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FORMULATION AND EVALUATION OF *IN SITU* GEL CONTAINING CHLORHEXIDINE DIACETATE LOADED MICROSPHERE FOR THE TREATMENT OF PERIODONTITIS

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ABSTRACT

In situ gel loaded microsphere system combines the benefits of sustained release microspheres with the advantages of *in situ* gelling systems that form a gel upon administration to periodontal pockets. Chlorhexidine diacetate is an anti-microbial drug used to treat periodontitis. Microspheres were prepared by solvent- evaporation method with six different formulations containing different concentrations of polymer i.e., Eudragit S-100 and Ethyl cellulose. Then the formulations were evaluated for various parameters like particle size, zeta potential, % entrapment efficiency, drug content and *in vitro* drug release study. Among the six formulations, F1 found to have highest % entrapment efficiency (83.79±0.81%.), particle size of 456µm and *in vitro* release of 81.64±0.03%. It was then incorporated into *in situ* gel forming system using Polaxomer 407 by using cold method. The prepared *in situ* gel containing chlorhexidine diacetate microsphere was evaluated for homogeneity, pH, gelation temperature, viscosity, drug content, *in vitro* drug diffusion study and kinetic study. The prepared *in situ* gel was found to be homogenous with 81.6±0.02% of drug content and %cumulative drug release of 77.62±0.04 % at the end of 11 hrs and showed zero order release. The developed microsphere *in situ* gel found to be stable and obtained results showed that chlorhexidine diacetate loaded microsphere *in situ* gel could be effective strategy in the treatment of periodontitis.

KEYWORDS: Periodontitis, *In situ* gel, Cold method, poloxamer 407, Chlorhexidine diacetate.

INTRODUCTION

Systemic administration and local administration are both important methods of drug delivery. In the past half century, the application of systemic administration in the treatment of oral infections has shown some beneficial effect. However, systemic delivery of drugs may lead to problems such as dysbacteriosis and poor biodistribution. Moreover, a high dose is usually administered in order to achieve and maintain an effective concentration, which may result in toxicity, gastrointestinal intolerance, and drug resistance. Owing to these obvious disadvantages of systemic administration, it is of great necessity to use LDDS (Local drug delivery system) for improving the prevention and treatments of periodontitis.[1]

Periodontitis is the most common diseases affecting many people in the world. Periodontitis is inflammation of the gingival and is the condition that affects the supporting structure of the teeth leading to the formation of pocket due to which tooth loss may occur.

Periodontal treatment aims to cure inflamed tissue, reduce the number of pathogenic bacteria, and eliminate the diseased pockets. Therapy is aimed primarily to reduce or eliminate inflammation, thereby allowing gingival tissues to heal.[2]

Effective delivery of drug to the periodontal pockets is still met with various challenges and more effective systems are needed for recognizing the periodontal pockets that are deep and small spaces around infected teeth. Therefore, it is difficult for drug to reach and retain in the periodontal pockets. In order to overcome this problem, *in situ* gel can be introduced in the periodontal pockets without the use of injectable devices.[3]

The advantages of *in situ* gel include ease in application of the dosage and even protection of drug from change in environmental conditions. The major drawback of *in situ* gel is, it shows rapid release of drug due to high water content and large pore size.[4,5]

Microspheres are small spherical particles with diameter ranging from 1 to 1000µm. These are novel drug delivery system, which shows sustained release property by adhering to the target site, and thereby increases the bioavailability. [6,7]

Chlorhexidine belongs to class II and is an antimicrobial agent, strong base and dicationic at pH levels above 3.5, with two positive charges. It prevents plaque accumulation, hence used as antiplaque and anti-gingivitis agent.[8]

To overcome above mentioned problems of *in situ* gel, microspheres loaded with suitable drug are prepared first and then incorporated into the *in situ* gel. This will enhance the duration of action in order to avoid the frequent administration, and improves patient compliance.[9]

Hence, aim of the present work was to formulate *in situ* gel containing chlorhexidine diacetate loaded microsphere to provide controlled release of drug by reducing dosing frequency, enhance bioavalialibility and patient compliance.

MATERIALS AND METHODS

Materials

Chlorhexidine diacetate, Polyvinyl Alcohol, Eudragit S 100, Polaxomer 407 and sodium chloride were purchased from Yarrow chemical, Mumbai, India. Ethyl cellulose, Dichloromethane and Chloroform were purchased from Loba Chemie Products, Mumbai, India. All ingredients used for the research were of analytical grade.

Methods

Formulation of Chlorhexidine diacetate loaded microsphere[10]

Microsphere formulations were prepared by Solvent evaporation method. Desired quantity of polymers (Eudragit S100 & Ethyl cellulose) was dissolved in dichloromethane to form a homogenous polymer solution. drug was added to the polymer solution and mixed thoroughly. The resulting mixture was then added to aqueous phase containing polyvinyl alcohol and agitated at 500 rpm. The drug and the polymer mixture were transformed to fine droplets which solidified into rigid microsphere by solvent evaporation. Microspheres were collected by filtration, washed repeatedly with distilled water and petroleum ether and dried at room temperature for 24 hours to get free flowing microspheres.

Table No. 01: Formulation table for preparation of Microspheres.

Formulation Code	Drug (mg)	Eudragit S 100(mg)	Ethyl cellulose (mg)	Poly vinyl alcohol (mg)	Dichloro methane (ml)	Distilled Water (ml)
F1	100	100		300	10	100
F2	100	200		300	10	100
F3	100	300		300	10	100
F4	100		100	300	10	100
F5	100		200	300	10	100
F6	100		300	300	10	100

CHARACTERIZATION OF CHLORHEXIDINE DIACETATE LOADED MICROPSHERES

Particle size[11]

The sample of prepared microspheres was randomly selected and their size was determined using Biovis particle size analyzer.

Zeta Potential[12]

The electrophoretic mobility of the charged vesicles was observed to measure the zeta potential using Zeta sizer (Malvern Instrument, Malvern, UK). All measurements were done at 25°C in triplicate after dilution of the formulations.

Surface morphology[13]

The surface morphology of the prepared microsphere was examined using optical microscopy for structural attributes such as uniformity of size and shape.

Entrapment efficiency[14]

The % entrapment efficiency of prepared formulation was obtained using ultracentrifugation method. Prepared formulation was centrifuged at 3000 rpm, 4°C for 60mins, this causes the separation of free drug (appearing as supernatant) from drug loaded vesicles (settled at bottom). This supernatant was taken and mixed with phosphate buffer pH 6.8 and absorbance was determined by UV-spectrophotometer. % Entrapment efficiency was calculated following formula;

$$\%\text{Entrapment Efficiency} = \frac{\text{Total Drug} - \text{unentrapped drug}}{\text{Total Drug}} X\ 100$$

Drug content[15]

A microsphere equivalent to 100mg of drug were taken and crushed in mortar and pestle and dissolved in 100 ml of phosphate buffer pH 6.8. After 12hrs the solution was filtered and the filtrate was analyzed at using UV spectrophotometry. The experiment was done in triplicate and results were calculated.

In vitro release studies[16]

Microspheres (25mg of drug) were suspended in 1ml phosphate buffer pH 6.8 and placed within a dialysis bag. The sample within the dialysis bag was dipped in the dissolution medium containing 50 ml of phosphate buffer, which was rotated at 50 rpm. The whole assembly

was maintained at 37°C using a thermostatic water bath. The amount of drug released was determined by withdrawing each time 3 ml aliquots at the selected specific time intervals. The volume withdrawn was replenished with an equal volume of fresh and prewarmed phosphate buffer pH 6.8 at 37°C. Samples were analyzed by UV spectrophotometer using phosphate buffer as the blank.

FORMULATION OF MICROSPHERE LOADED *IN SITU* GEL[17]

Based on the results, prepared microspheres (F1) were incorporated into *in situ* gel using polaxomer 407 and NaCl.

Cold Mixing Method: The gelling polymer and tonicity modifier was accurately weighed and slowly added to 50ml of ice-cold distilled water. Mixing was done by stirring at 1000 rpm for 15 min. Then the required amount of microspheres containing Chlorhexidine diacetate were loaded in to the *in situ* gelling system. The formulation was stored in the refrigerator for upto 24 hrs for further evaluation studies.

Table No.02: Composition of *in situ* gel containing Chlorhexidine Diacetate loaded microsphere.

Formulation Code	Polaxomer 407(%)	NaCl (%)
F1	17%	0.53%

CHARACTERIZATION OF *IN SITU* GEL

Homogeneity[18]

Homogeneity of *in situ* gel was tested through visual inspection for presence of any aggregates.

Physical appearance [18]

The clarity of gelling solutions was determined visually against white and black background.

Measurement of pH[19]

The pH of prepared *in situ* gel was measured using digital pH meter.

Gelation Temperature[20]

A magnetic bead and 10 ml of the sample solution were put into a 30 ml of beaker. A thermometer was placed in the sample solution. The solution was heated at the rate of 1°C/min with the continuous stirring using magnetic stirrer. The temperature at which the magnetic bead stops moving due to gelation was considered as gelation temperature.

Viscosity[21]

The viscosity of prepared *in situ* gel formulations at room temperature (25±5˚C) was noted using Brookfield viscometer DV-I-LV. The measurements were done by using spindle no.62 at a speed of 50 rpm. The procedure was repeated three times.

Drug Content[22]

1ml of prepared *in situ* gel formulation was transferred in 100 ml volumetric flask and completely dissolved in 50 ml of phosphate buffer of pH 6.8. Final volume was adjusted to 100 ml with phosphate buffer pH 6.8 and solution was filtered. The drug concentration in filtered solution was determined spectrophotometrically.

In vitro diffusion study[23]

The *in vitro* release study of Chlorhexidine diacetate from *in situ* gel was determined using a Franz‑diffusion cell. The cellophane membrane was fixed on the receptor cell. The donor cell was filled with 1 g of *in situ* gel formulation. The receptor compartment was filled with phosphate buffer pH 6.8 and constantly stirred at speed of 200‑250 rpm and temperature was maintained at 37 ± 0.5°C. The 0.5 ml samples were withdrawn at specified time intervals and were replaced with same volume of pH 6.8 phosphate buffer to maintain the sink condition. Samples were analyzed using UV ‑ visible spectrophotometer.

Stability studies[24]

The *in situ* gel was stored for 3 months at 4±3°C and 25±2°C / 65±5% RH. Samples were withdrawn after specific time intervals (0, 30, 60, and 90 days). The samples were analyzed for any changes in the physical appearance, % entrapment efficiency and *in vitro* drug study.

RESULTS AND DISCUSSIONS

Particle size and surface morphology of Microspheres

The particle size of prepared microsphere formulation was determined by Biovis particle size analyzer. Obtained result is shown in table number 03. The formulation F1 showed smallest particle size i.e., 212µm.The particle size increase with increase in the polymer concentration.

Table No.03: Particle size of prepared batches of microspheres.

Formulation Code	F1	F2	F3	F4	F5	F6
Particle Size(µm)*	212±0.02	348±0.04	456±0.06	215±0.02	324±0.03	447±0.04

*(*Data represented as mean ±Standard deviation and n=3)*

The surface morphology of microsphere was studied by using the optical microscope. Below figure no.01 demonstrates the surface morphology of chlorhexidine diacetate loaded microsphere which indicated that the vesicle was small in size with round shape.

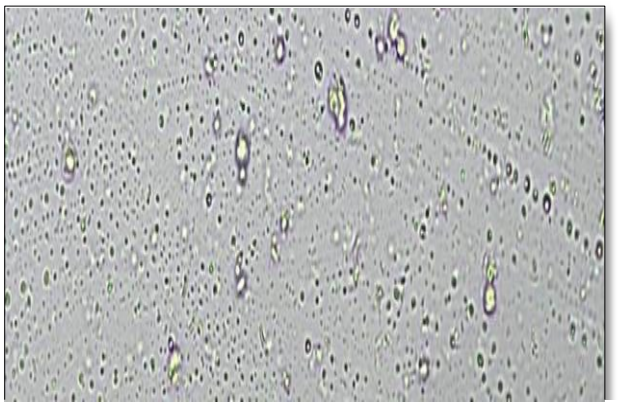

Fig No.01: Optical microphotograph of Prepared F1 formulation.

Zeta Potential of microsphere

Zeta Potential of the F1 formulation was found to be -23.7mV (Fig.no:21) which indicated a stable formulation.

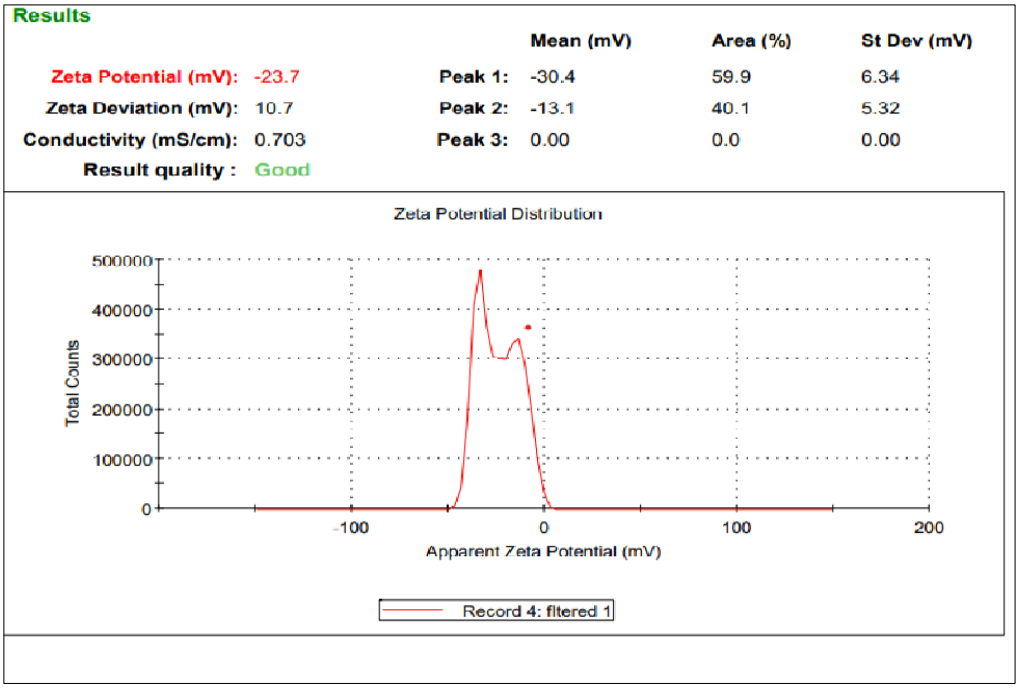

Fig. No. 02: Zeta potential of Prepared F1 formulation.

Entrapment efficiency of prepared microspheres

The %EE of all the formulation (F1-F6) was found in the range of 79.33±0.72%- 83.79±0.81% as shown in figure 03. The highest entrapment efficiency was found in the F1 i.e., 83.79±0.81%. As the polymer concentration increases the %EE of formulation decreases.

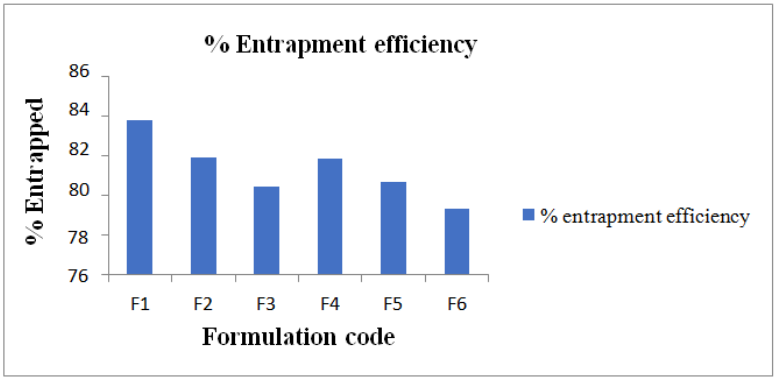

Fig No.03: Entrapment efficiency of Microsphere containing chlorhexidine diacetate.

Drug Content of microspheres

The drug content was ranged from 82.91±0.68% to 86.66±0.43% as shown in table No.11. F3 showed highest drug content i.e., 86.66±0.43%.

Table No.04: Drug content of prepared formulation.

Formulation Code	F1	F2	F3	F4	F5	F6
Drug Content* (%)	84.16±0.2	85.41±0.81	86.66±0.43	82.91±0.6	83.75±0.7	83.33±0.5

*(*Data represented as mean ± standard deviation and n=3)*

***In vitro* drug release studies**

All the prepared formulation found to show rapidly release in the initial hours (4hrs) due to the unentrapped drug adsorbed on the surface of microsphere and eventually slower in 11hrs. The cumulative percent drug release after 11hrs was found to be in the range of 75.92±0.02% to 81.64±0.03%.

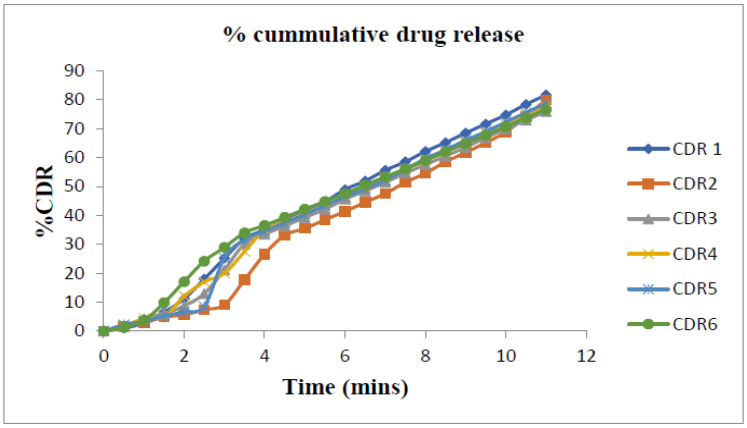

Fig No. 04: *In vitro* drug release study of Microsphere containing Chlorhexidine diacetate formulation (F1-F6).

Physical characteristics of *in situ* gel containing microsphere

Prepared in situ gelling system was studied for following parameters. Obtained results are given in table No.05.

Table No.05: Physical characteristics of *in situ* gel containing microsphere.

Homogeneity	Physical appearance	pH	Gelation temperature (◦C)	Viscosity (cPs)	Drug content (%)
No aggregates	Colourless	6.7±0.01	35±0.02	460	81.6±0.02%

The gelation temperature of prepared microsphere gel formulation was found to be 35±0.02°C. Hence 33–37°C is considered as optimum temperature for development of thermo sensitive *in situ* gelling formulation.

Fig no. 05: Formulation before and after gelation temperature.

***In vitro* diffusion study:** The *in situ* gel containing microsphere formulation with Chlorhexidine diacetae showed 77.62±0.04% of drug release at the end of 11 hours of study.

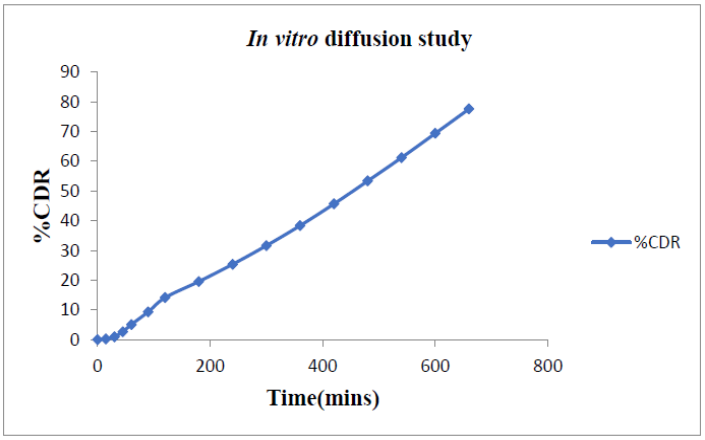

Fig no.06: *In vitro* diffusion study.

Stability studies

The Stability study was carried out for the prepared in situ gel formulation, kept for refrigerated condition 4 ±30C and at room temperature 25±2oC at 60±5%RH for 90 days. The in situ gels of microsphere formulation were analysed for physical changes such as colour, drug content and in vitro studies. The formulations was found to be stable during the period of study.

Table No.06: stability study of *In situ* gel.

Evaluation parameter		Colour	Drug content (%)	*In vitro* release
Initial days	4°±3°C	colourless	81.66±0.43%	77.62±0.04%
	25±2°C / 65±5% RH	colourless	81.66±0.43%	77.62±0.04%
After 30days	4°±3°C	colourless	80.52±0.33%	77.60±0.05%
	25±2°C / 65±5% RH	colourless	79.92±0.32%	76.5±0.02%
After 60days	4°±3°C	colourless	79.53±0.31%	76.81±0.03%
	25±2°C / 65±5% RH	colourless	78.76±0.30%	76.31±0.04%
After 90days	4°±3°C	colourless	78.36±0.27%	75.98±0.03%
	25±2°C / 65±5% RH	colourless	77.97±0.25%	74.93±0.02%

CONCLUSION

In situ gel containing Chlorhexidine diacetate loaded microsphere formulation were successfully formulated. From the results obtained, the formulation F1 was chosen as best formulation, as it was found to have good entrapment efficiency and *in vitro* release study. The same formulation was then formulated into in situ gel. The formulated *In situ* gel was found to be homogeneous with good viscosity and had *in vitro* release of 77.62±0.04 % at the end of 11 hours. Hence, from the current study it could be concluded that *In situ* gel containing Chlorhexidine diacetate loaded microsphere can be successfully used for prolonging the drug delivery in controlled manner and to enhance the duration of action thereby improve patient compliance.

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