International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article Open Access

Development and Characterization of Bioadhesive Gel Incorporated Microencapsulated Ketorolac Tromethamine for Periodontal Therapy

P. Parveen*, Dr. A. Seetha Devi

Department of pharmaceutics, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India

A B S T R A C T

To develop and characterize bioadhesive gel incorporated Ketorolac tromethamine loaded microspheres in order to sustain, localize and target drug action in periodontal pockets for more than 6hrs. Ketorolac tromethamine loaded microspheres was prepared by emulsion cross linking method using two types of solvents like liquid paraffin or sesame oil by glutaraldehyde cross linking. Tween 80, span 80 was used as emulsifying agents. Gelatin, sodium alginate, HPMC E15 and ethyl cellulose are used as polymers. The prepared microspheres were incorporated in the bioadhesive gel using different polymers like HPMC, NaCMC and Carbopol-934 in various proportions i.e., 300, 400 and 500mg by using triethanolamine as gelling agent. Effect of drug: polymer ratio, stirring rate, glutaraldehyde amount, emulsifying agents ratio and type of polymer on preparation of microspheres was investigated. The microspheres were characterised for % yield (98.5%), % drug loading (85%), particle size (52.14µm), scanning electron microscopy (SEM) and showed 92.8% drug release at the end of 6^{th} hr. The bioadhesive gel was characterised for pH (6.47-6.78), viscosity (55.9-635cps), drug content (92.1-99.7%), bio adhesive strength(2.49-15.7gms) and drug release (98.9%) at the end of 6^{th} hr. Bioadhesive gel loaded with microspheres of Ketorolac tromethamine produces prolonged and localized delivery system for the treatment of periodontitis.

Keywords: Gelatin microspheres, Bioadhesive dosage form, Ketorolac tromethamine, Periodontal pocket, Glutaraldehyde cross linking

A R T I C L E I N F O

CONTENTS

Article History: Received 06 December 2014, **Accepted** 10 January 2015, **Published Online** 15 March 2015

***Corresponding Author**
P. Parveen
Department of pharmaceutics, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India
Manuscript ID: IJCTPR2440

PAPER-QR CODE

Citation: P. Parveen and Dr. A. Seetha Devi. Development and Characterization of Bioadhesive Gel Incorporated Microencapsulated Ketorolac Tromethamine for Periodontal Therapy. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(2): 806-816.

1. Introduction

Periodontitis is a severe pathological condition affecting the tooth supporting structures. It is characterized by a destruction of periodontal ligament, a sorbtion of the alveolar bone and the migration of the junctional epithelium along the tooth surface which leads to changes in the morphology of gingival tissues, bleeding as well as periodontal pocket formation this results in growth of pathogenic bacteria and leads to inflammation and tissue distruction of periodontal tissue[1]. Antimicrobial and Anti – inflammatory agents are used to treat periodontal diseases. These agents are associated with drawbacks like short biological half life and inability of the drug to reside locally for a sufficient period of time. Thus to avoid these drawbacks and obtain a sustained and targeted delivery, drugs are encapsulated in microspheres, which are then formulated as a gel[2]. Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug (NSAID) chemically related to indomethacin and tolmetin having analgesic activity [3]. Its anti inflammatory effects are due to inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) which leads to the inhibition of prostaglandin synthesis [4]. The half life of the Ketorolac tromethamine is 2.5 hrs. Oral use of this drug is associated with side effects like gastrointestinal disturbance, nausea, vomiting and diarrhoea. Topical application of this Ketorolac prevents these side effects and offers potential advantage of delivering the drug at the site of action. The objective of the present study is to develop and characterize bioadhesive gel incorporated Ketorolac tromethamine loaded microspheres formulation as the novel technique to sustain and localize the drug action in the periodontal pockets for an effective treatment.

2. Materials and Methods

2.1 Materials

Ketorolac tromethamine was obtained as the gift sample from devis laboratories, Gelatin, HPMC, NACMC, liquid paraffin sesame oil were obtained from Loba chemise laboratory reagents and fine chemicals Ltd, Mumbai. Other reagents and solvents used were of pharmaceutical or analytical grade.

2.2 Preparation of microspheres by emulsion cross linking method

3g of polymer (Gelatine/Sodium alginate/ HPMC E15/ Ethyl cellulose) were accurately weighed and mixed in 10 ml of distilled water, preheated to 60ºc, followed by the addition of Tween 80 (0.1%w/v). To this, 1 g of Ketorolac tromethamine was added and thoroughly mixed to obtain a homogeneous solution. The mixture was maintained at 50ºc, and than added drop wise into 100 ml of liquid paraffin and sesame oil respectively containing Span 80 (0.1 %w/v) preheated to 60 ºc at constant stirring with 3-blade stirrer in order to form w/o emulsion. Glutaraldehyde was added drop wise to the emulsion and stirring and stirred for 1 hr at room temperature to stabilize the microspheres. The mixture was then left to cool at between 5-10 ºc for 30 min to enhance settling of the microspheres. Microspheres were collected by filtration using Whatman filter paper and washed with 3×10 ml of chloroform followed by 2×10 ml of 5 %w/v sodium metabisulphite, dried at room temperature and transferred to glass vials[5].Various process variables used in preparation of microspheres was shown in the Table no:1,2,3,4,5,6.

Process variables in microspheres preparations

Table 1: Effect of process variables on microsphere properties

S. No	Process variables	Values
1	Stirring rate (rpm)	500, 1000, 1500
2	Cross linking agent amount (ml)	0.5, 1.0, 1.5
3	Drug : polymer ratios	1:1, 1:2, 1:3, 1:4
4	Continuous phase amount (ml)	100,150,200
5.	Emulsifying agents Tween 80(ml)	0.01 , 0.02
	Span 80(ml)	0.1 , 0.2

Table 2: Batches for selection of drug: polymer ratio

Batch	Drug : polymer ratio	Glutaraldehyde amount (ml)	Stirring rate (rpm)	Continuous phase amount (ml)
F1	1:1	0.5	500	100
F2	1:2	0.5	500	100
F3	1:3	0.5	500	100
F4	1:4	0.5	500	100

Table 3: Batches for selection of glutaraldehyde amount and stirring rate

Batch	Drug: Polymer ratio	Glutaraldehyde amount (ml)	Stirring Rate (rpm)	Continuous phase amount(ml)
F5	1:3	1.0	500	100
F6	1:3	1.5	500	100
F7	1:3	0.5	1000	100
F8	1:3	1.0	1000	100
F9	1:3	1.5	1000	100
F10	1:3	0.5	1500	100
F11	1:3	1.0	1500	100
F12	1:3	1.5	1500	100

Table 4: Batches to determine effect of continuous phase amount on microspheres

Batch	Drug: Polymer ratio	Glutaraldehyde amount (ml)	Stirring Rate (rpm)	Continuous Phase amount (ml)
F13	1:3	1.0	1000	150
F14	1:3	1.0	1000	200

Table 5: Batches to select emulsifying agent and its ratio for microspheres preparation

Batch	Drug: Polymer ratio	Glutaraldehyde amount (ml)	Stirring Rate (rpm)	Continuous Phase amount (ml)	Tween 80(ml)	Span 80(ml)
F15	1:3	1	1000	100	0.02	0.2
F16	1:3	1	1000	100	0.01	0.1

Table 6: Batches to select suitable polymer

Batch	polymer	Drug: Polymer ratio	Glutaraldehyde amount (ml)	Stirring Rate (rpm)	Continuous Phase amount (ml)	Tween 80(ml)	Span 80(ml)
F16	Gelatin	1:3	1	1000	100	0.01	0.1
F17	Sodium alginate	1:3	1	1000	100	0.01	0.1
F18	HPMC E15	1:3	1	1000	100	0.01	0.1
F19	Ethyl cellulose	1:3	1	1000	100	0.01	0.1

2.3. Characterization of microspheres:

2.3.1. Determination of percentage yield

The microspheres prepared were collected and weighed. The measured weight of microspheres was divided by total amount of all non-volatile components, which were used for the preparation of microspheres[6]. The % yield was calculated by following formula

% yield = Wrec/Weight (drug + polymer)x100

Where Wrec = Weight of microspheres recovered

2.3.2. Determination of percentage of drug loading

The percentage of Ketorolac tromethamine loading in microspheres can be estimated using.

L=Qm/Wm x100

where, L is the % loading of microspheres, Qm is the quantity Ketorolac tromethamine present in Wm of microspheres and Wm is the weight of the microspheres in grams [7].

2.3.3. Fourier Transform Infrared Spectroscopy

Drug polymer interactions were studied by FTIR spectroscopy [8]. The spectra were recorded for pure drug and drug loaded microspheres using FTIR and scanned between wave number 4000–400 cm^{-1} using a Shimadzu Model 8400 FTIR.

2.3.4. Particle size analysis

The particle size analysis was carried out by using electron microscopy. The microspheres were analyzed for size and size distribution by dispersing them in 20 % v/v isopropyl alcohol. About 100 microspheres were selected randomly and their size was determined [9].

2.3.5. Scanning electron microscopy

SEM analysis was carried out in order to determine the size and shape of the microspheres [10].

2.3.6. Evaluation of in vitro release by static method

Microspheres, equivalent to 10 mg of Ketorolac tromethamine, were accurately weighed and transferred to conical flask containing 100 ml phosphate buffer (pH 6.8). The flask was kept in an incubator maintained at 37 °C, Five minutes before each sampling, the flask was shaken manually to minimize any concentration gradient within the release medium. The microspheres were allowed to settle down and clear supernatant medium was withdrawn for drug analysis. 1 ml of sample solution was withdrawn at regular intervals of time and after suitable dilution, the amount of drug released was determined using a spectrophotometer at 240 nm. For each sample withdrawal, 1 ml of fresh phosphate buffer of pH 6.8 was added to the release medium to replenish it [11].

2.4. Preparation of gel

Accurately weighed Ketorolac tromethamine (100 mg) was added to 15 ml of water in a beaker and stirred well to dissolve the drug, Carbopol-934/HPMC/NACMC with 300,400,500 mg of varying proportions was dissolved in this drug solution. Gelatin microspheres loaded with 100 mg of Ketorolac tromethamine was added to the drug polymer solution and mixed well. Methyl hydroxybenzoate (0.15 %w/w), propyl hydroxybenzoate (0.05 %w/w) and sodium metabisulphate (0.1 %w/w)) were then added to microsphere/drug/polymer mixture, triturate slightly in mortor and pestle to obtain a homogenous mixture. Stirring was continued until a lump-free suspension was obtained. Thereafter, 0.3 ml of triethanolamine was added to produce a gel. This was followed by the addition of a sweetening agent (saccharin sodium, 0.1 %w/w) and more water to make up to 20 g of gel[12]. Different bioadhesive gel formulations were prepared from microspheres obtained by two types of solvent system shown in table no:7.

FGL=Formulated gels prepared from microspheres obtained from liquid paraffin solvent

FGS= Formulated gels prepared from microspheres obtained from sesame oil solvent

Table 7: Formulation table of bioadhesive gel

Formulations	Polymer concentration(mg)		
	Carbopol-934	**HPMC**	**NA CMC**
FGL1	300	-	-
FGL2	400	-	-
FGL3	500	-	-
FGL4	-	300	-
FGL5	-	400	-
FGL6	-	500	-
FGL7	-	-	300
FGL8	-	-	400
FGL9	-	-	500
FGS10	300	-	-
FGS11	400	-	-
FGS12	500	-	-
FGS13	-	300	-
FGS14	-	400	-
FGS15	-	500	-
FGS16	-	-	300
FGS17	-	-	400
FGS18	-	-	500

2.5. Evaluation of bioadhesive gel incorporated microspheres

2.5.1. Surface pH of the gel:

An acidic or alkaline formulation is bound to cause irritation on mucosal membrane and hence this parameter assumes significance while developing a bioadhesive formulation. A digital glass electrode pH meter was used for this purpose. pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min[13].

2.5.2. Viscosity Study:

Viscosity of gels was studied on Brookfield viscometer by using spindle number 42 at 0.5 revolutions per minute at constant temperature [14] .

2.5.3. Estimation of drug content in formulated gels:

Formulations containing 1 mg of drug was taken in 10 ml volumetric flask, dissolved in pH 6.8 phosphate buffer made up the volume to 10 ml with pH 6.8 phosphate buffer and then filtered. Absorbance values were measured at 240 nm for the drug. Concentrations of drug were calculated from the standard calibration curve prepared in pH 6.8 phosphate buffers [15].

2.5.4. Evaluation of in vitro release by dynamic method

In vitro release studies of Ketorolac tromethamine from the gels were carried out at 37 °C using phosphate buffer of pH 6.8 as release medium. Gel (1 g) containing Ketorolac tromethamine was accurately weighed and transferred to receptor compartment of franz diffusion cell. The gel was gently pushed down to the surface of the egg membrane with the help of a stainless steel spatula to ensure that all the gel was in contact with the membrane. 1ml of Phosphate buffer of pH 6.8 was added to the reservoir compartment to wet the gel. Egg membrane is just immersed in the phosphate buffer which acted as the receiving compartment. The receiving compartment was magnetically stirred at 150 rpm, maintaining at 37 °C. 1ml of sample was withdrawn from the receiving compartment at regular intervals and the amount of Ketorolac tromethamine released from the gel was determined using a spectrophotometer at 240 nm. After each withdrawal of sample, equal quantity of phosphate buffer was added to the receiving compartment to replenish it [16].

2.5.5. Bioadhesion study:

In the present study, goat cheek pouch was used as a model mucosal surface for bioadhesion testing. The goat cheek pouch was procured from slaughter house, then excised and trimmed evenly from the sides. It was then washed with phosphate buffer of pH 6.8 and used immediately and tired tightly with the mucosal side upwards using rubber band over an inverted vial. Bioadhesive gel was applied to the mucosal surface and adhered with the surface of another vial and kept below the left hand set up of the balance. The balance was kept in this position for 8 min and then sand was added in the right pan by the spatula. The addition of sand was stopped as soon as the detachment of two surfaces was obtained. Weight of sand was measured. This gave the bioadhesive strength of the formulation in grams [17].

2.5.6. *Ex-vivo* permeation studies

The modified Franz diffusion cell was used for permeation studies. It consists of two compartments, one is donor compartment and another is receptor compartment of 10 ml capacity. The separated buccal epithelium was mounted between the chamber, and the receptor compartment was filled with 10 ml of phosphate buffer of pH 6.8 phosphate buffer. A Teflon‑coated magnetic bead was placed in the receptor compartment, and the whole assembly was placed on the magnetic stirrer, and buccal epithelium was allowed to stabilize for a while. After stabilization, samples of 1 ml were withdrawn at regular intervals, suitably diluted, and were analyzed spectrophotometrically at 240 nm[18].

***2.5.7. In-Vitro* Diffusion Study**

In-vitro diffusion study of optimized formulation of bioadhesive gel incorporated Ketorolac tromethamine loaded microspheres was compared with *in-vitro* diffusion study of Ketorolac tromethamine loaded microspheres.

2.5.8. Characterisation of drug in bioadhesive gel formulation

FTIR studies were conducted for characterisation of drug in bioadhesive gel of selected formulation. The IR spectrum was recorded using Fourier Transform Infrared Spectrometer. The IR spectrum of pure drug Ketorolac tromethamine and best formulation was taken, interpreted and compared with each other.

2.5.9. Release kinetics

The data obtained from in vitro experiments was fitted to various mathematical models that are employed to assess the drug release kinetics. Based on the R^2- value or n-value, the best-fitted model was selected.

Zero - order kinetic model - Cumulative % drug release versus time.

First - order kinetic model - Log cumulative percent drug remaining versus time.

Higuchi's model [19]- Cumulative percent drug released versus square root of time.

Erosion model[20] - cubic root of unreleased fraction of the drug versus time.

Korsmeyer equation / Peppa's model 21,22- Log M_t/ M_∞ versus log time.

3. Results and Discussions

Compatibility Studies:

From the FTIR spectra of physical mixture of the drug, polymer, and other ingredients, it was observed that the peak of the major functional groups of Ketorolac tromethamine were present in the spectrum of pure drug (Fig no :1,2).All the peaks were remained unaltered in the IR spectrum of physical mixture of drug and excipients this revealed that there was no interaction.

Ketorolac tromethamine pure drug

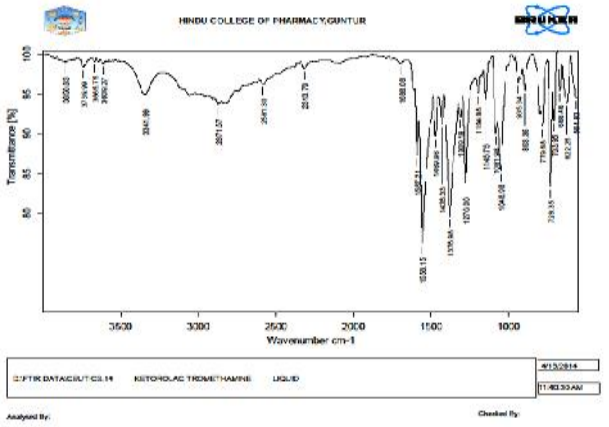

Figure 1: FTIR spectrum of pure drug Ketorolac tromethamine

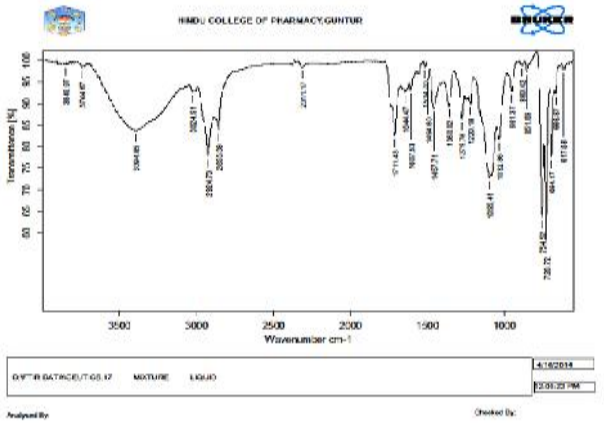

Figure 2: FTIR spectrum of Mixture

For All the formulations used in the microsphere preparation only for F 16 formulation microspheres are obtained thus it is selected as optimized formulation for preparation of Ketorolac tromethamine loaded microspheres depending on stirring rate 1000rpm, 1ml glutaraldehyde amount, 100ml continuous phase, 1:3 drug: polymer ratio, amount of emulsifying agents ratio and type of polymer used. Two types of solvents i.e liquid paraffin or sesame oil are used in the preparation of optimized F16 formulation microspheres thus resulting two types of microsphere formulations.

FL16 = Microspheres prepared from liquid paraffin solvent
FS16 = Microspheres prepared from sesame oil solvent

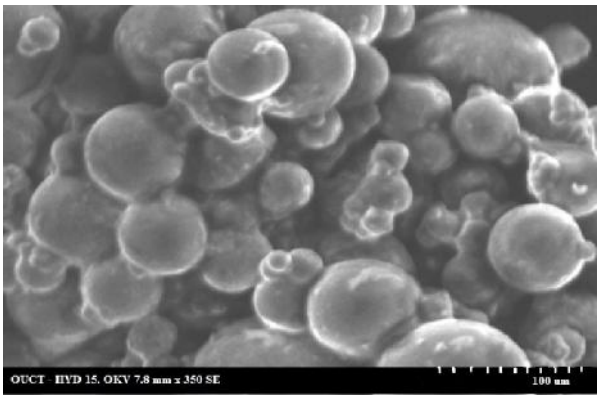

Figure 3: Scanning electron microscope photograph of microspheres

These microspheres appeared single, spherical, discrete with smooth surface by SEM studies and electron microscopy (fig no:3,4) .

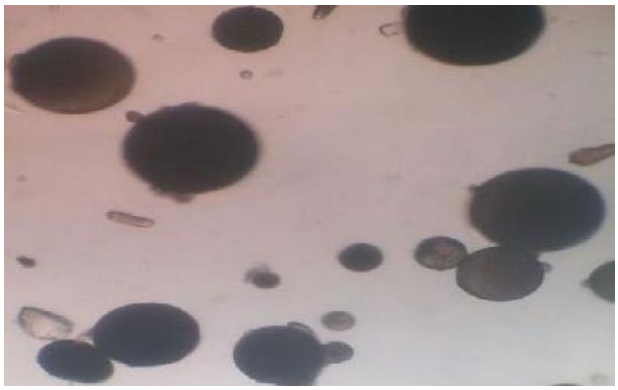

Figure 4: Electron microscope images of microspheres

The microspheres were characterised for the two formulations FL16 and FS16 by percentage yield , percent drug loading, particle size and %drug release by static method at the end of 6th hr(table no:9).The percentage yield of microsphere formulations were 97.52 %(FL16) and 98.5 %(FS16).It indicates good yield. The percentage drug loading of microsphere formulation were 95.7 %(FL16) and 85 %(FS16).It indicates formulations shows good percent drug loading. The results obtained indicated that the increase of drug: polymer ratio was associated with increase in both the %dug loading and the microspheres production yield. The average particle size of microspheres was 61.92um(FL16) and 52.14um(FS16) .The particle sizes in the two formulations is within the microsphere particle size range of 1-1000um which ensured the uniformity of particle sizes necessary for operations like mixing and preparation of gels and also provides more uniform drug release . All the formulations from SEM studies were single, spherical, discrete with smooth surface. Also average particle size of the prepared microspheres was within the size range of 50 to 90 um. The results indicate that microsphere formulations are useful for uniform mixing of microspheres in preparation of gels and produces uniform drug release (table no:8).

Table 8: Evaluation parameters of Ketorolac tromethamine loaded microspheres

Evaluation tests	FL16	FS16
% yield	97.52±0.105	98.5±0.23
%drug loading	95.7±0.34	85±0.42
Particle size(um)	61.92±0.56	52.14±0.65
%drug release at the end of 6th hr	92±0.32	92.8±0.13

Drug is released from microsphere formulation in sustained for prolonged period of time i.e>6hr(fig no:5).FT-IR spectrum of microsphere formulation reveals that all the peaks were remained unaltered in the IR spectrum of selected microspheres and there was no chemical interaction of drug with polymer and other ingredient in prepared microspheres (Fig.no :6).

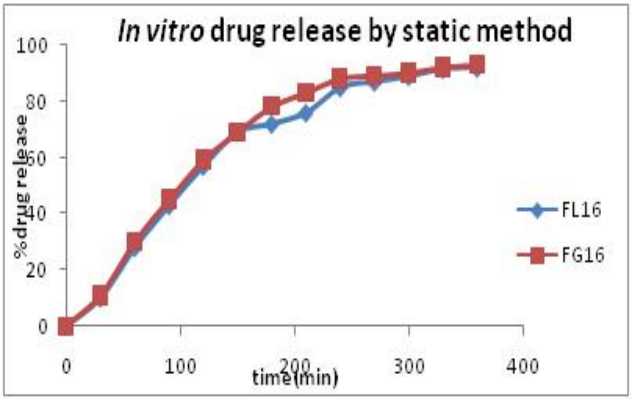

Figure 5: Drug release profile of formulated microspheres

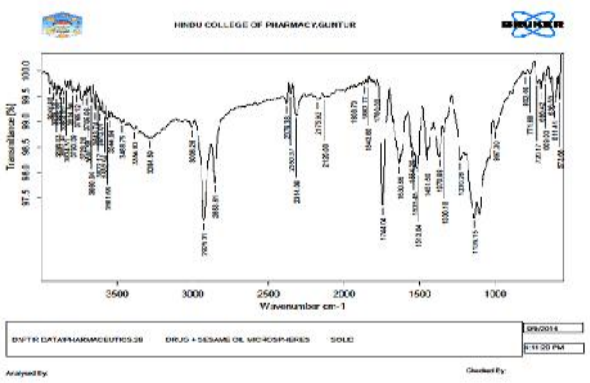

Figure 6: FT-IR of formulation microspheres

The bio adhesive gel from formulations FGL1-FGS18 were characterised for pH of gels was within the range of 6.47-6.78 which reflects that the gel will be non irritant to periodontal pocket mucosa,Viscosity of prepared gel formulations ranges between 55.9-635 cps. FGL7 showed lowest viscosity and FGS12 showed highest viscosity. Formulations prepared from carbopol-934 polymer with microspheres prepared from sesame oil vehicle showed high viscosity and gels prepared from sodium CMC polymer showed lowest viscosity. The data represent that with increase in the concentration of polymer viscosity was increased. The drug content of the prepared gel formulations was in the range of 92.1-99.7 %. All the formulations exhibited fairly uniform drug content. This indicates that the drug was uniformly distributed throughout the formulations.FGL7 showed lowest drug content and FGS12 showed highest drug content. Formulations prepared from carbopol-934 polymer showed high drug content compared with other formulations. This reveals that improved drug content has been observed with increase in polymer concentration. Bioadhesive strength was conducted on the goat buccal mucosa in fig no:7,8,9,10 and table no:9. Bio adhesion properties of prepared gels (FGL1-FGS18) is between 2.49-15.7gms. FGL7 showed lowest bio adhesion and FGS12 showed highest bio adhesion. The result showed that all the formulations had good bio adhesive values. The formulation prepared from carbopol-934 polymer showed the highest bio adhesive property when compared to other formulations which may be due to greater polymeric cross linking structure and greater cohesive force between polymeric gel and buccal mucosal membrane. Increase in polymer concentration increases bio adhesive strength of the gel which reflect that the bio adhesive gel can be retained in periodontal socket for prolonged period of time which will improve the patient compliance.

Figure 7: Goat cheek pouch for bioadhesion study

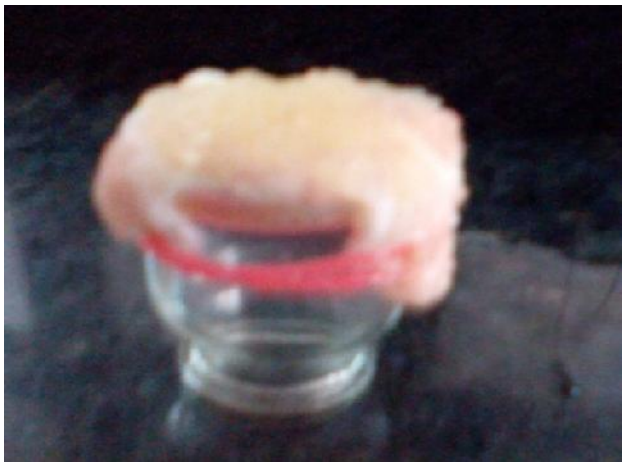

Figure 8: Bioadhesive gel on the goat mucosa tired over an inverted vial

Figure 9: Addition of sand in the right pan

Figure 10: Detachment of two vial surfaces

Drug release by dynamic method (98.9%for FGS10) at the end of 6th hr in fig no 11,12. Drug release was within 4hrs in case of gels prepared from sodium CMC polymer and 5hrs in case of gels prepared from HPMC polymer thus drug release was sustained for prolonged period of time in case of gels prepared from carbopol-934 polymer. *In vitro* drug release profiles of various gel formulations by dynamic method reported that decrease of polymer proportion resulted to increase of release rate. It was found out that gels prepared from carbopol-934 showed highest drug release when compared to other formulations.

Table 9: Evaluation parameters of bio adhesive gel

S.No	Formulation	pH	%drug content	Viscosity(cps)	Bio adhesive strength(gms)
1.	FGL1	6.60±0.13	97.1±0.13	327	10.50±0.12
2.	FGL2	6.73±0.14	97.3±0.12	450	10.52±0.43
3.	FGL3	6.40±0.23	97.5±0.10	550	10.54±0.32
4.	FGL4	6.50±0.14	92.8±0.13	180	3.10±0.22
5.	FGL5	6.51±0.13	93.2±0.17	210	4.12±0.67
6.	FGL6	6.50±0.12	93.7±0.16	240	5.10±0.33
7.	FGL7	6.48±0.11	**92.1±0.12**	**55.9**	**2.49±0.22**
8.	FGL8	6.60±0.16	92.46±0.16	61.2	3.15±0.32
9.	FGL9	6.52±0.18	92.6±0.14	72	4.30±0.45
10.	FGS10	**6.78±0.2**	98.4±0.12	620	15.55±0.45
11.	FGS11	6.72±0.23	98.5±0.17	630	15.64±0.67
12.	FGS12	6.50±0.12	**99.7±0.18**	**635**	**15.7±0.78**
13.	FGS13	6.51±0.14	95.6±0.19	190	3.43±0.55
14.	FGS14	6.50±0.14	95.7±0.13	230	4.28±0.55
15.	FGS15	6.48±0.12	95.8±0.12	250	5.37±0.45
16.	FGS16	**6.47±0.15**	92.5±0.11	59	2.58±0.65
17.	FGS17	6.50±0.17	92.6±0.13	63	3.28±0.89
18.	FGS18	6.51±0.18	92.7±0.19	74	4.42±0.76

\

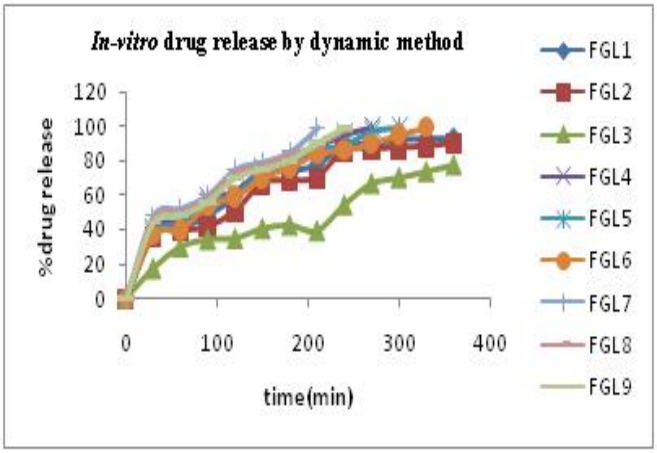

Figure 11: Drug release of gel formulations by dynamic method from FGL1-FGL9

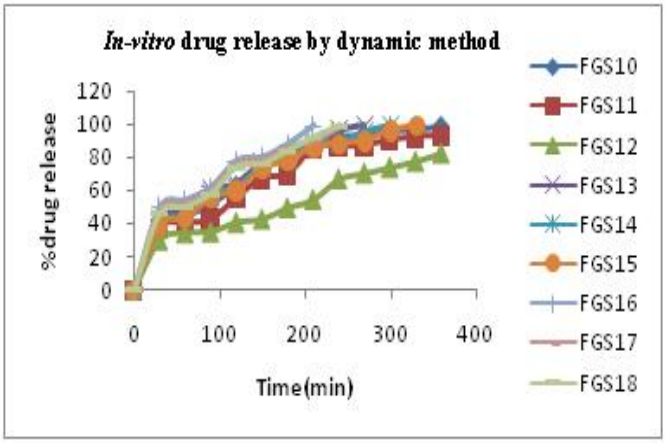

Figure 12: Drug release of gel formulations by dynamic method from FGS10-FGS18

In-vitro diffusion study of bio adhesive gel incorporated Ketorolac tromethamine loaded microspheres (FGS10) was compared with Ketorolac tromethamine loaded microspheres (FS16) in Figure: 13. Drug from the microspheres was released in a controlled manner for more than 6hr. The release pattern was biphasic with an initial burst release of 11% of the loaded drug in the first 30min.Thereafter release was slow but steady and by the end of the 6th hr, 92.8%of the loaded drug was released. In bio adhesive gel incorporated Ketorolac tromethamine loaded microspheres of FGS10 the release pattern was biphasic with the initial burst release of 38.6% by the end of first 30 min subsequently release was steady and by the end of the 6th hr, 98.9 % of the drug was released. *In vitro* – diffusion study indicates drug release from both microspheres and gel formulations showed sustained release pattern for prolonged period of time i.e >6hr in the periodontal socket for the treatment of periodontal inflammation.

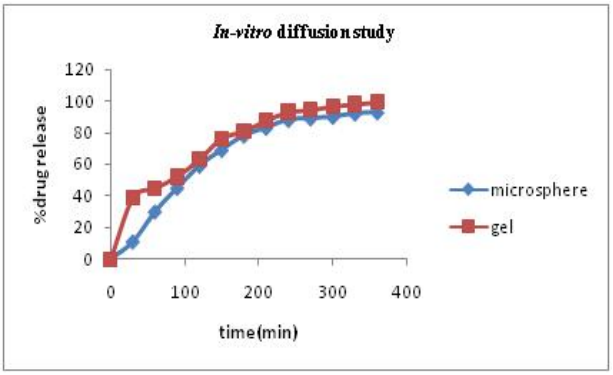

Figure 13: *In-Vitro* Diffusion Study of FS16 and FGS10 formulations

FT-IR of bio adhesive gel incorporated Ketorolac tromethamine loaded microspheres reveals that all the peaks were remained unaltered in the IR spectrum of selected optimized gel formulation thus there was no chemical interaction of drug with polymer and other ingredient in prepared bio adhesive gel(Fig no: 14) .

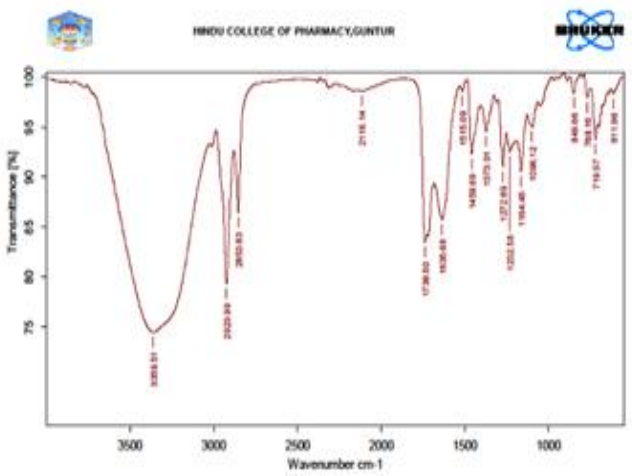

Figure 14 : FTIR spectrum of FGS10 bioadhesive gel formulation

Release Kinetics:

In order to study the exact mechanism of drug release from bio adhesive gel formulation drug release data were analysed according to Zero order, First order, Higuchi square root Erosion and Korsmeyer-peppas equation. Drug release from all the formulations obeyed Higuchi kinetic equation (Table.no:10). Regression coefficients of zero order and first order was compared it is found that R^2 values of first order was greater thus the formulation follows first order kinetics in order to know exact drug release mechanism regression values of Higuchi and Erosion were compared it was found that R^2 values of Higuchi was greater thus drug release was diffusion controlled and n value is between 0.5-1 that indicates drug release follows non fickian diffusion where drug release is controlled by combination of diffusion and polymer chain relaxation mechanisms.

Table 10: Regression values of gel formulations

Formulations	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Erosion (R^2)	Korsmeyer peppas	
					n	R^2
FGL1	0.6033	0.8104	0.9736	0.4687	0.7523	0.9515
FGL2	0.6729	0.8306	0.9683	0.4817	0.7415	0.9528
FGL3	0.7746	0.8659	0.9231	0.5567	0.7101	0.9678
FGL4	0.748	0.8386	0.9911	0.511	0.802	0.9584
FGL5	0.734	0.8566	0.9923	0.502	0.785	0.9601
FGL6	0.701	0.8633	0.9913	0.491	0.768	0.9581
FGL7	0.695	0.7357	0.9725	0.516	0.8437	0.9514
FGL8	0.6636	0.8523	0.9776	0.496	0.8186	0.949
FGL9	0.7235	0.7599	0.9816	0.510	0.818	0.953
FGS10	0.5834	0.9683	0.9737	0.4639	0.761	0.9513
FGS11	0.6576	0.8742	0.968	0.4808	0.7491	0.9522
FGS12	0.7982	0.8892	0.9598	0.5066	0.7069	0.9546
FGS13	0.7085	0.8836	0.9889	0.5008	0.803	0.9548
FGS14	0.6734	0.8707	0.9881	0.486	0.7848	0.9533
FGS15	0.646	0.8647	0.9787	0.477	0.7678	0.9517
FGS16	0.6608	0.805	0.9693	0.508	0.8492	0.9507
FGS17	0.6176	0.8802	0.9705	0.486	0.8221	0.9458
FGS18	0.6854	0.7727	0.9792	0.502	0.8219	0.9512

Best formulation obtained from in vitro drug release studies was selected for ex-vivo drug permeation studies (FGS10) using goat cheek pouch between donar and receptor compartment. FGS10 showed 75% drug release at the end of 6hr (Fig no: 15). The result from ex vivo permeation indicates drug release through biological membrane is sustained for prolonged period of time.

Stability studies:

The Optimized bioadhesive gel formulation (FGS10) was stored in three different temperatures 4 ± 2oC, 25 ± 2oC, 45±2oC. The stored formulation was evaluated periodically for drug content, pH and viscosity at predetermined time interval. There was no significant change in %drug content, pH and viscosity of bioadhesive gel formulation hence FGS10 was stable even after 30 days of study(table no:11).

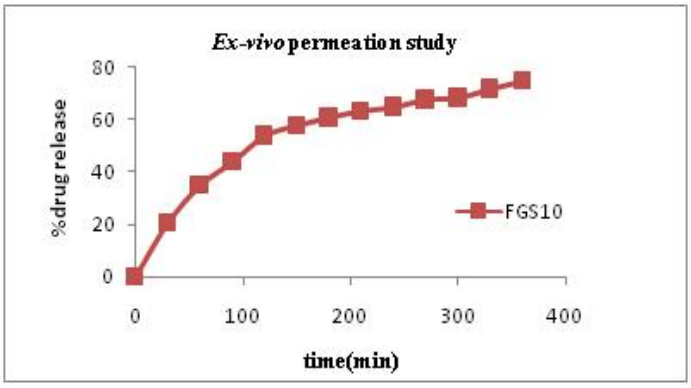

Figure 15: Ex-vivo drug release profile of FGS10 formulation

Table 11: Stability studies of optimized formulation (FGS10)

S.No.	Temp.	Drug Content (%)			pH			Viscosity (cps)		
		Days								
		0	15	30	0	15	30	0	15	30
1	4-8^{0}c	96.1	95	94.12	6.72	6.65	6.62	620	621	623

4. Conclusion

The formulated Ketorolac tromethamine loaded gelatine microspheres for sustain release was found to be potential and effective in terms yield, encapsulation efficacy, particle size and *in-vitro* release characteristics. The gel formulation , which consisted of drug loaded gelatine microspheres, showed sustain release of Ketorolac tromethamine ≥ 6 hr, thus indicating their suitability for the sustained delivery of the drugs for the treatment of inflammation in periodontitis. However, further studies, including clinical tests are required to confirm the gel's therapeutic efficacy.

5. References

1. http://www.nidcr.nih.gov/OralHealth/Topics/GumDiseases/PeriodontalGumDisease.htm.
2. Palak V Patel, Dhiren J Daslaniya, Upendra L Patel and Ragin R.Shah , Formulation and Evaluation of Bioadhesive Gel Incorporated Amoxicillin Trihydrate Loaded Microspheres for Periodontal Therapy, International Journal of Pharmaceutical Innovations, May- June **2013**, 3(3).
3. Rooks WH II, Maloney PJ, Shott LD et al. The analgesic and anti-inflammatory profile of ketorolac and its tromethamine salt. *Drugs Exp Clin Res*., **1985**, 11: 479-92.
4. Wakode R and Bajaj A. Gelatin microspheres for topical delivery of Vitamin A palmitate. NHSM Journal of Pharmacy and Healthcare Management **2011**, 2: 61-65.
5. K Karthikeyan, R Durgadevi, K Saravanan, K. Shivsankar, S. Usha and M Saravanan Formulation of Bioadhesive Carbomer Gel Incorporating Drug-Loaded Gelatin Microspheres for Periodontal Therapy Tropical Journal of Pharmaceutical Research June, **2012**, 11(3): 335-343
6. Hemlata Kaurav, S. L. Hari Kumar and Amanpreet Kaur Mucoadhesive Microspheres as carriers in Drug Delivery: a Review *International Journal of Drug Development & Research April-June,* **2012** 4(2).
7. P Chinna Reddy, K S C Chaitanya, Y Madhusudan RaoA review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods journal of Faculty of Pharmacy, Tehran University of Medical Sciences, **2011**, 19(6): 385-403.
8. Sanjay Singh, Rabinarayan Parhi, and Anuj Garg Formulation of Topical Bioadhesive Gel of Aceclofenac Using 3-Level Factorial Design Iran J Pharm Res., **2011** Summer; 10(3): 435–445
9. Nayak Bhabani Shankar*a, Rout Prasant Kumara, Nayak Udaya Kumarb and Bhowmik* Benoy Brata*c* Development and Characterization of Bioadhesive Gel of Microencapsulated Metronidazole for Vaginal Use Iranian Journal of Pharmaceutical Research, **2010**, 9 (3): 209-219
10. Karavana, Sinem Yaprak · Güneri, Pelin · Ertan, Gökhan Benzydamine hydrochloride buccal bioadhesive gels designed for oral ulcers: preparation, rheological, textural, mucoadhesive and release properties. Journal Pharmaceutical development and technology, **2009.**
11. Hitesh Chavda, Ishan Modhia, Anant Mehta,Rupal Patel and Chhagan Patel Development of Bioadhesive Chitosan Superporous Hydrogel Composite Particles Based Intestinal Drug Delivery System BioMed Research International **2013,** 5(3): 215-221.

12. P. Bottenberg, R. Cleymaet, C. P. Muynck. Development and testing of Bioadhesive, fluride containing slow release tablets for oral use, J Pharm Pharmacol, **1991**, 43: 457-464.
13. S. Senel, G. Ikinci, H. Akincibay, S. Kar, S. Erics, C. G. Wilson, et al. Effect of chitosan on periodontal pathogen porphyromonas gingivalis, Int J Pharm., **2002**, 235: 121-127.
14. P. Nagaraja, K. R. Sunitha, R. A. Vasantha, H. S. Yathirajan. Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations, Journal of Pharmaceutical and Biomedical Analysis, **2002**, 28(3-4): 527-535.
15. G. D. Gupta, R. S. Gaud. Release rate of nimesulide from different gallants, Indian J Pharm Sci., **1999**, 61(4): 227-230.
16. G. Alka, S. Garg, R. K. Khar. Measurement of bioadhesive strength of mucoadhesive buccal tablet: Design of an in-vitro assembly, Indian Drugs, **1992**, 30(4): 152-155.
17. Shoaib MH, Tazeen J, Merchant HA and Yousuf RI. Evaluation of drug release kinetics from ibuprofen matrix tablet using hpmc. Pak. Journal of Pharmaceutical Science, **2006**, 19: 119-124.
18. T. Higuchi, "Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices," Journal of Pharmaceutical Sciences, vol. 52, pp. 1145–1149, 1963.
19. R. W. Korsmeyer, R. Gurny, and E. Doelker, "Mechanisms of solute release from porous hydrophilic polymers," International Journal of Pharmaceutics, 1983, 15(1), pp. 25–35.
20. N. A. Peppas and J. J. Sahlin, "A simple equation for the description of solute release. III. Coupling of diffusion and relaxation," International Journal of Pharmaceutics, **1989**, 57(2): 169–172,.
21. G. W. R. Davidson and N. A. Peppas, "Solute and penetrant diffusion in swellable polymers V. Relaxation-controlled transport in P(HEMA-co-MMA) copolymers," Journal of Controlled Release, **1986**, 3(1–4): 243–258.