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Development of Controlled Release Lipid Based Topical Hydrogel of Gentamicin Sulphate

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ABSTRACT:
The present work aimed to formulate topical lipid-based gels for effective delivery of Gentamicin Sulphate. Achieving a desirable percutaneous absorption of drug molecule is a major concern in formulating dermatological products. For this purpose we are going to formulate lipid based nano particle of Gentamicin Sulphate. It is an alternative carrier system to tradition colloidal carriers, such as, emulsions, liposomes, and polymeric micro and nanoparticle. Solid lipid nanoparticles of Gentamicin are prepared by using lipids (glyceryl monostearate and glyceryl monooleate) with stabilizers (tween 80, poloxamer 407, and span 20. Entrapment efficiency is an important parameter for characterization solid lipid nanoparticle.in order to attain optimal encapsulation several factors will varied, including the type and concentration of the lipid and surfactant material used. The resulting lipid nano particle will be separated and formulated as Hydrogel of Gentamicin Sulphate. Swelling and mechanical features of hydrogel polymers have enabled them to find extensive applications in traditional, modern, and novel pharmaceutical area.
Keyword - Gentamicin Sulphate,Archaeosomes , nanoparticles,tween 80, liposomes, antibiotics, solubility, Ir spectroscopy

I. INTRODUCTION

The present work aimed to formulate topical lipid-based gels for effective delivery of Gentamicin Sulphate. Achieving a desirable percutaneous absorption of drug molecule is a major concern in formulating dermatological products. For this purpose we are going to formulate lipid based nano particle of Gentamicin Sulphate. It is an alternative carrier system to tradition colloidal carriers, such as, emulsions, liposomes, and polymeric micro and nanoparticle. solid lipid nanoparticles of Gentamicin Sulphate are prepared by using lipids (glyceryl monostearate and glyceryl monooleate) with stabilizers (tween 80, poloxamer 188, and span 20. Entrapment efficiency is an important parameter for characterization solid lipid nanoparticle in order to attain optimal encapsulation several factors will vary, including the type and concentration of the lipid and surfactant material used. The resulting lipid nano particle will be separated and formulated as Hydrogel of Gentamicin Sulphate.

Gentamicin Sulphate is an aminoglycoside antibiotic commonly used topically in the control of severe Gram positive and Gram negative microbial infections especially in burns and wounds as well as for treating bone and soft tissue infections (Chang et al., 2006). Topical Gentamicin Sulphate is often used in the treatment of impetigo, infected bed sores, burns, nasal staphylococcal carrier state, pyodermata, infections of the external eye and adenexa (Nishijima and Kurokawa, 2002). Despite its benefits, bacterial barriers and adverse effects such as nephrotoxicity, ototoxicity and neurotoxicity upon prolonged use limit Gentamicin Sulphate daily dosage (Robert and Walters, 1998). In fact, many clinicians are reluctant to use it, even for a short term (Drusano et al., 2007). Efforts have been made to determine its optimal therapeutic regimens in order to increase its overall efficacy while minimizing drug toxicity. These include liposomes (Jia et al., 2008), solidified reverse micellar drug delivery systems (Umeyor et al., 2011, 2012a, b), hydrogels (Eljarrat-Binstock et al., 2004; Changez et al., 2003; Ayhan and Özkan, 2007; Sokmen et al., 2008) and more recently, Gentamicin Sulphate transdermal microgels (Nnamani et al., 2013), and Gentamicin Sulphate -gold nanospheres for antimicrobial drug delivery to Staphylococcal infected foci (Ahangari et al., 2013). Topical hydrogels could be employed as an alternative low dose regimen aimed not only at reducing toxicity associated with prolonged use of Gentamicin

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Sulphate but also assuring proper utilization of the benefits ofGentamicin Sulphate , especially its rapid bactericidal activity, particularly in blood stream infections. These hydrogels can retain large quantities of Gentamicin Sulphate solution and can be directly applied to the skin without need for sophisticated equipment. Thus, this work seeks to design a Gentamicin Sulphate sulphate -loaded topical hydrogel and evaluate its physicochemical characteristics in an attempt to achieve predictable and reproducible Gentamicin Sulphate delivery.

1.1.1 Emulsion

Micro emulsions, self emulsifying drug delivery system, nanoemulsions and pickering emulsions are novel emulsion systems with many advanced applications.

Micro-emulsions

Micro-emulsions are transparent, less viscous, thermodynamically stable, optically isotropic system of oil and water stabilized by an interfacial film of amphiphilic compounds such as surfactant and co-surfactant. In contrast to ordinary emulsions, micro-emulsions form upon simple mixing of the components and require low shear rate as at higher shear conditions there is an abrupt breakdown of the bicontinuous structure, resulting in flow-induced phase separation6. The main difference between emulsions and microemulsions lies in the size and shape of dispersed particles as microemulsions have size of smaller magnitude (10 – 200 nm) than those of conventional emulsions (1 – 20 μm). Also emulsions consist of roughly spherical droplets whereas microemulsions constantly evolve between various structures ranging from droplet-like swollen micelles to bicontinuous structures.

Nanoemulsions

Nanoemulsions are composed of oil and water and are stabilized by surfactants and alcohol within a size range of 200-600nm. In contrast to microemulsions, nanoemulsions are metastable and can be diluted with water without changing the droplet size distribution.Nanoemulsion stability is influenced by environmental parameters such as temperature and pH which changes upon Nanoemulsion delivery to patients.

Pickering Emulsions

A Pickering emulsions are lipid-based emulsions with internal nanostructures stabilized by solid particles such as silica, clays, calcium carbonate, titanium dioxide, latex and many others. Solid particles added, will bind to the surface of the interface and prevent the droplets from coalescing thus making emulsion more stable. Properties such as hydrophobicity, shape, and size of the particle can have an effect on the stability of the emulsion.Additionally, it has been demonstrated that the stability of the Pickering emulsions can be improved by the utilization of amphiphilic particles so-called Janus particles due to the higher adsorption energy of the particles at the liquid-liquid interface. The skin absorption of caffeine from silica stabilized pickering emulsion was three fold higher than emulsifier stabilized emulsion attributed to the higher adhesion potential of pickering emulsions.

1.1.2 Vesicular Drug Delivery Systems

Newer vesicular systems are evolved every day. Lipid vesicular system includes:

Liposomes

Liposomes are nanosized artificial vesicles with lipid bilayer composed of phospholipids and cholesterol. Liposomes have many drawbacks like tendancy to be taken up by the RES system, modification of system for delivery to special sites, cost etc lead to development of newer drug delivery systems like transfersomes, ethosomes etc.

Phytosomes

Phytosomes are lipid vesicles formed from the reaction of a stoichiometric amount of the phospholipid (phosphatidylcholine) with the standardized extract or polyphenolic constituents (like simple flavonoids, tannins,) in an aprotic solvent. Phytosomes provide a new basis for delivery of phytoconstituents by improving its bioavailability which is attained by reducing the polarity of active substance, enhancing their rate and the extent of solubilisation into aqueous intestinal fluids and their capacity to cross biomembranes. They have been used to deliver liver-protecting flavonoids because they can be made easily bioavailable by phytosomes.

Transfersome

Transfersomes are ultradeformable, self optimized aggregates for transdermal application containing a mixture of lipids and biocompatible membrane softeners. Though basic organization is broadly similar to a liposome, the Transfersome differs by its softer, more deformable, and better adjustable artificial membrane they posses. Transfersome penetrate the stratum corneum by either intracellular route or the transcellular route by the generation of “osmotic gradient” due to

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evaporation of water. Thus a transfersome vesicle, when applied on an open biological surface, such as nonoccluded skin, tends to penetrate its barrier and migrate into the water-rich deeper strata to secure adequate hydration. As the vesicles are elastic, they can squeeze through the pores in stratum corneum (though these pores are less than one-tenth of the diameter of vesicles).

Ethosomes

Ethosomes are soft, malleable vesicles comprised of hydro alcoholic or hydro/alcoholic/glycolic phospholipids in which the concentration of alcohol is high (20-50%).35 Ethosomes are mainly proposed for transdermal drug delivery as they permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison with other lipid vesicles.

Archaeosomes

Archaeosomes are nano-sized vesicles prepared from total polar lipids (TPL) either extracted from the selected genera and species of the Archaea domain or synthetic archaeal lipids. Archaeal-type lipids consist of archaeol (diether) and/or caldarchaeol (tetraether) core structures wherein regularly branched and usually fully saturated phytanyl chains (20-40carbons in lengths), are attached via ether bonds to the sn-2,3 carbons of the glycerol backbone41.There are remarkable structural differences from liposomes: the archaeosomes surface is highly entropic, possessing half the surface tension than that of liposomes and its permeability to protons and sodium cation is nearly one third of that determined for liposomes; the inclusion of macrocyclic archaeols and caldarchaeols further impairs archaeosomes permeability to water and small solutes.

Vesosomes

Vesosomes are multicompartment structures which has distinct inner compartments separated from the external membrane. In simple terms it can be said as a larger vesicle that deliberately encapsulates many smaller vesicles in it. Each compartment of vesosome can encapsulate different materials and have different bilayer composition. In addition, while it has proven difficult to encapsulate anything larger than molecular solutions within lipid bilayer by conventional vesicle self-assembly, the vesosome construction process lends itself to trapping colloidal particles and biological macromolecules relatively efficiently.The disadvantage of conventional liposomes is that many important drugs are released faster than optimal in vivo.

II. EXPERIMENTAL

2.1. Preformulation studies

Preformulation testing is the first step in rational development of dosage forms of a drug substance. Preformulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence, preformulation studies were performed for the obtained sample of drug for identification and compatibility studies.

2.1.1. Determination of melting point

Melting point of Gentamicinsulphate was determined by Capillary tube method.

2.1.2. Solubility

Solubility is an important consideration in formulations as if the drug is not properly soluble in the vehicle it creates problem in various parameters such as bioavailability, firmness of the formulation which are essential requirements. The solubility of Gentamicinsulphate was tested in various solvents such as distilled water, ethyl alcohol, 2 propanol and acetone.

2.1.3. IR Spectroscopy

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug.

2.1.4.Thin layer chromatography

Mobil phase The lower layer obtained by shaking togetherequal volumes of strong ammonia solution, chloroform andmethanol and allowing to separate.Test solution. Dissolve 0.5 g of the substance underexamination in 100 ml of water.

Reference solution A 0.5 percent w/v solution of GentamicinsulphateApply to the plate 10 µl of each solution. After development,dry the plate in air, spray with ethanolicninhydrin solutionand heat at 110° for 5 minutes. The three principal spots in thechromatogram obtained with the test solution

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correspond tothose in the chromatogram obtained with the referencesolution.

2.2. Drug-Excipeints Interaction studies (By DSC)

The Drug-Excipeints interaction studies were performed by differential scanning calorimetry (DSC) graph. For Drug-Excipeints interaction studies drug and polymer mixed together in equal amount with the help of mortar-pestle. These mixtures were wrapped in aluminum foil and kept in environmental chamber for 30 days. The temperature should be 40^0C and the relative humidity (RH) should be 75%. The interaction can be identified by taking graph of these mixtures after 30 days on DSC. These graph compared with individual graph of drug if any interaction persist than the characteristic peak of drug or polymer deviate from its original position. For preparation of sample the mixture weight approximately 2mg and seal into a aluminum plate. This plate was place into furnace chamber and run up to the melting point of the drug. Alumina takes as a standard.

2.3. Formulation development

2.3.1. Selection of Vehicle

The solubility of Gentamicinsulphate was tested in various buffers such as acetate buffer I.P. (pH 6.0 & 6.5), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of Gentamicinsulphate in the above buffers were prepared to test its solubility at the dosage level desired (0.3%, w/v).

Methodology for formulations preparation:

2.3.2 Preparation of solid lipid microparticles

Gentamicin loaded Solid lipid microparticle were prepared by hot homogenization method followed by ultrasonication method. Gentamicin and monoglycerides were dissolved in a mixture of methanol and chloroform (1:1). Organic solvent were completely removed using a flash rotary evaporator. The embedded lipid layer was melted by heating to 5^0C above the melting point of the lipid. An aquous phase was prepared by dissolving the stabilizers in distilled water sufficient to produce 50ml and heating to the same temperature of the oil phase. The hot aquous phase was added to the oil phase and homogenization was performed (at 2500 RPM and 70^0C) using a mechanical stirrer for 30 min. the coarse oil in water emulsion so obtained was sonicating usingprobsonicator for 25 min. Gentamicin loaded solid lipid microparticle was finally obtained by allowing the hot non emulsion to cool to room temperature and was stored at 4^0C in the refrigerator.

2.3.3 Methodology for Hydrogel preparation:

- For the preparation of Poloxomer 407 based hydrogel all the ingredients were sieved from sieve no 44.
- Then despersion of gentamicinsulphate loaded solid lipid micro particles was prepared in acetate buffer 5.0 I.P.
- The despersion was cooled in a ice bath and poloxomer 407 F127 was added slowly with continuous stirring.
- Then the resulting solution was kept in a refrigerator under 4^0C for 24h. this storage was help in dissolving the poloxomer 407 completely.
- After 24h carbopol 934 and other exepients with continuous stirring. The stirring should be continued to 2-3 hours for proper mixing and avoid slug formation.
- pH was adjusted by 0.5N NaOH to 7.4
- The resulting formulation kept on probe sonicator to remove air bubble. All formulations were stored in LDPE (Low Density Polyethelene) bottles for further use. All the containers stored in refrigerator.

Table1: Composition Lipid Based Topical Hydrogel of GentamicinSulphate

Formulation	Gentamicin	Poloxomer 407	Carbopol 934	Triethanolamine	PEG 4000	Lipid base
F1	0.3%	20	0.2	0.1ml	3	QS
F2	0.3%	18	0.2	0.1ml	2	QS
F3	0.3%	16	0.2	0.1ml	1	QS

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F4	0.3%	20	0.3	0.1ml	3	QS
F5	0.3%	18	0.3	0.1ml	2	QS
F6	0.3%	16	0.3	0.1ml	1	QS
F7	0.3%	20	0.4	0.1ml	3	QS
F8	0.3%	18	0.4	0.1ml	2	QS
F9	0.3%	16	0.4	0.1ml	1	QS

2.4. Evaluations of formulations

- Appearance
- Determination of pH
- Rheological studies
- Drug content
- Entrapment efficiency
- Skin irritation study
- Microbial assay of Gentamicin
- In-vitro drug release
- Stability studies

2.4.1. Appearance

Clarity/non-gritty is one of the most important characteristic features of topical preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

2.4.2. Drug content

The assay of drug Gentamicin was performed by colorimetric method. The method was based on theninhydrin reaction with primary and secondary amines present in the gentamicin. This reaction produces a purplecolor [32].

2.4.3. Entrapment efficiency

The entrapment efficiency of solid liposphere was determined by the centrifugation method. The dispersion (containing an equivalent to a 5mg of drug) was centrifuge at 2000 rpm for one hour in a centrifuge to collect supernatant liquid. The collected liquid was filtered to measure the free drug concentration after suitable dilution with a fresh phosphate buffer saline pH 7.4. The absorbance was measured at 400nm in a UV Visible spectrometer after derivatization with Nynhydrin reagent.

2.4.4. pH

pH is one of the important parameter involved in the topical skin formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of topical formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Topical formulations for skin should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter.

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Figure1: pH Meter

2.4.5. Viscosity study

For viscosity studies thepH of formulations were raised to pH 7.4 by the addition of 0.5M NaOH.and the temperature was raised to 37^0C.The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study.

Figure2: Viscosity measurement by Brookfield viscometer

2.4.6. Skin irritation study

Guinea pigs (400-500 g) of either sex were used for testing of skin irritation. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard conditions. Hair was shaved from back of guinea pigs and area of 4 cm2 was mark done both the sides, one side served as control while the other side was test. Gel was applied (500 mg / guinea pig) twice a day for 7 days and the site was observed for any sensitivity and the reaction if any, was graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema and severe erythema with or without edema, respectively.

2.4.7. Microbial assay of Gentamicinsulphate

The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity. Two general methods are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method. The cylinder-plate method (Method A) depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a Petri dish or plate to an extent such that growth of the added micro-organism is prevented entirely in a zone

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around the cylinder containing a solution of the antibiotic.

2.4.7.1Media

Prepare the media required for the preparation of test organism inocula from the ingredients listed in Table. Minor modifications of the individual ingredients may be made, or reconstituted dehydrated media may be used provided theresulting media have equal or better growth-promotingproperties and give a similar standard curve response.Dissolve the ingredients in sufficient water to produce1000 ml and add sufficient 1 M sodium hydroxide or 1 Mhydrochloric acid, as required so that after sterilization the pH is as given in Table.

Table 4– Media: Quantities in g of ingredients per 1000 ml

S. No	Ingredient	Medium(gm)
1.	Peptone	6.0
2.	Pancreatic digest of casein	4.0
3.	Yeast extract	3.0
4.	Beef extract	1.5
5.	Dextrose	1.0
6.	Agar	15.0
7.	Final pH	7.8-8.0

6.4.7.2 Buffer Solutions Prepare by dissolving the followingquantities given in Table 2 of dipotassium hydrogen phosphateandpotassium dihydrogen phosphate in sufficient water toproduce 1000 ml after sterilisation, adjusting the pH with 8 Mphosphoric acid or 10 M potassium hydroxide.

Table: 5 Composition of buffer B2

Buffer no	Dipotassium hydrogen phosphate K2HPO4 (g)	potassium dihydrogen phosphate KH2PO4(g)	pH adjustedafter sterilisation to
2	16.73	0.523	8.0 ± 0.1

2.4.7.3. Preparation of the Standard Solution To prepare a stock solution, dissolve a quantity of the Standard Preparation of a given antibiotic, accurately weighed and previously dried where so indicated in Table 3, in the solvent specified in the table, and then dilute to the required concentration as indicated. Store in a refrigerator and use within the period indicated. On the day of assay, prepare from the stock solution five or more test dilutions, the successive solutions increasing stepwise in concentration, usually in the ratio 1:1.25 for Method A or smaller for Method B. Use the final diluents specified and a sequence such that the middle or median has the concentration specified in Table 6.7.

2.4.7.4 Preparation of the Sample Solution: From the information available for the substance under examination (the "unknown"), assign to it an assumed potency per unit weight or volume, and on this assumption prepare on the day of the assay a stock solution and test dilution as specified for each antibiotic in Table 6.7 but with the same final diluents as used for the Standard Preparation. The assay with 5 levels of the Standard requires only one level of the unknown at a concentration assumed equal to the median level of the standard.

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Table:6 Antibiotic and assay micro-organism with Atcc Number

Antibiotic	Test Organism	ATCC1 No.
Gentamicin	Staphylococcus epidermidis	12228

2.4.7.5. Preparation of inoculum. Prepare the microbial suspensionsfor the inoculum for the assay. If the suspensions are prepared by these methods, growth characteristics are sufficiently uniform so that the inoculums can be adequately determined by the trials given below.

Table:7 Preparation of standard and test dilutions for microbial assay

Antibiotic	Standard Stock Solution					Test Dilution		
	Assay method	Prior drying	Initial solvent (furtherdilu ent, if different)	Final stock concent ration per ml	Use before (number of days)	Final diluent	Median dose µg or units per ml	Incubatio n tempretur e (ºC)
Gentami cinSulph ate	a	Yes	B2	1mg	30	B2	0.1µg	36-37.5

2.4.7.6 Preparation of standard curve

For preparing the standard curve, use a total of 12 Petri dishes or plates to accommodate 72 cylinders or cavities. A set of 3 plates (18 cylinders or cavities) is used for each dilution. On each of the three plates of a set fill alternate cylinders or cavities with solution S3 (representing the median concentration of the standard solution) and each of theremaining 9 cylinders or cavities with one of the other 4 dilutions of the standard solution. Repeat the process for theother 3 dilutions of the standard solution. For each unknown preparation use a set of 3 plates (18 cylinders or cavities) and fill alternate cylinders or cavities with the sample solution and each of the remaining 9 cylinders of cavities with solution S3. Incubate the plates for about 18 hours at the specified temperature and measure the diameters or the zones of inhibition.

2.4.8.In-vitroDrug diffusion study

The in vitro release of GentamicinSulphate from the formulationswas studied through cellophane membrane. The dissolution medium used was artificial tear fluidfreshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight inthe dissolution medium, was tied to one end of a specifically designed glasscylinder (open at both ends and of 5 cm diameter). A 1gm of theformulation was accurately pipetted into this assembly. The cylinder was attachedto the metallic driveshaft and suspended in 50 ml of dissolution mediummaintained at 37± 1°C so that the membrane just touched the receptor mediumsurface. The dissolution medium was stirred at 50 rpm using magnetic stirrer.Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replacedby an equal volume of the receptor medium.

Figure3:In Vitro drug diffusion study

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2.4.8.1 Preparation of Calibration curve of GentamicinSulphate

For preparation of GentamicinSulphate solution of different concentrations from 30-100µg/ml were prepared in phosphate buffer pH 7.4. 5 ml solution of these concentrations were taken into 10 ml volumetric flask and add 0.5ml Nynhydrin reagent as a derivatizing agent. Resulting solutionwas heating on water bath on 95^0C for 15 miniute, after cooling the solution filter it and taking reading at 400nm.

Table:8 Preparation of calibration curve of GentamicinSulphate in buffer 7.4 pH at 400nm by Colorimetry

Concentration (µg/ml)	Absorption
30	0.098
40	0.156
50	0.218
60	0.279
70	0.349
80	0.415
90	0.486
100	0.565

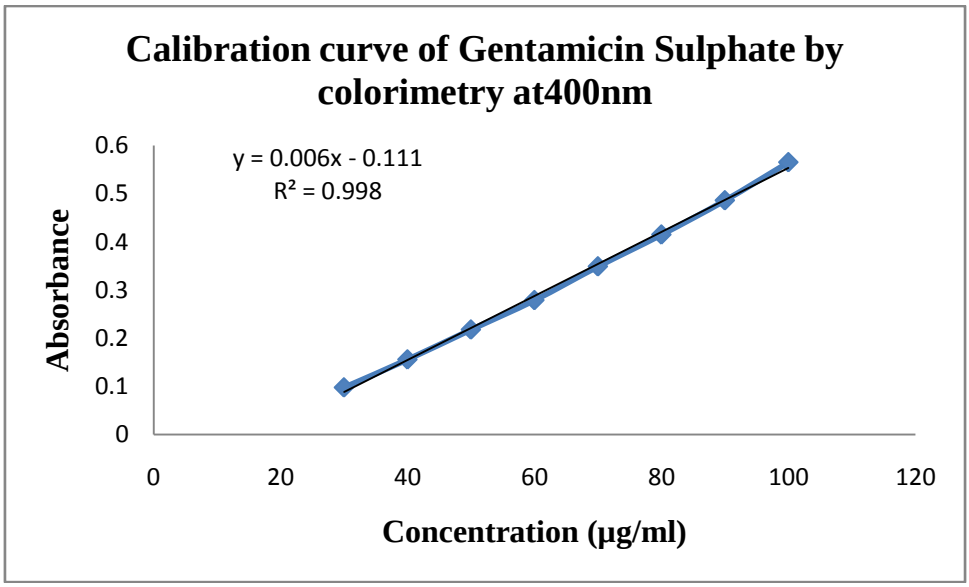

Figure4: Standard curve of GentamicinSulphate by colorimetry at400nm

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2.4.9 Stability studies[35]

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the five formulations were subjected to stability studies at ambient humidity conditions at 2°C to 8°C, ambient temperature and 40±1°C for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for following parameters.

Packs which are to some degree permeable to moisture (as are most plastics) will lose or gain moisture according to whether they are exposed to a high or low relative humidity respectively. 40°C/75% RH may be particularly severe on a fully exposed blister pack and give an artificially low shelf-life prediction. The same condition may offer little challenge to moisture loss as the vapour pressure inside the pack may virtually be at equilibrium with the external atmosphere (plastic containers).

The formulations were further evaluated for evaluation parameters after each sampling period

Table9: Storage conditions for Stability Studies according to ICH guidelines

Study	Storage Condition	Minimum Time Period
Long term	25ºC±2ºC, 60%±5%RH Or 30ºC±2ºC, 65%±5%RH	12 Months
Intermediate	30ºC±2ºC, 65%±5%RH	6 Months
Accelerated	40ºC±2ºC, 75%±5%RH	6 Months

III. RESULT AND DISCUSSION

3.1MeltingPoint

MeltingpointofGentamicinsulphatewasdeterminedbyCapillarytubemethodanditwasfoundtobe 246.5^{0}C-249.1^{0}C.

DSCgraphofGentamicinsulphatepossessed248.37^{0}C ofitsmeltingpointwhichischaracteristicspeakinDSC graphforGentamicinSulphate.

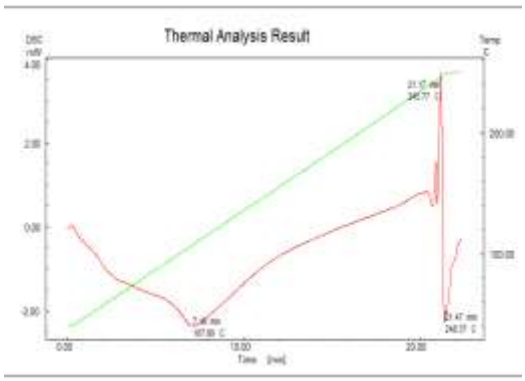

Figure 5:DSCspectraofGentamicinSulphate

3.2Solubilityanalysis

ThesolubilityofGentamicinsulphatewastestedinvarioussolventssuchasdistilledwater,ethylalcohol,andacetoneandsolubilityfoundtobechartedasfollow:

Table10:Solubilityanalysis

Solvent	Solubility
Water	Freelysoluble
Ethylalcohol	Practicallyinsoluble
Acetone	Slightlysoluble

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3.3FT-IRstudy

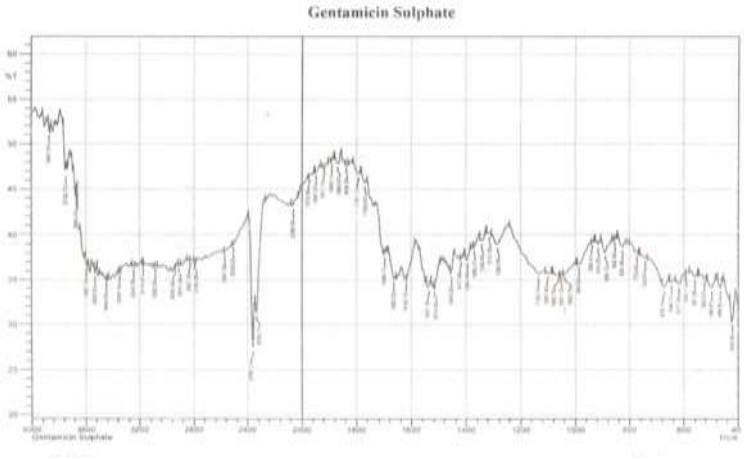

Figure 6:FT-IRspectraofGentamicinsulphate

Table11:CharacteristicpeakofGentamicinsulphateinIRspectra

S.No.	Peak	WaveNo.
1	N-HStretching	3444
2	N-HBending	1618.
3	C-NStretching	1284
4	CH_3Stretching(sym.)	2827
5	CH_3Stretching(asym.)	2935
6	C-O-CStretching	1132
7	O-HSTRECHING	3348
8	C-OHBending	1454

3.4.ThinlayerChromatography(TLC)

ThinLayerChromatographywasperformedaccordingtoIP2007.TheTLCplatewasdevelopedandexaminedunderUVchamber,3spotwereobservedandRFvaluewascalculatedforthem.

Figure 7:TLCplateofgentamicin

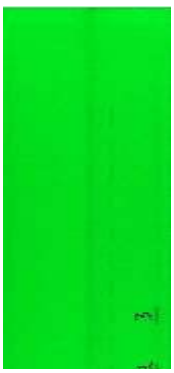

Figure 8 . TLCplateofgentamicininUVchamber

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Table12:RfvalueofTLCplateofGentamicinsulphate

Spot	Rfvalue
1	0.65
2	0.79
3	0.87

3.5.Drug-ExcipeintInteractionstudies(ByDSC)

DSCgraphofGentamicinsulphatepossessed 248.37^{0}CofitsmeltingpointwhichischaracteristicspeakinDSCgraphforGentamicinSulphate.Thenthemixtureofdrugandexcipeintwhichwaskeptinacceleratedconditionoftemperature(40^{0}C)andRH(75%)for15daysandanalyzedforDSCanalysis.Thecharacteristicpeakof Gentatamicinsulphatenotdeviatesfromitspositionof248^{0}Cthatshowsnointeractionbetweendrugandpolymers.TheDSCgraphsareasfollow.

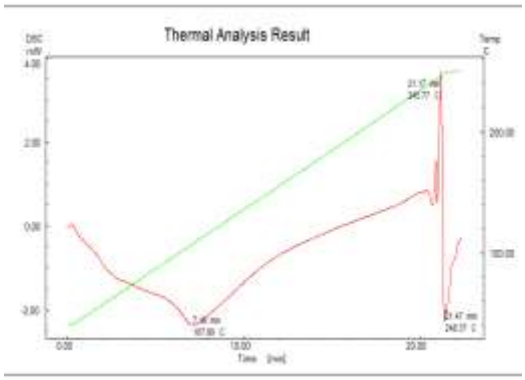

Fig 9 DSC spectraofGentamicin(Drug)

3. **6.Evaluationparameter**

3.6.1Appearance

Formulationswereevaluatedforappearance byvisualobservationagainstablackandwhitebackground.Someformulationshadproblemofprecipitationof carbopolduringstorage,theproblemovercomebyincreasingthestirringtimeupto2-3hoursduringformulation.

Table13:Appearancetestofgelformulations

Formulationcode	Appearance
F1	Clear
F2	Precipitateobserved
F3	Clear
F4	Clear
F5	Clear
F6	Clear
F7	Precipitateobserved
F8	Clear
F9	Clear

3.6.2.pHDetermination

ThedevelopedformulationswereevaluatedforpHbyusingdigitalpHmeter.ThepHofformulationswasdecreasesfrombufferpH5.0becauseofacidicgroupsofcarbopolsothatthepHwasadjustedto5.0byusing0.5NNaOH.

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Table14:pHDetermination

Formulation	pH	Adjustto	pHafter30daysstorage
F1	5.0	7.4±0.1	7.3
F2	4.9	7.4±0.1	7.2
F3	4.8	7.4±0.1	7.4
F4	5.1	7.4±0.1	7.4
F5	4.9	7.4±0.1	7.3
F6	4.8	7.4±0.1	7.3
F7	5.2	7.4±0.1	7.4
F8	4.9	7.4±0.1	7.3
F9	4.9	7.4±0.1	7.1

3.6.3Angularviscosityofformulations

Viscosityofformulationwasdeterminedbefo reandaftergelationbyusingBrookfield'sviscometerin thesmallvolumeadaptorandtheangularvelocitywasin creasedgraduallyfrom10,20,40,50,60,70,80,90AND 100RPM.Thecomparativestudyofviscositywasdone at50RPM.F4,F5,andF7showcomparativelybettervis cosityandgoodconsistencygel.

Table15:Angularviscosityofformulations

RPM	ViscosityF1	ViscosityF2	ViscosityF3	ViscosityF4	ViscosityF5	ViscosityF6	ViscosityF7	ViscosityF8	ViscosityF9
10	12561	13012	9056	14112	12600	9924	8886	12765	10598
20	9877	10032	7721	11872	11565	8199	7291	10396	9300
30	6987	7298	6392	9604	9359	6826	5935	9248	7144
40	6134	6457	5781	8112	7889	5487	5276	7992	5631
50	5687	6234	4601	7565	6723	4476	4487	7174	4567
60	5622	5904	4333	6498	6132	3998	4102	6574	4298
70	5539	5855	4153	5680	5643	3572	3821	6006	4151
80	4768	4667	3890	4967	5128	3171	3666	5489	3855
90	3988	4128	3505	4252	4555	2953	3501	4821	3561
100	3156	3510	3008	3987	4013	2538	3246	4521	3277

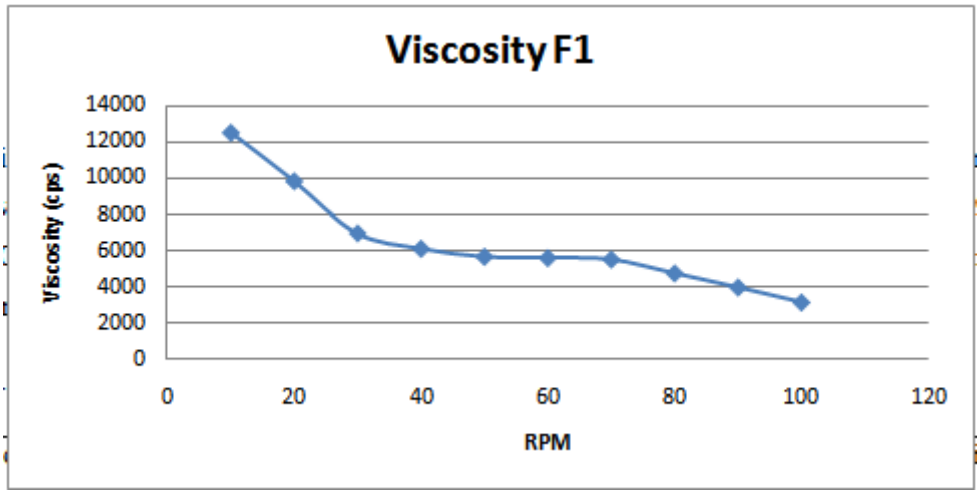

Figure 10: Angular viscosity of F1

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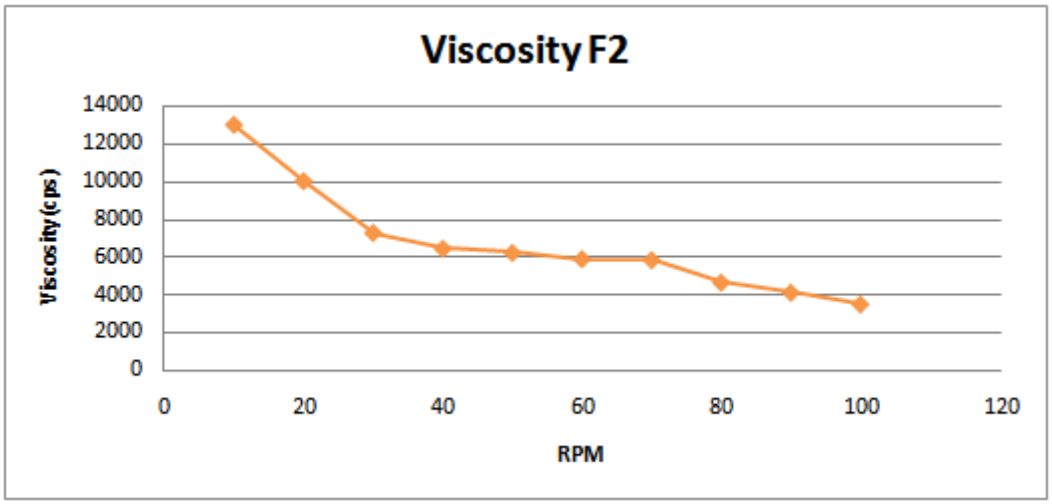

Figure 11: Angular viscosity of F2

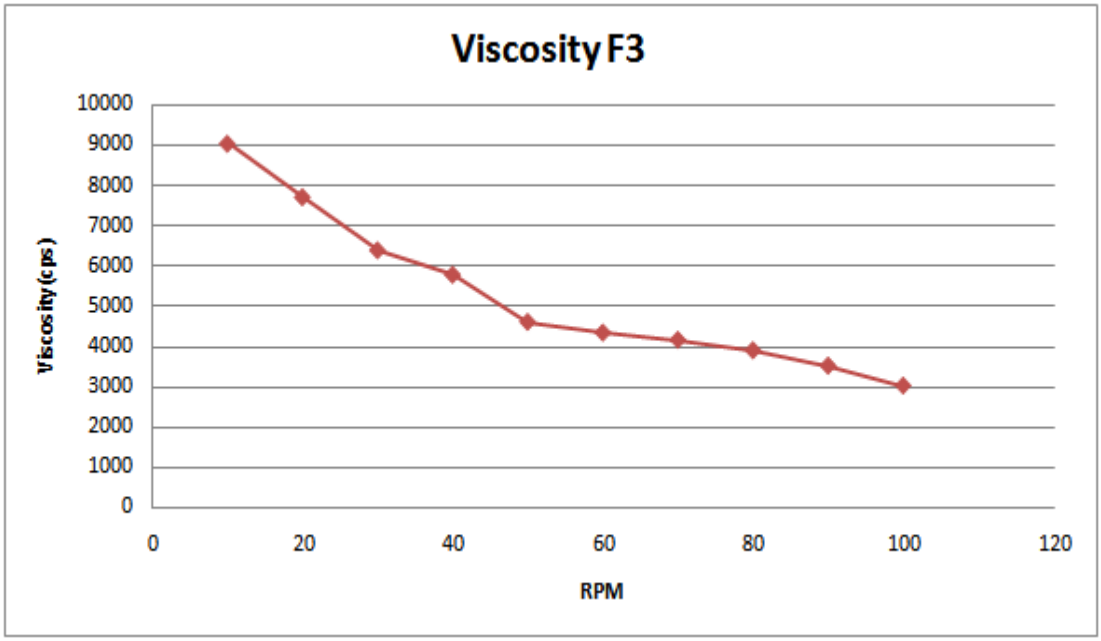

Figure12:AngularviscosityofF3

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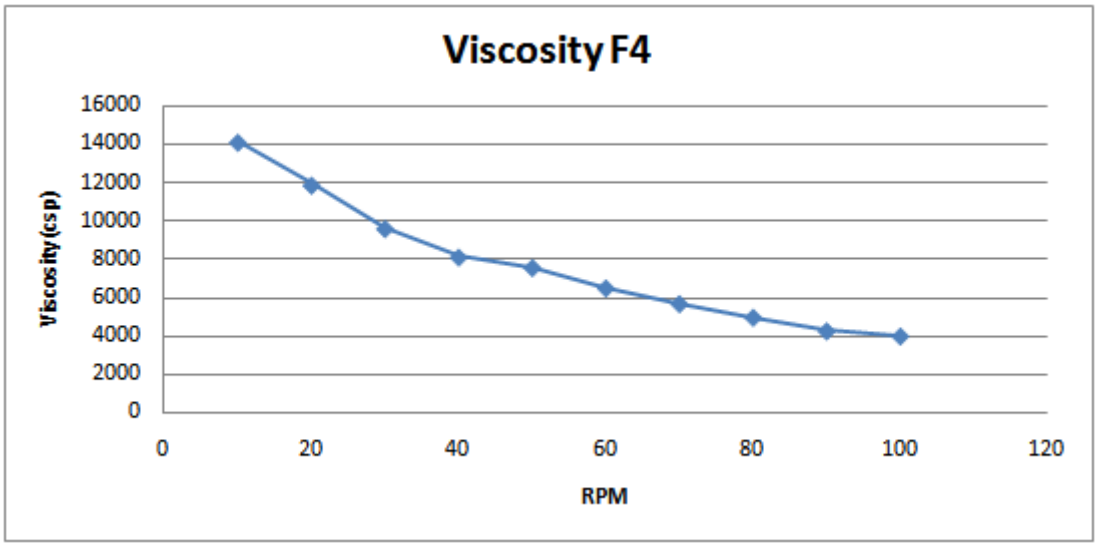

Figure 13: Angular viscosity of F4

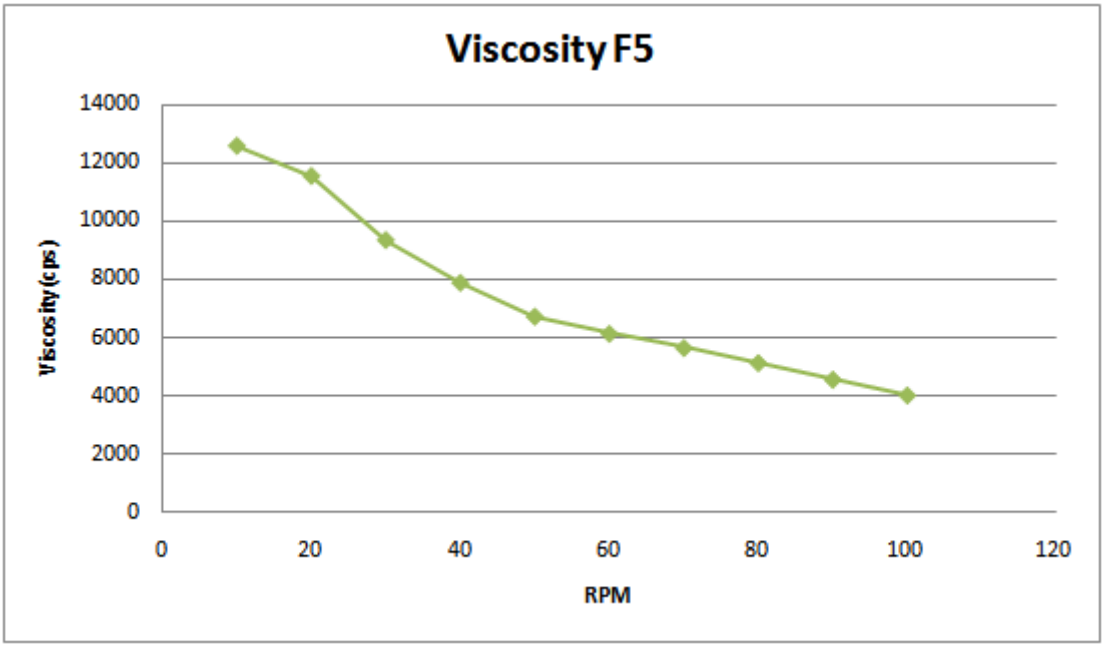

Figure 14: Angular viscosity of F5

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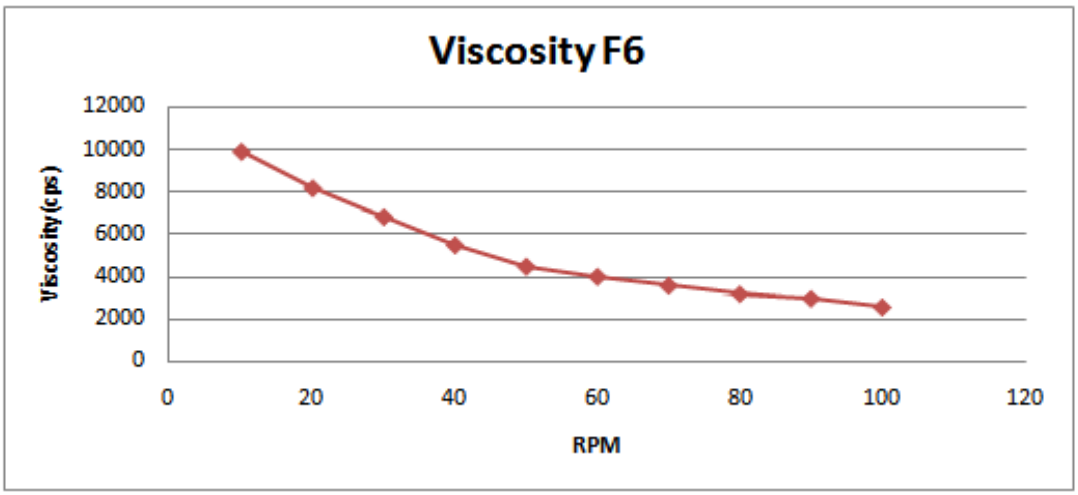

Figure 15: Angular viscosity of F6

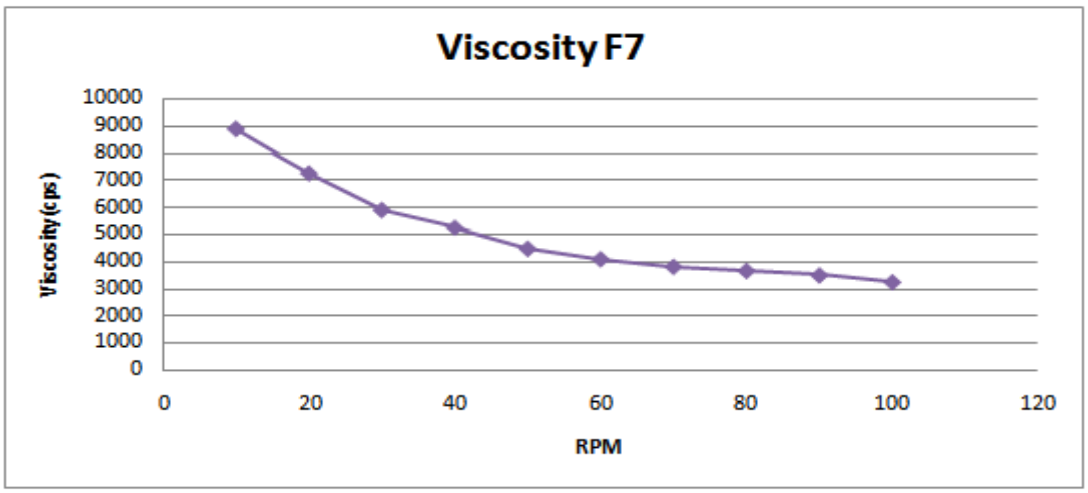

Figure 16: Angular viscosity of F7

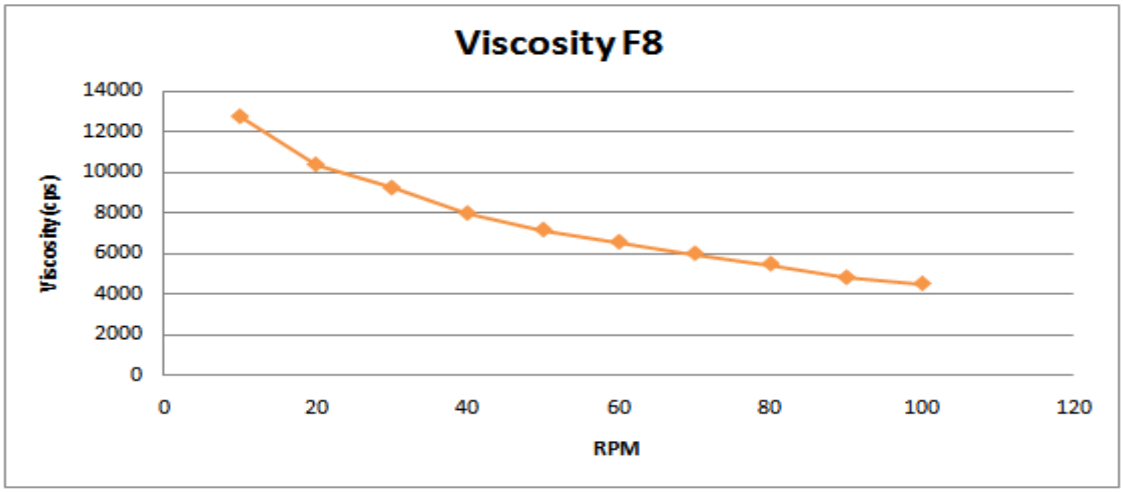

Figure 17: AngularviscosityofF8

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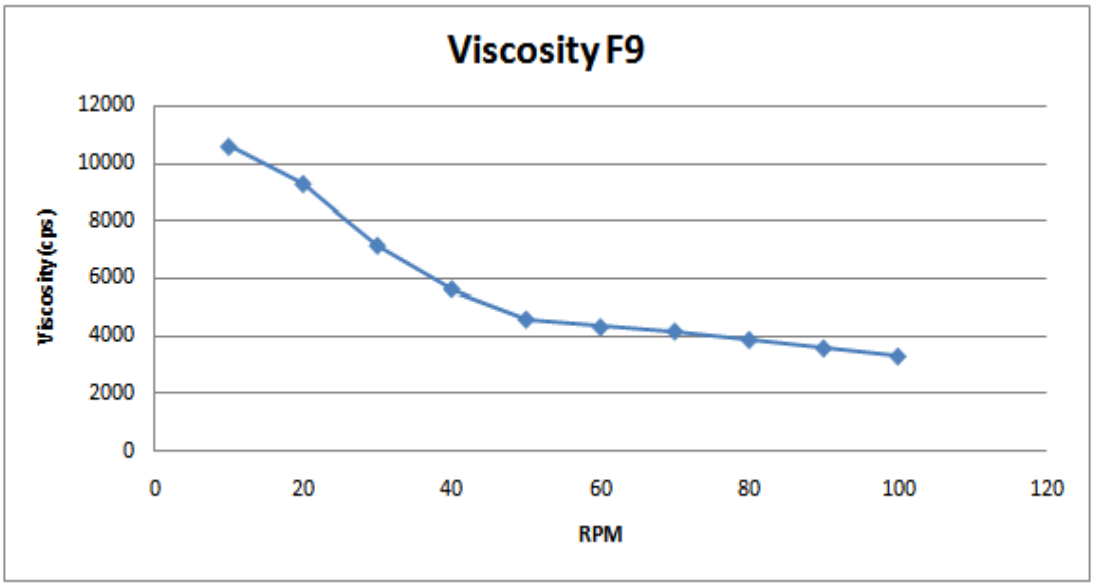

Figure 18: AngularviscosityofF9

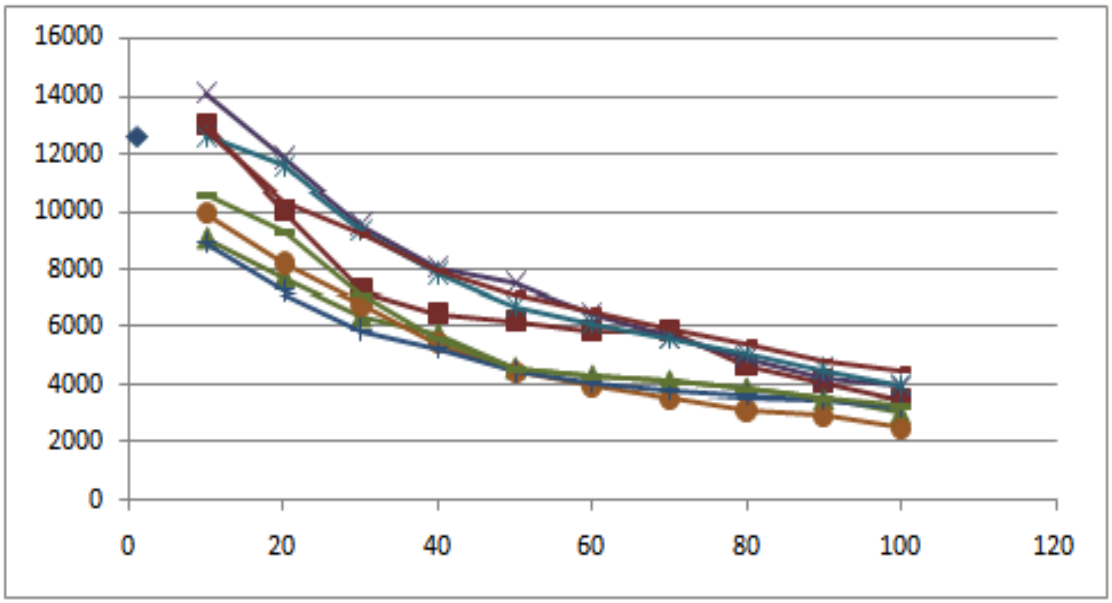

Figure 19: Comparison of angular viscosities of formulations

3.6.4Skinirritationstudy

Guineapigs(400-500g)ofeithersexwereusedfortestingofskinirritation. Theanimalsweremaintainedonstandardanimalfeedandhadfreeaccesstowater.Theanimalswerekeptunderstandardconditions.Hairwasshavedfrombackofguineapigsandareaof4cm2wasmarkdonebothsides,onesideservedascontrolwhiletheothersidewastest.Gelwasapplied(500mg/guineapig)twiceadayfor7daysanddthesitewasobservedforanysensitivityandthereactionifany,wasgradedas0,1,2,3fornoreaction,slightpatcherythema,slightbutconfluentormoderatebutpatcherythemaandsevereerythemawithorwithoutedema,respectively

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Table16:Resultforskinirritationtest

S.No	Formulationcode	Result
1	F1	0
2	F2	0
3	F3	1
4	F4	0
5	F5	0
6	F6	1
7	F7	0
8	F8	0
9	F9	1

0-noreaction,
1-slightpatchyerythema,
2-slightbutconfluentormoderatebutpatchyerythemaand
3-severeerythemawithorwithoutedema
Note:AsearlierparameterssuchasAppearance,Rheologystudy,viscosityandskinirritationtestsweren otsatisfactoryfortheformulationsF3,F6,F7,F9,sotheywerenotevaluatedforfurtherparameters.

3.6.5DrugContent
ThedrugcontentofGentamicininformulationswasdetermenedbycolorimetricmethod.Themethodwasbasedontheninhydrinreactionwithprimaryandsecondaryaminespresentinthegentamicin.Thisreactionproducesapurplecolour.

Table17:Drugcontent

Formulation	DrugContent(%)
F1	98.22
F2	98.02
F4	97.22
F5	98.65
F8	95.51

3.6.6MicrobialassayofGentamicinSulphate
ThemicrobialassayofGentamicinwasfollowedaccordingtoIP2007.mediumDwaspreparedforpreparationofPetriplateswithadditionofspecificmicro-organism(staphylococcusepidermidis).Allthedilutionsofstandarddrugandformulationswerepreparedin B2(buffer2).40µlofeachsolutionwereintroducedintothealternativetomedianconcentrationsolutionS_3.Afterincubationof18hourstherdiameterofZOI(Zoneofinhibition)measuredbyscaleandinterpritatedforcalculation.

Table18:PreparationofstandardcurveofMicrobialassay

Concentration	Meandiameter(ZOI)mm
S_1(0.05µg/ml)	14.4
S_2(0.075µg/ml)	16.6
S_3(0.1µg/ml)	18.3
S_4(0.125µg/ml)	21.7
S_5(0.15µg/ml)	22.9

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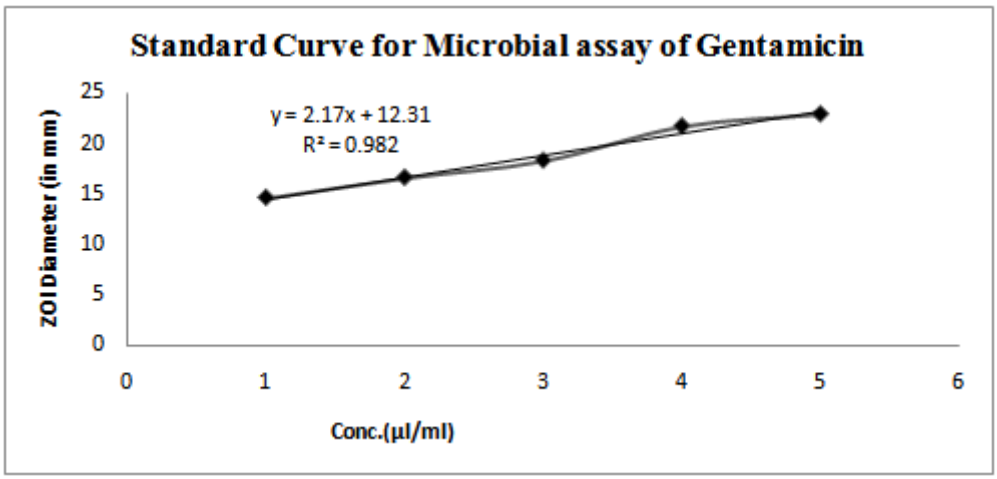

Figure20:StandardcurveforMicrobialassayofGentamicin

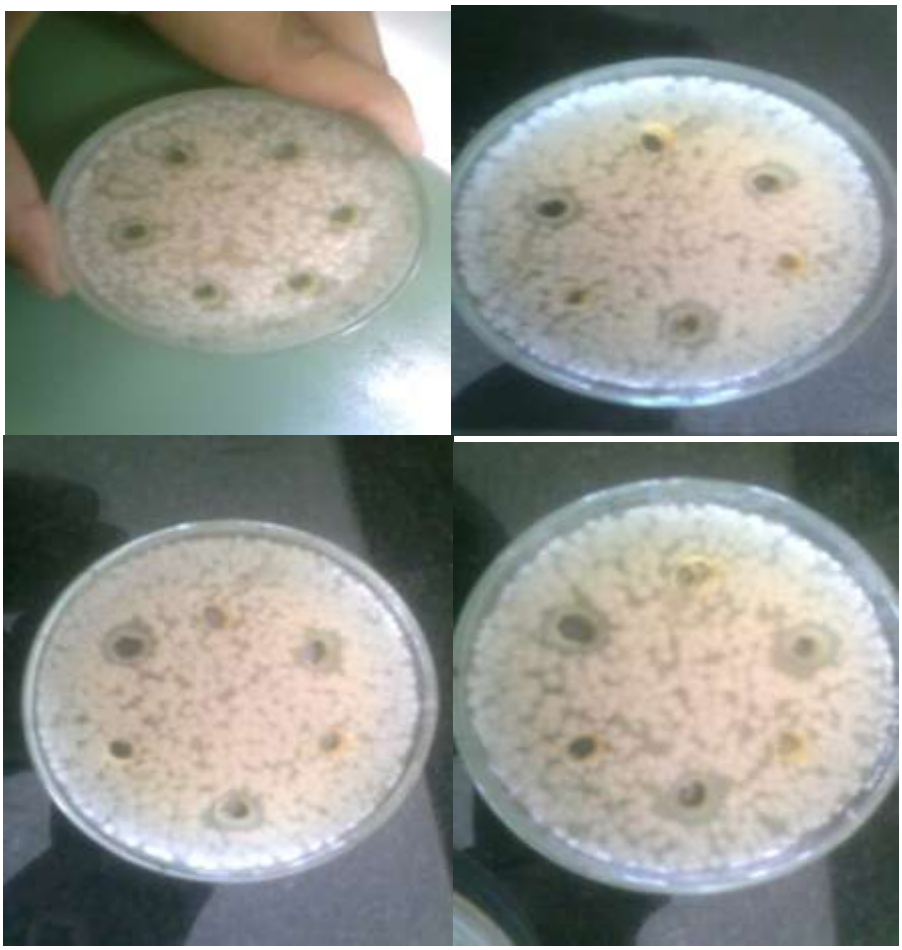

Figure21:PhotographsofMicrobialassay

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Table19:PercentagepotencyofdrugGentamicinSulphateinformulations

Formulation	MeanZOIStandards(S_3)	MeanZOITest	CorrectionFactor	PercentagePotency
F1	18.6	17.1	0.3	91.8
F2	17.7	18.8	0.6	99.45
F4	17.3	17.3	1.0	100
F5	18	17.3	0.3	97.7
F8	18.4	17.1	0.1	92.89

3.6.7Entrapmentefficiency

Approximately0.5%w/vdispersionoftheSLMsindistilledwaterwasprepared,allowedtoequilibratefor48hatroomtemperature,shakenandfiltered.Thefiltratewasadequatelyanalyzedfor Gentamicincontentspectrophotometrically(UV/VisSpectrophotometer)at400nm.Theamountofdrugencapsulatedin theSLMswascalculatedwithreferencetoastandardBeer'splotforgentamicinobtainEEusing

EE(%)=(Da/Dt)100..……..

Table20:%Entrapmentefficiency

Formulation	**%EE**
F1	**89.22±0.88**
F2	**88.25±1.58**
F4	**91.68±1.65**
F5	**94.67±1.99**
F8	**92.20±0.99**

7.6.8.In-vitrodrugreleasestudy

7.6.8.1PreparationofCalibrationcurveofGentamicinSulphate

ForpreparationofGentamicinSulphatesolutionofdifferentconcentrationsfrom30-100µg/mlwerepreparedinphosphatebufferpH7.4.5mlsolutionsoftheseconcentrationsweretakeninto10ml volumetricflaskandadd0.5mlNynhydrinreagentasaderivatizingagent.Resultingsolutionwasheatingonwaterbathon95^{0}Cfor15miniute,aftercoolingthesolutionfilteritandtakingreadingat400nm.

Table21:PreparationofcalibrationcurveofGentamicinSulphateinbuffer7.4pHat400nmbyColorimetry

Concentration(Inµg/ml)	**Absorption**
30	0.098
40	0.156
50	0.218
60	0.279
70	0.349
80	0.415
90	0.486
100	0.565

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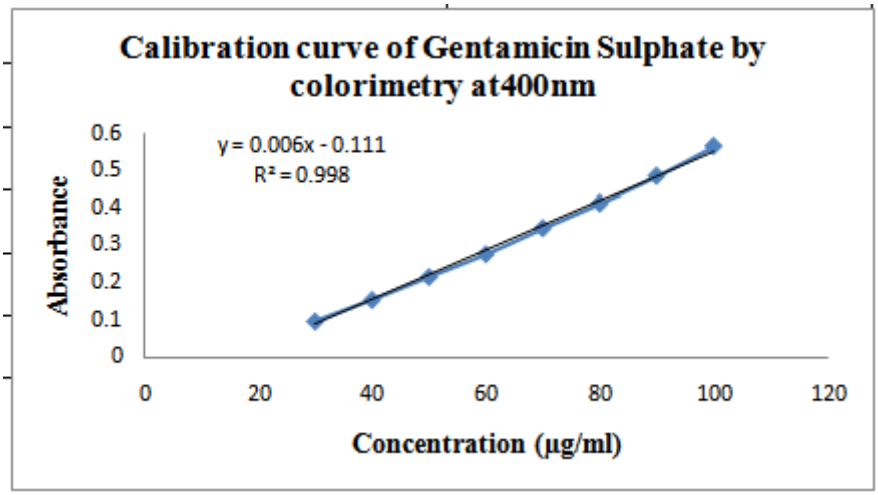

Figure22: StandardcurveofGentamicinSulphatebycolorimetryat400nm

Table22:InVitrodrugreleaseprofileofGetamicinsulphatefromFormulationF1

Time	Absorption	Concentration(µg/ml)	Dilution*10	Concentrationmg/ml	CumulativeConcentrationmg/ml	Concentrationmg/100ml	CumulativeConcentrationmg/100ml	%DrugRelease
0	0	0	0	0	0	0	0	0
1	0.413	87.36	873.6	.8736	.8736	87.36	87.36	29.12
2	0.549	110.34	1103.4	1.103	1.976	110.3	112.27	37.42
3	0.669	130.29	1302.9	1.303	3.279	130.3	133.57	44.52
4	0.792	150.6	1506.0	1.506	4.785	150.6	155.38	51.79
5	0.899	168.48	1684.8	1.685	6.47	168.5	174.97	58.83
6	1.024	189.33	1893.3	1.893	8.363	189.3	197.66	65.88
7	1.143	209.01	2090.1	2.090	10.45	209.0	219.45	73.15
8	1.244	225.99	2259.9	2.260	12.71	226	238.71	79.57

Table23:InVitrodrugreleaseprofileofGetamicinsulphatefromFormulationF2

Time	Absorption	Concentration(µg/ml)	Dilution*10	Concentrationmg/ml	Cumulative Concentrationmg/ml	Concentrationmg/100ml	CumulativeConcentrationmg/100ml	%DrugRelease
0	0	0	0	0	0	0	0	0
1	0.329	73.35	733.5	0.733	0.733	73.35	73.35	24.45
2	0.553	110.7	1107	1.107	2.947	110.73	113.67	37.89
3	0.736	141.3	1413	1.413	4.360	141.3	145.72	48.57

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4	0.890	166.9	1669	1.669	6.029	166.9	172.92	57.64
5	1.030	190.2	1902	1.902	7.931	190.2	198.13	66.04
6	1.188	216.6	2166	2.166	10.097	216.6	226.6	75.56
7	1.275	231.1	2311	2.311	12.40	231.1	243.5	81.16
8	1.330	240.3	2403	2.403	14.80	240.3	255.10	85.03

Table24:InVitrodrugreleaseprofileofGetamicinsulphatefromFormulationF4

Time	Absorption	Concentration (µg/ml)	Dilution* 10	Concentration mg/ml	CumulativeConcentrationmg/ml	Concentrationmg/100ml	CumulativeConcentration mg/100ml	%Drug Release
0	0	0	0	0	0	0	0	0
1	0.430	90.3	903	.903	.903	90.3	90.3	30.15
2	0.516	104.5	1045	1.045	1.94	104.5	106.4	35.48
3	0.610	120.3	1203	1.203	3.143	120.3	123.44	41.14
4	0.852	160.6	1606	1.606	4.749	160.6	165.3	55.11
5	1.020	188.6	1886	1.886	6.635	188.6	195.23	65.07
6	1.114	204.3	2043	2.043	8.678	204.3	212.97	70.99
7	1.138	208.2	2082	2.082	10.76	208.2	218.96	72.98
8	1.114	209.2	2092	2.092	12.85	209.2	222.05	74.01

Table25:InVitrodrugreleaseprofileofGetamicinsulphatefromFormulationF5

Time	Absorption	Concentration(µg/ml)	Dilution*10	Concentrationmg/ml	CumulativeConcentration mg/ml	Concentrationmg/100ml	Cumulative Concentrationmg/100ml	%DrugRelease
0	0	0	0	0	0	0	0	0
1	0.365	79.35	793.5	.793	.793	79.35	79.35	26.45
2	0.659	128.4	1284	1.284	2.007	128.4	130.40	43.46
3	0.793	150.6	1506	1.506	3.513	150.6	154.11	51.37
4	1.037	191.4	1914	1.914	5.427	191.4	196.82	65.60

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5	1.129	221.8	2218	2.218	7.645	221.8	237.09	79.03
6	1.370	246.9	2469	2.469	10.114	246.9	257.01	85.67
7	1.438	258.3	2583	2.583	12.69	258.3	270.99	90.33
8	1.505	269.4	2694	2.694	15.38	269.4	284.78	94.92

Table26:InVitrodrugreleaseprofileofGetamicinsulphatefromFormulationF8

Time	Absorption	Concentration(µg/ml)	Dilution*10	Concentration mg/ml	CumulativeConcentrationmg/ml	Concentrationmg/100ml	CumulativeConcentrationmg/100ml	%DrugRelease
0	0	0	0	0	0	0	0	0
1	0.275	64.35	643.5	.6435	.643	64.35	64.35	21.45
2	0.461	95.37	953.7	.9537	1.597	95.37	96.96	32.32
3	0.611	120.4	1204	1.204	2.801	120.4	123.20	41.06
4	0.855	161.1	1611	1.611	4.412	161.1	165.51	55.17
5	1.098	201.5	2015	2.015	6.427	201.5	207.92	69.30
6	1.300	235.3	2352	2.352	8.779	235.3	244.07	81.35
7	1.369	246.7	2467	2.467	11.246	246.7	257.94	85.98
8	1.436	257.9	2579	2.579	13.825	257.9	271.72	90.57

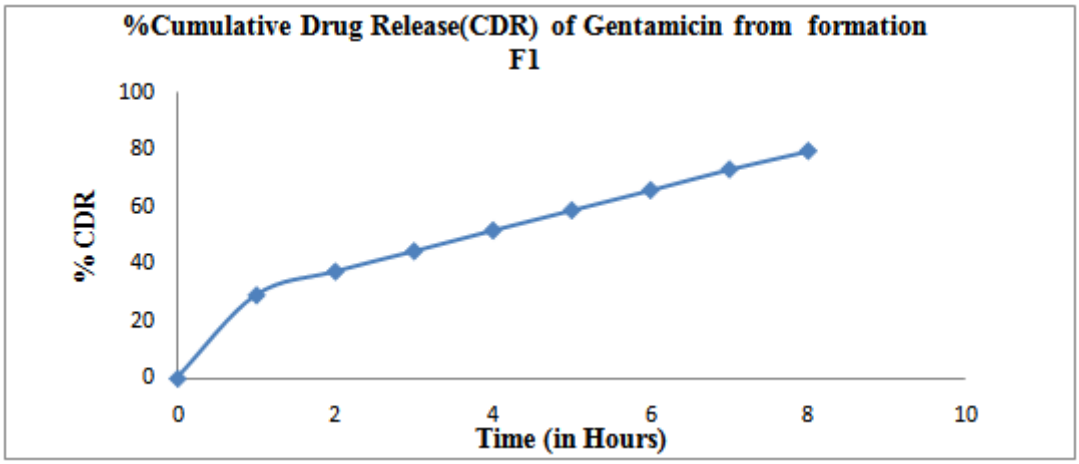

Figure 23:%CumulativeDrugRelease(CDR)ofGentamicinfromformationF1

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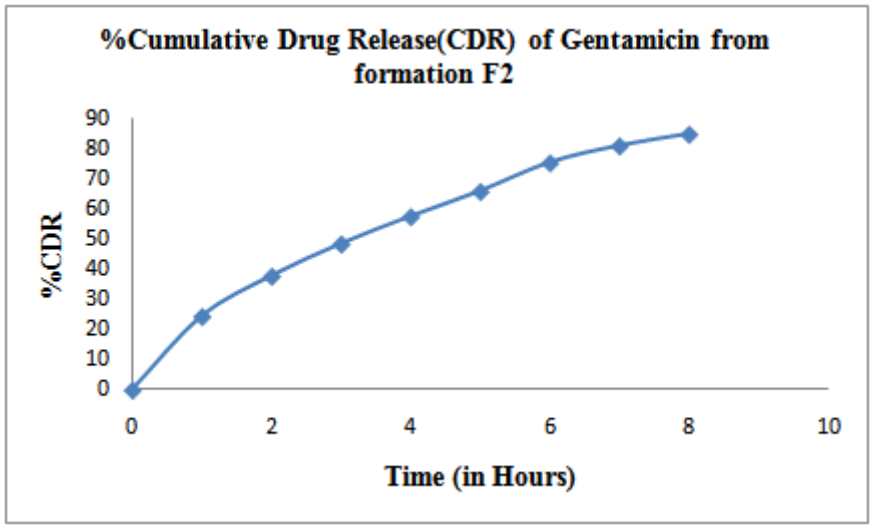

Figure 24:%CumulativeDrugRelease(CDR)ofGentamicinfromformationF2

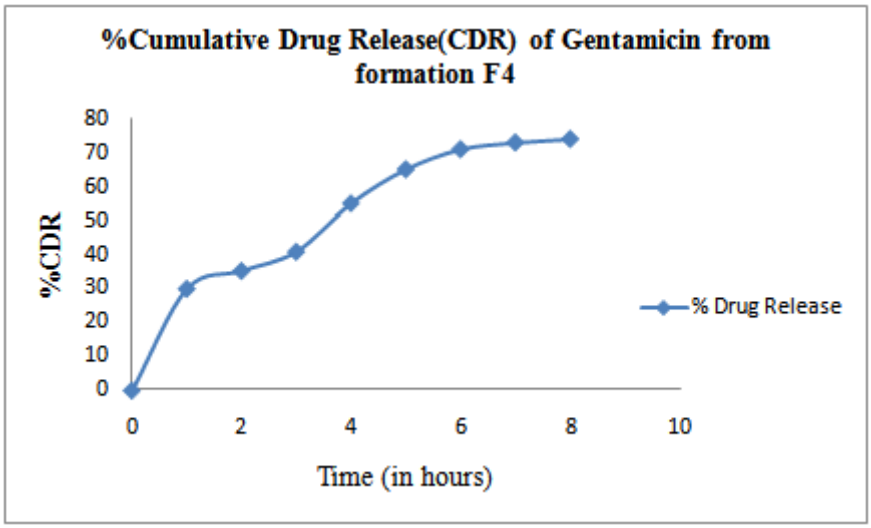

Figure 25 :%CumulativeDrugRelease(CDR)ofGentamicinfromformationF4

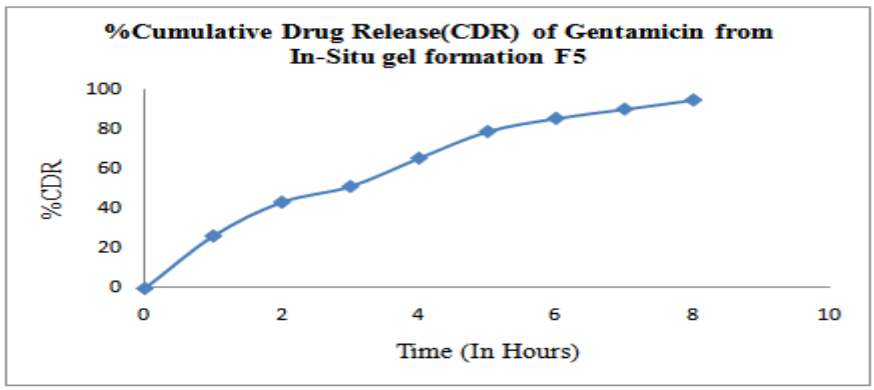

Figure 26:%CumulativeDrugRelease(CDR)ofGentamicinfromformationF5

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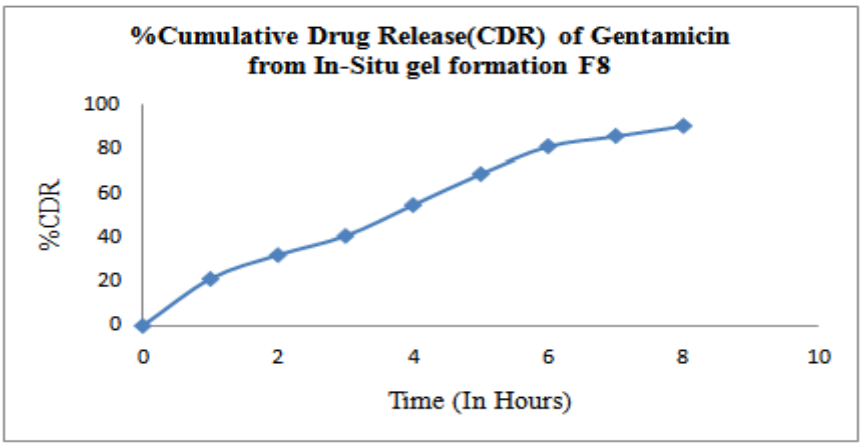

Figure 27: %CumulativeDrugRelease(CDR)ofGentamicinfromformationF8

Table27:Comparative%CDRofformulationsF1,F2,F4,F5,F8

Time(inhrs)	%CDRF1	%CDRF2	%CDRF4	%CDRF5	%CDRF8
0	0	0	0	0	0
1	29.12	24.45	30.15	26.45	21.45
2	37.42	37.89	35.48	43.46	32.32
3	44.52	48.57	41.14	51.37	41.06
4	51.79	57.64	55.11	65.60	55.17
5	58.83	66.04	65.07	79.03	69.30
6	65.88	75.56	70.99	85.67	81.35
7	73.15	81.16	72.98	90.33	85.98
8	79.57	85.03	74.01	94.92	90.57

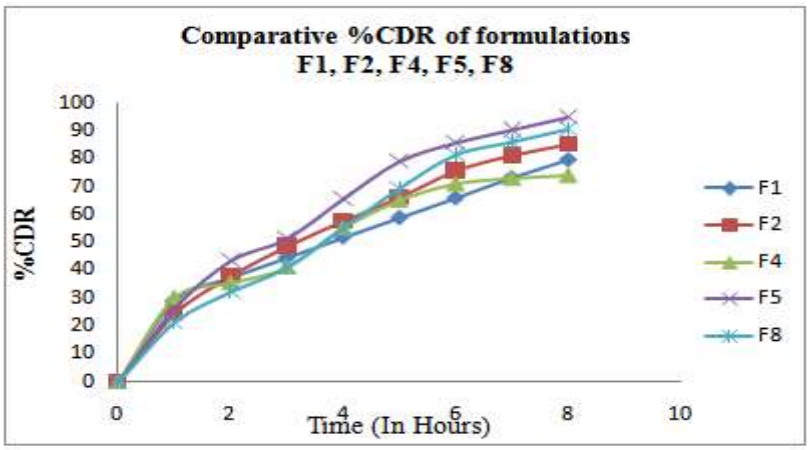

Figure 28:Comparative%CDRofformulationsF1,F2,F4,F5,F8

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7.7Stabilitystudies

Table28:Stabilitydatasheet

Formulation	Parameters evaluated														
	15days					30days					45days				
	Drugcontent	pH	Appearance	Viscosity	Skinirritation	Drugcontent	pH	Appearance	Viscosity	SkinIrriation	Drugcontent	pH	Appearance	Viscosity	Skinirritation
F1	97.32	Nochange	Nochange	5671	nil	94.18	Nochange	Nochange	5621	nil	96.45	Nochange	Nochange	5583	nil
F2	97.98	Nochange	Nochange	6200	nil	96.88	Nochange	Nochange	6192	nil	95.96	Nochange	Nochange	6125	nil
F4	96.48	Nochange	Nochange	7489	nil	94.38	Nochange	Nochange	7428	nil	94.81	Nochange	Nochange	7377	nil
F5	97.58	Nochange	Nochange	6616	nil	96.22	Nochange	Nochange	6587	nil	96.28	Nochange	Nochange	6536	nil
F8	95.11	Nochange	Nochange	7072	nil	91.88	Nochange	Nochange	6990	nil	94.61	Nochange	Nochange	6804	nil

IV. CONCLUSION

Fromtheexperimentalresultsitcanbeconcludedthat:-

DSCstudiesofGentammicinsulphate,aloneandtheirphysicalmixturewithPluronicF127,HPMC15cps,Carbopol934revealedthat,GentamicinSulphateiscompatiblewithallthepolymersused.

OphthalmicinsitugellingsystemofGentamicinSulphatewassuccessfullyformulatedusingpolymericcombinationofgellingagentsPluronicF127,Carbopol934as, temperaturesensitiveandpH-sensitiverespectivelyalongwithHPMC15cpsasviscosityenhancingagent.

Theclarityofthepreparedformulationswasfoundsatisfactorybutprecipitateobservedinformulationduringstorage.

ThepHofallformulationswasfoundtobe5.0.

Thedrugcontentofthepreparedformulationwaswithintheacceptablerange,andensuresdoseuniformity.TheformulationF5showedmaximumdrugcontent.

AlltheformulationsexceptF3,F6andF9showedinstantaneousgelationwhenformulatedformulationF4andF5showedbestgelationpropertyamongstallother.

FormulationF4,F5andF9showedsustaineddrugreleaseforaperiodof8hour.FormulationF9showedmostsustaineddrugrelease.

Theresultsofinvivoreleasestudiesrevealedthat,allthehydrogel

Theresultsoftheskinirritationstudiesindicatethatallexceptthreeformulations F3,F6andF9wereshowedslightpatchyerythemarestallshowedexcellentskintolerance.

Thestabilityofinsitugellingformulationswasobservedat40±1°Cand4°C(significantdecreaseindrugcontent).FormulationF5wasmorestablethanotherformulation.

Presentworkwasasatisfactorypreliminarystudyindevelopinginsitugellingsystemof GentamicinSulphate.Theformulationdevelopmentwasstartedwith9formulationbutformulationF3,F6andF9wasn'tshowgoodgellingcapacityinsimulatedtearfluid(STF),formulationF7hadsomestabilityproblemduringstorage,F4possessedirritationineyeirritationstudy,sowecanconcludethat**F5**mightbethebestformulationintermofInSitugelation,viscosity,Skinirritancy,stabilitythantheotherformulation.

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