Research Article

Characterization and In-vitro Evaluation of Drug Implants

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ABSTRACT

Recently subcutaneous implants are considered as a useful drug delivery system since they provide greater assurance of patient compliance and better therapeutic outcome than conventional drug therapies, especially in chronic disorders. Literature survey revealed that numerous studies have been conducted on subcutaneous implants to investigate use of different polymers for controlling the drug release for prolonged therapy, the pharmacokinetics and pharmacodyanamics of subcutaneous implants, the safety of the polymers. The science and engineering approaches in the development of implantable therapeutic systems have been well described in the literature.

In this investigation, it was planned to prepare biodegradable subcutaneous implants of Naproxen, the NSAID by using gelatin as polymer and glycerin as a plasticizer, under aseptic conditions. The subcutaneous implants, weighing 10 mg were hardened by exposing them to hardening agents such as Glutaraldehyde. The formulated implants were evaluated for thickness, wt variation, drug content uniformity, free Glutaraldehyde, drug polymer interaction and sterility. In vitro drug release studies were conducted in phosphate buffer pH 7.4. The stability studies were carried out at ambient temperature for 3 months.

Keywords: Naproxen, gelatin, subcutaneous implants.

INRODUCTION

Pellets were formulated without any binders, diluents, to permit total dissolution and absorption of the pellet from the site of implantation[1]. Lafarge pioneered, in 1861, 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 clinical use of implantable pellets for human health cares has declined in recent years. Currently, there are only a few steroid pellets still commercially available for medication: (a) testosterone (oreton pellet/ schering) (b) deoxycorticosterone acetate (pereorten pellet/ ciba) and (c) estradiol (progyon pellet/ schering)[6] on the other hand, the laboratory use of implantable pellets for experimental purpose has become quite popular[7].

MATERIALS AND METHODS

Naproxen was obtained as a gift sample from Divi's lab Pvt. Ltd. Hyderabad. Gelatin was purchased from S.D. Fine Chemicals Ltd; Mumbai. Glycerin and Glutaraldehyde were purchased from Ranbaxy Laboratories Ltd. Punjab. Glutaraldehyde was purchased from Loba Chemicals Mumbai. All other chemicals used were of analytical grade.

Preparation of implants[7]

Table 1: Formulae of Implants

Ingredients	Formulations		
	F_1	F_2	F_3
Naproxen	1 gm	1 gm	1 gm
Gelatin	20 gm	20gm	20 gm
Glycerin	10 ml	10 ml	10 ml
Water QS	100 ml	100ml	100 ml

F1 - Gelatin Grade-I, **F_2 -** Gelatin Grade-II,
F_3 - Gelatin Grade-III

Gelatin implantable discs containing Naproxen were prepared under aseptic conditions. Three formulations with different grades of gelatin are prepared. (Table 1).

Weighed quantity of gelatin i.e. Grade –I, II, and Grade –III Were sprinkled on the surface of the water and stirred well to avoid formation of lumps and allowed to hydrate for 30 minutes. Glycerin (10% as a plasticizer, was added to each formulation. It was heated on a water bath at 60^0c with continuous stirring until gelatin was dissolved in water completely. Weighed quantity of Naproxen for each formula was taken and dissolved separately in a small quantity of Methanol and this solution was mixed with FI, F2, & F3 each. This solution was poured in to a glass Petri dish to a 3 mm height and allowed to gel for 30 minutes by placing the glass moulds on ice and then dried at room temperature for 48 hours. After drying, the implants were cut into spherical discs of 6 mm size by specially designed stainless steel cutter.

Hardening of implants[8]

Glutaraldehyde solution (37% v/v) was transferred in a Petri dish and placed in an empty glass dessicator. A wire mesh containing the implantable discs was kept on the top of the Petri dish and immediately the dessicator was closed. The discs were made to react with Glutaraldehyde vapors for different time intervals i.e. 1, 3,6,12 & 24 hours. Then they were removed and air-dried for 72 hours so that the reaction between Glutaraldehyde and gelatin was completed. Afterwards the discs were kept in an open atmosphere in aseptic conditions for a week, to make sure that the residual Glutaraldehyde gets evaporated.

Fig. 2: Models of prepared disc shaped implants

3. Evaluation of sub dermal implants

1. Procedure for drug content uniformity test[11]

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 Naproxen.

The implant was cut in to small pieces and were taken in 25 ml volumetric flask and methanol was added and heated at 60^0 C to dissolve the drug after cooling.

Table 4: Drug content uniformity in gelatin based subdermal implants (mg)

Formula name	Drug content in (mg)			Mean	SD	CV
	I	II	III			
F1	10.05	10.09	10.15	10.09	0.002	0.020
F2	10.25	10.21	10.19	10.21	0.001	0.009
F3	9.87	9.77	9.97	9.87	0.010	0.100
F3 –G,(hardened with Glutaraldehyde)	9.72	9.69	9.75	9.72	0.001	0.010

2.Thickness measurement of implants[9]

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.

Table 2: Thickness of implants Hardened with Glutaraldehyde

Hardening time intervals in hours	Thickness in mm					
	I	II	III	Mean	SD	CV
1	3.01	3.06	3.03	3.03	0.0006	0.0208
3	2.99	2.95	3.05	2.99	0.0026	0.0650
6	3.10	3.04	3.00	3.04	0.0041	0.1359
12	2.97	2.95	3.03	2.98	0.0017	0.0581
24	3.09	2.99	3.01	3.03	0.0028	0.0924
48	3.10	3.12	3.09	3.10	0.0010	0.0322

3. Weight Variation[10]

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

Table 3: Weight uniformity of implants hardened with Glutaraldehyde [F3H]

Hardening time intervals in hours	Weight of discs in (mg)			Mean	SD	CV
	I	II	III			
1	70.09	71.01	71.00	70.70	0.2790	0.3940
3	71.05	71.01	71.00	71.02	0.0007	0.0009
6	71.20	70.80	71.50	71.16	0.1230	0.1730
12	72.50	72.00	71.90	72.13	0.1030	0.1430
24	74.00	73.80	74.20	74.00	0.0400	0.0540
48	75.80	74.90	75.10	75.26	0.4460	0.5930

4. Tests for sterility[12]

The sterility test was conducted by membrane filtration method on soyaben- casein digest medium and found to be implants are sterile.

Analytical instrument and method

For determination of drug content uniformity, the implants were analyzed by using UV spectrophotometer 1700 Shimadzu at wavelength of 260 nm. The drug content was determined with the help of previously established standard curve.

1.Qualitative test for free Glutaraldehyde[13]

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 40^0 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.

2. Drug polymers interaction study[14]

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

The Sub dermal implants of Naproxen prepared with Gelatin hardened with Glutaraldehyde were tested for compatibility of the drug with the excipients like, Gelatin, Glycerin hardening agents by I.R. Study. The I.R. Spectrum of the pure drug and the formulated discs are shown in the figures.

The I.R. Spectrum of Naproxen with excipients Gelatin is similar to that spectra of pure drug (Naproxen). All the characteristics bands of drug are retained in the I.R. spectra gelatin formulation indicating that the drug has not reacted with the excipients present in it.

Hence, from the I.R study it is confirmed that there is no interaction between the drug and excipients used.

3. Stability studies (Effect of Temperature)[15]

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

Procedure for in-vitro drug release study[16,17,18]

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 $37^0C \pm 5^0C$. 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 261 nm after suitable dilution with phosphate buffer pH 7.4.

Table 5: In vitro release of naproxen in phosphate buffer of pH 7.4 from discs prepared using f2 without hardening

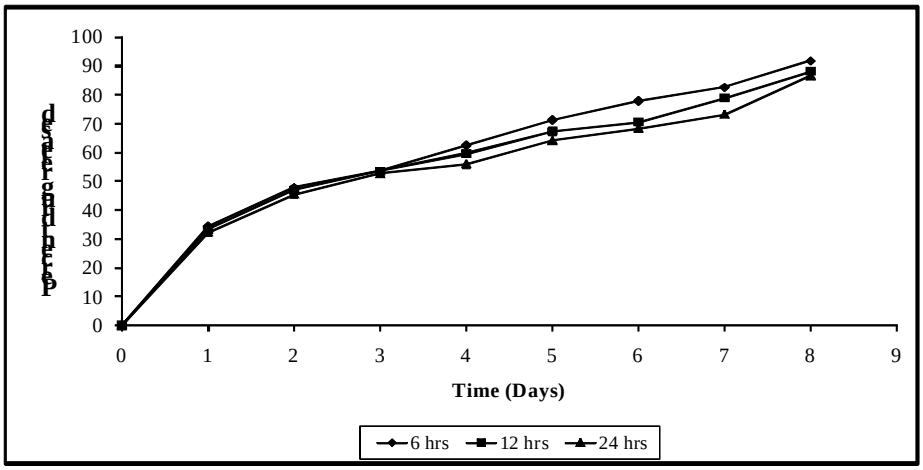
Time in min	Square root of time in min.	Percentage of drug released	Percentage of drug retained	Log Percentage of drug retained
30	5.477	16.10	83.90	1.9237
60	7.745	26.04	73.96	1.8689
90	9.486	39.63	60.37	1.7808
120	10.954	53.25	47.75	1.6603
150	12.247	60.49	42.51	1.6284

* Each reading is a mean of three replicates

Table 6: In vitro release of naproxen in phosphate buffer of ph 7.4 from discs prepared using f3 and hardened for 6 hours by using glutaraldehyde

Time in days	Square root of time in days	Percentage of drug released	Percentage of drug retained	Log Percentage of drug retained
1	4.80	34.33	65.67	1.8173
2	6.92	47.89	52.11	1.7169
3	8.48	53.53	43.09	1.6343
4	9.79	62.48	37.52	1.5743
5	10.95	71.30	28.70	1.4578
6	12.00	77.86	22.14	1.3451
7	12.96	82.67	18.33	1.2631
8	13.85	91.77	8.23	0.9153

* Each reading is a mean of three replicates.



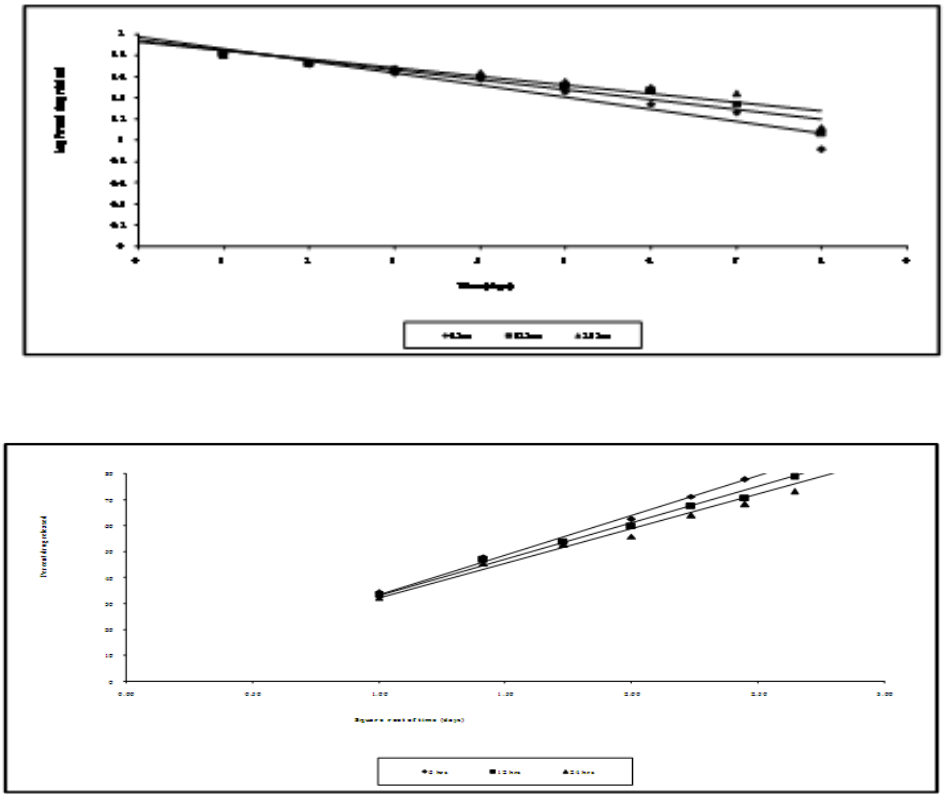


Fig. 1: In-vitro release of Drug in phosphate buffer of pH 7.4 from discs prepared using F3 and hardened for 6 hours, 12 hours & 24 hours by using Glutaraldehyde

RESULTS AND DISCUSSION

The results indicated that all the prepared discs were uniform in the shape i.e. circular and uniform in diameter i.e. 6 mm.

The thickness measurement studies were conducted on plain discs i.e. without hardening and hardened implants. The results indicated that exposure to the hardening agent did not produce an appreciable shrinkage in the implants.

The results of weight uniformity test indicated that the wt of plain implants and hardened implants did not show appreciable variation in the weight of implants.

The drug content uniformity results revealed that there was no appreciable variation in drug present in each implantable disc and found to be uniform in all Naproxen implants.

The results for free Glutaraldehyde test revealed that the implants passed the test since the sample solution was not more intensely colored than the standard solution that meant less than 20 mcg of free Glutaraldehyde was present in 25 discs.

The IR spectral studies depicted three peaks at similar wavelengths in case of pure drug and in implantable discs hardened with Glutaraldehyde and Glutaraldehyde, indicating absence of interaction between Naproxen and the excipients used. The comparative R_f values of the pure drug Naproxen and its two formulations on the chromatogram indicated that there was no interaction between drug and the carriers in implantable discs.

At intervals during the incubation period, and at its conclusion, when the media was examined for macroscopic evidence of microbial growth, no evidence of micro-organisms was found. Thus the implants passed the test for sterility.

The In-vitro dissolution studies indicated that the drug release was minimum and slow in case of F3, further the implants from F3 hardened with Glutaraldehyde for 6 hours showed sustained release of the drug for 8 days. The mechanism of drug release was found to be diffusion and it followed first order rate kinetics. In addition to diffusion, the gelatin implants were found to erode slowly giving out the drug. It was also observed that the drug release was sustained as exposure time to the

hardening agent increases. Gelatin based implantable discs hardened with Glutaraldehyde for 6 hours was found to be optimum.
Stability studies of the prepared drug implants has clearly indicated that even after a period of three months, there was no change in weight, colour, size and drug content of the polymeric discs. It revealed that the prepared implants were stable at ambient temperatures.

CONCLUSION

Gelatin based sub dermal implants containing Naproxen can be prepared. The release of drug from the gelatin implantable discs can be modulated by varying the concentration of the polymer (gelatin), the nature of cross linking/hardening agent and the time of exposure to hardening agent. The prepared sub dermal implants of Naproxen provide sustained release of drug for 8 days. Thus they are more useful in pre/post operative conditions and during the treatment of musculoskeletal disorders where drug action is required for prolonged period.

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