Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (3):889-896
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Thermoreversibal anesthetic gel for periodontal intrapocket delivery of mepivacaine hydrochloride

Aarti V. Daithankar*[1] and Mahendra R Shiradkar[2]

[1]*Department of Pharmaceutics, Pravara Rural College Of Pharmacy, Loni, Ahemadnagar, (MS), India*
[2]*Dr. Reddy's Lab Ltd, kukttpally, Hyderabad (AP), India*

ABSTRACT

Objective of the present study was to develop and evaluate thermoreversible in situ gelling drug delivery system for periodontal anasthesia. This system improves patient compliance by giving anesthesia at a site without distress of needle insertion. Thermoreversible gels were prepared by combining different concentration of pluronics F127 and F68. Formulations were evaluated in terms of their viscosity, gel strength, gelation temperature, stability and in vitro drug release. The formulations were stored at different temperature and humidity. The formulation has amide group anesthetic agent for delivery into periodontal pocket containing mepivacaine hydrochloride as a model drug. The prepared formulations are biocompatible and give effective release of anasthetic agent. The result for this study suggests that mepivacaine hydrochloride thermoreversible gel may offer an alternative to injection anasthesia.

Key Words: Mepivacaine hydrochloride, periodontal anesthesia, Pluronic F127, Pluronic F68.

INTRODUCTION [1, 2, 3]:

Periodontal disease are group of condition, including gingivitis and periodontitis, which affecting the supporting structure of the teeth such as gums, periodontal ligaments, alveolar bone and dental cementum. Plaque can spread and grow below the gum line. Toxins produced by the bacteria in plaque irritate the gums. The toxins stimulate a chronic inflammatory response in which the body in essence turns on itself and the tissues and bone that support the teeth are broken down and destroyed. Gums separate from the teeth, forming pockets (spaces between the teeth and gums) that become infected. As the disease progresses, the pockets deepen and more gum tissue and bone are destroyed.

The treatment of periodontal disease begins with the removal of sub-gingival calculus (tartar) and biofilm deposits. A dental hygienist procedure called scaling and root planning is the common first step in addressing periodontal problems, which seeks to remove calculus by mechanically scraping it from tooth surfaces [4, 5].

Scaling and root planning procedures is unpleasant and painful. The practice of modern dentistry is inconceivable without the application of local anesthesia. Anesthetic techniques used for this are nerve block/infiltration an aesthesia in combination with topical anesthesia. For effective anaesthetic effects anaesthetic agents should retained in periodontal pockets throughout the procedure. To improve residence time insitu gels show promising effect.

Advantage of thermoreversible gel has brought a new phase into dentistry especially into periodontology and pediatric dentistry where its use has drawn lot of success and patients acceptance.

Poloxamers or pluronic are the triblock copolymers which forms micelles at low concentration and clear thermoreversible gel at a high concentration. The concentrated solution (16-30%) is transformed from low viscosity

transparent solution at a 5^0C to a solid on heating at body temperature. By modulating the gelation temperature of different solutions of F127 and F68 liquid bases for periodontal use can be formulated that form a gel in a periodontal pockets at a body temperature resulting in the enhancement of the residence time in the periodontal pockets.

Mepivacaine hydrochloride, a tertiary amine used as a local anesthetic, is 1-methyl-2´, 6´ pipecoloxylidide monohydrochloride. It stabilizes the neuronal membrane and prevents the initiation and transmission of nerve impulses, thereby effecting local anesthesia.

The objectives of the present study are to develop insitu thermoreversible gelling system of mepivacaine hydrochloride suitable for periodontal pocket administration, which would enable a patient to have painless treatment without distress of injection.

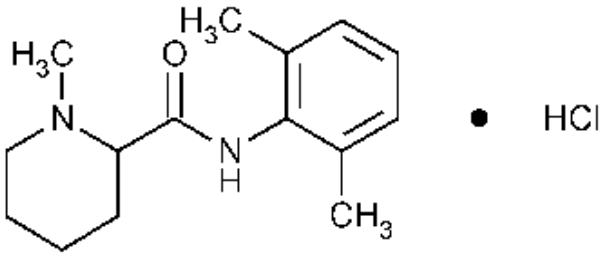
MATERIALS AND METHODS

To accomplish the objectives, gel forming solutions by phase transition (sol-gel transition), mediated by temperature were formulated using F127 and F68 polymer. Poloxamer is a triblock polymer consisting of polyoxyethylene–polyoxypropylene– polyoxyethylene units, and is used both internally and externally in various products that are designed for animal and human uses.

Mepivacaine hydrochloride (Mepivacaine HCl) was obtained from sigma Aldrich India. Pluronic F127 and F68 was obtained as gift sample from Signet chemical corporation Pvt. Ltd. Mumbai. Methyl paraben and Triethanolamine were procured from Loba Cheme, Mumbai, India. All other reagents used were of analytical grade and were used as procured.

Mepivacaine hydrochloride
Mepivacaine Hydrochloride, a tertiary amine used as a local anesthetic, Mepivacaine is an anesthetic that blocks the nerve impulses that send pain signals to brain.

Mepivacaine is used as a local anesthetic for an epidural or spinal block. It is also used as an anesthetic for dental procedures.



Chemical name: 1-methyl-2', 6' - pipecoloxylidide monohydrochloride
Molecular formula - 1-methyl-2', 6' - pipecoloxylidide monohydrochloride
Mol. Weight – 282.81

Preparation of Gel [6]:
Pluronic gels were prepared according to the “cold method” first described by Schmolka. The main advantage of this “cold method” compared to procedures using elevated temperatures, is the suitability for thermo-labile drugs, which can be incorporated at any preparation stage.

Table1: Composition of formulations

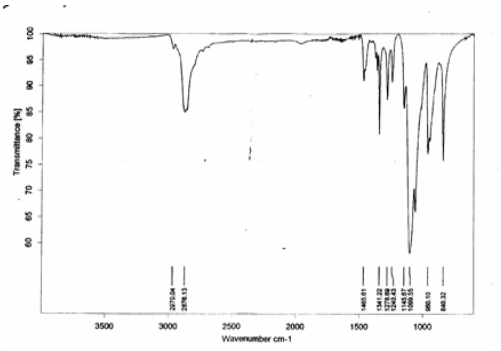
Ingredients (% W/W)	M1	M2	M3	M4	M5	M6
F 127	15	17	18	20	25	30
F 68	10	10	10	10	10	10
Drug	5	5	5	5	5	5
Preservative	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Water (Ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Pluronic F127 and F68 are more soluble in cold water than hot water. In situ gel containing 5% mepivacaine HCl was prepared using different concentrations of F127 and F68 by cold method. Various concentrations of pluronic F127 (15%, 17%, 18%, 20%, 25%, 30%) was mixed with constant concentration of F68 (10%) gel was slowly added to cold water, constant stirring was maintained. Each dispersion was refrigerated at 4°C to complete polymer desolvation until a clear solution was formed. Preservative was added and formulation was adjusted to neutral pH with triethanolamine (quantity sufficient). All gels were stored and cold temperature and evaluated within 48 hr.

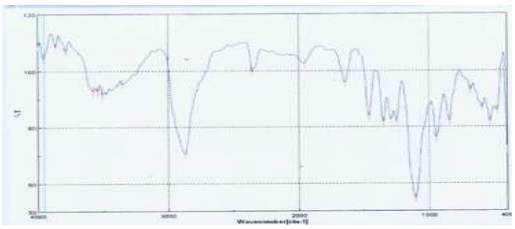
EVALUATIONS OF GEL FTIR SPECTROPHOTOMETRY [7-17]-

IR of Polymer and Drug

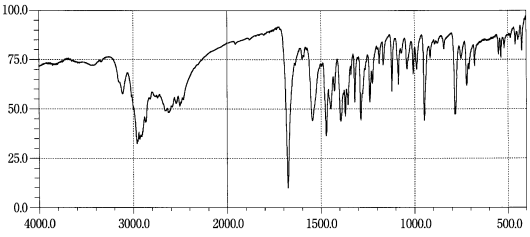
To evaluate the purity and compatibility of the drug in the formulations, IR spectra of the drugs and polymer were obtained by FTIR spectrophotometer.



IR Spectra of PF 127



IR Spectra of PF 68



IR Spectra of Mepivacaine Hydrochloride

Figure 1: IR Spectra of Drug and Polymer

Determination of Drug Content

The prepared formulations (M1 to M6) were analyzed for drug content by taking 1 mL of gel in 100 mL volumetric flask, dissolved and the volume was made upto 100 mL with 7.4 phosphate buffers. From the above solution 4 mL was pipetted out into a 10 mL volumetric flask and volume was adjusted with 7.4 phosphate buffer. Absorbance was measured at 214nm. Each formulation was subjected to pH measurement using pH meter (manufactured by Toshwan Industries Ltd, Ajmer.) which was previously calibrated using standard buffers of pH 4 and pH 7.

Determination of Gelation Temperature by Miller and Denovo Tech

A 2 ml aliquot of gel was transferred to test tubes immersed in water bath at 4^0C and sealed with aluminum foil. The temp of water bath was increased in increments of 1^0C.The samples were examined for gelation when the meniscus would no longer move upon tiling through 90^0C.

Measurement of Gel Strength
Gel strength was measured by method reported by Choi et al. 50 g of gel was placed in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A weight of 35g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink through the gel down by 5cm was measured.

Viscosity Studies
The viscosity studies of all the formulations (M1 to M6) were measured by using Brookfield digital viscometer (Brookfield DV II+, USA) with spindle number 94 at 50 rpm. Viscosity was measured at 4°C±1 and at 37±1°C using a thermo stated water jacket.

***In vitro* Release Studies**
Dialysis bag prepared from cellulose tubing with diameter of 2.3 cm and extension of 3.5 cm were filled with 1 g of gel. Gingival crevicular fluid (GCF), inflammatory exudate flows continuously in the pocket. The pH of GCF is 7.2 to 7.6 and a mouth saliva pH 6.4 to 7.4. Hence in the present study, phosphate buffer pH 7.4 was used for the in-vitro drug release studies of the gel. The bag was individually immersed in the recipient containing 60 mL of the receptor media. The media was maintained at 37 °C. At appropriate time intervals samples were taken from the receptor media and assayed by HPLC to determine the amount of bupivacaine released from the gel. Samples were analyzed spectrophotometrically at 214 nm.

Analysis of drug release data[18]
The data obtained from the *in vitro* release experiments were analysed by the following commonly used exponential equation.

$M_t/M=kt^n$ ……. [1]
Log M_t/M= log k + n log t

Where,
M_t/M= the fraction of released drug at time t k: release constant incorporating structural and geometric characteristics of the drug/polymer system. n: release exponent indicative of the release mechanism.

When n is equal to 0.5, the drug is released from the polymer with a Fickian diffusion mechanism (Higuchi model). If 0.5 < n <1 this indicates anomalous or non-Fickian release, while if n=1 this indicates zero order release.

***In vivo* study [19-21]**
Clinical evaluation of formulation was randomized, parallel- group ,double blind study using comparing anesthetic gel and placebo gel in pain sensitive patient. The study was approved by Institutional ethical committee of Pravara Institute of Medical Sciences, Loni and performed in department of Periodontology, Rural Dental College. Participants were require to present with quadrant with minimum of five teeth that had not received periodontal debridement in the last twelve months. The selected quadrant were require to have one or more periodontal pocket with depth of 5mm or greater.

Methodology of Evaluation
Fourty patients were screened for scaling and root planning. During the treatment, the chosen quadrant had undergone SRP along with either anaesthetic /placebo gel. The anaesthetic/placebo gel was placed into the periodontal pocket with a blunt ended, needle shaped applicator. (23 gauge, 0.6mm). Following a wait period of 3 -5 min SRP was performed. If the patient had any discomfort, a second application of the gel was done. A maximum of two applications of either anaesthetic /placebo gel was done per tooth and later SRP was carried out. If the SRP procedure was still painful after reapplication, no further application of the gel was done and the subject was classified as patient requiring rescue anaesthesia, which was an efficacy parameter in the study. At the end of the SRP the patient was asked to rate the overall pain perception on a visual analog scale (VAS) and verbal rating scale (VRS). Adverse events were monitored throughout the treatment period and at follow up visit.

EVALUTION PARAMETER OF PAIN MEASUREMENT
Visual Analog Scale (VAS)
After the SRP the overall pain was assessed by the subject using a 100 mm (10 cm) horizontal scale, with the left end point marked “no pain” and the right end point marked “worst pain imaginable” as the primary efficacy parameter.

Verbal Rating Scale (VRS)

As the secondary efficacy parameter the overall pain from the SRP was assessed using a 5-point verbal rating scale: no, mild, moderate, severe and very severe pain. After the SRP pain had been assessed by VAS, the patient was asked to rate the overall pain on the VRS in response to the question, "How much pain did you feel during the SRP procedure? The alternative that best describes the pain was chosen." The assessment of the VAS pain score (primary efficacy parameter) was always made before the VRS pain score (secondary efficacy parameter) to avoid influence of an already expressed verbal statement. Statistical methods Descriptive statistics were performed on demographic characteristics; age, gender. t–Test was performed on baseline scores, VAS and VRS scores. The test was 2- tailed, with a confidence interval of 5%.

RESULTS

Drug Content and pH of Gel and Measurement of Gel Strength

Drug content of all formulation was found to be in range of 98-100%. It is known that the normal physiological pH of GCF is 7.2 to 7.6 and a mouth saliva pH 6.4 to 7.4. The pH of all gel formulation was found to be in a range of 6.5-7.5 that is between physiological ranges of pH of mouth saliva. The gel strength of anesthetic gel formulation at 37^0C increased as the concentration of Pluronic increased. The mechanism of the increase gel strength might be related to hydrogen bonding between Pluronic and in the gel. It is observed that the thickening power of poloxamer in water increases as the hydrophobe molecule weight increases and as the ethylene oxide/propylene oxide ratio increases IR spectra of drug and polymer show purity and compatibility of drug and polymer in formulation. Drug content of all formulation was in the range of 98.19 to 100.12%. Content uniformity studies showed that the drug was distributed uniformly in all formulations. Gelation temperature of formulation was in the range of 21 ± 0.7^0c to above 41±.3 ^{0}c. Viscosity study of all formulation was in the range of 422±0.58to 8056±0.82

Table 2: Drug Content, Gelation Temperature, pH, Gel strength and Viscosity of formulation

Formulation	Gelation temperature	pH	Viscosity in (cps) at37 ± SD	Drug content %	Gel strength in seconds
M1(15%/10%)	41±.3	6.5	422±0.58	99.82±.12	42
M2 (17%/10%)	34.2 ± 0.5	6.7	3258±0.25	99.68±.26	76
M3 (18%/10%)	36 ± 0.5	6.9	6934±0.27	100±.12	98
M4 (20%/10%)	28 ± 0.5	6.3	7345±0.52	98.19±.45	108
M5 (25%/10%)	24 ± 0.7	6.8	7785±0.36	100±.62	140
M6 (30%/10%)	21 ± 0.7	6.6	8056±0.82	98.78±.86	170

Stability Study

The formulation M3 showing optimum gelation and viscosity at body temperature was selected for the stability study. A sufficient quantity of gel solution in glass vials was stored in desiccator's containing saturated solution of sodium chloride to maintain an approximate relative humidity of 60 ± 5%. The desiccator was kept at room temperature (30 ± 2°C) and samples were withdrawn at 0, 30, 60, 90 days. The physical stability of the gel was inspected periodically by checking clarity, gel temperature, viscosity, pH, and drug content.

Table3: Stability study of optimized formulation

Sr no	Days	Drug content	P^H	Viscosity	Gelation temperature
1	0	99.98	6.9	6934±0.27	36 ± 0.5
2	30	99.95	6.9	6936±0.27	36 ± 0.8
3	60	99.93	6.8	6933±0.27	36 ± 0.3
4	90	99.91	6.7	6938±0.27	36 ± 0.2

Rheological Studies

Viscosity measurement of the formulations at 4^0 and 37^0c temperatures showed that there was increase in viscosity with increase in temperature. This indicated the formation of temperature induced gel structure of pluronic. At constant concentration, abrupt changes in viscosities were observed due to sudden rise in micellar concentration. At low temperature region the liquid shows a very slight decrease in viscosity which was attributed to the dehydration of PPO blocks of the unimers. With rise in temperature the unimers start to form spherical micelles causing increase in intrinsic viscosity as a result of extremely high salvation in the micellar shell.

***In vitro* Release Analysis**

Among the different experimental protocols proposed for the determinations of drug release profiles, the dialysis method was chosen for its simple experimental procedure and high degree of reproducibility. The dialysis technique could reproduce the situation of a formulation applied into the periodontal pocket; in this case, the carrier is

presumably surrounded by a stagnant layer, causing a slow diffusion of the drug (i.e., non-sink conditions). Kinetic analyses of in vitro drug release data for all formulation were studied. The drug releases was diffusion control as best fit model was Higuchi's diffusion model. All the cumulative percent release vs. square root of time plots were straight lines with correlation coefficients ranging from 0.9973to 0.9999 (Figure 2, Table 4). Gelation temperature studies showed that formulation M3 with 18% F 127 and 10% of F68 was found to be most suitable with gelation temperature near to body temperature. It was reported that gelation temperature decreased with increase in concentration of F 127. The results of in vitro release studies showed that with increase in concentration of F 127, the rate of drug release decreased. The release of drug from the formulated gels was following diffusion controlled without swelling. One of the important objectives of this research was that anaesthetic effect should last for longer duration for effective completion of SRP and gelation temperature should be near to body temperature. So the formulation M3 was selected as optimized formulation for clinical evaluation.

Table 4: Correlation Coefficient of Higuchi's And Peppas Model

Formulation Code	Zero Order	Higuchi's model	Pappas's model	"n" values
M1	0.8496	0.9881	0.9689	0.4396
M2	0.8688	0.9988	0.9658	0.4595
M3	0.8499	0.9999	0.9678	0.4994
M4	0.8731	0.9998	0.9702	0.4815
M5	0.8943	0.9973	0.9756	0.4899
M6	0.8680	0.9979	0.9726	0.4739

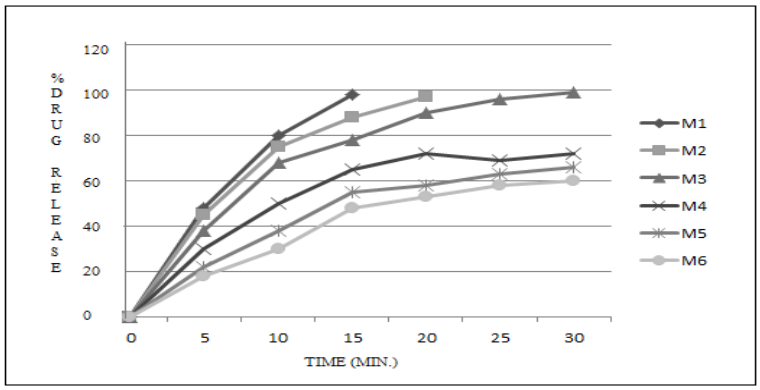


Figure 2: Drug Release Profile of Formulations

Table 5: Clinical finding in patient undergoing SRP

Base line parameter	Formulation group0{N=20}	Placebo group {N=20}
Median age { years}	38 (18-58)	39 (21-57)
Gender (numbers) **Male** **Female**	 12 08	 14 06
Mean pocket depth (mm)	4.7±0.3	4.9±0.1
Percent pocket with bleeding	41±28	42±27
Percent hypersensitive teeth	11±23	11±23
Percent pocket with Pus	0±0	0±0

Clinical Evaluation

The mean age ± standard deviation for the group receiving the active gel was 38±10 yrs. and that of receiving the placebo gel was 39±10 yrs. The median age (range) for active was 38 (18-58) yrs. and for placebo was 39 (21-57) yrs. The patients in the active gel group had 2 to 5 teeth in the treated quadrant with a mean pocket depth of 4.7±0.3mm. In the placebo group, the patient had 2 to 5 teeth in the treated quadrant with a mean probing depth of 4.9±0.1 mm. The mean percentage of pockets with bleeding on probing was 41±28 in active gel group and 42±27in placebo group. Overall VAS pain score, assessed after completion of SRP in the selected quadrant, was 7mm in anaesthetic gel group and placebo gel group was found to 21mm. The statistical analysis indicated a trend towards higher VAS pain scores in placebo group during probing. But, no such observation was in active gel group and no patient required rescue anaesthesia where in placebo gel group 12 out of 20 patients required rescue anesthesia. Anesthesia also helps in reducing bleeding at operating site due to its action of constriction of blood vessel. From

the VRS 75% of subject of test group reported no or mild pain. While in the placebo group nearly all patients reported having moderate to severe pain.

DISCUSSION

Various formulations of different concentrations of pluronic F127 and F68 was prepared. The gelation temperature was important criteria in optimization of formulation. It is observed that the gelation of F127 and F68 was found dependent on aqueous solubility of the polymer. Above 16% w/w concentrated aqueous solutions F127 and F68 exhibit reverse thermal gelation. As the temperature increases, micellar entanglement is promoted, leading to gel formation and hence overall increase in bulk viscosity. Temperature plays an important role in the micelle formation of F127 and F68 through the temperature-dependent hydration of the ethylene oxide units. Water is good solvent for PEO as well as PPO chains of polymer at low temperatures. However, at higher temperature the solubility of PPO is reduced and micelle formation occurs. The decrease in the gelation temperature exhibited as F127 and F68 concentration increased. It may be attributed to the higher number and volume occupied by micelles at lower temperature. As the concentration of F127 and F68 increases, the gel structure becomes more closely packed with the arrangement in a lattice pattern. In turn, the disruption of the lattice melting of gel occurs at higher temperatures. Temperature dependent changes in micellar properties have been related to the reversible thermal gelation of F127 and F68. The subsequent dehydration and increased end chain friction causes the gel formation. The phenomenon may be mediated through modification of micellar association of the F127 and F68 molecule. The gender distribution was similar between the active group and placebo group. The statistical analysis of baseline characteristics in both the groups (active gel group and placebo group), indicated that both the groups were almost similar in all the parameters. The statistical analysis showed that VAS and VRS pain scores in the active gel group were significantly lower than those in the placebo group. So, clinical studies of formulationM3 showed that, local anaesthetic gel 5% was overall significant and more effective than placebo. The results suggested that mepivacaine HCl gel 5% was clinically effective in reducing SRP pain for those patients who perceive the procedure to be painful.

There is good compatibility between pluronic F127 and F68 and mepivacaine hydrochloride In vitro diffusion of mepivacaine through the Pluronic gel was slow. As the Pluronic F127 and F68 consist of a large population of micelles in aqueous phase, the incorporated drug may be released by diffusion through gel matrix. Drug release can be affected by the viscosity of the gel, the size of the aqueous channels and the distribution of the drug between the micelles and the aqueous phase. Mepivacaine release from the gel follows the Higuchi square root law (Higuchi, 1962) because drug release concentrations increase with the square root of the time ($r > 0.99$). In this kinetic model, the release process is dependent on formulation characteristics. The concentration of Pluronic F127 and F68 in the gel has a strong influence on the drug release rate. Increasing the amount of polymer in the gel increases its viscosity and reduces the drug release rate and diffusion coefficient. M4 and M5 formulation show very slow release rate. M3 formulation was considered as optimized formulation as gelation temperature, release rate and duration of action of anasthesia was sufficient for intended purpose. M3 formulation is easy to apply and does not interfere with SRP shows no clinical signs of mucous membrane irritation and its taste does not affect the patient willingness to have the gel at their next visit.

CONCLUSION

The optimized formulation is well tolerated for local anasthesia of the peridontium. In the study population, the active gel was overall statistically significantly more effective in reducing pain associated with periodontal debridement for those patients who perceive the procedure to be painful. Pluronic gel proved to be a promising carrier for prolong and effective release of mepivacaine hydrochloride throughout the dental procedure. The result for this study suggests that mepivacaine hydrochloride gel may offer an alternative to injection anasthesia.

Acknowledgements
The authors are grateful to sigma Aldrich (AstraZeneca) and Signet chemical corporation Pvt. Ltd. Mumbai for providing us drug and polymer as a gift sample respectively. We are also thankful to Pravara Medical Trust and Pravara Rural Dental College for providing essential facility for in vivo study.

REFERENCES

[1] Listgarten M. A., *J. Periodontol Res*. 22(**1987**): 172-178
[2] Vyas S. P., sihorkar V., Mishra VJ. *Clin. Pharma Ther*. 25(**2005**):21-42
[3] Linden ET, Abrams H, Matheny J, Kaplan AL, Kopczyk RA, Jasper SJ *Jr. J Periodontol* **1986**; 57:637-42.
[4] Dunsky JL, Moore PA. *J Endod* **1984**; 10:457-60.

[5] Reader A, Nusstein J, Hargreaves KM: In: Cohen S, Hargreaves KM: Pathways of the pulp, 9th Edition. St, Louis: CV Mosby, **2006**: pp. 700.
[6] Schmolka, I. R., *J. Biomed. Mater. Res.*, 6, 571-582, **1972**.
[7] Akimoto T and Nagase Y (**2002**). *Dept. Applied Chem.*, pp.1259-1292.
[8] Vadnere N., Amidon G. Lindenbaum S., et al. *Int. J. Pharma* 22(**1984**):207-218.
[9] Pravin Kumar, Awasthi R, Rai PK, Manish Kumar MK, Pramod Kumar TM Scholars Research Library *Der Pharmacia Lettre*, **2010**; 2(4): 28-39.
[10] Cho CW, Choi JS, Shin SC, *Pak. Journal Pharm. Sci*., January **2011**; .24(1):87-93.
[11] Wu C, Qi H, Chen W, Huang C, Li L, Chen C, et al. *Int J Pharm*, **2007**;337: 178-87.
[12] Kramaric A., Resman A., Kofler B., Zmitek J., **1992**. European patent 0551, 626
[13] *J. Phys. Chem*. 95, **1991**; 1850-1858.
[14] Miller S. C., Drabic B. R. *Int. J. Pharm*, 18, **1984**: 269-276.
[15] Ricci EJ, Lunardi L, Nanclares DMA, Marchetti JM. *Int J Pharm*. **2005**; 288:235Y244.
[16] Ahuja A., Ali J., Rahman S., *Pharmazie*. **2006**; (61): 25-29.
[17] Bohorquez, M., Koch, C., Trygstad, T., and Pandi, N., *J. Colloid Interface Sci.*, 216, 34-40, **1999**.
[18] Peppas NA. *Pharm Acta Helv*. **1985**; 60:110-1.
[19] Peter Svensson, Jens Kolsen Petersen, Helle Svenssont, Anesth Prog 41:35-39, **1994**.
[20] Marjorie K. Jeffcoat, Nico C. Geurs, Ingvar Magnusson, Simon R. Macneill, Nancy Mickels, Frank Robinson, Afshin Salamati, Ray Yukn *J Periodontol* july **2001**, 895-900
[21] Ingvar Magnusson, Nico C. Geurs, Pearl A. Harris, Arthur F. Hefti, Angelo J. Mariotti, Sally M. Mauriello, Lina Soler, Steven Offenbacher, *J Periodontol* May **2003**, 597-602.