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DESIGN AND CHARACTERIZATION OF *IN-SITU* NASAL NANOEMULSION GEL CONTAINING METOCLOPRAMIDE

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ABSTRACT

A nasal *in-situ* nano-emulsion gel formulation was developed using metoclopramide, primarily 7 nano emulsions were prepared with varying ratios of the tween 20 and alcohol mixture, here the oleic acid is the oil phase, and the distilled water is aqueous phase, these preparations are subjected to the high speed homogenization using a hand held homogenizer. The obtained nano-emulsion is transformed into an *in-situ* gel using respective thermo-responsive and mucoadhesive polymers using the cold fusion method. Pre-formulation studies of metoclopramide and evaluation of the formulation were also conducted. In the pre-formulation studies solubility, melting point, absorbance maxima and compatibility study of the drug and excipients is conducted and various evaluation parameters such as pH, viscosity, drug content, drug release study and gelling temperature were studied. FTIR study on the mixture of polymers revealed that there was no interaction between drugs and excipients. All the formulations showed drug content ranged between 65% to 72% and cumulative drug release was ranged between 71.2 to 79.3 %. Among the five formulations F4 was selected as the most suitable candidate because it showed optimum gelation temperature and had an ideal pH and viscosity and showed an enhanced cumulative drug release.

KEYWORDS: *In-situ, Metoclopramide, mucoadhesive, nano-emulsion, thermo-responsive.*

INTRODUCTION

Novel drug delivery system (NDDS) refers to the approach, technologies and system for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects.

Evolution of an existing drug molecule from a conventional form to a novel delivery system can significantly improve its performance in terms of patient compliance, safety and efficacy. In this form of a NDDS an existing drug moiety can get a new life.[1]

Oral route is the most used and preferred route for both local and systemic drug delivery as it is the safest and highly patient compliant route. Even with all these advantages oral delivery of drugs is a challenging task due the highly acidic environment of stomach. To overcome these drawbacks of oral route a new drug delivery system has been proposed. Nasal route avoids the significant side effects and helps to overcome the circumstantial disadvantages of oral route.[2]

Nasal drug delivery system

Nasal delivery is a very dependable route of delivery in the novel drug delivery system. These delivery route bypasses the hepatic metabolism, hence greatly enhancing the bioavailability. The nasal route has large surface area and is highly vascularized, which enables the drugs to be absorbed to the systemic circulation via the venous blood flow.[3] The muco-adhesion in the nasal cavity can be achieved to promote the absorption of drug and also to increase its retention time in the site of absorption. By utilizing the properties of mucoadhesive polymers these can be achieved.[4] And these forms are having many advantages over the conventional system such as quicker onset of action, reduced side effects and reduced frequency of dosing.

All these advantages have made the nasal route an attractive route for developing formulations. The biggest hurdle in this route is mucociliary clearance, to overcome it increasing the residence time of the drug in nasal cavity by using lipidbased drug formulations such as emulsions, nano-emulsions, microemulsions. NE mixtures with nano-metric droplet size between 20 to 200nm which offers several advantages such as high solvent capacity, small particle size and excellent stability.[5] The in-situ forming gel systems are in the trend now, in-situ forming gels are liquid aqueous solutions before the administration and turn to gel under the physiological conditions. There are several

mechanisms by which *in-situ* gels can be achieved, one of them is temperature modulation, the formulation after administration into the nasal cavity, the difference in the temperature enables the formation of gel. The formulation thus elevates the residence time of the active ingredient. Hence the drug gets absorbed into the CNS via the route of olfactory mucosal epithelium. The mucosal interaction and *in-situ* gelling property of the formulation thus enhances the effectiveness and safety of the drug.[6]

Characteristics of an Ideal Nasal *In-situ* Gelling System[7]

Should be safe and not cause irritation or adverse reaction to the nasal mucosa.

Should have precise gelling mechanism in human body temperature.

Should provide controlled and sustained release of the drug.

Should adhere well to the nasal mucosa to prevent premature clearance.

Should be easy to administer via a nasal spray or dropper, should not be difficult for the patients to use.

Should be non-toxic and should not degrade or release harmful substances when in contact with nasal tissues.

The gelled formulation should gradually get cleared from the nasal cavity after the drug has been absorbed.

Thermo-reversible polymers

Thermo-reversibility of some materials is the ability to form gel when conditioned to a change in temperature from aqueous solution. The change in the temperature is achieved by the difference between room temperature and body temperature in human body, thus elevating the retention time of the formulation.[8] This property of some polymers has been used to develop dosage forms such as nasal *in-situ* gels, rectal *in-situ* gels and *in-situ* forming implants. the thermo-gelling property is displayed by the class of amphiphilic block copolymers the gelling property depends upon the molecular weight, chemical composition, polymer concentration. when applied to formulations the temperature responsive nature of polymer can be tuned to induce an *in-situ* gelation.[9]

Nasal *in-situ* gels

Nasal *in-situ* gel dosage forms which gets transformed from sol to gel form which adheres to the nasal cavity and increases retention time and enhances the absorption of the active ingredient into the CNS.

The important property of the nasal in-situ gels, among the various other *in-situ* gels is that increased retention time, which enables the quick access of drug to the CNS, compared to other formulations such as tablets.

The nasal *in-situ* gels have various advantages in treating CNS related problems using those drugs which shows high first pass metabolism or other gastric related problems.[8]

Metoclopramide

Metoclopramide is an agent used to control nausea and vomiting in chemotherapy patients. Metoclopramide is a dopamine receptor antagonist and has been approved by the FDA to treat nausea and vomiting in patients with gastroesophageal reflux disease or diabetic gastroparesis by increasing gastric motility and patients under chemotherapy. Additionally, some FDA unapproved; metoclopramide can be administered prophylactically to prevent nausea and vomiting in postoperative patients when nasogastric suction is contraindicated or unavailable and has shown surprising success in treating migraines, also used to treat hyperemesis gravidarum in pregnant patients, also shown evidence of metoclopramide`s efficacy in treating Diamond Blackfan syndrome.[10]

Metoclopramide is an anti-emetic agent which exerts both central and peripheral actions in the treatment. Centrally its mechanism of action is at the chemoreceptor trigger zone(CTZ) to suppress the vomting reflex by inhibiting 5HT3 and D2 receptor. Receptors are found in the CTZ in the area postrema of the fourth ventricle, this area lies outside the BBB and hence susceptible to drugs. metoclopramide is able to achieve its antiemetic effect specifically by inhibiting the vomiting pathway (CTZ).[11]

The conventional dosage forms of the metoclopramide like tablets are not effective and also have high frequency of administration, and the bio-availability of the drug administered oral dosage forms are not met. Hence to overcome these problems nasal *in-situ* nano-emulsion gel has been considered.

METHODOLOGY

Materials and equipment's

Table No. 01: List of Materials.

Ingredients	Company	Uses
Metoclopramide	Yarrow chem products, Mumbai	Antiemetic Drug
Oleic acid	Loba chemie, Mumbai	Oil phase

Tween 20	Loba chemie, Mumbai	Surfactant
Ethanol	Coating and coating's pvt ltd, Mumbai	Co-surfactant
Chitosan	Yarrow chem products, Mumbai	Mucoadhesive polymer
HPMC (E 15)	Yarrow chem products, Mumbai	Mucoadhesive polymer
Poloxamer 407	Yarrow chem products, Mumbai	Thermo-responsive polymer
Distilled water	Laboratory, Srinivas college ofPharmacy, Mangalore	Solvent

Table No. 02: List of Equipment's.

Equipment's	**Company**
Electronic Balance	Axpert enterprise, Gujarat.
Magnetic stirrer	IKA RH digital and REMI 1MLH,Bengaluru
Digital pH meter	Systronics, Bengaluru.
Handheld Homogenizer	TOUHE handheld homogenizer
Double beam UV-vis spectrophotometer	Jasco V-630, Japan.
Franz Diffusion Cell	Dolphin, Pune.
Fourier transform Infrared Spectroscopy (FTIR)	Jasco FTIR, Japan
Brookfield viscometer	Brookfield engineering laboratories, USA.

Preformulation Studies

In pre-formulation studies, active substances and excipients are assessed to determine how they may influence formulation and process design. A pre-formulation studies enable the development of a suitable formulation based on a foundation of knowledge. In addition, it outlines the nature of the drug substances and provides framework information for combination therapies using pharmaceutical excipients. As a result, pre-formulation studies were conducted on Metoclopramide HCl.[12]

Organoleptic properties

Solubility

Melting point

Organoleptic properties

The color and odor of Metoclopramide was evaluated and recorded using descriptive terminologies.

Solubility

It is determined by dissolving Metoclopramide in water and phosphate buffer pH 6.4 The solubility study was conducted by taking excess quantity of the drug in 10ml of solution. Then the samples were subjected to mixing in a magnetic stirrer for 1 hour at room temperature till

a saturated solution is obtained. The sample was filtered and analyzed spectrophotometrically. The concentration of metoclopramide was determined using respective standard graph.[13], [14]

Melting Point

Determination of melting point of Metoclopramide was carried out by using Capillary method. Melting point was determined by taking small amount of Metoclopramide in a capillary tube closed at one end. The capillary tube was placed in an electrically operated melting point apparatus and the temperature at which the drug melts was recorded. This was repeated three times and average value, and the standard deviation were recorded.[15], [16], [17]

Spectroscopic Studies-Construction of Standard Calibration Curve

Calibration Curve

Preparation of Phosphate Buffer pH 6.4

27.218 g of Disodium hydrogen phosphate was added into 1000ml volumetric flask anddissolved using distilled water. 11.6ml of 0.2M sodium hydroxide solution was added and dissolved. Final solution was made uptown 1000ml with distilled water.

Determination of Absorbance maximum (λ_{max})

Most of the drugs absorb light in UV wavelength region of 200-400nm. Hence the λ_{max} was determined by using UV spectrophotometer. The solution containing 10 µg/ml concentration of Metoclopramide was prepared in phosphate buffer pH 6.4 and scanned over the range of 200- 400nm against phosphate buffer pH 6.4 as blank. The wavelength which gives maximum absorbance was recorded as λ_{max} of the pure drug.[18], [19]

Preparation of stock solution of Metoclopramide using phosphate buffer of pH 6.4[20]

Stock Solution A

100mg of Metoclopramide was accurately weighed and transferred to 100ml volumetric flask. It was then dissolved in small quantity of phosphate buffer pH 6.4 and the volume was made up to 100ml to get a stock solution A (1000 µg/ml)

Stock solution B

5 ml of stock solution A was transferred to volumetric flask and the volume was made up to 100ml with phosphate buffer of pH 6.4 to get a stock solution B (50µg/ml).

Standard Calibration Curve

From the above stock solution 1ml, 2ml, 3ml, 4ml, 5ml and 6ml were diluted upto 10ml using phosphate buffer 6.4 pH to get concentration of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml and 30µg/ml respectively. The absorbance of these solutions was measured at λ_{max} against the reagent blank using UV-Visible spectrophotometer.

Drug-Excipient Compatibility Studies

FTIR absorption spectra of pure drug and physical mixture of drug with polymers were recorded in the range of 4000 to 400 cm^{-1} by using FTIR spectrophotometer. FTIR spectra of physical mixture of drug with all polymers were compared with FTIR spectra of pure drug and peak matching was done to detect any appearance or disappearance of peaks.[21]

Formulation of Nasal *In-situ* Nanoemulsion Gel of Metoclopramide

Step 1: Preparation of Nanoemulsion[5],[22],[23],[24]

The nano-emulsions were prepared by high-speed homogenization method.

Oleic acid (oil phase) was measured and taken in a glass beaker.

⇩

Oil phase added the specified amount of drug (Metoclopramide) while continuously stirring in a magnetic stirrer

⇩

After the drug has been completely solubilized in the oleic acid added the S-mix (surfactant mix) with continuous stirring at 500-600 rpm

⇩

After a clear solution is obtained the mixture is removed from the magnetic stirrer and kept aside for 15 minutes

⇩

This mixture of oleic acid, drug and S-mix Table no:4 is then subjected to high-speed homogenization using a tissue homogenizer

⇩

Distilled water (aqueous phase) is added dropwise to this mixture.

⇩

Homogenization is carried out at 10,000-15,000 rpm for 30 minutes or until the mixture undergoes phase inversion, which is indicated by the conversion of turbid solution to clear.

⇩

The clear solution is allowed to settle overnight to remove any remaining air bubbles.

Composition of Nano-Emulsion

Table no. 03: Composition of Metoclopramide HCl Nano-emulsion.

NE Formulas	MET HCL %W/V	Oleic acid oil %v/v	surfactant	cosurfactant	S-mix ratio	S-mix %v/v	DDW % v/v
F1	0.5	10	Tween20	Ethanol	1:1	60%	30%
F2	0.5	10	Tween20	Ethanol	1:2	60%	30%
F3	0.5	10	Tween20	Ethanol	1:3	60%	30%
F4	0.5	10	Tween20	Ethanol	2:1	60%	30%
F5	0.5	10	Tween20	Ethanol	3:1	60%	30%
F6	0.5	10	Tween20	Ethanol	4:1	60%	30%
F7	0.5	10	Tween20	Ethanol	5:1	60%	30%

Table no. 04: Composition of Nano-emulsion for 50ml of formulation.

NE Formulas	MET HCL (mg)	Oleic acid oil (ml)	Tween20 (ml)	Ethanol (ml)	DDW (ml)
F1	250	5	15	15	15
F2	250	5	10	20	15
F3	250	5	7.5	22.5	15
F4	250	5	20	10	15
F5	250	5	22.5	7.5	15
F6	250	5	24	6	15
F7	250	5	25	5	15

Step-2: Evaluation of Nano Emulsions[25]

The clarity, uniformity and phase separation of the prepared nano-emulsions was evaluated

⇩

Organoleptic analysis was performed for the determination of clarity, uniformity and phase separation

⇩

The most stable nano-emulsion formulations which do not show any changes in the clarity, uniformity and phase separation were selected for the preparation of *in-situ* gels

Step-3: Determination of Optimal Polymer Concentration[26],[27],[28]

The composition of both the mucoadhesive polymers and the thermos-responsive polymer was determined so that the *in-situ* gel preparation gives the optimum mucoadhesive and the thermos-responsive properties.

The varying concentrations of the polymers were prepared and incorporated into the prepared nano-emulsion formulas

⇩

The polymer incorporated nano-emulsions were then subjected to organoleptic analysis for the clarity, uniformity and phase separation.

⇩

Additionally, the gelling temperature of these preparations was also determined using invert test tube method

⇩

The stability of these polymer incorporated nano-emulsions were measured for a period of six days.

⇩

The most stable polymer dispersion with optimal clarity, uniformity and thermo-responsive property was selected for the incorporation into nano-emulsions to prepare *in-situ* gels

Table no. 05: Determination of optimal polymer concentration.

Ingredients	Concentration % w/v				
	IG-1	IG-2	IG-3	IG-4	IG-5
Poloxamer 407	15	10	5	20	12
Chitosan	0.2	0.1	0	0.5	0.1
HPMC E15	0.5	0.5	0.5	1	0.5

The most stable polymer dispersion which satisfies all the conditions like mucoadhesive and thermos-responsive property and uniformity was selected as the optimal polymer concentration for the preparation of nasal *in-situ* gel of metoclopramide HCl.

Step-4: Preparation of Nasal *In-situ* Gel[29]

In-situ gels were prepared by incorporating the mucoadhesive and thermo-responsive polymers into the nano-emulsions by cold fashion method

Specified amount of HPMC E15 and Chitosan were added with constant stirring in a magnetic stirrer at 650 rpm until a clear solution is obtained.

⇩

This mixture is then kept in a refrigerator until it reaches a temperature of 4^{o}C

⇩

To this cold dispersion, poloxamer 407 (thermos-responsive polymer) is added with continuous stirring at 650 rpm

⇩

This dispersion is kept overnight in the refrigerator at 4^{o}C and the final formulation is obtained

Evaluation of Nasal *In-situ* Nano-Emulsion Gel of Metoclopramide HCl

Metoclopramide HCl Nasal *in-situ* gels were evaluated for following properties Physical appearance.[29]

The prepared nasal *in-situ* gel formulations were checked by visual inspection for clarity, uniformity and any forms of phase separation by organoleptic methods.

Determination of pH

The required amount of formulation to be tested was taken in a glass beaker. pH was measured using digital pH meter. The pH was noted after bringing the electrode of the p H meter in contact with the surface of the formulation and allowing equilibration for 1 min. This was repeated three times and average value, standard determination was recorded.[29],[30]

Determination of gelling temperature

The test tube inversion method was employed for the determination of gelation temperature. 2ml of the formulation was placed in a test tube and immersed in a water bath, the temperature was gradually increased, and the gelation temperature was recorded when the formulation stops flowing upon the inversion of the test tube to 90º, this temperature was recorded as gelation temperature. The average of three readings were taken and recorded.[28],[31]

Viscosity

The viscosity of the nasal *in-situ* gels was determined by Brookfield viscometer. Appropriate quantity of the formulations was taken, and the viscosity was measured at 20 rpm for all the five formulations. The pre-gelling viscosity of the nasal *in-situ* nano emulsion gel was determined using spindle no.27 and the post-gelling viscosity was determined using spindle no. 64, and the viscosity was noted.[32], [33]

Determination of Drug content

The measured amount of 1ml of nasal *in-situ* nano emulsion gel was dissolved in 100 ml mixture of phosphate buffer of pH 6.4 and methanol in the ratio 7:3, from this mixture 1 ml was taken and further diluted upto 10ml with phosphate buffer of pH 6.4. The drug content was determined spectrophotometrically using UV visible spectrophotometer at specific wavelength of 273nm, employing the same mixture of phosphate buffer and methanol as blank. The calculation of drug content was performed using the linear regression equation derived from the standard curve in phosphate buffer of pH 6.4.[34]

***In-vitro* drug release studies**

The drug release of the nasal *in-situ* nano-emulsion gel of Metoclopramide HCl was measured using Franz diffusion cell apparatus. Assembly was set and the temperature was maintained at 37±0.5°C. 1ml of formulation was placed over the donor compartment, which was separated by the receptor compartment with the cellophane membrane. 1ml aliquots of samples were withdrawn at regular time intervals 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300, 360 minutes and replaced with an equal volume of phosphate buffer pH 6.4 as fresh receptor medium. The samples were appropriately diluted with Phosphate buffer pH 6.4 and analyzed spectrophotometrically (Double beam UV-visible spectrophotometer) at 273 nm.[34], [35], [36]

RESULTS AND DISCUSSION

Preformulation Studies of Metoclopramide

Firstly, the pre-formulation studies for the drug were carried out, followed by preparation of formulation and their evaluation. The results of the following pre-formulation studies are as follows.

Table no. 06: Pre-formulation studies of Metoclopramide.

PROPERTIES	REPORTED		RESULT
Odor	Odorless		odorless
Melting point	171-173^{o}C		172 °C
Appearance	White crystalline powder		White crystalline powder
Identification	273nm		273nm
Solubility	Water	0.2mg/ml	0.2mg/ml
	Phosphate buffer	1.4mg/ml	1.4mg/ml

Organoleptic Evaluation

Organoleptic properties like physical appearance, color, odor and taste of Metoclopramide were evaluated. It was found that Metoclopramide is a white crystalline powder which was odorless in nature.

Solubility Study

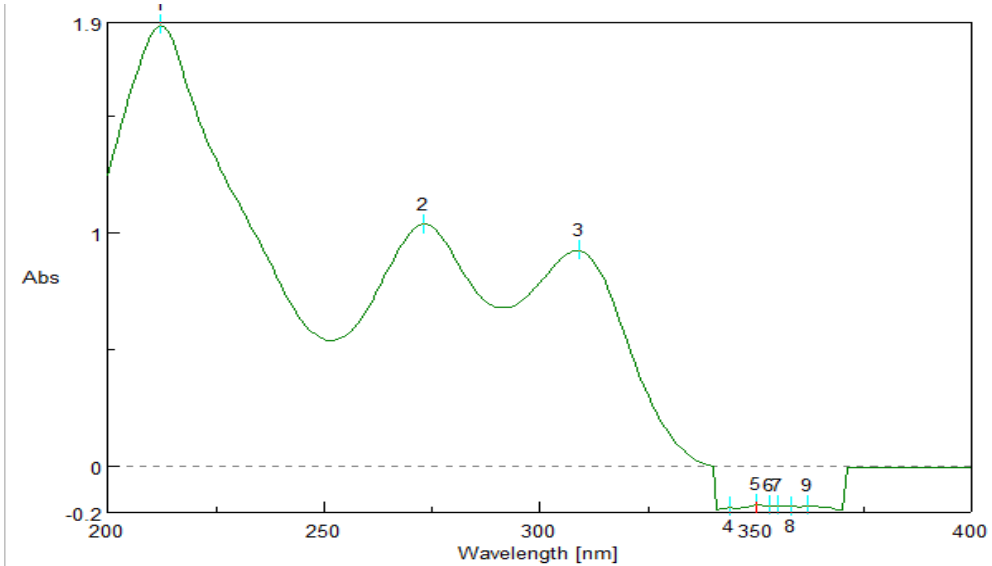
Metoclopramide was found to be poorly soluble in water with solubility of 0.2mg/ml and completely soluble in 6.4 pH phosphate buffer with solubility of 1.4mg/ml.

Melting Point

The melting point of Metoclopramide was carried out and found that the drug melted at 172°C temperature which is in the reported range of 171-173°C and thus was within the reported literature limits, and hence indicates that the drug is pure.

Determination of λ_{max} of Metoclopramide

From the obtained spectrum the absorption maximum of Metoclopramide was found to be 273nm which was selected for the present study. The same wavelength was used for further analysis.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	212	1.8904	2	273	1.03756
3	309	0.922505	4	344	-0.175107
5	350	-0.164326	6	353	-0.167402
7	355	-0.16743	8	358	-0.171472
9	362	-0.168227			

Fig no. 01: Determination of λ_{max} of Metoclopramide.

Standard Curve of Metoclopramide in Phosphate Buffer of ph 6.4

The absorbance values of pure Metoclopramide at of different concentration range (5-30ug/ml) in phosphate buffer (6.4 pH) are scanned at λ_{max} 273 nm. The values of absorbance at different concentrations are given in Table no.07 and the standard plot is shown in Fig-02.

Table no. 07: Standard curve of Metoclopramide in phosphate buffer of pH 6.4.

Sl. No	Concentration(µg/ml)	Absorbance*
1	5	0.179±0.000
2	10	0.396±0.005
3	15	0.566±0.005
4	20	0.730±0.000
5	25	0.893±0.005
6	30	1.036±0.005

(*Data represented as mean ± standard deviation and n=3)

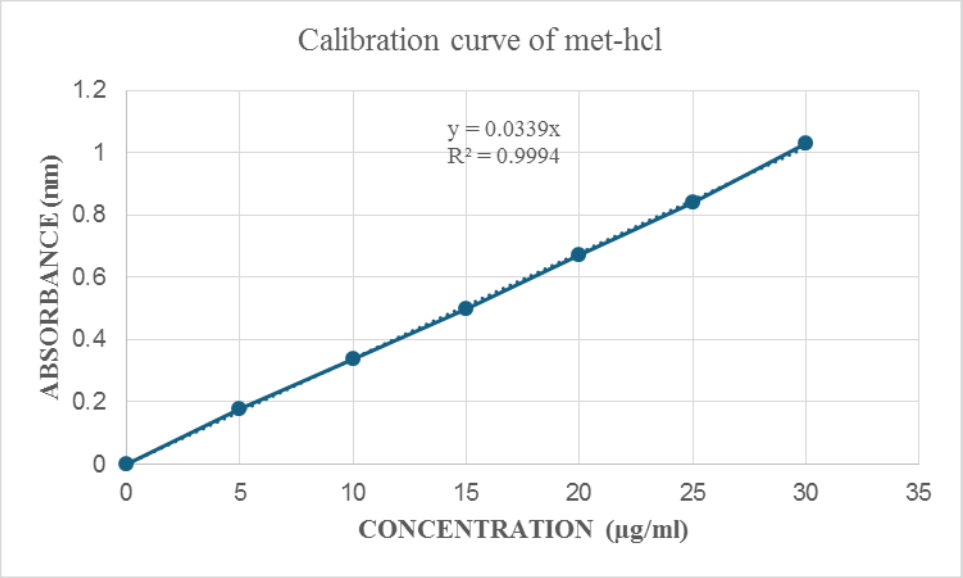


Fig. No. 02: Standard graph of Metoclopramide.

The absorbance value remained linear and obeyed Beer Lamberts law in the range of 5-30µg/ml with the Regression coefficient (R^2) value 0.9998 and slope 0.03.

Drug-Excipient Compatibility Study

Compatibility studies were performed using FTIR spectroscopy. The peaks obtained in the spectra of physical mixture were correlated with peaks of drug spectrum.

FTIR Spectra of Metoclopramide

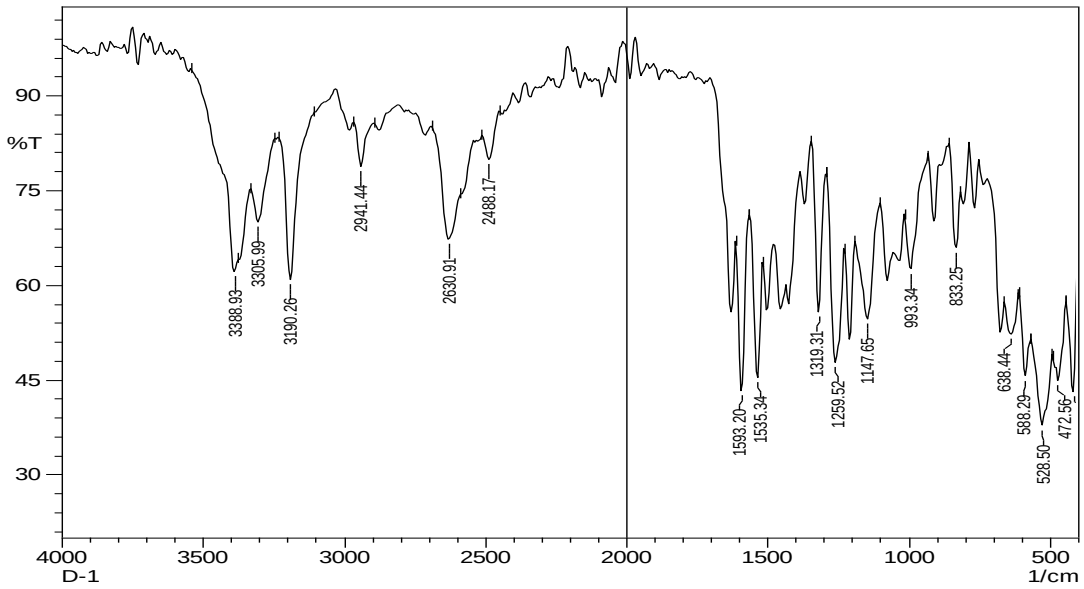


Fig. No. 03: FTIR spectrum of Metoclopramide.

Metoclopramide and HPMC E15

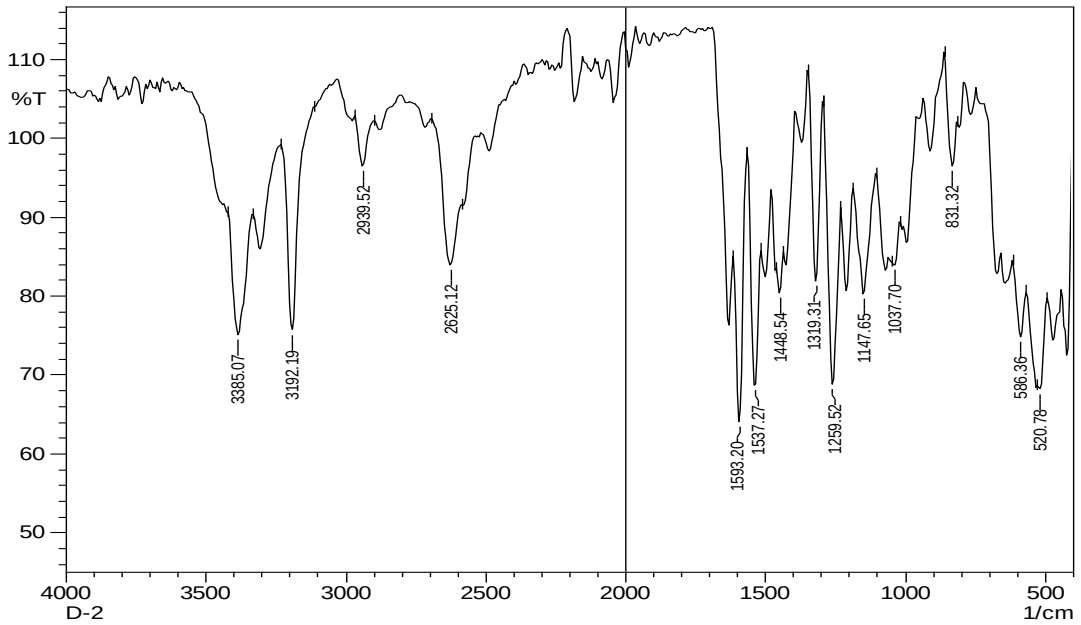


Fig. No. 04: FTIR Spectra of mixture containing Metoclopramide and HPMC E15.

Metoclopramide and Chitosan

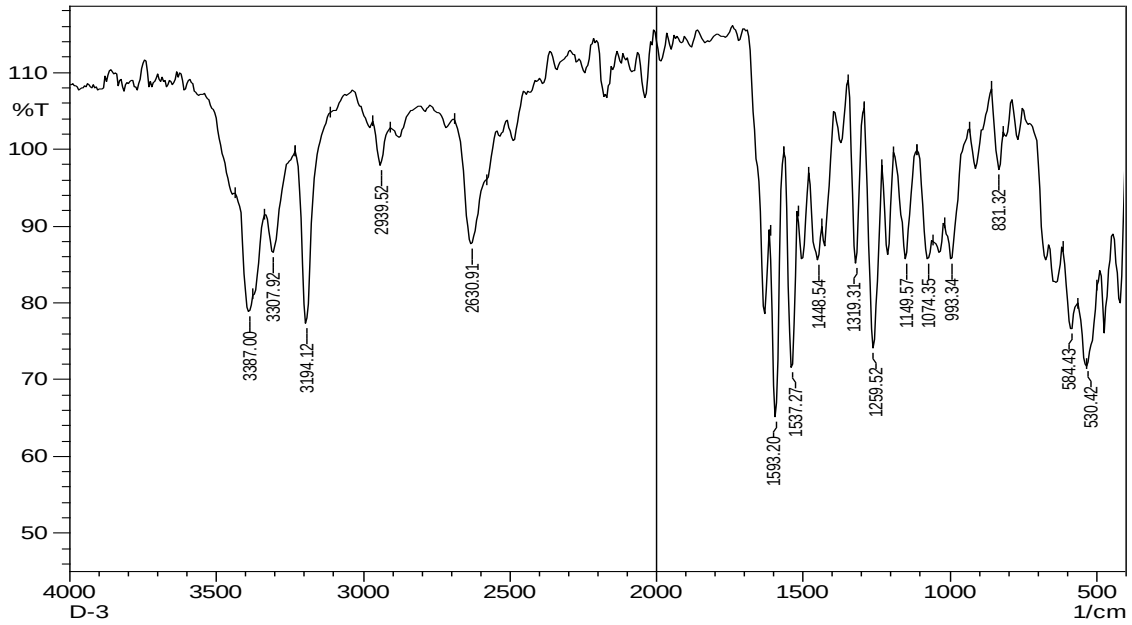


Fig. No. 05: FTIR Spectra of mixture containing Metoclopramide and Chitosan.

Metoclopramide and Poloxamer 407

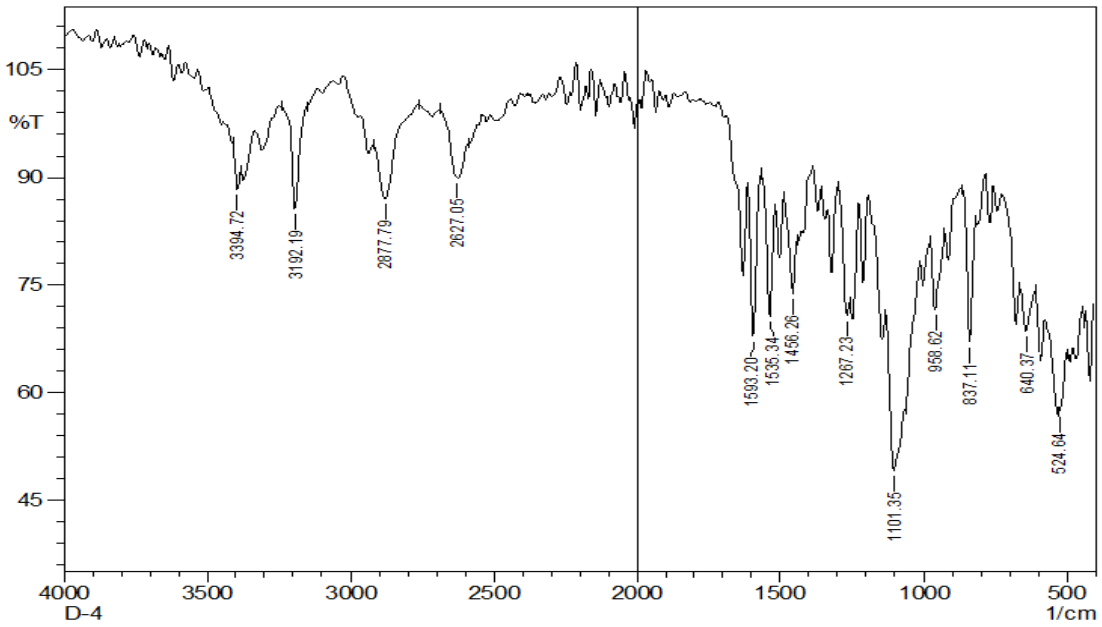


Fig No. 06: FTIR Spectra of mixture containing Metoclopramide and Poloxamer 407.

Metoclopramide, Poloxamer 407, Chitosan and HPMC E15

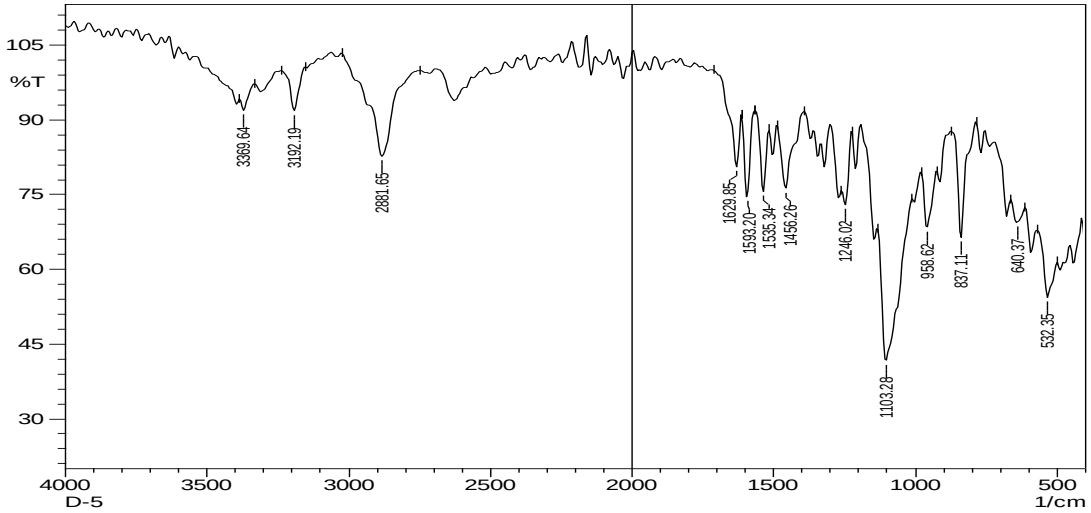


Fig.No.07: FTIR Spectra of mixture containing Metoclopramide, Poloxamer407, Chitosan and HPMC E15.

Table no. 08: Interpretation of FT-IR spectra.

Charecteristic range cm^{-1}	Functional group	Pure drug cm^{-1}	Drug + HPMC E15 cm^{-1}	Drug + Chitosan cm^{-1}	Drug + poloxamer 407 cm^{-1}	Drug + Polymer mixture cm^{-1}
3300-3400	N-H stretching	3388.93	3385.07	3387.00	3394.72	3369.64
2840-3000	C-H stretching	2941.44	2939.52	2939.52	2827.79	2881.66
1566-1650	C=C stretching	1535.34	1537.27	1537.27	1535.34	1593.20
1250-1310	C=O stretching	1259.52	1259.52	1259.52	1267.26	1246.02
550-850	C-Cl stretching	833.25	831.32	831.32	837.11	837.11

The interpretation of FTIR spectra showed that there are no significant interactions between the drug and the polymers that are used in the study, hence the drug and the polymers are compatible with each other.

Evaluation of Nano-emulsions

Clarity and Uniformity of Nano emulsions

The prepared nano-emulsions were evaluated for their clarity, uniformity and any forms of phase separation and it was observed that the formulations F1,F4,F5,F6,F7 shows optimum clarity and uniformity with no phase separation and remains stable, whereas the formulations F2 and F3 shows phase separation, hence the five stable formulations will be considered as best formulations and the polymers will be incorporated and the evaluation will be performed for these five formulation.

Determination of Optimal Polymer Concentration for Nasal *In-situ* Nano-Emulsion Gel

Organoleptic analysis

All the dispersions were subjected for the organoleptic analysis, out of which the dispersion IG-2 produces the clearest dispersion with desired clarity.

Gelling temperature

The gelling temperatures of all the polymer dispersions were determined and given in table no:09, and it is determined that the gelling temperature of polymer dispersion IG-2 is the closest to the body temperature, hence it is considered as the optimal in terms of thermo-responsive property.

Table no: 09: Gelling temperature of polymer dispersions.

Polymer Dispersion	Gelling Temperature ^{o}C*
IG-1	31.0±0.36
IG-2	37.8±0.20
IG-3	45.0±0.15
IG-4	26.0±0.11
IG-5	38.3±0.53

(*Data represented as mean ± standard deviation and n=3)

From the organoleptic and gelling temperature analysis of the polymer dispersions it is determined that the dispersion IG-2 (Table no:10) possess the optimum clarity, uniformity and the thermo-responsive property hence this polymer dispersion will be selected for the incorporation into the selected nano-emulsions to prepare nasal *in-situ* nano-emulsion gel of metoclopramide HCl.

Table no. 10: Composition of IG-2 polymer dispersion.

Ingredients	Composition %w/w
Poloxamer 407	10
Chitosan	0.1
HPMC E15	0.5

Evaluation of Nasal In-situ Nanoemulsion Gel of Metoclopramide HCl

pH

The pH of all the selected formulations were determined and three values were recorded for each formulation and determined their average and standard deviation as shown in Table-11

Table no. 11: Determination of pH of nasal *in-situ* nano-emulsion gel of MET-HCl.

Formulation	pH*
F1	4.82±0.08
F4	4.82±0.08
F5	5.1±0.26
F6	5.24±0.09
F7	5.52±0.05

(*Data represented as mean ± standard deviation and n=3)

The pH of all the formulations were found to be uniform with minimal variation and was compatible to be administered to the nasal cavity and falls within the literature limits.

Gelling temperature

Gelling temperature is the temperature at which liquid phase gets converted into gel phase at a particular temperature. The gelling temperature of all the formulations is given in the Table no:12

Table no. 12: Gelling temperature of nasal *in-situ* gel of MET HCL.

Formulation code	Gelling Temperature ^{O}C*
F1	37.36±0.20
F4	36.85±0.48
F5	36.13±0.25
F6	36.76±0.35
F7	36.23±0.40

(*Data represented as mean ± standard deviation and n=3)

Viscosity

i. Pre-gelling viscosity

The pre-gelling viscosity values of all the formulations was measured given in table no.13.

Table no. 13: pre-gelling viscosity of nasal *in-situ* gel of metoclopramide HCl.

Formulation	Viscosity (cps±SD)*
F1	484.6±5.03
F4	507±2.64
F5	502±3.60
F6	463.67±2.3
F7	414.33±2.08

(*Data represented as mean ± standard deviation and n=3)

It is determined by the present study that the pre-gelling viscosity of all the five formulations are uniform and falls within the literature limits of nasal *in-situ* gel preparations.

ii. Postgelling viscosity

The post gelling viscosity is determined after the formation of gel at a specific temperature, and the viscosity of all the formulations at different rpm is given in table no.14.

Table no. 14: Post-gelling viscosity of nasal i*n-situ* nano-emulsion gel of MET HCl.

Formulation	Viscosity (cps±SD)*
F1	10506.66±5.77
F4	10588.33±.50
F5	10687±4.16
F6	10536.7±4.16
F7	10544.7±1.15

(*Data represented as mean ± standard deviation and n=3)

It is determined by the present study that the post-gelling viscosity of all the five formulations are uniform and falls within the literature limits of nasal *in-situ* gel preparations.

Drug Content

The drug content was determined by measuring the absorbance of all the formulation in a mixture of phosphate buffer pH 6.4 and methanol at 273nm. The absorbance of all the formulations was measured and recorded and the amount was calculated as shown in the table no 15.

Table no. 15: Determination of drug content of nasal *in-situ* nano-emulsion gel of Metoclopramide HCL.

Formulation	Drug content(%)*
F1	68.0±0.01
F4	72.0±0.01
F5	65.0±0.11
F6	67.3±0.04
F7	71.2±0.11

(*Data represented as mean ± standard deviation and n=3)

The drug content in all formulations was evaluated and found to be between 65-72% indicating uniform distribution. F4 exhibited highest drug content.

***In-vitro* drug release studies**

The drug release rate of Metoclopramide was determined by using Franz diffusion cell. The percentage drug release of all the formulations at different time intervals are given in the table no 16.

Table no. 16: *Invitro* drug release studies of nasal *in-situ* nano-emulsion gel of Met HCl.

Time (min)	Cumulative drug release %*				
	F1*	F4*	F5*	F6*	F7*
5	14.8±0.32	15.6±0.1	15.2±0.25	13.6±0.1	14.2±0.3
15	18.6±0.55	20.2±0.11	19.6±0.40	17.5±0.26	17.9±0.45
30	24.2±0.45	26.8±0.2	22.5±0.15	19.5±0.35	19.6±0.75
45	27.3±0.37	28.6±0.17	26.3±0.2	21.4±0.30	22.1±0.50
60	32.5±0.65	34.7±2.3	32.5±0.11	26.5±0.5	27.6±1.6
75	36.3±0.62	39.3±0.5	36.5±0.25	31.5±0.65	32.2±0.5
90	41.5±0.81	43.7±0.7	40.2±0.3	34.2±0.25	35.9±0.5
105	46.9±0.66	49.5±0.76	46.3±0.25	38.3±0.52	40.5±0.64
120	53.5±0.80	56.5±1	54.1±0.5	44.5±0.68	46.8±0.57
180	61.3±0.78	64.3±1.04	61.5±1.55	49.8±0.51	52.4±0.11
240	68.5±0.47	71.5±1.04	67.2±0.55	56.7±0.66	58.6±0.76
300	73.2±0.25	75.3±0.04	72±0.40	65.9±0.55	68.8±0.57
360	77.8±0.35	79.3±0.76	76.9±0.52	71.2±0.52	73.5±0.28

(*Data represented as mean ± standard deviation and n=3)

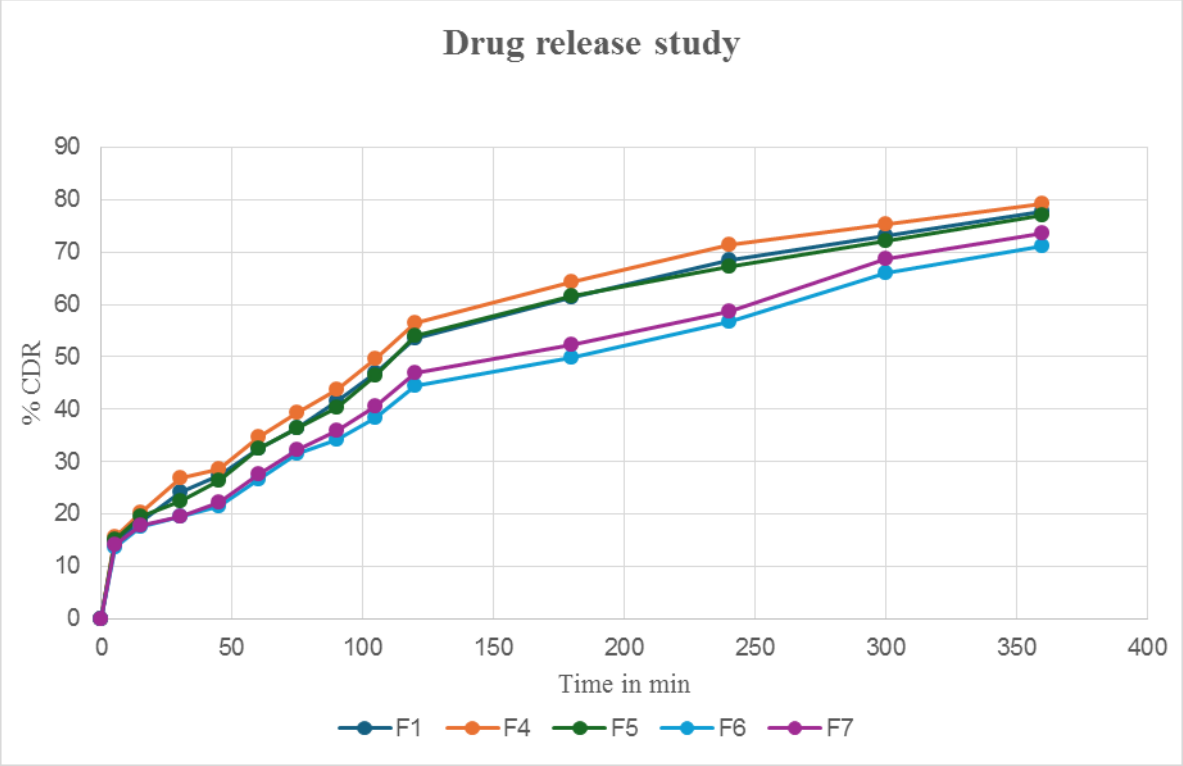


Fig. No. 08: Drug release study of nasal *in-situ* gel of MET-HCl.

The present study indicated that the formulation F4 shows the maximum drug release of 79.3% at the end of 6 hours whereas the other formulations also show comparable drug release at the end of 6 hours.

SUMMARY AND CONCLUSION

Nasal *in-situ* gel offers a controlled and extended therapeutic effect, decreasing the need for frequent dosing and thereby enhancing patient compliance. In this study, a nasal *in-situ* gel containing Metoclopramide for treating nausea and vomiting was successfully developed.

Five distinct Nano-emulsions, denoted as F1, F2, F3, F4 and F5 were prepared by High speed homogenizer in varying concentrations of Smix respectively., Tween 20: Ethanol (1:1, 2:1, 3:1, 4:1, 5:1). These Nano-emulsions were incorporated into gel with fixed concentrations of Polymers (Poloxamer 407, Chitosan, HPMC E15) using cold method.

The λ_{max} and calibration curve for the drug were plotted, showing a linear relationship within the concentration range of 10-30µg/ml at a maximum wavelength of 273nm. The slope of the curve was 0.306 and the regression coefficient was 0.9966 (R^2 value), indicating that the drug follows Beer-Lambert's Law.

An FT-IR study was performed on the physical mixture of the drug and polymer, and the results were compared with the IR spectra of the pure drug. The analysis showed no significant changes in the bands compared to the pure drug, confirming that there are no interactions between the drug and the polymer in the formulations.

All the prepared *in-situ* gels were evaluated through various parameters, including spectroscopic analysis, pH, viscosity, gelation temperature, clarity, drug content and in-vitro drug release studies.

The formulations demonstrated pH values suitable for the nasal mucosa, ensuring they did not cause irritation. Each nano-emulsion was clear and stable.

In-situ nano-emulsion gel formulations showed gelation within the temperature range of 36.13- 37.36 ^{o}C, which aligns with the nasal physiological temperature range of 30-37^{o}C.

The viscosity of all formulations was assessed both before and after gel formation. The pre-gelling viscosity ranged from 414.33 to 507 cps and the post gelling viscosity ranged from 10,506.66 to 10,687 cps.

The drug content was ranged between 65% to 72%. The percentage of cumulative drug release for all formulations over 6 hours varied between 71.2 % and 79.3%.

Among the five formulations, F4 was selected as the most suitable candidate because it showed optimum gelation temperature, had an ideal pH, viscosity and drug content and showed an enhanced cumulative drug release.

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