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Formulation and Evaluation of Novel Biodegradable Sustain Released Matrix Implant of Gentamicin Sulphate.

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ABSTRACT

The aim of this work was to formulate and evaluate GMS (Glycerol monosterate) based matrix implant of Gentamicin Sulphate (GS). GMS based implants were prepared by using combination of PEG (Polyethyleneglycone) and GMS as hydrophobic biodegradable sustain released matrices along with different percentage of Sorbitol and Tween80 as erosion enhancers and by using 10% Gentamicin sulphate as model drug for local delivery in the treatment of bone infection. Seven formulations were prepared (K1-K7) by melt granulation followed by compression to form disc shaped implants. GMS based implants were evaluated for physiochemical parameters, swelling study, matrix erosion, in-vitro drug release by rotating vial method, accelerated stability study and Histocompatability study. The effect of different percentage of erosion enhancers on drug release profile of Gentamicin sulphate from hydrophobic matrices was studied. Formulation K4 which contains 10% Sorbitol as erosion enhancer and shows excellent cumulative drug release profile and it does not completely lose its physical shape up to 28 days. This formulation has highest R^2 value (0.9926) and low fluctuations in drug release profile thus this formulation conclude to be optimum formulation among the all GMS based implant formulations (K1-K7). All GMS based Gentamicin sulphate implant formulations best fitted with Korsmeyer Peppas kinetic model and thus shows the biphasic drug release pattern. This includes initial burst release profile followed by slow release of drug for prolonged time. Histocompatability study shows good tissue compatibility profile. Formulation K4 shows excellent drug release profile up to 28 days thus this formulation can be effectively used in the treatment of bone infection.

Keywords: Gentamicin sulphate, Glycerol Monosterate, Biodegradable, Bone infection, Implant.

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INTRODUCTION

Osteomyelitis is as such an historic infection which is still remains challenging and difficult to treat, despite of advances in antibiotics and new operative techniques. Osteomyelitis is an orthopedic disease caused by bacterial infection of the bone medullary cavity, cortex and/or periosteum leading to bone loss.[1-3] Most commonly bacterial isolates in patients with chronic osteomyelitis are *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Proteus mirabilis*.[4] Commonly used antibiotics to treat osteomyelitis are fluroquinolones, ß-lactums and aminoglycosides.[4-5] *Staphylococcus aureus* (80-85%) is the major organisms associated with Osteomyelitis. The treatment of Osteomyelitis requires large doses of antibiotics administered by systemic routes for a period of 4-6 weeks. However, the disadvantages of systemic therapy are that only a small fraction of any given dose actually reaches the infection site, producing low-therapeutic tissue levels; and that an antibiotic overdose often has various adverse systemic effects.[6] Local antibiotic administration is therefore considered necessary. Thus development of implantable drug delivery is essential to overcome bone infection and to provide effective local treatment with minimal systemic side effects.

Amino glycoside antibiotics like Gentamicin sulphate is excellent broad spectrum antibiotics given through systemic route because it do not absorb through oral route. Gentamicin sulphate can reduce about 99% of infection causes due to *S. aureus* .Thus Gentamicin sulphate can be employed as suitable drug candidate in bone infection. [7] Gentamicin poly(methyl methacrylate) (PMMA) beads have been employed clinically to prevent or treat osteomyelitis.[8-10] However, since PMMA is a non-biodegradable material, secondary surgery is required to remove the beads after Gentamicin has been released. Thus GMS based Gentamicin sulphate implants can be effectively used as local delivery of antibiotics in the treatment of bone infection. GMS and PEG combination well adapted particularly to the implantable dosage forms because they biodegrade quickly to an acceptable level after delivery of drug.[11] Thus GMS and PEG can be used as biodegradable hydrophobic matrices in the development of local biodegradable sustain release implantable drug delivery systems (IDDS) for effective management of bone infection.

The aim of this investigation was to develop and characterize biodegradable implants, composed of GSM –PEG and drug blends, as drug delivery systems that could provide local bactericidal concentrations of Gentamicin sulfate (GS) for at least 4–6 weeks. In this work in-vitro drug release study was carried out by using rotating vial method and effect of different percentage of erosion enhancer on drug release profile was studied. Gentamicin was selected in this study because it is widely used for the treatment of osteomyelitis due to its broad spectrum characteristics. This methodology may prove effective to develop high drug loaded various GSM-antibiotic implantable bone-delivery systems with the property of antibiotic releasing for several weeks, which may enable clinicians to achieve effective antibiotic therapy at infected bone site, thereby facilitating individualized chemotherapy for local bone-delivery to treat bacterial bone infections like osteomyelitis.

MATERIALS AND METHODS

Materials

Gentamicin Sulphate was obtained as gift sample from Agio pharmaceuticals (Bhosari, Pune, India), Glycerol Monosterate, PEG6000, Sorbitol, and Tween80, were purchased from research-lab fine chem. industries (Mumbai, India), Ninhydrin was purchased from Merk limited (Mumbai, India). All other chemicals used were of analytical grade.

Methods

A] Drug-Excipient compatibility study

The samples of binary mixtures of Gentamicin Sulphate and excipients (1:1) were analyzed by FTIR, DSC and isothermal stress testing. These samples were observed for any change in physical appearance and physiochemical interactions between Gentamicin sulphate and excipients which indicates compatibility between the ingredients.[12-15]

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B] Preparation of implants

Preparation of GMS based implant was carried out in two steps including melt granulation (Thermoplastic granulation) and compression of granules to form disc shaped matrix implants. This process was carried out as follows-

Formulations K1-K7 was prepared with different ratio of GMS, PEG6000 and various levels of erosion enhancers as shown in table (1).At the first all excipients were passed through #60 mesh screen to obtain uniform particle size powder blend. The Gentamicin sulphate and other excipients were mixed by using glass mortar & pestle. This powder blend was then transferred in to glass petridish and melted at 80℃ on a thermostatic water bath [Biotech. India] maintained at constant temperature. The molten mixture was allowed to cool and solidified at room temperature for 10min followed by solidification in freezer for 1hrs. The solidified mass was pulverized in mortar and sieved through a 18 # screen. The granules were evaluated for flow properties by determining bulk density, tapped density, angle of repose, Hausner's ratio and percentage compressibility. These granules are compressed in to disc shaped implants by using flat-faced 8-mm punches in ten station rotary press [General machine, Bombay, India]. The prepared implants (3× 2mm)a are shown in fig. (1). Prepared implants were stored at room temperature in an airtight amber colored glass vials with cotton packing for further study.

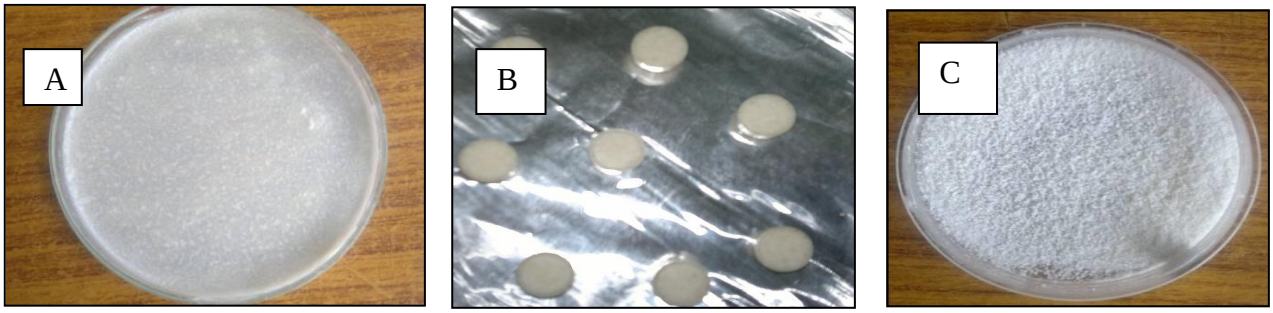

Fig: 1 Stages in implant preparation process
A] Molten mass of drug-excipient blend B] GMS based granules prepared by using melt granulation process
C] GMS based Implants (Disc shaped implants)

Table 1: Composition of Different formulations of GMS based implant.

	K1	K2	K3	K4	K5	K6	K7
Drug [%]	10	10	10	10	10	10	10
GMS [%]	60	60	60	60	60	60	60
PEG 6000 [%]	30	25	22.5	20	25	22.5	20
Sorbitol[%]	-	5	7.5	10	-	-	-
Tween 80 [%]	-	-	-	-	5	7.5	10

C] Evaluation of prepared Implants:

GMS based implants were evaluated by using following evaluation parameters.

a) Physiochemical evaluation

1] Dimensional analysis: **[16]** –

It includes evaluation of thickness and diameter of compressed implants (n=10). Ten implants from each batch of formulation were randomly selected & subjected for dimensional analysis. Thickness & diameter

of implants were determined by using micrometer screw Gauge [Yamayo classic micrometer] and the mean thickness and diameter with respective S.D. were calculated for each batch of formulation.

2] Hardness: [16]

The implants (n=6) from each batch of formulation were randomly selected & subjected for hardness test. Hardness was determined by using Monsanto hardness tester (Cadmach, Ahmadabad, India).

3] Drug content uniformity: [16] -

The implants (n=3) were randomly selected from each batch of formulation and subjected for content uniformity test. The implants were taken and milled separately by using Glass mortar and pestle then powder equivalent to 10mg of drug were accurately weighed and transfer 50ml of phosphate buffer solution (PBS, pH 7.4) and stirred at 80 rpm for 1hrs by using magnetic stirrer. Resulting solution was filter through whatmman filter paper and the final volume adjusted with PBS (pH 7.4) up to 100ml. Then the suitable dilutions were prepared and samples were analyzed by using validated colorimetric Ninhydrin assay method for Gentamicin sulphate at 400nm. This method developed by Frutos et al. (2000), briefly 5ml stock solution mixed with 1.5 ml of Ninhydrin solution (1.25%) and heated at 95℃ for 15 min. This solution then cooled in ice bath and analyzed at 400nm.

The results of evaluation of implants for physical appearance, thickness, Diameter, hardness, and drug content were shown in result section.

b] % water Uptake and matrix erosion study: [17-19]

Initially implants were weighed (t=0), then placed into the vial containing 15 ml phosphate buffer [pH 7.4] and kept in orbital incubator shaker which is operated at 60rpm speed and 37℃ temperature. At predetermined time intervals [1, 2, 4, 8, 24hrs, 7, 14, 21 and 28 day] and the implants were withdrawn from the release medium. The implants were blotted dry by using filter paper to remove excess surface water. The systems accurately weighed [wet mass (t)] and dried to constant weight in an oven at 37℃ [dry mass (t)]. The water content (%) (t) and matrix erosion (%) (t) Were calculated as follows:

$$\text{Water Uptake (\%) (t)} = \frac{\text{Wet mass (t)} - \text{dry mass (t)}}{\text{Wet mass (t)}} \times 100$$

$$\text{Matrix Erosion (\%) (t)} = \frac{\text{Dry mass (do)} - \text{Drug released(t)-Dry mass (t)}}{\text{Dry mass (do)}} \times 100$$

Where, Dry mass (do) denotes the dry implant mass at t = 0, Drug released (t) the cumulative amount of drug released at time (t), Wet mass (t) = mass of implant after treated with release medium, Dry mass (t) = mass of implant after drying at time t.

c] In-vitro Drug release evaluation: [20, 21]

In-vitro drug release profile was determined by rotating vial method as follows-

The implants from various formulation batch were subjected to short term dissolution study (1,2,4,8,24hrs) and Extended in vitro drug release study (2,4,7,14,21,28 days).The implants in triplicate were tested over 28 days using rotating vial method. The implants were individually placed into 20ml glass vials containing 15 ml phosphate buffer (7.4 pH) with 0.1% w/v sodium azide as preservatives to prevent microbial growth. The vials are kept in orbital incubator shaker and agitated to 60rpm at 37℃. At predetermined time

interval 1, 2, 4, 7, 10, and 14 days and there after once a week up to 28 days the release medium was completely replaced with fresh phosphate buffer pH 7.4 to maintain sink condition. The 2ml aliquots of sample was withdrawn with help of syringe and diluted up to 10ml with PBS. The amount of GS release was determined spectrophotometrically by Frutos method as described above. The percentage cumulative release and drug release kinetics was determined by PCP Disso India. The release profiles from various implant formulations are shown in fig (5, 6) and % cumulative drug release given in table (3). In order to investigate the mode of release from the implants the release data were analyzed with the mathematical models (zero order, first order, Higuchi, Korsmeyer-Peppas model, and Hixson Crowell).

d] Selection of formulation for sterilization, stability and biocompatibility study:

The formulation showing good result drug release profile with minimum burst effect has been selected for sterilization, biocompatibility and stability study.

e] Sterilization of implants and sterility test: [22, 23]

Optimized formulation of GMS based implants was sterilized by Gamma sterilization process. The sterility testing GMS based implants were carried out as per Pharmacopoeia of India (2007). Inactivation of antimicrobial property of Gentamicin sulphate was carried out by diluting the implant sample (below MIC value of Gentamicin Sulphate, 16-32µg /ml) as per IP 2007 Procedure. Three units (n=3) sterilized by gamma radiation were subjected to sterility test.

f] Biocompatibility study: [24-27]

The aim of this study was to evaluate the subcutaneous biocompatibility of GMS based Gentamicin sulpha[A]plants. As per the protocol approved by ethical c[B]tee; in vivo Histocompatability study of implan[illegible]s carried out in Wistar rat. Eight, 2-3 month-old male Wistar rats weighing 200 to 250g were randomly selected and divided into 4 experimental groups (n=2/group), for GMS based implants. The animals were anesthetized by inhalation anesthesia with diethyl ether after inhalation anesthesia; general anesthesia was induced by intraperitoneal Injection of ketamine (75 mg/kg). The implant was placed in to subcutaneous space as shown in fig (). All groups were subdivided into 3 evaluation periods Group 1: control [normal], Group 2: for 7 day implantation, Group 3: for 14 day implantation, Group 4: for 28 day implantation. At predetermined time period implant was removed with the surrounding tissue. The tissue samples were mounted on glass slide and stained with Hematoxylin and eosin. Each specimen was analyzed at ×400 magnifications with a light microscope. The samples were evaluated for Cellular inflammatory responses, Necrosis, Capsule thickness, Ulcer formation, Cellular infiltration, edema, migration of inflammatory cells at implantation site and other foreign body tissue reactions. The microscopical view of tissue specimen is shown in fig (7) and results are shown in table (4).

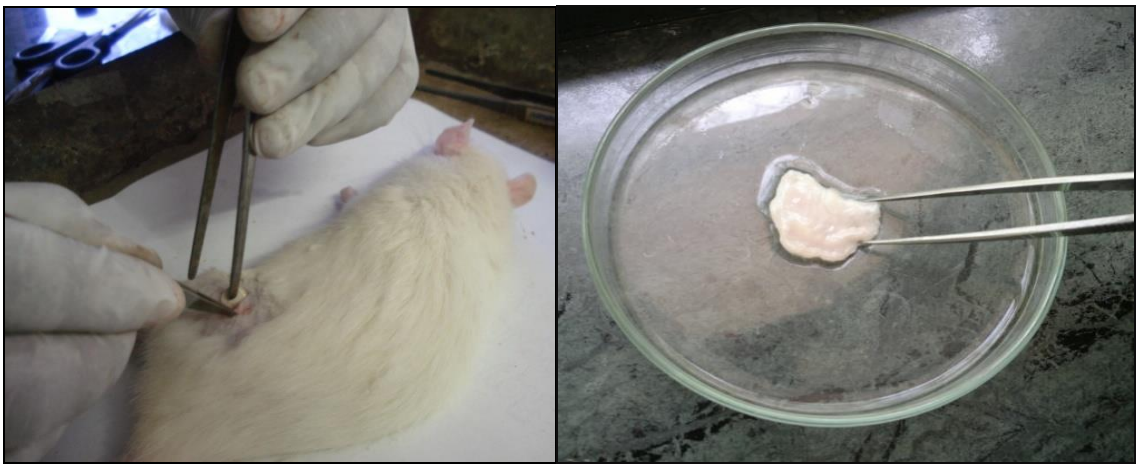

Fig: 2 Photographic views of various stages of biocompatibility study,
A] Insertion of implant in subcutaneous tissue and B] Tissue Specimen

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g] Stability study: [28-30]

Stability study was conducted as per the ICH Guidelines. Formulation K4 (n=10 units) of GMS based implant was wrapped in aluminum foil, this implants were placed in amber colored vials, sealed and kept for stability studies as per ICH guidelines under accelerated conditions 40 ± 2 °C / 75 ± 5 % RH for 3 month period. At one month intervals sample from each formulation was withdrawn and evaluated for physical properties, drug concentration and 24hrs dissolution study. The results are shown table (5).

RESULTS AND DISCUSSION

Drug-excipient compatibility study

Drug- excipient study was carried out by using thermal technique (DSC and Isothermal stress testing) and nonthermal technique (FTIR). From drug excipient study it is found that drug-excipient mixture have better compatibility and thus this combinations can be effectively used for development of GMS based implants.

Preparation and evaluation of GMS based granules

GMS based granules were prepared by thermoplastic granulation and granules were evaluated for flow properties parameters such as bulk density, tapped density, angle of repose, Hausner's ratio, and percentage compressibility. From results it is found that GMS based Granules has good flow properties and it complies with reported standard values.

Evaluation of prepared Implants

Physiochemical Evaluation of implants

GMS based implants were evaluated for Physiochemical parameters like Physical appearance, Thickness, Diameters, Hardness, and Drug content. The results are shown in table (2).

Table 2: Physiochemical Evaluations of GMS Based Implants.

Sr. no.	Parameters	K1	K2	K3	K4	K5	K6	K7
1.	Physical Appearance	Disc shaped implant	Disc shaped implant	Disc shaped implant	Disc shaped implant	Disc shaped implant	Disc Shaped implant	Disc shaped implant
2.	Thickness (mm, mean±SD)	3.35±0.03	3.32±0.11	3.32±0.02	3.12±0.17	3.25±0.15	3.27±0.14	3.29±0.13
3.	Diameter (mm, mean±SD)	8.27±0.12	8.32±0.04	8.34±0.13	8.18±0.13	8.36±0.06	8.34±0.04	8.39±0.03
4.	Hardness (kg/cm^{2}mean±SD)	3.2±0.20	3.0±0.09	3.1±0.29	3.06±0.09	2.9±0.29	2.78±0.20	2.9±0.41
5.	%Drug content (mean±SD)	95.2±2.2	98.7±1.25	96.6±3.33	96.5±1.5	94.75±2.75	102±2.4	96±2.08
6.	%RSD +of Drug content	2.31	1.26	3.44	1.55	2.90	2.35	2.16

The results for physiochemical evaluation are shown in table. Thickness of the Implants for all the formulations were found to be between 3.12 mm and 3.35 mm, with the average of 3.27mm. The maximum standard deviation in thickness was up to 0.17. Diameter of implants for all the formulations was found to be between 8.18 mm and 8.39mm, with the average of 8.31mm. The maximum standard deviation in diameter was up to 0.13. The hardness of the implants was found to be in the range 2.78 kg/cm^2 to 3.2kg/cm^2 and maximum standard deviation in Hardness was found to be up to 0.41. The % Drug content for all the formulation was found to be between 95.2% and 102% and % RSD for all formulation was found to be within

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1.26 to 3.44 which is within USP limit (RSD less than or equal 6.0%).Thus all Formulations complies with USP standards.

% water uptake & matrix erosion study

The results for water uptake and matrix erosion studies are shown in fig. (3, 4). The formulation K1 contains GMS and PEG and does not contain any erosion enhancer and shows low water uptake capability and low % matrix erosion profile. Formulation K2-K4 contains Sorbitol as erosion enhancer and K5-K7 contains Tween80 as erosion enhancer in different amount (as shown in table 1). The formulations K2-K4 shows lower water uptake and % matrix erosion profile than formulations K5-K7. Formulation K7 shows highest water uptake and matrix erosion profile among all formulations. This may be due to high concentration of Tween80 (10%) in K7 formulation. Formulation K4 contains 10% Sorbitol as erosion enhancer and shows high water uptake and matrix erosion profile than the K2 and K3 but shows lower water uptake profile than the K7 this may be due to low erosion enhancing activity of Sorbitol than the Tween80.

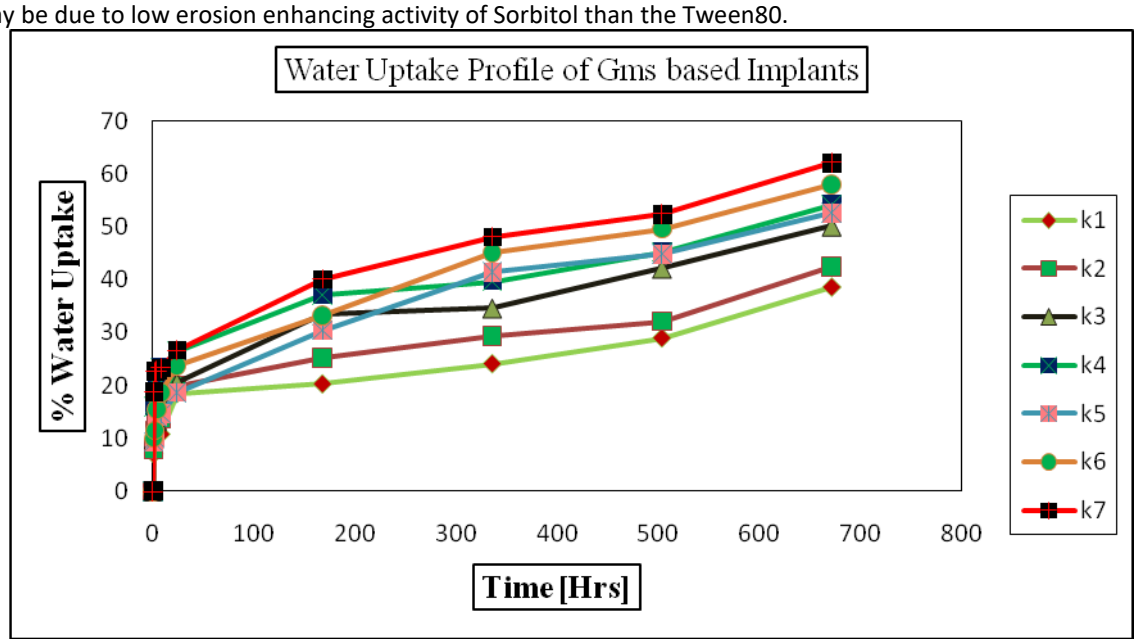

Fig 3: %Water Uptake Profile of GMS based Implants [K1-K7]

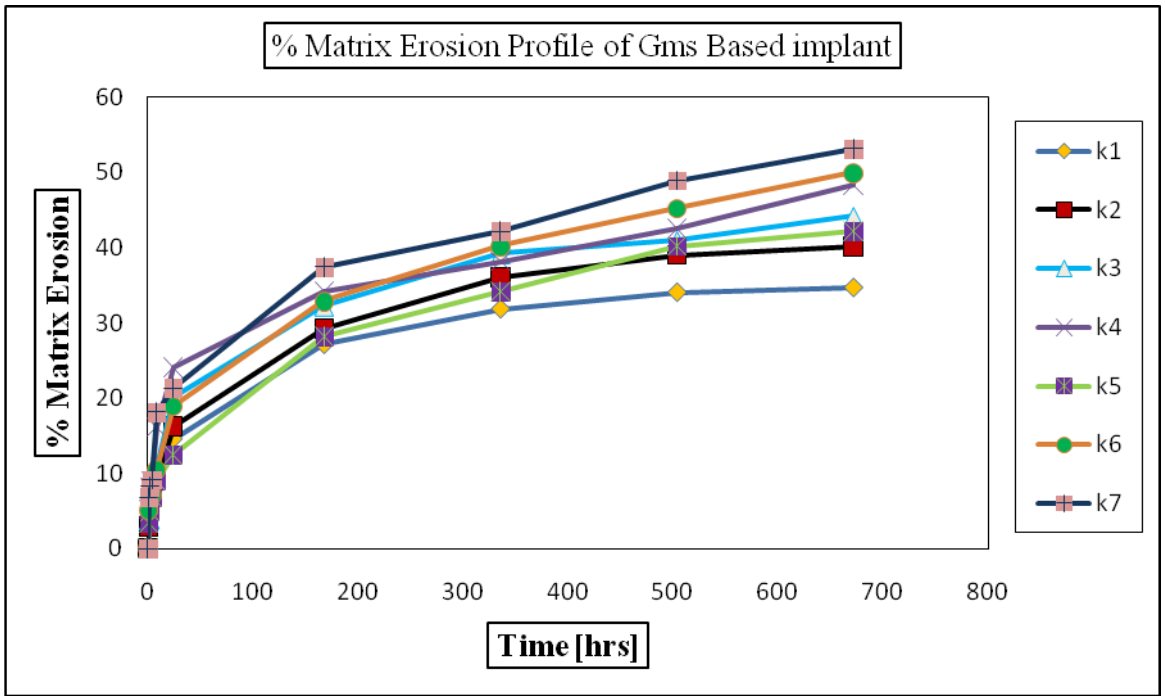

Fig 4: %Matrix Erosion Profile of GMS based implants [K1-K7]

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In-vitro Drug release evaluation

Gentamicin sulphate release from the formulations from different batches of GMS based implants was studied in triplicate under sink conditions to determine the release uniformity within formulations. The drug release profiles are shown in fig (5, 6) and results are shown in table (3).

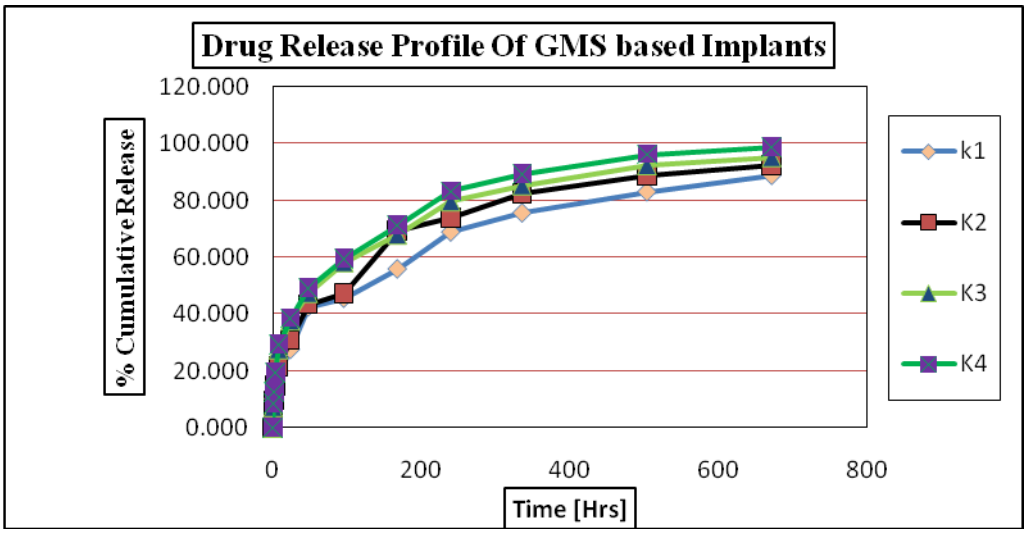

Fig 5: Drug release profile of GMS based Implants [K1-K4]

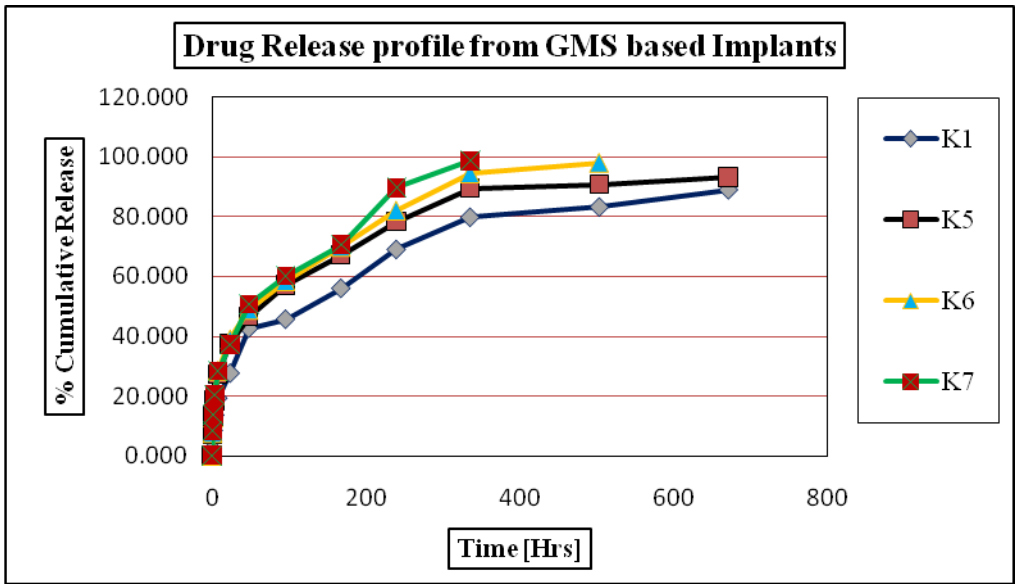

Fig 6: Drug release profile of GMS based Implants [K1, K5-K7]

Table 3: % Cumulative Release from GMS based implants (n=3)

	% Cumulative Drug Release (mean± SD)						
Time	**K1**	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**
1	5.26±0.19	6.50±0.58	7.37±0.16	8.67±0.94	7.30±0.57	7.68±0.18	8.36±0.82
2	8.56±0.35	9.84±0.68	13.48±0.36	12.68±2.1	13.46±0.12	12.84±0.83	13.87±2.4
4	13.68±0.95	14.95±0.22	19.76±0.49	19.47±0.34	18.44±0.54	20.36±0.44	20.42±0.74

8	19.33±0.62	21.70±0.37	27.59±1.23	29.50±0.28	27.39±0.35	28.44±0.40	28.36±0.28
24	27.69±0.43	32.94±0.83	37.70±0.39	38.53±0.26	37.51±0.98	40.92±0.62	43.14±0.47
48	42.66±0.82	43.42±0.28	47.37±0.25	49.21±0.64	46.63±0.22	48.76±0.42	50.60±1.25
96	45.72±1.52	47.37±0.56	58.20±0.62	59.63±0.82	57.31±0.46	58.35±0.38	60.21±2.14
168	56.03±0.67	69.36±1.84	67.94±0.72	71.16±0.96	67.27±0.78	69.76±0.98	70.64±1.45
240	69.01±0.21	73.80±0.92	79.63±0.56	83.28±1.7	78.56±0.66	81.89±0.73	89.69±3.2
336	75.75±2.1	82.49±0.98	85.20±0.86	89.40±0.38	89.68±0.16	94.32±1.2	98.79±2.8
504	83.18±1.82	88.64±0.84	92.26±1.8	96.36±0.42	90.92±3.5	97.827±2.8	-
672	88.95±2.6	92.43±3.24	95.13±2.4	98.85±0.95	93.41±0.72	-	-

In-vitro release study of GMS based implants was carried out by using Rotating vial method. The % cumulative release for short term (24hrs) and long term release study (28days) is shown in table (3). From the above result it is found that formulation K1 which does not contains any erosion enhancers shows low cumulative drug release profile this may be due to low water uptake profile and low matrix erosion profile. The formulations K2-K4 shows lower water uptake profile than formulations K5-K7 and thus shows lower % cumulative release than formulation K5-K7. Formulation K2-K4 contains different concentration of Sorbitol as erosion enhancer and thus shows different % cumulative drug release profile. Among these formulations K4 which contains high concentration of Sorbitol (10%) shows high cumulative drug release profile than K2 and K3. Formulations K1-K4 shows initial burst drug release followed by slow drug release profile up to 28 days. As concentration of Tween80 increases water uptake and matrix erosion profile of K5-K7 increases this results into increase in % cumulative drug release profile. Formulation K6 contains 7.5% of Tween80 and shows high water uptake profile thus shows high % cumulative drug release profile than K5. Formulation K6 shows initial burst release followed by slow drug release up to 21days and this formulation loses its physical shape after 21 days. Formulation K7 shows highest water uptake profile among all formulations and shows highest cumulative drug release profile than all formulations. K7 shows initial burst release followed by slow drug release up to 14 days and this formulation loses its physical shape after 14 days. This may be due to high concentration of Tween80 in K6, K7 formulation. Thus these formulations cannot be effective in treatment of osteomyelitis.

All GMS based Gentamicin sulphate implant formulations best fitted with Korsmeyer Peppas kinetic model and thus shows the biphasic drug release pattern. This includes initial burst release profile followed by slow release of drug for prolonged time. All formulations shows 'n' (Release exponent) value in range of 0.4172 to 0.5901 indicates that all formulation follows anomalous (non-Fickian) diffusion ($0.45 < n < 0.89$) as drug transport mechanism except K1 formulation which has n value 0.4172 which shows diffusion as drug transport mechanism.. Thus drug release from all formulations was found to be diffusion and erosion controlled. Formulation K4 which contains 10% Sorbitol as erosion enhancer and shows excellent cumulative drug release profile and it does not completely lose its physical shape up to 28 days. This formulation has highest R^2 value (0.9926) and low fluctuations in drug release profile thus this formulation conclude to be optimum formulation among the all GMS based implant formulations (K1-K7).

Sterilization and Sterility testing

Sterilization of implant was carried out by gamma sterilization and Sterility test of GMS based implants were performed as per IP 2007. Implants were observed periodically throughout the 14 days incubation period. The test samples does not show any evidence of macroscopical changes (no change in turbidity) thus it was concluded that test sample does not show any microbial growth and thus test sample is consider to be sterile and complies test for sterility as per IP 2007.

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Biocompatibility study

The microscopical view of tissue specimen is shown in fig (7). and results are shown in table (4).

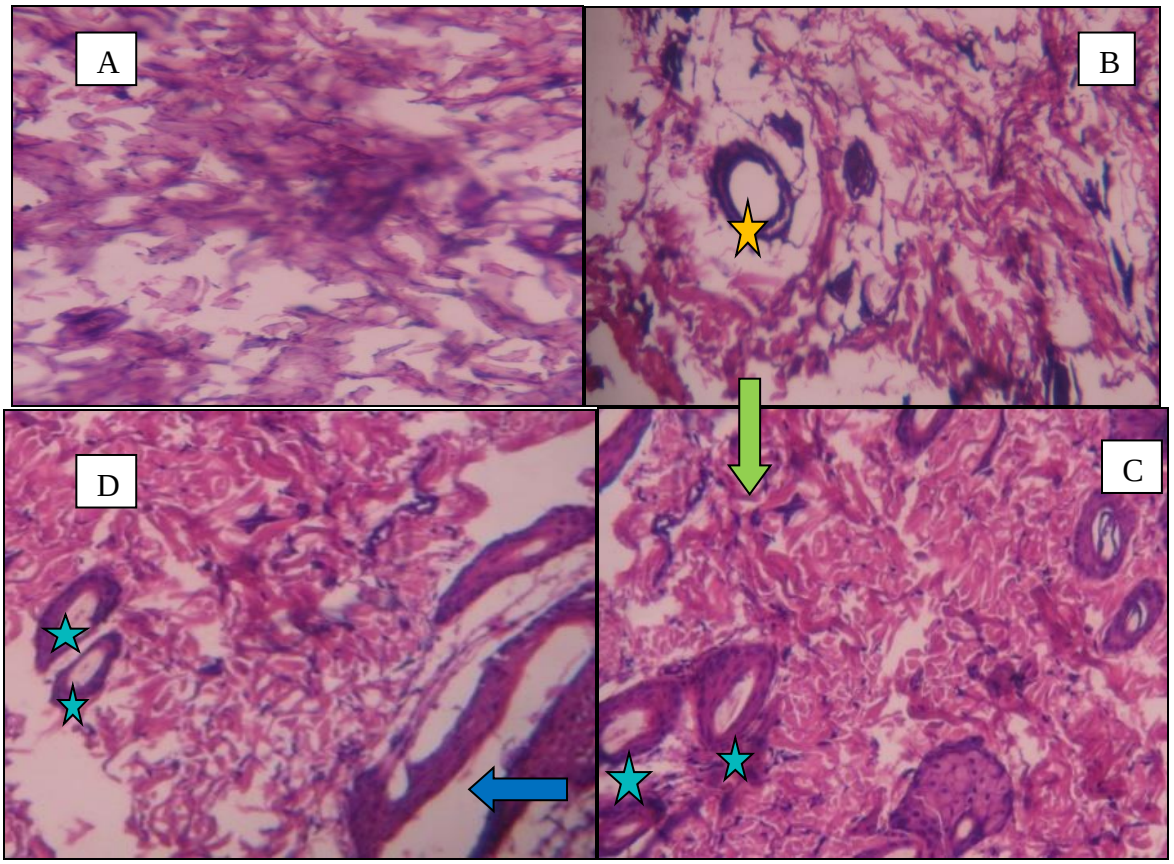

Fig 7: Micrographs of rat subcutaneous tissue response to GMS based Gentamicin sulphate implant A] Control group (Normal tissue sample), B] Day 7 histological response C] Day 14 histological response D] Day 28 histological response.

Table 4: Evaluation of Histopathological parameters

Group	Necrosis	Cellular infiltration	Edema hyperemia	Fibrous Tissue/capsule formation	Ulceration	Appearance of Giant cell
Group1(control)	-*	-	-	-	-	-
Group 2 (Day 7)	-	+*	+	-	-	-
Group 3(Day14)	-	-	-	+	-	-
Group 4(Day28)	-	-	-	+	-	+

*** - =** Absence of inflammatory response or Absence of any histopathological response
+ = Mild inflammatory response or mild histopathological response
++ = Moderate inflammatory response or moderate histopathological response
+++ = Severe inflammatory response or severe histopathological response

Macroscopic and microscopic studies of the implanted site were performed and various groups were evaluated for various histopathological response. Control group does not show any sign of histopathological

response. Other groups were observed for histopathological responses at different time interval as shown in table (4).

Second group evaluated for histopathological response and it was found that this group shows formation of mild edema and cellular infiltration. This may be due to initial surgical trauma. Third group shows formation of fibrous tissue surrounding to implant pieces and formation of thin fibrous capsule formation. Fourth group shows formation of fibrous tissue surrounding to implant pieces and appearance of macrophagic giant cell. From above results it was found that the evaluated GMS based implant formulation (K4) shows mild histopathological response and local tissue necrosis was never observed. It was observed that GMS based implant formulation degrades in a controlled fashion and decrease proportionality in size but it does not loose shape over long period of time thus it was concluded to be completely biodegradable in nature. Mild inflammatory reactions with normal wound healing response and fibrous encapsulation were evident and absence of necrosis demonstrating good tissue compatibility after 28 days.

Stability study

An optimized formulation from GMS based implants (K4) was subjected to Accelerated stability study (40±2°C/75±5% RH). The Implants were analyzed for hardness, thickness, diameter, drug content and drug release (24hrs in-vitro dissolution test) after keeping for 3 month at 40± 2^0C. The results obtained were compared with that of initial sample reading from the same formulation which was evaluated at room temperature (0 month readings).

Table 5: Evaluation of K4 implants as part of accelerated stability study

Sr. no.	Parameters		Initial (0 month)	1 month	2 month	3 month
1.	Physical appearance		Disc shaped implants	No change	No change	No change
2.	Hardness (kg/cm^2, mean±SD)		3.06±0.09	3.06±0.84	3.0± 0.28	3.0±0.42
3.	Thickness (mm, mean±SD)		3.12± 0.17	3.12± 0.47	3.11± 0.58	3.10± 0.38
4.	Diameter (mm, mean±SD)		8.18± 0.06	8.15± 0.74	8.10± 0.26	8.09± 0.82
5.	Drug content (%) (mean±SD)		98.5 ± 1.5	98.4± 0.96	97.9 ± 1.8	97.45± 1.14
6.	%Cumulative Drug Release (mean±SD)	Time (hr)				
		1	8.67±0.94	7.26±0.34	8.75±0.35	8.24±0.74
		2	12.68±2.1	10.25±0.64	12.7±0.28	14.54±0.24
		4	19.47±0.34	18.36±0.52	19.65±0.96	20.95±0.92
		8	29.50±0.28	25.42±0.12	27.27±0.72	32.69± 0.67
		24	38.53±0.26	34.38±1.2	37.16±0.68	39.85± 1.5

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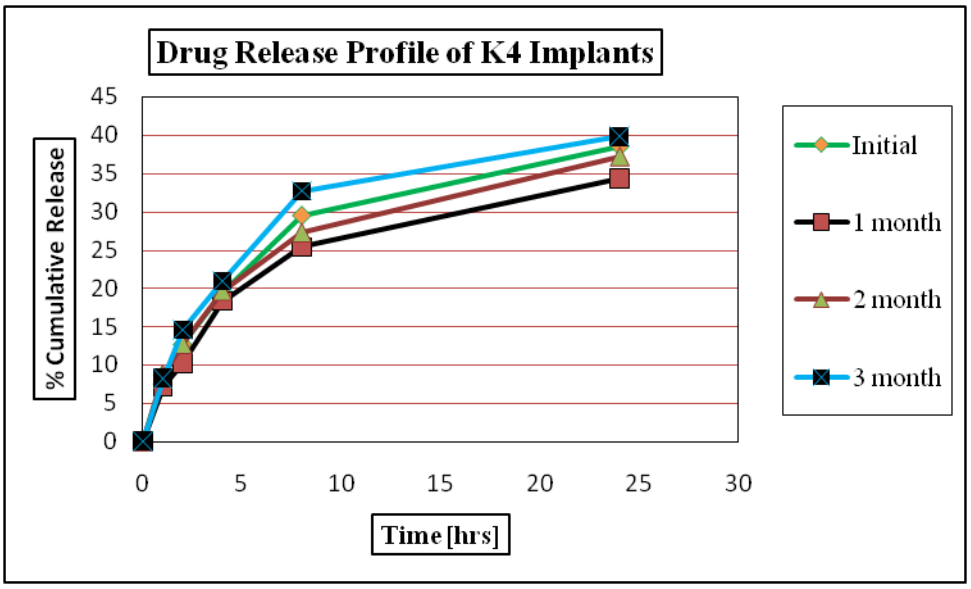

Fig 8: Drug release profile from optimized K4 formulation at different time interval in accelerated stability study

The implants (K4) exhibited no changes in the physical appearance, hardness, thickness, diameter and drug content at 40±2°C/75±5% RH during the whole testing period. All the implants maintained initial physical properties like colour, texture and diameter during stability testing period. There was little change in the drug content and dissolution profile after 3 month of storage at accelerated stability conditions. Thus from above results it is evident that the formulation K4 having good stability in terms of both drug content and dissolution stability trough out the 3 month evaluation period.

CONCLUSION

Preformulation study in development of implants includes identification of drug and drug excipient compatibility study. These studies were carried out as part of preformulation study and results are complies with standard reported values. Drug excipient studies in the development of GMS based implants shows excellent drug excipient compatibility profile. A result of physiochemical evaluation complies with standard reported values. Formulation K4 which contains 10% Sorbitol as erosion enhancer and shows excellent cumulative drug release profile and it does not completely lose its physical shape up to 28 days. Stability study shows good stability profile at accelerated storage conditions. In-vivo biocompatibility study shows good tissue compatibility. Thus developed GMS based implant formulation (K4) can be effectively used in the treatment of bone infection.

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