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STUDIES ON BIODEGRADABLE IMPLANTABLE DRUG DELIVERY DEVICE OF MELOXICAM FOR POST OPERATIVE ANIMAL 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, a nonsteroidal anti-inflammatory agent used in treatment of orthopaedic patient care. Implant formulated with 30%W/W Gelatin 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 showed there were no changes in appearance and the in-vitro drug release rates. Implants were found to erode slowly with diffusion mechanism. In vivo studies in sheep 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, Gelatin, Glutaraldehyde, Subdermal implant.

INTRODUCTION

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, interluckin-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 Meloxicam, the NSAID, by using gelatin for sustained release. The subcutaneous drug implants are hardened by exposing them to glutaraldehyde at different time intervals. The fabricated implants are studied for various physio-chemical parameters like weight variation, thickness, drug content uniformity, presence of free glutaraldehyde, drug polymer interaction, sterility test, in-vitro dissolution rate studies are performed on the implants by using phosphate buffer pH 7.4 at 362 nm. The implants are investigated for tissue polymer interaction by performing histopathological studies on sheep's thigh before and after implantation.

MATERIALS AND METHOD

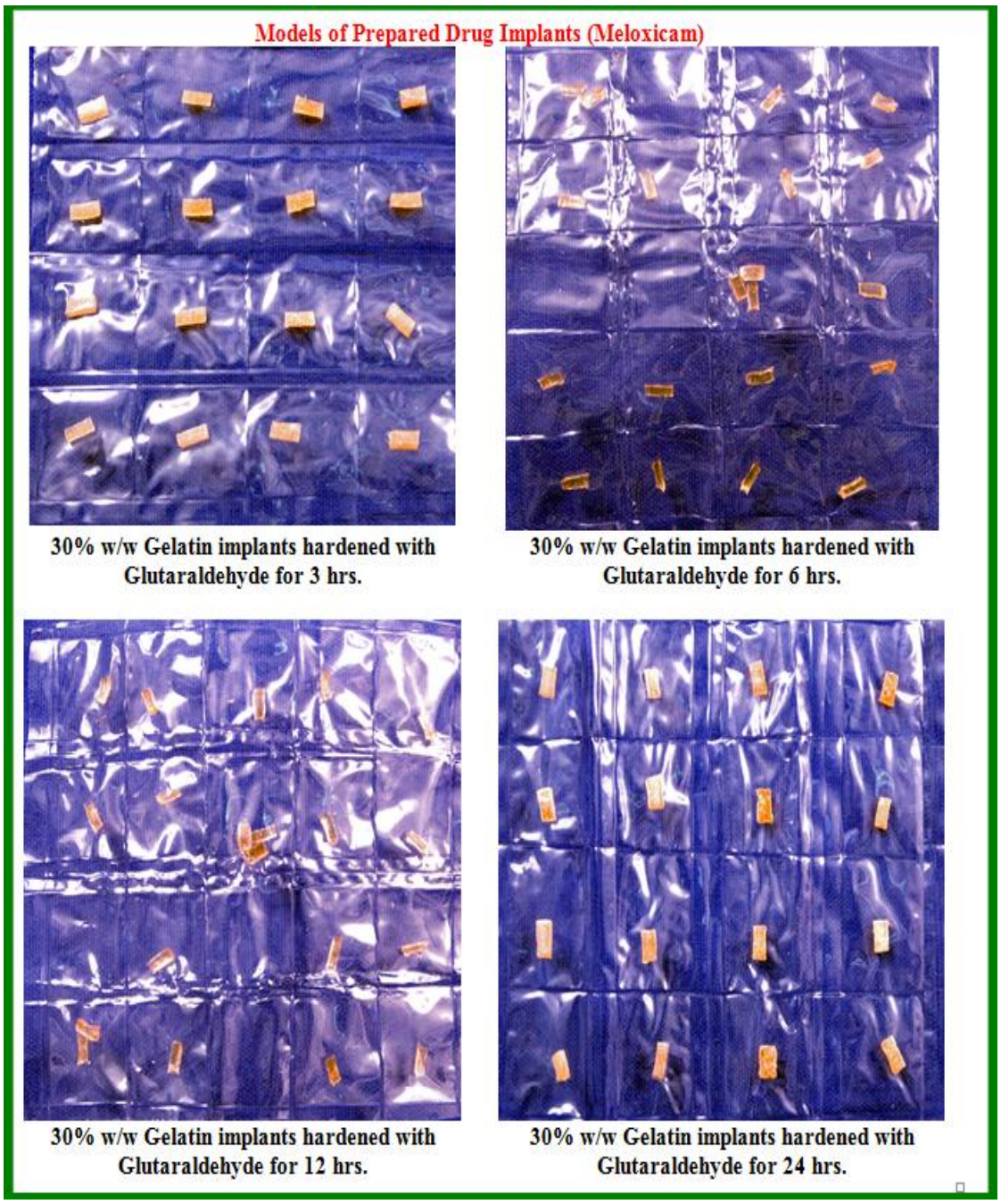
Meloxicam was obtained as a gift sample from Bio-vaccine Hi-tech formulation, Hyderabad (AP). Gelatin was purchased from S.D. Fine Chemicals Ltd., Mumbai. Glycerin and Glutaraldehyde were purchase from Ranbaxy Laboratories Ltd., Other chemicals used were of analytical grade.

Preparation of implants

Weighed quantity of polymer (15 gms) i.e Gelatin was sprinkled on the surface of water and kept aside for 30 minutes to hydrate. Glycerin (10 gms.) 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 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 an 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.[7]

Table -1: Formulation of Implants prepared.

Ingredients	Quantity
Meloxicam	4 gm
Gelatin	15 gms
Glycerine	10 gms
Water Q.S to	50 gms



Hardening of implants

A Petri-dish containing Glutaraldehyde solution (37% v/v) was placed in an empty glass desiccator. A wire mesh containing the implants was kept on the top of the Petri dish and the desiccator was closed immediately. The implants were made to react with glutaraldehyde vapors for different time interval such as 3,6,12 and 24 hours. Then they were removed from the desiccator and air-dried for 72 hours so that the reaction in between glutaraldehyde 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 glutaraldehyde gets evaporated.[8]

Evaluation of subdermal implants

Measurement of Thickness of Implants

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

Table-2: Thickness of implants prepared with 30% w/w Gelatin Hardened with glutaraldehyde.

Hardening time intervals in hours	Thickness (mm)					
	I	II	III	Mean	SD	CV
1	2.94	3.04	3.14	3.04	0.0102	0.3221
3	3.09	3.01	3.02	3.04	0.0109	0.3112
6	3.04	3.06	2.99	3.03	0.0016	0.3016
12	3.03	3.05	3.10	3.09	0.0033	0.0108
24	3.02	3.00	3.05	3.03	0.0013	0.0436

Weight Variation of implants

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

Table-3: Weight uniformity of implants prepared with 30% w/w Gelatin Hardened with Glutaraldehyde.

Hardening time intervals in hours	Weight of discs (mg)			Mean	SD	CV
	I	II	III			
1	127	127	127	127	0.015	0.080
3	128	127	126	128	0.006	0.041
6	127	127	124	127	0.031	0.481
12	127	127	127	127	0.371	0.642
24	128	129	128	128	0.039	0.031

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 362 nm in Phosphate buffer pH 7.4 on a UV spectrophotometer 1700 Shimadzu. The data was subjected to statistical analysis.[11]

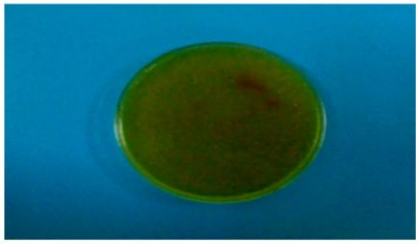
Table-4: Drug content uniformity in Gelatin based subdermal implants.

Formula name	Drug content (mg)			Mean	SD	CV
	I	II	III			
30% w/w gelatin discs	9.99	9.92	10.05	9.99	0.011	0.070
30% w/w gelatin discs hardened with glutaraldehyde	9.98	9.97	10.03	9.99	0.021	0.003

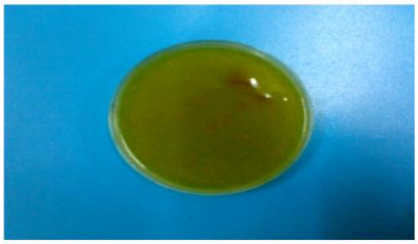
Tests for Sterility

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

STERILITY TEST OF IMPLANTS PREPARED



Agar Plate



Agar Plate with Implants after 72 Hours.

Observation: No growth of microorganism observed

Figure-2:

Test for Free Glutaraldehyde

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

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.[14,15]

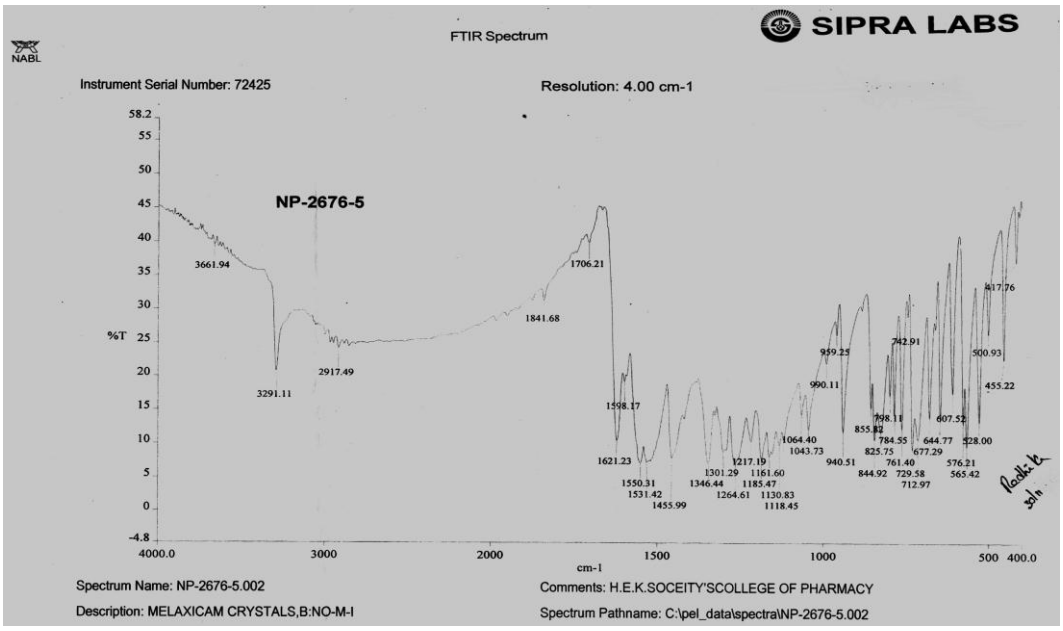


Figure-3: FTIR Spectrum of Meloxicam pure drug.

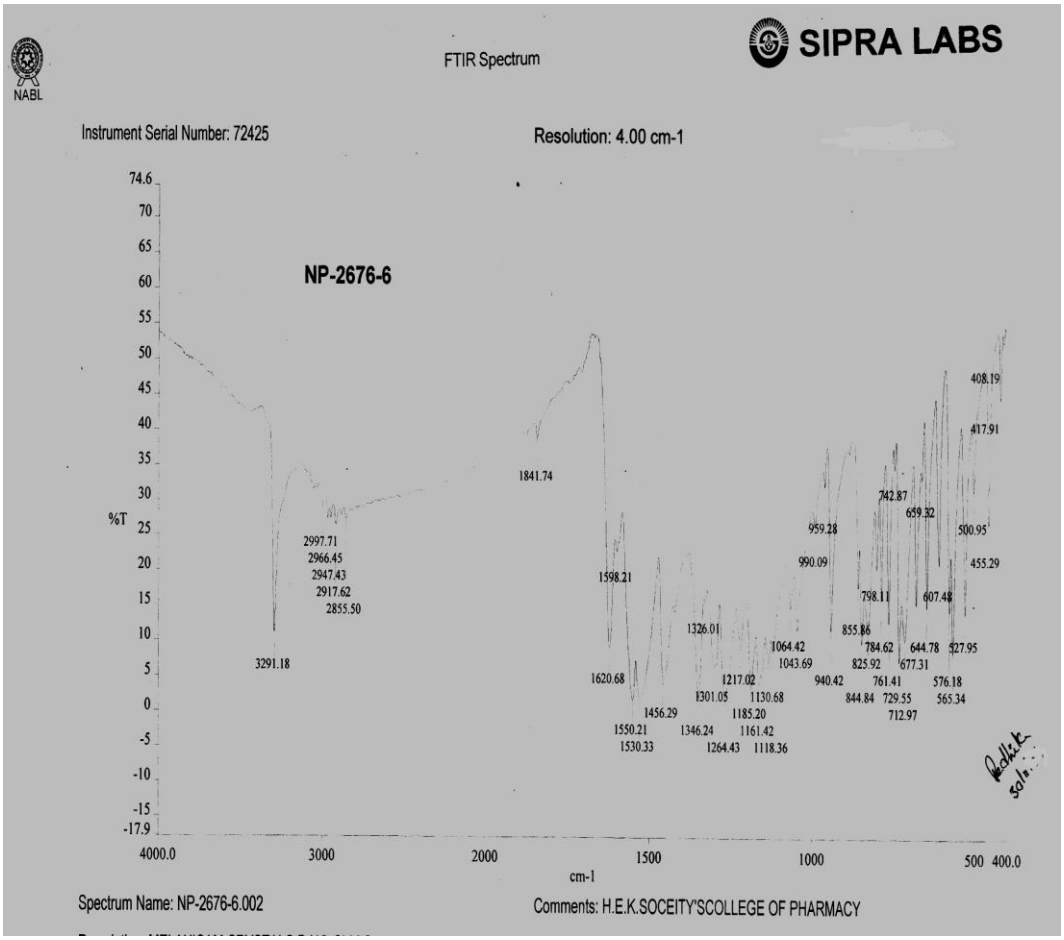


Figure-4: FTIR Analysis of Sample Gelatin implant hardened for 12 hours in Glutaraldehyde.

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 362 nm after suitable dilution with Phosphate buffer pH 7.4.[16,17]

Table-5: In-vitro Release of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Time in Hrs.	Percentage of Drug released with S.D. *			
	3 hours hardening	6 hours hardening	12 hours hardening	24hours hardening
0	0	0	0	0
1	19.45 ± .11	16.53 ± .08	16.14 ± .03	12.54 ± .03
2	24.37 ± .02	20.81 ± .03	17.89 ± .01	16.74 ± .06
3	27.11 ± .03	24.68 ± .02	20.62 ± .04	17.93 ± .11
4	28.71 ± .31	26.93 ± .06	22.99 ± .11	19.90 ± .08
5	35.50 ± .01	31.05 ± .07	23.61 ± .05	22.48 ± .04
6	40.22 ± .04	32.55 ± .02	31.38 ± .03	31.69 ± .02

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

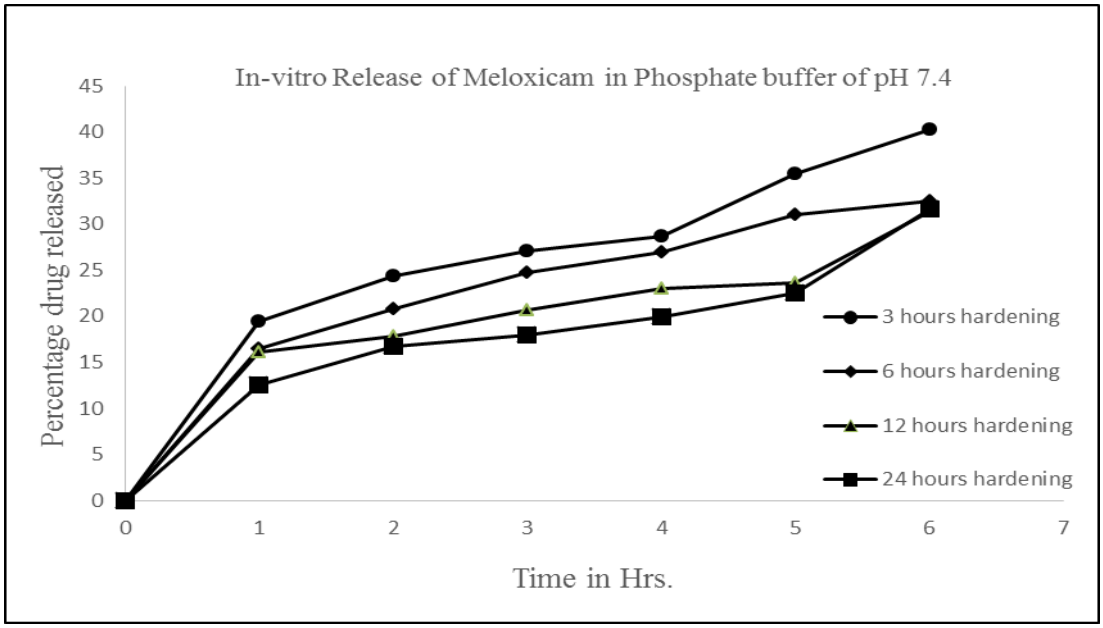


Figure-5: In-vitro Release of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Table-6: First Order Release of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Time in Hrs.	Log Percentage of Drug retained			
	3 hours hardening	6 hours hardening	12 hours hardening	24hours hardening
0	0	0	0	0
1	1.9061	1.9215	1.9284	1.9418
2	1.8787	1.8987	1.9144	1.9204
3	1.8627	1.8769	1.8997	1.9142
4	1.8538	1.8649	1.8865	1.9036
5	1.8420	1.8385	1.8830	1.8894
6	1.7766	1.8287	1.8532	1.8345

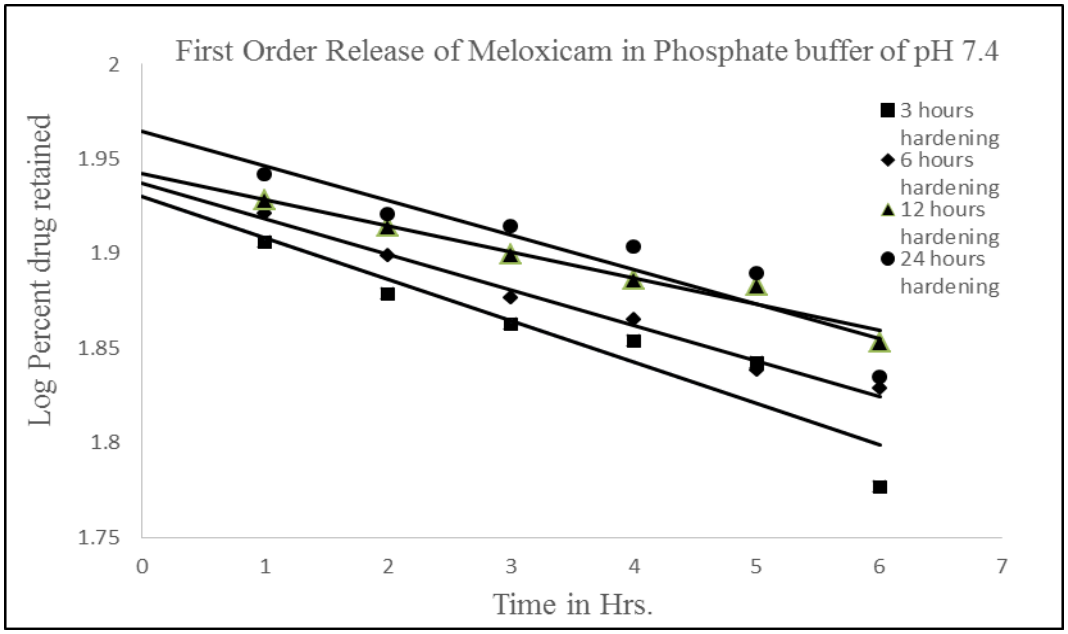


Figure-6: First Order Release of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Table-7: Higuchi's diffusion plot of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Time in Hrs.	Square root of time in hours	Percentage of Drug released with S.D. *			
		3 hours hardening	6 hours hardening	12 hours hardening	24hours hardening
0	0	0	0	0	0
1	1.0000	19.45 ± .11	16.53 ± .08	16.14 ± .03	12.54 ± .03
2	1.4142	24.37 ± .02	20.81 ± .03	17.89 ± .01	16.74 ± .06
3	1.7321	27.11 ± .03	24.68 ± .02	20.62 ± .04	17.93 ± .11
4	2.0000	28.71 ± .31	26.93 ± .06	22.99 ± .11	19.90 ± .08
5	2.2361	35.50 ± .01	31.05 ± .07	23.61 ± .05	22.48 ± .04
6	2.4495	40.22 ± .04	32.55 ± .02	31.38 ± .03	31.69 ± .02

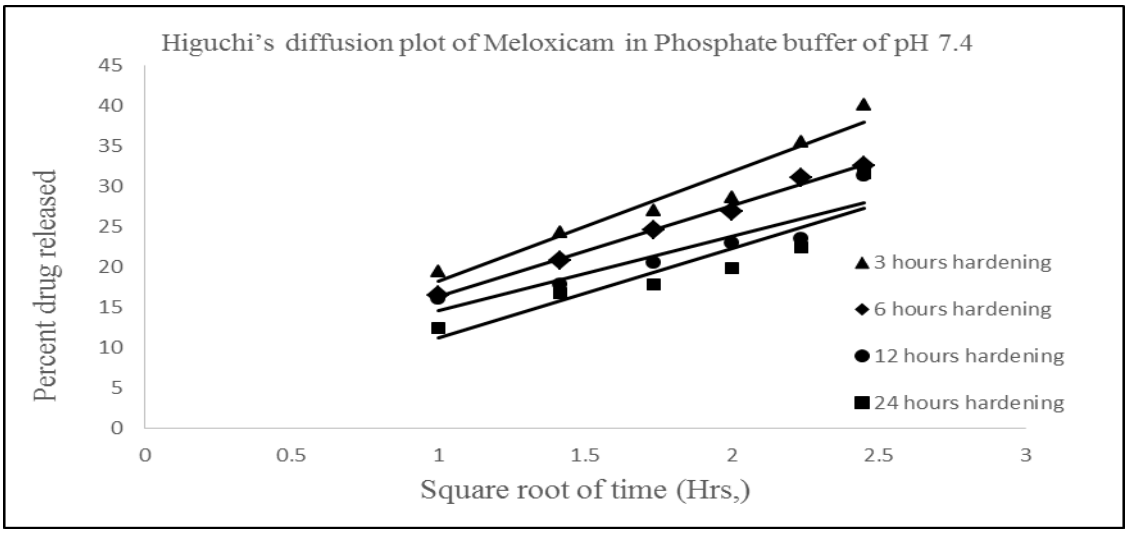


Figure-7: Higuchi's diffusion plot of Meloxicam in Phosphate buffer of pH 7.4 from implants prepared using Gelatin and Hardened with Glutaraldehyde.

Table-8: Statistical Analysis Values of Meloxicam in Phosphate Buffer of pH 7.4 from implants prepared with 30% w/w gelatin hardened with Glutaraldehyde.

Hardening time intervals of Glutaraldehyde		Zero Order	First Order	Higuchi Model
	r	0.9562	-0.2082	0.8810
3 hrs.	a	-5.9328	23.6400	-0.9260
	b	0.5114	-5.4550	3.1308
	r	0.9910	0.9640	-0.8687
6 hrs.	a	1.0240	51.6060	3.8408
	b	0.1600	-25.0690	-1.7591
	r	0.9650	0.0913	0.9200
12 hrs.	a	-2.8300	14.6220	0.3580
	b	0.3730	1.7690	2.5030
	r	0.9706	0.0462	0.9308
24 hrs.	a	-5.9466	1.4439	-0.6243
	b	1.0942	8.6206	4.7071

r = regression coefficient, a = intercept, b = slope

In Vivo Studies (Tissue-Polymer Interaction Studies)

In vivo studies were done in sheep. 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.[18,19]

In vivo studies in sheep through histopathological observations as shown in photo graphs of varied magnifications revealed that at subdermal thigh regions before and after one month of implantation of polymeric implants.

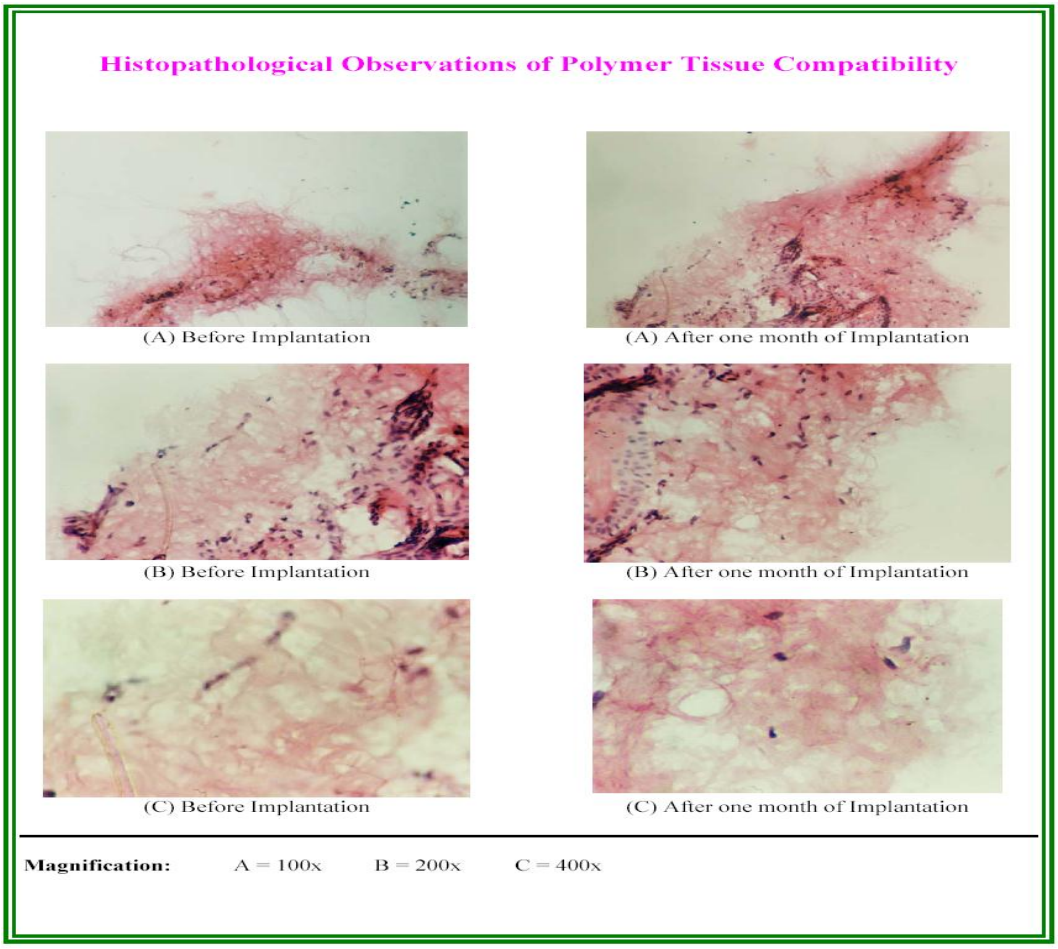


Figure-8: Histopathological Studies Thigh Region.

RESULTS AND DISCUSSION

Implants of meloxicam were prepared employing Gelatin (30% w/w) and hardened with glutaraldehyde for 3, 6, 12 & 24 hours (Table-1). Meloxicam rod shaped implants gave uniform results for thickness (Table-2), weight variation (Table-3), drug content (Table-4) and drug release characteristics. The data was subjected to statistical analysis (Table-8). 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 (Figure-2). The sample solution was not more intensely coloured than the standard solution inferring that less than 20 mcg of free glutaraldehyde is present in 25 implants. The I.R. reports of drug implants hardened with glutaraldehyde indicating absence of interaction between drug and the excipients used (Figure-3 & 4). The drug release studies of Meloxicam implants in phosphate buffer pH 7.4 indicated 40.22%, 32.55%, 31.38%, 31.69 % of drug release in 6 hours from glutaraldehyde hardened implants of 3 hrs., 6 hrs., 12 hrs., & 24 hrs. respectively (Table-5, Figure-5). The In-vitro dissolution studies revealed that implants hardened with glutaraldehyde show zero order rate kinetics (Table-6, Figure-6). 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 (Table-7, Figure-7). In-vivo studies in animals (sheep) 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 present. Thus, Gelatin was found to be compatible with the tissues at subdermal region (Figure-8).

CONCLUSION

Gelatin based subdermal implants of Meloxicam having uniform character can be prepared with minimum batch to batch variation. The subdermal implants containing Gelatin and hardened with glutaraldehyde for 12 hours are found to produce the most satisfactory sustained 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. The design of Meloxicam drug implants helps in the reduction of inflammation and pain in animals in the form of subdermal implants.

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