INTRODUCTION

Hydrogel nanoparticles are one among the promising delivery systems. They possess the properties and characteristics of hydrogels and nanoparticles simultaneously. The pharmacy will be benefited from both the hydrophilicity and biocompatibility of these particles, with all the advantages of the nanoparticles, such as, stability of the therapeutic agents and being actively or passively targeted to the desired biophase.[1] Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids, and polysaccharides have been developed and tested over the past decades. Among them Chitosan and carrageenan are very promising and widely exploited in the pharmaceutical field.

Carrageenan is a hydrocolloid obtained from Rhodophyceae (red sea weed). It consists chiefly of potassium, sodium, calcium, magnesium, and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. These hexoses are alternately linked at the α-[α]-1, 3 and β-[β]-1, 4 sites in the polymer. It is a water soluble polymer and has been biocompatible, biodegradable, anionic, and non-toxic in nature.[2] Nanoparticles can be formed with carrageenan by adding a polycationic solution. Poly-L-lysine can be used to form nanoparticles, but it has been found to be toxic and immunogenic if injected.[3] Therefore, chitosan was selected as an alternative, which is polycationic in nature. Chitosan is a polysaccharide comprising of co-polymers of glucosamine and N-acetyl glucosamine, and is nontoxic, biocompatible, and biodegradable in nature.

Mercaptopurine is an anticancer drug that is used to treat acute lymphoblastic leukemia. It has very poor bioavailability of 25 – 50%, due to its high first pass metabolism. Half-life is 60 – 120 minutes in the oral route and 20 – 90 minutes in the intravenous route.[4] The protein-binding capacity of mercaptopurine is 19% in the plasma. In order to overcome all the above parameters an alternative approach is required.

Hydrogel nanoparticles are formed by ionic interactions. Here cationic and anionic polymers are used to form Hydrogel nanoparticles. A cationic polymer solution like chitosan and an anionic polymer solution like carrageenan

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in the respective concentrations are stirred under high speed to form hydrogel nanoparticles. Nanoparticles are formed by means of the electrostatic interaction of positively charged amino groups of chitosan with the negatively charged sulfate groups of carrageenan.\([5]\)

**MATERIALS AND METHODS**

**Materials**
Chitosan was acquired from Sigma Chemicals (Mumbai) and Mercaptopurine from Sisco Research Laboratories (Mumbai). All other chemicals and agents used were of analytical grade.

**Preparation of Polymer solutions**

- **Preparation of chitosan solution**
An accurately weighed quantity of chitosan was dispersed in sodium acetate buffer solution (pH 4.0) and stirred vigorously for five hours, continuously, to obtain 0.25% w/v, 0.5% w/v, 0.75% w/v, and 1% w/v concentrations of chitosan solutions.

- **Preparation of carrageenan solution**
An accurately weighed quantity of carrageenan was dispersed in water and boiled at about 80°C to form 0.05% w/v, 0.1% w/v, and 0.2% w/v of clear carrageenan solutions.

Varied concentrations of polymers and stirring speed were used in order to study their effect on nanoparticle formation.

**Preparation of blank hydrogel nanoparticles**
Chitosan solution was taken in a beaker and carrageenan solution was added dropwise with magnetic stirring at 3000 rpm, which was continued for four hours. The resulting solution of nanoparticles was centrifuged at 3000 rpm for 15 minutes.

**Preparation of drug loaded nanoparticles**
Twenty-five milligrams of accurately weighed\([6]\) mercaptopurine was dissolved in the required amount of ethanol. This solution was added to the chitosan solution. To this mixture carrageenan solution was added dropwise, with magnetic stirring at 3000 rpm, which was continued for four hours. The resulting drug loaded nanoparticle solution was centrifuged at 3000 rpm for 15 minutes. Then it was subjected to freeze drying.

The compositions of various drug loaded nanoparticles are given in Tables 1 and 2.

**Physicochemical Properties**
The size and zeta potential of the nanoparticles were measured by photon correlation spectroscopy, which analyzed the fluctuations in light scattering due to Brownian motion of the particles using a Zeta sizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle. The samples were diluted 100 times with distilled water. The diluted samples were directly placed into the module and the measurements were made in triplicate after stirring for two minutes.\([7]\)

**Morphology of Nanoparticles**
External morphology of the nanoparticles was determined by using scanning electron microscopy (Cambridge S360). The samples were diluted with ultra purified water, to obtain a suitable concentration. Next the samples were spread on a sample holder and dried using vacuum, and then examined.\([8]\)

**Estimation of Drug Content**
Five milligrams of drug-loaded nanoparticles of each batch were dispersed in 5 ml of ethanol and boiled for some time. Next the resultant solution was centrifuged, the supernatant liquid was collected, and the absorbance was determined using an ultraviolet (UV) spectrophotometer at 331 nm, with ethanol as a blank. Drug content in the hydrogel nanoparticles was calculated using the following formula.\([9]\)

\[
\text{Drug content (\%w/w)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of nanoparticles}}
\]

**In Vitro Drug Release Studies**
The dialysis bag diffusion technique was used to study the *in vitro* drug release of mercaptopurine nanoparticles. The prepared nanoparticles were placed in the dialysis bag and immersed into 50 ml of Phosphate buffer pH (7.4). The entire system was kept at 37±0.5°C, with continuous magnetic stirring. The samples were withdrawn from the receptor compartment at predetermined intervals (0.5, 1, 1.5, 2, 3, 4, 8, 10, 12, 24 hours) and replaced by fresh medium. The samples were withdrawn and diluted to 10 ml with Phosphate buffer pH (7.4) and the amount of drug dissolved was analyzed by a UV-Spectrophotometer at 331 nm.

**Determination of Kinetics of Drug Release**
In order to predict and correlate the release behavior and mechanism of release from the polymer matrix, it is

| Table 1: Composition of batches F1 to F3 |
|-----------------------------------------|
| **Batch** | **Chitosan (0.75% w/v)** | **Carrageenan (0.1% w/v)** | **Mercaptopurine (mg)** |
| F1        | 8 ml                      | 24 ml                      | 25                      |
| F2        | 8 ml                      | 28 ml                      | 25                      |
| F3        | 8 ml                      | 32 ml                      | 25                      |

| Table 2: Composition of batches F4 to F6 |
|-----------------------------------------|
| **Batch** | **Chitosan (0.5% w/v)** | **Carrageenan (0.1% w/v)** | **Mercaptopurine (mg)** |
| F4        | 8 ml                      | 24 ml                      | 25                      |
| F5        | 8 ml                      | 28 ml                      | 25                      |
| F6        | 8 ml                      | 32 ml                      | 25                      |
necessary to fit the in vitro release data into a suitable model. Hence, the dissolution data was fitted in various well-known exponential equations like zero order kinetics \( C = k_0 t \), First order kinetics \( \log C = \log C_0 - kt / 2.303 \), Higuchi kinetics model \( Q = K t^{1/2} \), and the Korsemeyer – peppas kinetics model \( Mt / M_\infty = K t^n \).[10]

### RESULTS AND DISCUSSION

#### Preparation of Hydrogel Nanoparticles

Chitosan / carrageenan hydrogel nanoparticles were formed successfully using 0.75% w/v and 0.5% w/v concentrations of chitosan and 0.1% w/v of carrageenan, by means of an electrostatic interaction between the positively charged chitosan amino groups and the oppositely charged sulfate groups of carrageenan; 0.25% w/v and 1% w/v concentrations of chitosan and 0.05% w/v and 0.2% w/v concentrations of carrageenan formed micro range particles and free polymer was also present. With an increase in the concentration of chitosan, the particle size was also increasing. The particle size dramatically reduced when the stirring speed was increased from 1000 rpm to 3000 rpm. A further increase in stirring speed had no effect on the particle size. The preparation of mercaptopurine-loaded chitosan / carrageenan nanoparticles was relatively difficult due to the hydrophobic nature of the drug. A successful entrapment of the drug in the chitosan / carrageenan nanoparticles was achieved by dissolving mercaptopurine in alcohol prior to its incorporation in the chitosan solution, followed by the addition of carrageenan solution. The resulting drug-loaded hydrogel nanoparticles were centrifuged at 3000 rpm for 15 minutes and subjected to freeze drying.

#### Morphology and physiochemical characterization of hydrogel nanoparticles

Figure 1 displays the SEM image of chitosan / carrageenan nanoparticles, which were almost spherical in size. The particle sizes ranged from 373 nm to 803 nm, as shown in Table 3.

#### Estimation of drug content and entrapment efficiency

The drug content in the hydrogel nanoparticles ranged from 9.65 to 17.62, as shown in Table 4. It was found that with an increase in the concentration of chitosan, there was an increase in drug loading in the hydrogel nanoparticles.

#### In Vitro Release Studies

In vitro release studies that were performed using a dialysis membrane in a phosphate buffer of pH 7.4, showed a release of 69.48 to 81.31% mercaptopurine from hydrogel nanoparticles, as shown in Figure 2. The release of mercaptopurine was dependent on the chitosan concentration used in the formation of hydrogel nanoparticles. An increase in the concentration of chitosan resulted in the decreased release of mercaptopurine from hydrogel nanoparticles.

### Table 3: Showing particle size of batches F1 to F6

| Formulation | Particle size |
|-------------|--------------|
| F1          | 803 nm       |
| F2          | 665 nm       |
| F3          | 712 nm       |
| F4          | 589 nm       |
| F5          | 373 nm       |
| F6          | 435 nm       |

### Table 4: Showing % drug content of batches F1 to F6

| Formulation | % drug content |
|-------------|----------------|
| F1          | 17.2           |
| F2          | 12.61          |
| F3          | 15.7           |
| F4          | 14.27          |
| F5          | 9.65           |
| F6          | 11.21          |

**Figure 1:** Showing SEMa image of Hydrogel nanoparticles

**Figure 2:** Showing in vitro release of batches F1 to F6
Determination of the Zeta Potential
From all the results obtained F1 was found to be the ideal batch, for which the zeta potential was found to be 26.6 mV. This indicates the moderate stability of the batch.

Release Kinetics
The release kinetics was also calculated for F1. The $R^2$ values were found to be 0.996, 0.9844, and 0.9305 for zero order, first order, and the higuchi plot, which are shown in Figures 3 and 4. For the Korsemeyer – peppas slope, value ‘n’ was found to be 0.8281. The best correlation was found in the zero order, which indicated that the drug release rate was independent of its concentration. The Korsemeyer – peppas plot indicated a slope value ‘n’ of 0.8281 which was indicative of an anomalous diffusion mechanism or diffusion coupled with erosion. Hence, the drug release was controlled by more than one process.

CONCLUSION
The study confirms that the counter polymer gelation technique was suitable for the preparation of Mercaptopurine hydrogel nanoparticles. This formulation approach, with hydrophilic polymers like chitosan and carrageenan, increases the life span of nanoparticles in systemic circulation by preventing the opsonization of nanoparticles, which generally takes place when a formulation is given through an intravenous route. As mercaptopurine half life is less than 90 minutes it can benefit from the long life span characteristic of hydrogel nanoparticles and its therapeutic efficacy can be increased. With these formulations better patient compliance is provided as they shown sustained release behavior.

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Source of Support: Nil, Conflict of Interest: Nil.