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Formulation and Evaluation of sub dermal Implants containing NSAID

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ABSTRACT

The concept of implantable therapeutic system for the long term therapy is a newer and developing area in drug research programmes Subcutaneous route is selected for the sustained and prolong release of drugs, non steroidal anti-inflammatory drugs like, Piroxicam are model drugs used subcutaneous route is selected because it contains less no. of nerve network which should be helpful for implantation this route contains max. rate of drug absorption with less no. of side effects .It avoids first pass effect metabolisms, & other type of drug loss through different organs of the body. In the present piece of investigation an attempt has been made to design and develop some biodegradable sub dermal implants. Piroxicam a non steroidal anti inflammatory drugs has been selected to prepare rod shaped implants with the help of Gelatin and Chitosan its cross linking nature with glutaraldehyde. The implants were evaluated for drug content uniformity, thickness, weight variation, in vitro studies and stability studies at ambient temperature for three months implants were found to erode slowly with diffusion mechanism.

Key words: implants, chitosan, gelatin, Piroxicam.

INTRODUCTION

Historically, the subcutaneous implantation of drug pellets is known to be the first medical approach aiming to achieve prolonged and continuous administration of drugs. The pellets can be readily implanted into subcutaneous tissue by means of a Kearns pellet injector or by making a small incision. pellets were formulated without any binders, diluents, to permit total dissolution and absorption of the pellet from the site of implantation [1]. the concept of implantable therapeutic systems for long-term, continuous drug administration with the development of a subcutaneously implantable drug pellet. The technique was then rediscovered in 1936 by densely and parkes [2] who administrated crystalline hormones in the form of solid steroid pellets to mimic the steady, continuous secretion of hormones from an active gland for hormone substitution therapy[3]. The subcutaneous release rate of steroids from the pellets implantation was found to be slowed and hormonal activities were prolonged by dispersing the steroids in cholesterol matrix during pellet fabrication [4] .unfortunately, it was observed that the subcutaneous absorption of steroids from the cholesterol pellets varies greatly from one condition to another. The subcutaneous drug administration by pellet implantation method was then subjected to modification by several investigators[5].

The present work concentrate on making polymeric implants employing, gelatin chitosan, glycerine along with other excipients. Piroxicam(NSAID) was chosen as a model drug. implants containing Piroxicam were prepared and evaluated for various physico-chemical parameters like weight variation, thickness, drug content uniformity,

presence of free glutaraldehyde, drug polymer interaction, sterility test. In vitro dissolution rate studies were performed on the implants by using phosphate buffer pH 7.4

MATERIALS AND METHODS

Piroxicam was obtained as a gift sample from Bal Pharma Lab Pvt. Ltd; Bangalore. Gelatin was purchased from S.D. Fine Chemicals Ltd; Mumbai. Glycerin and Glutaraldehyde was purchased from Loba Chemicals Mumbai. All other chemicals used were of analytical grade.

Procedure for preparation of sub dermal implants [6]:

Chitosan and gelatin based rod shaped implants containing Piroxicam were prepared by using following method.

Weighed quantity of gelatin and chitosan was sprinkled on the surface of the 0.1% acetic acid in a beaker and stirred well to avoid formation of lumps and allowed to hydrate for 24 hours. Now glycerin as plasticizing agent was added. In other beaker Piroxicam was dissolved in a little quantity of methanol and the solution is dissolved in the above polymeric solution and kept a side for 24 hours and extruded into rod shaped implants by galaxy extruder and dried at room temperature for 48 hours. After drying the implants cut into rod shaped of 6mm in length and 3mm in width.

Tabel no.1 Formula of Implants Prepared [13]

Ingredients	formulations		
	70:30	80:20	90:10
Drug	4 gram	4 gram	4 gram
Polymer(gelatin chitosan)	30 gram	30 gram	30 gram
glycerin	20 ml	20 ml	20 ml
Distilled water Q.S. to	100ml	100ml	100ml

Hardening of implants [7]:

Procedure:

25 ml of 37% v/v of Glutaraldehyde solution was taken in a 100 ml beaker and kept in an empty desiccator. On the top of the beaker a wire mesh containing the implants was kept and immediately the desiccator was closed.

The implants were made to react with glutaraldehyde vapors for different time intervals (,6,12,24 & 48 hours). Then they were removed and air dried for 72 hrs so that complete reaction of the Glutaraldehyde with gelatin and chitosan will take place, afterwards the implants were kept in an open atmosphere for a week, to make sure that the residual Glutaraldehyde gets evaporated.

3. Evaluation of subdermal implants:

a) Procedure for drug content uniformity test [9] :

Drug content of implants from every batch was estimated. From each batch of implants, 3 samples of 6 mm in size and 3 mm thick were taken and analyzed for Piroxicam.

The implant was cut into small pieces and were taken in 25 ml volumetric flask and methanol was added and heated at 60⁰ C to dissolve the drug after cooling the solution is filtered and the volume was made up to 25 ml with methanol. This solution was suitably diluted with methanol and assayed for Piroxicam content by measuring the absorbance at 348 nm. Piroxicam contents were calculated using the calibration curve.

The drug content data were subjected to statistical analysis to test whether the drug content was uniformly distributed in the implants and the reproducibility of the method was possible, Mean, Standard Deviation and Coefficient of Variation (C.V.) were calculated using the equation given below.

b) Method for measurement of implants thickness and weight variation [9]:

i) Thickness measurement of implants:

The thickness of implants from every batch were measured with the help of screw gauge and were subjected to the previously mentioned statistical analysis, 3 samples were taken for study from each batch.

ii) Weight Variation:

Samples of implants from each batch (n=3) were taken and weighed individually. The average weight and standard deviations were calculated.

Table no.2 Various parameters of implants (90:10) hardened with glutaraldehyde

Sr.no.	Hardening time hrs	Wt of implants + S.D.	CV	Thickness of implants (mm) + S.D.	CV	Drug content mg + S.D.	CV
1	6	70.30+ 0.130	0.184	3.04+ 0.0041	0.1359	9.83 + 0.106	1.07
2	12	70.33+ 0.173	0.246	2.98+ 0.0017	0.0581	9.69+ 0.269	2.77
3	24	71.66+ 0.093	0.130	3.03+ 0.0028	0.0924	9.32+ 0.247	2.65
4	48	72.90+ 0.090	0.123	3.10+ 0.0010	0.0322	9.45+ 0.283	2.99

*Each reading is a mean of three replicates.

*Each implant contains 10 mg of drug .

c) Drug polymers interaction study [8]:

The IR spectra of Piroxicam and its formulations were obtained by KBr pellet method using Perkin Elmer FTIR series model 1615 spectrometer.

The Subdermal implants of Piroxicam prepared with Gelatin and Chitosan and hardened with Glutaraldehyde were tested for compatibility of the drug with the excipients like, Gelatin, Chitosan, Glycerin hardening agents by I.R. Study. The I.R. Spectrum of the pure drug and the formulated implants were recorded.

d) Qualitative test for free Glutaraldehyde [9]:

To 1ml of 1 in 10 dilution preparation to be examined in a test-tube, 4ml of water and 5ml of acetyl acetone solution were added. The tube was placed in a water bath at 40⁰ C for 40 minutes. The solution was not intensely colored than a reference solution prepared at the same time and in the same manner using 1ml of standard glutaraldehyde solution in place of the dilution of the preparation being examined. The comparison should be made by examining the tubes down their vertical axis.

RESULTS

The sample solution was not more intensely colored than the standard solution inferring that less than 20mcg of free glutaraldehyde is present in 25 rod shaped implants

e) Sterility test I.P.[10]:

The sterility test was conducted by membrane filtration method by soybean- casein digest medium.

f) Procedure for *in-vitro* drug release study[11]:**Static dissolution studies:**

Implants were placed separately into a 10 ml vials containing 10 ml of phosphate buffer pH 7.4. the vials were sealed with rubber stoppers and kept in incubator thermostated at 37⁰C ±5⁰C. The dissolution fluid was changed for given time intervals and replaced with fresh 10 ml phosphate buffer pH 7.4. The drug concentration in every dissolution fluid was analyzed spectrophotometrically at 348 nm after suitable dilution with phosphate buffer pH 7.4.

Table-3 Calibration Curve Data for Piroxicam in Methanol ($\lambda_{max} = 348$)

Sl. No.	Concentration (mcg/ml)	Absorbance
1.	0	0.000
2.	2	0.144
3.	4	0.296
4.	6	0.436
5.	8	0.582
6.	10	0.728

Figure-1: Calibration Curve Data for Piroxicam in Methanol ($\lambda_{max} = 348$)

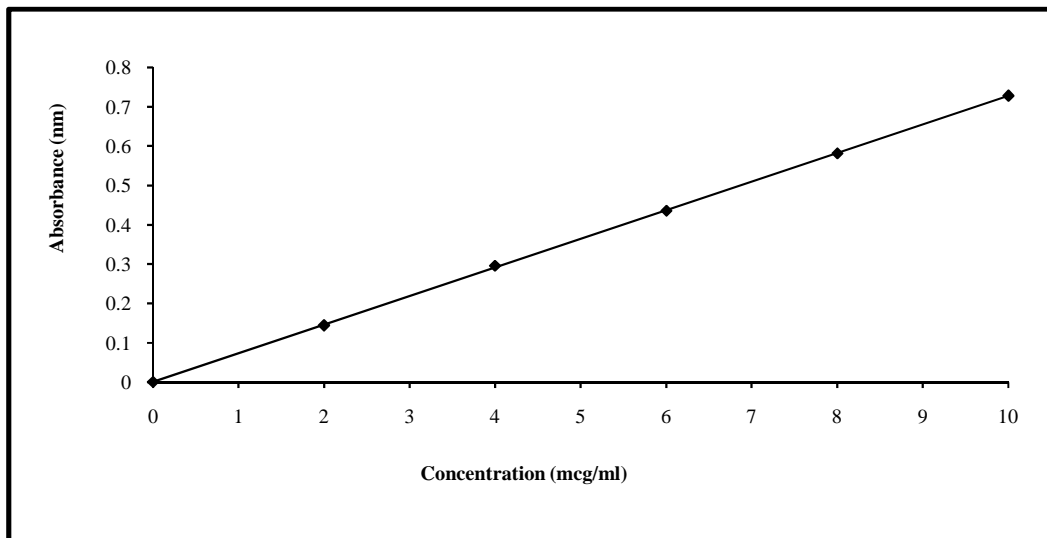


Table-4 Calibration Curve Data for Piroxicam in phosphate buffer pH 7.4 ($\lambda_{max} = 348$)

Sl. No.	Concentration (mcg/ml)	Absorbance
1.	0	0.000
2.	2	0.140
3.	4	0.287
4.	6	0.429
5.	8	0.574
6.	10	0.717

Figure-2: Calibration Curve Data for Piroxicam in phosphate buffer pH 7.4 ($\lambda_{max} = 348$)

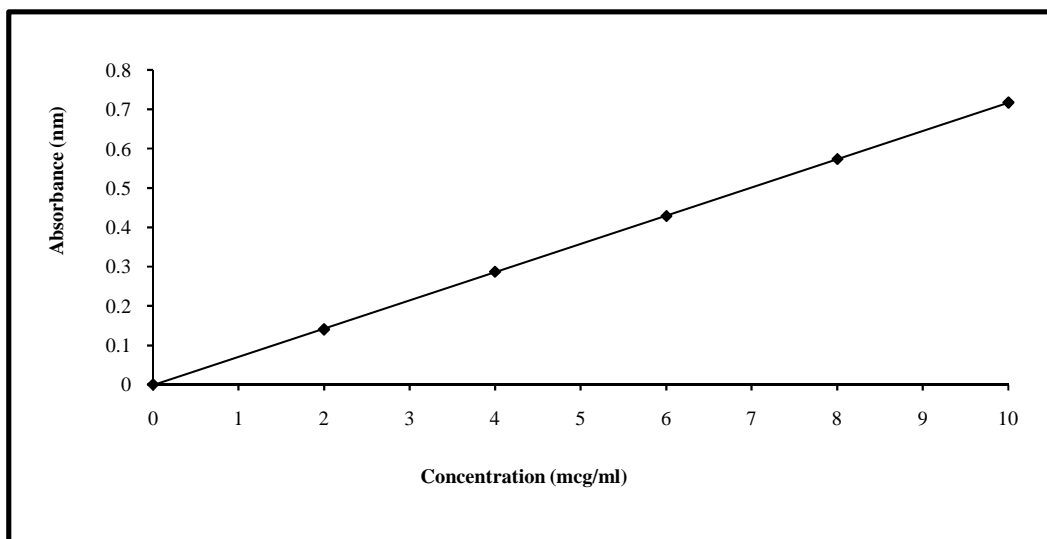


Figure-3: In Vitro Release of Piroxicam in Phosphate Buffer of pH 7.4 from implants prepared using 80:20 ratio of Gelatin and Chitosan and Hardened for 48 hours using Glutraldehyde

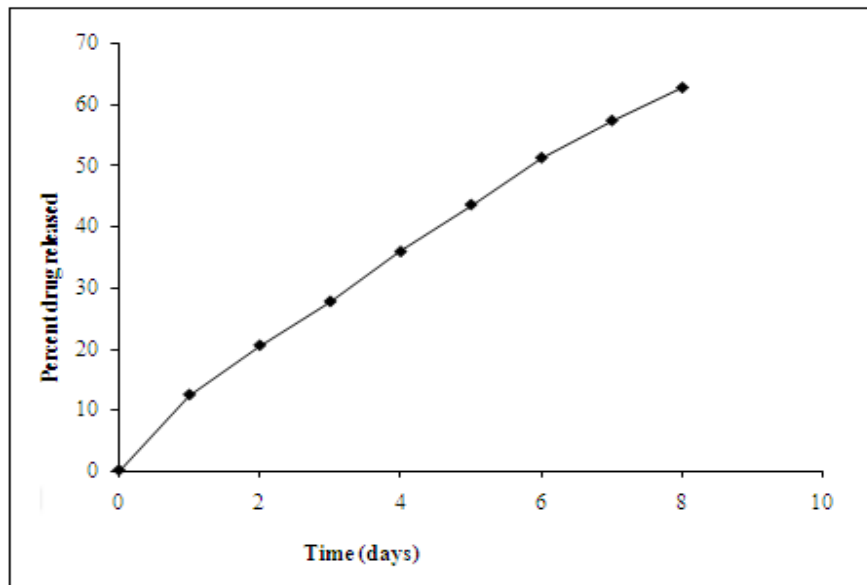


Figure-4: Higuchi's Diffusion Plot of Piroxicam in Phosphate Buffer of pH 7.4 from implants prepared using 80:20 ratio of Gelatin and Chitosan and Hardened for 48 hours using Glutraldehyde

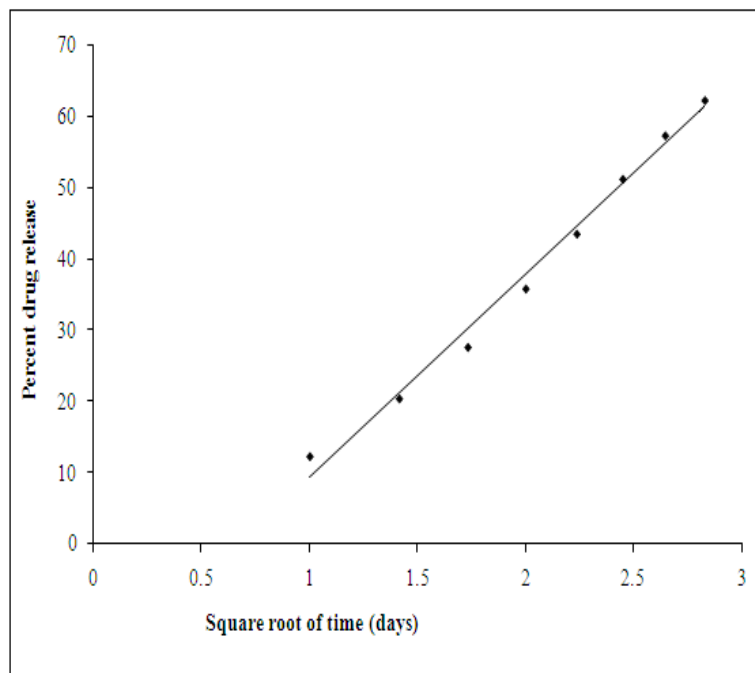


Figure-5: First Order Release Plot of Piroxicam in Phosphate Buffer of pH 7.4 from implants prepared using 80:20 ratio of Gelatin and Chitosan and Hardened for 48 hours using Glutraldehyde

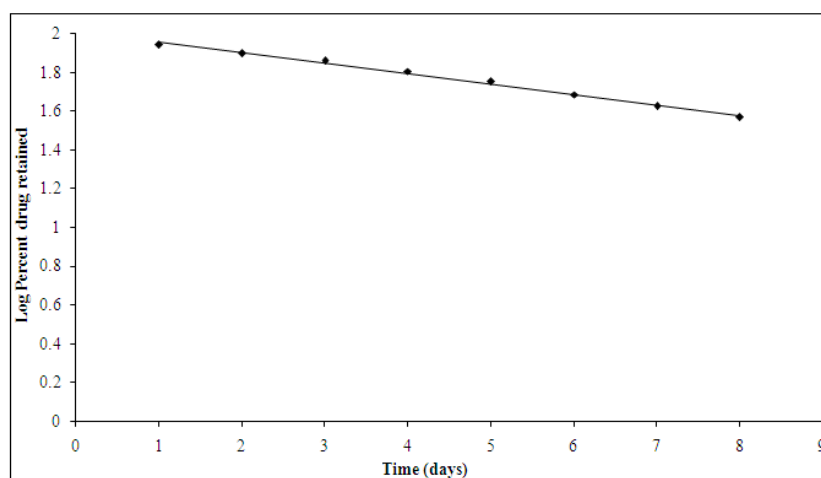


Table-5 IN VITRO RELEASE OF PIROXICAM IN PHOSPHATE BUFFER OF PH 7.4 FROM IMPLANTS PREPARED USING 80:20 RATIO OF GELATIN AND CHITOSAN AND HARDENED FOR 48 HOURS USING GLUTRALDEHYDE

Time (day)	Square root of time (day)	Absorbance	Concentration of drug (mcg/ml)	Percent drug released	Percent drug retained	Log percent drug retained
1	1.000	0.089	1.24	12.40	87.60	1.942
2	1.414	0.147	2.05	20.50	79.50	1.900
3	1.732	0.199	2.77	27.70	72.30	1.859
4	2.000	0.257	3.59	35.90	64.10	1.807
5	2.236	0.313	4.36	43.60	56.40	1.751
6	2.449	0.368	5.13	51.30	48.70	1.687
7	2.645	0.412	5.74	57.40	42.60	1.629
8	2.828	0.450	6.28	62.80	37.20	1.570

* Each reading is a mean of three replicates.

g) Stability studies [12] :

The stability of the discs was studied at ambient temperature. The discs (size 6 mm) were weighed individually and distributed in six sets (8 discs in each set.) The discs were wrapped individually in butter paper and placed in Petri dishes. The Petri dishes were stored at ambient temperature for a period of three months. The sample was analyzed for physical changes like colour, texture, appearance and the drug content was determined at an interval of fifteen days.

RESULTS AND DISCUSSION

Standard graphs of drug in methanol and phosphate buffer pH 7.4 has been shown in tables 3,4 and 5. Implants of Piroxicam were prepared employing Gelatin and Chitosan 80:20 ratio and hardened with glutraldehyde for 48 hours.

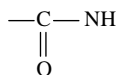
The Piroxicam implants prepared were evaluated for drug content, thickness, weight variation and drug release characteristics and results indicated as the implants were having uniform drug content 9.45mg and about 3.10 mm thickness with a mean weight of 72.90 mg.

The drug release studies of Piroxicam in phosphate buffer pH 7.4 indicated 62.80% of drug release was found in 8 days.

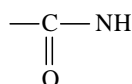
Gelatin and chitosan based subdermal implants contains a number of excipients apart the drug which is an active ingredient. So to check the purity of drug present in the formulation. IR spectral analysis has been carried out and compared with the pure drug sample.

Pure drug:

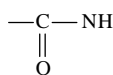
Broad peak at 3337.99 cm^{-1} is due O-H stretching and peaks at 1607.71 cm^{-1} and 1593.66 cm^{-1} are due to functional group confirm the drug.

**Implants without hardening:**

Broad beak at 3395.52 is due to – OH stretching and peaks at 1607.71 cm^{-1} and 1593.66 cm^{-1} are due for the functional group confirm the undisturbed drug in formulated.

**Implants hardened with glutraldehyde:**

Broad peak at 3405.59 cm^{-1} is due to O–H stretching and peaks at 1632.30 cm^{-1} and 1531.41 cm^{-1} are due to functional group confirm the undisturbed drug in formulation.

**CONCLUSION**

The following conclusions were drawn from the results obtained in the present study.

The implants prepared were translucent, smooth and elastic in nature.

Implants prepared were found to be uniform in diameter, thickness and weight of implants.

The I.R. study showed no interaction between the drug and excipient.

The stability study of implants prepared showed uniform drug content, thickness & weight.

In-vitro drug release from the implants of the present study in phosphate buffer pH 7.4 spread over a period of 8 days.

Drug release mechanism was found to be diffusion controlled following first order kinetics. Cross linking/hardening time of the implants shows prolonged release of the drug. Implants hardened with glutraldehyde gave sustained release. Thus the Gelatin and chitosan based subdermal implants containing Piroxicam could be prepared and the release can be modulated by varying the concentrations of polymers (Gelatin and Chitosan) and cross linking/hardening time with glutraldehyde.

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