



FORMULATION DEVELOPMENT OF CHITOSAN BASED SUB DERMAL IMPLANTS

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ABSTRACT

This present research study was carried out to develop and evaluate the subcutaneous implants of Aceclofenac sodium by using soluble Chitosan. Aceclofenac sodium, the NSAID by using Chitosan 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 formaldehyde and the formulated implants were evaluated for thickness, wt variation, drug content uniformity, free formaldehyde, 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. For studying the tissue polymer compatibility at sub dermal region, *in vivo* studies were carried out in rabbits under the supervision of Staff, Department of Pathology, M.R. Medical College, Gulbarga, with the permission from the Ethical committee.

Keywords: Aceclofenac sodium, Chitosan, Subcutaneous, implants.

INTRODUCTION

Rheumatic diseases are challenging task for orthopedic surgeons. Skeletal muscle disorders, affecting joints tendons, ligaments, bones, and muscle pains, can be broadly categorized either into Rheumatic diseases such as osteoarthritis, ankylosing spondylitis, or autoimmune diseases such as Rheumatoid arthritis, systemic lupus erythematosus.¹

The common symptoms of rheumatic diseases are inflammation in one or more joints, stiffness around the joint that lasts for at least one hour in the early morning, constant or recurring pain and tenderness in a joint, difficulty while using or moving the joint normally, warmth and redness in a joint.¹ The response of the body to the stress of tissue damage is known as inflammation. The inflammation is usually a defensive response of the body which involves a variety of chemical mediators such as histamin, prostaglandin, bradykinin, interleukin 1 (IL-1), tumor necrosis factor (TNF), nitric oxide, free oxygen radical.^{2,3}

NSAIDs are therefore the drugs of choice with occasional local treatment with steroids for the relief of pain and inflammation since NSAIDs modify the inflammation by reducing the levels of prostaglandins, bradykinins, and 5-HP⁴. The Vane hypothesis relates the anti-inflammatory action of NSAIDs to their ability to inhibit the enzyme Cox-2 Selectively.⁵ Subcutaneous

implants, 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 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.⁴

Chitosan, obtained by deacetylation of chitin, is a natural, hydrophilic, nontoxic, biocompatible, and biodegradable polysaccharide suitable for applications in pharmaceutical technology. Chitosan is soluble at acidic pH, forming gels; hydrogels are also formed in the presence of negatively charged drugs or polyanions, and represent a sustained drug release form. The bio adhesive nature of Chitosan can be attributed to the same type of ionic interactions with mucosal membrane components. Mucoadhesive formulations have been developed for ocular, nasal, buccal, gastrointestinal and vaginal drug administration. Chitosan is able to promote Trans mucosal absorption of small polar drugs, including peptides, inducing a transient opening of the tight junctions of the cell membrane. Due to its polymeric nature, Chitosan has been widely investigated for a variety of micro particular pharmaceutical forms. Chitosan is also a candidate for potential applications in the delivery of radiopharmaceuticals, genes and peptides.^{6,7}

To investigate the wound healing efficacy of two chitosan films, chitt-aa and chitt-la, in comparison with a commercial preparation, omiderm (r), using punch biopsy

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wounds in rate. The punch biopsy wounds were created in the abdominal region of male wistar rats. The films were evaluated in terms of transparency flexibility, adherence property, ease of removal from wounds without damaging underlying tissues and fluid accumulation. In addition, the wounds were examined for dryness, exudation, contraction, period of epithelialization and scar formation. Chitt-aa, chit-la and omiderm (r) films were comparable in terms of transparency, flexibility, adherence property, ease of film removal from wounds without damaging underlying tissues and fluid accumulation. Although there was no statistically significant difference in wound dryness and exudation between the film treated wounds and untreated wounds (control), a significant difference was obtained in complete wound closure ($t_{100\%}$), period of epithelialization and scar formation. Both chit-la and chit-aa were able to promote wound healing with minimal scar formation.⁸

MATERIALS AND METHODS

Acceclofenac sodium was obtained as a gift sample from Bio-Vaccine Hi-Tech Formulation, Hyderabad. Chitosan was purchased from S.D. Fine Chemicals Ltd; Mumbai. Glycerin and Formaldehyde were purchased from Ranbaxy Laboratories Ltd. Punjab. was purchased from Loba Chemicals Mumbai. All other chemicals used were of analytical grade.

Table 1: Formulae of Implants Prepared

Ingredients	Quantity
Drug	2 gram
Polymers (Soluble Chitosan)	15 grams
Glycerine	10 ml.
Distilled water Q.S. to	50 gms.

1. Preparation of Soluble Chitosan:

A) Preparation of N-trimethyl Chitosan chloride⁹:

A mixture of 2g. of sieved Chitosan (93% deacetylated), 4.8g. of sodium iodide, 11 ml. of a 15% aqueous sodium hydroxide solution and 11.5 ml. of methyl iodide in 80ml. of 1-methyl-2-pyrrolidinone was stirred on a water bath of 60°C for 1 h. (Le Dung et. al., 1994). Special care was taken to keep the methyl iodide in the reaction mixture by using a Liebig condenser. The product was precipitated using ethanol and thereafter isolated by centrifugation. The N-trimethyl Chitosan iodide obtained after this first step was washed twice with ether on a glass filter to remove the ethanol. It was dissolved in 80 ml. of 1-methyl-2-pyrrolidinone and heated to 60°C, thus removing most of the absorbed ether. Subsequently, 4.8 g. of NaI, 11 ml. of 15% NaOH solution and 7 ml. of methyl iodide were added with rapid stirring and the mixture was heated on a water bath at 60°C for 30 min. An additional 2 ml. of methyl iodide and 0.6 g. of NaOH pellets were added and the stirring was continued for 1 h.

The product, prepared as described above, was dissolved in 40 ml. of a 10% NaCl aqueous solution, instead of HCl to exchange the iodide. The polymer was precipitated with ethanol, isolated by centrifugation and thoroughly washed with ethanol and ether. In vacuum drying yielding a white, water-soluble powder.

Viscosity:

Viscosity of water soluble Chitosan is 409.94 cps measured by using Brookfield Viscometer DV-III Ultra Programmable Rheometer.

B) Preparation of Aceclofenac sodium rod shaped implants¹⁰:

Weighed quantity of Chitosan was dissolved in water and allowed to hydrate for 24 hours, Glycerin was added as a plasticizing agent and stirred for on hour. Aceclofenac sodium was dissolved separately in a small quantity of methanol and added to above polymeric solution the dough mass was extruded in to a 3mm diameter, rod shaped thread like implants are dried at room temp for 48 hours , After drying the implants were cut in to number of pieces of 6mm in length. The implants containing 10 mg of drug and have 155mg of total weight were prepared.

Hardening of rod shaped implants¹⁰:

25 ml of Formaldehyde solution (37% v/v) was transferred in a petridish and kept in an empty glass dessicator. A wire mesh containing the implantable discs was placed on the top of the petridish and immediately the dessicator was closed. The discs were made to react with formaldehyde vapors for 12hours. Then they were removed and air dried for 72 hours so that the reaction between formaldehyde and Chitosan was completed. After wards the discs were kept in an open atmosphere in aseptic conditions for a week, to make sure that the residual formaldehyde gets evaporated.

EVALUATION OF SUB DERMAL IMPLANTS

a) Measurement of implant thickness¹¹: [Table-2]

The thickness of implants was measured with a screw gauge on a sample of three implants.

b) Weight of Implants¹¹: [Table-2]

Weight variation was checked by weighing three implants individually.

c) Drug content uniformity test¹²: [Table-2]

Drug content of implants was estimated by removing a sample of three implants, of 6 mm in length and 3 mm thickness and analyzing the Drug content as follows. Each implant was cut in to small pieces and dissolved in a small quantity of methanol by heating at 60°C on a water bath. After cooling the solution was filtered and transferred to the 25 ml with methanol. This solution was diluted suitably with methanol and assayed for Aceclofenac sodium content by measuring the absorbance at 275 nm. Drug contents were calculated from the calibration curve.

Table 2: Measurement of implant -Thickness, Weight and Drug content

Thickness				Weight of Implants			Drug content		
SN	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
1	3.01	0.0100	0.332	155.01	0.0152	0.0098	9.72	0.0916	0.9423
2	3.02	0.0115	0.380	154.07	0.545	0.3522	9.646	0.277	2.87
3	3.02	0.0264	0.874	155.01	0.0152	0.0098	9.576	0.066	0.689

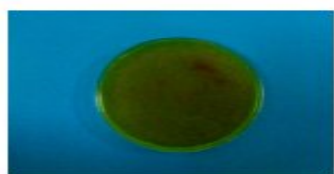
* Each reading is a mean of three replicates.

* Each implant contains 10mg. of drug

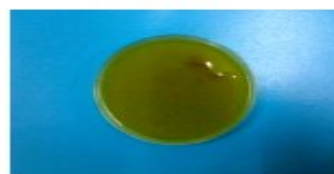
d) Tests for sterility IP¹³:

The sterility test was conducted by Membrane Filtration Method on soyabean-casein digest medium and found to be implants are sterile.

Sterility test of prepared Implants



Agar Plate



Agar Plate with Implants after 72 Hours.

e) Test for free formaldehyde¹⁴:

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

f) Drug-polymers interaction study¹⁵:

The sub dermal implants of Aceclofenac sodium prepared with Chitosan, hardened with formaldehyde was tested for compatibility of the drug with the excipients used like, Chitosan, Glycerin and hardening agents by I.R. Study. The IR spectra of Aceclofenac sodium and its formulations were obtained by potassium bromide pellet method using Perkin Elmer FTIR series model 1615 spectrometer.

g) In-vitro drug release studies¹⁶:

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 shaker thermo stated at 37⁰ + 0.5⁰ 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 295 nm after suitable dilution with phosphate buffer pH 7.4.

Table 3: Calibration Curve Data for Aceclofenac in Phosphate Buffer PH 7.4 ($\lambda_{MAX} = 275$)

Sl. No.	Concentration (mcg/ml)	Absorbance*
1.	0	0.000
2.	4	0.072
3.	8	0.140
4.	12	0.210
5.	16	0.275
6.	20	0.343

* Average of three determinations.

Figure 1: Calibration Curve Data for Aceclofenac in Phosphate Buffer pH 7.4 ($\lambda_{max} = 275$)

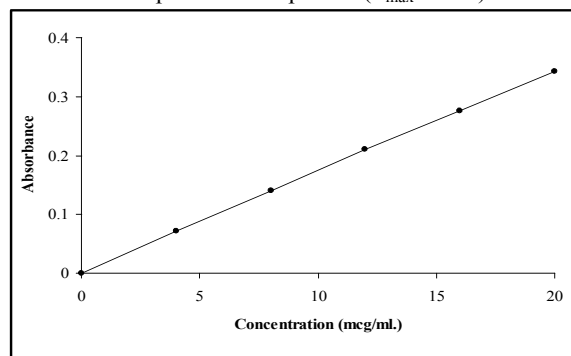


Figure 2: Comparative cumulative percent drug released versus time (zero order kinetic model) plots of implants prepared using gelatin and soluble Chitosan hardened with formaldehyde for 12 hours.

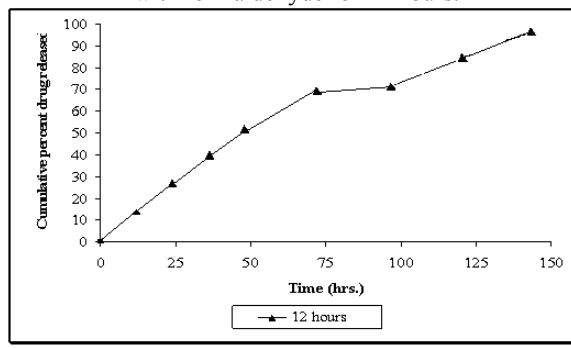


Table 4: *In vitro* release of Aceclofenac in phosphate buffer of pH 7.4 from implants prepared with soluble Chitosan and hardened for 12 hours using formaldehyde

Time (hrs.)	Square root of Time (hrs.)	Log time	Cumulative percent drug released	Log Cumulative percent drug released	Cumulative percent drug retained	Log Cumulative percent drug retained
0	0	0	0	0	0	0
12	3.464	1.079	13.91	1.143	86.09	1.934
24	4.898	1.380	26.41	1.421	73.59	1.866
36	6	1.556	38.91	1.590	61.09	1.785
48	6.928	1.681	51.41	1.711	48.59	1.686
72	8.485	1.857	69.91	1.805	36.09	1.557
96	9.797	1.982	71.41	1.853	28.59	1.456
120	10.954	2.079	83.91	1.923	16.09	1.206
144	12	2.158	97.41	1.988	2.59	0.413

* Each reading is a mean of three replicates.

* Each implant contains 10mg of drug

h) In- Vivo Studies (Tissue-Polymer Interaction Studies)^{17, 18}:

Two 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°C and 12 hr. lighting cycle). The animals were fed as usual 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, the animals were applied with lignocaine, a local anesthetic gel. No.5, skin punch biopsy stainless steel forceps was used to take the tissue sample from the thigh region for histopathological studies.

RESULTS & DISCUSSION

- Implants of Aceclofenac sodium were prepared employing Chitosan and hardened with formaldehyde for 12 hours. Prepared Implants gave the uniform results for drug content, thickness, weight variation and found to have uniform drug content 9.64 mg and 3.1 mm thickness with a mean weight of 155.01 mg. (Table 2) and in vitro drug release characteristics study. (Table 4).
- At intervals during the incubation period, and at its conclusion, when the media was examined for macroscopic evidence of microbial growth, no evidence of microorganisms was found. So the Aceclofenac sodium implants pass 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 was present in 25 implants.
- The same peaks as that of the pure drug Aceclofenac sodium were observed in implantable discs hardened with formaldehyde. The IR study showed there was no interaction between the Aceclofenac sodium and excipients used.
- The drug release studies of Aceclofenac sodium in phosphate buffer pH 7.4 indicated 97.41% of drug release in 6 days (table 4). Further the implants

hardened with formaldehyde show first order rate kinetics. The mechanism of drug release was found to be diffusion. Chitosan implants were found to erode slowly giving out the drug Aceclofenac sodium in addition to diffusion mechanism. (Fig 2,)

- In-vivo studies in animals (Rabbits), revealed that at sub dermal thigh region before and after one month of implantation, there was no inflammation at the site of implantation, foreign body granuloma formation was not observed, necrosis / hemorrhage was not present. Thus Chitosan was found to be compatible with the tissues at sub dermal region.

CONCLUSION

Chitosan based sub dermal implants of Aceclofenac sodium having uniform characters can be prepared with minimum batch to batch variation. The sub dermal implants containing Chitosan and hardened with Formaldehyde for 12 hours is found to produce the most satisfactory drug release.

The Aceclofenac sodium implants can be used for the treatment of arthritis as they meet the criteria such as better patient compliance, improved therapeutic outcome and minimum incidence of adverse effects.

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