Formulation and evaluation of Hydrogel for wound healing

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Abstract

The hydrogels are 3-D networks which consists of physically or chemically cross-linked bonds of hydrophilic polymers. The insoluble hydrophilic structures designate a potential to absorb wound exudates and allows oxygen diffusion to fasten healing process. The aim of the present study is to formulate and evaluate a hydrogel of Neomycin sulfate for wound healing. Neomycin sulphate is an antibiotic used to treat bacterial skin infections. It is good effective in treatment of infected cuts, wounds, ruptured tissue and fatigue as well as minor burns. The neomycin sulfate hydrogel for wound healing topical application was formulated using guar gum and Carbopol-940 and evaluation were performed. Proper selection of polymers and their proportions is a prerequisite for designing and developing a transdermal drug delivery system. The formulated hydrogel showed good homogeneity, good stability and better drug release rates when compared to marketed formulation.

Keywords: Hydrogel, transdermal drug delivery, Neomycin sulfate, guar gum, carbopol 940, *in-vitro* studies.

Introduction

A hydrogel can be defined as a hydrophilic polymer which links with each other and not dissolve in water. They are mostly as a polymer which are easily absorbed and form accurate shape in structures. The structure is such like that it hold a more amount of water in their well maintained structure also more swollen in water. The hydrogel is such like that they swells up more in liquid medium just like water, and links with each with one or another monomer.

They mainly soaks a large amount of liquid from functional groups hydrophillic attached to the support of more polymers, while their barrier are network to network chains. They are available in natural and synthetic.

Synthetic polymers can be easily modified to yield their degradability and functionality. They are stable in sharp and high degree of temperature also. They found in a number of chemical ways which comprise of only single step procedures like polymerization and cross-linking of more functional monomers, along with multiple step procedures. They have reactive groups and cross-linking, react with polymers which suits to maze liked polymer. Engineer can constructs its shape and make polymer through with molecular based control over structure They also have properties, such as biodegradation, provide strengthen properties, chemical and biological response.

Neomycin sulphate is an antibiotic used to treat bacterial skin infections. It is good effective in treatment of infected cuts, wounds, ruptured tissue and fatigue as well as minor burns. This medicine stops the growth of bacteria, which helps to cure symptoms and cure the under-lying infection. It stop the synthesis of vital proteins necessary for the survival of bacteria. It is very effective against skin infections such as boils, impetigo and infected hair follicles. It is used to cure infections in small cuts, ruptured wounds on skin. It shows some other effects and should cure the infections, as suggested by doctor.

PREFORMULATION STUDIES

Identification and Authentification

1. **UV spectrophotometric studies**: Accurately weighed 10 mg of drug, then dissolve in 10 ml of projected solution in 10 ml of volumetric flask and prepared suitable dilution. The spectrum of this solution was analyzed in 200-400 nm range in UV-visible spectrophotometer and compared with the standard.

Table No. 1: UV-Spectrum of drug

Wavelength (nm)	Interpretation	Inference
200-400 nm	Scanning range	Drug absorption maxima (λ max) at303.80 nm
303.80 nm	Highest peak	

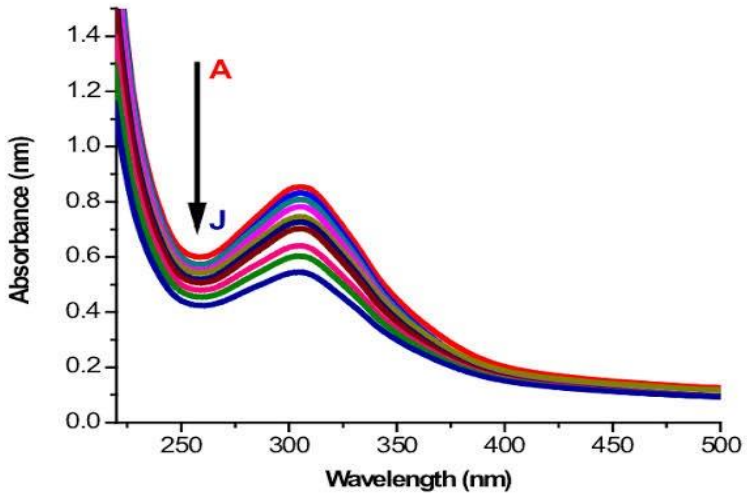


Figure No. 1: Standard UV spectra of drug sample in water

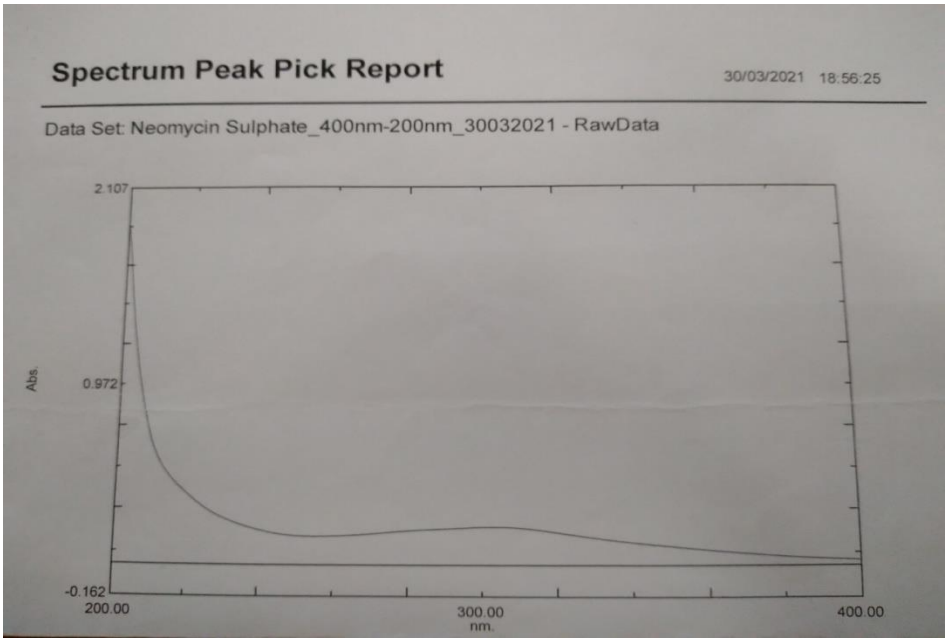
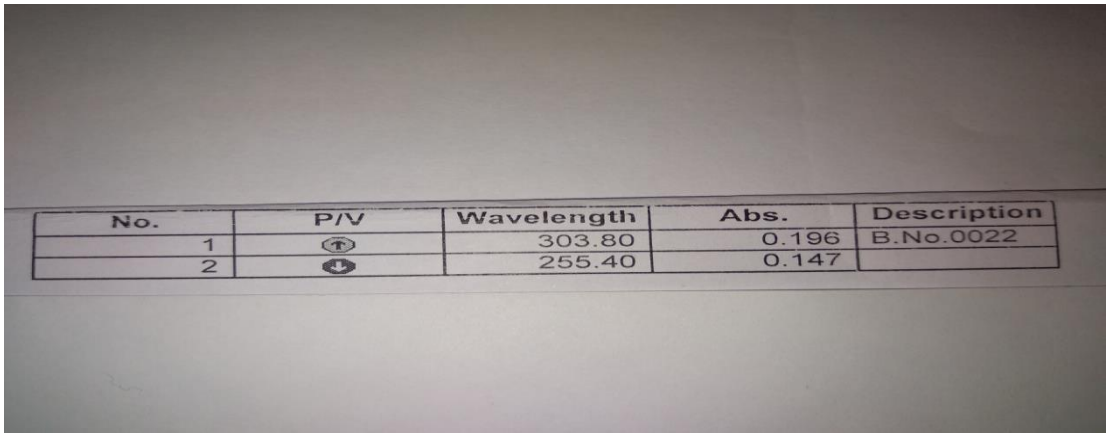


Figure No. 2: UV spectra of drug sample in water (UV-1800 series)



No.	P/V	Wavelength	Abs.	Description
1	↑	303.80	0.196	B.No.0022
2	↓	255.40	0.147	

Figure No. 3: UV spectra of drug sample test reading

2. **FT-IR spectrophotometric studies:** Infra red spectrum of any compound gives information about the group present in particular compound. An infrared spectrum of drug is taken by using KBr pellets. Small quantity of drug was mixed with oil and one drop placed between KBr pellets and spread uniformly. The pellets were placed in holder and infrared spectra been taken. Various peaks in infrared spectrum were interpreted for presence of different group in structure of drug.

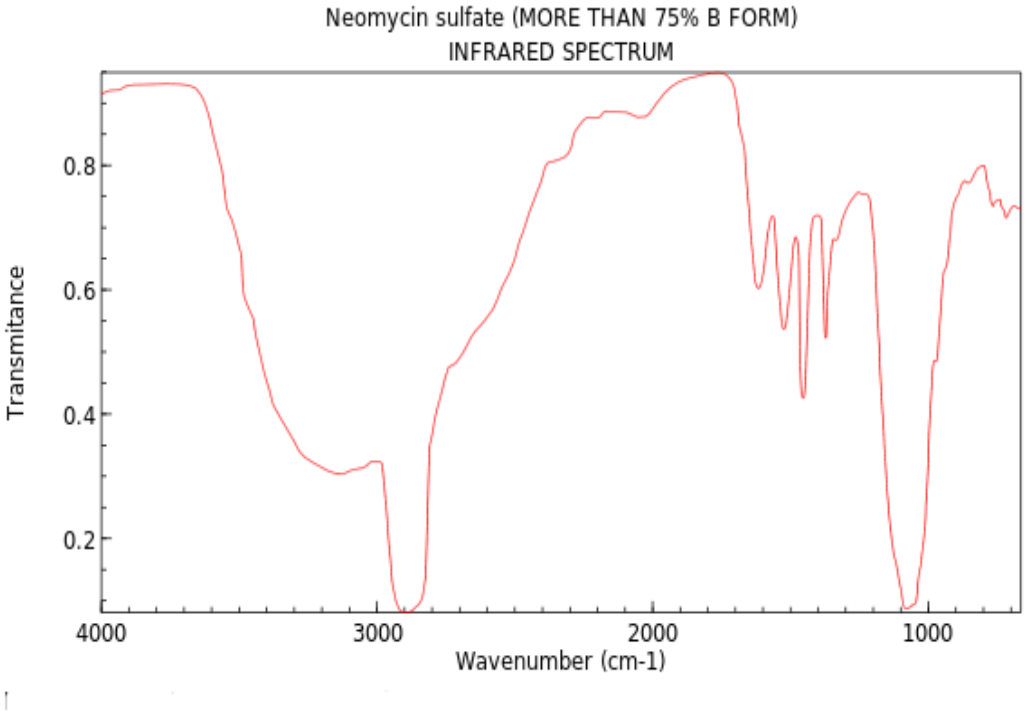


Figure No. 4: FT-IR Spectra of Standard drug sample

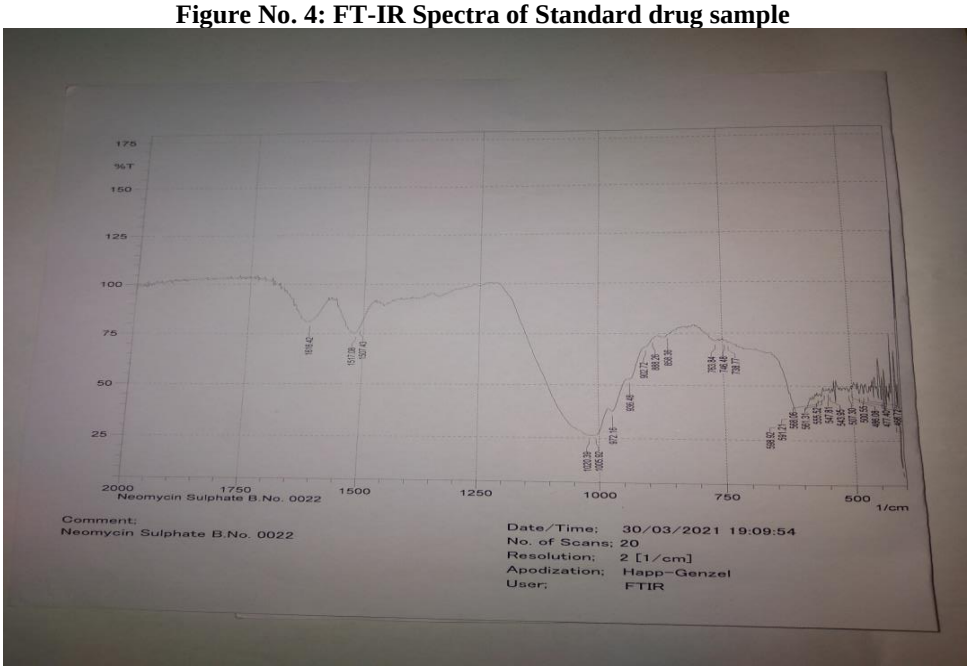


Figure No. 5: FT-IR Spectra of drug sample (Neomycin sulfate)

3. **Melting point range:** Melting point can be determined by taking Neomycin sulfate (drug) 50 mg accurately weighed using thiels tube. Take a capillary tube of 1 mm internal diameter and seal last end by heating it. To fill the capillary tube, make a heap of the powdered substance on the porous plate. Push open end of the capillary tube into the heap. Some of the powdered substance will enter the tube. Now, tap the sealed end of the tube on the porous plate slowly. Fill the tube upto 2-3 mm. Clamp the thermometer carrying the capillary tube to an iron stand and immerse them in the thiels tube containing liquid paraffin. Now, heat the beaker slowly with constant stirring to maintain uniform temperature. Then the temperature rises gradually. Note the temperature when the substance has completely melted.

Table No. 2: Melting point of sample

S.No.	Drug	Specification	Observation
1.	Neomycin sulfate	>187ºC (dec.)	187.6ºC

4. Physical description

Table No. 3: Physical description of sample

S.No.	Parameters	Standard	Observation
1.	Color	White to slightly yellow hygroscopic powder	Pale-yellow color
2.	Odor	Odourless	Odourless

5. Solubility analysis

- **Determination of solubility of drug in various solvent**

At a constant temperature as well as on a constant pressure the amount of substance that passes into a solution as well as form a equilibrium this is called as a solubility of a substance. A fix amount of a soluble is taken in test tube and then increases the solvent in small amount and shakes it and observed the solution. This is called as a quantitative determination.

Table No. 4: Solubility Analysis

S.No.	Solvent	Observation
1.	Distilled water	Freely soluble
2.	Chloroform	Freely soluble
3.	Ethanol	Insoluble
4.	Acetone	Insoluble
5.	Isopropyl Alcohol	Slightly soluble

OPTIMIZATION AND FORMULATION

Optimization has been defined as the implementation of systemic approaches to achieve the best combination of product and or process characteristic under a given set of conditions.

Optimization methodologies

It can be categorized into two classes i.e. Simultaneous optimization, where the experimentation is completed before the optimization takes place and Sequential optimization where experimentation continues as the optimization study proceeds.

Experimental design

Experimental design involves the arrangement of experiments in the design space such that the reliable and consistent information is achievable with minimum number of experiments. Experimental run or trail is a practical manipulation or series of manipulations carried out under defined conditions, resulting in the data for each of the response to be measured.

Formulation of Neomycin sulfate

Table No. 5 : Composition of Neomycin sulfate

S.No.	Ingredients	F1	F2	F3	F4	F5
1	Neomycin sulfate (Drug in mg)	100	100	100	100	100
2	Carbopol-940 (mg)	0.5	0.374	1.0	0.781	0.642
3	Guar gum (mg)	0.372	0.5	0.684	0.495	0.738
4	Isopropyl myristate (ml)	1.2	1.5	1.1	0.8	1.7
5	Isopropyl alcohol (ml)	0.20	0.28	0.16	0.32	0.14
6	Distilled water (q.s) (ml)	100	100	100	100	100

Method of preparation

Preparation of Neomycin sulfate hydrogel for wound healing:

1. Hydrogels were fabricated using different concentrations of polymeric dispersions.
2. Different concentrations of carbopol-940 colloidal dispersions were prepared using distilled water.
3. Different concentrations of guar gum colloidal dispersions were prepared using distilled water.
4. After complete dispersion, both the polymer solutions were kept in dark for 24 hours for complete swelling.
5. Dispersions of polymers were made using magnetic stirrer (500 rpm).
6. After dispersing carbopol-940 in distilled water, colloidal dispersion of guar gum was added to it under magnetic stirring.
7. 1% v/v isopropyl myristate and 0.25% w/v isopropyl alcohol were added.
8. Aqueous drug solution was added to the polymeric dispersion after addition of sodium hydroxide solution.
9. Finally, the remaining distilled water was added to obtain a homogeneous dispersion of gel under magnetic stirring.

EVALUATION PARAMETER

1 Physical characteristic

The prepared hydrogel formulations were inspected visually for their pH, colour, homogeneity, consistency, grittiness, texture and phase separation.

2 Determination of pH

The pH of hydrogel formulations was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. Then constant reading was noted. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3 Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

4 Extrudability study

The hydrogel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Table No. 6: Physical parameter of formulation

Formulation	Colour	Homogeneity	Consistency	Phase separation
F1	White	Excellent	Excellent	None
F2	White	Good	Good	None
F3	White	Average	Average	None
F4	White	Average	Average	None
F5	White	Good	Good	None

Table No. 7: Determination of pH

S.No.	Formulation	pH
1	F1	7.05 ± 0.01
2	F2	7.02 ± 0.02
3	F3	6.95 ± 0.05
4	F4	6.98 ± 0.01
5	F5	7.05 ± 0.03

Table No. 8: Result of washability and extrudability

S.No.	Formulation	Washability	Extrudability
1	F1	+++	++
2	F2	+++	+++
3	F3	+++	+++
4	F4	+++	++
5	F5	+++	++

Excellent: +++, Good: ++, Average: +, Poor: -

5 Spreadability

Two glass slides of standard dimensions (6×2) were selected. The hydrogel formulation whose spreadability had to be determined was placed over one of the slides.

$$\textbf{Spreadability} = m \times l / t$$

Where, **S =** Spreadability (gcm/sec), **m =** weight tied to the upper slide (20 grams),
l = length of glass slide (6cms), **t =** time taken in seconds.

Table No. 9 : Result of spreadability study

S.No.	Formulation	Spreadability (gcm/sec)
1	F1	12.24 ± 0.02
2	F2	13.38 ± 0.01
3	F3	14.52 ± 0.03
4	F4	13.20 ± 0.05
5	F5	14.54 ± 0.01

6 Viscosity

The measurement of viscosity of the prepared hydrogel was done using Brookfield digital Viscometer.

Table No. 10: Result of viscosity

S.No.	Formulation	Viscosity (cps)
1	F1	942 ± 2.4
2	F2	987 ± 2.1
3	F3	945 ± 1.5
4	F4	940 ± 2.5
5	F5	918 ± 1.5

7. Drug content

Accurately weighed equivalent to 100 mg of hydrogel was taken in beaker and added 20 ml of phosphate buffer pH 7.4. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 ml of filtered solution was taken in 10 ml

capacity of volumetric flask and volume was made upto 10 ml with phosphate buffer pH 7.4. This solution was analyzed using UV spectrophotometer at λ_{max} 303.80 nm.

Table No. 11: Result of drug content

S.No.	Formulation	Drug content
1	F1	96.4 ± 0.2
2	F2	94.8 ± 0.1
3	F3	95.4 ± 0.2
4	F4	92.3 ± 0.1
5	F5	89.1 ± 0.3

8 *In-vitro* drug release studies using the pre-hydrated cellophane membrane

The prepared hydrogel was evaluated for *in vitro* drug release. *In vitro* diffusion study was carried out in a Franz diffusion cell using cellophane membrane. The cellophane membrane was mounted on the Franz diffusion cell. Formulation was applied through donor compartment on the dialysis membrane. Reservoir compartment was filled with 25 ml phosphate buffer of pH 7.4. The study was carried out at 37 ± 1˚C and at a speed of 100 rpm for 8 hr. Samples were withdrawn from reservoir compartment at 1 hr. interval and absorbance was measured by spectrophotometric at 303.80 nm. Each time the reservoir compartment was replenished with the same quantity of 7.4 pH phosphate buffer.

Table No. 12: Cumulative % drug release of formulation F1-F5

S.No.	Time (min.)	F1	F2	F3	F4	F5	Marketed
1	5	73.3 ± 0.3	29.2 ± 0.4	75.5 ± 0.5	52 ± 1	45.8 ± 0.7	42.24
2	10	74.2 ± 0.2	38.1 ± 0.3	79.6 ± 0.2	58.3 ± 0.4	57.3 ± 0.05	65.54
3	15	76.5 ± 0.1	39.7 ± 0.3	81.3 ± 0.4	71.4 ± 0.2	64.2 ± 0.2	76.92
4	20	80.0 ± 0.1	47.8 ± 0.7	81.5 ± 0.2	73.3 ± 0.4	68.6 ± 0.5	78.54
5	30	80.9 ± 0.3	88.1 ± 0.5	82.4 ± 0.6	80.4 ± 0.3	75.2 ± 0.3	74.81

9. STABILITY STUDIES

The purpose of the stability testing is to provide evidence on the quality of a drug substance or its product, which varies with time under the influence of environmental factor such as temperature, humidity and light. Recommended storage conditions, re-test period and shelf life are to be established.

The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug substance and product" (QIA) describes the stability test requirement for drug registration application in the European Union, Japan and United States of America.

ICH specifies the length of study and storage conditions.

Long Term Testing: 25ºC ± 2ºC / 60% RH ± 5% for 12 months

Accelerated Testing: 40ºC ± 2ºC / 75% RH ± 5% for 6 months

Stability studies were carried out at 25ºC/60% RH, 30ºC/65% RH and 40ºC/75% RH for the selected formulation for 3 months.

9.1 Accelerated stability studies

Stability studies were carried out an optimized formulation according to International Conference on Harmonization (ICH) guidelines. The formulation packed in aluminium tube was subjected to accelerated stability testing for 3 months as per ICH norms at a temperature (40 ± 2ºC) and relative humidity 75 ± 5%. Samples were taken at regular time intervals and analyzed for the change in pH, spreadability and drug content and *in vitro* drug release by procedure stated earlier. Any changes in evaluation parameters, if observed were noted.

Table No. 13: Physical parameters after accelerated stability study of formulation F2

S.No.	Physical Parameter	Initial	After 1 month	After 2 month	After 3 month
1	pH	7.0 ± 0.06	6.9 ± 0.06	6.9 ± 0.06	6.9 ± 0.06
2	Viscosity	987 ± 2	991 ± 1.7	995 ± 2	997 ± 2.3

Temperature: 40 ± 2˚C; Relative humidity (RH): 75 ± 5% RH

RESULT AND DISCUSSION

The preformulation study is the first step in development of dosage forms of drug substance. These investigations may confirm that there are no significant barriers to dosage forms development. The preformulation study is performed as well as the results are perfect in which the identification of the drug is done as well as the compatibility study is also performed just according to standard procedure are used as well as the condition are also maintained just according to standard process by which we found that the drug is not in compatible with the drug. So we can also proceed to words, formulation as well as these all testing studies are also compared with the standard parameter of drug as well as the other excipients. Preformulation study was done initially and results directed for the further course of formulation. Organoleptic properties study, solubility study, loss on drying, identification and authentication of drug, partition coefficient, quantitative estimation of drug and compatibility study were carried out during preformulation study. These tests were performed as per procedure given in preformulation part. The results were found in table.

Organoleptic properties study of drug was performed for physical characterization.
Identification and authentication of drug sample was done by infrared spectroscopy, UV spectroscopy and melting point determination.
The identification of drug has been performed by IR spectra of sample matched with the reference spectra. The principle peak was obtained in sample spectra.
Firstly we have studied the organoleptic property of neomycin sulfate (drug) by physical characterization. The color of the neomycin sulfate (drug) was pale-yellow color and the odor of the drug was odorless.
Then we have observed the melting point determination of neomycin sulfate (drug) was 187.6ºC.
Then we have performed UV spectrophotometric study. The spectra was analyzed in 200-400 nm range in UV-visible spectrophotometer and compared with standard. So, we get Drug absorption maxima (λ max) of neomycin sulfate (drug) at 303.80 nm (highest peak).
Then we have performed FTIR study. The results were found in table from there we can easily checked.
Optimization is defined as the implementation of systemic approaches to achieve the best combination of product and/or process characteristics under a given set of conditions. Optimization techniques provide both a depth of understanding and an ability to explore and ranges for formulation and processing factors. A classical approaches to optimization is to investigate the effect of one experimental variable while keeping all others constant. Various processing parameters involved in the method were optimized.
Determination of pH of the hydrogel formulation was in the range of 6.95 to 7.05 which considered acceptable to avoid the risk of skin irritation upon application to skin.
Then comes to Stability testing of drug (neomycin sulfate) and drug products being as part of the drug discovery/ synthesis development / preformulation effort and ends only with the demise of the compound or commercial product. The purpose of stability testing is to provide evidence of how the quality of a drug substance such as temperature, light and humidity.
The ultimate goal of stability testing is the application of appropriate testing to allow the establishment of recommended storage condition, retest periods and shelf life.

CONCLUSION

The neomycin sulfate hydrogel for wound healing topical application was formulated using guar gum and Carbopol-940 and evaluation were performed. Proper selection of polymers and their proportions is a prerequisite for designing and developing a transdermal drug delivery system. The formulated hydrogel showed good homogeneity, good stability and better drug release rates when compared to marketed formulation.
UV spectrophotometric (UV-1800 series) of Neomycin sulfate (drug) in different solvents were obtained by making the solution of drug in different solvents and analyzing this solution using UV-visible spectrophotometer. The scanning range is 200-400 nm and we get the highest peak at the wavelength of 303.80 nm. So we get the drug absorption maxima (λ max) at 303.80 nm. The spectrum obtained was compared with the standard.
FT-IR Spectra of drug sample (Neomycin sulfate) was taken using KBr pellets. Small quantity of drug was mixed with oil and one drop placed between KBr pellets and spread uniformly. The pellets were placed in holder and from this we get infrared spectra. From this various peaks in FTIR were interpreted and gives the presence of different group structure in neomycin sulfate.
Melting point is that temperature when the substance has completely melted. The observation of Neomycin sulfate (drug) is 187.6ºC.
Physical description of Neomycin sulfate is Pale-yellow in color and its odor is odorless.
Solubility analysis of Neomycin sulfate in solvent distilled water is observed to be freely soluble. Then in solvent chloroform is also found to be freely soluble. Now in solvent both ethanol and acetone was found to be insoluble. At last, in solvent isopropyl alcohol is observed to be slightly soluble.
So at last we conclude that hydrogel shows a high degree of flexibility to penetrate into the natural tissue due to their significant water content. They have good transport properties and bio-compatible also. They also have a very good property is that they easily re-hydrate the wound bed and reduce wound pain also.

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