

## Formulation of Doxycycline Loaded Floating Film using Bio-Material Extracted from Tagetes Erecta

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### ABSTRACT

The aim of research work was to isolate novel biopolymer from the seeds of *Tagetes erecta* and to characterize its physicochemical properties along with the acute toxicity. The isolated polymer was subjected for screening its retardability by using as a bio nano carrier for formulating Doxycycline (model drug) loaded floating films. *Tagetes* is a genus of 56 species of annual and perennial mostly herbaceous plants in the sunflower family. The genus is native to North and South America, but some species have become naturalized around the world. *Tagetes* species vary in size from 0.01 to 2.2m tall. They have pinnate green leaves blooms are naturally in golden, orange, yellow and white colors, often with maroon highlights. *Tagetes* grow well in almost any sort of soil. It contains essential oils, fatty acids, carotenoids and lutein. *Tagetes erecta* has long been known for its medicinal use, especially for strengthening the heart, and for treating ailments like headaches, swellings and tooth aches. *Tagetes erecta* seeds were soaked in water and then washed with chloroform and ethyl acetate. Obtained 100 gm of fine powder was soaked in 100ml boiled water for 24 hours. The mixture was filtered and methanol was added in double. The solution was refrigerated for 24 hours and then centrifuged. Precipitate was collected as biopolymer and was dried. The separated biopolymer was subjected for various physicochemical parameters like color, texture, particle size, solubility, colour changing point. Spectral analysis such as IR spectroscopy was done to check the polymeric nature of biopolymers. Drug-polymer interaction and skin irritancy studies are also done to check the polymer safety. Doxycycline loaded floating films was formulated using biopolymer *Tagetes erecta* such as (FT 1 to FT 9) in different ratio (1:2 to 1:18). Then these floating films were subjected for various evaluation parameters as weight uniformity, surface pH, thickness, folding endurance, content uniformity, floating cum mucoadhesive study, in-vitro and ex-vivo drug release and in-vivo bioavailability and stability study. Based on comparison study of t50 and t80 FT4 were considered as the best formulation. Conclusion was drawn that isolated material from the seeds of *Tagetes erecta* was devoid of acute toxicity and shows polymeric nature which was confirmed by spectral studies. Apart from that biopolymer it also inbuilt retardability which was confirmed by formulating floating film.

**Keywords:** biopolymer, *Tagetes erecta*, bio nano carrier, Doxycycline, floating films.

### INTRODUCTION

Floating systems or dynamically controlled systems are low-density systems that have sufficiently buoyancy to float over the gastric

contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. This results in an increased gastric retention time and a better

**How to cite this article:** Kumar V, Madhav NVS, Negi RS, Formulation of Doxycycline Loaded Floating Film using Bio-Material Extracted from *Tagetes Erecta*, PharmaTutor, 2013, 1(2), 106-128

control of the fluctuations in plasma drug concentration. Many buoyant systems have been developed based on granules, powders, capsules, tablets, laminated films and hollow microspheres<sup>[1][2]</sup>.

Gastroretentive drug delivery is an approach to prolong gastric residence time, thereby targeting site-specific drug release in the upper gastrointestinal tract (GIT) for local or systemic effects. Gastroretentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the gastric retention time (GRT) of drugs. Over the last few decades, several gastroretentive drug delivery approaches being designed and developed, including: high density (sinking) systems that is retained in the bottom of the stomach, low density (floating) systems that causes buoyancy in gastric fluid, mucoadhesive systems that causes bioadhesion to stomach mucosa, unfoldable, extendible, or swellable systems which limits emptying of the dosage forms through the pyloric sphincter of stomach, superporous hydrogel systems, magnetic systems etc. The current review deals with various gastroretentive approaches that have recently become leading methodologies in the field of site-specific orally administered controlled release drug delivery systems<sup>[3],[12]</sup>.

The real challenge in the development of an oral controlled-release drug delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form within the gastrointestinal tract (GIT) until all the drug is completely released at the desired period of time. Indeed, gastric drug retention has received significant interest in the past few decades. Most of the conventional oral delivery systems have shown some limitations related to fast gastric-emptying time<sup>[4]</sup>.

This triggered the attention towards formulation of stomach specific (gastro retentive) dosage forms. This dosage forms will be very much useful to deliver 'narrow absorption window drugs. Several approaches

are currently utilized in the prolongation of the GRT, including floating drug delivery systems (FDDS), swelling and expanding systems, polymeric bioadhesive systems, high-density systems, modified-shape systems and other delayed gastric emptying devices<sup>[5]</sup>.

#### NATURAL BIO-POLYMERS USED IN FLOATING DRUG DELIVERY SYSTEM

The aim of this work is tried to give a brief overview to the role of natural bio-polymers in the development of floating drug delivery system. The use of bio-polymer is valuable based on biocompatibility and safety. Bio-polymers are generally employed in floating drug delivery system so as to target the delivery of drug to a specific region in the gastrointestinal tract i.e. stomach. Moreover, these bio-polymers are safe and nontoxic<sup>[6]</sup>.

#### ADVANTAGES OF BIO-POLYMERS

The various advantages<sup>[6]</sup> of natural plant based materials include.

- Biocompatible and non-toxic;
- Low cost;
- Environmental-friendly processing;
- Local availability;
- They have better patient tolerance as well as public acceptance.

There is less chance of side and adverse effects with natural materials compared with synthetic one.

#### BASIC GIT PHYSIOLOGY

Anatomically the stomach is divided in to three regions Fundus, Body and Antrum (pylorus)<sup>[1]</sup>fig.2. The proximal part made of Fundus and body acts as a reservoir for undigested materials, whereas the antrum is the main site for mixing motions and acts as a pump for gastric emptying by propelling actions. Gastric emptying occurs in both the fasting and fed states. During the fasting state

an interdigestive series of electrical events take place which cycle both through stomach and intestine every 2-3 hrs, which is called as interdigestive myoelectric cycle or migrating myoelectric cycle (MMC) which is further divided in to four phases After the ingestion of a mixed meal, the pattern of contractions changes from fasted to that of fed state which is also termed as digestive motility pattern<sup>[7]</sup>.

1. Phase 1-(Basic phase)-last from 30-60 minutes with rare contractions.
2. Phase 2-(Preburst phase)-last for 20-40 minutes with intermittent action potential and contractions.
3. Phase 3-(Burst phase) - last for 10-20 minutes which includes intense and regular contractions for short period.
4. Phase 4-last for 0-5 minutes and occurs between phase 2 and 1 of 2 consecutive cycles (Fig1).

After the ingestion of a mixed meal, the pattern of con-contractions changes from fasted to that of fed state which is also termed as digestive motility pattern.

#### MUCOADHESIVE DRUG DELIVERY SYSTEM

Mucoadhesive drug delivery system prolong the residence time of the dosage form at the site of application or absorption and facilitate an intimate contact of the dosage form with the underline absorption surface and thus contribute to improved and / or better therapeutic performance of the drug. In recent years many such mucoadhesive drug delivery systems have been developed for oral, buccal, nasal, rectal and vaginal routes for both systemic and local effects<sup>[8]</sup>.

#### DEFINITION

Adhesion can be defined as the bond produced by contact between a pressure - sensitive adhesive and a surface. The American Society of testing and materials has defined it as the state in which two surfaces are held together by

interfacial forces, which may consist of valence forces, interlocking action or both.<sup>[8]</sup>

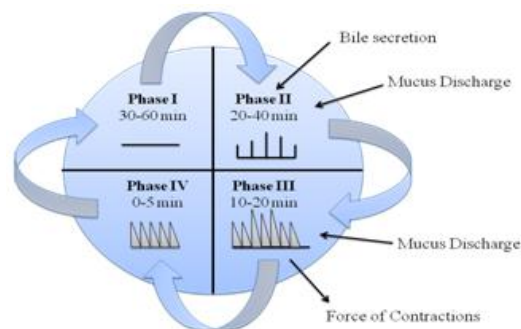


Figure 1: Gastrointestinal motility pattern

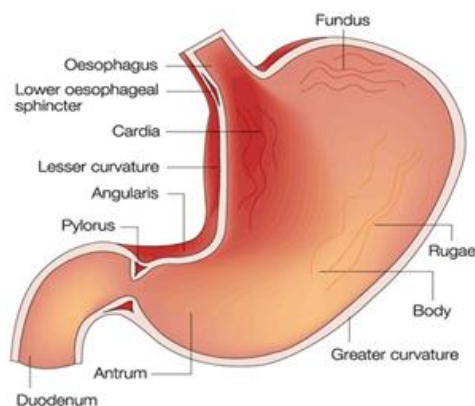


Figure 2: Structure of stomach

#### CONCEPT OF ADHESION

In biological systems, four types of bioadhesion could be distinguished:

1. Adhesion of a normal cell on another normal cell.
2. Adhesion of a cell with a foreign substance.
3. Adhesion of a normal cell to a pathological cell.
4. Adhesion of an adhesive to a biological substance.

For drug delivery purpose, the term bioadhesion implies attachment of a drug carrier system to a specific biological location. If

adhesive attachment is to a mucus coat, the phenomenon is referred to as mucoadhesion.<sup>[8]</sup>

#### THE MUCUS LAYER:

Mucus is a translucent and viscid secretion, which forms a thin, continuous gel blanket adherent to mucosal epithelial surface. The mean thickness of this layer varies from about 50-450  $\mu\text{m}$  in humans. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini. The exact composition of the mucus layer varies substantially, depending on the species, the anatomical location and pathological states. However, it has general composition. Mucus thickness was measured with a micropipette before and after mucus removal by suction. The mucus layer was translucent and continuous; It was thickest in the colon (approx. 830 $\mu\text{m}$ ) and thinnest in the jejunum (approx. 123 $\mu\text{m}$ ). On mucus removal, a continuous firmly adherent mucus layer remained attached to the epithelial surface in the corpus (approx. 80 $\mu\text{m}$ ), antrum (approx. 154 $\mu\text{m}$ ), and colon (approx. 116 $\mu\text{m}$ ). In the small intestine, this layer was very thin (approx. 20 $\mu\text{m}$ ) or absent. After removal, there was a continuous increase in mucus thickness with the highest rate in the colon and the lowest rate in the stomach<sup>[8]</sup>.

#### a) Composition of mucus:

Sr No.	Components	% Amount
1	Water	95
2	Glycoprotein and lipids	0.5-5.0
3	Mineral salts	1
4	Free proteins	0.5-1.0

#### b) Function of mucus layer: -

The primary functions<sup>[9]</sup> of the mucus layer are:

- **Protective:** - Resulting particularly from its hydrophobic.

- **Barrier:** - The role mucus layer as barrier in tissue absorption of drugs and other substances is well known as its influence the bioavailability of the drugs

- **Adhesion:** - Mucus has strong cohesive properties and firmly binds to the epithelial cells surface as continuous gel layer.

- **Lubrication:** - An important role of the mucus layer is to keep the mucosal membrane moist. Continuous secretion of mucus from the goblet cells is necessary to compensate for the removal of mucus layer due to digestion, bacterial degradation and solubilization of mucin molecules.

At physiological pH, the mucus network may carry a significant negative charge because of the presence of sialic acid and sulphate residues and this high charge density due to negative charge contributes significantly to the bioadhesion.

#### GASTRIC MUCOSA

The gastric mucosa<sup>[9]</sup> is the mucous membrane layer of the stomach which contains the glands and the gastric pits. In men it is about 1 mm thick and its surface is smooth, soft, and velvety. It consists of epithelium, lamina propria, and the muscularis mucosae.

In its fresh state, it is of a pinkish tinge at the pyloric end and of a red or reddish-brown color over the rest of its surface. In infancy it is of a brighter hue, the vascular redness being more marked.

It is thin at the cardiac extremity, but thicker toward the pylorus. During the contracted state of the organ it is thrown into numerous plaits or rugae, which, for the most part, have a longitudinal direction, and are most marked toward the pyloric end of the stomach, and along the greater curvature. These folds are

entirely obliterated when the organ becomes distended.

When examined with a lens, the inner surface of the mucous membrane presents a peculiar honeycomb appearance from being covered with funnel-like depressions or foveolae of a polygonal or hexagonal form, which vary from 0.12 to 0.25 mm. in diameter. These are the ducts of the gastric glands, and at the bottom of each may be seen one or more minute orifices, the openings of the gland tubes. Gastric glands are simple or branched tubular glands that emerge on the deeper part of the gastric foveola, inside the gastric areas and outlined by the folds of the mucosa.

There are three types of glands: cardiac glands (in the proximal part of the stomach), oxyntic glands (the dominating type of gland), and pyloric glands. The cardiac glands mainly contain mucus producing cells. The bottom part of the oxyntic glands is dominated by zymogen (chief) cells that produce pepsinogen (an inactive precursor of the pepsin enzyme). Parietal cells, which secrete hydrochloric acid are scattered in the glands, with most of them in the middle part. The upper part of the glands consist of mucous neck cells; in this part the dividing cells are seen. The pyloric glands contain mucus-secreting cells. Several types of endocrine cells are found in all regions of the gastric mucosa. In the pyloric glands contain gastrin producing cells (G cells); this hormone stimulates acid production from the parietal cells. ECL (enterochromaffine-like) cells, found in the oxyntic glands release histamine, which also is a powerful stimulant of the acid secretion. The A cells produce glucagon, which mobilizes the hepatic glycogen, and the enterochromaffine cells that produce serotonin, which stimulates the contraction of the smooth muscles.

The surface of the mucous membrane is covered by a single layer of columnar epithelium. This epithelium commences very abruptly at the cardiac orifice, where there is a sudden transition from the stratified epithelium of the esophagus. The epithelial lining of the gland ducts is of the same character and is continuous with the general epithelial lining of the stomach.

#### **Advantages<sup>[7]</sup> of FDDS**

1. Floating dosage forms such as tablets or capsules will remain in the solution for prolonged time even at the alkaline pH of the intestine.
2. FDDS are advantageous for drugs meant for local action in the stomach eg: Antacids.
3. FDDS dosage forms are advantageous in case of vigorous intestinal movement and in diarrhea to keep the drug in floating condition in stomach to get a relatively better response.
4. Acidic substance like aspirin causes irritation on the stomach wall when come in contact with it hence; HBS/FDDS formulations may be useful for the administration of aspirin and other similar drugs.
5. The FDDS are advantageous for drugs absorbed through the stomach eg: Ferrous salts, Antacids.

#### **Limitations<sup>[7]</sup> of FDDS**

1. Floating systems are not feasible for those drugs that have solubility or stability problems in gastric fluids.
2. Drugs such as Nifedipine, which is well absorbed along the entire GI tract and which undergo significant first-pass metabolism, may not be suitable candidates for FDDS since the slow gastric emptying may lead to reduced systemic bioavailability. Also there are limitations to the applicability of FDDS for drugs that are irritant to gastric mucosa.
3. One of the disadvantages of floating systems is that they require a sufficiently high level of

fluids in the stomach, so that the drug dosages form float therein and work efficiently.

4. These systems also require the presence of food to delay their gastric emptying.

## MATERIALS AND METHOD

### Tagetes erecta seeds

Scientific classification <sup>[11]</sup>	
Kingdom	Plantae
Order	Asterales
Family	Asteraceae
Genus	Tagetes
Species	Erecta

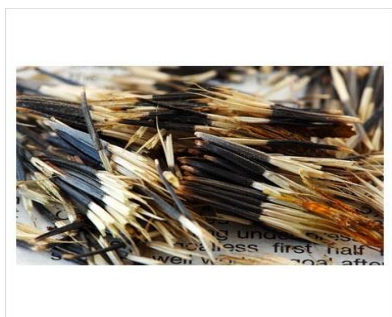


Fig 3: Seeds of *Tagetes erecta*

*Tagetes*<sup>[10],[11]</sup> is a genus of 56 species of annual and perennial mostly herbaceous plants in the sunflower family (Asteraceae or Compositae). The genus is native to North and South America, but some species have become naturalized around the world. One species, *T. minuta*, is considered a noxious invasive plant in some areas. The leaves are pinnate-compound, 3 to 20 cm long, with 9-20 linear to lanceolate small leaves, 1-3 cm long and 0.3-1.5 cm broad, with sharp apices and indented margins. The flowers of the outer ring, called ray florets, in number of 5-8, are feminine with obovate corolla, 1-2.5 cm long and 1-2 cm broad, formed by five petals joined together of an intense yellow colour in the wild, and also from orange to

brown-red and in some instances bicolor, yellow and brown-red, in the countless cultivated varieties. The fruits, containing one seed only and called achenes (or, more precisely, cypselae) in the *Asteraceae*, are thin, 0.6-1 cm long, black coloured, surmounted by the pappus, the modified calyx of the flower, formed by 1-2 pointed scales long 0.6-1.2 cm and by 2-4 scales, distinct or united at the base, oblong or linear, 0.2-0.6 cm long. It easily reproduces by seed, which germinates in one week on average, at the temperature of 18-22° C; the blooming begins after about three months and lasts until late autumn. It has a good resistance to the diseases; attacks by fungi (*Botrytis*) may occur in the wet climates and among the most frequent parasites stand the mites (the classical "spider mite"). The contact with the leaves may originate dermatitis in the most sensitive subjects.

### Description<sup>[11]</sup>:

*Tagetes* species vary in size from 0.01-2.2 m tall. They have pinnate green leaves. Blooms are naturally in golden, orange, yellow, and white colors, often with maroon highlights. Floral heads are typically (0.1-) to 4-6 cm diameter, generally with both ray florets and disc florets. In horticulture they tend to be planted as annuals, although the perennial species are gaining popularity. Depending on the species, *Tagetes* grow well in almost any sort of soil. Most horticultural selections grow best in soil with good drainage.

### Nutritional value:

It contains essential oils, fatty acids, carotenoids and lutein. Among carotenoids 93% utilizable pigments (detected at 450 nm), consisting of all-trans and cis isomers of zeaxanthin (5%), all-trans and cis isomers of lutein, and lutein esters (88%) are present. Less than 0.3% of lutein oxidation products are also present. It is found to have 26 components, accounting for 89% of the total oil. The major constituents are (Z)-β-

ocimene (42.2%), dihydrotagetone (14.3%), (Z)-tagetone (8.3%), limonene (7.3%), and (E)-ocimenone (6.1%) and (Z) ocimenone (5.3%).

#### Uses:

- Tagetes are often used in companion planting for tomato, eggplant, chili pepper, tobacco and potato. Some of the perennial species are deer-, rabbit-, rodent- and peccary-resistant.

- *T. minuta* (khakibush or huacatay), originally from South America, has been used as a source of essential oil for the perfume industry known as tagette or "marigold oil", and as a flavourant in the food and tobacco industries. It is commonly cultivated in South Africa, where the species is also a useful pioneer plant in the reclamation of disturbed land.

- The florets of *Tagetes erecta* are rich in the orange-yellow carotenoid lutein and are used as a food colour (INS-Number E161b) in the European Union for foods such as pasta, vegetable oil, margarine, mayonnaise, salad dressing, baked goods, confectionery, dairy products, ice cream, yogurt, citrus juice and mustard. In the United States, however, the powders and extracts are only approved as colorants in poultry feed.

- Marigolds are recorded as a food plant for some Lepidoptera caterpillars including the Dot Moth, and a nectar source for other butterflies. They are often part of butterfly gardening plantings.

#### Applications of *Tagetes erecta* (marigold) in pharmacy:

- The marigold has long been known for its medicinal use, especially for strengthening the heart, and for treating ailments like headaches, swellings and toothaches.

- Marigolds were used to treat wounds as well prevent them from getting infected during the American Civil War as well as the First World War. This was done by applying infused oil on wounds made from marigold extracts.

- The medicinal value of the marigold is largely because of the anti-fungal and anti-septic properties present in this flower.

- Marigold is often used by aroma therapists to cure many diseases like viral infections, eczema, cracked skin, scars, inflammation and rashes.

- It is also used in both conventional medicine and homeopathy, especially as an ointment for treating cuts and bruises.

- The petals of marigold flowers have their use too as eyewash.

- Drinking a tea or infusion made from marigold can help heal colitis, mouth ulcers and stomach ulcers. The infusion helps to stimulate the lymphatic system of the body; it reduces swelling; and also helps to cleanse toxins from the body.

- *Tagetes erecta* seeds are natural pesticide having food colourings and medicinal virtues.

#### DOXYCYCLINE<sup>[13],[14],[15]</sup>

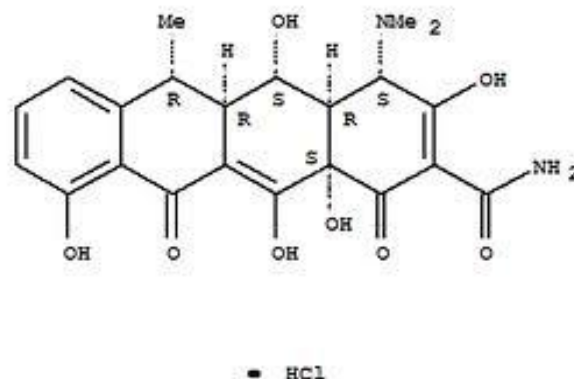


Table 1: Drug Profile of doxycycline

S.No.	Drug properties	Characteristics
1.	IUPAC name	(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide
<b>PHYSICAL AND CHEMICAL PROPERTIES</b>		
1.1.	Formula	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>8</sub>
1.2.	Molecular mass	462.46 g/mol
1.3.	Appearance	Yellow crystalline powder
1.4.	Melting Point	198-201°C
1.5.	Solubility	Freely soluble in <i>water</i> and in <i>methanol</i> ; sparingly soluble in <i>ethanol</i> (95%); practically insoluble in <i>chloroform</i> and in <i>ether</i> . It is soluble in solutions of alkali hydroxides and carbonates.
<b>2. PHARMACOKINETIC DATA</b>		
2.1.	Bioavailability	100%
2.2.	Protein Binding	>90%
2.3.	Metabolism	Hepatic
2.4.	pKa	3.5, 7.7, 9.5 (20 °C)
2.4.	Half-life	18-22 hrs.
<b>3. THERAPEUTIC DATA</b>		
3.1	Route	Oral

Doxycycline is a member of the tetracycline antibiotics group, and is commonly used to treat a variety of infections. Doxycycline is a semi synthetic tetracycline invented and clinically developed in the early 1960s by Pfizer Inc. and marketed under the brand name Vibramycin. Vibramycin received U.S. Food and Drug Administration (FDA) approval in 1967 becoming Pfizer's first once-a-day, broad-spectrum antibiotic.

### Mechanism of Action

**1)** Doxycycline interferes with the third stage of bacterial protein synthesis. After amino acids are activated and attached to t-RNA (transfer RNA), the resulting amino acyl-t-RNA migrates to the bacterial ribosome for synthesis of proteins. Doxycycline binds to the 30S subunit on the ribosome and inhibits binding of the aminoacyl-t-RNA molecules.

**2)** There is also some evidence that doxycycline may cause alterations in the cytoplasmic membrane, thereby allowing leakage of nucleotides and other compounds from the cell. This would explain the rapid inhibition of DNA replication that ensues when cells are exposed

to concentrations of doxycycline greater than that needed to inhibit protein synthesis.

**3)** In higher concentrations, doxycycline inhibits mammalian protein synthesis and may aggravate pre-existing renal function impairment. The drug may interfere with parenteral nutrition in post-operative patients by inhibiting utilization of amino acids for protein synthesis.

**4)** In periodontal disease, a doxycycline 20 milligram twice daily reduces the elevated collagenase activity which results from neutrophil response to the occurring inflammatory processes. Collagenase



contributes to the pathogenesis of periodontal breakdown.

### Pharmacokinetics

Drug Concentration Level

**1) ORAL:** The average C<sub>max</sub> reported with a 200-mg dose is 2.6 mcg/mL

**2) INTRAVENOUS:** A C<sub>max</sub> of 5 to 10 mcg/mL was reported with a 200-mg dose.

### ADME

#### Absorption

Doxycycline is almost completely absorbed from the gastrointestinal tract following oral administration. Several studies have been done comparing different dosage forms (capsules, tablets, solution) of doxycycline and different manufacturers. Studies have confirmed that there are no differences in the bioavailability of different doxycycline preparations.

#### Distribution

**a) Distribution Sites:** Protein Binding (80% to 93%)

**b) Distribution Kinetics:** Volume of Distribution (0.75 L/kg)

The apparent V<sub>d</sub> appears to be higher in geriatric patients than in younger patients.

#### Metabolism: Liver (50%)

**Excretion:** Renal Excretion (35% to 45%)

#### Elimination Half-life: 15 to 24 hours

**1)** In patients with renal dysfunction, the half-life of doxycycline ranges from 18 to 25 hours. Although a slight increase in the half-life in patients with renal dysfunction has been reported, others have found no significant changes in the half-life.

**2)** The half-life may be urine pH dependent with a range of 11 to 12 hours in alkaline urine (average 11.6 hr) and a range of 13 to 18 hours in acidic urine (average 14.6 hr).

### PHARMACOLOGY:

Doxycycline, a long-acting tetracycline derived from oxytetracycline, is used to inhibit bacterial protein synthesis and treat nongonococcal urethritis and cervicitis, exacerbations of bronchitis in patients with COPD, and adult periodontitis. Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis. Doxycycline prevents the normal function of the apicoplast of *Plasmodium falciparum*, a malaria causing organism.

**DOSE:** The equivalent of 200 mg of doxycycline on the first day followed by 100 mg daily.

#### USES:

**Antiprotozoal:** It is used in prophylaxis against malaria. It should not be used alone for initial treatment of malaria, even when the parasite is doxycycline-sensitive, because the antimalarial effect of doxycycline is delayed. This delay is related to its mechanism of action, which is to specifically impair the progeny of the apicoplast genes, resulting in their abnormal cell division. It can be used in a treatment plan in combination with other agents, such as quinine.

**Antibacterial:** It is used in the treatment and prophylaxis of *Bacillus anthracis* (anthrax). It is also effective against *Yersinia pestis* (the infectious agent of bubonic plague), and is prescribed for the treatment of Lyme disease, ehrlichiosis and Rocky Mountain spotted fever. In fact, because doxycycline is one of the few medications shown to be effective in treating Rocky Mountain spotted fever (with the next-best alternative being chloramphenicol), doxycycline is indicated even for use in children for this illness. Otherwise, doxycycline is not indicated for use in children under the age of eight years. Doxycycline, like other antibiotics,

will not work for colds, influenza, or other viral infections.

**Anthelmintic:** Elephantiasis is the end-stage condition of lymphatic filariases caused by one of two genera of filarial nematodes (roundworms): *Wuchereria* or *Brugia* (primarily *Wuchereria bancrofti*). Elephantiasis is characterized by permanently swollen limbs or genitals and permanent damage to the lymph system (often accompanied by severe secondary fungal and bacterial infections). This results from blockage of lymph flow caused by immune response against dead or dying adult worms in the lymphatics. This condition affects over 120 million people worldwide, with 1 billion at risk. Previous antinematode treatments have been limited by poor levels of effectiveness, drug side effects and high costs. Doxycycline was shown in 2003 to kill the symbiotic *Wolbachia* bacteria in the filarial worms' reproductive tracts, rendering them sterile, thus reducing transmission of the disease. Field trials in 2005 showed doxycycline almost completely eliminates the release of microfilariae when given for an 8 week course. However, doxycycline only reduces transmission and the relatively light pathology associated with microfilaraemia; there is currently no cure for lymphatic filariasis

#### TOXICITY:

Symptoms of overdose include anorexia, nausea, diarrhoea, glossitis, dysphagia, enterocolitis and inflammatory lesions (with monilial overgrowth) in the anogenital region, skin reactions such as maculopapular and erythematous rashes, exfoliative dermatitis, photosensitivity, hypersensitivity reactions such as urticaria, angioneurotic oedema, anaphylaxis, anaphyl-actoid purpura, pericarditis, and exacerbation of systemic lupus erythematosus, benign intracranial hypertension in adults disappearing on discontinuation of the medicine, haematologic

abnormalities such as haemolytic anaemia, thrombocytopenia, neutropenia, and eosinophilia. LD<sub>50</sub>=262 mg/kg (I.P. in rat).

#### STORAGE:

Store in tightly-closed, light-resistant containers in a cool place. If the substance is intended for use in the manufacture of injectable preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

#### CHROMATOGRAPHY STUDIES:

Carry out the method for *thin-layer chromatography*, using *silica gel H* as the coating substance and a mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water* as the mobile phase. Spray the plate evenly with a 10% w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with *10M sodium hydroxide*. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply separately to the plate 1 ml of each of three solutions in *methanol* containing (1) 0.05% w/v of the substance being examined, (2) 0.05% w/v of *doxycyclinehydrochloride RS* and (3) 0.05% w/v each of *doxycyclinehydrochloride RS* and *tetracycline hydrochloride RS*. After removal of the plate, dry it in a current of air and examine under *ultra-violet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

#### CAUTIONS AND SIDE EFFECTS:

Cautions and side effects are similar to those of other members of the tetracycline antibiotic group. However, the risk of photosensitivity skin reactions is of particular importance for those intending long-term use for malaria

prophylaxis, because it can cause permanent sensitive and thin skin. Unlike some other members of the tetracycline group, it may be used in those with renal impairment. Previously, doxycycline was believed to impair the effectiveness of many types of hormonal contraception due to CYP450 induction. Recent research has shown no significant loss of effectiveness in oral contraceptives while using most tetracycline antibiotics (including doxycycline), although many physicians still recommend the use of barrier contraception for people taking the drug to prevent unwanted pregnancy. Food, including dairy products, does not interfere with the absorption of doxycycline, unlike most other tetracycline antibiotics.

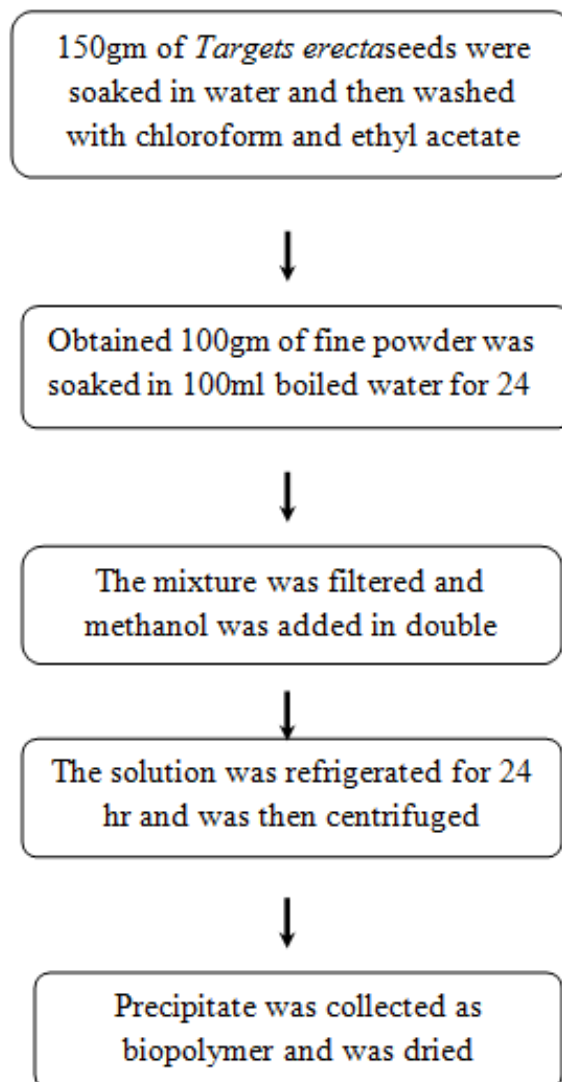
## RESEARCH METHODOLOGY

### Isolation of Bio-material from Natural edible sources:

#### Isolation from *Tagetes erecta* seeds (marigold seeds):

150 gm of marigold seeds were collected and were soaked in 100ml of water and was then washed with chloroform and ethyl acetate. It was then dried and crushed to convert into powder form. It was then sieved through sieve no. 120 to obtain fine powder which was further processed to extract out biopolymer. 100 gm of fine powder was taken in a 250 ml of beaker and was washed with soaked in 100ml of boiled water and was kept for 24 hr. Supernatant was obtained. Acetone was added in supernatant solution in double quantity and the solution was kept for refrigeration for 24 hrs. The upper solvent was discarded and the precipitate was centrifuged and it was then collected, dried and weighed. The extraction process was optimized by repeating the procedure six times with similar quantities of materials and practical yield was calculated a brief representation of the extraction procedure is drawn in the flowchart.

### Flow chart for the isolation of Bio-material from *Targets erecta* seeds:



### Physicochemical Characterization of Isolated Bio-material:

The isolated bio-material from various natural edible sources was subjected for various physical and chemical properties. Physical properties include appearance, odour, taste, melting point, state and solubility. Following chemical tests<sup>[16]</sup> were performed to check the presence or absence of carbohydrate, protein, and starch etc.

### 1. Test for carbohydrates :

**Molish Reagent Test:** In this test the polymeric solution was treated with molish reagent solution in equal quantity and then concentrated sulfuric acid was added slowly in few drops from the side of the test tube and observed for reddish brown precipitates.

**Fehling's Test:** In this test the polymeric solution was treated with Fehling's reagent A and B solution in equal quantity and was then boiled and observed for colour change from green to yellow to orange to red.

### 2. Test for proteins:

**Biuret Test:** The polymeric solution was treated with biuret reagent (1% of NaOH Solution) followed by few drops of aqueous copper II sulfate was added and observed for violet colour.

**Ninhydrin Test:** In this test the polymeric solution was treated with 0.1% ninhydrin solution and was then boiled and cooled and observed for blue colour.

### 3. Test for starch :

In this test 1-2 drops of iodine solution was added to the polymer solution and observed for the appearance of purple colour.

### Drug Polymer Interaction Studies:

Although excipients are considered to be pharmacologically inert, but excipients can initiate, propagate or participate in chemical or physical interactions with drug compounds, which may compromise the effectiveness of a medication. Excipients may also contain impurities or form degradation products that in turn cause decomposition of drug substances.

Drug interaction study<sup>[17]</sup> was performed by taking three different ratios of drug and biomaterial 1:1, 1:2, 1:3. The U.V. absorbance of the three ratios was taken and compared with the absorbance of pure drug.

**a) Dry method:** The drug and standard polymers and isolated biomaterials are to be mixed in various ratios (1:1, 1:3, 3:1, 1:20) in watch glass at room temperature and the results are to be analyzed.

**b) Wet method:** The drug and standard polymers and the isolated biomaterials are to be mixed in various ratios (1:1, 1:3, 3:1, 1:20) in a suitable solvent and kept at 50°C and sampling is done in every hour upto 3hrs and then TLC is to be performed of the various samples. Then R<sub>f</sub> value is to be calculated as:

**R<sub>f</sub> value = Distance travelled by solute/ Distance travelled by solvent**

### Preparation of Standard Curve<sup>[16]</sup>:

**1. Preparation of Hydrochloride buffer pH 1.2 :** 50 ml of 0.2M HCL and 50 ml of 0.2M KCL was taken separately in a 200 ml volumetric flask and volume was make upto 200 ml with distilled water.

#### a) Preparation of 0.2M HCL :

7.292 ml of concentrated HCL was taken in a 1000 ml volumetric flask and volume was make upto 1000 ml with distilled water.

#### b) Preparation of 0.2M KCL :

14.91 gm of KCL was taken in a 1000 ml volumetric flask and volume was make upto 1000 ml with distilled water.

### 2. Preparation of Phosphate buffer pH 7.4 :

50 ml of 0.2M Potassium Dihydrogen Phosphate and 39.1ml of 0.2M NaOH was taken in a 200 ml volumetric flask and volume was make upto 200 ml with distilled water.

#### a) Preparation of 0.2M Potassium Dihydrogen Phosphate:

27.218 gm Potassium Dihydrogen Phosphate was placed in a 1000 ml volumetric flask and volume was make upto 1000 ml with distilled water.

#### b) Preparation of 0.2M NaOH:

8 gm of NaOH was placed in a 1000 ml volumetric flask and volume was made upto 1000 ml with distilled water.

### 3. Preparation of Stock Solution:

10 mg of accurately weighed Doxycycline was taken in a 100 ml volumetric flask and was first dissolved in 50 ml of distilled water, it was then subjected to sonication for complete solubility of drug in water, and after that 50 ml distilled water was added to make a solution containing 100 µg/ml.

### 4. Determination of UV Absorption Maxima:

1 µg/ml solution of Doxycycline was prepared by addition of 0.1 ml of stock solution in a 10 ml volumetric flask in pH 1.2 HCL and was scanned for absorbance between 200-400nm using UV/Visible spectrophotometer. Doxycycline exhibits UV (UV-1800, Shimadzu) absorption maxima ( $\lambda_{max}$ ) of 344 nm. The method was optimized by repeating the same procedure for six times.

### 5. Preparation of Calibration Curve:

0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 ml of stock solution were transferred to 10 ml volumetric flasks and were serially diluted with pH 1.2 HCL, upto the mark to obtain 1,2,3,4,5,6,8,10,12,14,16,18 and 20 µg/ml concentration of Doxycycline respectively.

Absorbance of each solution was measured at  $\lambda_{max}$ . 344 nm using UV/Visible spectrophotometer against pH 1.2 HCL as a blank. Same procedure was repeated for preparing the standard curve in phosphate buffer 7.4 and water.

### preparation of floating BIOFILMS:

Doxycycline loaded Floating Biofilms were prepared by **SolventFilm Casting method**<sup>[18]</sup>. In this method the polymeric solution was prepared by dissolving the required quantity of polymer and dextrose in water. Dextrose is mainly used as an osmotic agent and film initiator. After triturating and centrifuging this solution glycerol which is used as plasticizer and drug (50 mg) was added. The prepared viscous solution was poured in a petri dish and was allowed to dry in a hot air oven for 4 hrs at 40°C. After drying, the films were removed with the help of a sharp blade. It was peeled off and cutted into patches of 1cm<sup>2</sup> for performing various evaluation parameters. Different formulations were prepared with varying ratios of the polymer, dextrose and glycerol.

Standard formulations were prepared by the same method as specified above, by using different ratios of NaCMC: Sodium alginate and NaCMC: Agar in place of biopolymer as mentioned in table no.2.

**Table 2: Formula for Floating Biofilms using *Tagetes erecta* Biopolymer:**

Ingredients	FT1	FT2	FT3	FM4	FT5	FT6	FT7	FT8	FT9
Doxycycline(mg)	50	50	50	50	50	50	50	50	50
<i>Tagetes erecta</i> (mg)	100	200	300	400	500	600	700	800	900
Dextrose(mg)	100	100	100	100	100	100	100	100	100
Glycerol(ml)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Distilled Water(ml)	10	10	10	10	10	10	10	10	10

**Evaluation of floating films:**

The prepared Doxycycline loaded floating biofilms were evaluated for the following parameters<sup>[19], [20]</sup>:

Floatability, surface pH, thickness, weight variation, folding endurance, tensile strength, content uniformity, water vapour transmission, moisture content, moisture uptake, and *in-vitro*, *ex-vivo* and *in-vivo* release study.

**Floating properties:**

The floating properties<sup>[21]</sup> of the floating biofilms were determined in the closed medium-filled flasks placed in a horizontal shaker (model OS1473VBA, Revco Scientific Inc., USA) (medium; 150 mL of 0.1 N HCl, 37 °C, 50 rpm). A patch of 1 X 1 cm of floating biofilm is placed in the medium and the time to float and duration of floating (floating time) were determined by visual observation.

**Surface pH:**

Anagar plate was prepared by dissolving 2 % (w/v) agar in warmed HCl buffer of pH 1.2 with continuous stirring and then pouring the solution into a petridish till gelling at room temperature. Then the patches of films were left to set for 2 hr on the surface of the agar plate. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. The mean of three readings was recorded<sup>[22]</sup>.

**Percentage moisture content:**

The prepared biofilms films of 1×1cm<sup>2</sup> were weighed individually and were kept in desiccator containing fused calcium chloride at room temperature for 24 hr. After 24 hr, the films were reweighed and the percentage moisture content<sup>[22]</sup> was determined by the formula mentioned below:

$$\% \text{ moisture content} = \left[ \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \right] \times 100$$

**Film Weight:** Film weights were evaluated by taking three patches of size 1 x 1 cm from every formulation and were weighed individually on a digital balance (ESSAE, Goa, and DS-852J). The average weights were calculated.

**Film Thickness:**

The thickness of the film was determined by Digital vernier caliper (Mitutoyo Corp., CD-6"CSX) at different position by placing the film in between two glass slides with known thickness and average thickness was calculated.

**Drug content uniformity:**

All the films were cut into patches of size 1 x 1 cm<sup>2</sup> and were taken in 100 ml of pH 1.2 HCl buffer separately and were sonicated and kept for 24 hr. After 24 hr it was again sonicated. After filtration and dilution, UV absorbance of the samples was found out at 344 nm. The average of drug content of the films was taken as final reading<sup>[23]</sup>.

**Folding endurance:**

Folding endurance of the film was determined by repeatedly folding one patch at the same place till it broke or folded manually, which was considered satisfactory to reveal good film properties. The number of times of film could be folded at the same place without breaking gave the value of the folding endurance<sup>[23]</sup>.

**Percentage moisture uptake:**

The prepared biofilms films of 1×1cm<sup>2</sup> were weighed individually and were kept in desiccator containing saturated solution of potassium chloride in order to maintain 75% RH for 24 hr. After 24 hr, the films were reweighed and the percentage moisture uptake<sup>[19]</sup> was determined by the formula mentioned below:

$$\% \text{ moisture content} = \left[ \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \right] \times 100$$

**In-vitro Drug Release Study:**

*In-vitro* dissolution studies <sup>[24]</sup> were carried out in HCl buffer pH 1.2 for 24 hours. In order to find out the order of release and the mechanism, which was predominantly influences the drug release from membrane, the *in-vitro* drug dissolution data was subjected to the different modes of graphical treatment. *In-vitro* release study was performed by using **USP dissolution apparatus**.

In this method a small piece (3-4cm) of freshly cut goat intestine membrane was stucked to the rotating basket and a film of 1 x 1 cm<sup>2</sup> was adhered to this membrane. This assembly was then immersed in the dissolution medium composed of 900ml of HCl buffer of pH 1.2 maintained at 37°C and rotated at 50 rpm. After regular intervals 3 ml of sample withdrawn from the basket and was replaced with 3ml Of fresh buffer. The study was continued for 24 hr. This procedure was repeated with all the formulations to get number of samples. All the samples were subjected for U.V. and absorbance readings were recorded at  $\lambda$  max 344nm.

**Ex-Vivo Drug Release Study:**

*Ex-vivo* studies <sup>[25]</sup> were carried out in HCl buffer pH 1.2 for 24 hours. *Ex-vivo* release study was performed by using USP dissolution apparatus. In this method a small piece (3-4cm) of freshly cut goat stomach membrane was stucked to the rotating basket and a film of 1 x 1 cm<sup>2</sup> was adhered to this membrane. This assembly was then immersed in the dissolution medium composed of 900ml of HCl buffer of pH 1.2 maintained at 37°C and rotated at 50 rpm. After regular intervals 3 ml of sample withdrawn from the basket and was replaced with 3ml Of fresh buffer. The study was continued for 24 hr. This procedure was repeated with all the formulations to get number of samples. All the samples were subjected for U.V. and absorbance readings were recorded at  $\lambda$  max 344nm.

**Stability Studies:**

Stability studies <sup>[26]</sup> were conducted as per ICH Guidelines at various conditions of temperature and relative humidity. The stability study of all the formulations were performed by keeping 1x1 cm<sup>2</sup> patch of all formulations in refrigerator, at room temperature and in oven and comparing it in three of the different temperature conditions, for 3 months.

**i) At 4-8°C in Refrigerator :**

1x1 cm<sup>2</sup> patches of the selected formulations were kept in the refrigeration and observed weekly for any change in physicochemical properties (e.g. colour, odour), as well as drug content and in vitro drug release.

**II) At room temperature :**

1x1 cm<sup>2</sup> patches of the selected formulations were kept similarly at room temperature as specified above and any change in the physicochemical properties as well as in vitro drug release was observed.

**III) At 40°C and  $\pm$  45% RH :**

1x1 cm<sup>2</sup> patches of the selected formulations were kept in the vials with an aluminium foil covering, the formulations were kept in oven and were observed weekly for any change in the physicochemical properties as well as drug content, and in vitro drug release.

## RESULTS AND DISCUSSION

**Isolation of the biomaterial**

Biomaterial from *Tagetes erecta* was isolated and optimized six times. The % yield was calculated and was found to be 19 $\pm$ 4.2%. The method was found out to be reproducible, economic and can be scale up by increasing the amount of *Tagetes erecta* for bulk production.

Physicochemical Properties of Isolated

**Biomaterial:-**

The biomaterial isolated from *Tagetes erecta* was brown in colour having characteristic taste. When the biopolymer was melted, it showed colour change from brown to black in range of  $160\pm 6^\circ\text{C}$ . It was insoluble in water, freely soluble in DMSO and slightly in mixture of ethanol: water (1:1) and mixture of methanol: water (1:1). The biopolymer was found to be odorless.

**Acute Toxicity Study:**

Acute toxicity was conducted to know whether the isolated biopolymer was safe and non-toxic in nature or not, so that it can be used for further study. Based on the observation as after 24 hours, the rat didn't show any sign of infection or any type of allergy symptom like itching, redness, rashes etc. and on regular observation for 14 days, the rat was not found to have any change in body weight and behavioral pattern. This shows that the biopolymer was safe and non-toxic to use as it was obtained from natural edible sources which are harmless in nature.

**Drug-Polymer Interaction Study**

Drug polymer interaction study was performed by wet and dry method by using UV spectrophotometric analysis and TLC method. The UV spectrophotometric analysis and the TLC method showed that there was no significant interaction between the drug and the biopolymers in different ratios, as the  $\lambda_{\text{max}}$  values (340-349) are very close to the  $\lambda_{\text{max}}$  value of pure drug (344nm) as shown in Table no 3. Hence no changes were found in the  $\lambda_{\text{max}}$  value of both the biopolymer as compared to pure drug this shows that both the biopolymer and drug was safe in nature so they can be used for further studies i.e. for formulating films as they are obtained from natural edible sources.

**Preparation of standard graph of Drug**

Standard graph of Doxycycline was prepared in water, pH 7.4, pH 1.2. Dilutions were prepared from 1 to 20  $\mu\text{g/ml}$  and observed under UV spectrophotometer. Absorbance was noted at  $\lambda_{\text{max}}$  344 which was found out by scanning the highest concentration. Standard graph was plotted between absorbance versus concentration. The regression analysis was applied and the slope and  $R^2$  value of standard graph in pH 1.2 were found to be 0.016 and 0.989 respectively. The equation of the graph was found out to be  $y = 0.016x - 0.012$ . On the other hand the slope and  $R^2$  value of standard graph in pH 7.4 were found to be 0.014 and 0.943 respectively. The equation of the graph was found out to be  $y = 0.014x - 0.004$ . Doxycycline was found to be stable in pH 1.2, pH 7.4, water and absorbance was found to be proportional to the Concentration as it followed Beer-Lambert's Law as shown in Figure 4-9.

**Preparation of Floating Biofilms by Solvent Casting Method:**

9 formulations with each biopolymer isolated from *Tagetes erecta* (FT1-FT9) in a ratio of 1:2 to 1:18 were prepared with doxycycline. The composition of which is shown in the Table no.2. The preparation method was simple, reproducible and economic for use. The method can be scale up by increasing amount of biopolymer and drug. The prepared Floating biofilms were subjected to various evaluation parameters like thickness, drug content, folding endurance, film weight, surface pH, % moisture absorption, in vitro drug release studies etc.

**Evaluation Parameters of the formulated Floating films:**

The prepared films were thin, flexible, smooth and uniform which were subjected to various physicochemical characteristics such as weight uniformity, drug content, thickness, folding endurance and surface pH. The physicochemical evaluation data was summarized in Table no 4 and 5. The weight uniformity was found to be in



a range of  $2.3 \pm 0.98$  –  $2.7 \pm 0.29$  for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9).

Thickness of the floating films mainly affects the floatability of a film. The thickness of floating films was found to be in the range of  $0.496 \pm 0.081$  mm to  $0.589 \pm 0.089$  mm.

In order to evaluate the flexibility, the films were subjected to folding endurance study. The folding endurance was measured manually by folding the film repeatedly at a point till it break. The number of time the film folds until it break gave the value of folding endurance. The folding endurance was found to be 180-296 for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9),

The drug content was found to be in a range of  $74.1 \pm 0.26$  –  $92 \pm 0.01$  for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9). This shows that the drug is evenly distributed throughout the films and there is no significant loss in the concentration of the drug in various formulations which means formulations will show there maximum bioavailability in systemic circulation without any loss of drug.

Surface pH of films was evaluated in order to examine whether they are safe or not for administrating to the stomach. It was found to be in a range of  $5.5 \pm 0.11$  –  $6.41 \pm 0.73$  for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9). From above data of surface pH it can be considered that these films are non-irritant and safe for administrating through stomach as the biopolymer were obtained from various natural edible sources.

#### ***In-vitro* drug release studies:**

*In vitro* drug release studies were performed for all the prepared formulation by using HCl buffer pH 1.2 as dissolution medium and measuring drug concentration UV spectrophotometrically at 344 nm. The studies were performed for a period of 24 hr. The results of the in-vitro

release study from different floating biofilms are shown in the Table no 6. As the polymer concentration increases, the release pattern from formulation FT1 to FT9 was found to be in the order of FT6(1:12) > FT8(1:16) > FT7(1:14) > FT3(1:6) > FT2(1:4) > FT4(1:8) > FT5(1:10) > FT9(1:18) > FT1(1:2) on the basis of  $R^2$ ,  $t_{50}$  and  $t_{80}$  values which are shown in the Table no 7, which shows the percentage drug release of 73.63636 to 90.66102%.

#### **Drug release mechanism and kinetics:**

The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi, and Korsmeyer Peppas and finding the  $R^2$  values of the release profile corresponding to each model. The best fit model for all the formulations were found to be the Korsmeyer Peppas and Higuchi-matrix model and maximum formulations follows the Anomalous transport and some follows Supercase II transport mechanism for release kinetics. The data of  $R^2$  values, best fit model and mechanism of release kinetic for all the formulations is shown in Table no. 6.

#### **Stability Studies:**

The stability studies showed that the formulations containing *Tagetes erecta* biopolymer (FT1-FT9) were stable at room temperature, at 4-8°C in Refrigerator, at 40°C and  $\pm 45\%$  RH. There was no change occur in the colour, odour and also no significant changes were observed in their *in-vitro* release pattern when compared with the data of *in-vitro* release. Hence, these floating films are safe to use and are stable in various stability conditions as biopolymer are obtained from natural edible sources.

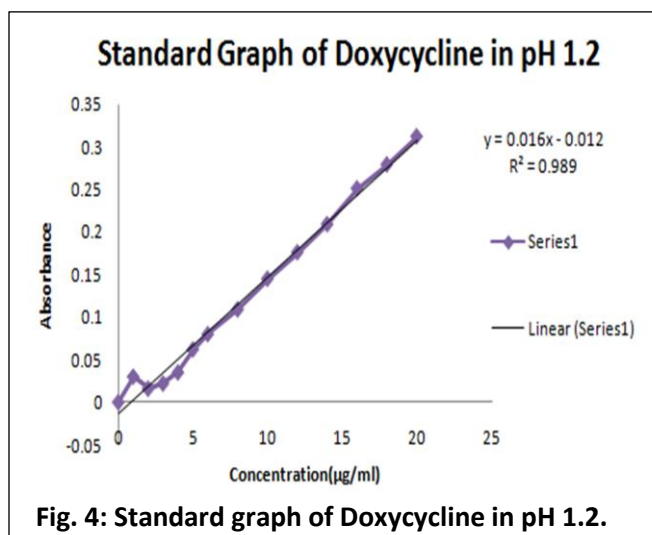


Fig. 4: Standard graph of Doxycycline in pH 1.2.

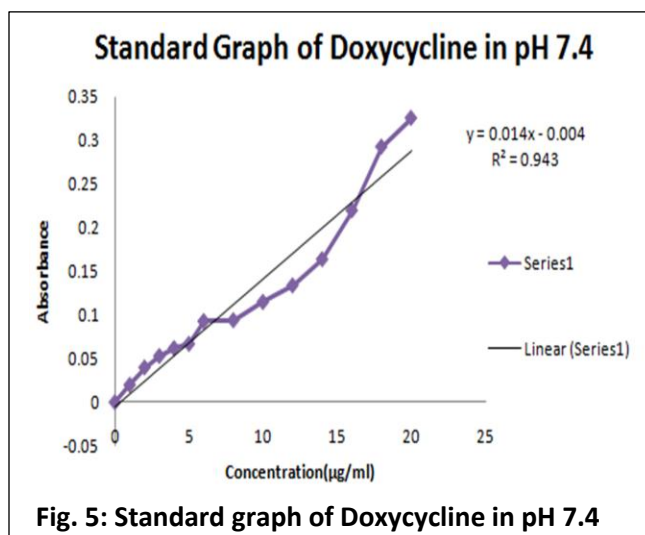


Fig. 5: Standard graph of Doxycycline in pH 7.4

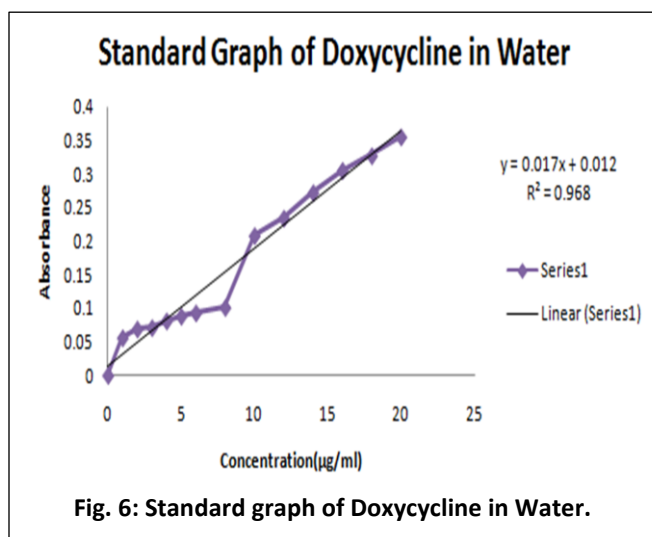


Fig. 6: Standard graph of Doxycycline in Water.

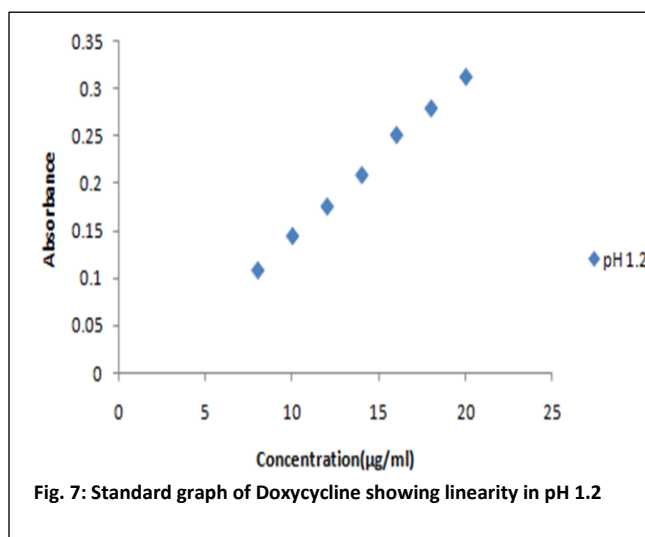


Fig. 7: Standard graph of Doxycycline showing linearity in pH 1.2

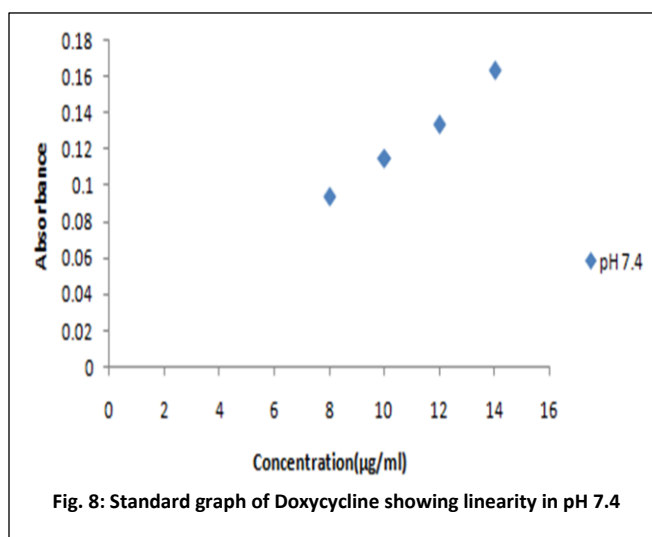


Fig. 8: Standard graph of Doxycycline showing linearity in pH 7.4

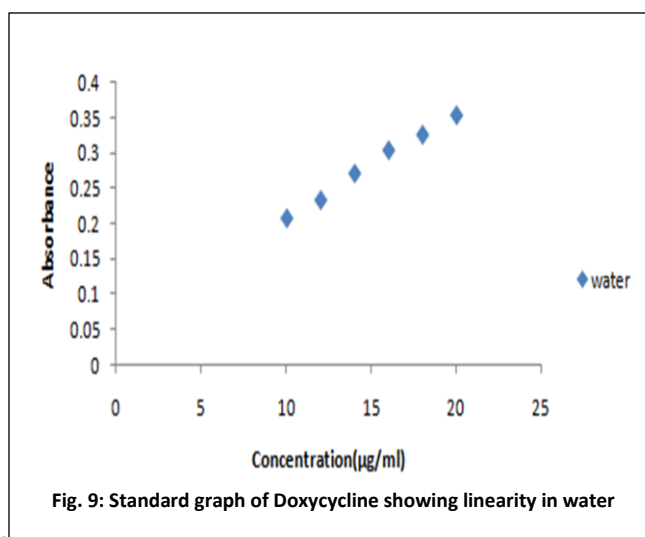


Fig. 9: Standard graph of Doxycycline showing linearity in water

**Table no.3:** Drug Interaction study for popped Zea mays biopolymer by UV spectrophotometric analysis.**i) BY DRY METHOD**

S.No.	Different Ratios	$\lambda_{\text{max}}$ . after 1 hr	$\lambda_{\text{max}}$ . after 2 hr	$\lambda_{\text{max}}$ . after 3 hr	$\lambda_{\text{max}}$ . of Pure Drug
1	1:1	368	368	368	344- 369
2	1:3	370	370	369	344- 369
3	3:1	368	368	369	344- 369
4	1:20	369	369	372	344- 369

**ii) BY WET METHOD**

S.No.	Different Ratios	$\lambda_{\text{max}}$ . after 1 hr	$\lambda_{\text{max}}$ . after 2 hr	$\lambda_{\text{max}}$ . after 3 hr	$\lambda_{\text{max}}$ . of Pure Drug
1	1:1	369	368	369	344- 369
2	1:3	369	368	368	344- 369
3	3:1	368	369	368	344- 369
4	1:20	369	369	369	344- 369

**Table no. 4:** Evaluation Parameter of Floating Films:

Formulations	Weight Uniformity (mg)	Thickness	Folding Endurance
FT1(1:2)	2.3±0.89	0.5016	189
FT2(1:4)	2.3±0.12	0.5099	206
FT3(1:6)	2.6±0.75	0.5298	231
FT4(1:8)	2.4±0.45	0.5121	193
FT5(1:10)	2.6±0.01	0.5611	180
FT6(1:12)	2.7±0.78	0.5691	296
FT7(1:14)	2.6±0.90	0.5710	261
FT8(1:16)	2.5±0.61	0.5888	249
FT9(1:18)	2.7±0.69	0.5801	289

**Table no. 5:** Evaluation Parameter of Floating Films:

Formulations	Surface pH	Drug Content (%)	Floating Cum Mucoadhesion (hrs)
FT1(1:2)	6.2±0.45	88±0.01	3
FT2(1:4)	5.8±0.12	81±0.82	2.5
FT3(1:6)	5.5±0.11	74.1±0.26	4
FT4(1:8)	5.7±0.78	86±0.53	3.5
FT5(1:10)	6.4±0.73	91±0.23	8
FT6(1:12)	5.6±0.31	92±0.01	9.5
FT7(1:14)	6.3±0.34	89±0.54	8.5
FT8(1:16)	5.8±0.78	79±0.78	9
FT9(1:18)	6±0.12	90±0.32	7

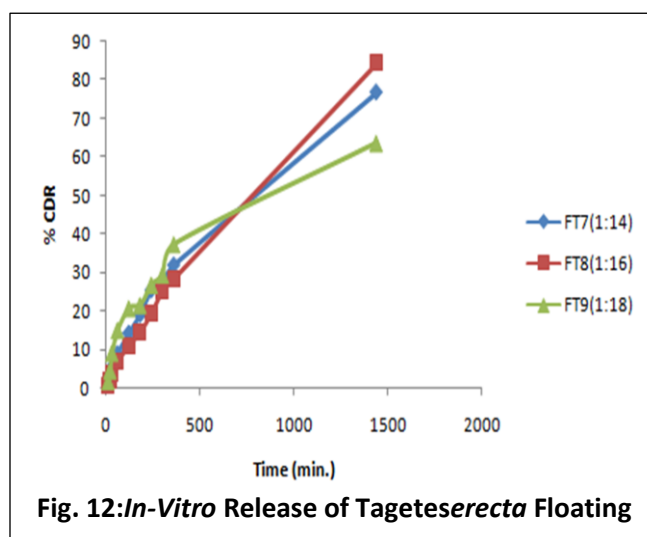
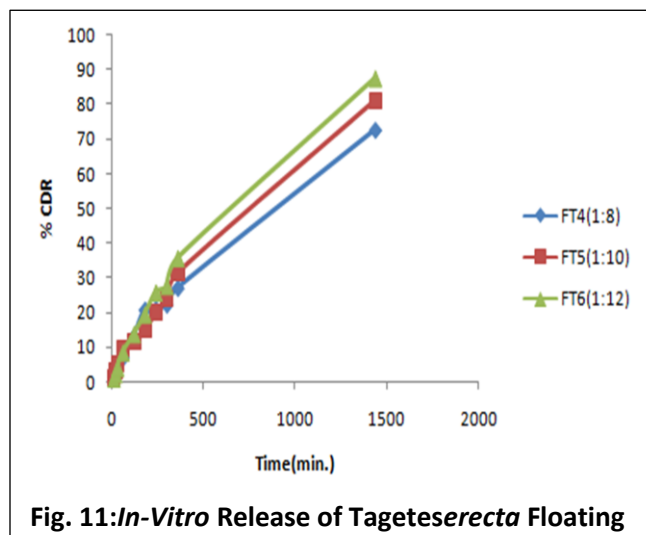
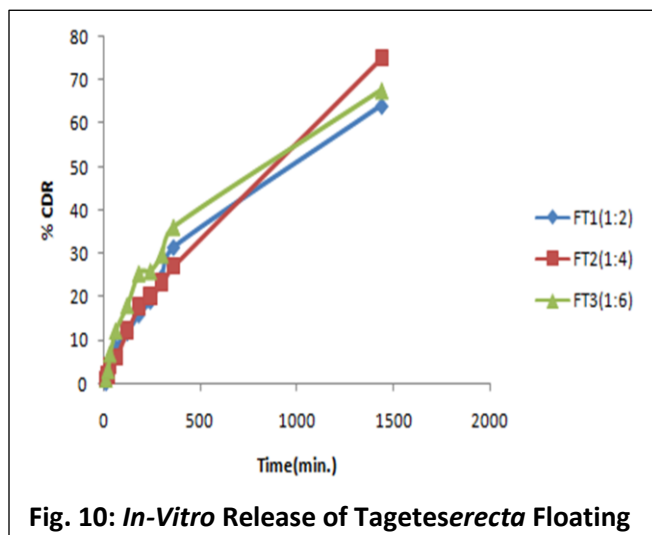


Table no. 6: In-Vitro Release Kinetic Data

S.No.	Zero Order	1 <sup>ST</sup> Order	Higuchi Matrix	Peppas	Hix. Crow.	Best Fit Model	Mechanism of Release
FT1(1:2)	0.7445	0.9029	0.9264	0.9168	0.8539	Higuchi-Matrix	Supercase II Transport
FT2(1:4)	0.7263	0.8487	0.9315	0.9376	0.8089	Peppas Korsmeyer	Supercase II Transport
FT3(1:6)	0.7306	0.8894	0.9250	0.9385	0.8399	Peppas Korsmeyer	Anomalous Transport
FT4(1:8)	0.7041	0.8402	0.9211	0.9352	0.7964	Peppas Korsmeyer	Anomalous Transport
FT5(1:10)	0.7454	0.8654	0.9211	0.9151	0.8367	Peppas Korsmeyer	Anomalous Transport
FT6(1:12)	0.7438	0.9260	0.9317	0.9574	0.8717	Peppas Korsmeyer	Anomalous Transport
FT7(1:14)	0.7482	0.9054	0.9276	0.9453	0.8572	Peppas Korsmeyer	Anomalous Transport
FT8(1:16)	0.7514	0.8676	0.9296	0.9567	0.8305	Peppas Korsmeyer	Anomalous Transport
FT9(1:18)	0.6790	0.8624	0.9281	0.9316	0.8031	Peppas Korsmeyer	Anomalous Transport

Table no. 7:  $t_{50}$  and  $t_{80}$  data of Floating films.

S.No.	$t_{50}$ (hrs)	$t_{80}$ (hrs)
FT1(1:2)	14.71	23.59
FT2(1:4)	16.21	24.81
FT3(1:6)	13.61	23.21
FT4(1:8)	16.58	25.10
FT5(1:10)	14.67	23.51
FT6(1:12)	18.56	30.27
FT7(1:14)	14.9	23.51
FT8(1:16)	16.02	24.82
FT9(1:18)	13.71	23.31

## CONCLUSION

In the present work a novel approach was designed with dual concept of mucoadhesion cum floating for gastro retentive drug delivery. For this approach the biomaterials was isolated from edible sources *Tagetes erecta* which was optimized and was used to formulate Doxycycline loaded floating films. Initially preformulation studies were carried out which included solubility characterization of drug and preparation of standard curves of the drug. Drug was found to be freely soluble in water and methanol, sparingly soluble in *ethanol* (95%); practically insoluble in *chloroform* and in *ether* and the  $\lambda_{max}$  was observed to be 344 nm in water. The standard curves shows linearity in different dissolution medium between 6  $\mu\text{g/ml}$  - 10 $\mu\text{g/ml}$  indicating that between these concentrations it was following Beer-lambert law. The isolation procedure for isolation of biomaterials was simple, reproducible and economic for use. The methods can be scale up by increasing the amount of all natural sources used for isolation of biomaterial. They were characterized for various physicochemical properties including solubility, color, and texture, color changing point, odour and chemical test. Calibration curve of Doxycycline was prepared in water, pH 1.2 and pH 7.4 using uv-vis Spectrophotometer

(shimadzu -1800). The drug-excipient interaction studies and the acute toxicity studies reveals that the polymer was safe for internal use as it not showed any toxic or side effects and no interaction was seen in drug excipient study between drug and polymer.

Doxycycline loaded floating films were prepared using biopolymer from *Tagetes erecta* FT1-FT9 by solvent casting method. Then these floating films were subjected for various evaluation parameters as weight uniformity, surface pH, thickness, folding endurance, content uniformity, floating cum mucoadhesive study, *in-vitro* and *ex-vivo drug release* and stability study.

The folding endurance was found to be 180-296 for formulations containing *Tagetes erecta* bio-polymer(FT1-FT9),

The drug content was found to be in a range of 74.1 $\pm$ 0.26 - 92 $\pm$ 0.01 for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9),

Surface pH was found to be in a range of 5.5 $\pm$ 0.11 - 6.41 $\pm$ 0.73 for formulations containing *Tagetes erecta* bio-polymer (FT1-FT9),

Almost all prepared Floating films were found to show Anomalous transport of drug release with some films showing SuperCase II transport of drug release.

The stability studies showed that the formulations were stable at room temperature, at 4-8°C in Refrigerator, at 40°C and  $\pm$  45% RH. No significant changes were observed in the colour, odour and *in-vitro* release pattern. Hence, these floating films are safe to use and are stable in various stability conditions as all biopolymer are obtained from various natural edible sources.

Finally a smart conclusion was drawn out that the isolated biopolymer *Tagetes erecta*, showed

there in-built ability as a novel film former and mucoadhesive property. The biopolymer isolated from *Tagetes erecta*, was found out to be safe, biodegradable, have good film forming ability. There is a great scope for the further study and development of the Floating Biofilms loaded with doxycycline. This work can be further extended in future for pharmacokinetic studies, *in-vivo* studies on higher animals and controlled clinical studies on human beings.

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