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Formulation and evaluation of chitosan-based hydrogel matrix of licorice for targeting *helicobacter pylori*

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ABSTRACT

In present investigation an attempt has been made to formulate and evaluate chitosan-based hydrogel of matrix of Licorice for targeting Helicobacter pylori. Licorice was evaluated for its physical characteristics, analytical profiles and drug polymer compatibility study. The prepared Hydrogel granules were evaluated for pre-formulation characteristics like Angle of repose, Bulk density, Tapped density and Carr's index. The results obtained were found to be satisfactory and within the specified limits. A Stomach retentive licorice loaded chitosan hydrogel was prepared successfully by chemical crosslinking method. Glutaraldehyde was used as chemical crosslinking agent. After compression parameters like Thickness, Hardness, Weight variation, Friability, content uniformity and *In-Vitro* release studies were evaluated. Mucoadhesive study showed that, licorice loaded hydrogel have good mucoadhesion property and retained in gastric environment of stomach for prolonged period of time. The results of *In-vitro* studies showed that by chitosan concentration the extent of swelling and rate of drug release can be modulated. In the present study the effect of concentration of polymer are studied through *In-Vitro* drug release.

Keywords: Licorice, Helicobacter pylori, chitosan, Hydrogel

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a successful pathogen that can persist in the stomach of an infected person for their entire life. It provokes chronic gastric inflammation that leads to the development of serious gastric diseases such as peptic ulcers, gastric cancer and Mucosa associated lymphoid tissue lymphoma. It is known that these ailments can be avoided if the infection by the bacteria can be prevented or eradicated.[1-5]Currently, numerous antibiotic-based therapies are available. However, these therapies have several inherent problems, including the appearance of resistance to the antibiotics used and associated adverse effects, the risk of re-infection and the

high cost of antibiotic therapy. Herbal medicine has been opened its way in therapy of gastric ulcer, among them, (Licorice) was shown to have anti *H. pylori* effects derived from the roots and stolon's of *Glycyrrhiza* species. Oral site-specific drug delivery systems that could increase the longevity of the treatment agent at the target site might improve the therapeutic effect and avoid side effects.[6-8]*Helicobacter pylori* lives deep within gastric mucus layer and prolonged local application of drug is needed efficiently to diffuse bacteria. It has been demonstrated that *H. pylori* is one of the major causative microorganisms for peptic ulcer disease. This bacterium release enzyme urease, which convert urea into ammonia and bicarbonate, which aids in neutralizing acidic medium and allow the bacteria to colonize in gastric mucosa.[9-13]

Gastro retentive drug delivery systems potentially prolong the gastric retention time and controlled/sustained release of a drug, thereby increasing the concentration of the drug at the application site, potentially improving its bioavailability and reducing the necessary dosage. Site-specific controlled release systems offer many distinctive advantages over classical method of drug delivery.[14-19]These include localized delivery of the drug to a particular part of the body. Controlled release systems that have been developed so exhibit pH-dependent drug release. Hydrogels are three dimensional, hydrophilic, polymer networks capable of imbibing large amounts of water or biological fluids. Chitosan, a natural polysaccharide, exhibits favourable biological properties such as biocompatibility, biodegradability and. For several years' chitosan has been largely evaluated as a potential vehicle for oral dosage forms. Chitosan have been much investigated as a stimulus sensitive drug release system and glutaraldehyde is the most common Crosslinking agent chosen for Chitosan-based hydrogels.

MATERIALS AND METHODS

Licorice drug was gift sample from Amsar private limited, Gurgaon, India. Chitosan (MW=3.5 ×105, >80% deacetylated) was gift sample from Loba Chemie laboratory. Glutaraldehyde and Magnesium stearate, Talc, Starch and Mcc are purchased from Fourrts India Laboratories.

Characterization of drug and hydrogel Fourier Transform Infra-Red Spectroscopy (FTIR)

The prepared chitosan hydrogel pieces were subjected to Fourier transform infrared (FTIR) analysis by KBr hydrogel method using FTIR spectrophotometer, [8201 PC (4000-400/cm), Shimadzu, Japan]. This was employed to ascertain the compatibility of drug with excipients.

Swelling study of hydrogel

The pH-dependent swelling property of hydrogel was studied by chitosan hydrogels in the pH (1.2) HCL buffer for8 hr. After regular intervals of time, hydrogels were removed from the aqueous solution, excess surface water was removed with filter paper, weighed, and returned to the same container until equilibrium was observed. The degree of swelling (Wt) was calculated at different times by means of following equation:

$$\% \text{ swelling} = \frac{\text{Initial weight of the hydrogel} - \text{Final weight of the hydrogel X 100}}{\text{Initial weight of the Hydrogel}}$$

Scanning electron microscopy

The shape and surface characteristics of chitosan hydrogel were determined by SEM using gold sputter technique (ZEISS EV40, Carl Zeiss NTS, North America). Samples of chitosan hydrogel were dusted onto a double-sided tape on an aluminium stub. The stubs containing the sample were coated with gold using a cool sputter coater (Polaran E 5100) to a thickness of 400 A0. Photomicrographs were taken at the accelerated voltage of 20 kV and chamber pressure of 0.6 mmHg.

Particle size and zeta potential analysis

The mean particle size and zeta potential of the Licorice-loaded chitosan hydrogel formulations were determined using Malvern Zetasizer Nano ZS90 (Malvern Instruments Limited, Worcestershire, UK). All the measurements were made in triplicate after dilution (1:200) with

distilled water at room temperature using 90° scattering angle.

Determination of amount of drug entrapped

The amount of drug entrapped in the hydrogels was determined by an indirect method. After the gel preparation washings are collected, filtered with a 0.45µm milipore filter and analyzed by UV spectrophotometry at 254 nm. The difference between the amount of drug initially employed and the drug content in the washing is taken as an indication of the amount of drug entrapped.

% Drug entrapment = A2 / A1 × 100

where,

A1 – Amount of drug initially loaded.

A2 – Amount of drug in washings.

Preparation of Licorice hydrogel

Licorice hydrogel was prepared by chemical crosslinking process of chitosan polymer. Initially chitosan gel was form by mixing chitosan in distilled water and dissolved in 2% acidic acid solution under constant stirring at 50 rpm for 60 min. Simultaneously, the drug solution was added into the chitosan gel and prone to homogenization at 50rpm for 30 min. The different concentration of glutaraldehyde was added. The above mixture was placed in Petri dish and placed in room temperature to form gel. Then prepared gel was washed with acetone solution to remove any unreacted chitosan and crosslinking agent. The solution was filtered and hydrogel was collected. Hydrogels were then dried in air and vacuum, and stored for further use.

Table: 1 Formulation and Composition of Licorice hydrogel

Ingredients	F1 mg	F2 mg	F3 mg	F4 mg	F5 Mg
Licorice	200	200	200	200	200
Chitosan	200	300	400	500	600
Glutaraldehyde	7.5 ml	7.5 ml	10 ml	10 ml	15 ml
Acetic acid	2 %	2 %	2 %	2 %	2 %

Preparation of Licorice Hydrogel Matrix tablet

A total number of 5 formulations were prepared by direct compression method. Controlled release matrix tablet of Licorice was prepared by using the drug and various concentrate of prepared hydrogels. Talc, Starch, Mg. stearate were added as glidant and lubricants, while microcrystalline cellulose was used as diluents. All ingredients were passed through a # 80 sieve, weighed and blended. The lubricated formulations were compressed by direct compression technique. Each tablet weighing 750 mg was formulated.

Table: 2 Licorice Hydrogel Matrix tablet

S.No	F1 Mg	F2 mg	F3 mg	F4 mg	F5 Mg
Hydrogel (equivalent 200 mg of licorice)	200	200	200	200	200
Mcc	125	100	50	20	20
Starch	125	100	50	15	15
Mg.Stearate	50	25	25	7.5	7.5
Talc	50	25	25	7.5	7.5
Avg. wt	750	750	750	750	850

EVALUATION OF PRE-COMPRESSION AND POST COMPRESSION

Swelling study of hydrogel matrix tablet

The pH-dependent swelling property of hydrogel tablet was studied by chitosan hydrogels tablet in aqueous solutions of the pH (1.2) HCL buffer for8 hr. After regular intervals of time, hydrogels were removed from the aqueous solution, excess surface water was removed with filter paper, weighed, and returned to the same container until equilibrium was observed. The degree of swelling (Wt) was calculated at different times by means of following equation.

$$\% \text{ swelling} = \frac{\text{Initial weight of the tablets} - \text{Final weight of the tablets X 100}}{\text{Initial weight of the tablets}}$$

Disintegration time

The disintegration test for hydrogel matrix tablets was carried out using USP XXIII disintegration tester. Six tablets were placed in each tube of the apparatus; the disintegration test was performed in Acidic buffer pH 1.2 as a medium. The temperature of the water bath was maintained at 37 ± 5°C throughout the test. The disintegration time for the tablets was recorded in seconds.

Mucoadhesion study of hydrogel

The mucoadhesive property of prepared chitosan hydrogel was evaluated by *in vitro* mucoadhesive testing method known as wash off method as reported previously. A rat stomach mucosa was tied on the glass slide using a thread. About 25 hydrogel pieces were spread on to wet rinsed tissue specimen and prepared slide was hung on to one of the grooves of a USP tablet disintegration apparatus. By operating the disintegrating test apparatus, he tissue specimen was given a slow regular up and down movement in the test fluid at 37±1°C. At every 1hr-intervalthe equipment was stopped and the number of pieces still adhering to tissue was counted. Percent mucoadhesion was given by the following formula.

$$\% \text{ Mucoadhesion} = P1/P2 \times 100$$

where,

P1- no. of adhered hydrogel pieces

P2- no. of applied hydrogel pieces

Mucoadhesion study of hydrogel Tablet

The mucoadhesive property of prepared chitosan hydrogel Tablet was evaluated by *in vitro* mucoadhesive testing method known as wash off method as reported previously by Shantha and Harding. A rat stomach mucosa was tied on the glass slide using a thread. About 5 hydrogel Tablet were spread on to wet rinsed tissue specimen and prepared slide was hung on to one of the grooves of a USP tablet disintegration apparatus. By operating the disintegrating test apparatus the tissue specimen was given a slow regular up and down movement in the test fluid at 37±1°C. At every 1hr-intervalthe equipment was stopped and the number of Tablet still adhering to tissue was counted. Percent mucoadhesion was given by the following formula.

$$\% \text{ Mucoadhesion} = P1/P2 \times 100$$

Where,

P1- no. of adhered hydrogel tablet

P2- no. of applied hydrogel tablet

In vitro dissolution study

In vitro drug release study was carried out using a USP-1 rotating dissolution tester. The dissolution was measured at 37.0±0.5 °C and 100 rpm speed. The drug release from the tablets was studied in 900 ml acidic medium (pH 1.2) acidic buffer) for (1,2,3,4,6to 12) hrs. At predetermined time intervals, 5 ml aliquots were withdrawn and replaced with the same volume of fresh solution. The amount of drug released was analyzed using a UV-Visible spectrophotometer (Shimadzu-1700, Kyoto, Japan), at λ max of 254 nm.

RESULT AND DISSCUSION

Drug entrapment efficiency

The entrapment efficiency of different hydrogel formulation was calculated as percent total drug entrapped. The entrapment efficiency of licorice in different formulation of hydrogel was found to be 94.2%, 95.1%, 95.47%, 95.89% and 96.89% for F1, F2, F3, F4 and F5 respectively. According to the method of preparation of hydrogel the entrapment efficiency should be 100%, but the observations shows that entrapment efficiency is <100% in all the formulations. This may be due to loss of drug during washing of hydrogel.

Swelling study

The release of the entrapped drug from the hydrogels depends on the swelling behavior because the swelling opens up the pores of network and provides a gateway for drug release. The equilibrium swelling study of the hydrogel was carried out in acidic buffer of pH 1.2. It was observed that the swelling of hydrogel depends upon the concentration of chitosan used. The cross-linked-chitosan hydrogel(F5) was shown highest swelling rate (194.78%). The purpose of measuring swelling index is to determine the ability of hydrophilic polymers used in the formulation to take up water upon hydration. The hydration and swelling behavior of the polymer is crucial because it is necessary to have an intimate contact with the mucosal membrane. The rate of swelling affects the duration of adhesion with faster swelling resulting in adhesion of shorter duration.

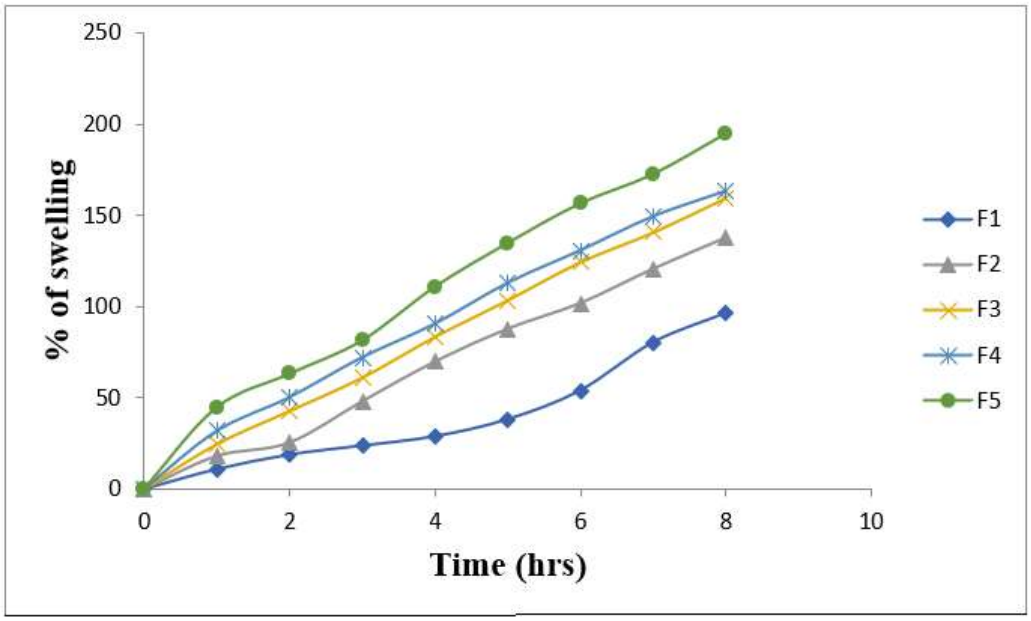

Fig 1 Swelling study of Licorice hydrogel

Scanning electron microscopy (SEM) of Licorice hydrogel

The morphology of the prepared hydrogel was examined using scanning electron microscopy. Figure 2 and 3 showed the surface morphology of the Licorice hydrogel under different magnifications. The SEM image showed that most of the hydrogel are rough and wavy morphology. The roughness of the surface of the hydrogels may be attributed to the presence of licorice.

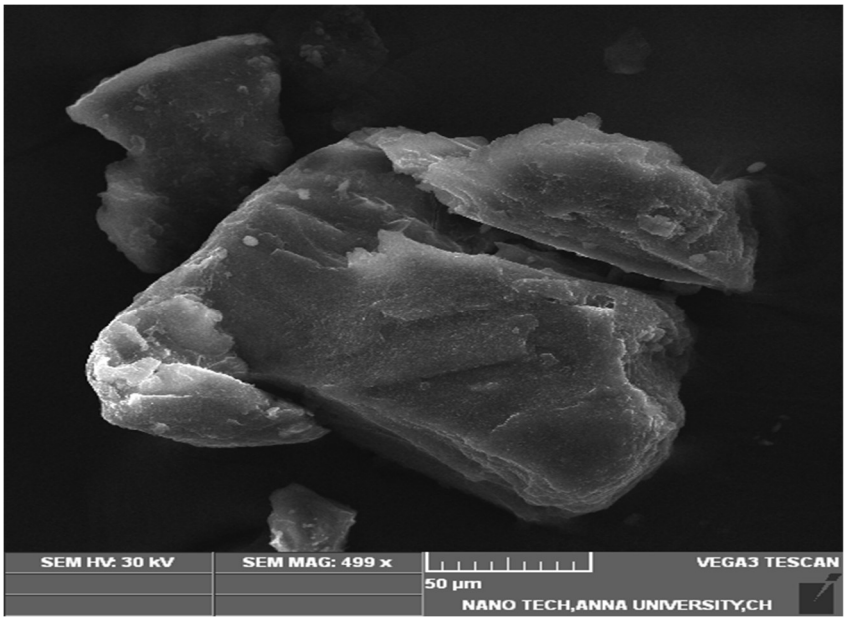

Figure 2 : SEM image of Licorice hydrogel

Particle size analyser

The particle size of hydrogels was found 461.3 µm and PDI was found 0.072. The relative charge beyond the hydrodynamically stagnant layer of the hydrogels was determined by zeta potential measurements. Formulation (F5) was shown negative potential (-3.07) due to Crosslinking with glutaraldehyde. The hydrogels with higher potential values have a higher charge density of the amino groups on the surface, as in the case of hydrogels produced with higher chitosan content. In the acidic region, the chitosan amino groups are protonated, resulting in relatively high values of potentials.

Compatibility study of FTIR

FTIR spectrum of Licorice was recorded and characteristic peaks were observed. The descriptions of the observed peak of chitosan spectrum exhibits band at 3379.05 (OH stretching) and 3436.91 (-NH2 stretching). The absorption band at 1118.64 (asymmetric stretching of C-O-C bridge) and 2923.88 (-CH2 stretching). The band at 1652.88 due the chitosan spectrum was attributed to the formation of C=N, due to imine reaction between amino group of chitosan and glutaraldehyde.

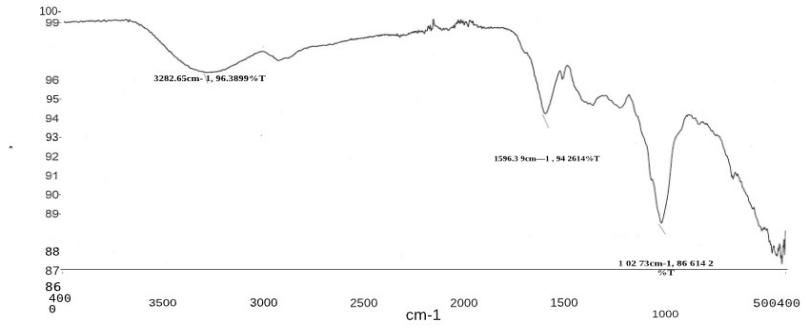

Figure 3. FTIR of Licorice Drug

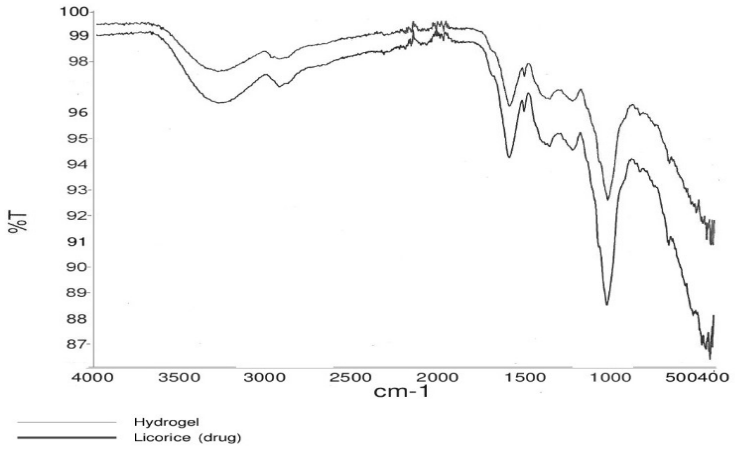

Figure 4. FTIR Test of Licorice loaded Hydrogel

Table 3. Post-compression evaluation parameters

Formulations	Thickness (mm)	Hardness	Weight Variations (%)	Friability (%)	Drug content (%)
F1	4.95± 0.11	5.4±0.11	751±5	0.197	97.80±0.32
F2	5.01± 0.04	6.3±0.14	748±5	0.292	98.01±0.15
F3	4.90±0.12	5.1±0.20	753±5	0.164	98.58±0.24
F4	5.03±0.02	6.5±0.18	746±5	0.158	99.12±0.84
F5	5.06±0.23	6.10±0.15	754±5	0.193	97.49±0.75

Swelling index of hydrogel matrix tablet

The swelling degree of the licorice hydrogel tablet was evaluated at pH 1.2 simulating gastric media. The results showed that the hydrogels tablet had different swelling degrees at pH 1.2 according to the concentration of chitosan. Fig. 3shows the average weight variation of the hydrogels tablet at (pH 1.2) among all the formulations, F5 showed highest swelling rate due to consist of higher concentration of chitosan. This result revealed that chitosan chains at low pH take expanded forms due to the intermolecular repulsions between the positively charged amino groups, leading to the network expansion.

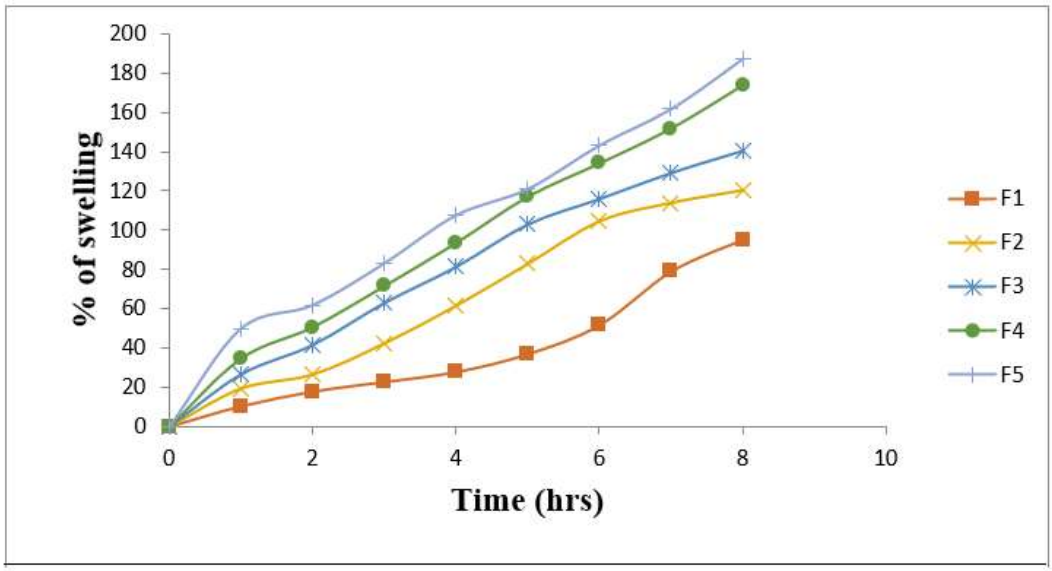

Figure 5. Swelling study of hydrogel matrix tablet

Mucoadhesion study of hydrogel

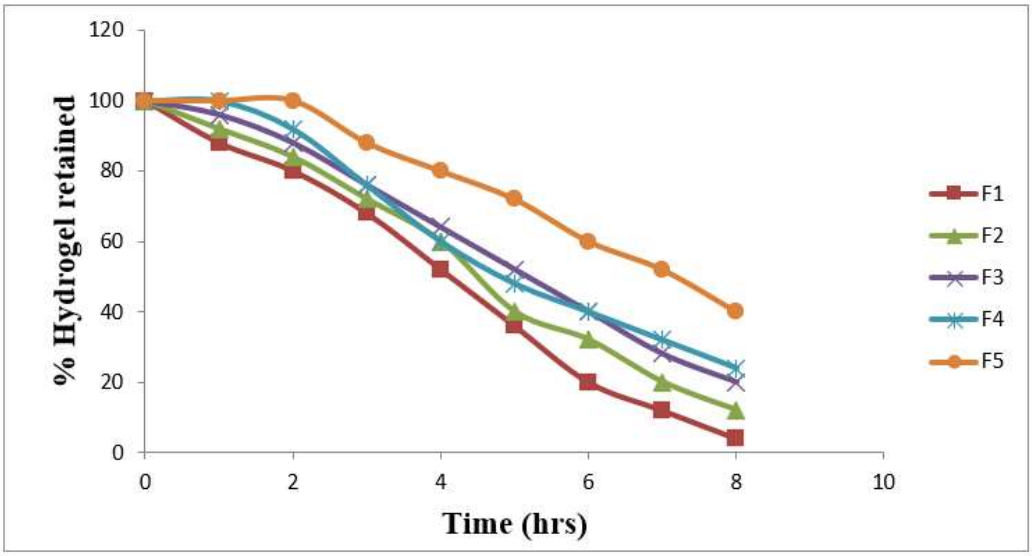

Figure 6. % Mucoadhesion of Licorice hydrogel

The mucoadhesive property of the hydrogels was evaluated by wash-off method. At the end of 08hr, % mucoadhesion was found to4%, 12%, 20%, 24%, 40%.for F1, F2,F3,F4,F5 formulations respectively. Formulation F5 shows highest % Mucoadhesion upto 8 hrs. The basis of mucoadhesion is that adosage form can stick to the mucosal surface. A salt bridge effect has been proposed for the interaction of the positively charged mucoadhesive hydrogel particles with the negatively charged mucous glycoprotein. Chitosan possesses OH andNH_2 groups that can give rise to hydrogen bonding. These properties are considered essential for Mucoadhesion. Further cationic polyelectrolyte nature of Chitosan could provide a strong electrostatic interaction with mucosal

surface. The rank order of mucoadhesion for formulations was to be F1 > F2 > F3 > F4>F5.

Mucoadhesion study of hydrogel tablet

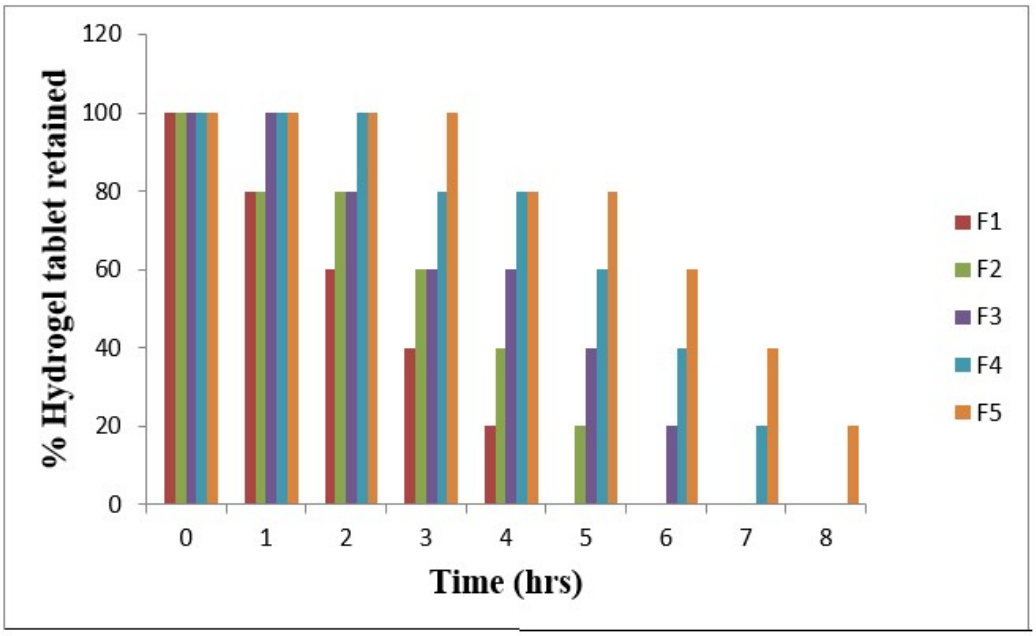

Figure 7. % Mucoadhesion of hydrogel tablet

The mucoadhesive property of the hydrogels Tablet was evaluated by wash-off method. Formulation F5 showed highest duration of mucoadhesion activity upto8 hrs. Whereas all others formulation could not adhere the membrane for long duration time period.

Mucus is a viscoelastic gel lining the mucosal tissues exposed to the external environment in gastrointestinal tract. Mucins are the main component of the mucus, which are glycoproteins responsible for its gel like characteristics. These glycoproteins are made of protein core to which carbohydrate side chains are covalently attached via o-glycosidic linkages. Conventional (non-mucoadhesive) formulations lack the ability to withstand the strong involuntary muscular movement as well as the extensive washing effects. The limitations lead to the loss of substantial amount of the administered drug at the site of applications. The mucoadhesive properties of the licorice hydrogel tablets may enhance the residence time of the drug, increases the concentration gradient at the site of action and this could lead to target H-Pylori efficiently.

In-vitro Dissolution study

The drug release studies of Licorice hydrogel matrix tablets were carried out in 0.1N HCl (pH 1.2) for 12 hr. The prolonged percentage of release of Licorice was found of formulation (F5), its containing higher the polymer and crosslink agent concentration. Due to concentration of chitosan polymer was increased. The rate of drug release was prolonged upto 12 hrs. The diffusion of Licorice from hydrogel containing chitosan was enhanced because of swelling at lower pH. The extent of release was increased as the hydrogel swelling increase at lower pH, which leads to ionization of amino groups. This selective release may ensure maximum availability of the drug in the stomach thereby maintaining bactericidal concentration of the antibiotic in the stomach. The formulation (F5) show best release profile and it is released about 96.15 in 12 hrs, so justifying itself as an optimized formulation in terms of drug release profile.

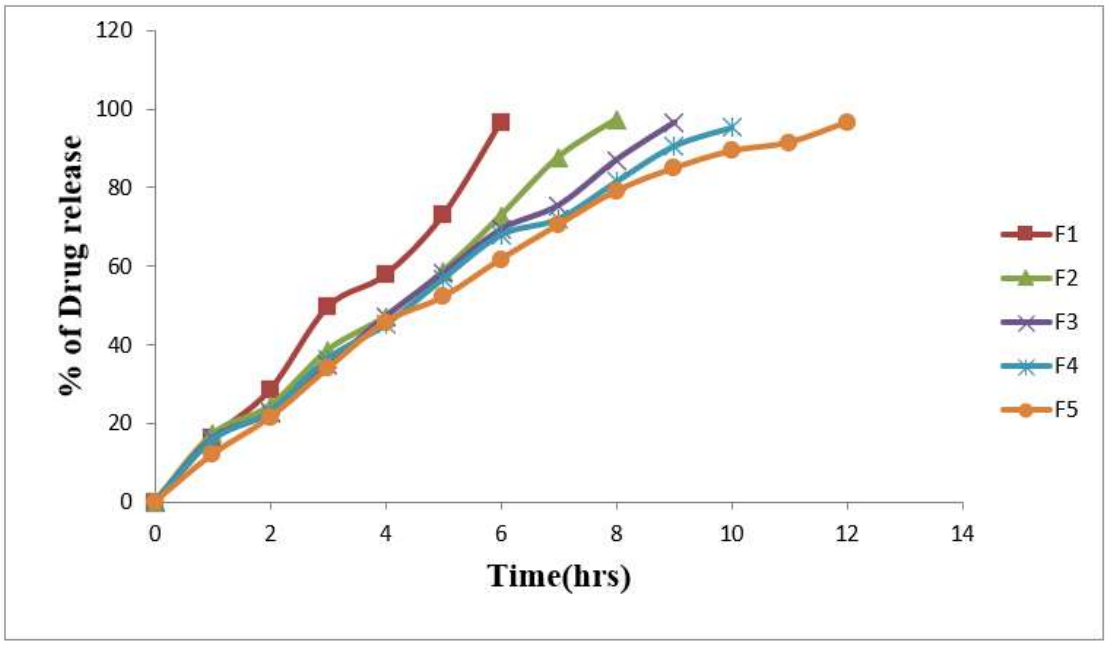

Figure 8. Dissolution profile for chitosan based Licorice hydrogel

CONCLUSION

It shows that increase in concentration of polymer leads to the controlled drug release from hydrophilic chitosan hydrogelfor12hrs, which means release rate from hydrophilic chitosan hydrogel depends on type and concentration of polymer used in the formulation. Hydrogel formulation (F5), containing chitosan and Crosslinking agent of Glutaraldehyde is probably showing release upto 96.2±0.65% within 12 hrs. The hydrogel prepared maintain drug concentration in stomach for prolonged period of time, can be used as a drug delivery system for treatment of *H. pylori* infection and in management of peptic ulcer. According to stability study it was found that there was no significant change in average weight, drug content and *in vitro* dissolution of optimized formulation (F5). This can be expected to reduce the frequency of administration and decrease the dose dependent side effects. The efficacy and safety of Licorice hydrogel dosage form are expected to offer optimum therapeutic efficacy and improved patient compliance.

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