FORMULATION AND COMPARISON BETWEEN TWO METHODS FOR 5-FLUOROURACIL-LOADED CHITOSAN MICROSPHERES

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ABSTRACT:

In order to achieve complete remission from most tumours, patients often need to undergo radiation therapy, have the tumour removed surgically, and undergo lengthy chemotherapy. The objective of this study was to find an alternate method of encapsulating 5-fluorouracil (5-FU) in microspheres that would improve the effectiveness of chemotherapy without having a detrimental effect on the patients' quality of life or causing as many severe adverse effects on the system as the current method does. In the present experiment, chitosan microspheres were cross-linked in two distinct ways utilizing 5-fluorouracil (5-FU). It was hypothesized that altering factors such as the concentration of chitosan, the type of the oily phase, the nature of the cross-linker, and the amount of time spent cross-linking would provide a diverse set of features for the microspheres. The concentration of chitosan, the viscosity of the oil phase, and the glutaraldehyde content all had an influence on the pace at which 5-FU was released from the microspheres. Chitosan microspheres containing 5-fluorouracil (5-FU) that have been cross-linked release their contents very quickly. The goal of this study was to identify the most effective technique for manufacturing porous microspheres, which have the potential to postpone the breakdown of medications by stomach acid for up to six hours.

Keywords: 5-Fluorouracil, Chitosan, Microspheres, Colon cancer

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INTRODUCTION:

Colorectal cancer, often known as colorectal cancer (CRC), is the third most frequent form of cancer overall and the second most lethal variety. It can affect either the colon or the rectum. It poses a significant danger to one's health. In the year 2020, colorectal cancer will be the underlying cause of 9.4% of all cancer fatalities. In spite of this, it is anticipated that the number of individuals living with colorectal cancer will more than double throughout the world by the year 2035, with the greatest increase occurring in less developed nations, where the number of cases discovered in older people is on the rise [1-3]. The genes that cause CRC are diverse, and the disease can originate in a variety of different ways. For instance, various degrees of gene expression in a large number of CRC cells were responsible for the development of hundreds of somaclonal mutations [1-4].

The chemotherapy medicine known as 5-fluorouracil (5-FU) is frequently used to treat patients who have cancer. Since 1957, it has been utilised in the treatment of malignancies of several locations, including the breast, the colon, the head and the neck. In addition to this, it is an essential component of the treatment for colon cancer. The anticancer medicine known as 5-fluorouracil belongs to a family of medications known as "antimetabolites." It does this by inhibiting the creation of nucleic acids and DNA, which ultimately results in the death of cancer cells. It is one of the cancer medications that is prescribed to patients the most frequently. As a treatment for solid tumours such colon, breast, liver, brain, and pancreatic cancer, it can be administered on its own or in combination with other medications. Since it was difficult for the body to absorb via the digestive tract and was not always successful when taken by mouth [5-8], 5-FU was frequently administered in clinics through the use of intravenous (IV) therapy.

The use of carrier technology is a clever method for the delivery of pharmaceuticals. The drug is fastened to a carrier particle such as microspheres, nanoparticles, or liposomes, for example, and this attachment modifies the manner in which the medication is absorbed and released into the body. Because to their diminutive size and capacity to perform admirably in the role as carriers, microspheres constitute a significant portion of these particulate DDS. Nevertheless, due to the fact that they only remain at the site of absorption for a brief period of time, these novel DDS have a restricted scope of use. It would be beneficial if the design allowed the DDS membranes and the absorbing membranes to be placed in close proximity to one another. It's feasible that this can be accomplished by developing brand-new delivery methods known as "mucoadhesive microspheres" and incorporating mucoadhesion into those systems. Microparticles and microcapsules with a drug core are known as mucoadhesive microspheres. These microparticles and microcapsules range in diameter from 1-1000 m and are constructed of a mucoadhesive polymer in either its entirety or in part. In spite of the fact that microspheres may be engineered to adhere to mucous membranes, there are still valid reasons to utilise them for the targeted and controlled release of drugs. It is feasible for the bioadhesive system to adhere to the mucosal layer [9–13]. Hence, the mucoadhesive drug delivery system might be designed for buccal, oral, vaginal, nasal, rectal, and ocular routes of administration.

Chitosan is a biodegradable, hydrophilic, biocompatible, natural linear biopoly-aminosaccharide that has a lot of potential for use in the medical field. It has a high charge density, is non-toxic, and has the ability to adhere to mucous membranes. Chitosan is a natural linear biopoly-aminosaccharide. Chitosan was also investigated as a potential means of encapsulating pharmaceuticals within microspheres. Chitosan molecules form bonds with one another after being subjected to reactions with trace levels of multivalent anions. The production of chitosan microspheres has seen extensive application of this crosslinking technique. These are the medication delivery techniques that have received the most investigation [14–16]. Controlling the release of medications such as antibiotics, antihypertensive pharmaceuticals, anticancer treatments, proteins, peptide

therapies, and vaccines are some of the applications for these devices.

MATERIAL AND METHODS:

Materials:

As a sign of good will, Khandelwal labs Pvt. Ltd. gave away a free sample of the drug (5-fluorouracil) (Thane, India). SD Fine Chemicals took our order for Span-80 chitosan (Mumbai, India). Sigma-Aldrich Chemie made the glutaraldehyde and the paraffin oil, while SD Fine Chemicals made the acetic acid. E. Merck provided calcium chloride, potassium dihydrogen phosphate, cyclohexane, Tween 80, methanol, and dichloromethane (Darmstadt, Germany). All of the other reagents were at least of analytical grade.

Method:

Method of preparation of Chitoson Microspheres I (CMS-I):

Microspheres can be made through chemical denaturation or, if that's too hard, through emulsification, which involves less phase separation. A 250 mL polypropylene beaker was filled with 100 mL of regular paraffin oil. In a beaker, oil was mixed with 1 mL of span-80. A 22-gauge hypodermic syringe was used to inject small amounts of a chitosan solution that was 3.5% w/v and had 50 mg of 5-fluorouracil in it. To make the delivery vehicle, 1% acetic acid was mixed with chitosan. During this method, a high-speed stirrer with propellers was used to stir paraffin oil at a rate of 2,000 revolutions per minute (rpm). After 15 minutes, 0.25 mL of glutaraldehyde was added to the oil, and then the chitosan solution was added. Even though everything else stayed the same, the speed of the stirring was cut in half, from 2,000 turns per minute to 1,000. At 30 minutes and 1 hour, 0.50 mL of glutaraldehyde was added while constantly stirring. After another hour of mixing, the last amount of glutaraldehyde was added, and the process was stopped. Before sinking to the bottom, the chitosan microspheres floated in paraffin oil for twenty-four hours. After the clear supernatant was taken away, microspheres were taken from the residue and washed four or five times in diethyl ether. After the microspheres were washed for the last time, they were left at room temperature to dry by air. The powder was then collected and kept at room temperature [17–19].

Method of preparation of Chitoson Microspheres II (CMS-II):

Emulsification and chemical cross-linking are two additional processes that may be used when producing microspheres for the purpose of phase separation. A polypropylene beaker with a capacity of 250 millilitres was given 35 millilitres of light paraffin oil and 25 millilitres of petroleum ether. Spinning the contents of the beaker allowed us to mix one milliliter of span-80 with one milliliter of oil. We did this using the beaker. Using a hypodermic syringe with a 22-gauge needle, a solution of chitosan that was 4% water by volume was introduced very slowly. In order to make this solution, chitosan was first diluted to a concentration of one percent using acetic acid. This combination had a 5-fluorouracil dosage of fifty milligramme total. This was then added to the oil, which was being stirred at a rate of 2,000 revolutions per minute by a high-speed stirrer that was fitted with a propeller. The oil was stirred for a further five minutes so that the chitosan solution would be well mixed. This was done to guarantee that the solution would be thoroughly blended. At the same time, 1.6 millilitres of glutaraldehyde-saturated toluene (GST) was added to the mixture, and it was thoroughly combined after each addition. After waiting for half an hour, add 1.6 more mL of GST to the mixture. After an hour, include 1.8 mL of aqueous aldehyde in the mixture. When the last SGST was added, the mixture was allowed to sit without being stirred for the next 1 hour and 30 minutes. After being retrieved, the chitosan microspheres were shown to be floating in the oil. It took them twenty-four hours to finally settle to the bottom. After discarding the clear supernatant, the microspheres were washed many times with petroleum ether, methanol, a 5% solution of sodium bisulfide, and acetone. After receiving one more washing, the microspheres were allowed to dry in the air. The remaining quantity of dry powder was kept in airtight containers and kept at room temperature [20, 22].

Evaluation Parameter

Determination of Percentage Drug Entrapment

How effectively each batch fared in capturing drugs was measured by the PDE (percentage of drug entrapment).

PDE = Practical drug loading × 100 Theoretical drug loading

We made the assumption that the end product would absorb all of the medicine that was present in the chitosan solution that was used to make the microspheres so that we could accurately calculate the potential drug loading.

It was concluded that the best way to load a drug would be to use 25 mg of chitosan microspheres, and a 25 mL volumetric flask was used to do this. The bottle was then filled with methanol (around 25 mL). After continuously agitating the suspension at room temperature for twenty-four hours, we made the decision to let it alone. After centrifugation at 2000-3000 rpm, the concentration of the supernatant was measured utilizing UV Spectrophotometry at 266 nm [23, 24].

Determination of Particle Size and Particle Size Distribution

The releasing properties of microspheres coated with eudragit are shown to be greatly impacted by the particle size of the microspheres. To evaluate the distribution of particles in a suspension of chitosan microspheres (10 mg) in sterile water, a particle size analyzer (Malvern Master Sizer, model E, UK) and laser light diffraction were employed. The particle size analyzer was manufactured in the United Kingdom (2 mL). [25, 26] It was said that the Z-average particle size was the typical value (d.nm).

Determination of Zeta Potential

The zeta potential is a representation of the surface charge that may be seen on microspheres. It takes a significant amount of zeta potential for mucoadhesive substances to disperse. Using laser Doppler anemometry on a Zeta master (Malvern, UK), the researchers were able to estimate the surface charge of microparticles that were suspended in deionized water [27].

IN-VITRO Wash-Off Test for Microspheres

The IN-VITRO wash-off procedure was utilised in order to assess the mucoadhesiveness of the microspheres. That was done by threading the mucosa that lined the rat's stomach onto a glass slide, and it covered an area that was one centimeter squared. Within the USP pill dissolving test apparatus, the sample was placed on a slide, which was then suspended from a groove. They were able to determine the pace at which microspheres dissolved in a tissue sample by using this procedure. Using disintegration testing equipment, tissue samples were often shaken while being held in a beaker containing phosphate buffer pH [28, 29].

Morphological Study of Microspheres

Digital microscopes are a type of optical microscope. With the use of photomicrographs, we were able to characterise the shape and cohesion of loaded chitosan microspheres. Microspheres made from chitosan solutions with concentrations of 3.5 and 4% remained mainly distinct after being spun at a speed of 2000-2500 rpm [30, 31].

IN-VITRO Release Study of CMS-1 and CMS-2

In a fluid model of the gastrointestinal system, drug release investigations from chitosan microspheres were conducted in the laboratory (SGF). The paddle technique (model Sentwin, India) was utilised to conduct dissolving experiments on microsphere-encapsulated drugs. These examinations were conducted in line with USP XXIII. Microspheres weighing a total of 100 milligramme were mixed with the dialysis membrane and the solubilizing solvent (SGF). The material was spun at a rate of 100 revolutions per minute at a temperature of 370 ° C +/- 0.50 ° C. The best circumstances for observing the dissolution of tablets were provided by the sink. We were able to reproduce the circumstances of gastrointestinal transit by adjusting the pH of the dissolving solution in minute increments at regular intervals. With the aid of 0.1 N HCL, the pH of the dissolving liquid was maintained at 1.2 during the course of two hours. To facilitate the dissolution of the substance, the pH of the medium was decreased to 4.5 using 1.0M sodium hydroxide. The mixture was then supplemented with 1.7 grammes of KH2PO4 and 2.2 grammes of Na2HPO4.2H2O. The study of the discharge's flow rate consumed an additional two hours. After 4 hours of incubation, the pH of the solubilizing media was

adjusted to 7.4 using 0.1 N sodium hydroxide. As soon as it reached that location, it remained there for the remainder of the next day. Using a pipette, samples were obtained at various stages of the procedure from the solutions used for dissolving and diluting the chemical in concern. Researchers substituted saline solution for soluble growth factor (SGF) because they understood the importance of maintaining a constant receptor size. Using UV soctrophometry, the researchers were able to determine the rate of FU release; they then created a release curve to illustrate how the rate of release altered over time [25-28].

RESULT AND DISCUSSION:

A number of processes, including as solvent evaporation, single emulsification (of the w/o type), and multiple emulsification (of the w/o/w type), were utilised throughout the manufacturing process of microspheres. The results are summarized in the table that can be seen below. The evaporation of liquid paraffin and acetone resulted in the formation of minuscule spheres. For the purpose of creating an emulsion, the drug solution that was dissolved in acetone was mixed with the chitosan solution and the liquid paraffin. To begin, we filtered the emulsion that included the microspheres. This was done in order to dry the object, which was followed by sterilizing it. Unfortunately, it does not appear that acetone is an efficient solvent for the dissolution of 5-FU. It was assumed that re-emulsifying would be ineffective and would result in the waste of solvent.

Because 5-FU and chitosan are soluble in a solution containing 5% acetic acid, there is no need to emulsify the mixture before attempting to form microspheres out of the two substances. As solvents, acetic acid and diethyl ether are typically utilised. In order to improve the stability of the emulsion, glutaraldehyde, which is a cross-linking agent, was added to it. The microsphere emulsion was prepared for use after being washed, filtered, and then baked in an oven at a temperature of 600 degrees for one hour. The microspheres that you make should be consistent throughout in terms of their size and shape, and they should be able to effectively capture individual particles. After that, we generated microspheres using two different methods, none of which involved emulsification. We were able to tailor the concentration of chitosan, the length of the oily phase, the concentration of the cross-linking agent, the amount of time spent cross-linking, and the washing solvent to our specifications by utilizing these methods. Table 1 contains an inventory of the components required for the production of chitosan microspheres using either Method I or Method II.

Table 1: Chitosan microspherespreparedby MethodI&MethodII

PROCESSVARIABLES	METHOD I	METHODII
Concentrationofchitosan	3.5%	4.0%
Typeofoilyphase	Lightliquidparaffin	Liquidparaffin&petroleumether
Typesofcross-linker	Glutaraldehyde	Glutaraldehydesaturatedtoluene
Cross-linkingtime	15, 30, 60min	60&120min
Washingsolvent	Diethylether	Petroleumether,methanol,acetone

For the purpose of this inquiry, we decided to use a method that combines traditional emulsification with chemical cross-linking. Our decision was based on the concerns outlined above. Because this method is versatile, it may be used to a wide variety of various types of polymers and solvents. It has the capability of capturing molecules that have a predisposition for either water or oil as their natural environment. Oil-in-water, or o/w, emulsions

are a delivery method that can be used for medicines that do not dissolve in water. Because of this, the manufacturing of hydrophilic pharmaceuticals must make use of emulsion or double emulsion technology. So, in the not-too-distant future, there will be an increased emphasis placed on research aimed at perfecting the straightforward chemical denaturation process of emulsification. In this method, a layer of chitosan that has been treated with acetic acid is shielded by a layer of liquid paraffin. Throughout processing, there were a number of elements that had an effect on the particle size. It was important to take into account the drug polymer ratio, as well as the stirring speed, cross-linker concentration, and cross-linking agent time. In Table 2, comparisons are made between microspheres made in two different ways with regard to their IN-VITRO wash-off efficiency, swelling profile, zeta potential, particle size distribution, and drug entrapment.

Table 2: EvaluationparametersofCMS-1&CMS-2

Sr.No.	Evaluationparameters	CMS-1	CMS-2
1.	Percentagedrugentrapment	25.89	14.57
2.	Particlesizeandsizedistribution	4352.53nm	2761.80nm
3.	Zetapotential	+41.0mV	+51.2mV
4.	Swellingproperties	Less	More
5.	*IN-VITRO*wash-offtest	>24hrs	<24hrs

It was discovered that Method 1 had a higher value for the amount of medication that was entrapped (25.89) than Method 2. (14.57). although if the total amount of chitosan used in all three methods is the same, the entrapment efficiency may be improved by increasing the polymer concentration. Nevertheless, the efficiency of the trapping reduces with rising drug concentration if there is not enough chitosan available to bind the drug. This is the case if there is not enough chitosan present.

The decreased entrapment efficiency in Process 2 needed longer cross-linking times to make up for it. This was necessary since there was a possibility that the emulsion did not form properly. In order to make these microspheres as effective as possible, method 1 was used because of their consistent size, high entrapment efficiency, and predictable release profile.

Even though the total volume of the polymer stayed the same, the microspheres produced by increasing the polymer concentration were much bigger. It was discovered that the concentration of the cross-linking agent had an effect on the size of the particles, with larger cross-linking concentrations producing smaller microspheres. A denser packing of the microspheres is produced as a result of the higher glutaraldehyde concentration in Step 2.

The fact that both formulations had positive zeta potentials suggests that the negatively charged sialic acid and fucose residues in stomach mucus interact electrostatically with the positively charged chitosan, which in turn extends the amount of time the medicine is present in the stomach. It is possible that the level of cross-linking can govern the quantity of free amino groups that are found on the surface of a positively charged chitosan microsphere. In fewer than 15 minutes, the cross-linking caused by glutaraldehyde caused the zeta potential of the microspheres to drop from around +55 mV to approximately +47 mV. Now the surface charge has reached a state of equilibrium [22-27].

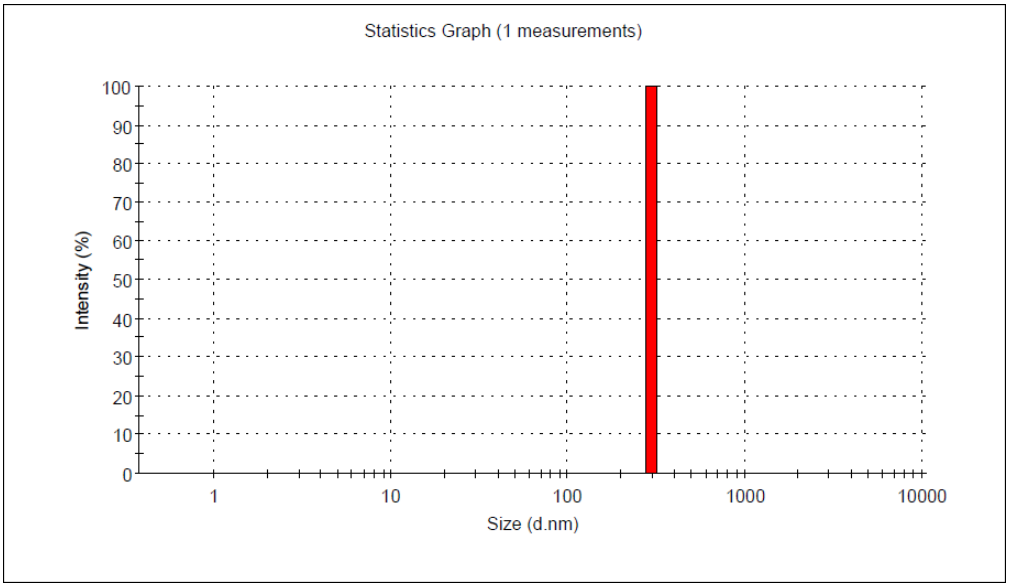

Figure 1:Particlesizedistributionofchitosanmicrospherespreparedby method-1

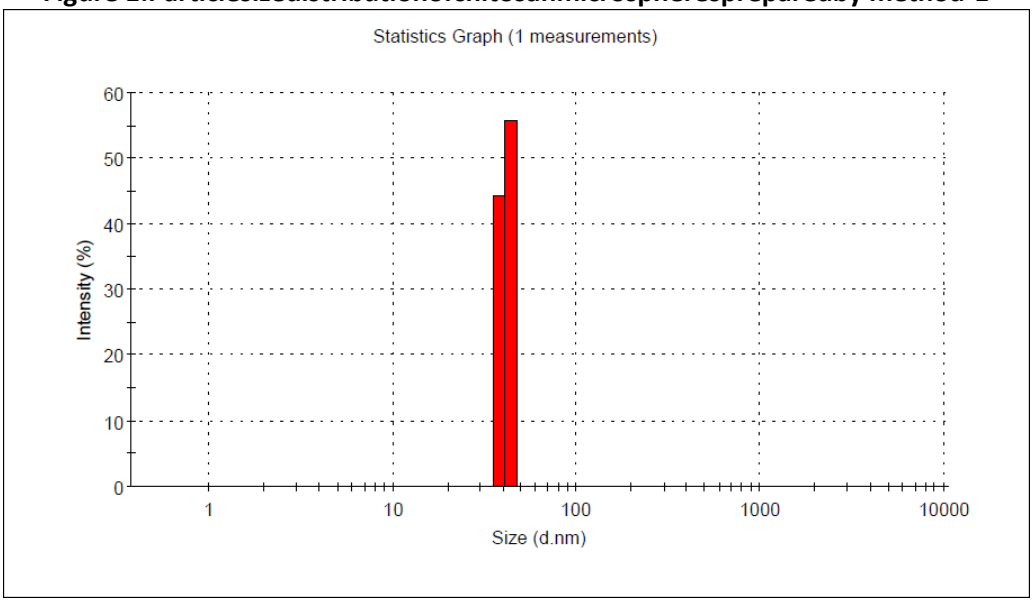

Figure 2: Particle size distribution of chitosan microspheres prepared by method-2

Morphological Study of Microspheres

Digital microscopes are a type of optical microscope. The shape and size of the unloaded chitosan microspheres were characterized by the use of photomicrographs, which revealed that the microspheres were spherical and grouped together. Microspheres made from a solution containing 1% chitosan and spun at 2000-2500 rpm were perfectly spherical.

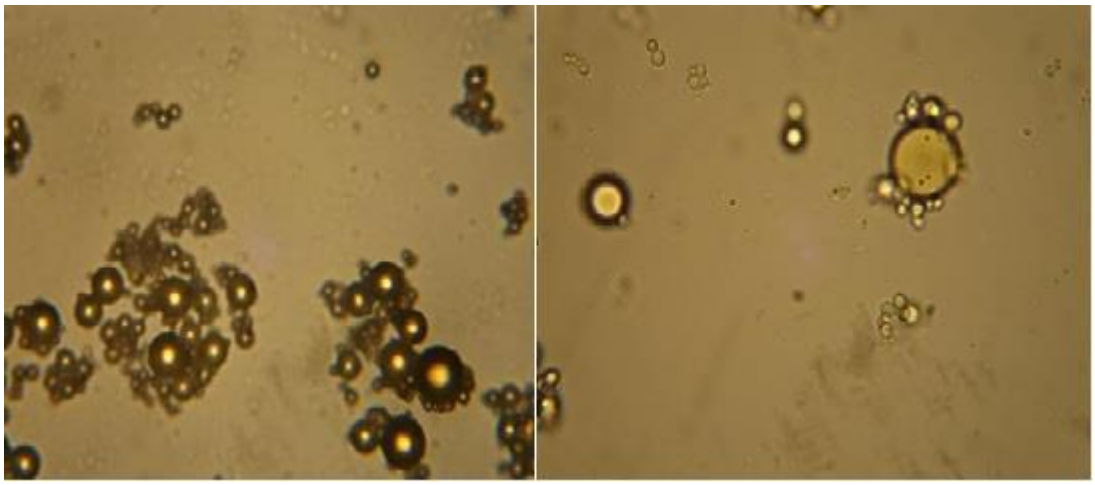

Figure 3: Photographsof unloaded chitosanmicrospheres

IN-VITRO ReleaseProfileofCMS-1 andCMS-2

Table 3: IN-VITRO release profileof5-FUfromCMS-1&CMS-2

Sr.No.	Time(hrs)	% DrugReleaseof CMS-1	% DrugReleaseofCMS-2
1.	1	8.96	7.56
2.	2	17.25	17.78
3.	3	22.64	21.34
4.	4	28.46	26.49
5.	5	33.49	31.56
6.	6	38.43	33.56
7.	7	44.37	40.78
8.	8	50.46	45.18
9.	9	57.64	48.64
10.	10	63.49	50.87
11.	11	67.18	55.67
12.	12	72.34	57.64
13.	24	80.64	60.49

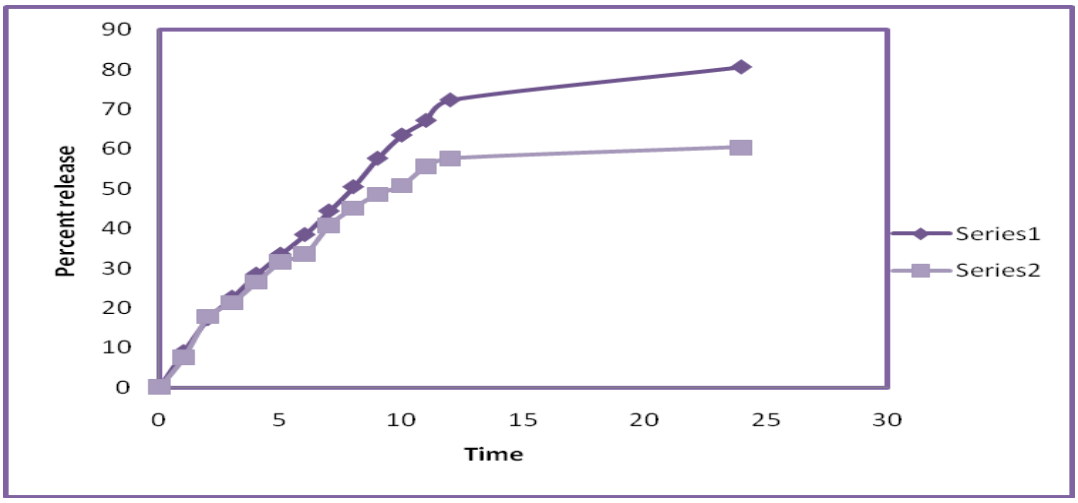

Figure 4: PercentreleaseofFluorouracilfromCMS-1&CMS-2

Because the appropriate amount of cross-linking agent was used, and the cross-linking process was quick, the microspheres that were created using Method 1 had a superb, linear release pattern. This was possible due of two factors. When the amount of time spent cross-linking increases, drug release decreases [26-31].

CONCLUSION:

The application of the emulsion method resulted in the successful manufacture of microspheres that contained 5-fluorouracil. In order to pack the chitosan microspheres with the maximum number of 5 FU feasible, the variables in the recipe were optimized. If you want to improve the efficacy of the cytotoxic drug at a lower dose, the evidence shows that Method B chitosan microspheres loaded with 5-fluorouracil (5-FU) may be superior to CMS-1 microspheres. It might be beneficial for injecting 5 FU into the lungs, which is where it is most likely to have a significant impact on the state of the patient. In order to keep the anticancer medicine 5 FU active for a longer length of time, a biodegradable carrier system was devised. According to the results of our research, CMS-1 has the potential to be a successful long-term treatment for cancer that is safer, less costly, and offers a lower danger to patients than the conventional approach of administering medications through parenteral route.

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Conflicts of interest

There are no conflicts of interest among all the authors with publication of manuscript.

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