COMPARISON OF STABILITY OF NATURAL AND SYNTHETIC SUSPENDING AGENTS IN THE FORMULATION OF SUSPENSION USING AZITHROMYCIN AS A MODEL DRUG TO MITIGATE STREP THROAT

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ABSTRACT
The aim of the present study is to formulate and evaluate azithromycin suspension using natural and synthetic suspending agent, and to find out the stability of natural and synthetic suspending agents in the formulation of azithromycin suspension. Trigonella foenum graecum Mucilage was used as natural suspending agent. Total 10 formulations were prepared by varying concentration of suspending agent from 0.5-2% in natural suspension. Prepared suspensions were evaluated by studying different parameters like pH, sedimentation volume, redispersibility, Flow rate (F), viscosity, degree of flocculation, effect of temperature etc. batches. FN3, FN4 were found to be best among all the natural formulations, and FN4, FN5 were found to be best formulations among all the synthetic formulations. As the concentration of suspending agent increased viscosity also get increased which reduces the sedimentation and contributes to the stability of suspension. Increase in viscosity avoids the particle aggregation so particles remain in a flocculated state. The use of natural suspending agent reduced the floccules formation and increased the sedimentation volume and produced a stable suspension. Hence the use of mucilage of fenugreek seeds increased the stability of suspension.

KEYWORDS: azithromycin, Suspension, Sedimentation volume, redispersibility, flocculation.

INTRODUCTION
Suspension: Suspension is a heterogeneous biphasic system consisting solid phase in liquid phase. The solid phase

Divided into small particles and dispersed in liquid medium.

Suspension is a dispersion system. it mainly contain two phases
1. Dispersion phase
2. Continuous phase

The term “Disperse System” refers to a system in which one substance is (Dispersed Phase) is distributed, in discrete units, throughout a second substance (continuous phase)

Definition
A Pharmaceutical suspension is a coarse dispersion in which internal phase (active ingredient) is dispersed uniformly throughout the external phase. The internal phase consisting of insoluble solid particles having a range of size(0.5 to 5 microns) which is maintained uniformly throughout the suspending vehicle with aid of single or combination of suspending agent. The external phase (suspending medium) is generally aqueous in some instance, may be an organic or oily liquid for non-oral use.

The reasons for the formulation of a pharmaceutical suspension

- When the drug is insoluble in the delivery vehicle
- To mask the bitter taste of the drug.
- To increase drug stability.
- To achieve controlled/sustained drug release.

Features Desired In Pharmaceutical Suspensions

- The suspended particles should not settle rapidly and sediment produced, must be easily re-suspended by the use of moderate amount of shaking.
- It should be easy to pour yet not watery and no grittiness.
- It should have pleasing odour, color and palatability.
- Good syringeability.
- It should be physically, chemically and microbiologically stable.
Parenteral / Ophthalmic suspension should be sterilizable.

**Classification of Suspension**

1. **Classification Based On General Classes**
   - Oral suspension E.g. Paracetamol suspension antacids,
   - Externally applied suspension E.g. Calamine lotion.
   - Parenteral suspension E.g. Procaine penicillin G Insulin Zinc

2. **Based on Proportion of Solid Particles**
   - Dilute suspension (2 to10% w/v solid) E.g. cortisone acetate.
   - Concentrated suspension (50% w/v solid) E.g. zinc oxide suspension.

3. **Based on Electro kinetic Nature of Solid Particles**
   - Flocculated suspension
   - Deflocculated suspension

4. **Based on Size of Solid Particles**
   - Colloidal suspensions: they are having sizes of suspended solid less than about 1 micron in size are called as colloidal suspensions.
   - Coarse suspensions: they are having sizes of greater than about 1 micron in size are called as coarse suspension.
     Ex: Coarse dispersion Barium sulphate 15
   - Nano suspensions: Suspensions are the biphasic colloidal dispersions of nano sized drug particles stabilized by surfactants. Size of the drug particles is less than 1 micron.

**Types of Suspensions**

**A. Flocculated Suspension**

In this type, solid particles appear as loose aggregates such that individual particles come in contact with each other to forms network like structure called as a floccule. These flocs are light, fluffy in nature, which are held together by weak van der waals force of attraction. Aggregation is achieved by adding flocculating agent. These suspensions will readily sediments. These suspensions possess better physical stability but less bioavailability as compared to deflocculated suspension due to dissolution of floccules.

**B. Deflocculated Suspension**

Deflocculated suspension in this type of suspension, individual particle exits as a separate entity, means particles carry finite charges on their surface. Hence particles approaches each other, they experience repulsive forces. These forces create a high potential barrier, which prevents an aggregation of particles. During storage, these suspension shows a sedimentation at slow rate, due to that particles forms a close packing arrangement. So that it is difficult to re-dispersed on agitation & forms a cake or claying which is hard in nature. This type of suspension has shorter shelf life but high bioavailability as compared to flocculated suspension.

**1. Advantages**
   - Suspension can improve chemical stability of certain drug.
     E.g. Procaine penicillin G
   - Drug in suspension exhibits higher rate of bioavailability than other dosage forms Solution > Suspension > Capsule > Compressed Tablet > Coated tablet
   - Duration and onset of action can be controlled. E.g. Protamine Zinc-Insulin suspension.
   - Suspension can mask the unpleasant/ bitter taste of drug. E.g. Chloramphenicol
   - They allow the development of liquid products containing an appropriate quantity of active ingredients in a reasonable volume
   - Resistance to hydrolysis and oxidation is generally good in suspension when compared with that in respective aqueous solutions.

**2. Disadvantages**
   - Physical stability, sedimentation and compaction can causes problems
   - It is bulky sufficient care must be taken during handling and transport.

**Theoritic Consideration of Suspensions:**

A knowledge of the theoretic considerations pertaining to suspension s technology ultimately help formulator to select ingredients that are Appropriate for suspension preparation. Some theoretic considerations are

- Sedimentation
- Brownian movement
- Electro kinetic Aggregation

1. **Theory of Sedimentation:**

Sedimentation means settling of particle (or) floccules occur under gravitational force in liquid dosage form. Velocity of sedimentation expressed by Stoke’s equation

\[ V_i = \frac{d^2(\rho_s - \rho_o)}{18\eta g} \]

Where,
- \(d\) = Diameter of particle
- \(V_i\) = sedimentation velocity in cm / sec
- \(\rho_s\) = density of disperse phase
- \(\rho_o\) = density of disperse media
- \(g\) = acceleration due to gravity
\( \eta = \) viscosity of disperse medium in poise

2. Sedimentation Parameters:- Sedimentation volume (F) or height (H) for flocculated suspensions. Sedimentation volume is a ratio of the ultimate volume of sediment \((V_u)\) to original volume of suspension before settling \((V_o)\)^5

\[
F = \frac{V_u}{V_o}
\]

Where,

\( V_u = \) final or ultimate volume of sediment
\( V_o = \) original volume of suspension before settling

F has values ranging from less than one to greater than one.

When \( F < 1 \) \((V_u < V_o)\)
When \( F = 1 \) \((V_u = V_o)\)

The system is in flocculated equilibrium and shows no clear supernatant.

When \( F > 1 \) \((V_u > V_o)\) Sediment volume is greater than the original volume due to the network of flocs formed in the suspension and so loose and fluffy sediment.

The sedimentation volume gives only a qualitative account of flocculation.

3. Degree of flocculation \((\beta)\) :- It is the ratio of the sedimentation volume of the flocculated suspension (F) to the sedimentation volume of the deflocculated suspension (F∞)^6

\[
\beta = \frac{F}{F_{\infty}}
\]

The minimum value of \( \beta \) is 1, when flocculated suspension sedimentation volume is equal to the sedimentation volume of deflocculated suspension.

4. Brownian movement (Drunken walk):- Brownian movement of particle prevents sedimentation by keeping the dispersed material in random motion. Brownian movement depends on the density of dispersed phase and the density and viscosity of the disperse medium. The kinetic bombardment of the particles by the molecules of the suspending medium will keep the particles suspending, provided that their size is below critical radius \((r)\). Brownian movement can be observed, If particle size is about 2 to 5mm, When the density of particle & viscosity of medium are favorable.^7

Brownian motion is given by equation:

\[
T_B = \eta R^2 \left(\frac{K_B T}{\pi N r^3}\right)^{-1}
\]

Where, \( R = \) gas constant
\( T = \) temp. in degree Kelvin
\( N = \) Avogadro’s number
\( \eta = \) viscosity of medium
\( t = \) time
\( r = \) radius of the particle

5. Electro kinetic Properties

Zeta Potential

The zeta potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and electro-neutral region of the solution.

As the potential drops off rapidly at first, followed more gradual decrease as the distance from the surface increases. This is because the counter ions close to the surface acts as a screen that reduce the electrostatic attraction between the charged surface and those counter ions further away from the surface.^8

Zeta potential has practical application in stability of systems containing dispersed particles since this potential, rather than the Nernst potential, governs the degree of repulsion between the adjacent, similarly charged, dispersed particles. If the zeta potential is reduced below a certain value, the attractive forces exceed the repulsive forces and the particles come together. This phenomenon is known as flocculation. The flocculated suspension is one in which zeta potential of particle is 20 to +20 mV. Thus the phenomenon of flocculation and de flocculation depends on zeta potential carried by particles.^9

6. Rheology of Suspensions

- The flow property of suspension depend upon their rheological characters
- The rheological properties of suspension decide the pourability, easy of injection, sedimentation, its redispersibility.
- The viscosity of flocculated suspension greater than deflocculated particles in same suspension.
- The flocculated suspension has yield value and behaves like a plastic or pseudo plastic system.

E.g. conc. parenteral suspension contain 40-70 % w/v of Procaine penicillin G.

<table>
<thead>
<tr>
<th>Ingredients used in formulation of suspensions</th>
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<tr>
<td><strong>Ingredients</strong></td>
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<tr>
<td>Wetting Agents</td>
</tr>
<tr>
<td>Flocculating Agents</td>
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<tr>
<td>Thickeners</td>
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<tr>
<td>Buffers</td>
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Pharmaceutical excipients play an important role in various pharmaceutical formulations. Recent trends towards the use of natural origin excipients demand the replacement of synthetic excipients with the natural ones. Naturally derived excipients are attractive alternatives to synthetic excipients, because of their better availability, biocompatibility, biodegradability, low toxicity, environmental friendliness and low price compared to synthetic excipients. Furthermore, they can be modified to obtain tailor made materials for drug delivery systems and they can compete with the synthetic agents available in the market. Ability to produce a wide range of excipients based on their properties and molecular weight, natural excipients became a thrust area in majorities of investigations related with development of drug delivery systems.

A large number of plant-based pharmaceutical excipients are available today. Researchers and formulators have explored the usefulness of these excipients in the development of various formulations. The rationale for increase in importance of natural plant-based material is that plant resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw materials.

Mucilages are naturally occurring, high molecular weight poly uronides consisting of sugars and uronic acid units. They are normal physiological metabolism products formed with in the cell or deposited on it in layers. Mucilages serve as food reserve, membrane thickener and in water storage and seed germination. Plant mucilages are important polysaccharides with wide range of pharmaceutical applications such as thickeners, binding agents, water retention agents, emulsion stabilizers, suspending agents, disintegrating agents, gelling agents and film formers.

Acacia, Tragacanth, Gum ghatti, Gum karaya etc are popular examples of plant mucilages, used in pharmacy. Thus, with the increase demand for these substances, it has been necessary to explore the newer sources to meet the industrial demands. Pharmaceutical suspension, is thermodynamically unstable, thus, making it necessary to include in the dosage form, a suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by increasing the consistency of suspending medium and by protective colloidal action.

There are several reports about the successful use of various plant mucilages, as innovative suspending agents like mucilages isolated from tamarind seed, fenugreek, Basella alba L. leaves, Spinacia oleracea L. leaves, etc.

Introduction to Natural Suspending Agent
Trigonella foenum-graecum L. is an annual herb grown in various countries around the world. It was thought to be indigenous to the countries bordering on the eastern shores of the Mediterranean, but now is widely cultivated in India, China, northern and eastern Africa, and parts of Europe and Argentina. The health promoting property of fenugreek has been long documented when it is taken as vegetables, food supplements or medicinal remedies. It has been used in many different cultures, but especially in Asia and the Mediterranean region.

Figure 3: fenugreek plant.

Active constituents
Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30% proteins high in lysine and tryptophan; 5-10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.3 6%), choline (0.5%), gentianine, and carpine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin, and isoquercitin; free amino acids, such as 4-hydroxyisoleucine (0.09%); arginine, histidine, and lysine; calcium and iron; saponins (0.6-1.7%); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol;...
vitamins A, B1, C and nicotinic acid; and 0.015% volatile oils (n-alkanes and sesquiterpenes), which are thought to account for many of its presumed therapeutic effects.

Pharmacological effects and mechanisms of action

Fenugreek is known to have several pharmacological effects such as: hypoglycemic and antilipidemic or hypocholesterolemic. However, the exact mechanism of action is still unclear.

The antidiabetic effect of Fenugreek was thought to be due to formation of a colloidal-type suspension in the stomach and intestines when the mucilaginous fiber of the seeds is hydrated, therefore affecting gastrointestinal transit, slowing glucose absorption. The antilipidemic effects of Fenugreek was thought to be due to inhibition of intestinal cholesterol absorption due to saponin-cholesterol complex formation, increased loss of bile through fecal excretion due to saponin-bile complexes, thus increasing conversion of cholesterol to bile by the liver, and effects of amino acid pattern of fenugreek on serum cholesterol. Furthermore, this plant has an antioxidant action, gastroprotective activity, appetite stimulation and antirheumatism.

The aim of the present work was to compare the use of natural and synthetic suspending agents in the formulation of azithromycin suspension. Azithromycin is an anti-infective drug (macrolide antibiotic) that is used to prevent or treat Mycoplasma avium complex (MAC) infection. Azithromycin may also be used to treat other types of infections such as bacterial pneumonia, toxoplasmosis, and sexually transmitted diseases (ie. chlamydia).

Azithromycin is an antibiotic useful for the treatment of a number of bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler’s diarrhea, and certain other intestinal infections. Along with other medications, it may also be used for malaria. It can be taken by mouth or intravenously with doses once per day.

Azithromycin is used to treat many different infections, including:

- Prevention and treatment of acute bacterial exacerbations of chronic obstructive pulmonary disease due to H. influenzae, M. catarrhalis, or S. pneumoniae. The benefits of long-term prophylaxis must be weighed on a patient-by-patient basis against the risk of cardiovascular and other adverse effects.
- Acute bacterial sinusitis due to H. influenzae, M. catarrhalis, or S. pneumoniae. Other agents, such as amoxicillin/clavulanate are generally preferred, however.
- Community-acquired pneumonia due to C. pneumoniae, H. influenzae, M. pneumoniae, or S. pneumoniae

- Acute otitis media caused by H. influenzae, M. catarrhalis or S. pneumoniae. Azithromycin is not, however, a first-line agent for this condition. Amoxicillin or another beta lactam antibiotic is generally preferred.
- Pharyngitis or tonsillitis caused by S. pyogenes as an alternative to first-line therapy in individuals who cannot use first-line therapy.
- Uncomplicated skin and skin structure infections due to S. aureus, S. pyogenes, or S. agalactiae
- Urethritis and cervicitis due to C. trachomatis or N. gonorrhoeae
- Trachoma due to C. trachomatis.
- Genital ulcer disease (chancroid) in men due to H. ducreyi
- In combination with ceftriaxone, azithromycin is part of the United States Centers for Disease Control-recommended regimen for the treatment of gonorrhea. Azithromycin is active as monotherapy in most cases, but the combination with ceftriaxone is recommended based on the relatively low barrier to resistance development in gonococci.

Mechanism of action

Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. Nucleic acid synthesis is not affected.

Pharmacokinetics

Azithromycin is an acid-stable antibiotic, so it can be taken orally with no need of protection from gastric acids. It is readily absorbed, but absorption is greater on an empty stomach. Time to peak concentration (T_{max}) in adults is 2.1 to 3.2 hours for oral dosage forms. Due to its high concentration in phagocytes, azithromycin is actively transported to the site of infection. During active phagocytosis, large concentrations are released. The concentration of azithromycin in the tissues can be over 50 times higher than in plasma due to ion trapping and its high lipid solubility.

Azithromycin's half-life allows a large single dose to be administered and yet maintain bacteriostatic levels in the infected tissue for several days. Following a single dose of 500 mg, the apparent terminal elimination half-life of azithromycin is 68 hours. Biliary excretion of azithromycin, predominantly unchanged, is a major route of elimination. Over the course of a week, about 6% of the administered dose appears as unchanged drug in urine.

MATERIALS AND METHODS

Materials

Azithromycin was procured inhouse. Fenugreek seeds, were purchased from local market. Methyl cellulose, aerosol were obtained from S. D. Fine chemicals. citrus flavour and amaranth were purchased from local market. All other solvents used were of analytical grade.

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Methods

Extraction of suspending agent from Trigonella foenum graecum (Seed)

Initially seeds of Trigonella foenum graecum were crushed and reduced in size using ball mill. The crushed seeds were soaked in distilled water for 12 hrs and boiled in water bath to prepare slurry. Further slurry was cooled and allows settling down unwanted material. Upper portion was collected and concentrated in water bath and after cooling acetone was added in it with continuous stirring. The precipitate was collected and dried at room temperature for 24 hrs. The air dried material further subjected to size reduction and passed through sieve no. 60 and stored in desiccators for further evaluation.\textsuperscript{[7,10]}

Formulation of synthetic suspension

Suspended agents used for the preparation of suspensions include the following colloidal silicon dioxide (0.5%), carboxy polymethylene (2%), methyl cellulose (2%). The azithromycin suspension was prepared by trituration method. F1 contains mixture of andsilsilicon dioxide and methyl cellulose, F2 contains mixture of polymethylene and methyl cellulose and, F3 contains mixture of silicon dioxide and methyl cellulose. F4 contains mixture of all the three, F5 contains only methyl cellulose sodium cnc. all the formulations were prepared in the following manner. The suspending agents were added in the mortar and pestle and they were triturated well to form a smooth paste. The drug was added to smooth paste along with the addition of tween 80 and methyl paraben. The simple syrup was added to the contents in the mortar and pestle. Finally the citrus flavour and amaranth were added and triturated well to form a suspension.

Formulation of natural suspension

Azithromycin natural suspension was prepared by trituration method using fenugreek seed mucilage as suspending agent. The mucilage was extracted from fenugreek seeds. The azithromycin was added to the mucilage and triturated well, in order to form a smooth paste to the mortar contents tween 80 was added followed by addition of simple syrup and methyl paraben. Finally the citurs savour, amarth were added and triturated well to form a suspension.

| Table 1: Formulation of Synthetic Suspension. |
|-------|---|---|---|---|---|
| S. no. | Ingredients | FS1 | FS2 | FS3 | FS4 | FS5 |
| 1      | Azithromycin | 20mg | 20mg | 20mg | 20mg | 20mg |
| 2      | Colloidal silicon dioxide (%) | 0.5 | - | 1 | - | 2 |
| 3      | Carboxy polymethylene (%) | - | 1 | - | 2 | 1.5 |
| 4      | Methyl cellulose (%) | - | - | 0.5 | 2 | 1.5 |
| 5      | Simple Syrup | 5ml | 5ml | 5ml | 5ml | 5ml |
| 6      | Tween 80 | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 7      | Citrus Flavour | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 8      | Methyl Paraben | 0.1 ml | 0.1 ml | 0.1 gm | 0.1 gm | 0.1 gm |
| 9      | Amaranth | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 10     | Distilled Water | q.s to 100ml | Q.s to 100ml | Q.s to 100ml | q.s to 100ml | q.s to 100ml |

| Table 2: Formulation of Natural Suspension. |
|-------|---|---|---|---|---|
| S. no. | Ingredients | FS1 | FS2 | FS3 | FS4 | FS5 |
| 1      | Azithromycin | 20mg | 20mg | 20mg | 20mg | 20mg |
| 2      | Fenugreek mucilage (%) | 0.5 | - | 1 | 2 | - |
| 3      | Simple Syrup | 5ml | 5ml | 5ml | 5ml | 5ml |
| 4      | Tween 80 | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 5      | Citrus Flavour | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 6      | Methyl Paraben | 0.1 ml | 0.1 ml | 0.1 gm | 0.1 gm | 0.1 gm |
| 7      | Amaranth | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| 8      | Distilled Water | q. s to 100ml | Q. s to 100ml | Q. s to 100ml | q. s to 100ml | q. s to 100ml |

Evaluation parameters

1. **PH:** The PH of the suspension was determined by using pH meter

2. **Redispersibility:** Fixed volume of each suspension (50 ml) was kept in calibrated tubes which were stored at room temperature for various time intervals (1, 2, 3, 4, 5 days). At regular interval one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any was recorded.

3. **Flow rate (F):** The time taken for 10ml sample of suspension to flow through a 10ml pipette was determined and the flow rate calculated using the following equation:

\[
F = \frac{Volume \ of \ pipette (ml)}{Flow \ time \ (sec)}
\]

4. **Determination of viscosity:** The viscosity of suspension samples was determined using the Brookfield viscometer at 100 rpm. All determinations were carried out in at least triplicates and results obtained were expressed as the mean values.

5. **Degree of flocculation:** Degree of flocculation (β) was determined using following equation


\[ \beta = \text{sedimentation volume of flocculated suspension/sedimentation volume of deflocculated suspension.} \]

Where, \((\nu_u) = \text{flocculated is ultimate sedimentation volume in flocculated suspension}\)

\((\nu_d) = \text{deflocculated is ultimate sedimentation volume in deflocculated suspension.} \]

6. **Effect of temperature**:- Further, the effect of the temperature (30˚c to 60˚c) was investigated on the viscosity of the suspension of all formulations.

7. **Particle size analysis**:- First calibrate the eye piece. Place a drop of lotion on a glass slide and was covered with a cover slip without any air bubbles and was observed under microscope. Each particle diameter was measured and recorded for at least 100 particles.

8. **In-vitro dissolution studies Parameters for dissolution process**

- Apparatus – paddle type dissolution apparatus
- Medium- pH 6 Sodium Phosphate Buffer
- Stirrer- paddle at 50 rpm
- Temperature- 37˚c ± 0.5˚c
- Duration - 30 min.
- Total no of samples = 6
- Wave length = 760nm

**Procedure:** Fill the vessel with pH 6 sodium phosphate buffer up to 900ml and maintain the temperature of the vessel at 3.7˚c ± 0.5˚c. Place 5ml of Ibuprofen suspension in the vessel. The samples of 5ml was collected at a predetermined time intervals. Replace the 5ml of sample with 5ml of pH 6 sodium phosphate buffer inorder to maintain the sink condition. The samples were diluted if necessary and the concentration of the sample was measured using UV visible spectro-photometer at 760 nm.

9. **Drug content** :- 5 ml of formulated Azithromycin suspension were dissolved in 25 ml of methanol in 50 ml of volumetric flask and made up to mark with PH6 Sodium phosphate buffer. Then that solution was properly diluted. To the diluted solution 2ml of Folin Ciocalteu reagent and 2 ml of 20% Sodium carbonate solution were added & mixed well and keptaside for 15 minutes for maximum development of color. Then concentration of the sample was measured at 760 nm in Visible spectrophotometer.

10. **Determination of sedimentation volume**:- Transfer the prepared azithromycin suspension into a measuring cylinder and was kept aside without disturbing observe the height of the sediment at a regular time intervals of 0, 10, 20, 30, 40, 50, 60 mins and calculate the sedimentation volume by using the following equation.

\[ F = 100 \frac{W_1 - W_2}{W_2} \times 100 \]

W1= Weight of tablet at time ‘0’

W2= Weight of tablet at time ‘t’

12. **Pytochemical tests for fenugreek** (*Trigonella foenum graecum*) :- Preliminary tests were performed to confirm the nature of mucilage obtained. In view of phytochemical test, fenugreek mucilage contains carbohydrates, alkaloids and proteins.

**RESULTS AND DISCUSSION**

1. **pH**:- The pH of all formulations was found to be in the range of 6.2-7.4

2. **Redispersibility**:- The redispersibility of suspensions were found to be in the range of 6-12 in case of natural suspension, 3-16 in case of synthetic suspension. Since the suspension sediment on storage, it must be radially dispersible so as to ensure a more uniform dosage administration of medicament after shaking. Suspension is called as caked if sediment remains after vigorous shaking. All the suspension was found to be easily redispersible after maximum 13 shaking after 45 days. Redispersibility was found to be faster for suspension with lower amount of suspending agent comparing to higher concentration. This may attribute to higher viscosity of these suspensions with higher concentration

3. **Flow rate**:- Flow rate was found to be decreased as concentration of suspending agent and viscosity of suspension increased. It is found in the range of 0.1 -0.05

4. **Determination of viscosity**:- Viscosity of all formulation was found to be decreased with increasing rpm indicated shear thinning nature of suspension.

5. **Degree of flocculation**:- Degree of flocculation was determined for all formulated suspension using different concentration of *Trigonella foenum graecum* mucilage. The values of degree of flocculation for all formulated suspension have been mentioned in table and it found to be increased at higher concentration of suspending agent. This is due to higher viscosity of suspension at higher concentration, which ultimately reduces the sedimentation of suspension.

6. **Effect of temperature**:- Increase in temperature reduces the viscosity for all formulation.

7. **Drug content**:- Drug content for all batches was found to be in the range of 95-98% in case of natural suspension. 85-94% in case of synthetic suspension.

8. **Particle size measurement**: Particle sizes of 20 particles of all formulated suspensions were determined and values are reported. the extract of fenugreek seeds possessed all the phytoconstituents.
Table 3: Cumulative % drug release of suspension using natural suspending agent.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>FN1</th>
<th>FN2</th>
<th>FN3</th>
<th>FN4</th>
<th>FN5</th>
</tr>
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<tr>
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<tr>
<td>5</td>
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<td>88.89</td>
<td>92.34</td>
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</tr>
</tbody>
</table>

Table 4: Cumulative % drug release of suspension using synthetic suspending agent.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Particle size of Natural Suspension</th>
<th>Particle size of synthetic Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Particle size distribution of natural as well as synthetic suspension.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>FS1</th>
<th>FS2</th>
<th>FS3</th>
<th>FS4</th>
<th>FS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>24.53</td>
<td>28.34</td>
<td>29.75</td>
<td>44.37</td>
<td>43.54</td>
</tr>
<tr>
<td>10</td>
<td>35.72</td>
<td>30.56</td>
<td>38.05</td>
<td>51.79</td>
<td>59.34</td>
</tr>
<tr>
<td>20</td>
<td>42.42</td>
<td>42.21</td>
<td>59.34</td>
<td>75.44</td>
<td>74.04</td>
</tr>
<tr>
<td>30</td>
<td>69.82</td>
<td>57.68</td>
<td>65.74</td>
<td>85.98</td>
<td>84.49</td>
</tr>
<tr>
<td>45</td>
<td>75.33</td>
<td>65.49</td>
<td>78.09</td>
<td>92.34</td>
<td>95.23</td>
</tr>
</tbody>
</table>

Table 6: Ratio of sedimentation volume of natural and synthetic suspension.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Formulation code</th>
<th>Average Sedimentation volume natural suspensions (Hu/Ho) at 24 hrs</th>
<th>Average Sedimentation volume of synthetic suspensions (Hu/Ho) at 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>0.97</td>
<td>0.92</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>0.98</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>0.98</td>
<td>0.92</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>0.92</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Table 7: Evaluation parameters of synthetic suspension.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Formulation code</th>
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<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PH</td>
<td>7.03</td>
<td>6.89</td>
<td>6.56</td>
<td>7.32</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
<td>Degree of flocculation</td>
<td>2.87</td>
<td>3.45</td>
<td>3.67</td>
<td>3.97</td>
<td>4.02</td>
</tr>
<tr>
<td>3</td>
<td>Viscosity(cps)</td>
<td>3.45</td>
<td>2.98</td>
<td>3.84</td>
<td>1.56</td>
<td>1.23</td>
</tr>
<tr>
<td>4</td>
<td>Redispersibility</td>
<td>9</td>
<td>11</td>
<td>12</td>
<td>05</td>
<td>06</td>
</tr>
<tr>
<td>5</td>
<td>Drug content</td>
<td>75.47</td>
<td>78.34</td>
<td>84.97</td>
<td>95.98</td>
<td>98.89</td>
</tr>
</tbody>
</table>

Table 8: Evaluation parameters of natural suspension.

<table>
<thead>
<tr>
<th>S. no.</th>
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<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
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<td>PH</td>
<td>6.79</td>
<td>5.89</td>
<td>7.56</td>
<td>7.32</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>Degree of flocculation</td>
<td>1.87</td>
<td>2.45</td>
<td>3.67</td>
<td>3.97</td>
<td>3.82</td>
</tr>
<tr>
<td>3</td>
<td>Viscosity(cps)</td>
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<td>2.98</td>
<td>1.84</td>
<td>1.56</td>
<td>3.67</td>
</tr>
<tr>
<td>4</td>
<td>Redispersibility</td>
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<td>10</td>
<td>3</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Drug content</td>
<td>75.47</td>
<td>68.34</td>
<td>94.97</td>
<td>85.98</td>
<td>68.89</td>
</tr>
</tbody>
</table>

Figure 1: Calibration Curve for Azithromycin.

Figure 2: Particle size of natural and synthetic suspensions.
CONCLUSION
Azithromycin suspension was formulated with natural suspending agent i.e. *Trigonella foenum graecum* showed superior stability over period of time compared to that of synthetic suspending agents. Increase in concentration of suspending agent increases the viscosity of suspension which ultimately reduces sedimentation and contributes to the stability of suspension. Hence natural suspension prepared by using fenugreek mucilage was found to be stable and easily redispersable compared to synthetic suspension.

REFERENCES