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Preformulation Studies of Biodegradable Drug Implants of Meloxicam for Orthopedic Patient Care

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Abstract

Post-operative surgical care of a orthopedic patient is utmost important for speedy recovery from injuries. Subcutaneous implantation is currently the most utilized route of the potential of controlled drug delivery system. Present investigation attempts to prepare biodegradable subcutaneous implants of Meloxicam; nonsteroidal anti-inflammatory agent used in treatment of orthopaedic patient care. Implant formulated with 30%W/W Gelatin-Na Alginate (90:10) 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:Meloxicam, Subdermal implant, Gelatin, Sodium Alginate

INTRODUCTION

Deformity is an alteration in the shape of a limb or spine. Deformities can be broadly grouped as congenital deformities and acquired deformities (Natrajan, 1994). Fracture is defined as a break in the bone. (Maheshwari, 2005). 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 (Adam and Hamblen, 1999).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, (Wilson and Giswold, 1998), 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 (foye,1995). 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 (Murthy, 2001). The present work aims at fabricating biodegradable subcutaneous implants of Meloxicam, the NSAID, by using gelatin for sustained release. The subcutaneous drug implants are hardened by exposing them to formaldehyde and glutaraldehyde at 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.

MATERIALS AND METHOD

Meloxicam was obtained as a gift sample from Bio-vaccine Hi-tech formulation, Hyderabad (AP). Gelatin was purchased from S.D. Fine

Chemicals Ltd., Mumbai. . Glycerin and formaldehyde were purchase from Ranbaxy Laboratories Ltd., Punjab.. Other chemical used were of analytical grade.

Preparation of implants;

Weighed quantity of polymer (30 gms) i.e Gelatin and Na Alginate (90:10%)was sprinkled on the surface of water and kept aside for 30 minutes to hydrate.. Glycerin (20 ml) was added as a plasticizing agent with continuous stirring & the solution was heated on a water bath at 60^0C until gelatin was dissolved. Meloxicam (4 gms) 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 shape of 3 mm width & 1.5 mm length by specially designed stainless steel cutter. (Gwen et al., 1996).

Hardening of implants:

A Petri-dish containing Formaldehyde solution (37% v/v) was placed in an empty glass dessicator. A wire mesh containing 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.(Swarbrick and Boylan.1988)

Evaluation of subdermal implants

Measurement of Implants Thickness

The thickness of a sample of three implants was measured with a screw gauge. (Saparia et al., 2002)

Weight Variation of implants

Weight variation was checked by weighing three implants individually. (Roohullah et at., 2003)

Drug content Uniformity

Meloxicam 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. Meloxicam content was calculated by measuring the absorbance at 293.37nm on a UV spectrophotometer 1700 Shimadzu. The data was subjected to statistical analysis. (Table.1)(Rao et al., 2007)

Table 1. Various experimental parameters of prepared implants hardened with formaldehyde

Hardening time hrs.	Weight of Implants (mg.) ± S.D	Thickness of Implants (mm) ± S.D	Drug Content mg. ± S.D
3	122.40 ± 0.65	3.02 ± 0.54	9.33 ± 0.65
6	123.22 ± 0.85	3.17 ± 0.013	9.67 ± 0.07
12	121.60 ± 0.25	2.35 ± 0.34	9.54 ± 0.41
24	1.24 ± 0.64	2.28 ± 0.11	9.56 ± 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. (IP, 1985)

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. (IP, 1985)

Drug-Polymer Interaction Study

The IR spectra of Meloxicam and its formulations were obtained by potassium bromide pellet method using Perkin Elmer FTR series model 1615 Spectrometer and compared.. (Lin et al., 2001, Tayade and Kale, 2004)

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 $37^0 \pm 0.5^0$ 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 266.0 nm after suitable dilution with Phosphate buffer pH 7.4. (Gokhan et al., 2005. USP, 1995)

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.. (Manavi et al., 1997. Siegel et al., 2002.)

RESULTS AND DISCUSSION

Implants of meloxicam were prepared employing Gelatin-Na Alginate (30% w/w) and hardened with formaldehyde for 12 hours. Meloxicam 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 indicating absence of interaction between drug and the excipients used. The drug release studies of Meloxicam implants in phosphate buffer pH 7.4 indicated 96.44 % of drug release in 144 hours. (Table.2). The *In vitro* dissolution studies revealed that implants hardened with formaldehyde show Zero 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 Meloxicam(Figure: 1,2,3). 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 was found to be compatible with the tissues at subdermal region.

Table 2. *In Vitro* Release of Meloxicam in Phosphate Buffer of pH 7.4 from implants prepared with Gelatin 30% w/w - Na Alginate drug implants hardened 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	28.81± 0.64	1.201	71.0	1.914
24	4.898	1.380	37.52± 0.16	1.453	62.48	1.861
36	6.000	1.556	52.75± 0.28	1.611	59.09	1.671
48	6.928	1.681	65.52± 21.0	1.727	46.59	1.542
72	8.485	1.857	76.61± 31.0	1.818	34.09	1.413
96	9.797	1.982	85.52± 0.51	1.865	26.59	1.471
120	10.954	2.079	95.41± 0.73	1.934	14.09	1.234

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

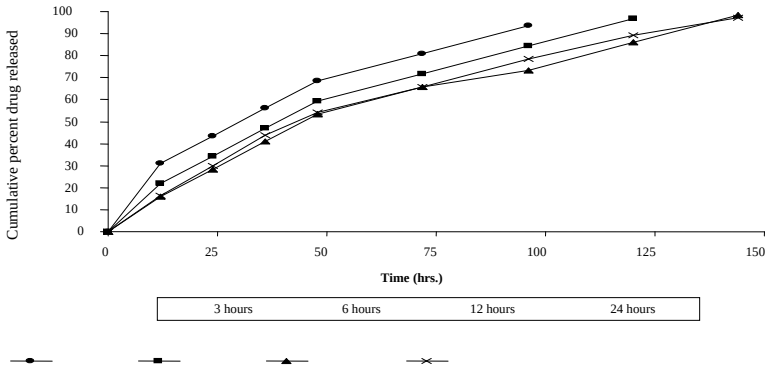

Figure 1. Comparative In-Vitro Drug Release of Implants in Phosphate Buffer pH 7.4 from implants hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

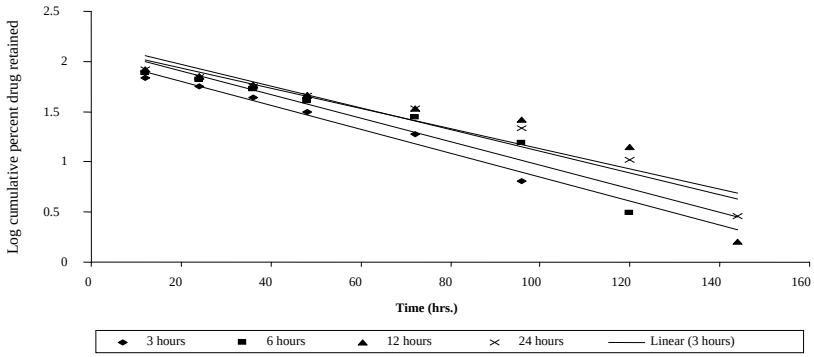

Figure 2. Comparative First order release plots of implants hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

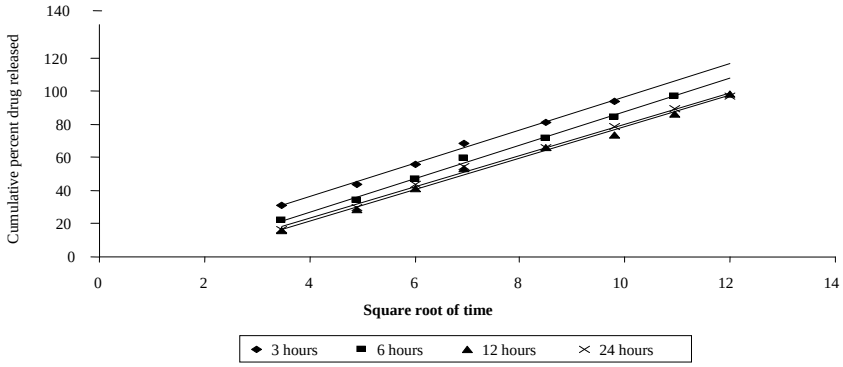

Figure 3. Comparative Higuchi square root plots of implants hardened with Formaldehyde for 3 hours, 6 hours, 12 hours and 24 hours

CONCLUSION

Gelatin-Na Alginate based subdermal implants of Meloxicam 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. Drug 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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