IJPSM

Rawal Snehal *et al*, International Journal of Pharmaceutical Sciences and Medicine (IJPSM), Vol.7 Issue. 3, March- 2022, pg. 1-11

ISSN: 2519-9889
Impact Factor: 5.721

Formulation and Evaluation of Ocular *in situ gel* of Neomycin Sulphate

Rawal Snehal; Patidar Sunita; Pawar Rajat
Swami Vivekanand College of Pharmacy, Indore

DOI: 10.47760/ijpsm.2022.v07i03.001

ABSTRACT: Conventional dosage forms of Neomycin sulphate suffer from different drawbacks like spillage, poor penetration, low bioavailability, etc. In situ ophthalmic gel can overcome these problems by improving bioavailability, decreasing spillage and diminish the need for recurrent application. Thus, the aim of the present work was to prepare and evaluate in situ ophthalmic gel of Neomycin sulphate for sustained ocular delivery. polymers Carbopol 940 were used in different concentrations for the preparation of Neomycin sulphate insitu gel all formulations were found to be transparent and clear; pH of the formulations was within drug content was found within 94.16% in all optimized in situ gelling systems. Optimized formulations F3 (Carbopol 940), were liquid before instillation to the eye and underwent rapid gelation upon instillation to the eye. Hence, from the above results we can conclude that in situ ophthalmic gels of Neomycin sulphate can be a good option for the treatment of various bacterial eye infections.

Keywords: Neomycin sulphate, carbol 940, *in situ gel*, ophthalmic gel

1. INTRODUCTION

Ocular drug delivery is an extremely important topic, especially with the recent development of new drugs for the treatment of different eye diseases. An ideal drug therapy achieves effective concentration of drug at the target for a specified period of time in order to minimize general and local side effects. Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. Since past few decades, there have been plenty of research reports exhibiting potential of controlled and sustained drug delivery systems. Moreover, various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs and the second one involves maximizing corneal drug absorption and minimizing pre-corneal drug loss Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently, it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal

ISSN: 2519-9889
Impact Factor: 5.721

suspension or using erodible or non-erodible insert to prolong the pre-corneal drug retention.[1]

The eye is a unique organ, both anatomically and physiologically, containing several widely varied structures with different physiological functions that render the organ highly impervious to foreign substances. The conventional drug delivery such as suspension, ointment, solution show some drawbacks like increase pre-corneal drainage, blurred vision, low bioavailability low residence time. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye, and by other concomitant factors like, drainage of the instilled solutions, lachrymation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, binding by the lachrymal proteins.[2]

2. MATERIALS AND METHOD

MATERIALS

Neomycin sulfate was obtained as Yarrow chem. pvt. Ltd. Carbopol 940, Benzolkonium chloride was obtained from loba chemicals.

EXPERIEMENTALS

2.1 Identification of Drug

2.1.1 By UV Spectroscopy : 100 mg drug dissolve in 100 ml dm water it's a obtained stock solution of 1000 microgram/ml .then stock solution are removed 10 ml and volume make up the 100 ml with dm water and scanned of UV was 200 to 400 nm wavelength.[3]

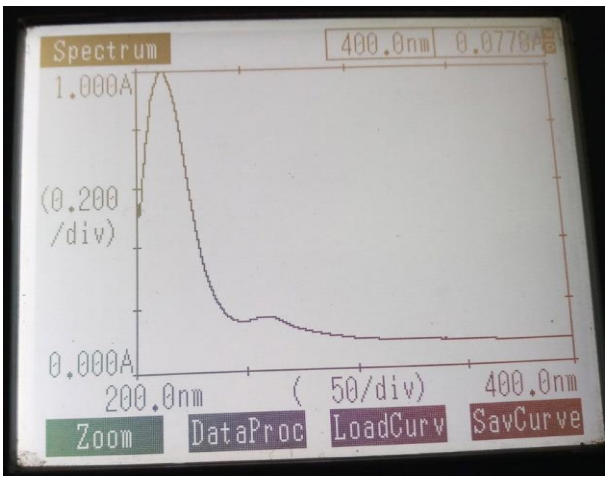

Figure 1: Spectrum of Neomycin sulfate by UV Spectroscopy

ISSN: 2519-9889
Impact Factor: 5.721

2.1.2 By melting point determination

Melting Point determination is one of the preformulation property in which the temperature at which it changes state from solid to liquid at atmospheric pressure. At the melting process the solid and liquid can exist equilibrium. The Melting point of Neomycin sulphate drug is determine by using two types of method one is Conventional method and another is Digital method.[4]

Table 1: Melting Point of Neomycin sulphate

Drug	Observed
Neomycin sulphate	187℃– 190℃

2.1.3 Preparation of Calibration Curve:

Preparation of calibration curve of drug with dm water

Calibration curve is determined by using UVspectrophotometric methods. In which 10 mg of drug dissolve in 100 ml dm water .to prepare different dilution (2,4,6,8,10) of above solution. Take absorbance at 304 nm.

Calibration curve of Neomycin sulphate with dm water

Table 2: Absorbance data of neomycin sulphate with calibration curve at 304nm.

s.no.	Concentration (µg/ml)	Absorbance
1	0	0
2	5	0.060
3	10	0.125
4	15	0.187
5	20	0.241
6	25	0.30

ISSN: 2519-9889
Impact Factor: 5.721

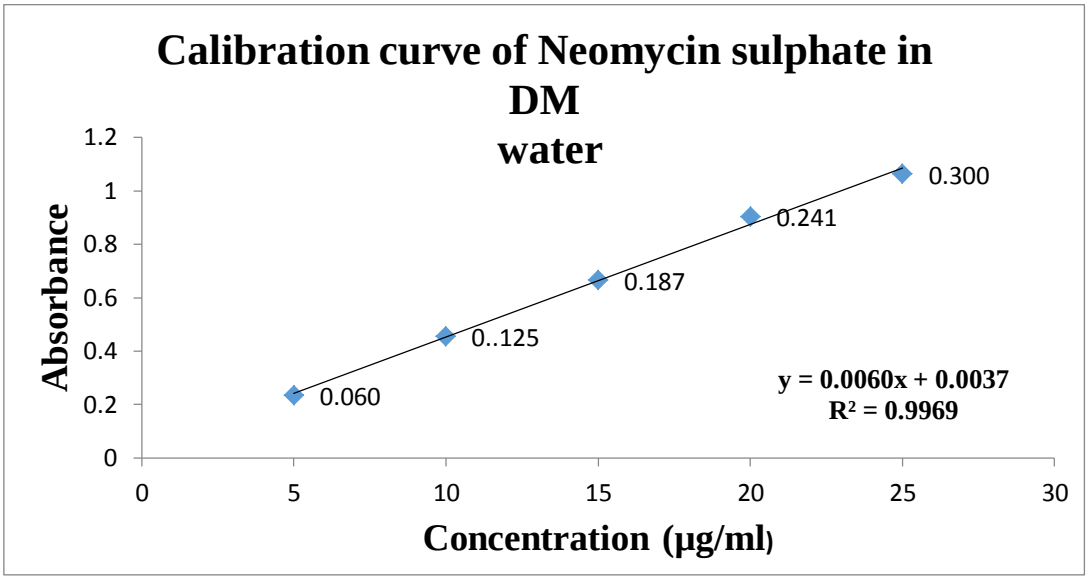

Fig no. 2 Calibration graph of Neomycin Sulphate in dm water

2.1.4 Preparation of calibration curve of drug with phosphate buffer (pH7.4)

Calibration curve is determined by using UV spectrophotometric methods. In which 10 mg of drug dissolve in 100 ml phosphate buffer solution (PH 7.4) .to prepare different dilution (2,4,6,8,10) of above solution . Take absorbance at 304 nm respectively.[5,6]

Calibration curve of Neomycin sulphate with PBS (7.4)

Table 3: Absorbance data of Neomycin sulphate with calibration curve at 304nm

s.no.	Concentration (µg/ml)	Absorbance
1	0	0
2	5	0.155
3	10	0.229
4	15	0.411
5	20	0.587
6	25	0.731

ISSN: 2519-9889
Impact Factor: 5.721

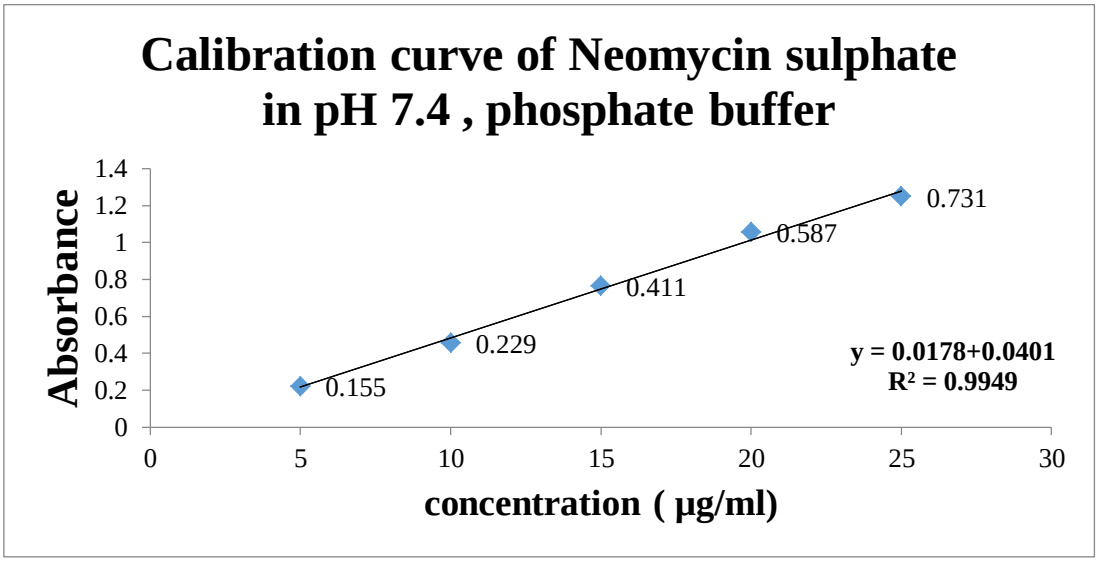

Fig no. 3 Calibration graph of Neomycin sulphate in phosphate buffer pH 7.4

2.1.4 Solubility studies of drug:

The Term Solubility is defined as maximum amount of solute that can be dissolved in a given amount of solvent to form a homogenous system at specified temperature and Specific Pressure to from Saturated Solution**.**

Procedure:

To Prepare a different solutions Water, pH 7.4 Phosphate Buffer. The drug material is added in to above solutions till Supersaturated Solution is from the Mixture can Placed in Orbital Shaker for 24 hrs. After 24 hrs. Filter the mixture Take Filtrate and Give Absorbance to detect the Concentration of Drug is Soluble in Different Solutions.

RESULT:

Table no. 4: Solubility data of Neomycin Sulphate

Solubility studies of Neomycin sulphate with different solvents

- Neomycin sulphate

s.no.	solvents	Solubility (mg/ml)	inference
1	DM water	0.0795	Slightly soluble
2	PBS (PH 7.4)	0.0456	Slightly soluble

ISSN: 2519-9889
Impact Factor: 5.721

2.1.5 Drug and Excipient Interaction Studies:

The Equal portion of Drug and Excipient (1:1 ratio) is added in Ampules and the Ampules are placed in Stability Chamber for one Weak, After One Weak the Drug Excipient Compatibility Study is Determine by using TLC (Thin Layer Chromatography). Different plates of silica gel was made and activated at 80℃ and hot air oven cool plates mark and pore 2 different sports with the help of capillary one is test solution and another is reference solution. Kept in mobile phase (Toluene : Ethyl acetate : glacial acetic acid) in ratio of (20:10:0.2) after some time remove plates from mobile phase and spray ninhydrin reagents over the plates observe and compare under UV chamber.[7,8]

RESULT:

Table 5: Drug and Excipient interaction studies

- NEOMYCIN SULPHATE

s.no.	Drug – Excipient	Initial physical state	observation (In days)	
			1st	7th
1	NMS	OWP	N	N
2	NMS +CARBOPOL 940	OWP	N	N
3	NMS + BKC	OWP	N	N

- NMS – Neomycin sulphate
- OWP – off white powder
- N - No change in color and physical state

The different formulation Excipients, drug and their physical mixtures were found to be stable under refrigerated condition, room temperature. As there was no change physical characteristics. Hence it was inferred that the selected excipients are compatible with drug.

3. Formulation

Selection of Excipient for Ocular *In Situ* Gel

Table 6: List of excipient used in *in situ* gel

S.NO.	NAME OF EXCIPIENT	USE
1	Neomycin Sulphate	Aminoglycoside Antibiotic
3	Carbopol 940	Gelling Agent , Emulsifying agent
4	Benzolkonium Chloride	Preservatives

ISSN: 2519-9889
Impact Factor: 5.721

3.1 Procedure of Ocular *In Situ* Gel

1. Require quantity of carbopol 940 was dissolved in dm water with the help of magnetic strirrer. carbopol 940 was sprinkled and allow to hydrate overnight the solution was stirred under an overhead strirrer.
2. Then add preservatives of benzolkonium chloride was added slowly slowly with the help of magnetic stirrer.
3. Drug was dissolve in dm water and added to the polymer solution purified water was added to make up the volume 100 ml.

Formulation of ocular *in situ* gel:

Table 7: Composition of ocular *in situ* gel

S.NO.	INGREDIENTS	F1	F2	F3	F4
1	Neomycin Sulphate	50 mg	50 mg	50 mg	50 mg
3	Carbopol 940	0.5 gm	0.75 gm	1.0 gm	0.5 gm
4	Benzolkonium chloride	0.1 ml	0.1 ml	0.1 ml	0.1 ml
5	DM-water	10 ml	10 ml	10 ml	10ml

4. Evaluation of Formulation

Physico-chemical characterization

Visual inspection and clarity: Visual appearance and clarity was tested under fluorescent light against a white and black back ground for presence of any particulate matter .[29]

pH determination: pH of the *in-situ* gels after addition of all ingredients were measured using digital pH meter[9]

RESULT:

Table 8: Physico-chemical characterization

s.no.	Formulation	Clarity	pH
1	F1	CLEAR	7.4
2	F2	CLEAR	7.4
3	F3	CLEAR	7.4
4	F4	CLEAR	7.4

ISSN: 2519-9889
Impact Factor: 5.721

Drug content: Drug content was determined by taking 1ml formulation and diluting it to 100ml dm water then 5ml solution was withdrawn and diluted 25ml with distilled water Neomycin sulphate concentration at 304nm by UV visible spectrophotometer [10]

RESULT:

Table 9: Physico-chemical characterization

s.no.	Formulation	Concentration of Neomycin Sulphate
1	F1	94.06 +/- 1.36
2	F2	92.66 +/- 0.84
3	F3	94.16 +/- 0.79
4	F4	92.53 +/- 2.10

The result of drug content are (shown in table). The *in situ* gel formulations with different batches (F1-F4) show good drug content are F3 batch then rest formulations

Gelling capacity

All prepared formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as in situ gelling systems. The gelling capacity was determined by placing a drop of the formulation in a vial containing 2 ml of artificial tear fluid freshly prepared (pH 7.4) and equilibrated at 37 °C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.[11]

Gelling capacity parameter:

parameter	Gelling capacity
+	Gelation within 50-60 second
++	Gelation within 60 second for 3hours
+++	Gelation within 60 second for 6 hours

RESULT:

Table 9 Gelling capacity parameter

s.no.	Formulation	Gelling Capacity
1	F1	++
2	F2	+
3	F3	++
4	F4	+

ISSN: 2519-9889
Impact Factor: 5.721

Composition of artificial tear fluid:

Table 10: Composition of tear fluid[2]

S.NO.	COMPOSITION
1	Sodium chloride
2	Sodium bicarbonate
3	Calcium chloride dihydrate
4	water

Sterility studies

Sterility: ophthalmic preparations must be sterile when prepared. Gram negative and Gram positive both bacteria may cause serious infection of cornea. It can cause complete loss of eye sight in 24-48 hrs. To maintain sterility in multi dose container, containing ophthalmic products, a suitable preservative is added. The preservative should be non-irritant and should be compatible with medicaments. Here we added boric acid used as preservative.

Procedure of Sterility:

The ophthalmic products are generally sterilized by autoclaving. Filtration through bacteria proof filters and addition of bacteria at low temperature. All formulation might pass through 0.45 micron filter with the help of vacuum pump. And sterility test performed in sterilized plate (5 plate) containing nutrient media (agar). We took sterile swab containing formulations. This sterile swab spreaded all over the agar plates. The test performed in sterile environment or under the laminar air flow. These plates kept in biological Oxygen Demand (BOD) incubator in 37+/-5c for 24-28 hr. After 24 hr. check the possible growth of microorganism. If growth occurred then sterility is negative. The preparation is contaminated. If there we no growth then results were positive and preparation were sterile.

Sterility testing should be carried out under strict conditions specifically designed to avoid microbial contamination of the material being tested.

Evaluated methods

In order to suggest methodologies for screening the antimicrobial activity, two different qualitative methods were evaluated as follows: agar diffusion test employing two different types of reservoirs (filter paper disc impregnated with compound test and wells in dishes). Besides we discussed the aspects of the micro dilution method used for the determination of minimum inhibitory concentration (MIC).

ISSN: 2519-9889
Impact Factor: 5.721

Agar diffusion well-variant

The bacteria inoculum was uniformly spread using sterile cotton swab on a sterile petri dish MH agar. Nine serial dilutions yielded concentration of 100, 80, 60, 40, 20, 10, 5, 2.5, and 1.25mg/ml for extracts and fractions and four serial dilutions yielded concentrations of 20, 15, 10, e 5 mg/ml for pure substances. 50µl were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The system were incubated for 24 h at 36C +/- 1C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in m.

Direct inoculation

Preparation should be examined during usage. Sterile media was pipette out by sterile pipette and with sterile syringe then aseptically transferred the specified volume of materials to a vessel and inoculate the media and incubated for 7 days**.**

5. Conclusion

Neomycin sulphate is a broad spectrum of aminoglycosides antibiotic, antibacterial agent used for treatment of ocular eye infection. It was formulated as ocular in situ gel forming eye drop are using polymers such as carbopol 940 as a gelling agent in combination of suitable preservatives. The characterization of drug sample was done using spectrophotometric analysis and melting point determination. All the observations and recorded data were identical to the values reported in literature. Calibration curves of Neomycin sulphate in distilled water and phosphate buffer (pH 7.4) were prepared using a double beam UV-visible spectrophotometer (Shimadzu 1800) It is a newer approaches to improve easy to eye instillation residence time and enhance bioavailability, prolonged and sustained drug release. Also important in the ease of administration afforded and the decrease frequency of administration resulting in the better patient compliance and acceptance. Therefore, thought worthwhile to develop an in-situ gel formulation using a suitable phase transition polymer to effectively deliver the drug into eyes with sustained and prolonged release and enhanced drug bioavailability. It is observed from the formulation F3 which shown drug content 94.16 % and have good gelling capacity. Thus, it can be concluded that the drug given in the form of ocular in situ gel of Neomycin sulfate providing better patient compliance and an effective mode of treatment.

Acknowledgement:

The author is thankful to the management of Swami Vivekanand College of Pharmacy, Indore. For providing necessary facilities to carry out the research work and heartily thankful to my Co-Guide and guide for providing all the support and encouragement to carry out this studies.

ISSN: 2519-9889
Impact Factor: 5.721

References

[1]. Patel. H. A, Dave, M.R. formulation and evaluation of sustained release in situ ophthalmic gel of neomycin sulphate . BPR (2015), 5(1); 1-5.
[2]. Dabir. Dpriyanka, shahi. S.R, deore. Swati v. ophthalmic in situ gel: a review. EJPMR (2016), 3(6); 205-215.
[3]. Savale KS. Formulation and evaluation of diclofenac sodium sustained released tablet. AJPAMC (2015), 3(4): 214-224.
[4]. Sravani D. formulation and evaluation of ocular in situ gel of acyclovir. IJRPC (2017), 7(2): 162-170.
[5]. Mandal S, Thimmasetty MK, Prabhushankar G, Geetha M. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. Int. J. Pharm. Investig. 2012;2(2):78-82.
[6]. Mishra DK, Jain DK. Formulation and evaluation of valsartan sustained release matrix tablets. Bull. Pharm. Res. 2014;4(2):81-5.
[7]. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. Int. J. Pharm. 2001;229(1-2):29-36.
[8]. Mohanambal E, Arun K, Abdul HasanSathali A. Formulation and evaluation of pH triggered in situ gelling system of levofloxacin. Indian J. Pharm. Educ. Res. 2011;45(1): 58-64.
[9]. Nagpal N, Arora M, Rahar S, Rageeb M, Swami G. Formulation and evaluation of sustained release floating microballoons of ketorolac trometamol. Bull. Pharm. Res. 2014;4(2):86-93.
[10]. Qi H, Chen W, Huang C, Li L, Chen C, Li W, Wu C. Development of a poloxamer analogs/carbopol-based in situ gelling and mucoadhesive ophthalmic delivery system for puerarin. Int. J. Pharm. 2007; 337(1-2):178-87.
[11]. Robinson JC. Ocular anatomy and physiology relevant to ocular drug delivery, In: A. K. Mitra (ed.). Ophthalmic Drug Delivery Systems, Marcel Dekker: New York, 1993; 29-57.
[12]. Sasaki H, Yamamura K, Nishida K, Nakamura J, Ichikawa M. Delivery of drugs to the eye by topical application. Prog. Ret. Eye Res. 1996;15(2):583-620.
[13]. Tinu T S, Thomas L, Kumar A. (polymers used in ophthalmic in situ gelling system). International Journal Of Pharmaceutical Sciences Review And Research, 2013; 20(1): 176-83.
[14]. Rajoria G, Gupta A. (In-Situ Gelling System : A Novel Approach for Ocular Drug Delivery).Am. J. PharmTech Res., 2012; 2(4): 25.

