulated with trial I can be used where drug release can be expected to be for 30 days.

Figure 7 shows comparative dissolution or drug release from formulations E-I.

In situ gel preparation and evaluation

pH

pH of all trials was well within neutral pH range. Trial G3 and G4 showed pH towards more acidic range due to acidic nature of polymer. Trial G5 and G6 showed pH towards neutral range, which indicated that trial G5 and G6 would be non-irritant to buccal cavity.

Gelation temperature

Poloxamer have the property to form thermoreversible gels. Mechanism of gel formation at higher temperature is mainly due to micelle formation. Micelle formation occurs at critical micellization temperature and on increasing temperature the micelles come into contact and no longer move and thus form gel. The gelation temperature of trial G1 was found to be 34°C which is near body temperature than trial G2. This can be due to increase in number and volume occupied by micelles. Thus, G1 was selected for poloxamer concentration for further trials as gelation temperature was near to body temperature. Furthermore, higher concentrations of poloxamer produce more viscous preparations and thus further sustain the drug release. Thus trial G1 would not provide a higher sustained drug release compared with trial G2. On addition of mucoadhesive polymers to poloxamer, gelation temperature was lowered,

which can be due to the effect of mucoadhesive polymers on formation of micelles.

Gelation time

The gelation time was recorded visually as the time required for conversion of sol to gel at 37° ±1C. The time required for trial G2 to form gel was less than that required for trial G1. Not much difference was observed in the gelation time of all trials.

Viscosity

Viscosities were measured by using Brookfield viscometer (DV II + Pro) spindle number 64 at 50 rpm. Viscosity of trial G2 was more compared with trial G1 mainly due to polymer concentration. Not much difference was observed between the viscosities of trial G3 and G4, trial G5 and G6. Viscosity of trials from G3 to G6 was more than trial G1 due to addition of mucoadhesive polymers. Viscosity of *in situ* gel should be optimum since highly viscous solutions can cause problem during administration.

Mucoadhesive strength

Hydrogen bonding is one of the major parts to determine mucoadhesion. Carboxylic group has the ability to provide hydrogen bonding. Carbopol has a very high percentage (58 68%) of carboxylic groups that gradually undergo hydrogen bonding with mucus membrane, resulting in formation of strong mucoadhesive bond between polymer and mucus membrane. Trial G3 and G4 showed good mucoadhesive strength compared to G5 and G6 due to Carbopol 974P.

Trial G3 showed good mucoadhesion and even gelation temperature near to body temperature. However, pH was more inclined towards acidic range and thus pH was required to be adjusted using triethanolamine. Since triethanolamine can cause skin irritation and can be harmful if absorbed through skin. There are chances that it can cause local irritation to buccal mucosa, which is already inflamed due to oral infection.

From the characteristics of *insitu* gel seen in Table 6, trial G5 was selected as optimized batch and was further selected for *in vitro* drug release study.

In vitro drug release of pure tetracycline from in situ gel

Figure 8 demonstrates the dissolution profile of pure tetracycline HCl dispersed in *in situ* gel. It was observed that *in situ* gel released 99.98% of drug in 8 h. Thus, it was observed that almost 100% of drug is released in 8 h and hence only dispersing drug in *in situ* gel did not provided a sustained drug release for sufficient period of time as per the need of study.

Evaluation of combination delivery system

Optimized microspheres formulation and optimized *in situ* gel formulation were selected for combination delivery. *In vitro* drug release was carried out for microspheres of formulation trial E which were dispersed in *in situ* gel of formulation G5 and further evaluated for *in vitro* drug release.

Table 5: Effect of polymer concentration on drug release from microspheres

Trial	E	F	G	H	I
Drug:Polymer	1:5	1:7	1:10	1:15	1:20
Drug release (%)	99.10	96.55	75.42	67.21	16.83
Time (days)	10	10	14	14	5

Table 6: Results for characteristics of *in situ* gel

Formulation	Gelation temperature (°C)	pH	Gelation temperature (s)	Viscosity (cps)	Mucoadhesive strength (newton/m²)
G1	34±1	6.5	180	1251.22	-
G2	29±2	6.6	240	1345.21	-
G3	32±1	5.1	295	1556.20	890.90
G4	30±1	5.3	300	1559.33	809.91
G5	33±2	6.2	220	1449.55	668.42
G6	32±1	6.4	210	1439.56	607.43

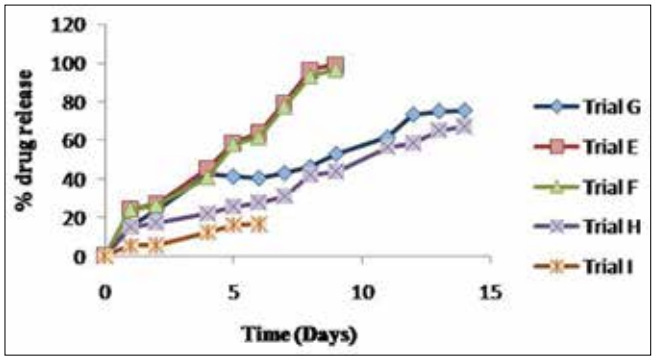

Figure 7: Comparative drug release from formulations (trial E-I)

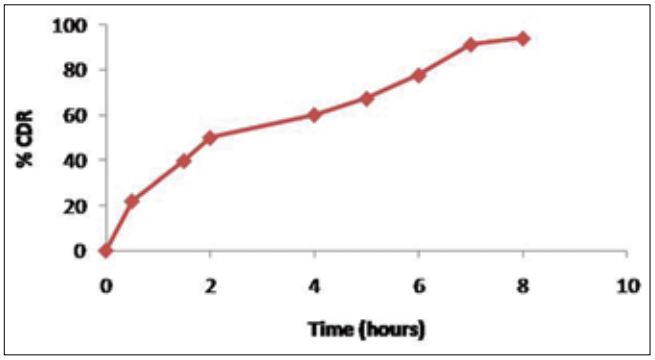

Figure 8: Plain tetracycline hydrochloride release from *in situ* gel

In vitro drug release

Figure 9 demonstrates the dissolution profile of combination delivery system. It was observed that combination delivery system released 98.96% of drug at end of 10 days and optimized formulation of microspheres (trial E) released 99.10% of drug in 10 days. Combination delivery system released 13% of drug on day 1 of study, whereas optimized formulation of microspheres (trial E) released 24% of drug on day 1 of study. Hence, release rate of drug from microspheres was not much affected by the use of polymers used in formulating *in situ* gel.

In vivo study

Patients of control and test group showed no significant difference in the duration required for wound healing. The wound healing was satisfactory in patients of both study groups after 7 days as mentioned in Table 7. This shows that local drug delivery can prove to be effective to treat oral infections with reduced dose, prolong duration of action, reduced side-effects, reduced microbial resistance and patient compliance due to reduced frequency of dose administration.

T = Test group; C = Control group, X = No; Y = Yes; NA = Not applicable.

It was observed that entire volume of 0.5 ml of *in situ* gel containing tetracycline HCl microspheres was not placed in the tooth socket. Duration for wound healing would have been reduced in Group II patients if entire dose of microspheres would have been placed in the extracted tooth socket and if there was no leakage of *in situ* gel from the sutured socket. Other possibilities to increase retention time of microspheres in the tooth socket can be to select a better carrier for dispersing microspheres, if the microspheres were compressed into mucoadhesive rectangular chips/patch it could help to retain the microspheres in the extraction socket or if the microspheres were made bioadhesive.[17] Futhermore, moldable and biodegradable, absorbable dressings can be used.[18,19]

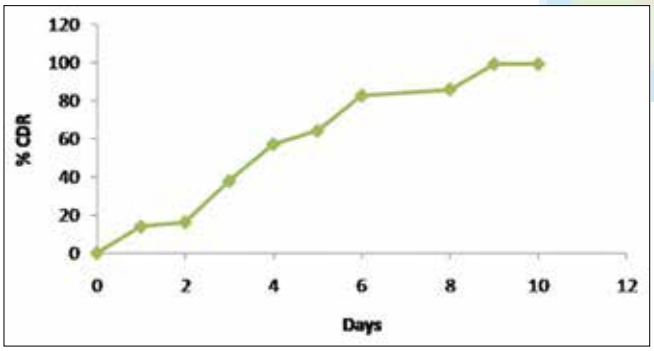

Figure 9: Dissolution profile of combination delivery system (tetracycline loaded microspheres dispersed in *in situ* gel)

Patients of test group showed no inflammation at extraction site after 7 days and no irritation was sensed by patient on placement of *in situ* gel containing tetracycline HCl microspheres in the extracted tooth socket. The placement of gel with help of syringe was easy in the extracted tooth socket.

Figure 10 and 11 shows extracted tooth socket of Group II patient on 1st and 7th day respectively.

CONCLUSION

Results obtained from *in vitro* and *in vivo* studies suggest that tetracycline loaded biodegradable microspheres can

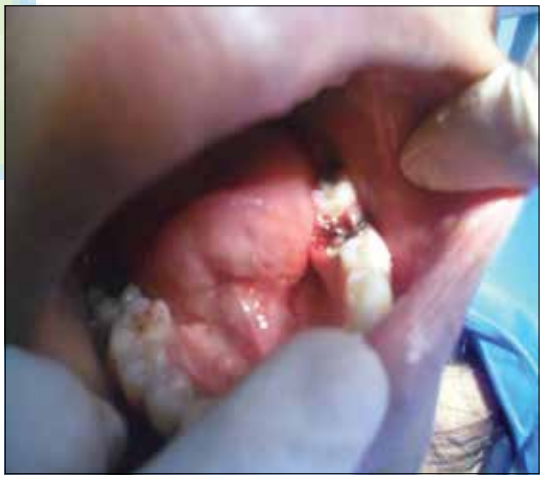

Figure 10: Extracted tooth socket of Group II patient on 1st day

Table 7: Clinical studies carried out for testing the efficacy of formulation applied locally against standard oral antibiotics

Patient no.	Gender	Age (years)	Addiction	Irritation	Pain	Taste sensation of drug	Ease of gel placement	Inflammation (on 7th day)	Wound healing (on 7th day)
1 (T)	Male	19	X	X	X	X	Y	X	Y
2 (T)	Female	44	X	X	X	X	Y	X	Y
3 (T)	Male	30	Smoking	Slight	X	X	Y	X	Y
4 (T)	Female	36	X	X	X	X	Y	X	Y
5 (C)	Male	21	Smoking	NA	X	NA	NA	X	X
6 (C)	Female	28	X	NA	X	NA	NA	X	Y
7 (C)	Male	47	X	NA	X	NA	NA	X	Y
8 (C)	Female	49	Tobacco	NA	X	NA	NA	X	Y

T: Test group, C: Control group, X: No, Y: Yes, NA: Not applicable

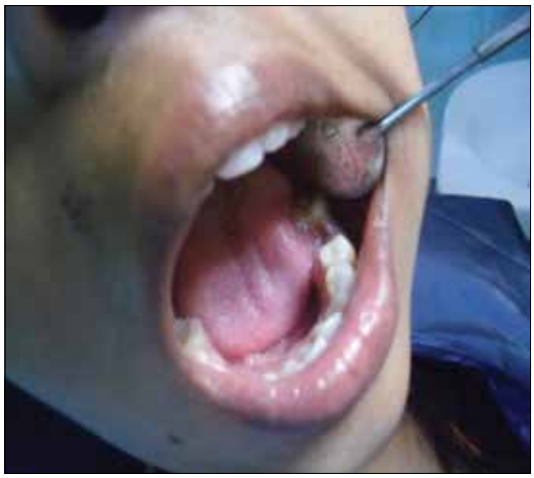

Figure 11: Extracted tooth socket of Group II on the 7th day of treatment

be effectively used for treatment of oral infections. Microspheres with smooth surface and spherical shape were obtained by utilizing W/O/W double emulsion solvent evaporation method. Microspheres obtained were in size range of 15-22 µm. Salt additions to external aqueous phase helped to increase encapsulation efficiency and even produce microspheres with smooth surface. *In vitro* drug release indicated a sustained drug release for period of 10 days. Clinical studies indicated that local drug delivery can be used to treat oral infections or to provide an antimicrobial action after tooth extraction since no significant difference was observed in patients of test and control group. There were no signs of irritation or inflammation in patients of test group. Thus if microspheres are delivered in the tooth socket with other possible methods explained in the article then local drug delivery can prove to be beneficial over systemic treatment. Developed formulation is site specific unit dose, which provides local delivery of tetracycline in a sustained manner, which may reduce side effects of systemic antibiotics, which may eliminate bacteria from nondental sites, risk for bacterial repopulation, dilution of the drug before it reaches the site of infection, super-infections, bacterial resistance, patient compliance and an inoculum effect where the total bacterial load in periodontal pockets may be too large relative to the maximal antibiotic concentrations needed for the adequate elimination of putative organisms.

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