FORMULATION AND EVALUATION OF CHITOSAN BASED HYDROGEL MATRIX OF LICORICE FOR TARGETING *HELICOBACTER PYLORI*

A Dissertation submitted to

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IN
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DECLARATION

I hereby declare that the thesis entitled, "**FORMULATION AND EVALUATION OF CHITOSAN BASED HYDROGEL MATRIX OF LICORICE FOR TARGETING *HELICOBACTER PYLORI***" has been originally carried out under the guidance and supervision of **Dr. Ubaidulla, M. Pharm., Ph.D.,** Department of Pharmaceutics, **C.L. Baid Metha College Of Pharmacy,** Chennai 97 and **Mr. M. THIRUMARAN, M. Pharm Manager (FR&D) Fourrts (India) Laboratories Pvt, Ltd., Kelambakkam Chennai-603103** during the academic year 2019. This work has not been submitted in any other degree at any other university and that all other sources we have used or quoted have been indicated and acknowledged by complete reference.

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1.INTRODUCTION

1.1 *Helicobacter pylori*

Helicobacter pylori are Gram negative, spiral, microaerophilic, multiflagellate bacterium found on the human gastric mucosa. *Helicobacter pylori* live and grow in an environment so acidic that for years gastroenterologists considered it to be sterile. *Helicobacter pylori* used a dual strategy in order to survive in the harsh conditions that prevail in the human stomach. First, the bacteria have multiple polar flagellate, tail-like structures with which to propel through the mucus layer lining the stomach until they can attach to the cells at the bottom of the lining**.[1]**

This is accomplished by Urease, a powerful enzyme made by *H. pylori*, which converts urea, a chemical made by stomach cells, to carbon dioxide and ammonia. Those chemical products formed by the enzymatic action of Urease neutralize the acidity in the mucus immediately surrounding the bacteria, creating a non-acidic micro zone that protects the bacteria. Helicobacter pylori are non-invasive, but colonize in the human stomach's antral region and gastric mucosal surfaces where they release pathogenic proteins that induce cell injury and inflammation **[2].** As a result of these changes, the stomach and duodenum become more vulnerable to damage from digestive juices, such as stomach acid.

1.2 *Helicobacter pylori* pathogenesis in humans

Helicobacter pylori infection is common (global prevalence – Asia: 50–80%, Africa: 70–90%, USA: 30%, eastern Europe: 70%, western Europe: 30–50% and Australia: 20%) and infects almost half the world's population. Age, ethnicity, gender, geography and socio-economic status are all factors that influence the incidence and prevalence of H. pylori infection.**[3]**

Approximately one in 10 *H. pylori*-infected individuals will develop disease, including peptic ulcer disease, and in the worst case 1 in 100 will develop gastric cancer. Whether infection will lead to symptoms (or not) is dependent both on the host and on the bacterial genetics. If remained untreated, the *H. pylori* infection lasts for a lifetime and involved in the pathogenesis of different gastroduodenal diseases, such as chronic active gastritis, duodenal and gastric ulcers, and gastric neoplasia**.[4]** The people infected with H. pylori could not get rid of it, but instead remained infected for life unless they were treated with antibiotics.

Because H.pylori produces chemical components in their cell walls that are very much like molecules made by the stomach cells of the host

1.3 Diagnosis testing of *Helicobacter pylori*

1.3.1 Invasive testing

Invasive testing includes histopathological diagnosis, biopsy urease testing, brush cytology and real time polymerase chain reaction (RT-PCR). Histology is indicated as at times proton pump inhibitors may reduce the sensitivity of other modalities of diagnosis. It is important to establish gastritis and even detect intestinal metaplasia or MALT lymphoma. *H. pylori* generally has variable distribution in the stomach and hence a combination of four biopsy sites (lesser and greater curvature of the mid antrum and the mid body) was deemed optimal for adequate detection. Biopsy urease testing is performed on the biopsy specimen and is less expensive than the histopathology. False positive is rare with 90 to 95& sensitivity and 95 to 100% specificity. Brush Cytology is rarely used. It can be considered in patients with bleeding disorders. Real-time polymerase chain reaction (RT-PCR). Biopsy specimens of refractory cases of *H. pylori* could benefit from RT-PCR and in situ hybridization techniques.**[5]**

1.3.2 Non-invasive testing

Non-invasive testing methods include urease breath testing, serology, stool testing, salivary assays, urinary assays and 13C-urea serodiagnosis testing. *H. pylori* produce urease that splits urea to ammonia and carbon dioxide. This carbon atom is tagged and detected on breath samples. For the urease breath test the patient should ideally be off antibiotics for 4 weeks and off PPi's for 2 weeks. The sensitivity is 88-95% while the specificity is 95-100%]. ELISA serology is used to detect IgG antibodies. The confirmation of *H. pylori* eradication is indicated in certain conditions but presently recommended to be performed in all cases due to the easy availability, inexpensive and noninvasive methods. As per the ACG (American College of Gastroenterology) guidelines from 2007the confirmation of eradication is indicated in Patients with *H. pylori* associated ulcer, Persistent dyspepsia after treatment, H. pylori associated MALT lymphoma or anyone who has undergone resection of early gastric

cancer. UBT performed after 4 weeks of antibiotic therapy is the test of choice. Stool antigen testing is less accurate**.[6]**

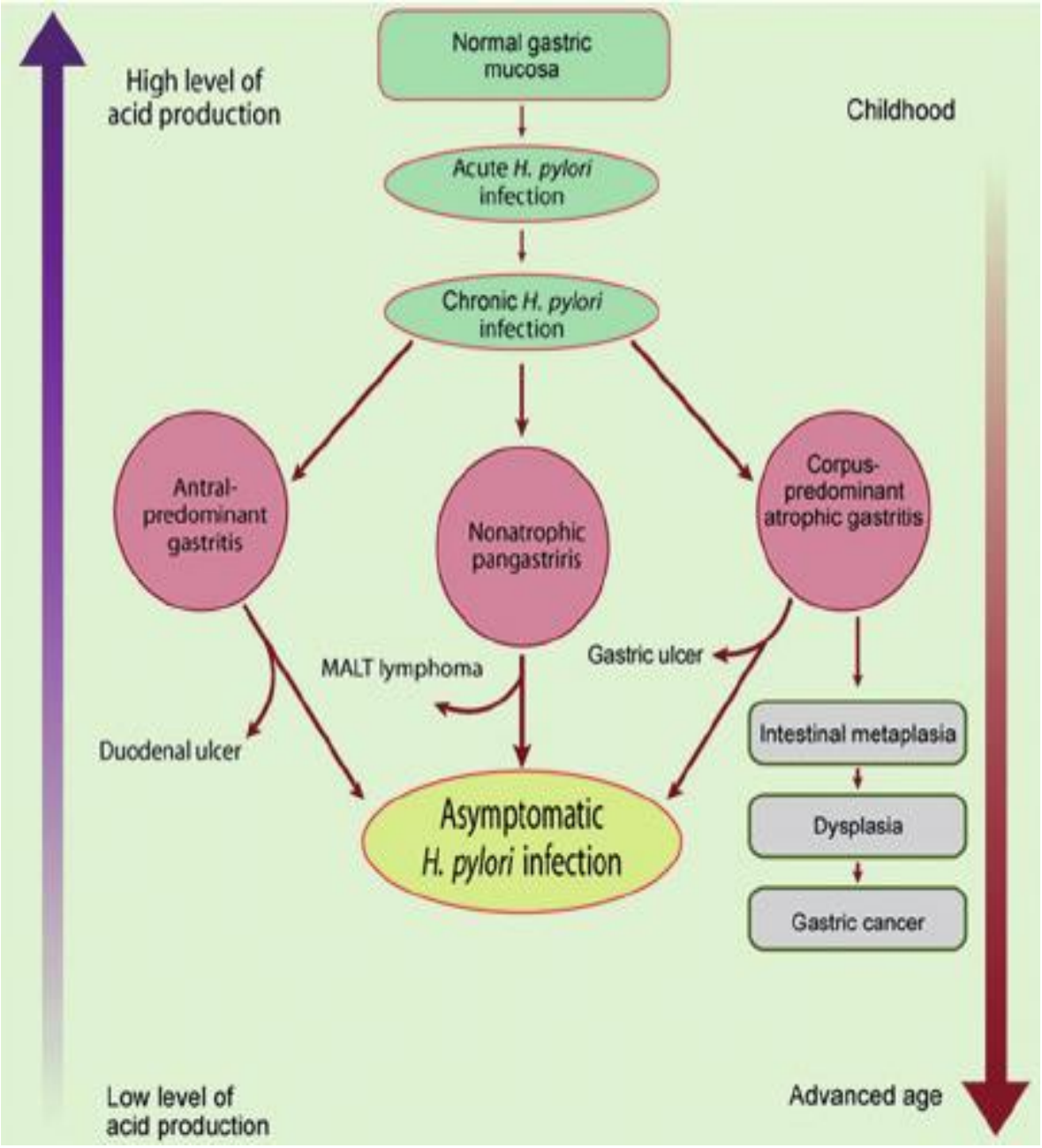


Fig No: 1 Asymptomatic of *Helicobacter Pylori* Cycle

1.4 Hydrogels

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical crosslink (tie-points, junctions), or physical crosslink, such as entanglements or crystallites [4±9]. The latter provide the network structure and physical integrity. These hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media**.[7]**

There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical sectors [13± 15]. Hydrogels resemble natural living tissue more than any other class of synthetic biomaterials. This is due to their highly water contents and soft consistency which is similar to natural tissue. Furthermore, the highly water content of the materials contributes to their biocompatibility**.[8]**Thus, hydrogels can be used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and drug delivery devices.

1.5 Classification of hydrogel products

The hydrogel products can be categorized as described below:

1.5.1 Classification based on source

Hydrogels can be classified into two groups based on their natural or synthetic origins

1.5.2 Classification according to polymeric composition

The technique of preparation leads to formations of principal classes of hydrogels. These can be represented as following**:[9]**

1.5.3 Homopolymer hydrogels

These are referred to polymer network which are derived from a single species of monomer, which is the basic structural unit comprising of any polymer network Homopolymers may have cross-linked skeletal structure dependent on the nature of the monomer and polymerization method.

1.5.4 Co polymeric hydrogels

These are consisted of two or more distinct monomer species with at least one hydrophilic component, assembled in a random, block oral treating configuration along the chain of the polymer network

1.5.5Multipolymer:

These are also called as interpenetrating polymeric chitosan based hydrogel, an important class of hydrogels, which is made of two independent cross-linked synthetic/or natural polymer component, confined in a network form. In semi-IPN hydrogel, one componentis a cross linked polymer and another component is a non-cross-linkedpolymer**.** **[10]**

1.5.6 Classification based on type of cross-linking

Hydrogels can be divided into two groups on the basis of their chemical or physical behavior of the cross-link junctions. Chemically cross-linked networks have stable junctions, while physical networks have temporary junctions that results from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds or hydrophobic interactions.

1.6 Physical, chemical and toxicological properties of hydrogels

1.6.1 Factors affecting swelling of hydrogels

The Crosslinking ratio is one of the most important factors that affects the swelling of hydrogels. It is defined as the ratio of moles of Crosslinking agent to the moles of polymer repeating units. The higher the Crosslinking ratio, the more Crosslinking agent is incorporated in the hydrogel structure. Highly cross-linked hydrogels have a tighter structure,

and will swell less compared to the same hydrogels with lower Crosslinking ratios. Crosslinking hinders the mobility of thepolymer chain, hence lowering the swelling ratio. Swelling of environmentally sensitive hydrogels can be affected by specific stimuli. Swelling of temperature-sensitive hydrogels can be affected by changes in the temperature of the swelling media. Ionic strength and pH affect the swelling of ionic strength- and pH-sensitive hydrogels, respectively there are many other specific stimuli that can affect the swelling of other environmentally responsive hydrogels**. [11]**

1.6.2 Dynamics of swelling

The swelling kinetics of hydrogels can be classified as diffusion-controlled (Fickian) and relaxation-controlled (non-Fickian) swelling. When water diffusion into thehydrogel occurs much faster than the relaxation of the polymer chains, the swelling kinetics is diffusion-controlled. Annice mathematical analysis of the dynamics of swelling is presented by Peppa sand Colombo.

1.6.3 Mechanical properties

However, a higher degree of Crosslinking creates a more brittle structure. Hence, there is a Mechanical property of hydrogels are very important for pharmaceutical applications. For example, the integrity of the drug delivery device during the lifetime of the application is very important to obtain FDA approval, unless the device is designed as a biodegradable system. A drug delivery system designed to protect a sensitive therapeutic agent, such as protein, must maintain its integrity to be able to protect the protein until it is released out of the system. Changing the degree of Crosslinking has been utilized to achieve the desired mechanical property of the hydrogel. Increasing the degree of Crosslinking of the system will result in a stronger gel. Optimum degree of Crosslinking to achieve a relatively strong and yet elastic hydrogel.

1.6.4 Cytotoxicity and in-vivo toxicity

Cell culture methods, also known as cytotoxicity tests, can be used to evaluate the toxicity of hydrogels. Three common assays to evaluate the toxicity of hydrogels include extract dilution, direct contact and agar diffusion**.[12]** Most of the problems with toxicity

associated with hydrogel carriers are the uncreated monomers, oligomers and initiators that leach out during application. Therefore, an understanding he toxicity of the various monomers used as the building blocks of the hydrogels is very important. The relationship between chemical structures and the cytotoxicity of acrylate and methacrylate monomers has been studied extensively. Several measures have been taken to solve this problem, including modifying the kinetics of polymerization in order to achieve a higher conversion, and extensive washing of the resulting hydrogel. The formation of hydrogels without any initiators has been explored to eliminate the problem of the residual initiator. The most commonly used technique has been gamma irradiation [52±56]. Hydrogels of PVA have been also made without the presence of initiators by using thermal cycle to induce crystallization. The crystals formas physical Crosslink's. These crystals will be able to absorb the load applied to the hydrogels.

1.7 Hydrogel technical features

- The highest absorption capacity (maximum equilibrium swelling) in saline.
- Desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
- The highest absorbency under load (AUL).
- The lowest soluble content and residual monomer.
- The lowest price.
- The highest durability and stability in the swelling environment and during the storage.
- The highest biodegradability without formation of toxic species following the degradation. pH-neutrality after swelling in water.
- Colorlessness, odor lessness, and absolute non-toxic,Photo stability.
- Re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it; depending on the application requirement (e.g., in agricultural or hygienic applications)**[13]**

1.8 Characterization hydrogels

Generally hydrogels are characterized for their morphology, swelling property and elasticity. Morphology is indicative of their porous structure. Swelling determines the release mechanism of the drug from the swollen polymeric mass while elasticity affects the mechanical strength of the network and determines the stability of these drug carriers (Khare and Peppas,1995). Some of the important features for characterization of hydrogels are as follows:

1.8.1 Morphological characterization

Hydrogels are characterized for morphology which is analyzed by equipment like stereomicroscope. Also the texture of these biomaterials is analyzed by SEM to ensure that hydrogels, especially of starch, retain their granular structures **[14]**

1.8.2 Swelling behavior

The hydrogels are allowed to immerse in aqueous medium or medium of specific pH to know the swell ability of these polymeric networks. These polymers show increase in dimensions related to swelling.

1.8.3 Rheology

Hydrogels are evaluated for viscosity under constant temperature of usually 4°C by using Cone Plate type viscometer **[15]**. Polymer solutions are essentially viscous at low frequencies, tending to fit the scaling laws: $G' \sim \omega^2$ and $G'' \sim \omega$. At high frequencies, elasticity dominates ($G' > G''$). This corresponds to Maxwell-type behaviour with a single relaxation time that may be determined from the crossover point and, this relaxation time increases with concentration.

1.8.4 Scanning Electron Microscopy (SEM)

SEM can be used to provide information about the sample's surface topography, composition, and other properties such as electrical conductivity. Magnification in SEM can be controlled over a range of up to 6 orders of magnitude from about 10 to 500,000 times. This is a powerful technique widely used to capture the characteristic ‘network’ structure in hydrogels.

1.8.5 FTIR

FTIR (Fourier Transform Infrared Spectroscopy) is a useful technique for identifying chemical structure of a substance. It is based on the principle that the basic components of a substance, i.e. chemical bonds, usually can be excited and absorb infrared light at frequencies that are typical of the types of the chemical bonds. The resulting IR absorption spectrum represents a fingerprint of measured sample. This technique is widely used to investigate the structural arrangement in hydrogel by comparison with the starting materials

1.9 Pharmaceutical applications of hydrogels

To provide sustained or controlled drug delivery into systems, the hydrogels are designed, modulated and characterized for the expected in-vivo results. These hydrogels have gained existence in drug delivery through parenteral, ocular, rectal, vaginal, dermal and nasal routes some of the important pharmaceutical applications of hydrogels are discussed below.

1.9.1 Brain drug delivery

Permanent functional loss usually occurs after injury to the spinal cord. Currently, a clinical strategy to promote regeneration in the injured spinal cord does not exist. In order to promote regeneration, a growth permissible substrate at the injury site is critical. Jain and co-workers have reported the utilization of an agarose scaffold that gels in situ conformally filling an irregular, dorsal over-hemi section spinal cord defect in adult rats. To simultaneously deliver multiple neurotrophies with individual release rates as vehicles for the delivery of growth factors to promote the regeneration of diseased or damaged tissue unique hydrogels composites have been developed. In the central nervous system, there are many instances, where the delivery of neurotrophies has great potential in tissue repair, especially

for treatment of spinal cord injury. Verreck and co-workers have developed a biodegradable drug-loaded nerve guide for peripheral nerve regeneration. **[17]**

1.9.2 Ocular drug delivery

In ocular drug delivery, many physiological constraints prevent a successful drug delivery to the eyes, due to its protective mechanisms, such as effective tear drainage, blinking and low permeability of the cornea. Thus, conventional eye drops containing a drug solution tend to be eliminated rapidly from the eye, and the drug administered exhibit limited absorption, leading to the poor ophthalmic bioavailability. Additionally, their shortterm retention often results in a frequent dosing regimen to achieve the therapeutic efficacy for a sufficiently long duration. These challenges have motivated researchers to develop drug delivery systems that provide a prolonged ocular residence time of drugs. Certain dosage forms, such as suspensions and ointments, can be retained in the eye, although these sometimes give patients an unpleasant feeling because of the characteristics of solids and semi-solids. Hydrogels based on weakly crosslinked HEMA have been used as re-loadable soft contact lenses for administration of timolol and have been characterized by determination of their swelling and drug release kinetics in 0.9% NaCl solution and Acidic buffer (1.2). Both water uptake and timolol release exhibited Fickian kinetics. The results indicated that the incorporation of methacrylic acid as co monomer increased the timolol loading capacity to therapeutically useful levels while retaining appropriate release characteristics. Due to their elastic properties, hydrogels can also represent an ocular drainage resistant device. In addition, they may offer better feeling, with less of a gritty sensation to patients**.[18]**

1.9.3Ear drug delivery

For certain patients who experience intense vertigo arising from unilateral vestibular lesions, the primary therapy is a vestibular nerve section, an intracranial surgical procedure. One alternative to this treatment is therapeutic ablation of vestibular function on the unaffected side using an ototoxic agent. Kelly and co-workers have prepared a biodegradable sustained release gel delivery system using sodium hyaluronate that can be administered into the middle ear using only a local anesthetic. The gel contain gentamycin sulphate, the ototoxic agent of choice for treatment of unilateral vestibulopathy, and it exhibited diffusion-controlled release of drug over a period of hours. The released gentamycin could then diffuse into the inner ear through the round membrane. By carefully controlling the dose, it is possible to inhibit vestibular function while minimizing hearing loss. In another observation

cross-linked hyaluronic acid hydrogels have been evaluated for their ability to elicit new micro vessel growth in vivo when preloaded with one of two cytokines, vascular endothelial growth factor or basic fibroblast growth factor. Hyaluronic acid film samples have been surgically implanted in the ear pinna's of mice, and the ear retrieved 7or 14 days post implantation have showed significantly more micro vessel density than control ears undergoing surgery but receiving no implant. Endo and co-workers have studied the efficacy of a biodegradable hydrogel as a sustained drug delivery of brain-derived neurotrophic factor into the cochlear fluid of the inner ear. The functional and histological protection of the auditory primary neurons by brain- derived neurotrophic factor applied through the hydrogel has significantly reduced the threshold elevation.

1.9.4 Rectal Drug delivery

The rectal route has been used to deliver many types of drugs, although patient acceptability is variable due to discomfort arising from administered dosage forms. Its primary application has been for local treatment of diseases associated with the rectum, such as hemorrhoids. Additionally, it is well known that drugs absorbed from the lower part of rectum drain into the systemic circulation directly. Thus, the rectal route is a useful administration route for drugs suffering heavy first –pass metabolism. Conventional suppositories hitherto adapted as dosage forms for rectal administration are solids at room temperature, and melts or soften at body temperature. A problem associated with rectal administration using conventional suppositories is that drugs diffusing out of the suppositories in an uncontrolled manner are unable to be sufficiently retained at a specific position in the rectum, and sometimes migrate upwards to the colon. This often leads to a variation of the bioavailability of certain drugs, in particular, for drugs that undergoes extensive first-pass elimination. In this context, hydrogels may offer a valuable way to overcome the problems in conventional suppositories, provided that they are designed to exhibit a sufficient bio adhesive property following their rectal administration**.[19]**

1.9.5 Transdermal drug delivery

Drug delivery to the skin has been traditionally conducted for topical use of dermatological drugs to treat skin diseases, or for disinfections of skin itself. In recent years, a transdermal route has been considered as a possible site for possible site for systemic delivery of drugs. The possible benefits of transdermal drug delivery include that drugs can

be delivered for a long duration at a constant rate, that drug delivery can be easily interrupted on demand by simply removing the devices, and that drug can bypass hepatic first -pass metabolism. Furthermore, because of their highly water contents, swollen hydrogels can provide a better feeling for the skin in comparison to conventional ointments and patches.

1.9.6 Subcutaneous drug delivery

A subcutaneous drug delivery system, which consists of a polymeric matrix of poly(hydroxyethyl methacrylate-bisglycol acrylate),has been developed for the delivery of 5-fuorouracil. Subcutaneously inserted exogenous materials may more or less evoke potentially undesirable body responses, such as inflammation, carcinogenicity and immunogenicity. Therefore, biocompatibility is a prerequisite that makes materials implantable. Due to their highly water content, hydrogels are generally considered as biocompatible material. They also provide several promising properties like minimal mechanical irritation upon in-vivo implantation, due to their soft,elastic properties; broad acceptability for individual drugs with different hydrophilic ties and molecular sizes**.[20]**

1.9.7 Wounds drug delivery

Healing under the wet environment of the hydrogel dressing has some advantages, which include faster healing rate, easier to change the dressing, i.e. hydrogel can be peeled off without any damage to the regenerated surface and no dressing material remains on the wound. Hydrogels also help to maintain a moist wound environment recognized as being beneficial in wound healing by promoting natural debridement, hydrating necrotic tissue and loosening and absorbing slough and exudates in a variety of wounds. Subcutaneous implantation studies in mice have shown in vivo the hydrogels are biocompatible since the foreign body reaction seen around the implanted hydrogel sample is moderate and became minimal upon increasing implantation time**. [21]**

2. LITERATURE REVIEW

1. **Radhika.et. al., (2019)[22]** reviewed hydrogels and their applications in targeted drug delivery. Conventional drug delivery approaches are plagued by issues pertaining to systemic toxicity and repeated dosing. Hydrogels offer convenient drug delivery vehicles to ensure these disadvantages are minimized and the therapeutic benefits from the drug are optimized. With exquisitely tunable physical properties that confer them great controlled drug release features and the merits they offer for labile drug protection from degradation, hydrogels emerge as very efficient drug delivery systems. The versatility and diversity of the hydrogels extend their applications beyond targeted drug delivery also to wound dressings, contact lenses and tissue engineering to name but a few. They are 90% water, and highly porous to accommodate drugs for delivery and facilitate controlled release. Herein we discuss hydrogels and how they could be manipulated for targeted drug delivery applications. Suitable examples from the literature are provided that support the recent advancements of hydrogels in targeted drug delivery in diverse disease areas and how they could be suitably modified in very different ways for achieving significant impact in targeted drug delivery. With their enormous amenability to modification, hydrogels serve as promising delivery vehicles of therapeutic molecules in several disease conditions, including cancer and diabetes.

2. **Liu Y.et. al., (2019)[23]** Investigated co-transplantation a chitosan (CS) thermo sensitive hydrogel with bone marrow-derived mesenchymal stem cells (BMSCs) could optimize and maximize the therapeutic of BMSCs in a mouse model of MI. The fate of transplanted BMSCs was monitored by bioluminescence imaging (BLI) and the recovery of cardiac function was detected by echocardiogram. Our results proved that CS hydrogel enhanced the BMSCs survival and the recovery of cardiac function by protecting the vascular endothelial cells. Further studies revealed that the increased number of vascular endothelial cells was due to the fact that transplanted BMSCs inhibited the inflammatory response and alleviated the pyroptosis of vascular endothelial cells. In conclusions, CS hydrogel improved the engraftment of transplanted BMSCs, ameliorated inflammatory responses, and further promoted functional recovery of heart by alleviating vascular endothelial cell pyroptosis.

3. **Oliveira Gonçalves.et. al., (2019)[24]** developed one promising adsorbent based on chitosan hydrogel scaffold modified with carbon nanotubes, for food dye removal in single and binary aqueous systems. The modified hydrogel scaffold was characterized in relation to the gel strength, swelling degree, surface attributes, and infrared spectrum. Adsorption isotherms were performed using dyes, food red 17 (FdR17) and food blue 1 (FdB1), in single and binary aqueous systems. The experimental data were adjusted to the Langmuir model and the thermodynamic parameters were estimated. The kinetic behavior was evaluated and, desorption studies were performed to verify the reuse capacity of the modified hydrogels scaffold. The results showed that maximum adsorption capacities were of 1508 and 1480 mg g^{-1} for the single system and of 955 and 902 mg g^{-1} for the binary system, for FdB1 and FbR17, respectively. The thermodynamic parameters indicated that the adsorption was the spontaneous, exothermic and favorable process. The model that best represented the kinetic data was that of Avrami. In desorption, the adsorbent can be used until four times and maintaining the adsorption capacity of the adsorbent in 71% of the initial capacity.

4. **Nie L.et. al., (2019)[25]** Studied the chitosan/gelatin hydrogel incorporated with biphasic calcium phosphate nanoparticles (BCP-NPs) as scaffold (CGB) for bone tissue engineering was reported in this article. Such nanocomposite hydrogels were fabricated by using cycled freeze-thawing method, of which physicochemical and biological properties were regulated by adjusting the weight ratio of chitosan/gelatin/BCP-NPs. The needle-like BCP-NPs were dispersed into composites uniformly, and physically cross-linked with chitosan and gelatin, which were identified via Scanning Electron Microscope (SEM) images and Fourier Transform Infrared Spectroscopy (FT-IR) analysis. The porosity, equilibrium swelling ratio, and compressive strength of CGB scaffolds were mainly influenced by the BCP-NPs concentration. *In vitro* degradation analysis in simulated body fluids (SBF) displayed that CGB scaffolds were degraded up to at least 30 wt% in one month. Also, CCK-8 analysis confirmed that the prepared scaffolds had a good cytocompatibility through in culturing with bone marrow mesenchymal stem cells (BMSCs). Finally, *In vivo* animal experiments revealed that new bone tissue was observed inside the scaffolds, and gradually increased with increasing months, when

implanted CGB scaffolds into large necrotic lesions of rabbit femoral head. The above results suggested that prepared CGB nanocomposite had the potential to be applied in bone tissue engineering.

5. **Mohammad ZadehPakdel P.et. al., (2018)[26]** reviewed, chitosan has been used as a raw material for synthesis of hydrogels in a wide range of potential and practical applications like wastewater treatment, drug delivery, and tissue engineering. This review represents an overview of the application of chitosan-based hydrogels for wastewater treatment and helps researchers to better understand the potential of these adsorbents for wastewater treatment. It covers recently used and prospected methods for synthesis and modification of these hydrogels. Chitosan-based hydrogels are modified physically and chemically through crosslinking, grafting, impregnation, incorporating of hard fillers, blending, interpenetrating, and ion-imprinting methods to improve adsorption and mechanical properties. Understanding of these methods provides useful information in the design of efficient chitosan-based hydrogels and the select of appropriate pollutants for removal. This review provides a brief outlook on future prospects of chitosan-based hydrogels for wastewater application

6. **Hamid Hamedi.et. al., (2018)[27]** reviewed advanced development of chitosan hydrogels has led to new drug delivery systems that can release their active ingredients in response to environmental stimuli. This review considers more recent investigation of chitosan hydrogel preparations and the application of these preparations for drug delivery in wound dressings. Applications and structural characteristics of different types of active ingredients, such as growth factors, nanoparticles, nanostructures, and drug loaded chitosan hydrogels are summarized.

7. **Rahul Rama Hegde.et al., (2016)[28]** Prepared by This gastro pathogen has been regarded as serious public health problem due to its association with dyspepsia, gastritis, gastroduodenal ulcers, mucus-associated lymphoid tissue lymphoma and gastric carcinoma. In vivo eradication of established *H. pylori* infections is difficult due to several factors such as gastric niche, coccid form due to sub-minimum inhibitory concentration of antimicrobials, bacterial load, primary antibiotic resistance, patient compliance and stability of therapeutics in gastric acid secretion.

8. **Wu JR.et. al., (2015)[29]** Prepared from polysialic acid (PSA) and carboxy methyl chitosan (CMCS) using glutaraldehyde as the cross-linking agent. The resulting PSA-CMCS hydrogel exhibited pH sensitivity, in which the swelling ratio under acidic conditions was higher than those under neutral or alkaline conditions. The swelling ratio of PSA-CMCS hydrogel at equilibrium depended on the medium pH, the cross-linking agent concentration, and the ratio of PSA to CMCS (w/w). Bovine serum albumin (BSA) and 5-fluorouracil (5-FU) were used as model drugs to prepare hydrogel delivery systems. The loading efficiencies of the hydrogel for BSA and 5-FU were 26.25 and 36.74%, respectively. Release behaviors of BSA and 5-FU were influenced by the pH. MTT assays confirmed that PSA-CMCS hydrogel has no cytotoxicity toward the NIH-3T3 cell line; in fact, the 100% aqueous extract of the PSA-CMCS hydrogel enhanced cell growth. These results suggest that PSA-CMCS hydrogel may be a promising pH-sensitive delivery system, especially for hydrophobic chemicals.

9. **RupeshPatil.et al., (2014)[30]** Formulated by the aim of the present work was to formulate and evaluate formulation of Miconazole nitrate as oral hydrogel to improve the efficacy &bioavailability. Miconazole Nitrate gel was prepared with Gellan gum and Carbopol 934P as gelling agents with polyethylene glycol as a penetration enhancer. The formulations were examined for pH, spreadability, consistency, viscosity, homogeneity, drug content and stability. In vitro drug release was evaluated using Franz diffusion cell. The viscosity of all formulation follows a pseudo-plastic flow behaviour.

10. **Manoj Sharma.et al., (2013)[31]** Reviewed by This study reports the development and characterization of hydrogel drug delivery system containing natural polymers was developed for stomach specific delivery of Nizatidine. Formulation and evaluation of in-situ gel by using different concentration of sodium alginate and sodium citrate with calcium chloride. These designs provide an effective means for studying the effect of various parameters on the dependent variables. Thus, factorial designs were applied to optimize the formulation and development of mucoadhesive microspheres and hydrogels.

11. **Sweta Garg.et. al., (2012)[32]** Studied by the Hydrogel are turning out to be very popular because of their distinctive properties such as highly water content, flexibility, elasticity and biocompatibility. as well as nature synthetic hydrophilic polymers can be physically or chemically cross-linked in order to fabricate hydrogels. The hydrogels are being employed for the fabrication of contact lenses, various hygienic products, tissue engineering scaffolds, drug delivery systems and wound dressings. The objective behind this review is to present a study of their main characteristics and biomedical uses.

12. **HidemiGoto.et al., (2011)[33]** In this present work of *Helicobacter pylori* (*H. pylori*) infection is a pathogenic agent of gastric diseases, but their mechanisms are unclear. Ammonia disturbed the collagen metabolism in the ulcer base. The involvement of anti-Lewis auto antibodies in the development of peptic ulcer might be unlikely. Moreover, *H. pylori*-specific IgA in gastric juice and TNFα gene polymorphism in persons infected with *H. pylori* were studied. According to *H. pylori*-specific IgA titer in gastric juice, persons were divided into two histologically and endoscopically different states of disease. *TNFA -857* single nucleotide polymorphism (SNP) may be associated withdrawl hyperplasic gastritis and gastric carcinomas without severe atrophy.

13. **KiarashGhazvini.et al., (2009)[34]** Formulated by the *Helicobacter pylori* is the causative agent of gastritis and peptic ulcer disease. Eradication of*H-pylori* is hard to achieve and often require multiple antibiotics regimens and the susceptibility of *H.*

pylori to the extract of licorice has been investigated in the present study. The agar dilution assay was used to test the susceptibility of the clinical isolates of *H. pylori* to the *Glycyrrhiza* extract at different concentration. The assay plates had 25, 50, 100, 150, 200, 300 and 400 mg/ml of licorice extract. The above preliminary experiments indicated strongly that licorice extract has some anti-*H.pylori* property and *H. pylori* shows susceptibility to licorice extract in concentration ranges that are achievable in the stomach.

14. **Ramesh.et. al.,(2008)[35]** Formulate and evaluate modified release oral Hydrogeland synthesized by physical cross-linking polymerization technique using, N,N-Ethylene is acryl amide as cross linker. The compatibility of drug with the polymers was confirmed by Fourier transform infrared spectroscopy (FT-IR) and Differential scanning calorimetric (DSC), studies were carried out to check the nature of the drug in the hydrogel formulations.

15. **Venkatasubbarao.et al., (2006)[36]** The Studied by Licorice Glycyrrhizaglabra is an important herb used in almost all systems of medicine. The author tries to present in this article a comprehensive review on all aspects of Licorice. The effect of licorice extract as oral preparation was evaluated on atopic dermatitis. The extract was standardized, based on Glycyrrhizinic acid by using a titrimetric method. Different hydrogels were formulated by using different co-solvents. After standardizing of preparations, the best formulations (1% and 2%) were studied in a double-blind clinical trial in comparison with base gel on atopic dermatitis over two weeks (30 patients in each group). Propylene glycol was the best co-solvent for the extract and Carbopol 940 as gelling agent showed the best results in final formulations.

16. **Sanjay Kumar.et al., (2005)[37]**Reviewed by the polymeric hydrogels has recently been playing a more important role as target specific drug delivery devices. These hydrogels can be synthesized to respond to various physiological stimuli in the body, for example pH, ionic strength and temperature. This article briefly summarizes drug delivery applications of hydrogels.

17. **Suh JK.et. al., (2000)[38]** reviewed Once damaged, articular cartilage has very little capacity for spontaneous healing because of the avascular nature of the tissue. Although many repair techniques have been proposed over the past four decades, none has successfully regenerated long-lasting hyaline cartilage tissue to replace damaged cartilage. Tissue engineering approaches, such as transplantation of isolated chondrocytes, have recently demonstrated tremendous clinical potential for regeneration of hyaline-like cartilage tissue and treatment of chondral lesions. As such a new approach emerges, new important questions arise. One of such questions is: what kinds of biomaterials can be used with chondrocytes to tissue-engineer articular cartilage. The success of chondrocytes transplantation and/or the quality of neocartilage formation strongly depend on the specific cell-carrier material. The present article reviews some of those biomaterials, which have been suggested to promote chondrogenesis and to have potentials for tissue engineering of articular cartilage. A new biomaterial, a chitosan-based polysaccharide hydrogel, is also introduced and discussed in terms of the biocompatibility with chondrocytes.

3.DRUG PROFILE[39]

LICORICE

IUPAC Name: 6-[6-carboxy-2-[(11-carboxy-

4,4,6*a*,6*b*,8*a*,11,14*b* heptamethyll-14-oxo-2,3,4*a*,5,6,7,8,9,10,12,12*a*, 14*a*-dodecahydro-1 *H*-picen-3-yl)oxy]4,5-dihydroxyoxan-3-yl] oxy-3,4,5-trihydroxyoxane-2-carboxylic acid

Synonyms: Glycyrrhizaglabra, Yoshimatsu Root

Molecular weight: 693.658 g/mol

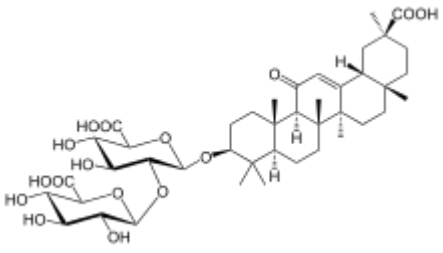
Molecular formula: $C_{35}H_{35}NO_{14}$

CAS Number: 1405-86-3

Therapeutic group: Ulcers, anti-inflammatory

Recommended dose: 200 mg/ day

Structure:



Physical and chemical parameters

Appearance:	Brown powder, glossy black externally
Odor:	Odorless
Taste:	Intensely sweet
Solubility:	Freely sol in water, alcohol, practically in sol in ether
pH:	pH ranging from (1.2 to 7.4).
pKa:	3.7
Melting point:	220°C
Stability:	Good stability

Pharmacokinetics:

Absorption:	90% (Uniformly absorbed Throughout the GIT)
Protein binding:	85% to 95% bound to plasma proteins
Volume of distribution:	4.3 ± 1.6 liter.kg-1
Bioavailability:	75% or more
Half-life:	2-6 hrs
Metabolism:	90% in liver
Elimination Rate constant:	0.1828hr -1
Renal clearance:	Should not depend on dose

Mechanism of Action

Glycyrrhizic acid can be found in the alpha and beta forms. The alpha form is predominant in the liver and duodenum and thus, it is thought that the anti-inflammatory liver effect of this drug are mainly due to the action of this isomer. Glycyrrhizic acid anti-inflammatory effect is generated via suppression of TNF alpha and caspase 3. It also inhibits the translocation of NFkB into the nuclei and conjugates free radicals. The antiviral activity of glycyrrhizic acid includes the inhibition of viral replication and immune regulation.

The mechanism involved in drug release includes either diffusion or dissolution. On exposure with aqueous solution hydration of matrix takes place as a result it swells to block up existing pores, dissolution of the contents takes place. Due to gel formation a viscous solution is formed which give rise to a positive pressure which opposes the liquid entry and causes the disintegration of matrix.

4. EXCIPIENTS PROFILE[40]

Chitosan

Nonproprietary Names

BP: Chitosan hydrochloride

PhEur: Chitosan hydrochloridum

Synonyms

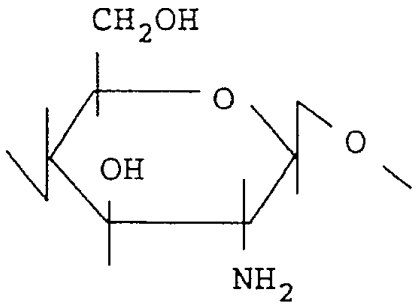
2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; deacetylated chitin;deacetylchitin;b-1,4-poly-D-glucosam

Chemical Name and CAS Registry Number

Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose [9012-76-4]

Empirical Formula and Molecular Weight

Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of N-deacetylation between chitin and chitosan has not been defined,(1–3) and as such chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80–85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10 000–1 000, and vary in degree of deacetylation and viscosity.



Structural Formula

Applications in Pharmaceutical Formulation or Technology

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems, and use for gene delivery. chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets, and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression, and conventional granulation processes.

Typical Properties

Acidity/alkalinity: pH = 4.0–6.0 (1% w/v aqueous solution)

Density: 1.35–1.40 g/cm3

Glass transition temperature: 203 C

Moisture content

Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility

Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in

mineral inorganic acids (except phosphoric and sulfuric acids). Upon dissolution, amine groups of the polymer become protonated, resulting in a positively charged polysaccharide (RNH3 þ) and chitosan salts (chloride, glutamate, etc.) that are soluble in water; the solubility is affected by the degree of deacetylation

Viscosity

A wide range of viscosity types is commercially available. Owing to its high molecular weight and linear, unbranched structure, chitosan is an excellent viscosity-enhancing agent in an acidic environment. It acts as a pseudo-plastic material, exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solutions increases with increasing chitosan concentration, decreasing temperature, and increasing degree of deacetylation.

Stability and Storage Conditions

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The PhEur 2005 specifies that chitosan should be stored at a temperature of 2–88C.

5. CHEMICAL PROFILE [41]

Glutaraldehyde

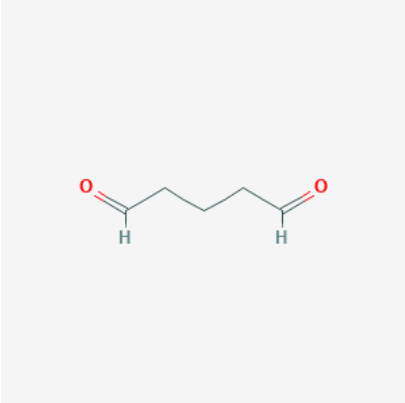
Chemical Name: Glutaraldehyde,Pentanediol,Glutaral

Synonyms: Diswart,Gludesin, Glutaral,Glutaraldehyde

Molecular Formula: $OHC(CH_2)_3CHO$

Molecular Weight: 100.117 g/mol

Structure



Melting Point: less than 20 ° F

Density: 1.062 to 1.124

Viscosity: 12.75 mm^2/s

Solubility

Greater than or equal to 100 mg/mL, Miscible with water, Soluble in ethanol, benzene, ether.

Stability

Stable under recommended storage conditions. Acid glutaraldehyde is more stable than alkaline glutaraldehyde Stable in light, oxidizes in air, polymerizes in heat.

6.OBJECTIVE OF THE STUDY

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. 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 (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.

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 ingastric mucosa.
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. These include localized delivery ofthe 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 favorable 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.

The aim of the present study was to prepare hydrogel matrix tablets of licorice forthe treatment of *H Pylori*. The objectives of the study are.

- Pre-formulation studies
- The licorice loaded hydrogels were prepared by chitosan polymer using crosslinking agent i.e glutaraldehyde (GL).
- To characterize the prepared hydrogel formulations.
- Hydrogel tablet formulations were prepared by direct compression method
- Pre and post compression parameters of hydrogel matrix tablets of licorice were evaluated.
- To evaluate the accelerated stability study of the formulations as per ICH guidelines.

7. PLAN OF WORK

- **Literature Review**

  - ✓ Selection of Drug based on Literature.
  - ✓ Selection of Polymer based On Literature.

- **Pre formulation Studies**

  Compatibility evaluation is carried out between drug and polymers in physical observation and by using FT- IR spectral study.

- **Development of dosage form**

  - Preparation of licorice loaded hydrogel using chemically cross linking of Chitosan polymer.
  - Characterization of hydrogel is carried out
    - Drug loading
    - Particle size analyzer
    - Surface morphology (SEM)
    - Swelling studies
  - Hydrogel tablet is prepared by direct compression method
  - Pre and Post compression parameters are evaluated

i) Pre compression test

- Angle of repose
- Apparent bulk density
- Tapped bulk density
- Percent compressibility

ii) Post compression test

- Tablet dimensions
- Hardness, Friability
- Weight variation
- Content uniformity of active ingredient
- Assay
- In-vitro dissolution study

- Accelerated Stability study of optimized batch is performed as per ICH guidelines

8. MATERIALS AND METHODS

MATERIALS

Table No: 1. Materials used

S.NO	INGREDIENTS	MANUFACTURER
1	Licorice	Amsarprivate limited.,
2	Chitosan	LobachemieLaboratory
3	Glutaraldehyde	SD fine-chemical limited.,
4	Acetic acid	Rankem Pvt limited
5	Mcc	S. D. Fine Chemicals Ltd.,
6	Starch	Astrra Chemicals
7	Magnesium stearate	LobaChemiePvt. Ltd.,
8	Talc	Astrra Chemicals

8.1 INSTRUMENTATION

Table No: 2.Instruments used

S.NO	INSTRUMENTS	MANUFACTURER
1	Weighing Balance	Shimadzu
2	Hot Air Oven	Technico
3	Moisture Analysers	Mettler Toledo
4	Monsanto Hardness Tester	Thermonik
5	Roche Friabilator	Elchem
6	Disintegration Tester	Elchem
7	Dissolution Test Apparatus	Electro Lab
8	UV Spectroscopy	Shimadzu
9	Ultra Sound Sonicator	Labman
10	Mechanical Stirrer	Remi Motor
11	Compression Machine	Cadmach

8.2METHODS

PREFORMULATION STUDY

RAW MATERIAL ANALYSISOF LICORICE

8.2.1. Solubility

The solubility of Licorice drug can be identified by dissolving the small amount of drug to fixed volume of solvent such as acetone, water, ethanol, methanol, acidic buffer (pH:1.2) and shake vigorously until a clear solution formed and examined visually for undissolved solute particles.

8.2.2. Calibration curve

The stock solution was prepared by taking 10 mg of Licorice in 100 ml of pH 1.2 acidic buffer. The drug was dissolved in a Buffer by using sonicator. Five different dilutions were prepared from stock solution having concentration as 1 mcg/ml,2 mcg/ml, 3 mcg/ml, 4 mcg/ml and 5mcg/ml respectively. These prepared dilutions were then analyzed by UV-Visible Spectrophotometer (Shimadzu, Japan) at 254 nm.

8.2.3. 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 ofdrug with excipients.

8.2.4. 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.[**42]**

8.2.5. Formulation and Composition of Licorice hydrogel

Table No: 3. Formulation and Composition of Licorice hydrogel

Ingredients	F1	F2	F3	F4	F5
	(mg)	(mg)	(mg)	(mg)	(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 %

8.3. CHARACTERIZATIONOF HYDROGEL

8.3.1. 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:

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

8.3.2. 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 aluminum 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.

8.3.3. 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

8.3.4. 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 0.45 µm 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.

8.4.FORMULATION OF LICORICE HYDROGELMATRIX TABLET

Table No: 4

S. No	F1	F2	F3	F4	F5
	(mg)	(mg)	(mg)	(mg)	(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

8.4.1.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 are shown in table No: 4

8.4.2. Flow Property Studies

The prepared hydrogel granules flow property was measured using bulk density and Tapped density, angle of repose, compressibility index (Carr's index) and Hausner's ratio.

8.4.3. Bulk density and Tapped density

The prepared hydrogel granules was transferred. The initial volume occupied by granules will be noted as bulk density volume. Then the measuring cylinder was tapped for (100) times and volume obtained after tapping was taken as tapped volume. The bulk density and tapped density was calculated using following formula.

Bulk density is denoted by (ρb)

$\rho b = m/vi$

Tapped density is denoted by (ρt)

$\rho t = m/vt$

m = mass of the blend

vi = initial volume

vt = tapped volume

8.4.4.Carr's Compressibility index (CI)

Compressibility is the ability of the powder to decrease in volume under pressure. Using untapped density and tapped density the percentage compressibility of granules was determined, which is given as Carr's compressibility index.

The Carr's Compressibility index was expressed in a percentage calculated using the formula.

$CI = (TD - BD / TD) \times 100$

8.4.5. Hausner's ratio

It is a measurement of frictional resistance of the drug. The ideal range should be 1.0 – 1.5. It is determined by the ratio of tapped density and bulk density.

Hausner's ratio =(TD / BD)

8.4.6. Angle of repose

A funnel with a wide outlet is affixed at a distance of 3 cm above the bench, where a piece of paper is placed directly beneath the funnel. The powder is added while the funnel is closed. Weighed quantity of the drug was passed through a funnel kept at a height 3 cm from the base. The powder is passed until it forms a heap and touched the tip of the funnel. The radius the base of the conical pile, and the height of the pile were measured and the angle of repose was calculated using the formula.

$$\theta = \tan^{-1} (h/r)$$

h = height of the pile

r = radius of the base of the conical pile

θ = angle of repose

Table No:5 Standard limitsfor Flow properties of granules

S.NO	Carr's index (%)	Hausner's ratio	Angle of repose (θ)	Properties
1	5-12	1.00-1.11	25-30	Free flowing
2	12-16	1.12-1.18	30-35	Good
3	18-21	1.19-1.25	35-40	Fair
4	23-33	1.32-1.45	40-55	Poor
5	35-38	1.46-1.59	55-60	Very poor
6	>40	>1.60	>60	Extremely poor

8.5. EVALUATION OF HYDROGEL MATRIX TABLET

8.5.1. Weight variation

The weight variation test was done by weighing 20 tablets individually, calculating the average weight and comparing the individual weights to the average weights.

8.5.2. Thickness

The thickness of the tablet is important for uniformity of tablet size. Thickness was measured using vernier calipers. It was determined by checking the thickness of ten tablets of each formulation.

8.5.3. Hardness

The hardness of the tablets was checked by using Monsanto hardness tester. The hardness was measured in terms of kg/cm². 10 tablets were chosen randomly and tested for hardness. The average hardness of 5 determinations was recorded.

8.5.4. Friability

10 tablets were weighed and the initial weight of these tablets was recorded and placed in Roche friabilator and rotated at the speed of 25 rpm for 100 revolutions. The tablets were removed from the friabilator, dusted off the fines and again weighed and the weight was recorded. Percentage friability was calculated by using the formula:

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

8.5.5. 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. [**43]**

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

8.5.6. 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.

8.5.7. 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 by Shantha and Harding. 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. [**44]**

% Mucoadhesion = P1/P2 × 100

where,

P1- no. of adhered hydrogel pieces

P2- no. of applied hydrogel pieces

8.5.8. 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 spreadon to wet rinsed tissue specimen and prepared slide washung 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.

% Mucoadhesion = P1/P2 × 100

Where,

P1- no. of adhered hydrogel tablet

P2- no. of applied hydrogel tablet

8.5.9. In vitro dissolution study

In vitro drug release study was carried out using a USP-23 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.

8.5.10. Release Kinetics

The results of *In-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows.

- Cumulative percent drug release versus time (zero order kinetic model)
- Log cumulative percent drug remaining versus time (first order kinetic model)
- Cumulative percent drug release versus square root of time (Higuchi's model)
- Log cumulative Percent Drug released versus log time (korsmeyers model)

Drug release kinetics-model fitting of the dissolution Data:

Whenever a new solid dosage form is developed or produces, it is necessary to ensure that drug dissolution occurs in an appropriate manner. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q = f (t). Some analytical definitions of the Q (t) function are commonly used such as zero order, first order, Higuchi, korsmeyers-peppas models.

Zero-order kinetics:

A zero-order release would be predicted by the following equation.

$Q\mathbf{t = Q_o + K_o t}$

Where,

Qt = amount of drug dissolved in time t

Q_o= Initial amount of drug in solution

K_{ot}= Zero-order rate constant (hr)

When the data is plotted as cumulative percent drug release versus time if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to k_o.

First-order kinetics:

A first order release would be predicted by the following equation.

$\text{Log } C = \text{Log } Co - K_t / 2.303$

Where

C = Amount of drug remained at time t

C_o = Initial concentration of drug

K = First-order rate constant

The data obtained rate plotted as log cumulative percentage of drug remaining versus time which would yield a straight line with a slope of -k/2.303.

Higuchi model

Drug release from the matrix devices by diffusion has been described by following higuchi's classical diffusion equation.

$$\mathbf{Q = [DE/ \tau(2A- ECs) C_{st}]}$$

Where

Q = Amount of drug release at time t

D = Diffusion coefficient of the drug in the matrix

A = Total amount of drug in unit volume of matrix

C_s = solubility of the drug in the matrix

E = Porosity of the matrix

T = Time in hrs at which q is the amount of drug is release

Equation-3 may be simplified if one assumes that D, Cs and A are constant. Then equation-3 becomes

$$\mathbf{Q = Kt^{½}}$$

When the data obtained were plotted as cumulative drug release versus Square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to k.

Korsmeyers Peppas model:

In order to understand the mode of release of drug from swellable matrices, the data were fitted to the following equation

$$\mathbf{M_t / M_{ά} = Kt^n}$$

Where,

$M_t / M_{ά}$= fraction of drug released at time 't'

K = Constant incorporating the structural and geometrical

Characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of release.

The above equation can be simplified by applying log on both sides we get

$$\mathbf{Log\ M_t/M_{ά} = Log\ K + n\ Log\ t}$$

When the data is plotted as a log of drug released versus log time, yields a straight line with a slope equal to n and the k can be obtained from y- intercept.

The value of n for a cylinder is <0.45 for fickian release, > 0.45 and < 0.89 for non-fickian release, 0.89 for the case 2 type release and > 0.89 super case 2 type release.

8.5.11. Stability study

Stability studies were conducted for the optimized formulations as per ICH guidelines. The storage conditions used for stability studies were (40°C±2°C/75%±5%RH; 25°C±2°C/60%±5%RH). Sample of chitosan hydrogel Tablet were analyzed after 1, 2 and 3 months for physical characters and assay were performed followed by *in vitro* dissolution.

9. RESULT AND DISSCUSION

9.1. Raw material analysis

9.1.1. Solubility:

Solubility of licorice was found in water, acidic buffer pH 1.2 and alcohol. The results showed that licorice is freely soluble in all the solvents.

9.1.2. Standard calibration curve of licorice in acidic buffer pH1.2

Standard calibration curve of licorice was draw by plotting absorbance v/s concentration. The absorbance value are tabulated in Table No:8 Standard calibration curve of licorice in the Beer's range between 1-5µg/ml

Table No: 6

9.1.3. Calibration curve of licorice in acidic buffer pH 1.2at 254nm

S.NO	Concentration (µg)	Absorbance
1	0	0
2	1	0.208
3	2	0.366
4	3	0.5
5	4	0.67
6	5	0.799

The linear regression analysis for standard curve in Acidic buffer 1.2

The linear regression analysis was done on absorbance data point. The result are as follow:

The slope :0.148

The intercept :0.062

The correlation coefficient :0.998

A straight tline equation (y= mx+c) was generated to facilitate the calculation for amount of drug. The equation is as follows.

Absorbance = 0.148 x concentration

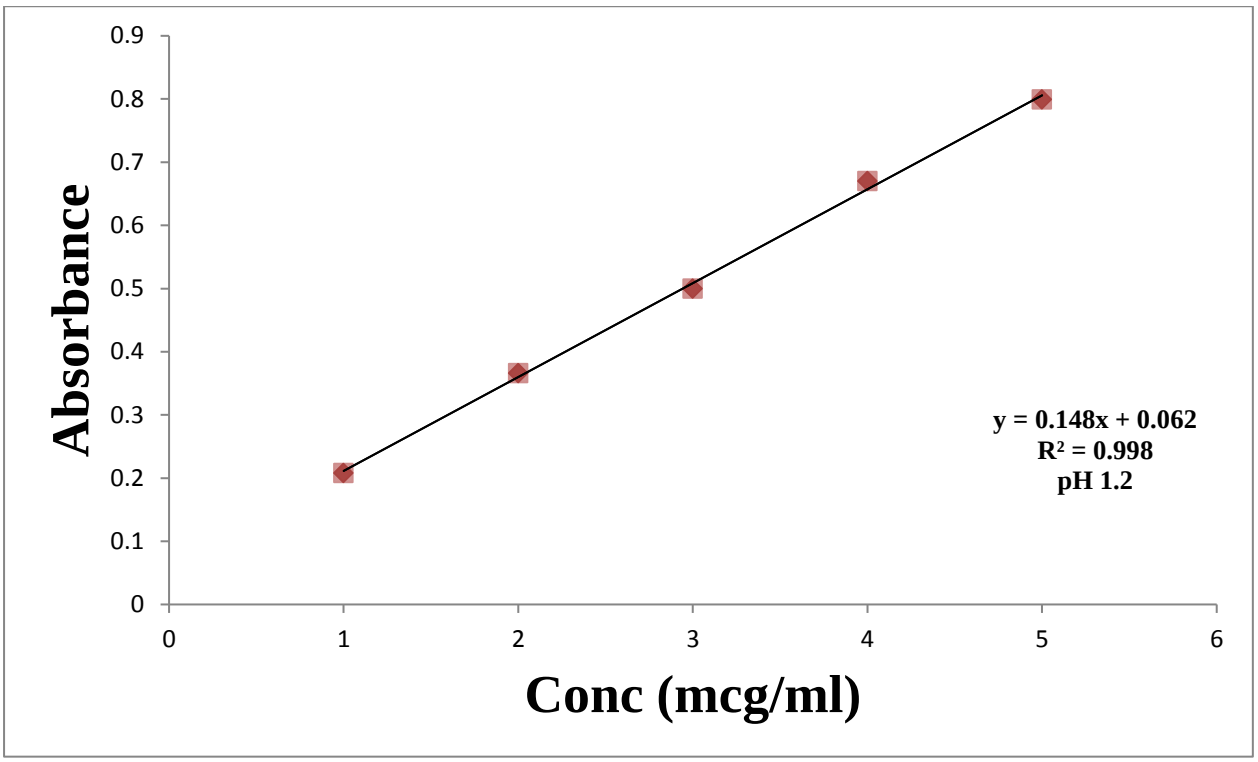


Fig No: 2Standard calibration curve of licorice in Acidic buffer pH: 1.2at 254nm.

9.1.4. Compatibility study of FTIR

FTIR spectrum of Licorice was recorded and characteristic peaks were observed. The descriptions of the observed peak was showed at Table No: 7

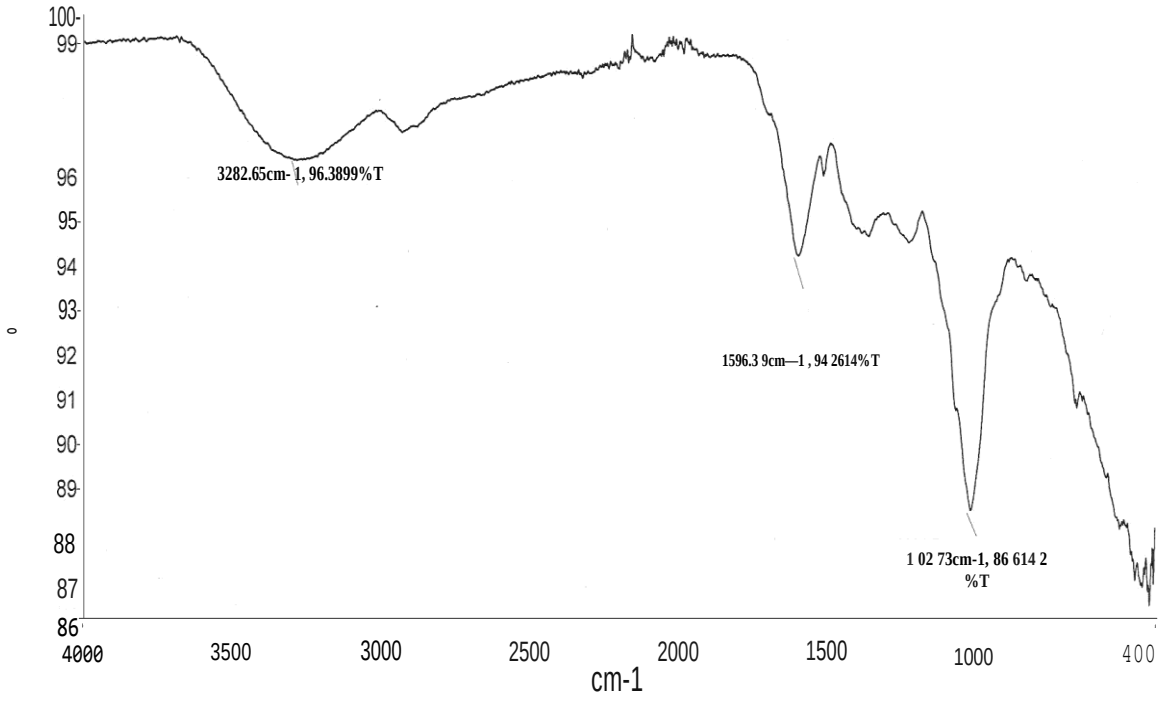


Fig No: 3 FTIR test of licorice

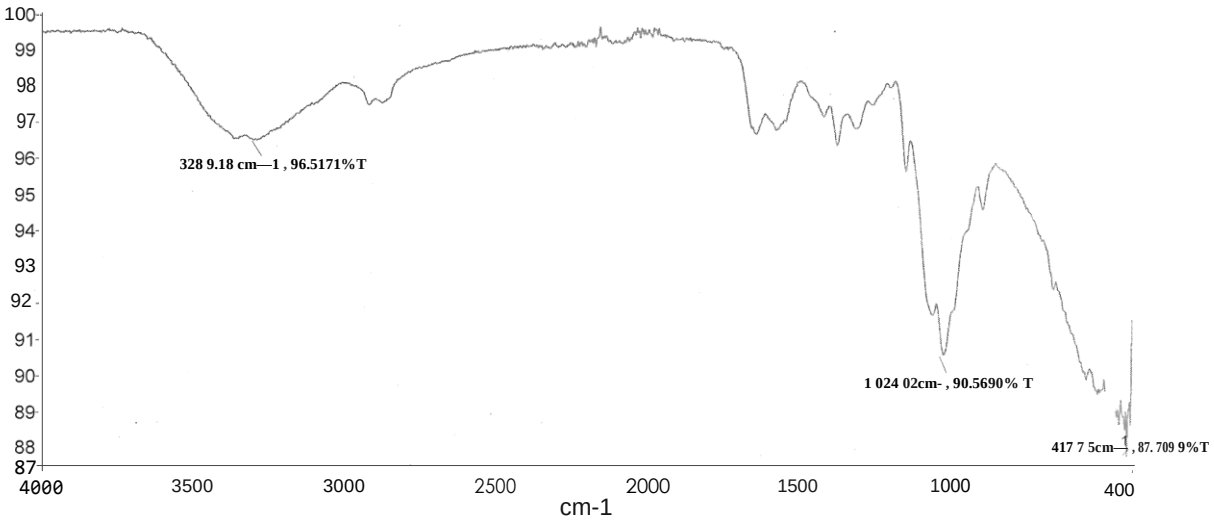


Fig no: 4 FTIR test of Chitosan

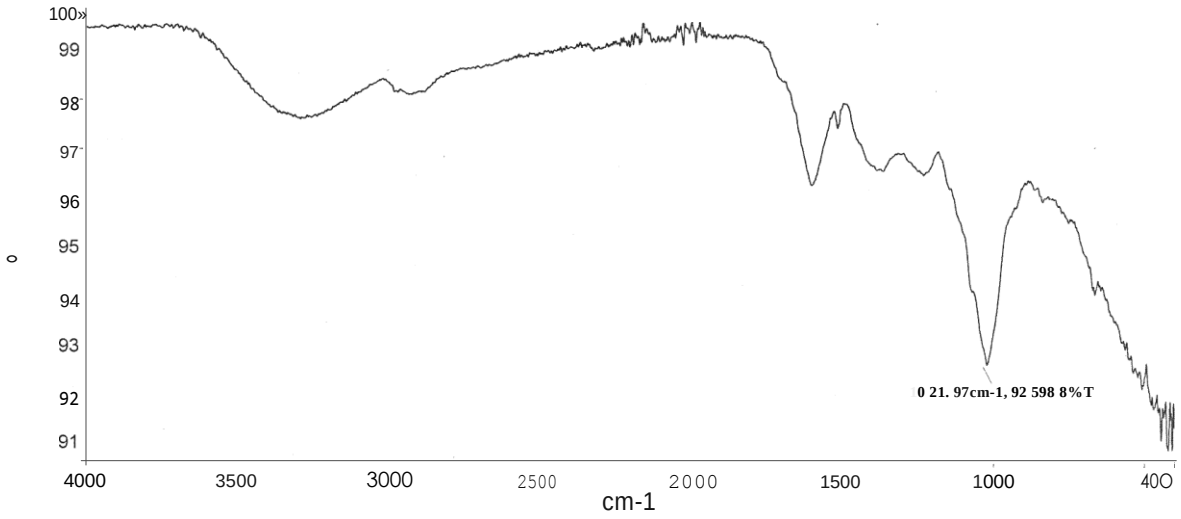


Fig No: 5 FTIR test of Licorice hydrogel

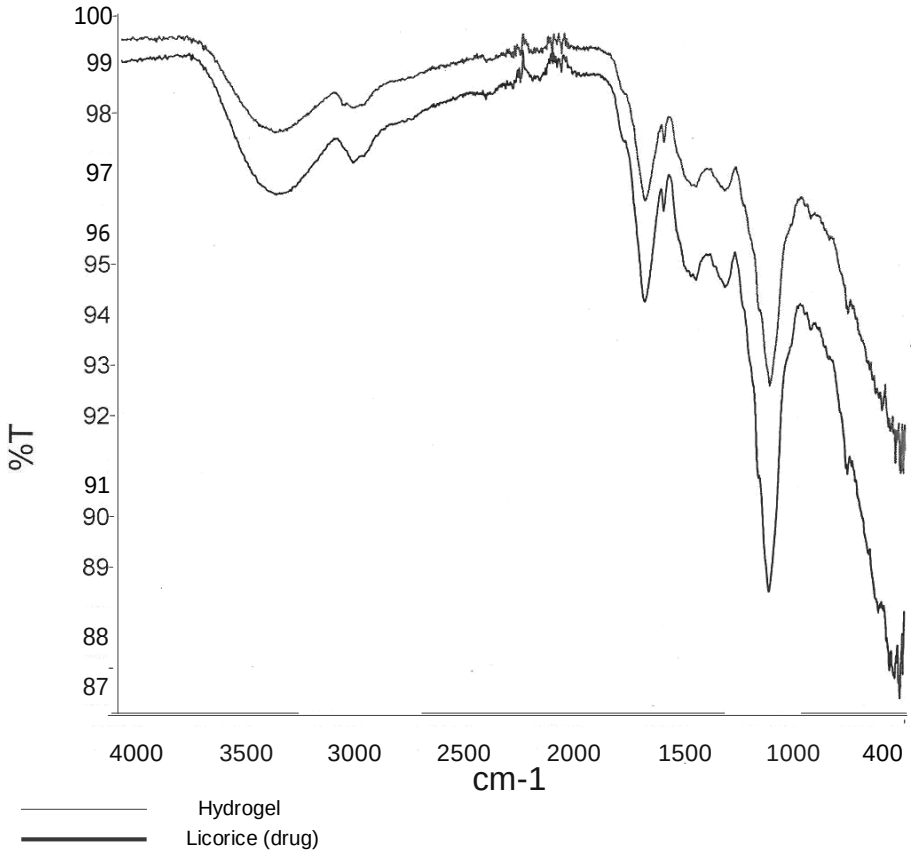


Fig No: 6 Correlation between hydrogel and licorice

The 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.

Table No: 7

Characteristics peak of FTIR spectrum

S.NO	Wave number	Bond
1	**3379**	**OH (Strecning)**
2	**3436**	**-NH2 (Strecning)**
3	**2923**	**-CH2 (Strecning)**
4	**1118**	**C-O-C(asymmetric stretching)**
5	**1652**	**C=N(Band)**

9.1.5. Flow property studies

Table No:8 Evaluation of Flow properties of Hydrogels

Formulation	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Carr's index (%)	Hausner's ratio	Angle of repose (θ)
F1	0.632 ±0.01	0.569±0.01	13.10	1.162	28.70'
F2	0.723 ±0.01	0.608±0.01	14.89	1.197	29.44'
F3	0.754 ±0.01	0.597±0.01	16.90	1.187	26.96'
F4	0.698±0.01	0.601±0.01	15.18	1.152	24.15'
F5	0.714±0.01	0.578±0.01	17.01	1.136	25.75'

Characterization of hydrogels

The hydrogel prepared for hydrogel matrix tablets were evaluated for their flow properties. The bulk density was in range of 0.632 to 0.754 gm/cm³. The tapped density was in range of 0.569 to 0.608 gm/cm³. The Carr's index was found to be in range of 13.10 to 17.01 %, which indicates some formulations were good flow and some formulations were fair flow. Hausner's ratio was found to be in range of 1.136 to 1.197, which indicates that the prepared granules exhibited fair flow properties. Angle of repose was in range of 24.15° to 29.44°, these values indicate the good flow property for all the formulations.

9.2. EVALUATION ON CHARACTERIZATION OF HYDROGEL

9.2.1. 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.

9.2.2. 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. [**43]**

Table No: 9Swelling study behavior of Licorice hydrogel

Time	F1	F2	F3	F4	F5
	%	%	%	%	%
0	0	0	0	0	0
1	10.5	18.02	24.51	31.45	44.78
2	17.86	25.43	42.75	50.18	63.89
3	22.78	47.95	61.03	72.04	81.74
4	26.82	69.72	83.41	90.40	110.8
5	37.09	87.13	103.47	112.56	134.87
6	52.12	101.70	124.41	130.56	156.63
7	78.86	120.47	140.63	149.28	172.45
8	95.45	137.51	159.62	163.78	194.78

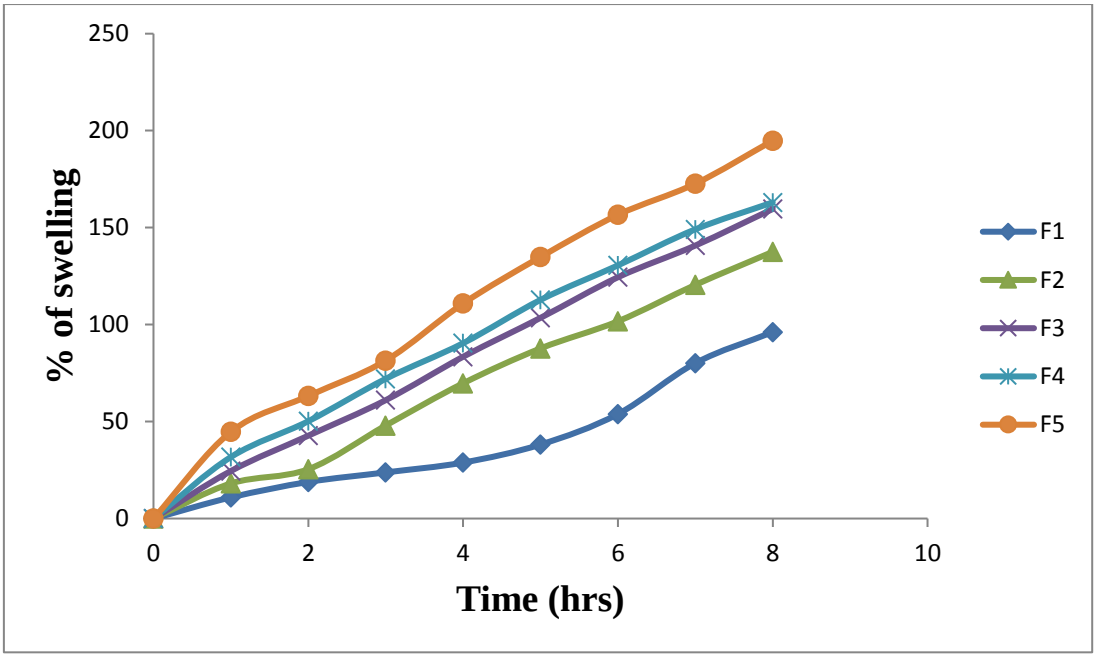


Fig No: 7Swellingstudy of Licorice hydrogel

9.2.3. Optical microscopic image of hydrogel

The optical image of all the prepared licorice loaded chitosan based hydrogel formulation (F1 to F5) was shown in Fig. 8 to 12.

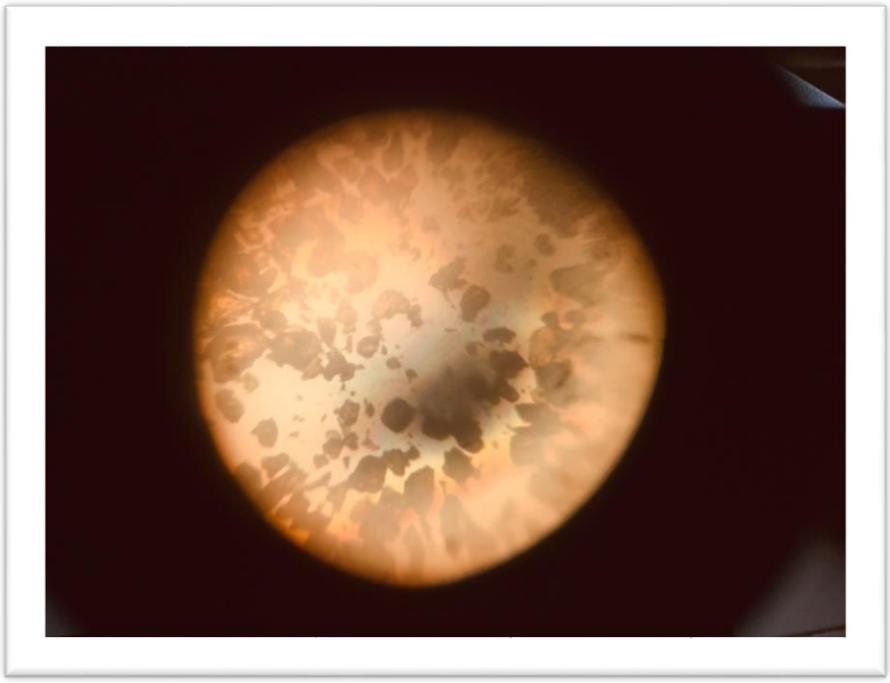


Fig No: 8 Optical microscopic image of Licorice hydrogel(F1)

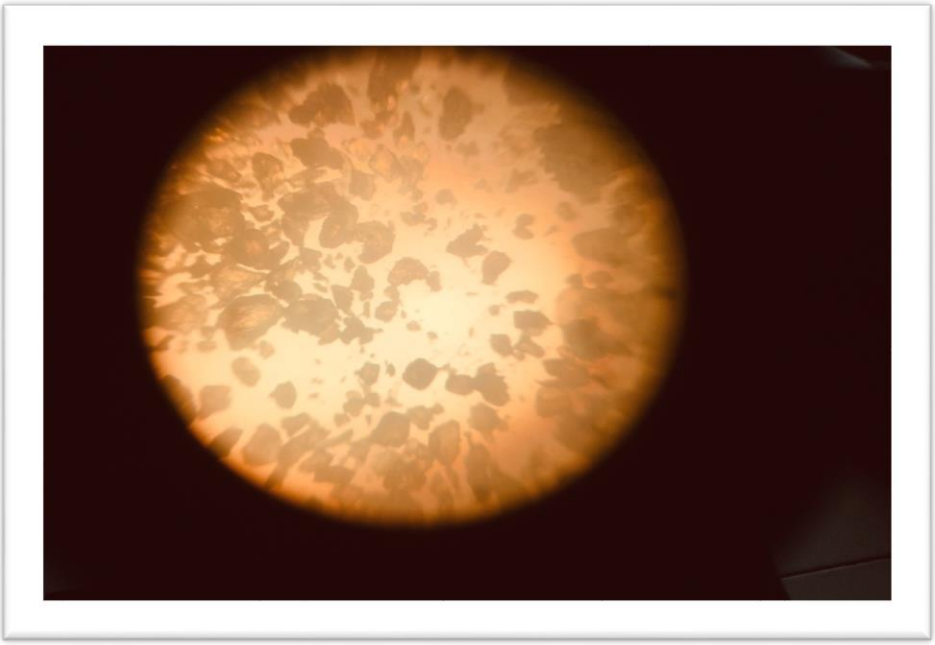


Fig No: 9 Optical microscopic image of Licorice hydrogel (F2)

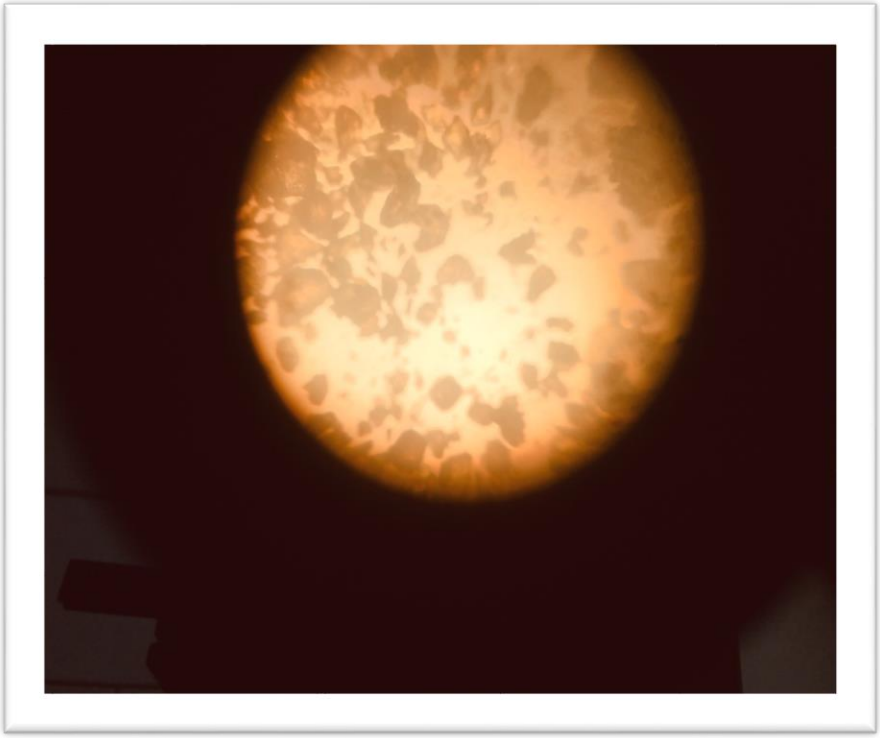


Fig No: 10 Optical microscopic image of Licorice hydrogel (F3)

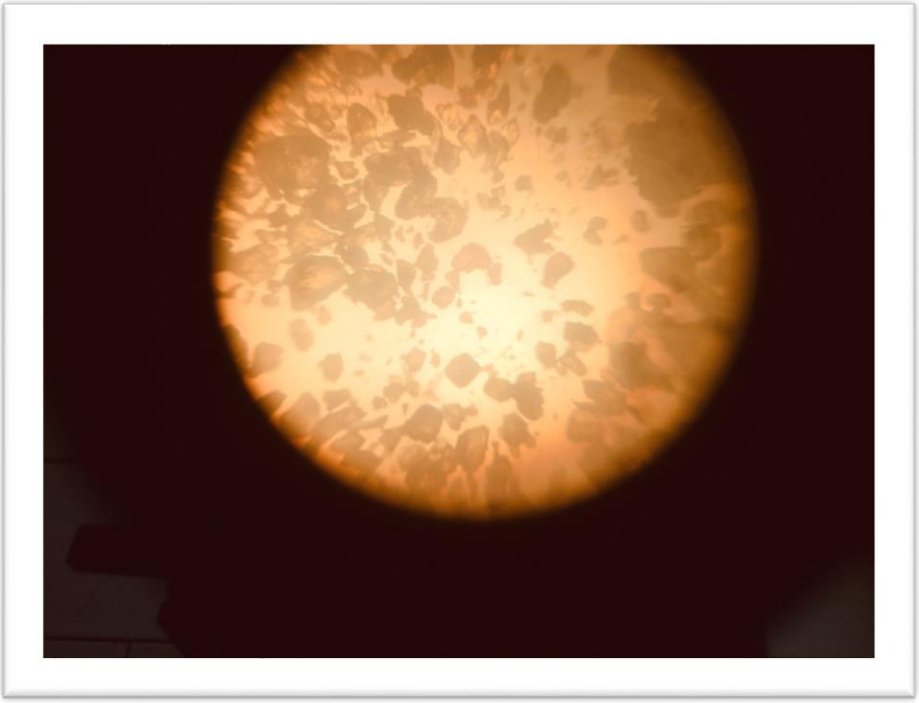


Fig No: 11 Optical microscopic image of Licorice hydrogel (F4)

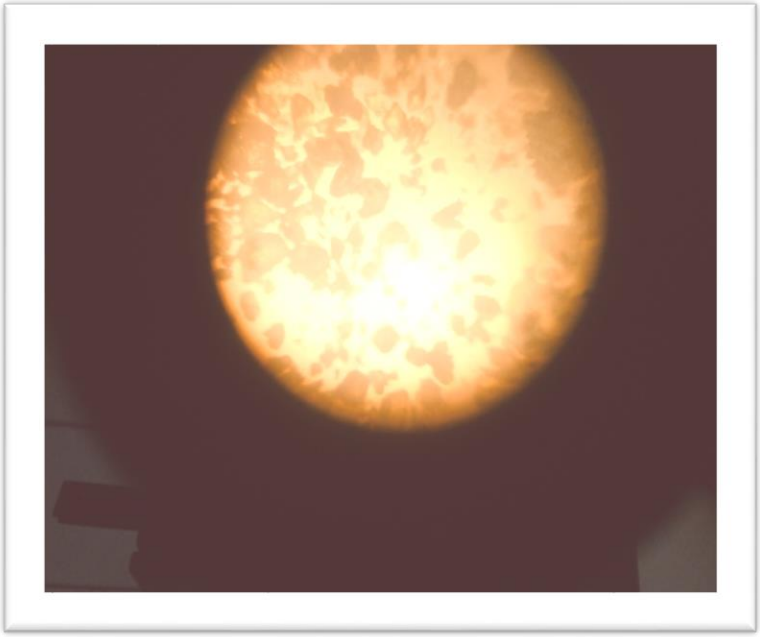


Fig No: 12 Optical microscopic image of Licorice hydrogel (F5)

9.2.4. Scanning electron microscopy (SEM) of Licorice hydrogel

The morphology of the prepared hydrogel was examined using scanning electron microscopy. Fig.13 to17 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.

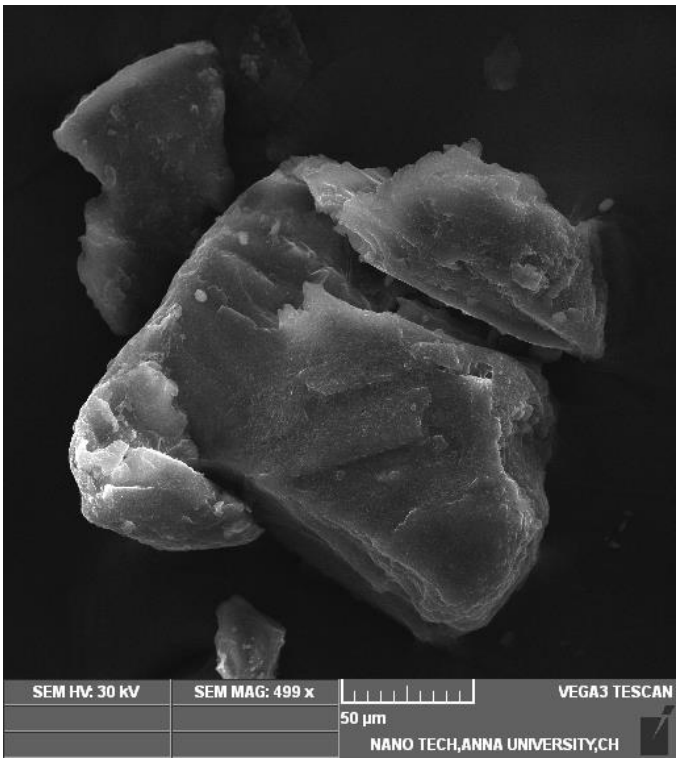


Fig No: 13. SEM image of Hydrogel (F1)

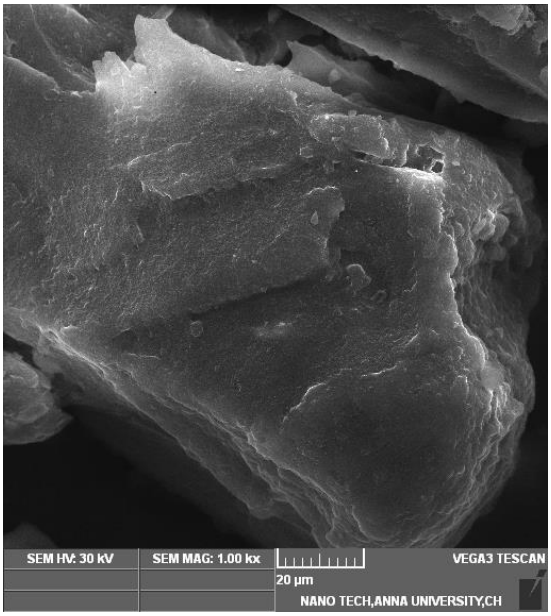


Fig No: 14. SEM image of Hydrogel (F2)

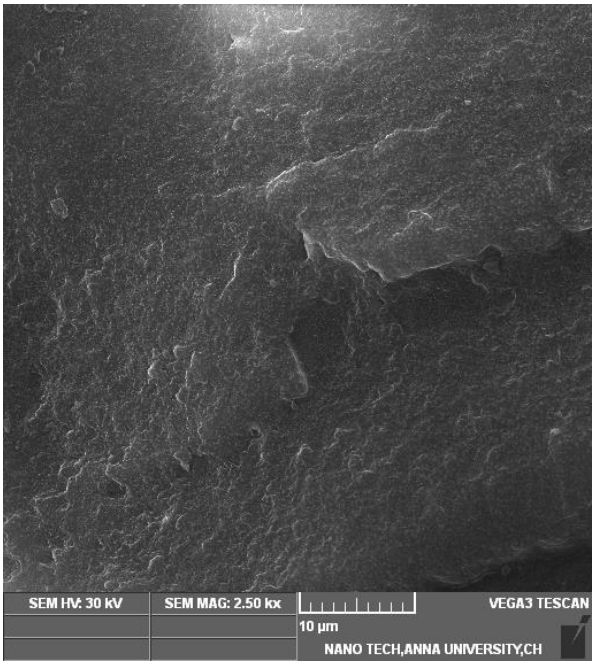


Fig No: 15. SEM image of Hydrogel (F3)

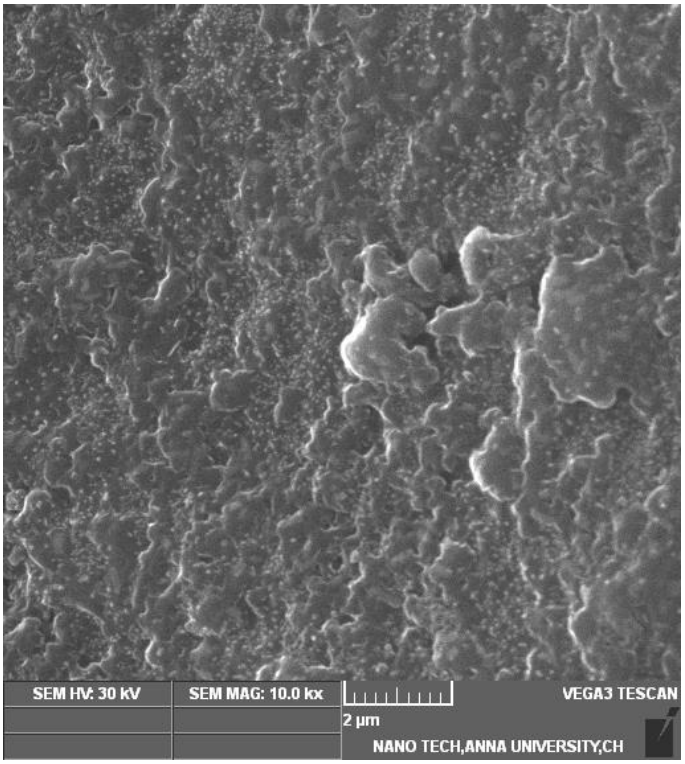


Fig No: 16. SEM image of Hydrogel (F4)

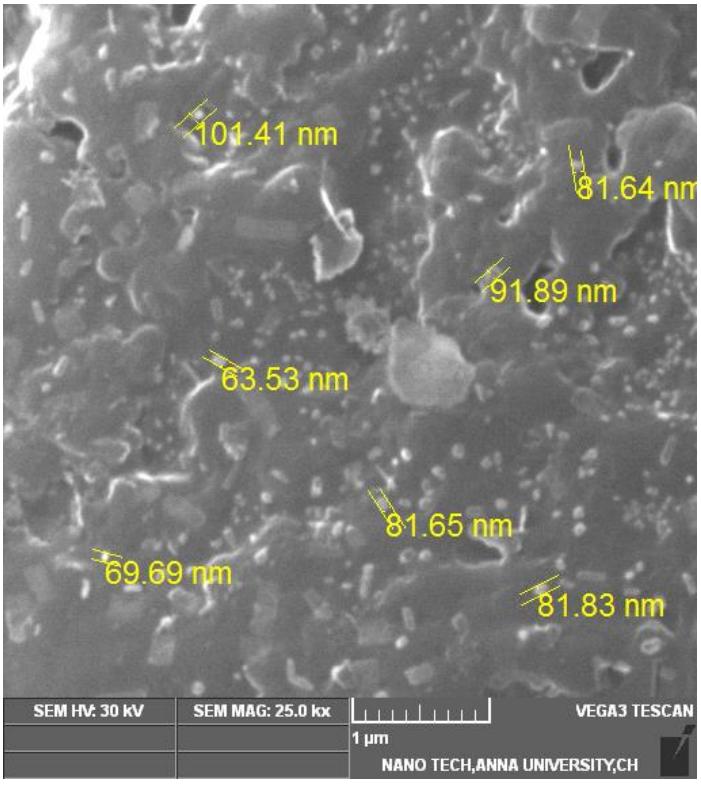


Fig No: 17. SEM image of Hydrogel (F5)

9.2.5. Particle size analyzer

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.

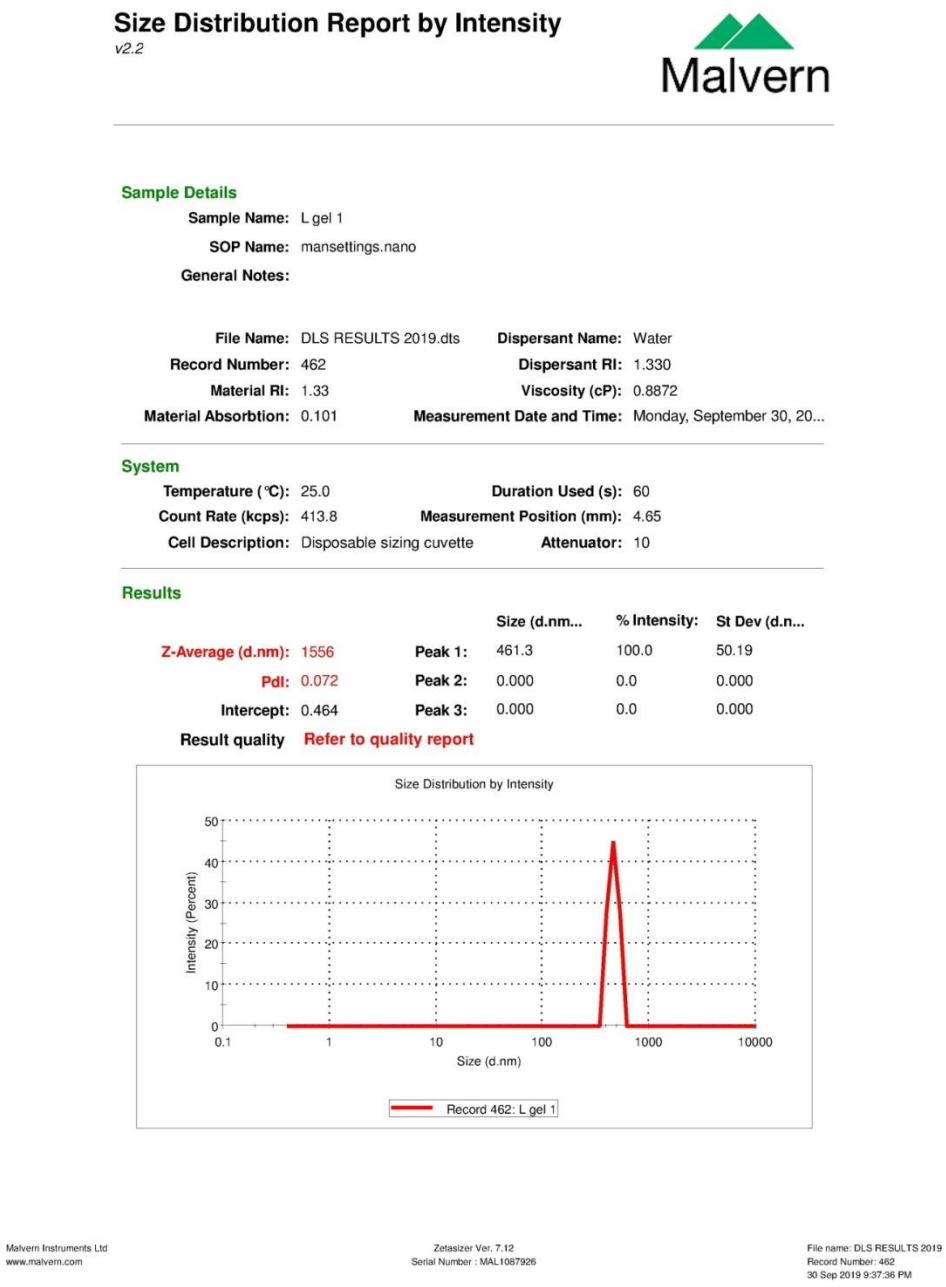


Fig No: 18. Size distribution of Licorice loaded hydrogel

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: L gel 1
SOP Name: mansettings.nano
General Notes:

File Name:	DLS RESULTS 2019.dts	**Dispersant Name:**	Water
Record Number:	463	**Dispersant RI:**	1.330
Date and Time:	Monday, September 30, 2019 ...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	10
Count Rate (kcps):	196.4	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	11

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-14.0	**Peak 1:**	-3.07	44.2	4.61
Zeta Deviation (mV):	12.6	**Peak 2:**	-30.1	28.4	3.91
Conductivity (mS/cm):	0.00584	**Peak 3:**	-14.7	27.4	4.40
Result quality	See result quality report				

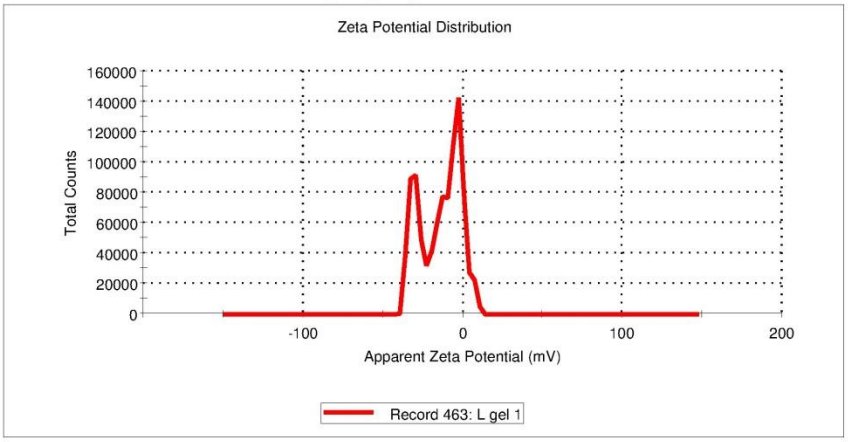


Fig No: 19. Zeta potential of Licorice loaded hydrogel

9.3. EVALUATION OF LICORICE HYDROGEL MATRIX TABLET

Table No: 10

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

9.3.1. Thickness Test

Thickness of the developed formulations F1 to F5 varied from 4.95 ± 0.11 mm to 5.06 ± 0.23 mm (Table 10) in all the formulation and the average thickness is within the range of ± 5%. Each sample was analyzed in triplicate.

9.3.2. Hardness Test

Hardness of the developed formulations F1 to F5 varied from 5.4 ± 0.11 to 6.10 ± 0.15 kg/cm^2 (Table 10) in all the formulation indicating good mechanical strength with an ability to withstand physical and mechanical stress condition while handling.

9.3.3.Weight variation Test

The maximum % deviation was found to be ±5% (Table 10) from all the formulations. As none of the formulation showed a deviation of more than ± 5% (I.P. limit) for any of the tablets tested, the prepared formulations comply with the weight variation test, thus it fulfills the I.P. requirements.

9.3.4. Friability

The loss in total weight of the tablets due to friability was in the range of 0.158 ± 0.05% to 0.292 ± 0.13% (Table 10) in all the formulation and the friability value is less than 1% which ensures that formulated tablets were mechanically stable.

9.3.5. Drug content

The percentage of drug content was found to be between the 97.80% - 99.12% of licorice, which within acceptable limits. (Table10) showed the results of drug content uniformity in each batch.

9.3.6. 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. [**43]**

Table No: 11Swelling study of hydrogel matrix tablet

Time	**F1**	**F2**	**F3**	**F4**	**F5**
	%	**%**	**%**	**%**	**%**
0	**0**	**0**	**0**	**0**	**0**
1	**10.4**	**19.45**	**26.71**	**34.7**	**49.26**
2	**17.86**	**26.54**	**41.72**	**50.70**	**61.45**
3	**22.78**	**42.36**	**63.14**	**71.63**	**83.65**
4	**27.82**	**61.47**	**81.54**	**93.15**	**107.68**
5	**36.09**	**82.74**	**103.16**	**117.84**	**120.48**
6	**51.12**	**104.34**	**116.75**	**134.96**	**143.67**

7	**79.86**	**113.67**	**129.43**	**151.45**	**161.78**
8	**95.45**	**120.09**	**140.73**	**173.65**	**187.58**

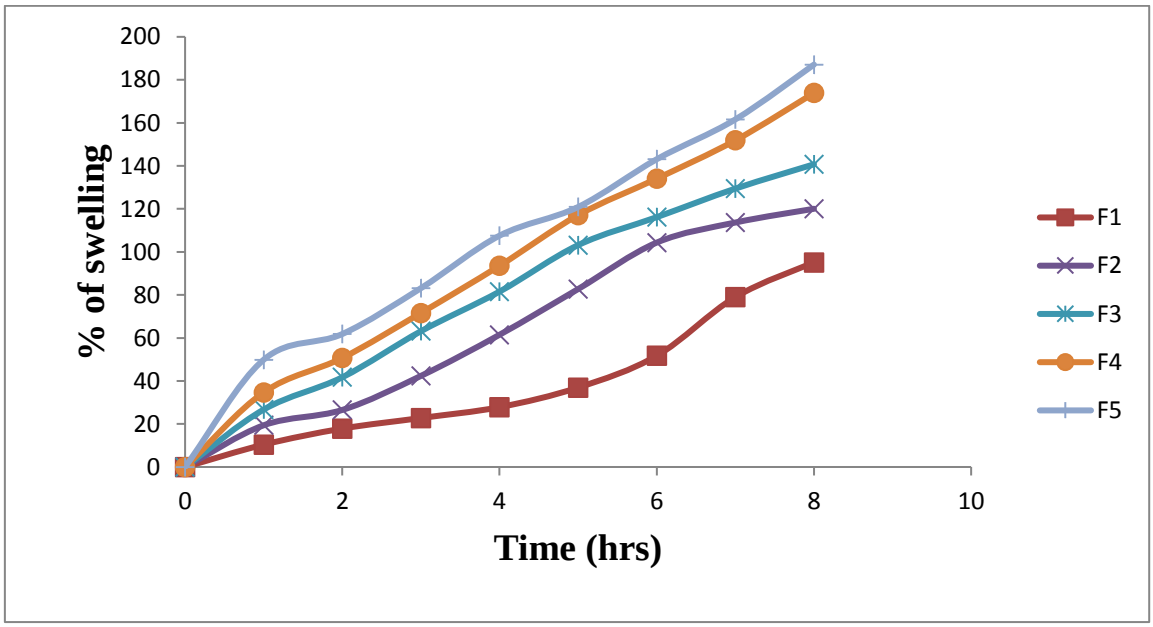


Fig No: 20Swelling study of hydrogel matrix tablet

9.3.7. Mucoadhesion study of hydrogel

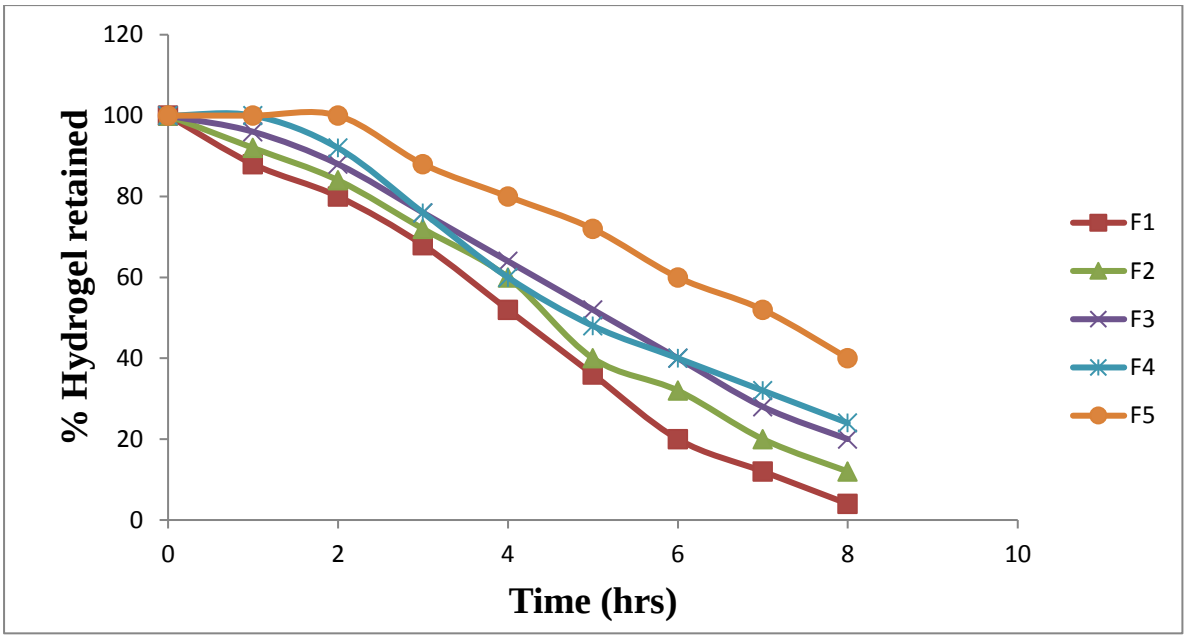


Fig No: 21% Mucoadhesion of Licorice hydrogel

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 a dosage 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 couldprovide a strong electrostatic interaction with mucosal surface. [**44]** The rank order of mucoadhesion for formulations was to be F1 > F2 > F3 > F4>F5.

9.3.8. Mucoadhesion study of hydrogel tablet

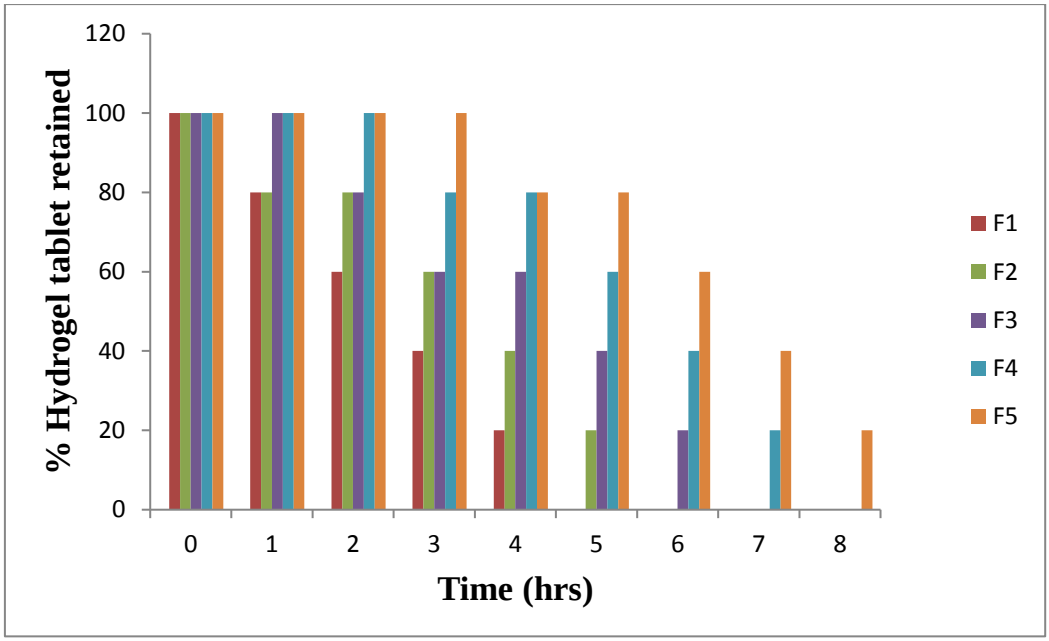


Fig No:22% Mucoadhesion of hydrogel tablet

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 visco elastic 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. **[44]**

9.3.9. In-vitro Dissolution study

Table No: 12

Time (hrs)	F1	F2	F3	F4	F5
	%	%	%	%	%
1	16.82	17.70	16.28	15.17	12.68
2	28.30	24.01	22.72	23.40	21.72
3	49.80	38.4	34.14	36.57	33.94
4	57.61	47.78	48.17	45.89	45.56
5	72.94	58.41	59.53	56.20	52.31
6	96.53	72.63	68.91	67.45	61.78
7	–	87.48	75.44	72.13	70.91
8	–	97.71	87.82	81.46	79.57
9	–	–	98.73	92.95	84.16
10	–	–	–	95.02	89.95
11	–	–	–	–	91.45
12	–	–	–	–	96.15

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 increase as the hydrogel swelling increase at lower pH, which leads to ionization of amino groups. [**45**] This selective release will ensure maximum availability of the drug in the stomach there by 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.

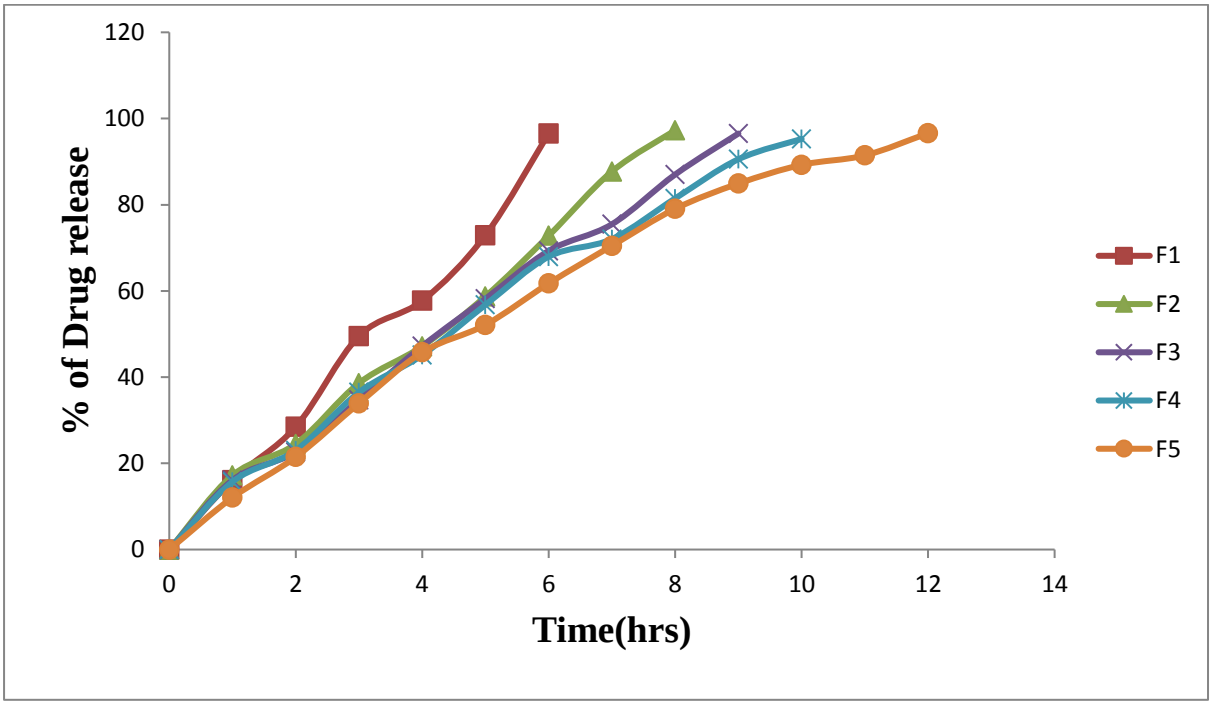


Fig No:24 Dissolution profile for chitosan based Licorice hydrogel

9.3.10. Kinetic Release

Table No:13 In-vitro drug release kinetics of Licorice hydrogel tablet

Time(hrs)	Cumulative % Drug release	Cumulative % drug Reaming	Log Cumulative % Drug release	Log cumulative %drug reaming	Square root	Log Time
0	0	0	0	0		
1	29.14	70.86	1.4644	1.8504	1	0
2	32.24	67.76	1.5083	1.8309	1.4142	0.3010
3	39.75	60.25	1.5993	1.7799	1.7320	0.4771
4	42.53	57.47	1.6286	1.7594	1.5	0.6020
5	48.20	51.8	1.6830	1.7143	2.2360	0.6989
6	53.15	46.85	1.7255	1.6714	2.4494	0.7781
7	59.07	40.93	1.7713	1.6120	2.6457	0.8450
8	65.73	34.27	1.8177	1.5349	2.8284	0.9030
9	71.32	28.68	1.8532	1.4575	3	0.9542
10	80.12	19.88	1.9037	1.2984	3.1622	1
11	86.09	13.91	1.9349	1.1433	3.3166	1.0413
12	96.70	3.3	1.9854	0.5185	3.4641	1.0739

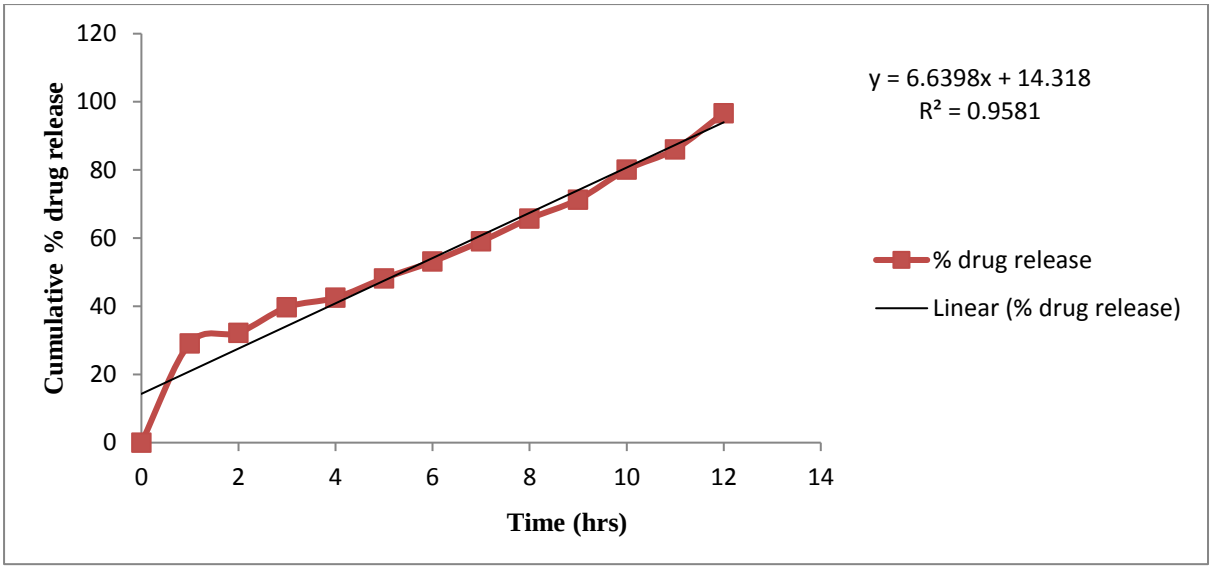


Fig No: 25 Zero Order Kinetic release

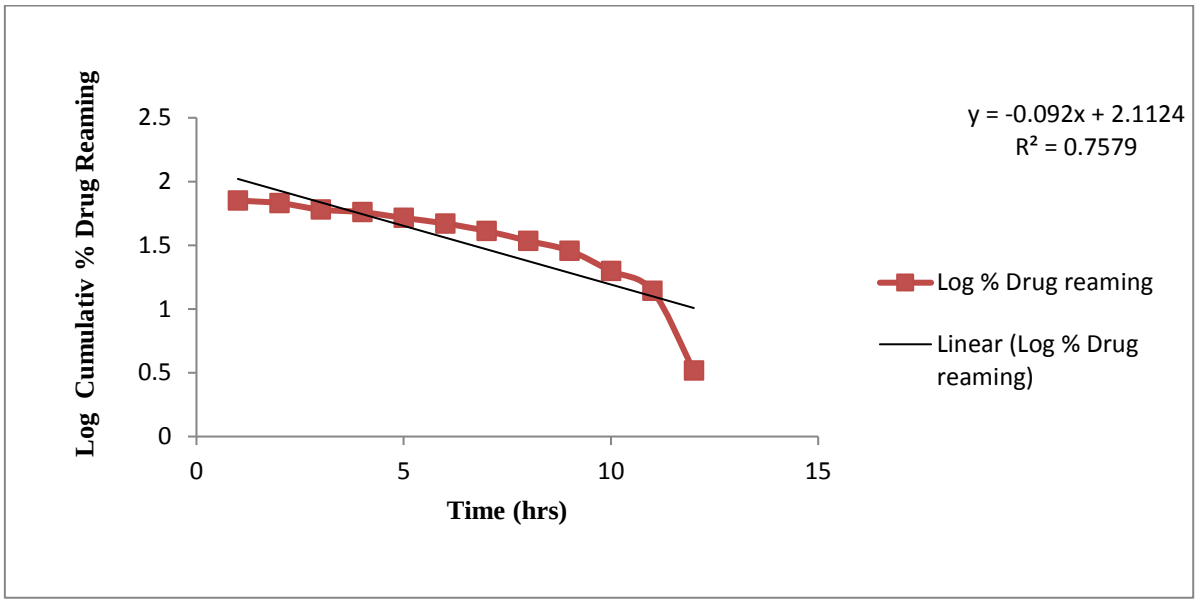


Fig No: 26First Order Kinetic release

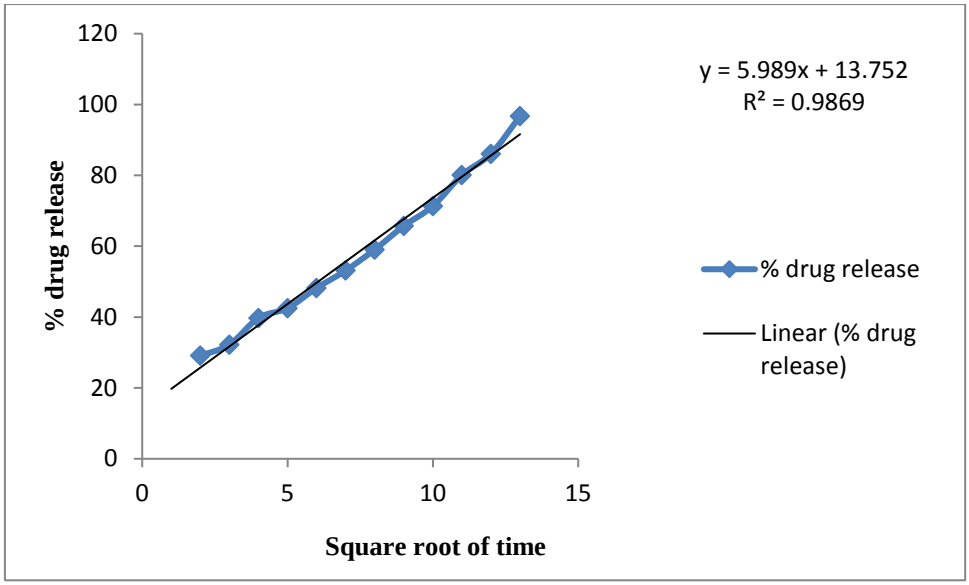


Fig No: 27 Higuchi Model release

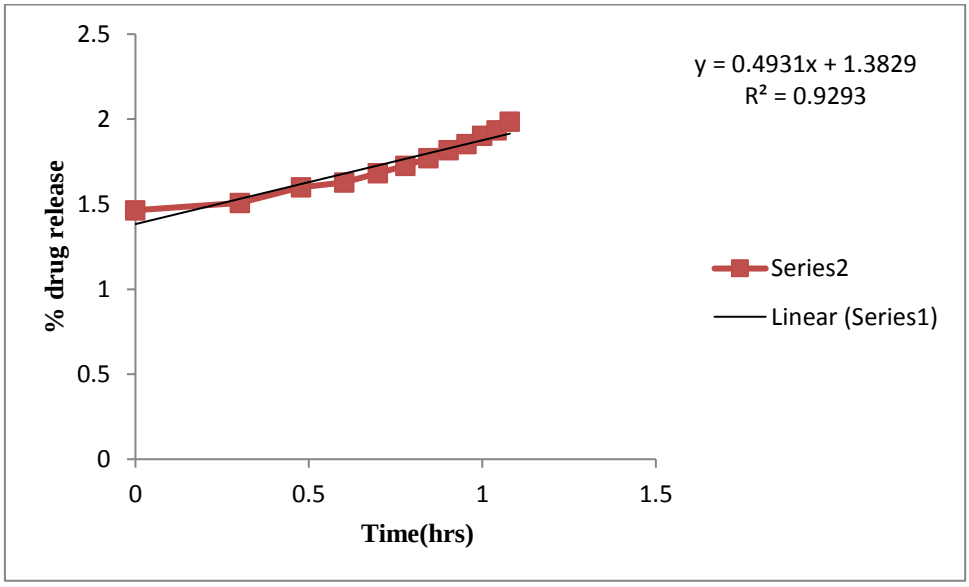


Fig No:28 Korsmeryer peppas model release

Kinetic release

In vitro drug release data was subjected to the goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, Higuchi's and Korsmeyer's and Peppa's models in order to determine the mechanism of drug release. When the regression coefficient values of zero order and first order plots were compared, it was observed that 'r2' values of zero order plots was found 0.959, indicating drug release from formulations(F5) was found to follows zero order kinetics. It is notable that the 'r^2' values of the linear regression for Higuchi's plot were found to be in the range of 0.986 indicating that the data fits the Higuchi's model well and the drug release were found to be predominantly controlled by a diffusion process. When the *in vitro* dissolution data was fitted to Korsmeyer's and Peppa's model, ther^2' values were found to be in the range of 0.929 in the formulations(F5), indicating the data fits the Korsmeyer's and Peppa's model well. The slopes (n) values of Korsmeyer's and Peppa's equation were found to be less than 0.5 indicated the release mechanism followed fickian diffusion due to swelling of gel matrix and high solubility of Licorice. [**46]**

9.3.11.Stability study

Tablet No: 14. Stability study report of Licorice loaded hydrogel tablet

Formulation	Conditions	Time Interval (Month)	Average Wt (mg)±5	Colour	Assay %	In vitro Dissolution					
						2hr	4hr	6hr	8hr	10hr	12hr
Licorice Hydrogel Matrix Tablet	25 °C±2 °C/75±5 % RH	0	750	Brown	99.80	98.75	99.62	98.45	98.42	97.35	98.45
		3	749	Brown	99	99.62	99.30	98.35	98.58	97.54	97.50
	40 °C±2 °C/75±5 % RH	0	753	Brown	99.52	99.84	99.52	98.54	97.12	97.85	98.43
		3	747	Brown	99.12	99.78	98.75	98.74	97.68	97.94	97.10

Stability studies were conducted for the optimized formulation of licorice loaded chitosan hydrogel tablets at 25±2ºC/60±5%RH, 40±2ºC /75±5% RH for 3 months. The hydrogel were analyzed for appearance, average weight, assay and *in vitro* drug release and report was shown in **Table 14**. The results revealed that no considerable different in all parameters were observed.

10.SUMMARY AND CONCLUSION

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 tof 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. It shows that increase in concentration of polymer leads to the controlled drug release from hydrophilic chitosan hydrogelfor12 hrs, 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 reduced 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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