Research Article

Development and Evaluation of Hydrogels of Kanamycin for the Purpose of Wound Healing

Chanchal Chaurasiya[1]*, Sachin Kumar[1], Rahul Kumar Verma[2]

1. Asst. Professor, NKBR College of Pharmacy and Research Centre, Meerut, India.
2. Executive, Lupin Limited, India.

***Corresponding author's E-mail:** chourasiachanchal1992@gmail.com

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ABSTRACT

The objective of present study was development and evaluation of kanamycin for the purpose of wound healing. Kanamycin is a first line antibiotic. For the Kanamycin that exhibit absorption from parentral route only, hydrogel improve its absorption from topical route. Hydrogel shows a better patient compliance over conventional dosage form for the purpose of wound healing. Carbomer (Carbopol) is used for the formulation of hydrogel. Five batches of hydrogel were prepared using different concentration of hydroxy propyl methyl cellulose and ethyl cellulose to increase the crosslinking of drug and to increase the physical properties of hydrogel. The kanamycin is insoluble in PEG-400, but as per the procedure it is must to dissolve kenamycin in PEG-400. Therefore, a co-solvent study performed. Evaluation parameter such as appearance, solubility study, swelling measurement, spreadibility, percent drug entrapment efficiency, ph detection, and in-vitro release study were performed for hydrogels of each batch and batch-3 was optimized best batch from all five batches. Batch-3 shows best solubility, swelling and percent drug entrapment properties. Batch-3 is able to release 91% drug release in 8 hr.

Keywords: Carbopol, Dangling Side Chain, Entanglement, ionizable.

INTRODUCTION

The term hydrogel describes three-dimensional network structures obtained from a class of synthetic and/or natural polymers which can absorb and retain significant amount of water[1]. Hydrogel is a hydrophilic mixture which has the properties of both solid and liquid[2,3]. Hydrogel structure consists of networks that are formed from randomly cross-linked macromolecules[4].

It contains three phases:

1) Polymeric-network matrix solid phase,

2) Interstitial fluid phase,

3) Ionic phase.

The solid phase includes a network of cross-linked polymeric chains. The cross-linked polymeric network can be formed physico-chemically, for example by van der Waal interactions, hydrogen bonding, electrostatic interactions and physical entanglements as well as by covalent bonds. The fluid phase fills in the pores of the polymeric matrix and makes that hydrogel has wet and elastic properties. The ionic phase consists of the ionisable groups that are bounded to the polymer chains and the mobile ions (counter-ions and co-ions). This phase exists due to the presence of electrolytic solvent[5, 6].

The hydrogel is called a carrier when it is loaded with a drug. As the swelling of the hydrogel increases, the chains of the cross linked network move further apart and the drug can diffuse more quickly through the hydrogel to the skin[7].

Application of hydrogels in wound care and healing benefits

Hydrogel dressings are a great way to provide hydration to wound. An excellent source for providing moisture to a dry lesion, hydrogel dressings act fast to help cool down a wound, as well as provide temporary relief from pain for up to six hours. Here are a few quick guidelines on when to use hydrogel dressings, its wound healing advantages and also when you should try to abstain from using hydrogel[8,9].

How they work

Hydrogel dressings consist of 90 percent water in a gel base, according to the medical journal Apple Bites, and serves to help monitor fluid exchange from within the wound surface. By keeping the wound moist, the hydrogel dressing assists in protecting your body from wound infection and promotes efficient healing[10].

Kanamycin is a first line antibiotic. For the Kanamycin that exhibit absorption from parentral route only hydrogel improve its absorption from topical route. kanamycin "irreversibly" binds to specific 30S-subunit proteins and 16S rRNA[11, 12].

MATERIALS AND METHODS

Materials

Drug Kanamycin and polymer carbopol were acquired from Macleods pharmaceutical limited. PEG 400 was acquired from Qualizens Pharma Pvt. Ltd. HPMC and EC were acquired from CDH.

Methods

Preformulation study

Prior to the development of dosage form it was essential that certain fundamental, physical and chemical properties of potential drug molecule and other derived properties of drug powder are determined[13].

Physical appearance

The physical appearance of drug includes its color, odor, taste and state of powdered drug. A drug sample was kept in a watch glass and observed by organoleptic study. The state of drug can be check by microscopy study.

Melting point

Melting point of a drug was determined by capillary melt method.

Solubility Analysis

Weighed accurately about 10 mg of drug in a weighing bottle using analytical weighing balance. Take 1 ml of solvent in a test tube. Add small amount of drug powder in different solvent. Add more quantity of powder till drug powder stop dissolution and solution become saturated. Weighed the remaining quantity of powder from the weighed powder and calculate the amount of drug powder soluble in taken amount of solvent. Then measure the solubility of drug powder as per IP 1996.

Partition Coefficient Study

Weigh accurately about 25mg of drug and added to each of n-Butanol and distilled water (1:1) in a separating funnel. The mixture was shaken continuously in a circular motion for 30 min until equilibrium reached and kept aside for 24 hr. The two phases were separated. Both the phases were analyzed for respective drug content by measuring the absorbance using UV spectrophotometer at suitable range. The partition coefficient of drug in both phases were calculated.

Identification of Drug

Identification of drug was done by UV Spectroscopy and FTIR study.

Formulation Design

The kanamycin is insoluble in PEG-400, but according to procedure we have to dissolve kenamycin in PEG-400. Therefore, a co-solvent study performed for preparation of hydrogel.

Cosolvent study

Water is chosen best as co-solvent for co-solvent study. Four batches were prepared with different concentration and procedures. Last batch with best stability is optimized best for formulation of hydrogel.

Method of Preparation

The solution of drug in specified amount of PEG-400 were prepared. The carbopol was soaked in efficient amount of distilled water. The required amount of HPMC was added to the soaked carbopol with gradual stirring with glass rod. Then after ethyl cellulose was added and stand for 1 hour. Drug solution was added dropwise and mixed uniformly. The pH (neutral-7) was measured and maintained by triethanolamine, and then finally the volume was make up with distilled water.

Evaluation of prepared Hydrogels

Appearance

The hydrogels formulated were observed for their Visual appearance, colour, texture, feel upon application such as grittiness, greasiness, stickiness, smoothness, stiffness and tackiness.

pH

The pH of the hydrogels was determined by immersing pH meter to a depth 0.5 cm in a beaker containing hydrogels. The determinations were carried out in triplicate and the average of three reading is recorded.

Solubility

Normally the hydrogel content of a given material is estimated by measuring its insoluble part in dried sample after immersion in deionised water.

Weigh accurately about dried hydrogel. Immerse this dried hydrogel in deionized water for 8 hr at room temperature[14]. The sample was prepared at a dilute concentration (typically ~ 1%) to ensure that hydrogel material is fully dispersed in water. filter hydrogel from mesh and weight this filter hydrogel by a stainless steel net of 3 meshes (681nm). The gel fraction is then measured by given equation-

$$\text{Gel Fraction (hydrogel \%)} = \left(\frac{W_d}{W_i}\right) * 100$$

Where, Wi is the initial weight of dried sample and Wd is the weight of the dried insoluble part of sample after extraction with water.

Swelling Measurement

The dry hydrogel was immersed in deionised water for 4 hours at room temperature. After swelling, the hydrogel is filtered by a stainless steel net of 3 meshes (681 nm). The swelling was calculated as follows[15].

$$\text{Swelling} = \frac{W_s - W_d}{W_d}$$

Where, Ws is the weight of hydrogel in swollen state and W_d is the weight of hydrogel in dry state. The terms 'swelling ratio'[16], 'equilibrium degree of swelling' (EDS)[17] or 'degree of swelling'[18, 19] has been used for more or less similar measurements.

Spreadibility

Place 1gm of hydrogel on a glass slide. Another slide of same diameter placed over it. 4 gm weight was placed over the slide having hydrogel. The time taken by hydrogel to cover the whole length of slide was recorded. Spreadibility of hydrogel was calculated by using following formula-

where S= spreadibility

W= weight added

L= length of glass slide

T= time taken by glass slide to cover glass slide

Percent Drug Entrapment Efficiency

1ml of hydrogel was taken and volume was made up to 10 ml with distilled water and centrifuged at 15000 rpm for 15min. The supernatant was collected and 1ml of supernatant diluted up to 10 ml with distilled water. The supernatant liquid was analyzed spectrophotometrically. From observed absorbance, concentration was determined from calibration curve. The amount of drug in supernatant (w) was then subtracted from the total amount of drug added (W). The % entrapment was calculated from the formula:

$$\% \text{ Drug Entrapment} = \frac{W\text{-}w \times 100}{W}$$

Where; W = the weight of drug added to the system w= the weight of drug in the supernatant.

In-vitro drug release study

Treatment of Egg membrane

An egg was dipped in a beaker, containing 100 ml concentrated HCl. White foam made over the surface of HCl, because HCl react with Calcium present in shell of egg and produce effervescent. Removed egg from HCl, when effervescent stopped, transferred egg in a beaker containing distilled water, washed at least two times. Egg membrane was separated from the egg. Egg membrane was tide in a two-side open glass tube.

Release studies

100 ml Phosphate buffer (pH 6.4) was used as acceptor phase to ensure sink conditions. The pH of the applied buffer approaches the natural pH value of human skin. Therefore, this kind of buffer is usually used as dissolution medium for the investigation of transdermal drug delivery. 0.50 gm Hydrogel was placed in the donor compartment prepared by closing the one side of a both open sided test-tube with the membrane. The effective diffusion surface area was 7.069 cm2 (approx.). The receptor compartment was filled with phosphate buffer, pH 5.4(40 ml). During the experiments, the solution in receptor side was maintained at 37°C ±0.5°C and stirred at 800 rpm with Teflon-coated magnetic stirring bars. At fixed time intervals (0.5, 1, 1.5,2,3,4,12 & 24 hrs) 5 ml sample was withdrawn from receptor compartment and analyzed by UV spectrophotometer.

RESULTS AND DISCUSSION

Preformulation study

Physical Appearance

Kanmycin was white, odourless, crystalline powder drug. It has salty and bitter mucilaginous taste.

Melting point

Melting point of kanamycin was found to be 248 ºC.

Solubility Analysis

Drug is found to be freely soluble in water. But found practically insoluble in PEG-400, Acetone, Chloroform, and in ethanol (95%).

Partition Coefficient Study

The partition coefficient of drug was found to be 0.032.

Spectroscopic Study

A) UV Spectroscopy:

The wavelength maximum of kanamycin was found at 205nm.

B) **Construction of Calibration Curve of Kanamycin:**

UV absorbance of sample solution of different concentration is given in following table (Table 1) and calibration curve of kanamycin is shown in figure 1:

Table 1: UV absorption of sample solutions

Concentration (mcg/ml)	Absorbance
2	0.027
4	0.047
6	0.058
8	0.070
10	0.082
12	0.096
15	0.110

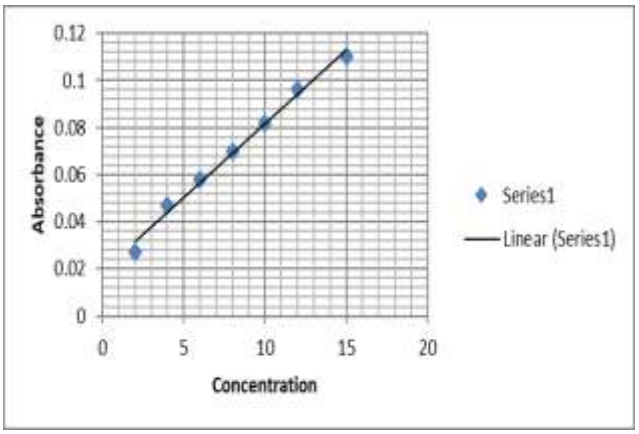

Figure 1: Calibration curve of kanamycin

C) IR Spectroscopy-

IR spectra of kanamycin is shown in Figure 2.

Formulation Design

Co-Solvent study

Co- Solvent study is presented in Table 2.

Precautions- PEG-400 was place in water bath containing chilled water/ice cubes. And the solution of drug should be added dropwise at the time of study 3 and 4.

Formulation of Hydrogel

Formulation of different batches of hydrogel is presented in Table 3.

Evaluation of Prepared Hydrogel

Appearance

Different parameters of appearance are shown in Table 4. And Figure 3.

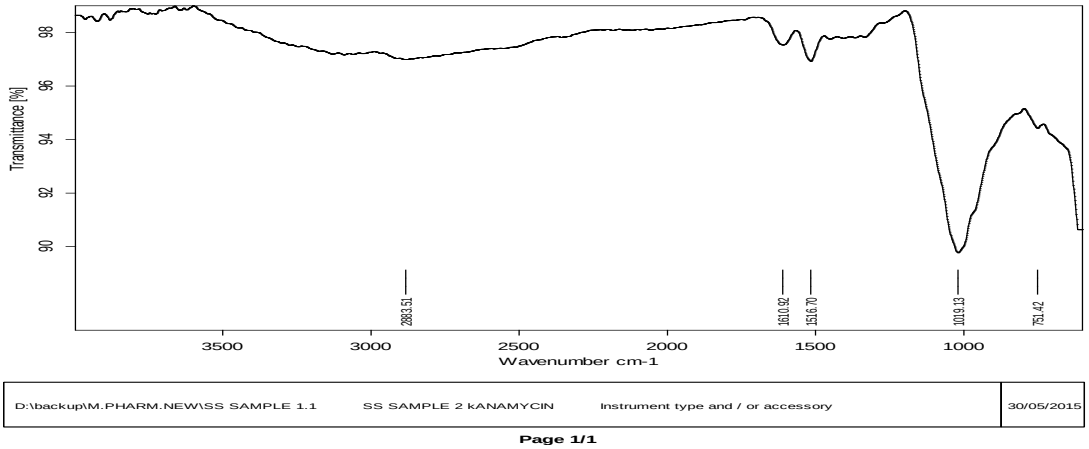

Figure 2: IR Spectra of kanamycin

Table 2: Co-Solvent study

Study no.	Test	observation	Stability study	Result
Study-1	70 mg of drug dissolve in 4 ml water. Then add 10ml PEG-400 in this solution.	A white milky solution occurs with heat generation.	Not stable. Drug precipitates out from solution and drug stick on the wall of beaker.	Fail
Study-2	Dissolve 5mg drug in 5ml water (1mg/ml) then add 5ml PEG-400 in this solution, dropwise.	Till the addition of 1.5 of drug solution in PEG-400 no precipitation occurs. But on more than 1.5ml precipitation occurs.	Solution stands for 2 hours at room temperature, at high temperature in hot air oven (40˚C), and at freezing temperature. Stable	Unsatisfactory
Study-3	Dissolve 5mg drug in 5ml water (1mg/ml). Add this solution in 10 ml of PEG-400, dropwise.	No precipitation occur, but solution become quit milky white	Solution stands for 2 hours at room temperature, at high temperature in hot air oven (40˚C), and at freezing temperature. Stable	Satisfactory
Study-4	Dissolve 250mg of drug in 10ml water, add this solution in 25ml PEG-400, dropwise	No precipitate occurs, milky white solution	Solution stands for 2 hours at room temperature, at high temperature in hot air oven (40˚C), and at freezing temperature. Stable	Pass

Table 3: Formulation of hydrogel

S.No.	Ingredient	B1	B2	B3	B4	B5
1	Kanamycin	250	250	250	250	250
2	Carbopol	500	500	500	500	500
3	Hydroxypropymethyl cellulose (HPMC)	--	250	500	750	1000
4	Polyethylene glycol (PEG)-400	25	25	25	25	25
5	Ethyl cellulose (EC)	--	100	150	200	250
6	Distt. Water	40	40	40	40	40

Table 4: Appearance of Prepared hydrogel

Property	B1	B2	B3	B4	B5
Color	Transparent	Transparent	Whitish to transparent	Whitish	White
Odour	Odorless	Odorless	Odorless	Odorless	Odorless
Texture	Gel like	Gel like, spreadable	Amorphous, Adhesive, Spongy, Smooth	Amorphous, Adhesive, Thick	Crystalline, Thick, Less adhesive

Table 5: Time V/s % Drug release study of prepared Hydrogels

S.No.	Time interval (hr)	% Drug Release				
		B1	B2	B3	B4	B5
1	0	0	0	0	0	0
2	0.5	52.71	20.98	16.70	13.28	10.28
3	1	99.85	51.85	25.20	21.42	17.15
4	1.5	--	77.57	31.71	28.28	25.71
5	2	--	96.85	39.85	35.14	32.58
6	3	--	--	55.71	49.71	36.0
7	4	--	--	69.85	60.85	54.85
8	6	--	--	80.57	75.42	66.0
9	8	--	--	91.28	84.00	78.0

Figure 3: Prepared hydrogel

pH

ph of Batch 1 to Batch 5 was found to be 6.8, 6.8, 6.6, 6.7 and 6.6 respectively.

Solubility

Solubility of Batch 1 to Batch 5 was found to be 78.61 %, 59.27 %, 36.2 %, 21.25% and 12.13% respectively.

Swelling Measurement

Swelling measurement of Batch 1 to Batch 5 was found to be Nil, 35.6 %, 50.20 %, 22.34 % and 15.31 % respectively.

Spreadibility

Spreadibility of Batch 1 to Batch 5 was found to be 5.78, 1.44, 0.96, 0.59 and 0.59 respectively.

Percent Drug Entrapment Efficiency

Percent Drug Entrapment Efficiency of Batch 1 to Batch 5 was found to be 95.14, 94.95, 94.62, 94.42 and 94.71 respectively.

In-vitro Release Study

In-vitro release study drug is presented in Table 5.

CONCLUSION

Hydrogels of kanamycin were successfully prepared. The prepared hydrogel displayed good evaluation property and good stability during storage. The hydrogel would be potential dosage form for drug delivery for various purposes.

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