

## PREFORMULATION STUDIES OF PILOCARPINE HYDROCHLORIDE AS NIOSOMAL GELS FOR OCULAR DRUG DELIVERY

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### ABSTRACT

**Objective:** Pilocarpine hydrochloride is a direct acting cholinergic parasympathomimetic agent which directly stimulates cholinergic receptor ( $M_3$ ). It is a drug used in the treatment of chronic open-angle glaucoma for over 100 years. Designing a new ophthalmic dosage form for pilocarpine hydrochloride compels performing preformulation studies for drug. Therefore, the current aim of the study was to investigate some of the important physicochemical properties of pilocarpine hydrochloride which can help to select subsequent approaches during the development of niosomal gel for ocular use.

**Methods:** Preformulation studies of drug were carried out for identification (physical appearance, melting point, and UV spectrophotometric analysis), solubility profile, lipophilicity (partition coefficient), compatibility studies by Fourier-transform infrared (FTIR) spectroscopy, and thermal behavior by differential scanning calorimetry (DSC).

**Results:** The melting point of pilocarpine hydrochloride was found to be  $204 \pm 3^\circ\text{C}$ . The log P value was found to be  $1.12 \pm 0.02$ , from which it can be interpreted that drug is highly hydrophilic in nature. The scanned  $\lambda_{\text{max}}$  was found to be 215 nm. No significant changes were found when FTIR spectra of physical mixture compared with FTIR spectra of pure drug and excipients. This indicates absence of any possible interaction between the drug and excipients which confirms the identity and purity of drug. DSC thermogram of pure drug showed a sharp exothermic peak at  $191.923^\circ\text{C}$  (area=68.890 mJ,  $\Delta H = 22.963 \text{ J/g}$ ), indicating the crystal melting point of the drug.

**Conclusion:** These results suggest that the pilocarpine hydrochloride serves as suitable candidate for ocular drug delivery system.

**Keywords:** Pilocarpine hydrochloride, Preformulation, Ocular delivery, Spectrometric analysis, Compatibility.

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### INTRODUCTION

Preformulation study is an important tool for the determination of physical and chemical properties of the drug before the development of dosage form. The nature of the drug highly affects the processing parameters such as method of preparation, entrapment efficiency, compatibility, and pharmacokinetic response of the formulation. Preformulation studies are indispensable protocol for the development of safe, effective, and stable dosage form. Thus, to ensure optimum condition for clinically beneficial delivery system, preformulation studies were carried out. A thorough understanding of these properties ultimately provides a rational for formulation design. Characterization of drug and drug-excipient compatibility studies was done in this phase to provide a useful support in the development of dosage form [1].

Pilocarpine hydrochloride is a drug used in the treatment of chronic open-angle glaucoma for over 100 years [2]. It is a parasympathomimetic alkaloid obtained from the leaves of tropical South American shrubs from the genus *Pilocarpus* [3]. It is a non-selective muscarinic receptor agonist which acts therapeutically at the muscarinic acetylcholine receptor  $M_3$ , found on the iris sphincter muscle, causing the muscle to contract resulting in pupil constriction (miosis). Pilocarpine hydrochloride also acts on the ciliary muscle and causes it to contract. When the ciliary muscle contracts, it opens the trabecular meshwork through increased tension on the scleral spur. This action facilitates the rate that aqueous humor leaves the eye to decrease in intraocular pressure, which ultimately helpful in treating open-angle glaucoma [4].

The major drawbacks associated with pilocarpine HCl, administered as an eye drop, were its low ocular bioavailability (1–3%) and short precorneal residence time. Niosomes, administered as an

ophthalmic gel, containing bioadhesive polymer (locust bean gum and Carbopol 934), are capable of localizing and maintaining drug activity at its site of action with prolonged precorneal residence time and improved bioavailability [6].

Therefore, the current aim of the study was to investigate some of the important physicochemical properties of pilocarpine HCl which can help to select subsequent approaches during the development of niosomal gel for ocular use.

Preformulation studies were carried out for identification (physical appearance, melting point, and UV spectrophotometric analysis), solubility profile, lipophilicity (n-octanol-water partition-coefficient determination), spectrometric fingerprints, and compatibility studies by Fourier transform infrared (FTIR) and thermal behavior analysis by DSC. The use of preformulation parameter maximizes the chances of getting a formulation which is safe, efficacious, and stable product and at the same time provides optimization of the drug product quality [7].

### Characterization of pilocarpine HCl

#### Organoleptic properties

Organoleptic properties of the pilocarpine HCl were characterized on the basis of appearance, color, odor, and taste by visual inspection.

#### Melting point determination

The melting point of a drug was determined using digital melting point apparatus (DB- 31354, Decibels Instruments, Perfit, India). In this method, a tiny amount of drug was introduced into a small capillary tube, attaching this to the stem of a thermometer centered in a heating bath, heating the bath slowly, and observing the temperatures at

which melting of drug begins and is completed. The melting point was recorded and compared with literature value.

### Determination of solubility

#### Qualitative solubility

Qualitative solubility of pilocarpine HCl in different solvents was determined according to USP NF, 2007 [8]. Pilocarpine HCl (1 mg) was accurately weighed and transferred into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each) such as distilled water, phosphate buffer saline (pH 7.4), methanol, ethanol, acetic acid, acetic anhydride, and diethyl ether. The solubility (mg/ml) was observed by visual inspection and compared with that available in literature.

#### Quantitative solubility

Quantitative solubility analysis of drug was done by taking 5 ml of each solvent and drug in gm(s) into the solvent till saturation of solvent. Solutions were filtered and absorbance was recorded using UV spectrophotometer and the concentration of drug dissolved in respective solvents was calculated [9]. Different solvents such as distilled water, phosphate buffer saline (pH 7.4), and simulated tear fluid pH 7.4 were used for the solubility determination. This is done to determine the capacity of the solvent for dissolving the drug in it.

### Lipophilicity (partition coefficient)

The partition coefficient of a chemical compound provides a thermodynamic measure of its hydrophilicity-lipophilicity balance. The partition coefficient of a substance between n-octanol and water is referred to as  $\log P_{o/w}$ , which corresponds to the negative logarithm of the ratio of the concentration of the substance in the aqueous and hydrophobic phases [10]. The partition coefficient of pilocarpine HCl was carried out in water: octanol (1:1) using shake flask procedure.

### METHODS

Before a partition coefficient is determined, the phases of the solvent system were mutually saturated by shaking at the temperature of the experiment. To do this, high purity analytical grade n-octanol and water were taken into a separating funnel in 1:1 ratio. Then, separating funnel was shaken for 30 min to allow complete mixing and then the funnel was allowed to stand for 24 h to develop two phases which were saturated with each other after that the drug in minimum quantity (not more than 0.01 mol/liter) was added to one of the phase and the funnel was again shaken for 30 min and then allow to stand for 1 h after that the amount of drug in both phases (n-octanol and water) was determined spectrophotometrically.

The partition coefficient is a ratio of concentrations of unionized compound between the two solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is adjusted such that the predominant form of the compound is unionized. The logarithm of the ratio of the concentrations of unionized solute in the solvents is called  $\log P$  [11,12].

$$\log P_{\text{oct/wat}} = \log \left( \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}^{\text{un-ionized}}} \right)$$

### UV-visible spectrophotometric analysis

#### Determination of $\lambda_{\text{max}}$ of pilocarpine HCl in phosphate buffer solution (PBS) (pH 7.4)

A standard stock solution of pilocarpine HCl was prepared by dissolving 100 mg of drug in a 100 ml volumetric flask and the volume was made up to 100 ml using PBS of pH 7.4 to get the concentration of 1000  $\mu\text{g/ml}$  of standard pilocarpine HCl. From the standard stock solution, 10 ml was pipette out into 100 ml volumetric flask and the volume was made up to 100 ml with PBS of pH 7.4 to get the concentration of 100  $\mu\text{g/ml}$ . From this solution, 1 ml was pipette out into 10 ml volumetric flask and the volume was made up to 10 ml with PBS of pH 7.4 to get the concentration of 10  $\mu\text{g/ml}$ . Maximum wavelength ( $\lambda_{\text{max}}$ ) was obtained by scanning the resulting solution (14  $\mu\text{g/ml}$ ) in the wavelength region

between 200 nm and 400 nm using UV-visible spectrophotometer (UV1700 PharmaSpec, Shimadzu, Japan).

### Preparation of standard curve of pilocarpine HCl in PBS of pH 7.4

From the above prepared stock solution, five dilutions were made using PBS of pH 7.4 which has ultimate concentration 12  $\mu\text{g/ml}$ , 14  $\mu\text{g/ml}$ , 16  $\mu\text{g/ml}$ , 18  $\mu\text{g/ml}$ , and 20  $\mu\text{g/ml}$ . Then, check the pH of the diluted solutions to confirm that the diluted solutions were ranges in the pH of 7.4. The absorbance was measured at  $\lambda_{\text{max}} \times 215$  nm using UV-visible spectrophotometer.

### FTIR spectroscopy

FTIR spectra of the pure drug were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). Sample was ground thoroughly with KBr powder in mortar and pestle, in a weight ratio of 1:100 and then pressed the mixture in dies set in pellet press under a hydraulic pressure of 15 tons for a minute. Release the pressure by rotating the side valve in anticlockwise direction to take of the pellet from the dies set. Then, the pellet was placed in the sample holder and spectral scanning was taken in the wavelength region between 4000 and 400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  and scan speed of 2  $\text{mm/s}$  [13].

### Drug excipient compatibility screening by FTIR

FTIR spectra of locust bean gum, Carbopol 934, and a physical mixture of locust bean gum: Carbopol 934:pilocarpine HCl in a weight ratio of 1:1:1 were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). Each sample was ground thoroughly with KBr powder in a weight ratio of 1:100 and then pellets were prepared using a hydraulic pellet press under a hydraulic pressure of 15 tons for a minute. Then, the pellet was placed in the sample holder and spectral scanning was taken in the wavelength region between 4000 and 400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  and scan speed of 2  $\text{mm/s}$ . IR spectra of the physical mixture were then compared with the IR spectra of pure drug and polymer to find out the evidence of any compatibility [14]. Fig. 8.

### Differential scanning calorimetry (DSC)

DSC analysis was performed on the pure drug using PerkinElmer instrument (Pyris-1, Osaka, Japan), available at the Department of Textile Technology, Indian Institute of Technology, New Delhi, India. Initially, the moisture was removed by heating the samples, and then, each sample (about 3–7 mg) was accurately weighed into platinum crucible 40  $\mu\text{l}$  aluminum pan in hermetically sealed condition, where alpha alumina powder used as a reference. Thermograms were recorded from 50°C to 300°C at the heating rate of 20°C/min under a constant flow of an inert nitrogen gas atmosphere with the flow rate of 20  $\text{ml/min}$  [15]. The DSC spectra used to find out the exotherm peak position or any change in their position compared with the standard spectra.

## RESULTS AND DISCUSSION

### Characterization of drug

#### Organoleptic properties

Organoleptic properties of the drug sample were found to be as given in Table 1. The physical properties were found as similar as reported in literature that proves the identity of drug.

#### Melting point determination

The melting point of drug was determined in triplicate and their mean values with standard deviation are shown in Table 2. The melting point of pilocarpine HCl was found to be  $204 \pm 3^\circ\text{C}$ , which corresponds to the literature value of 204–205°C that proves the identity and purity of drug.

**Table 1: Organoleptic properties of pilocarpine HCl**

Organoleptic properties	Results
Physical form	Slightly hygroscopic crystalline powder
Color	White to off-white
Odor	Odorless
Taste	Tasteless

### Determination of solubility

#### Qualitative solubility

The qualitative solubility data of pilocarpine HCl in different solvents at room temperature are shown in Table 3.

#### Qualitative solubility

Results of quantitative solubility data of the drug in different solvents at room temperature are given in Table 4.

These results indicated that the available pilocarpine HCl form is freely soluble in water and there is no noticeable difference between the solubility of the pilocarpine HCl form used and the solubility of the reference pilocarpine HCl.

#### Partition coefficient

The log P value of drug was determined in triplicate and their mean values with standard deviation are shown in Table 5. The log P value was found to be  $1.12 \pm 0.02$  and reported value was 1.15 from which it can be interpreted that drug is highly hydrophilic in nature. Hence, the

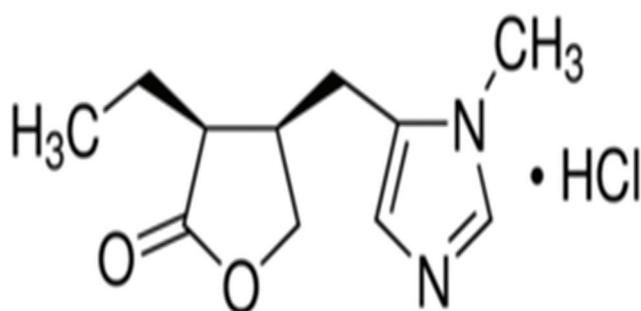


Fig. 1: Chemical structure of pilocarpine HCl [5]

Table 2: Melting point of pilocarpine HCl

S. No.	Melting point (°C)	Mean±S.D. (°C)
1.	205	204±3°C
2.	203	
3.	204	

Table 3: Qualitative solubility of drug in different solvents at 37°C

S. No.	Solvent (1 ml)	Solubility of the drug (1 mg)
1.	Distilled water	Freely soluble
2.	Phosphate buffer saline pH 7.4	Freely soluble
3.	Methanol	Freely soluble
4.	Ethanol	Freely soluble
5.	Acetic acid	Freely soluble
6.	Acetic anhydride	Sparingly soluble
7.	Diethyl ether	Insoluble

Table 4: Quantitative solubility of drug in different solvents at 37°C

S. No.	Solvent	Concentration of drug in solvent (mg/ml)
1.	Distilled water	2.02
2.	PBS pH 7.4	2.13

Table 5: Partition coefficient of pilocarpine HCl

S. No.	Log p-value	Mean±SD
1.	1.12	1.12±0.02
2.	1.13	
3.	1.11	

corneal epithelium is expected to be the rate-limiting barrier for ocular absorption [16]. This is an incentive to consider niosomes (surfactant/lipid-based system) for the ocular delivery of pilocarpine HCl.

### Standard curve of pilocarpine HCl

#### Determination of $\lambda_{max}$ of pilocarpine HCl in PBS (pH 7.4)

UV spectrophotometric study was carried out to determine the  $\lambda_{max}$  of pilocarpine HCl in PBS of pH 7.4.  $\lambda_{max}$  of pilocarpine HCl was found to be 215 nm, as shown in Table 6. Fig. 2 shows the peak at 215 nm of pilocarpine HCl in PBS of pH 7.4. The scanned  $\lambda_{max}$  was found to be similar as that of reported  $\lambda_{max}$  (215 nm).

#### Preparation of standard curve of pilocarpine HCl in PBS of pH 7.4

The concentration and absorbance data of pilocarpine HCl in PBS of pH 7.4 are given in Table 7. This absorbance was plotted on Y-axis against concentration on X-axis and slope of the standard curve was obtained that is shown in Fig. 3. The slope and intercept were found to be 0.0213 and 0.0016, respectively, as shown in Fig. 4.

### FTIR spectroscopy

FTIR spectra of the pure drug were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan) and are presented in Fig. 5. The interpretation of FTIR spectra of pilocarpine HCl is shown in Table 8. Pilocarpine HCl showed the principle IR peaks at  $3217.10 \text{ cm}^{-1}$  resulted from N-H stretching, the peak at  $1764.75 \text{ cm}^{-1}$  resulted from C=O stretching, the peak at  $1612.38 \text{ cm}^{-1}$  resulted from C=N stretching, the peak at  $1552.59 \text{ cm}^{-1}$  resulted from N-H bending, and the peak around  $3400.27 \text{ cm}^{-1}$ , indicating stretching of hydroxyl group. All the principal peaks of pilocarpine HCl are present in the spectra, which confirm the purity and identity of drug.

### Drug-excipient compatibility screening by FTIR

FTIR spectra of locust bean gum, Carbopol 934, and a physical mixture of locust bean gum: Carbopol 934:pilocarpine HCl in a weight

Table 6: Scanned  $\lambda_{max}$  and absorbance of pilocarpine HCl in PBS (pH 7.4)

S. No.	Strength ( $\mu\text{g/ml}$ )	Scanned $\lambda_{max}$ (nm)	Absorbance
1.	14	215	0.313
2.	14	215	0.180
3.	14	215	0.076

Table 7: Standard curve data of pilocarpine HCl in PBS pH 7.4

S. No.	Concentration ( $\mu\text{g/ml}$ )	Absorbance
1.	0	0.000
2.	12	0.252
3.	14	0.294
4.	16	0.344
5.	18	0.373
6.	20	0.432

Table 8: Interpretation of FTIR spectra of pilocarpine HCl

S. No.	Functional group	Reported frequency ( $\text{cm}^{-1}$ )	Observed frequency ( $\text{cm}^{-1}$ )
1.	N-H stretching	3400-3250	3217.10
2.	C=O stretching	1900-1600	1764.75
3.	C=N stretching	1700-1600	1612.38
4.	N-H bending	1700-1500	1552.59
5.	C-H bend in plane	1500-1300	1483.16
6.	C-C stretching	1200-800	1180.35
7.	N-H rocking	900-700	759.90
8.	C-Cl stretching	800-600	626.82
9.	O-H stretching, H-bonded	3500-3200	3400.27

ratio of 1:1 were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan) and are presented in Figs. 6 and 7, respectively, and the interpretation of FTIR spectra is shown in Table 9-11, respectively.

No significant changes were found when FTIR spectra of physical mixture compared with FTIR spectra of pure drug and excipients. This

indicates the absence of any possible interaction between the drug and excipients.

**Differential scanning calorimetric (DSC)**

DSC thermogram tracings of pilocarpine HCl are shown in Fig. 9. It showed a sharp exothermic peak at 191.923°C (area=68.890 mJ, delta H = 22.963 J/g), indicating the crystal melting point of the drug. This result is in contrary to that of the reference melting point of pilocarpine HCl which is 204–205°C. The marked difference between the observed melting point and the reference one is attributed to crystallization form of the drug.

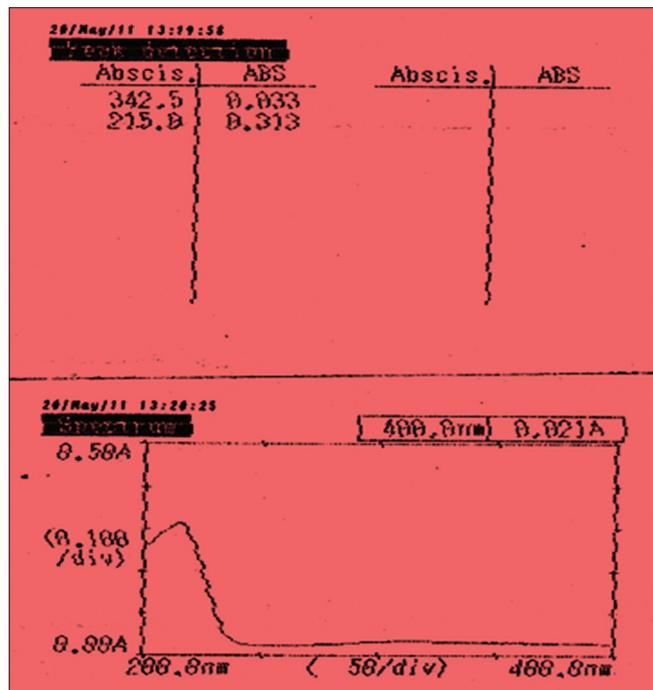


Fig. 2: Peak of pilocarpine HCl in phosphate buffer solution of pH 7.4

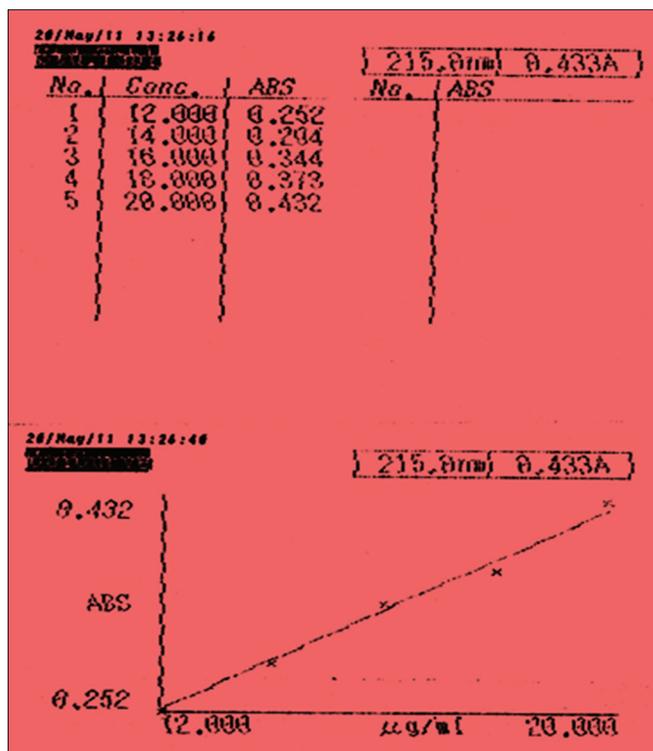


Fig. 3: Standard curve of pilocarpine HCl showing in PBS pH 7.4

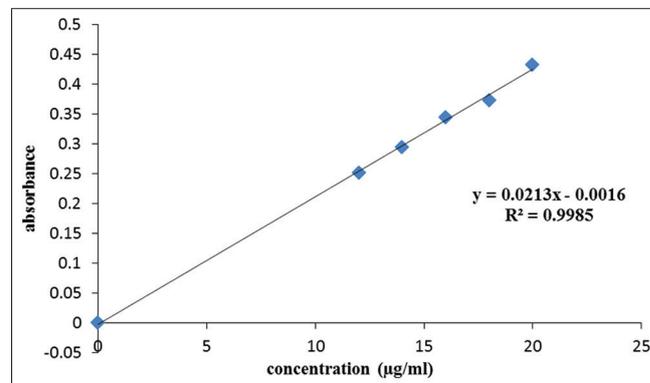


Fig. 4: Standard curve of pilocarpine HCl in PBS pH 7.4

Table 9: Interpretation of Fourier transform infrared spectra of locust bean gum

S. No.	Functional group	Reported frequency (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
1.	O-H stretching, H-bonded	3500-3200	3458.13
2.	C-O-C stretching in ring	1150-1000	1056.92
3.	C-H stretching in ring	3330-3000	3272.98
4.	C-H stretching	3000-2840	2923.88
5.	C-H bending	1470-1450	1440.73

Table 10: Interpretation of Fourier transform infrared spectra of Carbopol 934

S. No.	Functional group	Reported frequency (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
1.	O-H stretching	3640-3610	3640.07
2.	C-C stretching	1300-800	1240.14
3.	C-H stretching	3000-2840	2894.95
4.	C=O stretching	1725-1700	1716.53

Table 11: Interpretation of Fourier transform infrared spectra of physical mixture

S. No.	Functional group	Reported frequency (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
1.	N-H stretching	3400-3250	3217.10
2.	N-H bending	1650-1580	1540.13
3.	N-H wagging	910-665	632.26
4.	C=N stretching	1700-1600	1677.95
5.	C-H stretching	3000-2850	2937.38
6.	C-H bending	1470-1450	1427.23
7.	Aromatic C=C stretching	1675-1650	1637.45
8.	O-H stretching, H-bonded	3500-3200	3400.27
9.	C-O-C stretching in ring	1150-1000	1116.71

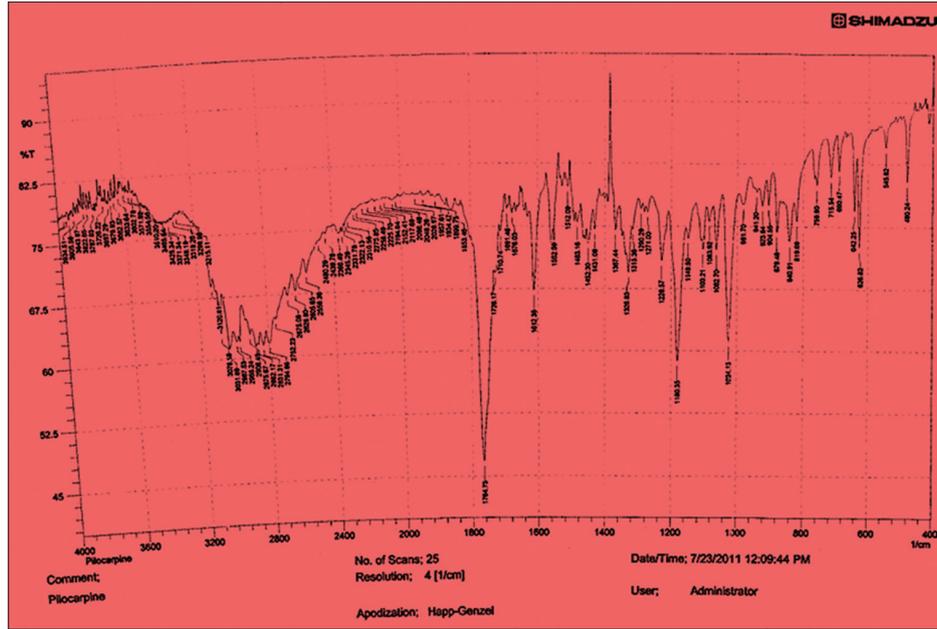


Fig. 5: Fourier transform infrared spectra of pilocarpine HCl

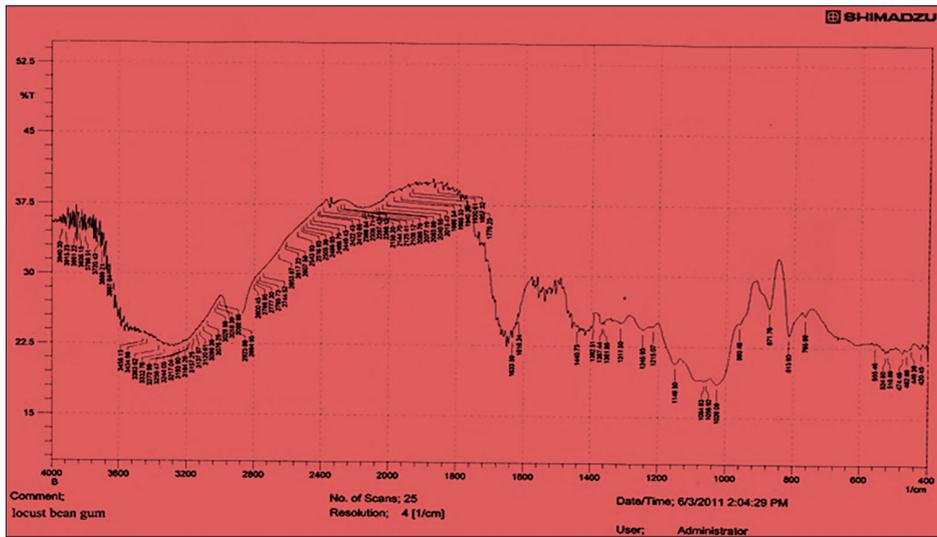


Fig. 6: Fourier transform infrared spectra of locust bean gum

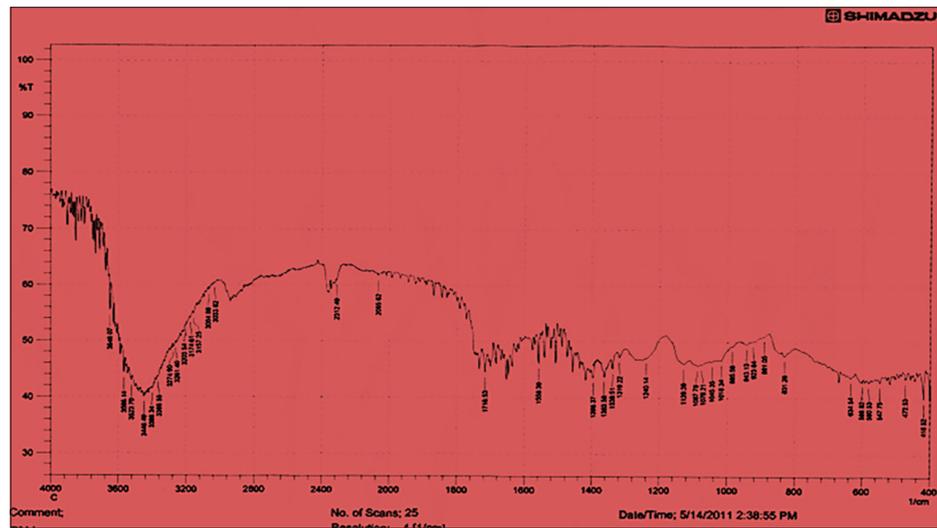


Fig. 7: Fourier transform infrared spectra of Carbopol 934

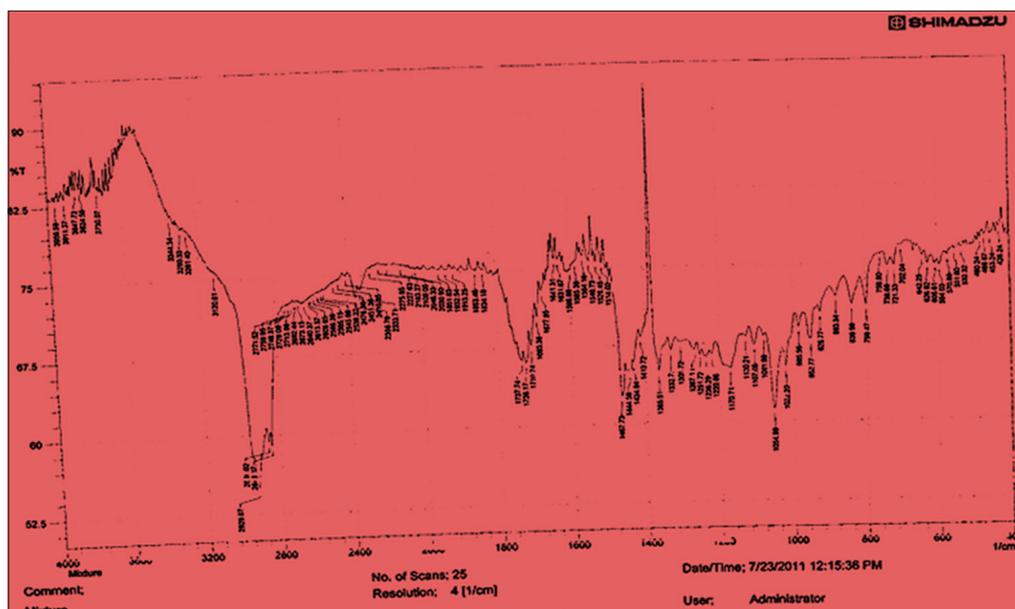


Fig. 8: Fourier transform infrared spectra of physical mixture

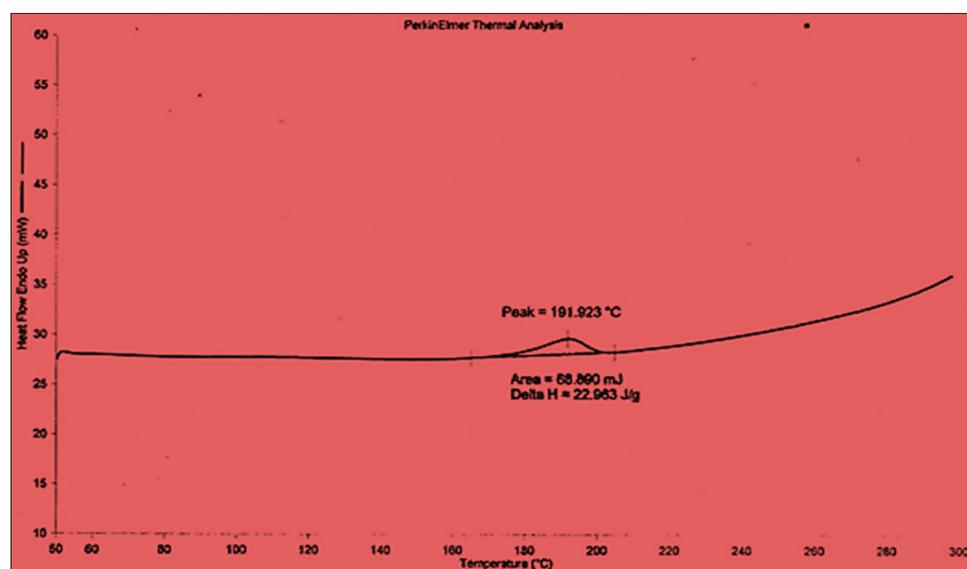


Fig. 9: DSC thermogram tracings of pilocarpine HCl

## CONCLUSION

The preformulation parameter such as melting point and UV spectrophotometric analysis, solubility profile, partition coefficient, spectrometric fingerprints, and compatibility studies by FTIR and thermal behavior analysis by DSC maximizes the chances of getting a formulation which is safe, efficacious, and stable product and at the same time provides optimization of the drug product quality. On the basis of these studies, it was concluded that the pilocarpine HCl serves as suitable candidate for niosomal gel for ocular use.

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## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

## AUTHORS' CONTRIBUTION

Neelam Jain: Concept, design, collection of the data, laboratory investigations, and drafting the final report. Anurag Verma: Supervised the findings of this work and discussed the results and contributed to the final manuscript.

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