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OPTIMIZATION FORMULATION AND EVALUATION OF IN-SITUGEL OF SOME ANTIULCER DRUG

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Abstract:

The purpose of this study was to develop and evaluate the effectiveness of novel in-situ gelling drug delivery systems that might be used for controlled administration of medication. The medicine that was tested in this study was called nizatidine. The effectiveness of in situ gels fabricated from a variety of environmentally sensitive polymers was evaluated using both in vitro and in vivo testing methodologies. The research was comprised of four distinct investigations, and we gathered the most significant data from each of those studies.

"In situ gelling polymers" is another term for a reaction that is driven by an external stimulus. This phrase relates more specifically to formulations that gel upon contact with mucosa. "In situ gelling polymers" Since these formulations are fluid-like before they come into contact with the human surface, the primary advantage that they offer is that they may be easily supplied as a drop, an injection, or a spray device. This makes it possible to administer the medication in a variety of ways.

Key Words : pH (potency of hydrogen), Nizatidine

Abbrevations : Fourier Transform Infrared Spectroscopy (FT-IR), Hydroxypropyl Methylcellulose (HPMC)

1. Introduction:

Hydrogels are three-dimensional polymeric networks that are hydrophilic and have the ability to absorb large quantities of water or other biological fluids. Hydrogels are also known as hydragels. Hydrogels absorb more water after being exposed to it, yet they do not disintegrate. These networks' insolubility can be attributed, impart, to the chemical and physical crosslinks that are present in the system, and they are constructed out of homopolymers or copolymers (entanglements or crystallites).

The second type maintains the network's consistency while also protecting it from phyiscal threats. Because of their thermodynamic compatibility with water, these hydrogels swell up when they are submerged in liquid. These hydrogels

offer a wide variety of potential applications, particularly in the biomedical and pharmaceutical industries. When compared to hydrogels, the majority of other types of synthetic biomaterials don't even come close to replicating the characteristics of genuine tissue. This is due to the fact that synthetic tissues are highly flexible and contain a significant amount of water, which is comparable to the flexibility of real tissue. A significant amount of water is included in the materials, which contributes to their biocompatibility.

2. DRUG PROFILE

NIZATIDINE

Structure:

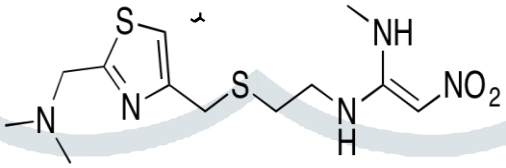

Fig 1: Nizatidine Drug structure

Chemical name	**:**	N-(2-[[(dimethylamino)methyl]thiazol-4- yl)methylthio]ethyl)-N-methyl-2-nitroethene- 1,1-diamine
Molecular formula	**:**	$C_{12}H_{21}N_5O_2S_2$
Molecular weight	**:**	331.5
Physical characteristics	**:**	White to off–white crystallinesolid.
Melting range	**:**	130-132ºC
Storage	**:**	Light resistance, temper proof, air tight container

Mechanism of action:

Histamine H_2 receptors, especially in the parietal gastric cells, are blocked very effectively and reversibly by nizatidine. Nizoralidine inhibits the effect that histamine has on stomach cells, which results in a decrease in the amount of acid produced by the stomach. Histamine and nizatidine are both capable of binding to H_2 receptors located on the basolateral membrane of parietal cells in the stomach; however, nizatidine has a modest edge in this regard. Inhibiting proteins have the ability to put a stop to the production of stomach acid at both the basal and nocturnal levels. As a result of taking this drug, the stomach's acid is less responsive to many stimuli, such as food, coffee, insulin, betazole, and pentagastrin.

Plasma half life **:** 1.5hrs

Disposition in the body:

The oral administration of nizatidine results in quick and almost total absorption of the drug, with peak plasma concentrations being reached within one to three hours. Eating raises the absorption rate, however using antacids like aluminium hydroxide gel or magnesium silicate lowers it by ten percent. The rate can be enhanced by eating. Even though a significant portion of its metabolism takes place there, it does not inhibit the mixed function oxidase system in the liver. Nizatidine N-2-oxide, nizatidine S-oxide, and N-2-monodesmethyl nizatidine are the three metabolites that have been found. Nizatidine N-2-oxide is responsible for sixty percent of the effect of nizatidine.

It has been discovered that the drug nizatidine travels to many different parts of the body and has even been detected in

breast milk (at a concentration of 0.1 percent of the dose). The vast majority of a drug's dose, approximately sixty percent, is eliminated in the urine in its original form. The remaining ten percent of the dose is eliminated in some other way. The amount of a dose that is lost in the bowel movement is less than six percent.

RATIONAL OF STUDY

A controlled medication delivery system involves dosing the medication in phases at specified intervals in order to treat or at the very least manage a medical condition in the shortest amount of time possible. This is done in order to maximise efficiency. As a direct result of the work that has been done in the field of controlled drug delivery over the course of the past ten years, researchers have created increasingly intricate strategies for maintaining drug delivery. It is now widespread information among the general public, regulators, healthcare professionals, and the pharmaceutical industry that a more comprehensive grasp of the therapeutic benefits of controlled drug delivery systems is now common knowledge. The majority of these systems are currently built on synthetic polymers, which can vary in how easily they erode, how much they swell, and how sensitive they are to the biological milieu in which they are located.

Increases in both the variety of medications and the variety of routes of administration point to a bright future for controlled drug delivery. There has been a significant increase in the number of studies looking into drug delivery systems that are capable of maintaining the drug's release into the circulatory system of the body. Devices for the regulated delivery of drugs have been created as a solution to the issue of unpredictability in drug levels that is generated by the use of standard methods of drug administration.

Controlled drug delivery systems have as their primary purpose the achievement and maintenance, for a predetermined amount of time, of a drug concentration that is within the therapeutically effective range. This range is required for the treatment. These systems have the ability to control the dosage, extend the duration of the therapeutic impact, and/or deliver the medication to the specific region of the body that is being targeted. In spite of the significant progress that has been accomplished, there is still a great deal to learn and put into practise regarding the treatment of a wide variety of clinical disorders.

Recent innovations in drug delivery technology have resulted in the creation of state-of-the-art drug delivery systems. These systems have radically changed the conventional method of dosing patients with medications and brought a variety of advantages, including those listed below. In-situ gels, also known as hydrogels, are a type of polymer that can absorb a significant amount of water without compromising its structure. Hydrogels are another name for in-situ gels. Both chemical and physical cross-linking methods can be utilised to produce hydrogels. In situ gels have a wide variety of applications, some of which include hydrogels for macromolecular therapeutics, hydrogels for proteins and genes, tissue barriers, and tissue engineering. The polymeric system in water undergoes a straightforward phase change known as the sol-gel transition, which does not include any kind of chemical reaction. This transformation results in the formation of hydrogels. This technology is user-friendly and safe to utilise in real life.

Controlled medicine distribution is one of the areas of study that is progressing at the fastest rate and providing some of the most significant advances to human healthcare. Both the range and depth of the pharmaceutical technology industry have expanded dramatically in recent years. Over the past few years, scientists have invested a great deal of time and energy into exploring the possibility of producing gels on-site. The advantages of in situ forming polymeric delivery systems, such as better patient compliance and comfort, simplified dosing, lower administration frequency, and tailored drug delivery, have sparked a fresh interest in the field.

This formulation is administered through injection as a liquid, but when being stimulated, it transforms into a gel, imitating the qualities of a semi-solid implant. Changes in pH, temperature, and the chemical make-up of the solvent can all play a role in the formation of gels in their natural surroundings. Other events, such as changes in pressure, can also play a role. In contrast to implants, these systems can be inserted without the need for surgical intervention because of their injectability. In situ gels increase the amount of medicine that is available at the site of action because they prevent the drug from being metabolised in the first pass and permit controlled release of the drug. Innovative in situ gelling techniques may help reduce the adverse effects of systemic or oral medicine administration, prevent the development of drug resistance, and save money on treatment.

3. MATERIAL & METHODS

Preformulation studies are required in order to ensure that a dosage form that is not only consistent but also safe and effective therapeutically may be manufactured. During this stage of drug development, the maturation process, the physical pharmacist will describe the physicochemical properties of the drug substance as well as how it interacts with the other components.

During preformulation studies, the process variables that affect the physical properties of the formulation in connection to the in vitro release profile and the in vivo drug absorption are optimised in an effort to maximise the effectiveness of the formulation. Preformulation studies are performed during the production phase to assess the demands of the formulation. These include the variability of the process, the selection of relevant ingredients, and the corresponding amounts. Once these are determined, the production phase can begin. In addition to discussing the medication's physical properties and the various ways it can be administered, scientists working in the pharmaceutical industry may also discuss the mechanistic function that particular mechanisms play in the distribution of drugs. This may take place in conjunction with the other topics.

TEST FOR IDENTIFICATION:

The following tests were used for identification of ranitidine bismuth citrate referred:

Physical appearance : A fine, amorphous powder that ranges in colour from white to off-white.

Melting point : The melting point of nizatidine hydrochloride was found to be 132^{o}C.

pH indication test : A 1.0% w/v solution of nizatidine hydrochloride in carbondioxide-free water has a pH of 5 when measured at 25±1^{O}C with a pH metre.

PROFILE OF SOLUBILITY

The solubility of nizatidine hydrochloride was investigated using a variety of different industrial and common household solvents. The following table provides information regarding the nizatidine hydrochloride's solubility in a variety of solvents.

PARTITION COEFFICIENT

As a substitute for a drug's lipophilicity and an indicator of its capacity to pass across biomembranes, the partition coefficient is a useful metric to have. When the organic and aqueous phases of the unionised drug are in equilibrium, this is the proportion of the former to the latter. To determine the partition coefficient of nizatidine hydrochloride between n-octanol and water (pH 7.0) and n-octanol and phosphate-buffered saline, we followed the procedure provided by Leo and Hansche115 (pH 7.4).

CALIBRATION CURVE OF NIZATIDINEHYDRO CHLORIDE IN SGF:

Addition of distilled water brought the total volume of a solution containing 3.2 grammes of pepsin, 2 grammes of sodium chloride, and 7.0 millilitres of highly concentrated hydrochloric acid to the desired 1000 millilitres. The pH was measured with a pH metre as we added 0.1N HCl until it read 1.2 (I.P.,1996).

STANDARD STOCK SOLUTION PREPARATION

A stock solution of 100 g/ml of the medication was prepared by dissolving a precisely weighed quantity of nizatidine hydrochloride (10 mg) in 100 µg/ml of SGF (pH 1.2).

ABSORPTION MAXIMA DETERMINATION

The UV/visible spectrophotometer was used to look for the absorption maxima between 200 and 400 nm after a standard stock solution was diluted to a concentration of 10 µg/ml with SGF (pH 1.2). (Shimadzu 1800, Japan). It was established that 228 nm is the wavelength at which absorption is at its peak.

Procedure

In a succession of 10 ml volumetric flasks, 0.2, 0.4,.....1.8, 2.0 ml aliquots of stock solution recovered were combined with varying quantities of SGF. A spectrophotometer was used to determine the maximum absorbance of each sample at λmax 228 nanometers.

NIZAIDAZINE HYDROCHLORIDE STANDARD ERROR OF MEASUREMENT (SIF)

CALIBRATION CURVE:

Preparation of SIF (pH 6.8)

Following the dissolution of 28.80 grammes of disodium hydrogen orthophosphate, 11.45 grammes of potassium dihydrogen orthophosphate, and 10.0 grammes of pancreatin in approximately 100 millilitres of pure water, the remaining 900 millilitres were filled with distilled water. The pH of the solution was brought up to 6.8 using either 0.1N hydrochloric acid or 0.1N sodium hydroxide, depending on what was required.

Standard stock solution preparation

In order to prepare a stock solution of nizatidine hydrochloride at a concentration of 100 µg/ml, 10 mg of the drug was metered out and then dissolved in 100 ml of intestinal fluid (pH 6.8).

Absorption maxima (λ_{max}) determination

Absorption maxima were established by UV/visible spectroscopic analysis of a 10 g/ml stock solution of a standard diluted in simulated intestinal fluid (pH 6.8) from 200 to 400 nm.

Procedure

Multiple 10-milliliter volumetric flasks' worth of 0.2-milliliter, 0.4-milliliter, 1.8-milliliter, and 2.0-milliliter aliquots of the aforesaid stock solution were diluted to yield 10 millilitres of intestinal fluid (pH 6.8). The resulting concentration of the solution was between 2 and 20 µg/ml. Absorbance at 313 nm was taken using a UV/visible spectrophotometer (Shimadzu 1800, Japan).

NIZATIDINE HYDROCHLORIDE PBS CALIBRATION CURVE

Preparation of PBS (pH 7.4)

The following were dissolved in 100 mL of distilled water: 2.38 g of disodium hydrogen orthophosphate, 0.19 g of potassium dihydrogen orthophosphate, and 8.0 g of sodium chloride. The pH was brought down to 7.4 with 0.1N hydrochloric acid or 0.1N sodium hydroxide prior to application.

Standard stock solution preparation

Using phosphate buffer, 10 mg of nizatidine hydrochloride was diluted to produce a 100 µg/ml stock solution (pH 7.4).

Absorption maxima (λmax) determination

Maximum absorption wavelengths were determined by scanning standard stock solutions diluted to 10 µg/ml in phosphate buffer (pH 7.4) in a UV/visible spectrophotometer from 200 to 400 nm (Shimadzu 1800, Japan).

Procedure

The aforementioned stock solution was used to obtain aliquots of 0.2, 0.4,.....1.8, and 2.0 ml, which were then diluted to a final volume of 10 ml in a series of 10 ml volumetric flasks that contained phosphate buffer (pH 7.4) As a direct consequence of this, the final concentration of the solution was somewhere in the range of 2 to 20 µg/ml. We measured the maximum absorbance of each solution at 325 nm with a UV/visible spectrophotometer manufactured in Japan by Shimadzu and using the model number 1800.

FORMULATION OF NIZATIDINE *IN SITU* GELS

Following the process of dispersing gellan gum in deionized water at a concentration ranging from 0.25% to 1.00% w/v and heating the mixture to 90 JC while agitating it, we cooled the solution to temperatures below 40 JC. HPMC E15LV was added to a solution of sodium citrate (0.25%±1.0% w/v) and calcium chloride (0.016%) dissolved in water. The resulting solution was then mixed thoroughly. The calcium carbonate and the drug were combined quite smoothly because to the constant mixing that took place. After they had been manufactured, gels had their pH levels measured and analysed. The formula chart is displayed here in this table.

Table 1:In situ gels containing nizatidine: a formulation guide

Formulation Code	Ingredients (% w/v)					
	Nizatidine	Gellan Gum	HPMC E15LV	CaCO3	Sodium Citrate	Calcium Chloride
L1	10	0.25	0.1	1.0	0.25	0.015
L2	10	0.5	0.1	1.0	0.25	0.016
L3	10	0.75	0.1	1.0	0.25	0.016
L4	10	1.0	0.1	1.0	0.25	0.016
L5	10	0.5	0.05	1.0	0.25	0.016
L6	10	0.5	0.1	1.0	0.25	0.016
L7	10	0.5	0.15	1.0	0.25	0.016
L8	10	0.5	0.2	1.0	0.25	0.016
L9	10	0.5	0.1	0.25	0.25	0.016
L10	10	0.5	0.1	0.5	0.25	0.016
L11	10	0.5	0.1	0.75	0.25	0.016
L12	10	0.5	0.1	1.0	0.25	0.016

IN-SITU GEL CHARACTERIZATION

Testing for Microorganisms (Sterility testing)

After 14 days of incubation at 37±1 JC and 25 JC in fluid thioglycollate media (including aerobic and anaerobic bacteria) and soya casein digest media, the sterility of the in situ gel was tested (containing fungal organisms). Bacteria and fungi were incubated, and their progress was tracked after.

Fourier transform infra red spectroscopy (FT-IR)

Interaction studies between the drug and the excipient were carried out so that the stability and safety of the drug could be determined once it was formulated. Infrared Fourier transform spectroscopy was utilised in order to investigate both the unadulterated medication as well as the improved formulations. The FT-IR spectra of the unadulterated drug as well as its many different formulations were collected by employing the KBr pellet technique. The samples were scanned at a range of wave numbers, from 400 to 4000 cm^{-1}, depending on the desired information.

Differential scanning calorimetry (DSC)

The manufactured medicine and the pure drug were both subjected to differential scanning calorimetry. The calorimetric measurements were compared to a standard, which consisted of empty cells that were discs of high purity alpha alumina. Dynamic scans were performed in an atmosphere composed primarily of nitrogen with a heating rate of 10JC min-1. The amount of energy was calculated using the joules per kilocalorie conversion factor.

Thermosensitivity Evaluation

Temperature of gelation

The solution was gelified by heating it in a glass tube with a thin wall (internal diameter = 10 mm, length = 82 mm, thickness = 0.6 mm) in a water bath kept at a specific temperature. This was done so that the in situ gelation temperature could be determined. Here are the exact measurements for the tube:

It has an internal diameter of 10 mm, an overall length of 82 mm, and a thickness of 0.6 mm. This experiment led us to conclude that a steady rate of 2 joules per degree Celsius per minute is the most efficient technique to steadily increase the water temperature. After inverting the test tube, we observed that there was no flow of liquid, which was conclusive evidence of gel formation.

Period of Gelation

In order to determine the amount of time necessary for in situ gelation, the tube inversion approach was utilised. It was necessary to alter the temperature of the water bath based on the gelation temperature, which had previously been estimated for in situ gels. Following the transfer of a 2 ml aliquot of the gel, the tube was positioned within a water bath that was heated to a particular degree. In order to determine whether or not the substance had gelled, the test tube was turned upside down. A test tube was inverted and a flow or no-flow criterion was used to determine how long the gelation process took.

The in situ gelation time of nizatidine was evaluated by gradually adding 10 ml of the formulation to a volume of 900 ml of pH 1.2 buffer solution that was maintained at 37JC. The solution was not stirred during this process. Recording the amount of time that passes as the solution transforms into a gel is something that is done.

CONTINUITY OF DOSAGE FORM AND MEDICATION

Gels formed *in situ* from nizatidine:

For thirty minutes, five millilitres of in-situ gel were sonicated in fifty millilitres of a pH 1.2 HCl buffer. It was brought up to 100 ml by adding HCl buffer with a pH of 1.2. After appropriate dilution, the sample's drug concentration was measured at 208 nm using UV-Visible spectrophotometer.

Syringe ability test

Following Yannic's protocol, a syringe ability test was performed. A 5.0 ml glass syringe filled with smart gel kept at 5±1JC and a pan resting on the piston of the syringe were used to assess the syringeability of the gels. The prop was an 18G needle attached to a syringe. The syringeability time was determined by timing how long it took for the gel to be expelled after a 0.5 kilogramme mass was placed on the pan.

Study of Rheological

In order to conduct rheological tests, thermostatically regulated universal stress Rheometer SR5s were utilised. In this geometry problem, a cone and a flat plate were used as the building blocks. The following is a rundown of the cone's dimensions: diameter of 25 millimetres, angle of 0.04 radians, and gap size of 1 millimetre. The 1.5 ml sample was put through its paces by being subjected to shear speeds ranging from 5.0 to 500 sec-1. Rheological investigations employing the temperature sweep method were utilised in order to characterise the temperature-dependent viscosity of the formulations. At both 8JC and 37JC, the viscosity of 125 duplicate samples of smart gels was measured.

Study for viscosity

The Brookfield viscometer was calibrated to the appropriate spindle (no. 5) and rotating speed in order to obtain accurate readings of the sample's viscosity (20, 30, 40, or 50 revolutions per minute). Before beginning the measurement, the samples were preheated to the desired temperature for ten minutes, and then the temperature control unit was inserted in the instrument itself. We measured the viscosity at two temperatures (n=3) using a thermostated water jacket 126. The temperatures we utilised were 8 ±1JC and 37±1DC.

Duration of floating and time spent floating

We poured 10 millilitres of the formulation into the dissolution vessel, which held 900 millilitres of the HCl buffer at a pH of 1.2 and 37 degrees Kelvin. This was done with as little stirring as possible. It was determined by monitoring the passage of time how long the formulation took to float to the surface of the dissolving media (the floating lag time), as well as how long it lasted at that level (the floating duration).

Assessment of the gel's capacity to absorb water

In situ, gel was generated in 40 millilitres of HCl buffer at a pH of 1.2 and it was employed. It was necessary to take the gel component out of the HCl buffer with a pH of 1.2 and then blot off any excess HCl solution from each batch. After determining the gel's initial density by weighing it before adding 10 ml of distilled water, we then decanted the water at intervals of 30 minutes and remeasured the gel's weight in order to evaluate the degree to which its density shifted.

IN VITRO DRUG RELEASE STUDIES

Static diffusion method

Dialysis membranes with permeabilities of 50 were used in the diffusion studies that were carried out. In order to hydrate a membrane, we used diffusion medium that had been soaked in water. The dialysis membrane sac (which had an area of approximately 1.4 cm^2) was then sealed on both sides after having 1 ml of in situ gel inserted into it. The membrane that was

used for dialysis had one millilitre of in situ gel added to it, and then it was placed in a glass beaker that contained diffusion medium. The investigation on the release was conducted at a temperature of 37 ±0.5^{0}c. We measured the drug concentration with a UV-Visible spectrophotometer by regularly removing a known amount of recipient solution and replacing it with an equivalent volume of fresh, warm buffer solution. This allowed us to get an accurate reading of the drug concentration.

***In vitro* drug release of Nizatidine *in situ* gels**

Pharmaceutical formulations' release rates were evaluated using the USP dissolving testing apparatus II (paddle type). The dissolvability test was conducted in a buffer at 37°±0.5°C, 50 rpm, and 1.2 HCl. An aliquot (ten millilitres) was taken from the sink at regular intervals (every 12 hours) to ensure a constant state. Fresh dissolving medium of the same volume as the removed samples was added in their place. Readings were taken at 448 nm using a UV-Visible spectrophotometer to determine sample composition.

Mechanism of drug release

The kinetics of drug release from dosage forms have been the subject of numerous mathematical models. The model that comes closest to explaining the data from experiments is the one that is chosen as the best fit. PCP dissolving v2.08 was used to create a mathematical model of the in vitro data to find out how the drug was released and how fast it was released. The kinetics of release from the formulations may be better understood if the plots that best fit the release data are located. Plots such as the Hixson-Crowell plot, the Korsmeyer-Peppas plot, and the Higuchi matrix were utilised. Higuchi developed several theoretical models to examine the release of both highly and weakly water-soluble medicines from semi-solid and/or solid matrices. This action was taken to learn more about the pharmaceutical leak. Inside this system, we modelled the spread of drugs as a Fickian diffusion process with a temporal dependence defined by the square root of its value. The dissolution of many distinct types of modified release pharmacological dose forms can be described using this relation.

Stability Studies

The testing of the stability of medicines begins at the very beginning of the medication development process and continues right up until the chemical or commercial product is no longer of any use. Stability testing for new pharmacological compounds and products is governed by standards established by the International Council for Harmonization (ICH). For drugs intended for human consumption to be approved for registration, certain conditions must be met. By analysing the effect of external influences on the product's quality over the course of time, the goal of stability testing is to identify the product's shelf life as well as the ideal storage conditions for the product.

Because drug disintegration or degradation can occur during storage as a result of chemical modification of the active components or product instability, it is essential to evaluate the stability of pharmaceutical preparations. This is because some of these transformations may cause the medication concentration in the dose form to drop. The purpose of stability studies is to estimate the length of time a product may be stored by accelerating the rate at which it decomposes. This is accomplished most frequently by subjecting the product to higher temperatures and higher levels of relative humidity (RH). When a pharmaceutical formulation meets all of the criteria listed below, we refer to it as stable.

- At least 90% of the labelled active component is present;
- Added preservatives, if any, are present at sufficient concentrations;
- No off-coloration, precipitation, or unpleasant odours occur;
- No irritation or toxicity occurs;

Studies were conducted by storing glass vials containing optimised in situ gels at 5± 2JC or 25± 2JC and 60% RH for 12 months. The vials were sealed with rubber bungs using aluminium crimping (long term storage) at 25°C, 60°F, and 60±5% RH, or 2,750

hours at 40°C, 60±5% RH (accelerated storage).

Samples should be taken every 3, 6, 9, and 12 months under standard storage circumstances, and every 3, 6, and 9 months under accelerated storage conditions, as suggested by the International Conference on Harmonization (ICH) Q1A (R2). This was done so that any alterations in the drug's outer appearance or chemical make-up could be identified. Samples were collected every three and six months for accelerated storage conditions. The plots that were utilised to determine whether or not there was statistical significance were created with Sigmaplot 12.0.

4. RESULTS

Table No 2.: An investigation of the degree to which nizatidine hydrochloride can dissolve in typical organic solvents

S.NO	Solvent (S)	Solubility indicator
1.	Distilled water	++
2.	Ethanol (95%).	++
3.	Methanol.	+++
4.	Isopropanol.	++
5.	Ethyl acetate.	++
6.	Acetone.	-
7.	Chloroform.	-
8.	Toluene.	-
9.	PBS (pH7.4).	++
10.	SIF(pH1.2).	+++
11.	Acetate buffer(pH5.0).	++

++++ Extremely soluble in less than 1 part
+++ Insoluble in water; soluble in a range of 1-10 parts
++ Solubility range: 30-100 parts per million
+ Mild solubility: 100-1000 parts per
- Hardly soluble: > 1000 parts per million

Nizatidine hydrochloride partition coefficient values

S. No.	Solvent system (s)	Partition coefficient (P)
1	n-octanol / water.	0.2347
2	n-octanol / PBS (pH 7.4).	0.2183

Nizatidine hydrochloride calibration curve at λmax 228nm in SGF (pH 1.2).

S.No.	Concentration (µg/ml)	Absorbance	Regressed Absorbance	Statistical parameter
1	2	0.010	0.0029	
2	4	0.025	0.0216	
3	6	0.038	0.0403	Correlation coefficient
4	8	0.052	0.0590	(R2) = 0.9951
5	10	0.072	0.0777	Slope (m) = 0. 0087
6	12	0.092	0.0963	Intercept(C)=-0.0008
7	14	0.112	0.1150	Equation of line :
8	16	0.141	0.1337	y = 0.0087x - 0.0008
9	18	0.159	0.1524	Beer's law range:
10	20	0.169	0.1711	2-20(µg/ml)

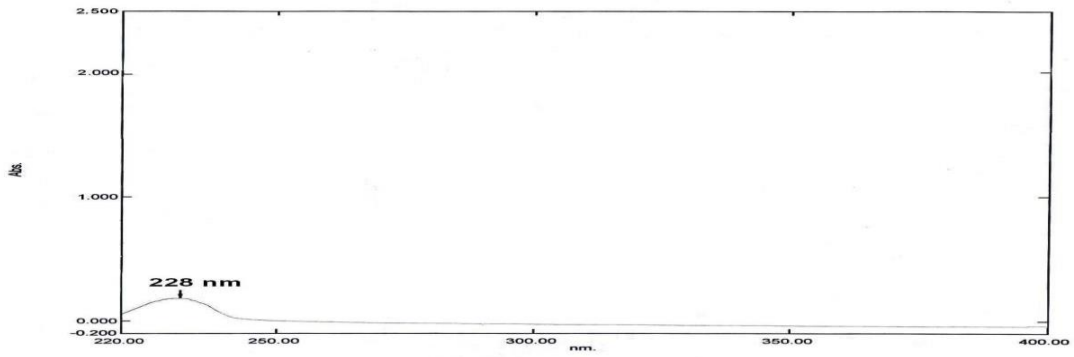

Fig:2 Maximum UV absorption of nizatidine hydrochloride at pH 1.2 is λmax 228 nm.

Nizatidine hydrochloride calibration curve in SIF (pH 6.8) at λmax 313 nm

S. No	Concentration (µg/ml)	Absorbance	Regressed Absorbance	Statistical Parameters
1	2	0.101	0.0939	
2	4	0.164	0.1837	Correlation coefficient:
3	6	0.244	0.2736	$(R^2) = 0.9947$
4	8	0.378	0.3634	
5	10	0.473	0.4533	Slope (m) = 0.045
6	12	0.553	0.5431	Intercept (C)= 0.0027
7	14	0.655	0.6330	
8	16	0.738	0.7228	Equation of line:
9	18	0.791	0.8127	y=0.045x + 0.0027
10	20	0.885	0.9025	

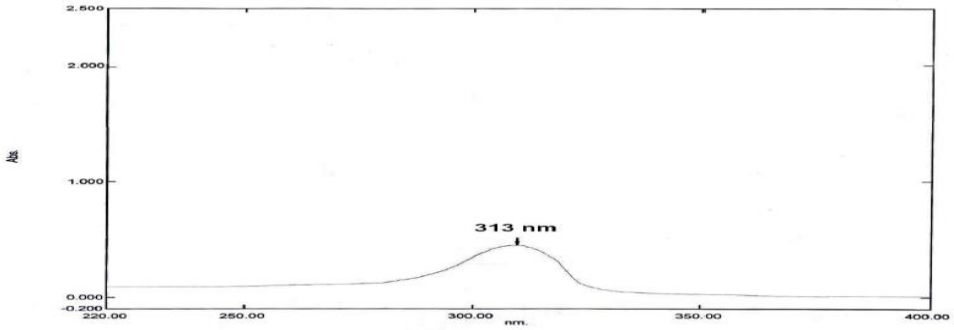

Fig 3: Nizatidine's peak UV absorbance in the SIF

PREPARATION OF NIZATIDINE *IN SITU* GELS

There were twelve (12) different attempts to develop gellan gum and nizatidine-based in situ gelling compositions. During preparation, quantities of gellan gum, high polymer methylcellulose (HPMC), and calcium carbonate (CaCO3) were varied to study their impact on the formulation and in vitro properties of in situ gels.

Under acidic conditions, calcium ion triggers a sol-gel transition in a gellan gum-based in situ gelling system. Sodium citrate aids in maintaining fluidity before to delivery; following administration, it is complexed with calcium chloride to release sodium at an acidic pH. Only incredibly minute amounts of divalent cations were required for their presence. The concentrations of sodium citrate and calcium chloride were the same across all of the formulations.

Every time, the procedure for producing in-situ gels was uncomplicated and uncomplicatedly simple. Using a calibrated digital pH metre, the pH of the completed formulations was determined while the temperature was set at 25 degrees Celsius.

The formulations all had a pH that was at least 7, which made it safe for the human body to consume them orally and digest them in the stomach. pH levels ranged anywhere from 7.97 to 8.38 in the various formulations.

Table No. 3: Measured pH values of Nizatidine *in situ* gels

Formulation Code	pH*
L1	8.06 ± 0.02
L2	8.22 ± 0.03
L3	8.31 ± 0.01
L4	8.38 ± 0.04
L5	8.15 ± 0.01
L6	8.24 ± 0.02
L7	8.29 ± 0.03
L8	8.33 ± 0.02
L9	7.97 ± 0.03
L10	8.03 ± 0.05
L11	8.18 ± 0.01
L12	8.23 ± 0.04

The primary purpose of this investigation was to create recipes for PF-127-containing floating in situ gels with a constant concentration of 20% weight-per-volume, as well as the inclusion of a suitable co-polymer, in order to produce a series of in situ gels that possessed the qualities that were being sought after.

Fourier Transform Infrared Spectroscopy (FT-IR)

In order to determine whether or not the medicine is compatible with polymers, the FT-IR method was utilised. FT-IR spectroscopy was utilised to examine both unadulterated Nizatidine as well as the L4 formulation in order to identify any potential drug-polymer interactions. The spectral peaks of pure nizatidine were compared to those of the L4 formulation, which revealed an almost exact correspondence between the two in terms of position. These peaks, which are characteristic of Nizatidine, did not relocate and stayed put in the regions where they were found initially. Because of this, we know that the medicine's active ingredient was not compromised, and we also know that the excipients utilised in the formulation were compatible with one another. Since the medication is already in its active form, it is simple to extract it from its bound state within the polymeric network. The table provides an extensive summary of the data as well as the spectra.

Table No. 4: Nizatidine with L4 formulation FT-IR spectrum data

Functional groups	Peak positions in pure drug	Peak positions
C-Cl	763.84	761.91
Ar-H	2955.04	2928.01
C-N Stretching	1257.63	1259.56
N=N Stretching	1581.68	1583.61
C-O primary alcohol	1072.46	1078.24
C=C Stretching	1458.23	1456.30
OH Stretching	3201.94	3394.83

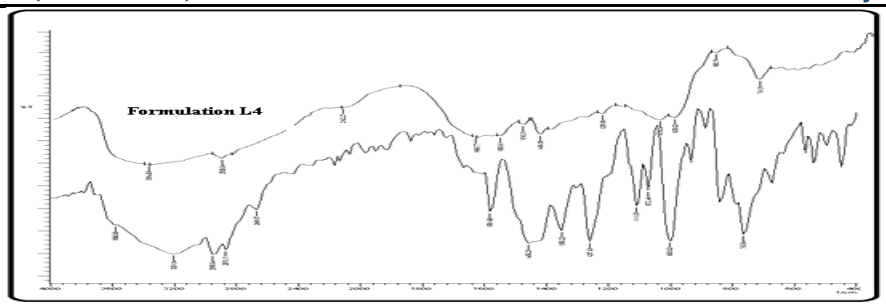

Fig 4: Nizatidine and L4 formulation FT-IR spectra

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The thermograms that were produced from the DSC analysis of pure Nizatidine and formulation L4 are displayed in the picture, and the data that corresponds to those thermograms may be found in the table. According to the readings on the thermogram, the unadulterated substance has a point of melting at 257 degrees Celsius. The temperature at which the drug melted coincided with the endothermic peak measured in Formulation L4, as was expected. The thermograms demonstrate that the formulation of Nizatidine as an in situ gel with gellan gum did not have any impact on the melting point of the compound. In addition, the DSC thermograms did not reveal any evidence of an interaction between the medicine and the excipient. Because the drug and the excipients that were used were discovered to be compatible with one another, it is now possible to safely eliminate the drug from the body.

Table No,5: Nizatidine and Formulation L4 pure and diluted DSC thermograms

Sample	To (oC)	Tm (oC)	Tc (oC)	Melting Range (oC)
Pure Losartan potassium	241	257	262	21
Formulation L4	225	260	275	50

Where, T_{o}- ñ Onset of melt, T_{m} - Melting point and T_{c}- ñ Completion of melt

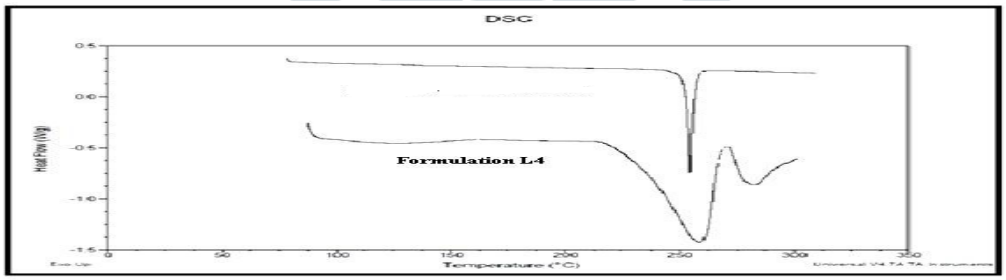

Fig 5: Analysis of pure Nizatidine and Fomrulation L4 using differential scanning calorimetry

Gelation time

The amount of time that must pass in order for a solution (sol) to turn into a gel when subjected to the conditions of a laboratory (pH 1.2). The time needed for gelation should be cut down significantly in order to prevent drug release while the formulations are still in the sol state. They have a three-dimensional network that, when they are changed into a gel (in situ), results in a slower release of the medication because it lengthens the path along which the drug can diffuse.

Changing the formulations from a sol to a gel immediately after placing them in a buffer with a pH of 1.2 prompted the change in phase to take place. The survey was completed by almost all of the different types of sol-gels. According to the findings of the inquiry, the length of gelation for formulation L4 was just 1.33 seconds, with the duration reaching a maximum of 5.33 seconds for formulation L9. The quick formation of a gel is made possible by the large concentrations of gellan gum and calcium carbonate found in these formulations.

Drug content uniformity

The amount of medication that was contained in each of the created sol-gels was evaluated. The goal of this was to establish the manner in which the illicit substances were combined with the various goods. Because the produced formulations are liquids, the patient will benefit from the uniform distribution of the medication, which will guarantee the availability of the proper dose at each administration . This will be the case because the created formulations are liquids.

The study of the drug's content revealed that the in situ gel formulations loaded with the medication consistently and disseminated the drug in the suitable manner. The completed formulations had medication concentrations that ranged from 97.63 percent to 99.63 percent of the entire therapeutic dose. Because of this, it is possible to conclude that the formulation that was produced had an appropriate distribution of the medicine. The data is presented in the following table.

Floating lag time

In all of the formulations, we found that there was only a small amount of floating lag time. After being introduced to the dissolution medium, the majority of the formulations rose to the surface of the medium within the space of just two minutes. The lag time demonstrated by the formulation L4 was the smallest, clocking in at 82 seconds, while the lag time demonstrated by the formulation L9 was the largest, clocking in at around 142 seconds. The primary reason for the difference in delay time is that the various formulations have varying levels of calcium carbonate in their ingredients.

Measurment of water uptake

After there has been an enough amount of water added to the system, the drug will be able to be extracted from the polymer matrix. It's possible that the medicine may diffuse or dissolve at the same time as water will diffuse through the matrix and release the medication. This would be a simultaneous process. In the current inquiry, a straightforward test was carried out for the in situ gel formulations; however, a thermo gravimetric analyzer may be utilised to determine the water content of the formulation at any given time.

Table No 6: Nizatidine in situ gels' prepared characteristics, flotation

Formulation Code	Drug Content* (%)	Gelation time* (sec)	Floating lag time* (sec)	Duration of floating*	% Water Uptake
L1	99.63±0.09	4.33±1	125±6	>12 h	13.0
L2	98.87±0.25	3.00±1	107±8	>12 h	7.9
L3	98.52±0.39	2.00±1	95±6	>12 h	6.7
L4	99.42±0.22	1.33±1	82±2	>12 h	4.6
L5	98.53±1.53	3.00±1	112±5	>12 h	10.7
L6	98.04±1.28	2.00±1	105±5	>12 h	8.7
L7	97.82±0.25	2.00±1	98±4	>12 h	7.4
L8	99.34±0.33	2.00±1	91±6	>12 h	5.2
L9	98.67±0.40	5.33±1	142±5	<12 h	11.2
L10	99.44±0.11	4.33±1	126±7	>12 h	9.1
L11	98.90±0.51	3.00±1	115±5	>12 h	8.8
L12	97.63±0.38	2.00±1	104±4	>12 h	8.7

* Mean ± Standard deviation, n=3

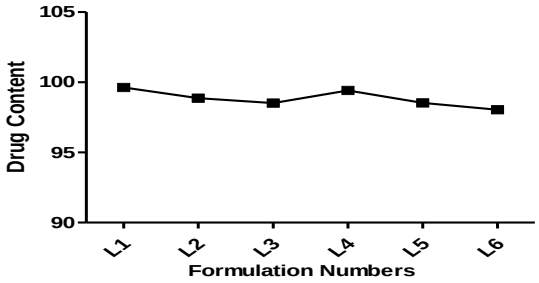

Fig 6:Nizatidine in situ gels' prepared characteristics, flotation

Studies of viscosity

Because these formulations (solutions) are meant to be consumed orally, the viscosity and rheological properties they possess are of utmost importance. Both the appropriate viscosity and the appropriate gelling capacity are necessary for in situ gelling devices in order for the liquid dose form to be suitable for oral administration (speed and extent of gelation). The formulation ought to have a viscosity that is low enough to allow it to be easily eaten as a liquid, and then it ought to rapidly transition into a solid state that is known as a sol-gel as a consequence of ionic interaction.

In order to accomplish this, the rheological features of sol-gels with various amounts of gellan, calcium carbonate, and HPMC were examined. At 25 degrees Celsius and using three different rotating speeds, a Brookfield viscometer was used to evaluate the consistency of each composition (20, 30, and 40 rpm, spindle no. 8).

It was discovered that the viscosity of the substance rose in tandem with the amount of gellan that was present. This greater viscosity can be explained by the fact that chain interaction increases as polymer concentration increases. This causes the chain to connect more strongly with one another. The viscosity of the formulation rose in proportion to the amount of calcium carbonate included in it at each of the polymer concentrations that were evaluated. Because calcium carbonate is insoluble, an increase in the concentration of calcium carbonate leads to a greater proportional rise in the number of dispersed particles, which in turn contributes to a greater increase in viscosity as a result of the calcium carbonate's insoluble dispersion in the formulations.

The formulation L4, which had the most gellan, was also the one that had the highest viscosity. When brought into contact with 0.1N hydrochloric acid, the formulations L1 and L9 resulted in the production of a slimy, scattered gel due to their low viscosity.

Table No 7: -situ gel formulations of Nizatidine and their viscosity measurements

Formulation code	20			30			40		
	SS	SR	cps*	SS	SR	cps*	SS	SR	cps*
L1	13.6	26.4	49.46±0.55	16.1	39.6	38.2±0.56	21.23	52.8	29.1±0.79
L2	26.4	26.4	79.46±0.83	29.8	39.6	63.2±0.36	34.1	52.8	54.1±0.64
L3	33.1	26.4	91.33±0.36	38.1	39.6	87.2±0.64	41.5	52.8	81.6±0.56
L4	42.2	26.4	101.96±0.81	47.2	39.6	97.1±0.45	50.5	52.8	92.1±0.35
L5	22.2	26.4	64.36±0.28	28.2	39.6	58.5±1.2	32.5	52.8	52.4±0.98
L6	25.9	26.4	75.7±0.53	31.9	39.6	64.6±0.81	36.1	52.8	58.6±0.87
L7	28.2	26.4	87.2±0.76	34.2	39.6	78.2±0.79	38.2	52.8	64.2±0.56
L8	34.1	26.4	98.4±0.74	45.1	39.6	88.6±0.65	49.4	52.8	77.2±0.65
L9	19.1	26.4	55.7±0.91	25.1	39.6	46.7±0.44	29.8	52.8	42.1±0.48
L10	22.6	26.4	58.73±0.51	29.6	39.6	51.9±0.32	35.8	52.8	45.87±0.58
L11	24.2	26.4	65.4±0.26	31.2	39.6	61.3±0.25	36.4	52.8	55.2±0.63
L12	28.1	26.4	82.46±0.28	33.1	39.6	82.5±0.78	39.3	52.8	74.5±0.65

*Mean ± Standard deviation, n=3

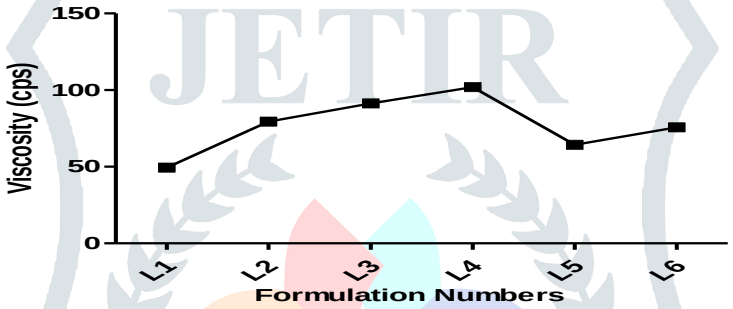

Fig 7 : Effect of Viscosity on 20 cps on different formulation

***IN VITRO* DRUG RELEASE STUDY**

In vitro drug release testing was performed on all of the in situ gelling formulations that were able to float. The results of the in vitro investigations are presented below in table 6.12. It was shown that the concentration of the polymer had an effect on the rate of drug release from the free-floating in situ gels (gellan and HPMC).

As the concentrations of the polymer or polymers in the formulations increased, the drug release rates decreased. After 10 hours, all of the medication contained inside formulations L1 and L9 had been released, whereas the release of medication contained within other formulations was still delayed after 12 hours. After 12 hours, almost 90.25 percent of the medication had been released from the L4 formulation.

Table No. 8: Data on Nizatidine release in vitro from suspended *In situ* gels (L1 to L6)

Time (h)	Cumulative % drug release					
	L1	L2	L3	L4	L5	L6
0	0	0	0	0	0	0
1	38.38±3.1	36.82±2.4	34.75±2.1	30.60±1.3	42.70±2.5	36.82±2.6
2	55.93±2.4	49.05±1.5	45.51±1.5	38.55±1.6	55.97±2.1	50.72±2.1
3	68.65±1.2	58.10±1.1	52.93±1.4	49.35±0.8	68.00±1.4	61.29±1.6
4	76.37±0.8	69.53±0.9	60.77±1.5	55.42±0.6	76.35±1.2	70.60±1.9
5	80.55±0.6	76.51±0.6	70.59±0.8	64.67±0.5	81.68±1.0	77.25±1.2
6	88.50±0.2	83.21±0.4	76.02±0.6	72.03±0.2	86.21±0.8	83.51±0.9
8	92.91±0.15	89.11±0.3	85.49±0.3	82.76±0.2	93.10±0.8	89.19±0.7
10	99.86±0.1	94.66±0.15	90.50±0.1	88.12±0.2	96.42±0.6	93.19±0.5
12	--	96.85±0.1	93.20±0.1	90.25±0.1	99.53±0.3	96.38±0.1

*Mean ± Standard deviation, n=3

Table No. 9: Reports of in vitro Nizatidine release from *In situ* gels in suspension (L7 to L12)

Time (h)	Cumulative % drug release					
	L7	L8	L9	L10	L11	L12
0	0	0	0	0	0	0
1	34.10±2.5	34.23±2.1	41.07±3.1	37.86±2.5	36.65±2.1	33.34±1.5
2	48.26±1.5	43.43±1.7	51.61±2.5	48.50±2.1	46.59±2.5	44.26±0.9
3	59.24±1.2	57.10±1.2	60.65±2.1	57.87±1.5	56.12±1.9	54.11±0.9
4	68.49±0.9	66.11±0.7	72.73±1.5	68.88±1.0	66.59±0.8	63.89±0.8
5	75.55±0.5	71.76±0.5	80.43±1.2	77.06±0.8	75.27±0.6	73.05±0.8
6	81.38±0.5	77.93±0.4	89.60±0.6	87.81±0.5	86.01±0.5	83.59±0.4
8	87.26±0.5	84.31±0.2	93.90±0.4	91.90±0.2	90.30±0.3	88.54±0.2
10	91.77±0.5	88.46±0.2	99.73±0.2	96.60±0.2	94.31±0.2	92.20±0.2
12	94.76±0.1	91.87±0.2	--	98.25±0.1	96.57±0.2	94.28±0.2

*Mean ± Standard deviation, n=3

The Impact of Gellan Gum on Drug Dissolution

The amount of drug released from *in situ* gels was significantly decreased when the polymer concentration was increased in in vitro experiments. This effect can be traced back to the presence of a polymer matrix with a higher density as well as longer diffusion pathways for the drug molecules. The drug release from these gels exhibited a sharp increase in the beginning, followed by a more steady decrease (burst effect). In spite of this, the remaining drug was discharged at a more gradual pace as the gel was being formed, and this was followed by a second phase of moderate discharge. The kinetics associated with matrix diffusion typically exhibit a biphasic release pattern. It was shown that there was a statistically significant decrease in medication release when the concentration of gellan gum in the gelling systems was increased.

THE IMPACT OF HPMC ON DRUG DISTRIBUTION

It was discovered that increasing the concentration of HPMC leads to increased viscosity values in in situ gels. After 12 hours, the in situ gel that had the highest concentration of HPMC (L8-91.87%) had the lowest amount of medication released from it.

Additionally, it has been established that HPMC is effective at inhibiting the initial (burst) release of the medication. By raising the concentration of HPMC from 0.05% to 0.2%w/v, the release was greatly reduced, and the percentage of release decreased from 42.27 to 32.23%. This could be due to the fact that HPMC has a natural inclination to gel, which produces a thick gel, contributes to an increase in viscosity, and inhibits medication release by prolonging the path along which molecules can diffuse.

Table No. 10: Nizatidine in situ gels: stability data

Sampling condition	Sampling interval (months)	Physical appearance	% Drug content (Mean ± SD*)
25±2&C/ 60±5% RH	0	Milky white gel	99.42 ± 0.22
	3	Milky white gel	99.22 ± 0.18
	6	Milky white gel	98.96 ± 0.24
	12	Milky white gel	98.78 ± 0.31
30±2&C/ 65±5% RH	0	Milky white gel	99.42 ± 0.22
	3	Milky white gel	99.10 ± 0.29
	6	Milky white gel	98.68 ± 0.16
	9	Milky white gel	98.36 ± 0.29
	12	Milky white gel	98.12 ± 0.26
40±2oC/ 75±5% RH	0	Milky white gel	99.42 ± 0.22
	3	Milky white gel	98.98 ± 0.24
	6	Milky white gel	98.22 ± 0.38

* Mean ± Standard deviation, n=3

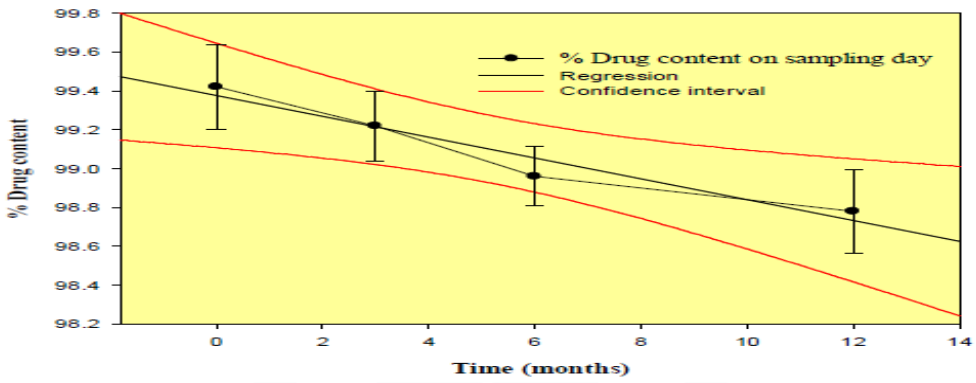

Fig.8: % Drug content in Nizatidine *in situ* gels when stored at 25±2˚C/60±5% RH for 12 months

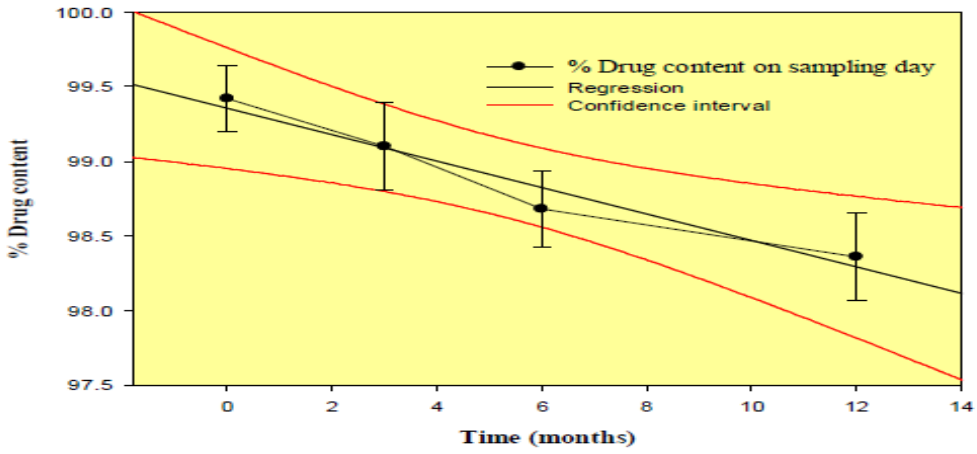

Fig.9: % Drug content in Nizatidine *in situ* gels when stored at 30±2˚C/65 ± 5 % RH for 12 months

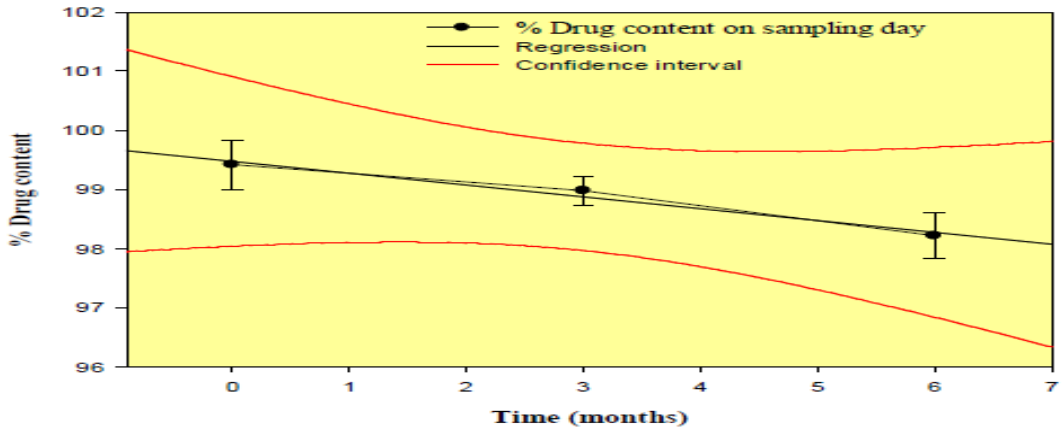

Fig.10: % Drug content in Nizatidine *in situ* gels when stored at 40±2**'**C/75 ± 5 % RH for 6 month

5. CONCLUSION

Nizatidine *in situ* gels

- To improve drug absorption and bioavailability, in-situ gels of nizatidine are created to lengthen the amount of time the drug spends in the stomach.
- $CaCO_3$ was added to the newly formed nizatidine in situ gels at concentrations ranging from 0.75 to 1 percent gellan gum and 0.05 to 0.2 percent HPMC in order to induce the production of gas.
- The majority of formulations gelled in less than 2 minutes, and the nizatidine in situ gels were buoyant and had a short floating lag time.
- When exposed to a solution with a pH of 1.2, nizatidine in situ gels formed almost immediately, with the L4 formulation demonstrating the quickest gelation time (1.33 seconds) and the L9 formulation demonstrating the sluggishest gelation time (5.33 seconds). In point of fact, they floated for a period of time greater than 12 hours.
- Gels were only able to absorb a small amount of water; after 2 hours, gel L1 had the highest rate (at 13%) while gel L4 had the lowest rate (at 4.6%) (2 h).
- The greater the quantity of gellan gum that was added, the more viscous the gel became. The viscosity of the formulation rose in direct proportion to the amount of calcium carbonate added no matter which polymer concentration was being evaluated.
- The fact that the viscosity of every formulation that was tested reduced noticeably when the rotating speed was increased provided evidence that the formulations exhibited shear thinning behaviour.
- The drug release from the in situ gels that floated was dependent on the polymer concentration (gellan and HPMC). The amount of medication released from the formulations reduced as the polymer content increased.
- First-order release was seen for all formulations, and the release mechanism was determined to be consistent with Peppas's model; fickian diffusion was uncovered to be the mechanism responsible for drug transport.
- To increase the amount of time the medicine spends in the stomach, a floating in situ gel can be made with the right proportions of gellan gum, high molecular weight polyethylene glycol, and calcium carbonate.
- Patients who have trouble swallowing can benefit from the easy and straightforward composition of the floating *in situ* gel, and drugs that are absorbed primarily by the stomach can also benefit from this delivery route.
- According to research conducted on the long-term stability of nizatidine *in situ* gels, it was discovered that these gels may be safely kept at room temperature for an extended period of time.

- According to the findings of this study, the use of in situ gels containing nizatidine as a gastroretentive medicine delivery approach can be beneficial.

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