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Designing of Diclofenac Sodium Biodegradable Drug Implant for Speedy Fracture Healing

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ABSTRACT

Subcutaneous implantation is currently the most utilized routes of the potential of sustained drug delivery system. Present investigation attempts to prepare biodegradable subcutaneous implants of Diclofenac Sodium; nonsteroidal anti inflammatory agent used in treatment of orthopaedic patient care. Implant formulated in varied ratios of Gelatin and Sodium alginate i.e. 70:30, 80:20 and 90:10 % w/w by heating and congealing method. The implants were evaluated for content uniformity, thickness, weight variation, IR, in vitro release studies and stability studies at ambient temperature for 3 months. Implants were found to erode slowly with diffusion mechanism. In vivo studies in Rabbits revealed that at subdermal thigh region before and after one month of Implantation of polymeric rod, there was no inflammation at the site of implantation, foreign body grannuloma formation, necrosis or hemorrhage.

Keywords: Diclofenac Sodium, Subdermal implant, Gelatin, Sodium Alginate.

INTRODUCTION

Orthopaedic is the art and science of the diagnosis and treatment of all diseases and disorder of the human locomotor system which could result in deformity in limbs or spine. Deformity is an alteration in the shape of a limb or spine. Deformities can be broadly grouped as congenital deformities and acquired deformities [1]. Fracture is defined as a break in the bone [2]. There

are different type of fractures such as Green stick fracture, Closed fracture, open fracture, Pathological fracture, Stress fracture, Birth fracture, Comminuted fracture, stellate fracture, Avulsion fracture and Depressed fracture [3].The response of body to the stress of tissue damage is known as inflammation. The inflammation is usually a defensive response of the body which involve a variety of chemical mediator such as histamine, prostaglandin, bradykinin, interleuckin 1 (IL-1), tumor necrosis factor (TNF), nitric oxide, free oxygen radical, [4], NSAID are therefore the drugs of choice with occasional local treatment without steroids for the relief of pain and inflammation since NSAID modify the inflammation by reducing the level of prostaglandins, bradykinins, 5-Hp [5]. A Subcutaneous implant of drug pellets is known to be the first medical approach aiming to achieve prolonged and continuous administration of drugs. Subcutaneous implantation is currently one of the most utilized routes to investigate the potential of sustained drug delivery system. This is because ready accessibility of drugs to unusual absorption sites such as tumor, bone marrow, slow absorption of drugs at a fixed rate through subcutaneous tissue, low reactive nature of subcutaneous tissue to the foreign material, easy removal of the device at any time, If needed [6]. The present work aims at fabricating biodegradable subcutaneous implants of Diclofenac sodium the NSAID, by using gelatin and sodium alginate for sustained release. The subcutaneous drug implants are hardened by exposing them to formaldehyde and glutaraldehyde for different time intervals. The fabricated implants are studied for various physico-chemical parameters like weight variation, thickness, drug content uniformity, presence of free formaldehyde, drug polymer interaction, sterility test, in-vitro dissolution rate studies are performed on the implants by using phosphate buffer pH 7.4. The implants are investigated for tissue polymer interaction by performing histopathological studies on rabbit's thigh before and after implantation.

EXPERIMENTAL SECTION

Materials

Diclofenac sodium was obtained as a gift sample from Bio-vaccine Hi-tech formulation, Hyderabad (AP). Gelatin was purchased from S.D. Fine Chemicals Ltd., Mumbai. Sodium alginate purchased from Bombay Research Lab, Pune. Glycerin and formaldehyde were purchase from Ranbaxy Laboratories Ltd., Punjab. Glutaraldehyde was purchased from Loba Chemicals, Mumbai. Other chemical used were of analytical grade.

Preparation of implant

Weighed quantity of Gelatin was sprinkled on the surface of water and kept aside for 30 minutes to hydrate. Sodium alginate was added in hydrated Gelatin. Glycerin was added (Table. I) as a plasticizing agent with continuous stirring & the solution was heated on a water bath at 60^0C until gelatin was dissolved. Diclofenac sodium was dissolved separately in a small quantity of acetone and added to the Gelatin and Sodium alginate Solution. The Solution was poured in a glass Petri dish upto 3 mm height and allowed to gel by placing the Petri-dish on ice for 30 minutes. Then they were dried at room temperature for 72 hours in aseptic cabinet. After drying the implants were cut into rod shaped of 3 mm width & 1.5 mm length by specially designed stainless steel cutter [7].

Table 1. Formulae of Implants Prepared

Ingredients	Formulations		
	70:30	80:20	90:10
Drug	4 gram	4 gram	4 gram
Polymers (Gelatin and Sodium alginate)	30 grams	30 grams	30 grams
Glycerin	20 ml.	20 ml.	20 ml.
Distilled water Q.S. to	100ml.	100 ml.	100 ml.

Hardening of implants:

A Petri-dish containing Formaldehyde solution (37% v/v) was placed in an empty glass dessicator. A wire mesh contained the implants was kept on the top of the Petri dish and the dessicator was closed immediately. The implants were made to react with formaldehyde vapors for different time interval such as 3, 6, 12 and 24 hours. Then they were removed from the dessicator and air-dried for 72 hours so that the reaction in between formaldehyde and gelatin was completed. Afterwards the implants were kept in an open atmosphere in aseptic conditions for a week to make sure that the residual formaldehyde gets evaporated. The same procedure was employed for the implants contained 70:30, 80:20, 90:10 % w/w gelatin and sodium alginate and hardened with Glutaraldehyde [8].

Evaluation of subdermal implants

Measurement of Implants Thickness

The thickness of a sample of three implants was measured with a screw gauge [9].

Weight Variation of implants

Weight variation was checked by weighing three implants individually [10].

Drug content Uniformity

Diclofenac sodium content of implants was estimated by removing a sample of three implants from every batch. Each implant was cut in to small pieces and dissolved in small quantity of methanol by heating at 60^0C on a water bath. After cooling the solution was filtered and suitably diluted with methanol. Diclofenac Sodium content was calculated by measuring the absorbance at 282.4 nm on a UV spectrophotometer 1700 Shimadzu. The data was subjected to statistical analysis [11] (Table.2).

Table 2. Various experimental parameters of prepared implants (90:10) hardened with formaldehyde

Sr. No.	Hardening time hrs.	Weight of Implants (mg.)± S.D	Thickness of Implants (mm)± S.D	Drug Content mg.± S.D
1.	3	124.90 ± 0.62	3.03± 0.54	9.35± 0.65
2.	6	125.41± 0.83	3.07± 0.013	9.68± 0.07
3.	12	123.11± 0.21	2.95± 0.34	9.84± 0.41
4.	24	126.27± 0.65	2.98± 0.11	9.71± 0.09

** Each reading is a mean of three replicates.; * Each implant contain 10 mg of drug*

Tests for Sterility

The sterility test was conducted by membrane filtration method on soybean-casein digest medium [12].

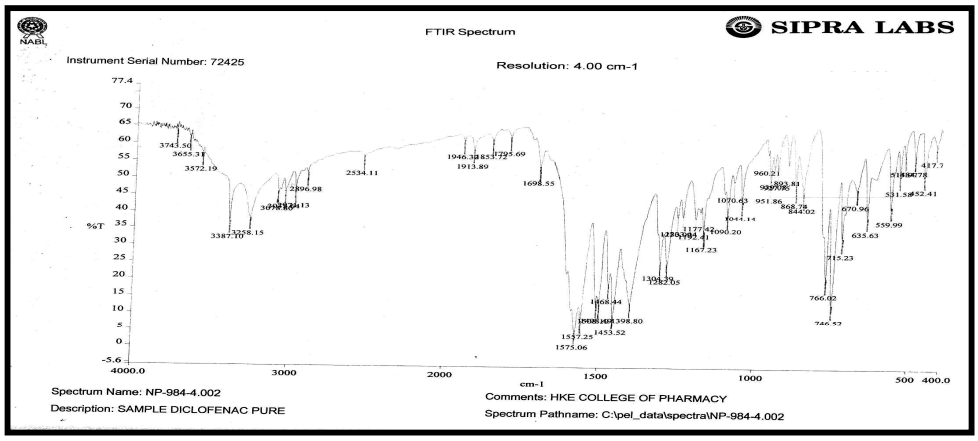

Figure 1: FTIR Analysis of pure Diclofenac sodium drug

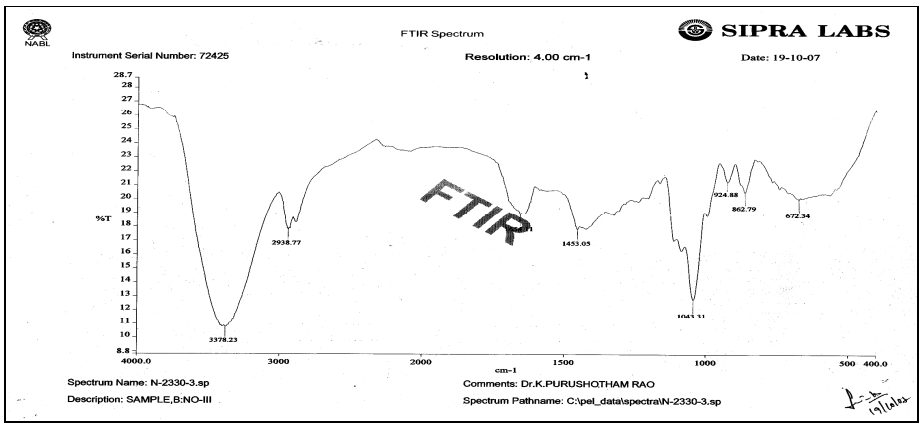

Figure 2: FTIR Analysis of Sample Gelatin: Sodium alginate (90:10) Diclofenac Sodium implant hardened for 12 hours in Glutaraldehyde

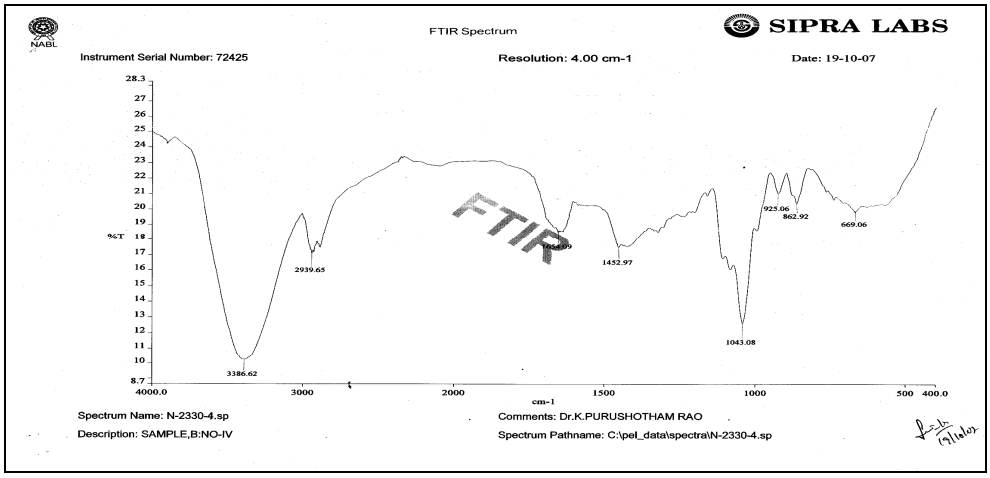

Figure 3: FTIR Analysis of Sample Gelatin: Sodium alginate (90:10) Diclofenac Sodium implant hardened for 12 hours in Formaldehyde

Test for Free Formaldehyde
To ascertain the absence of free formaldehyde, the implants were subjected to pharmacopoeial test for free formaldehyde. During the test the colour of 1ml of 1 in 10 dilution of implant preparation was compared with the colour of 1ml of standard formaldehyde solution [13].

Drug-Polymer Interaction Study
The IR spectra of Diclofenac Sodium and its formulations were obtained by potassium bromide pellet method using Perkin Elmer FTR series model 1615 Spectrometer and compared [14] (Figure: 1, 2, 3).

In vitro Drug Release Studies
Implants were placed separately into 10 ml vials containing 10 ml of Phosphate butter pH 7.4. The vials were sealed with rubber stoppers and kept in incubator shaker thermo stated at 37^0 + 0.5^0 C. 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 276.2 nm after suitable dilution with Phosphate buffer pH 7.4 [15], [16].

In Vivo Studies (Tissue-Polymer Interaction Studies)
Twelve male white rabbits weighing around 2.5 Kg were used for the study. The animals were housed individually in cages under environmentally controlled conditions (temperature 37^0 C and 12 hr lighting cycle). The animals were fed with a standard rabbit diet that is commercially available and had access to water ad libitum. On the day of implantation the skin at the site of implantation (thigh) was cleaned by alcohol swab. Before implantation lignocaine a local anesthetic gel was applied. The skin punch biopsy stainless steel forceps No.5 was used to take the tissue sample from the thigh region for histopathological studies (Figure: 4) [17], [18].

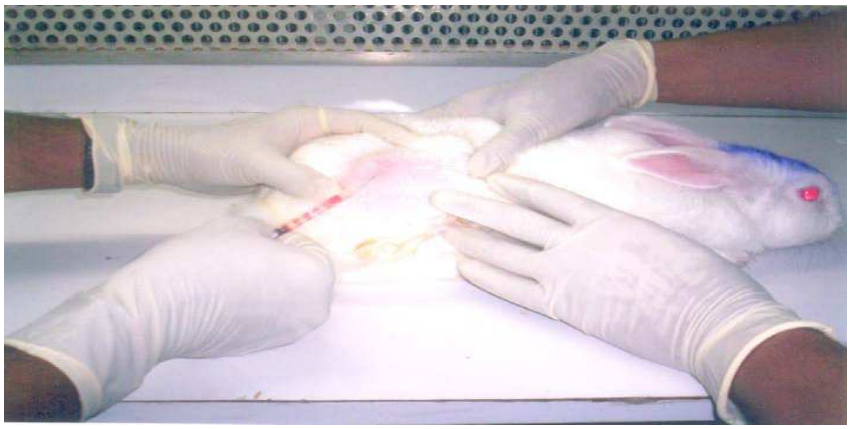

Figure 4. Polymer Implantation in Subdermal Region. (Thigh)

RESULTS AND DISCUSSION

Implants of Diclofenac Sodium were prepared employing Gelatin and Sodium Alginate (90:10% w/w) and hardened with formaldehyde for 12 hours. Diclofenac sodium rod shaped implants gave uniform results for thickness, weight variation, drug content and drug release

characteristics. The data was subjected to statistical analysis. At interval during the incubation period, and at its conclusion, when the media was examined for macroscopic evidence of microbial growth, no evidence of micro-organism was found. Thus the implants passed the test for sterility. The sample solution was not more intensely colored than the standard solution inferring that less than 20 mcg of free formaldehyde is present in 25 implants. The I.R. reports of drug implants hardened with formaldehyde and glutaraldehyde indicating absence of interaction between drug and the excipients used. The drug release studies of Diclofenac sodium implants in phosphate buffer pH 7.4 indicated 98.41 % of drug release in 144 hours. (Table.3).

Table 3. *In Vitro* Release of Diclofenac Sodium in Phosphate Buffer of pH 7.4 from implants prepared with Gelatin and Sodium Alginate (90:10) hardening for 12 hours using Formaldehyde

Time (hrs.)	Square root of Time (hrs.)	Log time	Cumulative percent drug released± S.D	Log Cumulative percent drug released	Cumulative percent drug retained	Log Cumulative percent drug retained
12	3.464	1.079	15.91±0.66	1.201	84.09	1.924
24	4.898	1.380	28.41±0.19	1.453	71.59	1.854
36	6	1.556	40.91±0.23	1.611	59.09	1.771
48	6.928	1.681	53.41±0.18	1.727	46.59	1.668
72	8.485	1.857	65.91±0.34	1.818	34.09	1.532
96	9.797	1.982	73.41±0.56	1.865	26.59	1.424
120	10.954	2.079	85.91±0.73	1.934	14.09	1.148
144	12	2.158	98.41±0.38	1.993	1.59	0.201

** Each reading is a mean of three replicates.; * Each implant contains 10mg. of drug*

The *In vitro* dissolution studies revealed that implants hardened with formaldehyde show first order rate kinetics. The mechanism of drug release was found to be diffusion. Implants were found to erode slowly, in addition to diffusion mechanism, giving out the drug Diclofenac sodium (Figure: 5, 6, 7). In-vivo studies in animals (Rabbits) revealed that at subdermal thigh region before and after one month of Implantation of polymeric rod, there was no inflammation at the site of implantation, no foreign body grannuloma formation, necrosis / hemorrhage was not present. Thus Gelatin-Sodium Alginate was found to be compatible with the tissues at subdermal region. (Figure.8)

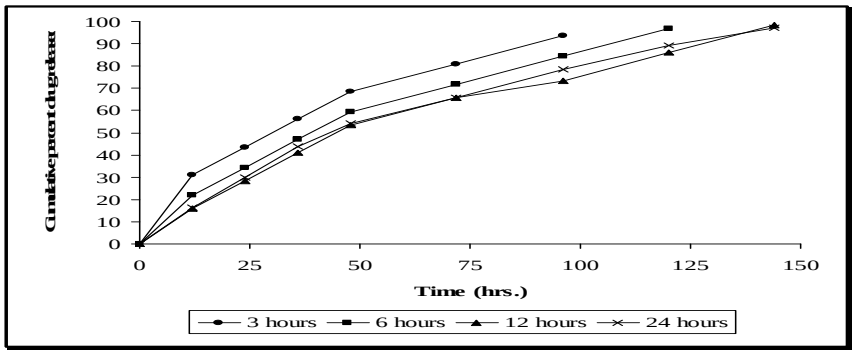

Figure 5. Comparative zero order plots of implants (90:10) hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

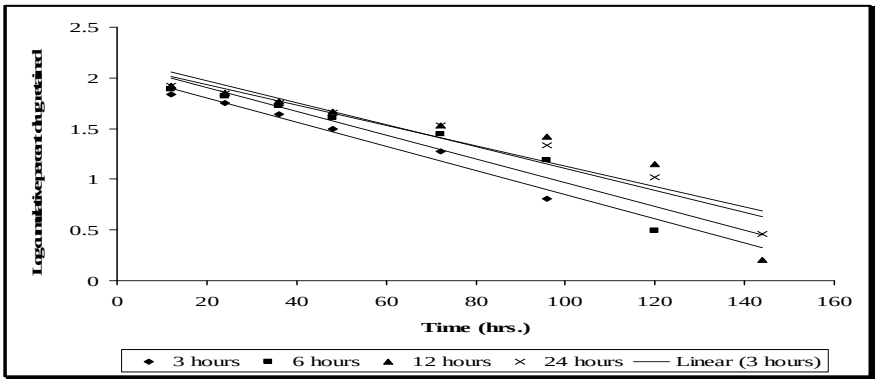

Figure 6. Comparative First order release plots of implants prepared using Gelatin and Sodium Alginate (90:10) hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

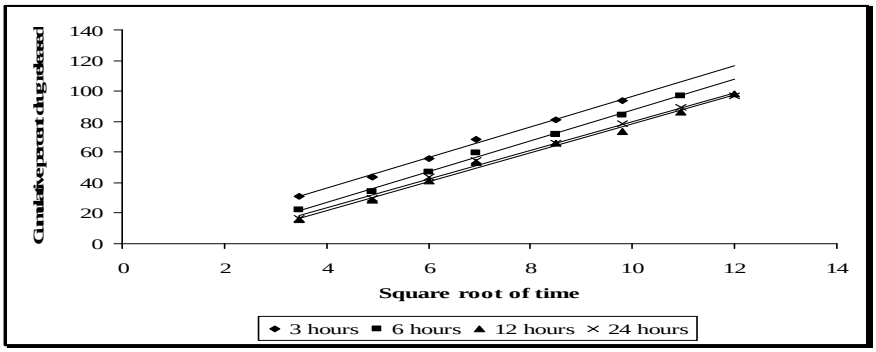

Figure 7. Comparative Higuchi square root plots of implants prepared using Gelatin and Sodium Alginate (90:10) hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

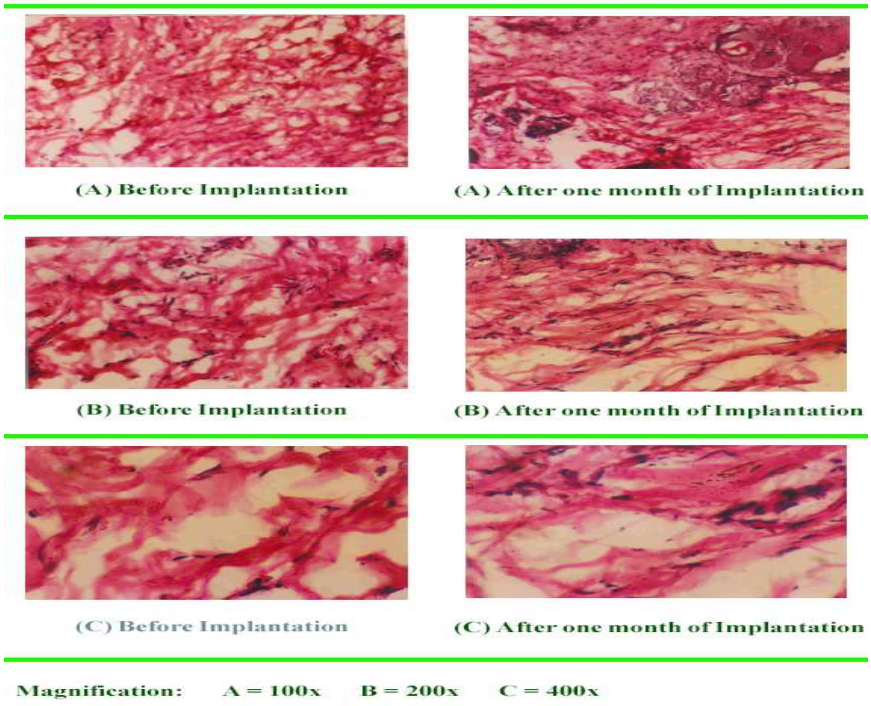

Figure 8. Histopathological study of prepared implants (90:10 %) hardened with formaldehyde

CONCLUSION

Gelatin Sodium Alginate based subdermal implants of Diclofenac sodium having uniform character can be prepared with minimum batch to batch variation. The subdermal implants containing 90:10 % w/w Gelatin: sodium alginate and hardened with formaldehyde for 12 hours are found to produce the most satisfactory drug release. Diclofenac sodium implants can be used for the treatment of orthopaedic patient care, bone fractures. As they meet the criteria such as better patient compliance, improved therapeutic outcome & minimum incidence of adverse effects.

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