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Design and evaluation of controlled release gentamycin incorporated gelatin-alginate matrices for wound management

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ABSTRACT

Natural polymers have gained profound importance as lead compounds for delivery of drugs in treatment of various infections. Gelatin and sodium alginate have proven to own wound healing properties individually. Hence the combination of these two polymers and incorporation of drugs into their matrices may show a synergistic improvement in wound healing activity. Hence, the objective of this study was to evaluate the efficacy of sustained release of aminoglycoside antibiotic gentamycin from gelatin-alginate matrix, in order to establish a new delivery system for local anti-infective therapy. Thus, the drug loaded Gelatin-alginate matrices were prepared with two different gelatin concentrations (3% and 5%) and evaluated for various in vitro evaluation tests like swelling capacity, drug loading efficiency, SEM, FT-IR, in- vitro drug release by diffusion studies, antibacterial activity, and wound healing studies. The obtained matrices were characterized by good sorption properties and integrity. The release kinetics of the gentamycin followed the pattern of Higuchi kinetics revealing the diffusion controlled release from the matrix. Thereby, this study confirms that the absorbable gelatin-alginate matrix has potential for use as a delivery vehicle for aminoglycoside antibiotics. Moreover, the relatively low cost of these commercially-available products makes them attractive compared with other expensive custom-made delivery matrices.

Keywords: Gelatin-alginate matrix, Aminoglycoside antibiotics, Localized therapy, Natural polymers, Drug release kinetics.

INTRODUCTION

Despite a reduction in the risk of infection due to improved material, implant, and clean room technique as well as preoperative antibiotic prophylaxis, still infections remain a feared complication in orthopedic, traumatic surgery etc [1]. Conventional therapy in the form of systemic administration of an antibiotic is often little effective and not free from numerous adverse effects. Moreover, insufficiency in local blood supply due to post-traumatic or post-operative tissue damage as well as inadequate tissue penetration at bony sites or bacterial resistance increases the local ineffectiveness of systemic antibiotic therapy, both in terms of preventive or curative drug administration[2].

In order to achieve therapeutic levels at the site of infection, topical or localized therapy is used alternatively with relatively good result. The first description of regional antibiotic delivery was by Buchholz and Englebrecht in 1970, who described the elution of gentamicin and other antibiotics from polymethyl methacrylate (PMMA) beadsdramatically decreased the quantity of antibiotic required as compared with systemic administration which further facilitates utilization of more expensive antibiotics[3]. But its major drawback was the beads have to be removed in a second operation which poses additional risks of infection[4].

This leads to search for drug carrier that would enable prolonged sustained release of the therapeutic levels in the infected tissue and at the same time not evoking tissue reaction and undergoing complete bioresorption. For this,

biomaterials are becoming popular; because biomaterials are natural polymers and are biodegradable, non-toxic,have low immunogenicities and in this aspect gelatin proved to be advantageous[6]. Gelatin is a natural polymer which is used widely in many pharmaceutical and medical applications, especially in the production of biocompatible and biodegradable wound dressings and drug delivery systems[7]. Due to its easy processability and gelation properties, gelatin has been manufactured in a range of shapes including sponges, injectable hydrogels and gelatin microspheres etc. Gelatin showed various interesting biological characteristics, since it contains arginine-glycine-aspartic acid (RGD)-like sequence which promotes cell activities. It also known to have mucoadhesive properties and is well tolerated on ocular administration and also shown to exhibit activation of macrophages and high haemostaticeffect[8].

Gelatin sponges or matrices have been utilized for many regional drug delivery systems among the other forms of gelatin. Absorbable gelatin sponge (AGS) was introduced by Correl and Weisman as absorbable hemostatic agent in 1945[9]. Studies have shown that these sponges have minimal cytotoxic and genotoxic characteristics and effectively induce platelet adhesion and release of α-granules in the formation of platelet aggregates[10]. The challenges encountered when using gelatin alone to elute drugs are excessively rapid release of the drug and quick deterioration of the sponge material. The most effective approach to prolonging drug elution appears to be the addition of various diffusion restrictors, one such approach involves crosslinking gelatin with alginate (a polysaccharide found in brown algae cell walls), which prolonged drug release by decreased sponge porosity and decreased water uptake ability[11]. Both alginate as well as gelatin has been used in a number of biomedical applications such as wound dressings, tissue engineering and drug delivery. There are reports suggesting that certain alginate dressings (e.g. Kaltostat) can enhance wound healing by the stimulation of human monocytes to produce elevated levels of tumor necrosis factors such as a-interleukin-6[12]. Addition of glycerol as plasticizer provides elasticity to inserts.

Because these drugs are eluted regionally, antibiotics which may not have been safe to use systemically become viable options with this route. One example is aminoglycosides which exhibits broad spectrum bactericidal activity against clinically critical microbes such as *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus species,* and *Enterobacteriacea* can be beneficial for multidrug-resistant infections, but have known systemic limitations due to renal tubular toxicity and ototoxicity[13].

Based on the tissue repair and hemostatic properties of gelatin and alginate matrices, the purpose of this study was to prepare and evaluate porous gelatin-alginate matrix as active substance carrier for sustained in vitro release of aminoglycoside antibiotic gentamycin for local anti-infective therapy, and extrapolate this data to determine the feasibility of a future in vivo prospective clinical study utilizing a animal model.

MATERIALS AND METHODS

Materials

Gelatin powder and sodium alginate was purchased from Molychem, Mumbai. Glycerol was obtained from SD fine CHEM limited, Mumbai. Gentamycin was obtained from Abott Company, Mumbai. All other chemicals and regents used in the work are of analytical grade.

Methods

Preparation of Gelatin-alginate Matrices

The gelatin-alginate matrices were produced by mixing a sterile solution containing 10%w/v, 9%w/v, 8%w/v, 6%w/v of gelatin with 0%w/v, 1%w/v, 2%w/v, 4%w/v solution of sodium alginate respectively to produce four batches. The ratio of gelatin to sodium alginate was maintained as 10 parts per weight ratio. The mixtures were produced in triplicate to substantiate reproducibility. The mixtures thus obtained were subjected to lyophilisation and the dry product was subjected to pulverization.

The required quantity of gelatin-alginate mixture which was previously size reduced was allowed to swell in purified water. The swollen matrix was dissolved with the aid of heat and the volume was adjusted to uphold 10% w/v gelatin concentration. About 1 g of aminoglycoside antibioticgentamycin were dissolved individually in a portion of water and added to the gelatin-alginate matrices on foaming. Then glycerolwas added to the solution in such an amount that their content was 3% and 5% respectively to all the four batches, so eight distinct formulations are prepared. The mixture was foamed by high-speed stirrer and was transferred to petri pans and lyophilized for 24 h. The dried gelatin-alginate matrices were cut in to equal sizes of 4 x 4 matrices and were subjected to further evaluations[14].

Determination of Drug Content

Drug loaded gelatin alginate matrices were accurately weighed to an amount equivalent to 50 mg of drug and transferred into a 100 ml volumetric flask. The volume was made up to 100 ml with distilled water and shaken for 6 hours continuously in an orbital shaker incubator at 50 strokes per minute. The solution was then filtered and diluted with water. The samples were analysed spectrophotometrically at 276 nm using water as blank[15]. All samples were analyzed in triplicate and the drug content was calculated according to the following equation.

Drug loading (%) = Weight of the drug loaded in the gel/The total weight of the gel x 100

Swelling Index

The swelling capacity is an important characteristic of wound healing dressing especially in exudating wounds. Due to their high fluid holding capacity they can absorb a moderate amount of the wound exudates by swelling, which leads to formation of a dry bed of wound which further aids into healing process. Swelling index was determined by soaking pre-weighed pieces (2 x 2 cm) of gelatin-alginate matrices in double distilled water. Soaked matrices were removed with blunt forceps and blotted to remove excess liquid from the medium at predetermined time (5, 10,20,30,60,120 min) and their weight was determined by using digital weighing balance and % swelling index was calculated by the following equation[16].

$$\% S = \frac{W2 - W1}{W1} * 100$$

Where, S is the percentage water adsorption of gelatin-alginate matrices at equilibrium.
W1 is the initial weight of the gelatin-alginatematrix.
W2 is the after immersion weight of the gelatin-alginate matrix.
Results were averaged on three independent runs.

Surface scanning study

Morphological analysis was carried out by a scanning electron microscope (SEM) JEOL model JSM-6390LV at 500 and 2000 resolution. Surface morphology of gelatin-alginatematrix can be observed by usingSEM. Gelatin-alginatematrix was mounted on aluminium pin stubs usingconductive self-adhesive carbon label. The specimens were sputter-coated with a layer of gold approximately 50 nm thick in a sputtercoater. All samples were examined in a Field emission gun-scanning electron microscope (model- JSM 7600F)[17].

Fourier Transform Infrared Spectroscopy

The FTIR spectra were recorded using KBr pellet in a FT-IR spectrophotometer (Shimadzu IR spectrophotometer, model 840, Japan). Gelatin, Sodium alginate, gentamycin and drug loaded gelatin alginate matrix (F7) were each separately grounded finely with KBr and FTIR spectra were recorded in the range of 4000-400 cm^{-1}[17].

Invitro drug release studies

Diffusion Study: Franz Cell Diffusion System

The antibiotic release from gelatin-alginate matrices was determined by using diffusion membrane for all eight formulations. The in-vitro release studies for the formulations were performed using Franz diffusion cells with the donor and receptor compartments and a sampling port to facilitate withdrawal of the sample. Dialysis membrane (molecular weight cut off 12000-14000 Da) was used and the membrane was soaked in distilled water for 12 hrs before mounting inside. Gel formulations containing drug equivalent to 50 mg was placed in the donor compartment and the receptor compartment was filled with diffusion medium (water). The content of the cell was stirred with the aid of magnetic stirrer and the temperature was maintained at 37ºC. Sample aliquots (1 mL) were withdrawn from the receptor compartment through the sampling port for analysis at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours and an equivalent amount (1 mL) of fresh diffusion medium was replaced to maintain constant volume. The collected samples were diluted and analysed spectrophotometrically using water as blank. All samples were analyzed in triplicates[14].

Antibacterial activity

Antibacterial activity of gentamycin impregnated gelatin-alginate matrix was evaluated by agar well diffusion method. The test microorganisms gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa* were inoculated into the Mueller Hinton agar plate at a density of 1 x 10^5cfu/ml by pour plate method. The zone of inhibition was determined by placing definite pieces of freshly prepared F7 matrix in the inoculated solidified agar medium in a petriplate; incubated at 37°C for 48 h. This was done in triplicate for each organism and an average diameter ofzone of inhibition was noted[17].

Wound healing activity

In wound healing study, healthy male wistar rats were selected and divided into six groups. The excision wounds were used for the study of rate of contraction of a wound on a wistar rat. The wistar rats were anaesthetized by giving intraperitoneal ketamine injection dose (50 mg/kg). The backside area of each wistar rat was shaved to create an excision and excision wound sized 2 cm and 2 mm depth was made by cutting out a layer of skin from the shaved area using a surgical scissor. Then 2 x 2 cm area of gelatin alginate matrix was cut and fixed on the excision area with the help of blunt forceps and blank matrix was used as control. The wound areas were measured on days 3, 12, 15, and 21 for calculation of wound contraction[16].

RESULTS AND DISCUSSION

A method to deliver controlled amounts of medications in a local environment is desirable for a number of reasons. Decreased systemic side effects, decreased medication quantities, and potential increased efficacy can be seen with such a delivery technique[18].Using biopolymers such as gelatin and sodium alginate, it is possible to obtain porous carriers for drugs which will undergo bioresorption.Blending two different polymers cangreatly improve the functional properties such as gellingand melting temperature, gel strength and room temperature stability[19].

Figure 1: Blank and drug loaded gelatin-alginate matrices

All the prepared gelatin-alginate matrices were creamy white in colour and had distinct porous structure (Figure 1). Impregnating gelatin-alginate matrices with antibiotics may be possible for placement within an infected wound environment or a location where systemically administered antibiotics may not penetrate well. Thus, in this study, a total of eight formulations were prepared with gentamycin incorporated gelatin alginate matrices changing the concentration of gelatin and alginate using 3% and 5% glycerol. F1 has 10% gelatin with 3% glycerol, F2 has 10% gelatin with 5% glycerol, F3 has 9% gelatin, 1% alginate with 3% glycerol, F4 has 9% gelatin, 1% alginate with 5% glycerol, F5 has 8% gelatin, 2% alginate with 3% glycerol, F6 has 8% gelatin, 2% alginate with 5% glycerol, F7 has 6% gelatin, 4% alginate with 3% glycerol, F8 has 6% gelatin, 4% alginate with 5% glycerol and evaluated to ascertain the applicability of prepared combination for wound management.

Table 1: composition and evaluation parameters of drug loaded gelatin-alginate matrices

Batch code	Gelatin	alginate	Glycerol	Drug content	Swelling index
F1	10	0	3	76	482
F2	10	0	5	65	465
F3	9	1	3	81	652
F4	9	1	5	73	635
F5	8	2	3	88	843
F6	8	2	5	75	821
F7	6	4	3	97	1012
F8	6	4	5	86	995

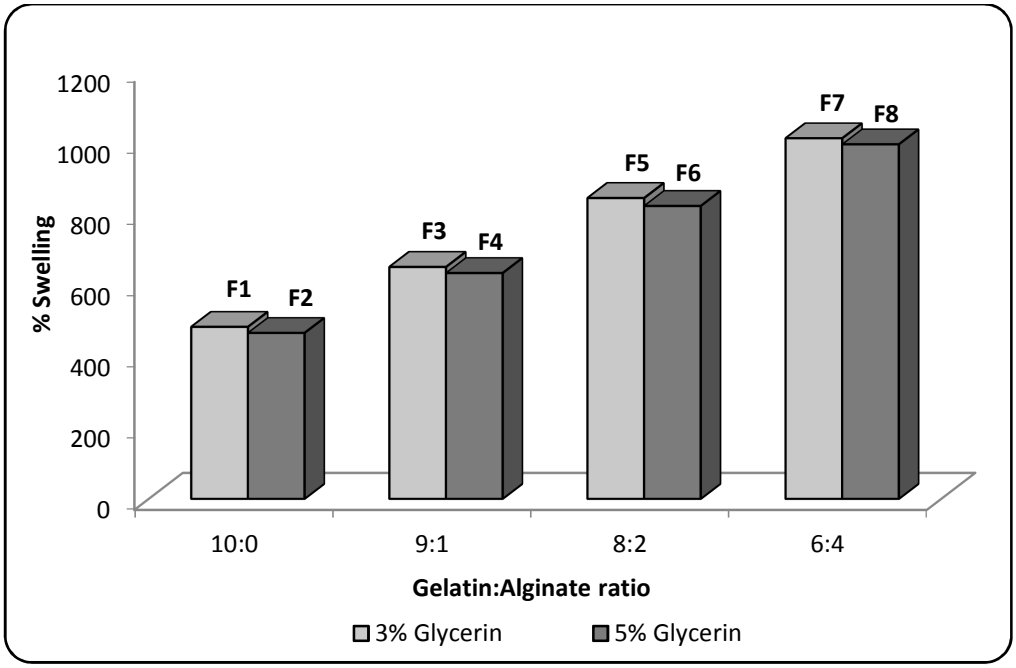
The results of Drug content were given in Table 1. From the results, drug loading was good for the formulations F5, F6, F7 and F8. The high drug loading efficiency for formulations containing gentamycin could be due to hydrophilic nature of the drug, sorption capabilities, integrity of the drug in matrix and polymer regularity of maintaining porous structure.

Swelling Tests

The prepared matrices were subjected to swelling study which ranged from 482 % to 1012 %.Water absorption capacity is an important factor for biological preparations and wound healing. It presents the capacity of matrix to

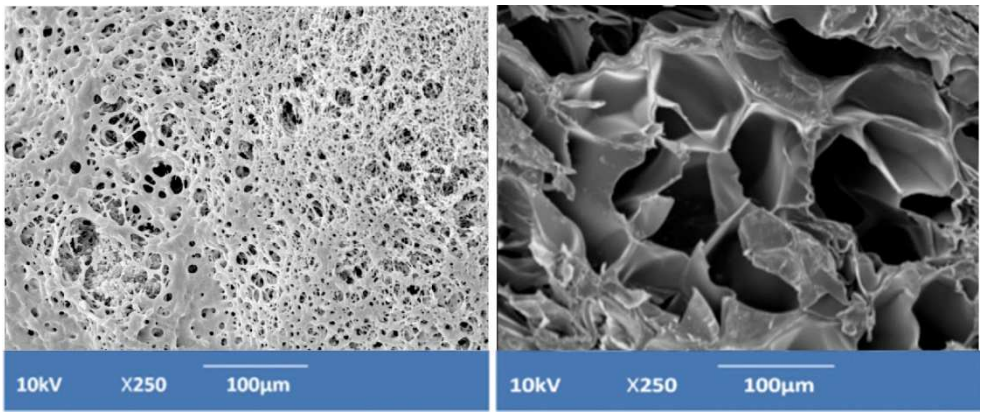
absorb wound exudates. As the gelatin content of the prepared matrices were increased, the water uptake capacities were significantly decreased. The maximum swelling ability increases with increase in sodium alginate content.

Figure 2: Swelling studies for diffusion using 3 % and 5% glycerol gel matrices



The matrices appear as a very porous structure and roughness of the surface decreased with increasing alginate content. The large pores were observed in the SEM images and the pore sizes were 29.8 µm for blank matrix and 31.5 µm for drug loaded matrix. The matrix with low gelatin level represent regular pore size and irregular pore size was observed with high gelatin content.

Figure 3: SEM images of Blank gelatin-alginate matrix and gentamycin loaded matrix



The chemical structures of the matrices were examined by FTIR spectra. Furthermore, the band of gelatin centered at about 3400cm^{-1}, which was the stretching vibration, broadened and coupled with –OH band of sodium alginate at 3450 cm^{-1}, included by the addition of sodium alginate to gelatin, implied the occurrence of hydrogen bonds between –OH groups of sodium alginate and –NH groups of gelatin molecules. FTIR spectra of pure drug and drug loaded matrices revealed the principle peaks at 3412 cm^{-1}and 3321 cm^{-1}corresponding to the amine stretch and –OH stretch, respectively. The IR spectrum of gelatin-alginate matrices also presented the peak characteristics of the drug, indicating no interaction between the drug and the polymer matrix.

The diffusion of drug from different formulations of gelatin-alginate matrices were carried out using Franz diffusion cells in water. The in-vitro release of gentamycin from the prepared gelatin-alginate matrix was studied by diffusion method. The release from the matrices varied between 64.91 to 97.83 at the end of 24 hours. The release of gentamycin was found to be dictated by the composition of the matrices, wherein the increase in the concentration of

alginate in the matrices led to a faster release. This observation is in correlation with the swelling and rate retarding abilities of alginate and gelatin respectively. The inclusion of glycerine as the elasticizing agent at two different concentrations such as 3% and 5% tend to influence the release in a timid manner. The formulations prepared with higher concentration of glycerine proved to decrease the release of gentamycin. This could be attributed due to increase in the sorption capabilities with the increase in glycerin concentration. Thus formulation F7 prepared with gelatin and alginate in the ratio 6:4 containing 3% of glycerin could release the entire amount of drug at the end of 24h in a controlled fashion.

Figure 4: FTIR spectra of alginate (A),gelatin (B), gentamycin (C) and gentamycin loaded gelatin-alginate matrix (D)

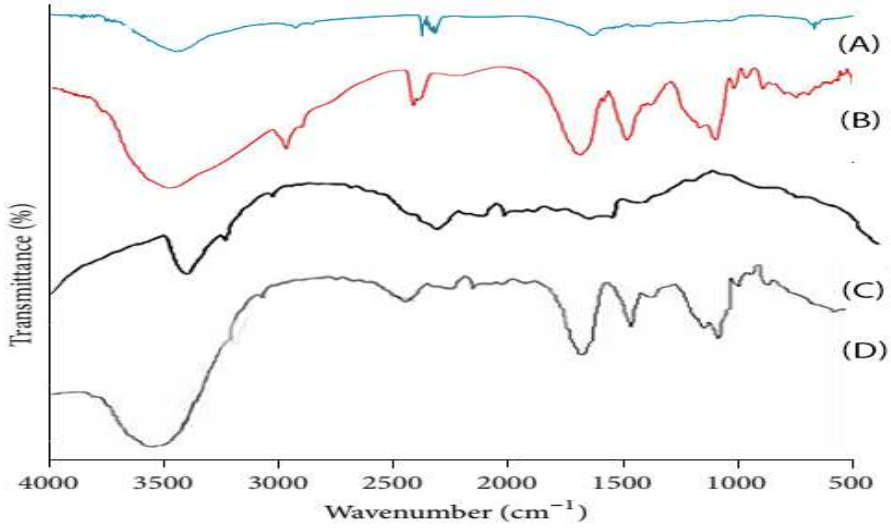
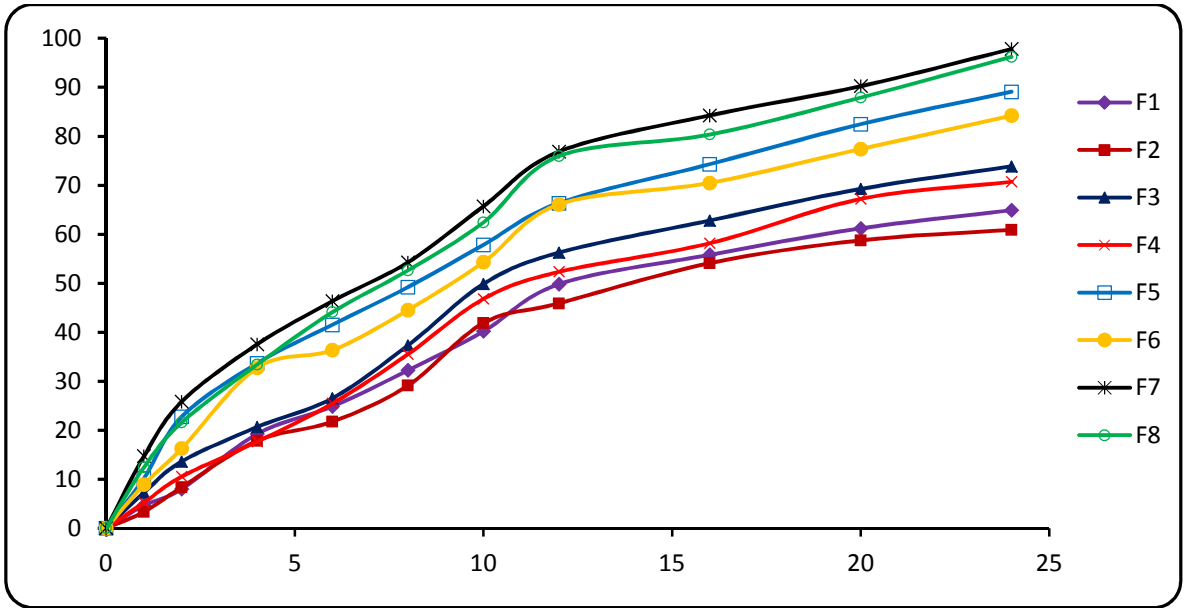


Figure 5:Comparative Release Profile of Gentamycin from 3% and 5% Gelatin-Alginate Matrices



The release profile of formulations was treated using various kinetic models including zero order, first order, Higuchi and Peppas. The mechanism of drug release from the matrix formulation was found to follow Higuchi pattern revealing diffusion as the prominent mechanism of drug release (r^2 = 0.9903).

Release Kinetic Studies

The release data from gentamycin aminoglycoside antibiotics were fitted to various kinetic equations and from the regression (r^2) values the possible release kinetics mechanism was derived.

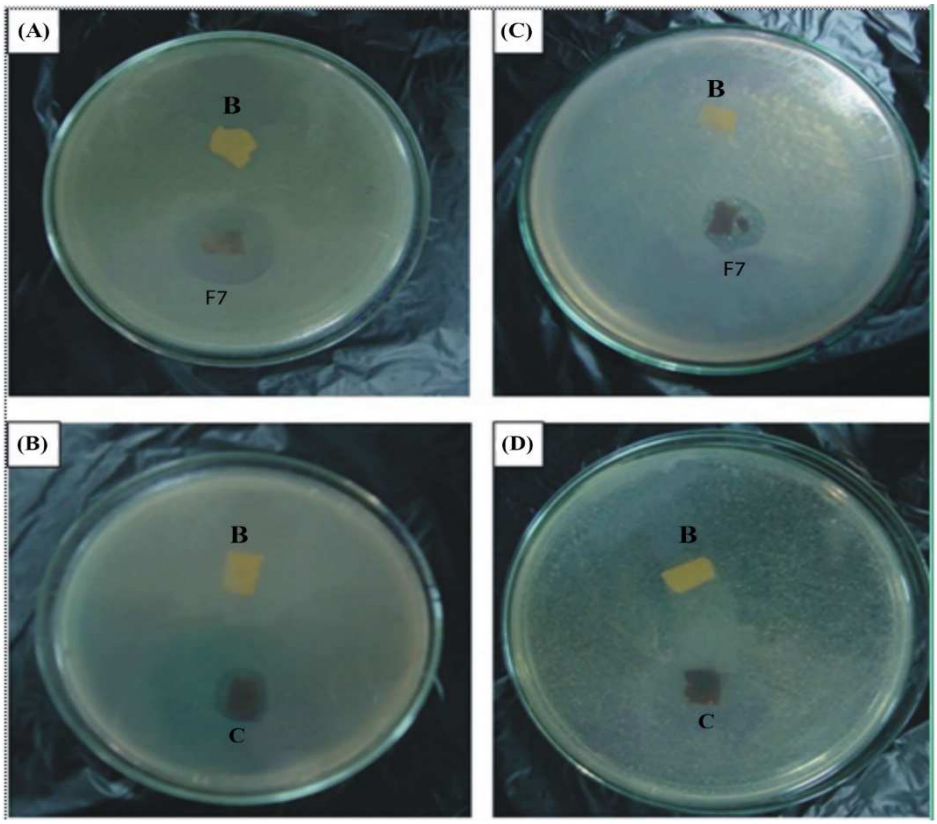
Table 2: Release Kinetics of Gelatin-Alginate Matrices

Formulation	Zero (r^2)	First (r^2)	Higuchi (r^2)	Peppa's (r^2)
F1	0.9417	0.9814	0.9675	0.8937
F2	0.9334	0.9702	0.9595	0.9119
F3	0.9367	0.9855	0.9681	0.8311
F4	0.9442	0.9876	0.9656	0.8796
F5	0.9248	0.9953	0.9926	0.7493
F6	0.9219	0.9911	0.982	0.7881
F7	0.9136	0.9459	0.9903	0.7064
F8	0.9216	0.9619	0.9862	0.745

From the table 2, the release of drug from all eight formulations was observed to follow first order release pattern revealing the diffusion controlled release from the matrix.

Bactericidal efficacy of drug incorporated gelatin-alginate matrix (F7) was examined. Two strains used for the study were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The matrices having the same composition, but without the drug was used as the control. The Muller Hinton agar was used for antibacterial activity testing by Agar diffusion method. Figure 6 [A&B] depict the activity exhibited by blank, control and drug loaded matrix against gram negative *P. aeruginosa* and Figure 6 [C&D]against gram positive *S.aureus*. The zone of inhibition of *P. aeruginosa* was approximately 30 mm diameter while for *S.aureus* it was 29 mm.

Figure 6: Antibacterial activity of prepared matrices against Pseudomonas aeruginosa (A) & (B) and Staphylococcus aureus (C) & (D) [B – Blank matrix, C – Control, F7 – Drug loaded matrix]

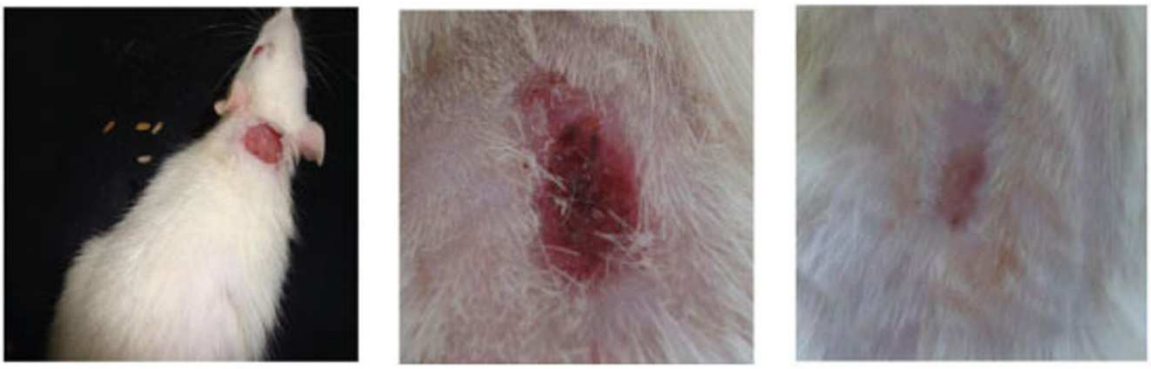


The optimised formulation was subjected to the wound healing study onmale wistar rats. The rate of wound contraction was taken as ameasure of the wound healing process. A fresh batch of F7 wasprepared and compared with commercially available povidone-iodine solution and blank matrix. The F7 gelatin alginate matrices showed excellent antimicrobial, anti-inflammatory activity and substantial sign of amendment in woundhealingi.e. 70.61% within 12 days.The mean healingtimeof 12daysand % of wound contraction were shown in table 3. The examination and findings depicted that when compared to commercially available product, gelatin-alginate matrix showed better epithellization and granulation of woundafter the 6^{th} day.

Table 3: Wound closure of animal treated with blank matrix and gentamicin loaded matrix

	Wound closure (%)			
Time	3rd day	12th day	15th day	21st day
Control	14.56	27.11	45.64	90.43
Blank matrix	12.68	42.73	77.35	93.56
F7	25.87	70.61	88.96	99.37

Figure 7: Wound healing study on male wistar rat



Day 0 **Day 12** **Day 21**

CONCLUSION

Despite various applications of Gelatin-alginate as a drug vehicle reported in the literature, it should be noted, however, that only a few drug delivery products are going in to clinical trials or are currently marketed. The use of gelatin and alginate in pharmaceutical and biomedical applications is particularly attractive because of its biocompatibility and biodegradability, together with the total absence of toxicity or allergic problems generally associated with the use of synthetic polymers. These benefits will carry the future developments and uses as indicated by intensified studies to utilize gelatin-alginate matrix in the growing field of tissue engineering, for delivery of growth factors or cells and as a favorable matrix for on-site drug delivery. The relatively low cost of these products makes them attractive compared with other expensive commercially-available delivery matrices.

Currently, drug delivery systems containing only a single antibiotic drug are available on the market. The next generation of gelatin-alginate matrix drug delivery system will be focused on both drug combination and different release profiles which will lead to better infection control. Moreover, together with this development and better understanding of benefits coming from local drug delivery, this delivery system may, in selected indications, even replace current standard of systemic antibiotic treatment.

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