Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.2S,
July-December 2024: P. 1243-1260

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

FORMULATION AND CHARACTERIZATION OF SACITUZUMAB-LOADED CHITOSAN NANOPARTICLES VIA IONOTROPIC GELATION FOR ENHANCED DRUG DELIVERY

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Abstract:

Sacituzumab loaded chitosan nanoparticles were produced by the ionotropic gelation method. This method exploits the electrostatic interaction between the negatively charged tripolyphosphate (TPP) and the positively charged chitosan polymer. The chitosan/TPP ratio significantly affects the size and efficiency of nanoparticle formation, aiming for a size below 250 nm. The highest levels of chitosan and TPP were found at a concentration of 8 mg/ml. Several formulations were assessed, and the chitosan/TPP ratio of 0.8% yielded the formation of the smallest particles (229±7 nm). The duration of sonication was also a crucial element, since the lowest particle size was obtained after 90 seconds. The zeta potential exhibited a range of +3 to +6 mV, indicating the stability of the colloidal system. The particle size was estimated to be about 100 nm, and the spherical shape was confirmed using FESEM and TEM investigations. The AFM investigation confirmed these results by revealing the presence of compact, almost spherical nanoparticles. The formulation labelled as F3 had the greatest level of entrapment efficiency (EE), ranging from 53.2±1.5% to 68.5±0.3%. An initial rapid and forceful release was detected in drug release studies conducted outside of a living organism, which was thereafter followed by a continuous and prolonged release. F3 attained a discharge rate of 98.2±1.4% during a 24-hour period. The stability and effectiveness of the nanoparticles

for possible therapeutic uses were verified using stability experiments done over three-month duration. These investigations demonstrated negligible changes in particle size and constant drug loading.
Keywords: *Sacituzumab, chitosan nanoparticles, ionotropic gelation method, cancer*

1. Introduction:

The global challenge of discovering novel and groundbreaking therapies for cancer persists. The proliferation of cancer treatment modalities and the advent of personalized medicine have significantly enhanced the therapeutic effectiveness of some malignant tumors. Chemotherapy is a standard and extensively used way for treating cancer. Chemotherapy exerts its effects via several routes, primarily by eradicating rapidly dividing cells, both cancerous and healthy. This indiscriminate action leads to significant side effects such as bone marrow suppression, alopecia, and gastrointestinal problems (Yao et al., 2020). As a result, a significant portion of research on cancer in the last several decades has been devoted to creating drugs that precisely target tumour cells rather than healthy ones. Targeted treatment has made great strides towards precision medicine, but there are still a number of unavoidable adverse effects, and the problem of drug resistance has continued to be problematic. As of right now, cancer is the second leading cause of death, and there are currently inadequate therapies available for many forms of the disease. As a result, more studies are concentrating on precisely treating cancer and developing solutions for drug resistance (Yao et al., 2020).

An increasing number of medical applications of nanotechnology have emerged in the last few decades. This has resulted in improvements in both diagnosis and therapy, as well as safer methods of targeting malignancies. Several benefits, including improved pharmacokinetics, targeted tumor cell delivery, fewer side effects, and decreased susceptibility to treatment resistance, have been shown by drug delivery systems created on nanoparticles into cancer therapy. The dimension and assets of nanoparticles (NPs) used into medicine delivery systems were often selected or created in a way that corresponds to the pathophysiology of tumors (Sarvari & Sarvari, 2023). Using the nanoparticle carrier effect and careful targeting after absorption, nano-carriers in cancer treatment selectively target tumor cells (Zitvogel et al., 2008). Subsequently, the cancer cells are treated with the medications to cause their death. There is hope for gene therapy and cytotoxic treatment using nano-carriers, which incorporate both nucleic acids and conventional chemotherapy drugs. Another potential use for NPs is as a vehicle for the encapsulation and circulation-transport of medicines with poor solubility (Zitvogel et al., 2012). Because of NPs' size, surface properties, and propensity to increase permeability and retention, nano-carriers may make it easier for nebulizers to accumulate within cancer tissues and extend the duration of nebulizer effects. In the meantime, the targeting system prevents cancer treatment's harmful side effects by shielding healthy cells from the cytotoxic effects of the medications. In contrast to free doxorubicin, PEGylated liposomes loaded with doxorubicin mitigated its cardiac damage (Zitvogel et al., 2011). Furthermore, nanoparticle albumin bound Sacituzumab had minimum side effects and permitted tolerable dosage increases when contrasted with solvent-based taxanes. Immunotherapy, ablation therapy, chemotherapy, gene therapy, and other cancer treatments have all shown the utility of nanoparticle meds in several studies. It is thought that the nanoparticle-based drug delivery method might improve immunotherapy efficacy by reversing

the immunosuppressive tumour environment (Yao et al., 2020).

Over the past few years, here have been a growing use of nanomaterials in the medical profession, namely for the purposes of diagnosis, therapy, and more precise targeting of tumors, resulting in improved safety and effectiveness. Nanoparticle (NP)-based drug delivery systems have shown several benefits in cancer therapy, including favourable pharmacokinetics, specific tumour cell targeting, mitigation of adverse effects, and overcoming drug resistance. The selection or engineering of nanoparticles (NPs) for medication transportation often takes into account the unique pathophysiology of various cancers, influencing their dimensions and characteristics (Dadwal et al., 2018). Small carriers are used in cancer therapy to selectively attack tumour cells by exploiting the carrier function of nanoparticles (NPs) and the positional influence of the desired drug when it has been consumed. The drugs are then supplied to the tumour cells with the aim of causing their death. The small carriers have the capacity to be used for both gene therapy and toxic uses, since they include DNA and typical chemotherapy medicines. Additionally, nanoparticles (NPs) may serve as a platform for enclosing drugs that have poor solubility, which helps in their distribution into the bloodstream (Lohcharoenkal et al., 2014). Nano-carriers may prolong the lifespan of medications and facilitate their accumulation in tumour tissues due to the small size and surface properties of nanoparticles (NPs) and their potential to enhance permeability and retention. Simultaneously, the targeting system safeguards healthy cells from the harmful effects of medications, so mitigating the negative consequences of cancer treatment. For instance, the use of PEGylated liposomes loaded with doxorubicin resulted in a decrease in cardiotoxicity when compared to the administration of free doxorubicin (Yao et al., 2020). In addition, nanoparticles albumin-bound Sacituzumab demonstrated less side effects and allowed for the use of greater tolerated dosages compared to taxes dissolved in solvents. Additional studies have shown the use of nanoparticles drugs in tumour immunotherapy and excision therapies, as well as chemotherapeutic and gene therapy. It is hypothesised that the immunosuppression environment in tumours may be countered by using a medicine delivery method that utilises particles. This technique is expected to improve the effectiveness of treatment (Akanda et al., 2023).

2. **Nanoparticles in cancer:**

The effectiveness of nano-drug delivery and, thus, the success of medical therapy, are greatly affected by the precise sizes, shapes, and surface properties of the nanoparticles (NPs) used. Nanoparticles (NPs) within the size series of 10 to 100 nm are often painstaking appropriate for cancer treatment because they may efficiently transport drugs and induce an improved permeability and retention (EEPR) effect. Phagocytes are capable of eliminating particles that exceed 100 nm in size from the bloodstream, but smaller particles (less than 1-2 nm) may readily evade the typical blood vessels and pose a threat to healthy cells (Kucuksayan et al., 2021). Particles with a diameter less than 10 nm may be easily removed by the kidneys by filtration. Moreover, the surface features of NPs may have an impact on their bioavailability and half-life (Edis et al., 2021). As a result, nanoparticles (NPs) are usually altered to become water-loving, which extends the time they stay in the bloodstream and enhances their ability to enter and gather in tumours. The overall effectiveness of nanoparticles in treating cancer depends on their many characteristics. Various nanoparticles for the treatment of cancer. The following text will describe the specific benefits of each treatment method in the management

of tumours (Palazzolo et al., 2017).

Deacetylation is a natural process that takes chitin the main component of the exoskeleton of crustaceans, lobsters, and prawns and turns it into chitosan, a carbohydrate polymer. Because of its ability to be digested by chitinases in the body, its low toxicity, and its exceptional biocompatibility, chitosan is an ideal material for use in therapeutic applications (Gong et al., 2017). Nanoparticles of chitosan are often made in a safe environment since the polymer dissolves easily in acidic water-based solutions when the temperature is room temperature. No high temperatures or toxic chemical solvents are needed. Medications that may be added with chitosan DDS include proteins, small compounds, and polynucleotides, among others [8]. Chitosan allows for the slow release of the encapsulated substances. According to, chitosan's free amine groups make it easier for ions to crosslink (Iqbal et al., 2018).

Ionotropic gelation is the procedure Calvo et al. were the first to create chitosan-Polyethylene oxide (PEO) nanoparticles using the ionotropic gelation technique (P. Zhang et al., 2016). When a negatively charged polyanion interacts with chitosan, a sol-gel transition occurs, which may lead to the creation of nanoparticles in some cases (Aghebati-Maleki et al., 2020). Chitosan is composed of amine groups that have a positive charge. Tripolyphosphate (TPP) is a commonly used polyanion. In their synthesis of chitosan, Calvo et al. dissolved it in solutions containing different concentrations of acetic acid. They did the same thing with chitosan; they dissolved it in water at concentrations of 0.05 wt%, 0.1 wt%, 0.5 wt%, and 1 wt% to make TPP (Sun et al., 2021). The best conditions for producing chitosan nanoparticles were identified by combining several concentrations of TPP with the chitosan solutions. Aqueous TPP solutions were mixed with chitosan solutions containing PEO and PEO-PPO in a continuous swirling motion to produce chitosan/PEO and chitosan/PEO-PPO nanoparticles (Chang et al., 2021). The chitosan nanoparticles obtained had a chitosan-to-TPP proportion of 5:1, and a minimum dimension of 260 nm. The nanoparticle particle sizes of chitosan/PEO and chitosan/PEO-PPO ranged among 300 and 1000 nm, according to the concentrations of PEO and PEO-PPO. That chitosan/PEO and chitosan/PEO-PPO nanoparticles have a reduced zeta strength compared with the chitosan nanoparticles, indicating that the latter two were stabler (Siddique & Chow, 2022).

Sacituzumab is an antibody-drug conjugate (ADC) predominantly used for treating certain forms of cancer, such as metastatic triple-negative breast cancer (mTNBC) and urothelial cancer. The composition consists of an antibody that specifically targets the Trop-2 protein, which is often found in excessive amounts on the outer surface of cancer cells. This antibody is connected to a chemotherapeutic drug known as SN-38, which is a very effective inhibitor of topoisomerase I (Bardia et al., 2021).

Sacituzumab govitecan is an antibody-drug combination consisting of an IgG1 kappa antibody that targets the antitrophoblast cell-surface antigen 2 (Spring et al., 2021). It is linked to SN-38, which is the action form of irinotecan and acts as a topoisomerase. The linkage between the antibody and SN-38 is made by a unique hydrolyzable linker. Trop-2 is a protein that spans the cell membrane and has a role in transmitting calcium signals (Bardia et al., 2019). It is shown to be highly expressed in several forms of tumors, such as breast cancer, with a

frequency of more than 90%.After being administered, the anti-Trop-2 monoclonal antibody binds to Trop-2, a protein found on the surface of tumour cells (Xie et al., 2023). This allows for the specific delivery of SN-38 to those cells. Due to its propensity to pass across cell membranes, free SN-38 might have antitumor effects in nearby cancer cells (known as the bystander effect) before the antibody-drug combination is taken up by the cells. This may happen either via the hydrolysis of the linker or by the release of SN-38 within the cell after internalization (O'Shaughnessy et al., 2022).

Mechanism of action: The mechanism of action of Sacituzumab involves the specific targeting and binding of its antibody component to Trop-2, facilitating the direct delivery of the cytotoxic SN-38 to the cancer cells. This focused strategy helps minimize harm to healthy cells while maximizing the effect on cancer cells (FDA, 2020).

Indication: Sacituzumab is suggested for individuals having metastatic a triple-negative breast carcinoma who have received at least two previous treatments for advanced disease. It is advisable for patients using urothelial cancer that has spread regionally or to other regions of the body, who have previously had therapy with platinum-based drugs and either a PD-1 or PD-L1 inhibitor (O'Shaughnessy et al., 2022).

Administration: Sacituzumab is given by an intravenous route, usually on days 1 and 8 of a 21-day therapy cycle.

Adverse effect: Neutropenia, tiredness, nausea, diarrhoea, and baldness are often seen as side effects. Patients are observed for these adverse effects and treated appropriately.

3. Material and method:

3.1 Preparation of Sacituzumab loaded nanoparticle:

Chitosan nanoparticles loaded with Sacituzumab were produced using the ionic gelation technique; through liquefying 0.4%.A solution containing 5 mg of chitosan (0.4%, 0.6% and 0.8% in glacial acetic acid 1% (v/v)) was introduced into the previously described solution under constant stirring using a magnetic field. Subsequently, 0.4% of the solution was added gradually in little drops. The sample was immersed in a 0.6% or 0.8% solution of TPP in water, and then subjected to sonication after 30 mins of uninterrupted magnetic stirring(Hanna et al., 2023) . In order to eliminate any surplus TPP and Sacituzumab that was not captured, the nanoparticle solution underwent centrifugation for duration of 30 minutes at a speed of 13,000 rpm at a temperature of 4^0C in an ultracentrifuge. The pellets were scattered in deionized water and subjected to lyophilization in a freeze dryer for duration of one day, in order to preserve them in a powdered form. The diagram depicts the procedure for manufacturing Sacituzumab CS-NPs by the use of the ionic gelation method (Fraguas-Sánchez et al., 2022).

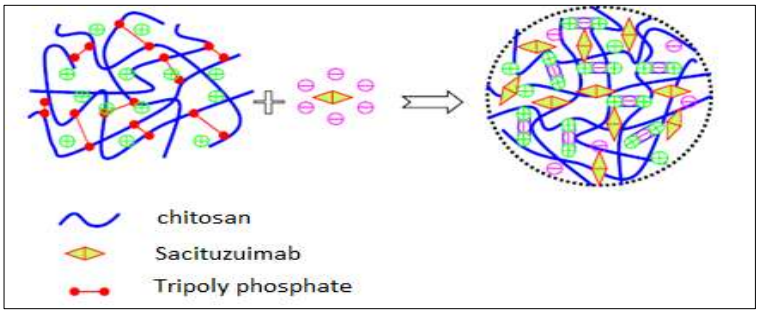

Figure1: Preparation of Sacituzumab loaded nanoparticle

3.2 Evaluation parameter of Sacituzumab loaded nanoparticle:

3.2.1 Characterization of particle sizes and determination of the polydispersity index: The Zetasizer ZS 90 was used to evaluate the average size of the particles and the polydispersity index (PDI) of the nanoparticle. The dynamic light scattering technique was implemented using a 532 nm laser at a 90° angle inside a cell with a diameter of 10 m, operating at a temperature of 25 ºC. The Stokes-Einstein equation is used in this approach to precisely estimate the diffusion of particles via Brownian motion and then transform it into a distribution of sizes. The average particle size of five Sacituzumab nanoparticles was determined by immersion sonication after dispersing them in two milliliters of double-distilled water. The average diameter, together with its standard deviation, is the outcome derived from three rounds of meticulous assessment (C. Zhang et al., 2020).

3.2.2 Assessment of zeta potential: The zeta potential of the NPs is calculated using the Helmholtz-Smoluchowski equation and the Zeta sizer ZS90. Following sonication in a bath sonicator, five Sacituzumab nanoparticles were evenly distributed in one milliliter of double-distilled water to obtain a uniform state. Experiments were made at a temperature of 25ºC using a disposable polystyrene cuvette and a zeta dip cell. The dimensions have been evaluated thrice, and the information is reported as the mean diameter ± standard deviation (Fraguas-Sánchez et al., 2022).

3.2.3 Assessment of Drug loaded content: 50 milligrams of lyophilized the sacituzumab nanoparticles were liquefied in acetonitrile, and the medication's concentration remained determined using ultraviolet light. The formula was used to determine the medication loading (Juan et al., 2020).

Drug loading content [%] = Weight of drug in nanoparticles x 100 Weight of nanoparticles

3.2.4 Assessment of Transmission electron microscopy (TEM): The high-resolution transmission electron microscopy (TEM) technique was used to study the surface morphology of five CS-NPs loaded with Salituzumab. The analysis was conducted at an electron beam energy of 80 kilovolts (kV).A sample holder was attached to the copper grid containing the dried nanosuspension, and the holder was placed within a vacuum chamber. Transmission electron microscopy (TEM) pictures were seen and recorded in a vacuum (Castillo-Tobías et al., 2023).

3.2.5 Assessment of Autonomic force microscopy (AFM): Atomic force microscopes reveal the three-dimensional surface characteristics of five sacituzumab-loaded nanoparticles. This was accomplished by using pyramidal cantilevers with resonance frequencies and force constants ranging from 0.35 to 6.06 N/m, which were driven by silicon probes in tapping mode. The frequency of the scan was two hertz. Prior to being placed on a glass slide, the samples were diluted by a factor of 10 using distilled water. Following a 24-hour vacuum drying period at a temperature of 25°C, the samples were examined. The height was computed using the AFM image analysis programme NTMDT, specifically the NTEGRA prime model (Petrilli et al., 2020).

3.2.6 Assessment of Entrapment efficacy (EE): The nanoparticles underwent ultracentrifugation for 45 minutes at a temperature of 4°C and a speed of 13000 revolutions per minute. The UV spectrophotometer may be used to identify any one of the five Sacituzumab residues in the nanoparticle supernatant. The entrapment efficiency (EE) was facilitated by

using the following formula:

$$EE = \frac{\text{Amount of total drug - Amount of free drug in supernatant}}{\text{Amount of total drug}} \times 100$$

3.2.7: DSC Examination: The DSC-60, a Shimadzu Thermal Analyzer, was used to perform thermal analysis on sacituzumab and its freeze-dried nanoparticle formulations. Aluminium containers were used to enclose samples weighing 2-4 mg for examination. The DSC thermograms were obtained by heating the sample at a rate of 10°C/min, starting at 30°C and ending at 300°C. An empty pan was used as a reference. The DSC experiment was performed with a nitrogen flow rate of 50 mL/min.

3.2.8 Study on the release of drugs in an artificial environment: The dialysis sac approach was used to investigate the in vitro release. The five Sacituzumab nanoparticles were freeze-dried in pH 7.4 phosphate solutions. Then, they were put on a dialysis membrane with a molecular weight cut-off of 10,000–12,000Da and sealed using membrane clips. The temperature of the beaker was maintained at 37ºC, and the membrane was consistently stirred using a magnetic stirrer. The nanoparticles represented a dosage of sacituzumab equivalent to two milligrams. The sacituzumab concentration was assessed by spectrophotometric analysis. At certain intervals of time (1, 2, 4, 8, 12, and 24 hours), 2-milliliter portions were extracted from the medium. The samples were substituted with an equal amount of recently prepared pH 7.4 phosphate buffers (Chowdhury et al., 2021).

4. Results:

Chitosan nanoparticles loaded by Sacituzumab are created using the ionotropic gelation method. This approach uses the electrostatic attraction among a positively charged polyelectrolyte polymer called chitosan and a negatively charged polyanion called tripoly phosphate. It also takes use of chitosan's capacity to form a gel and manage its interaction with tripoly phosphate. As a consequence, the solubility of chitosan in water is reduced. The study focused on investigating the creation of nanoparticles with a size below 250 nm by analyzing the influence of the Chitosan/tripoly phosphate ratio. The maximum concentration of Chitosan and tripolyphosphate is 8 mg/ml. The data is shown in a Table 1, which encompasses the measurement of the polydispersity index, zeta potential, and atom dimension. The findings exposed that the particle dimension was contingent upon the concentration of chitosan and tripolyphosphate, which is crucial for the production of nanoparticles with optimal efficacy (Kalyane et al., 2020).

4.1 Evaluation of Sacituzumab loaded nanoparticles:

4.1.1 Analysis of the distribution of particle sizes and calculation of the polydispersity index:

Table 1: Evaluation of Sacituzumab-loaded chitosan nanoparticles using the CS:PPT method.

Run	Drug (mg)	C.S.(%)	TPP (%)	Particle size (nm)	PDI (%)	Zeta potential (mV)
F_1	4	0.4	0.4	490±5	0.47±0.06	+3±6
F_2	4	0.6	0.6	380±2	0.33±0.03	+5±8
F_3	4	0.8	0.8	229±7	0.28±0.07	+6±2

F_4	4	0.4	0.4	1178±8	0.40±0.04	+3±1
F_5	4	0.6	0.6	1460±5	0.54±0.01	+4±3
F_6	4	0.8	0.8	1680±3	0.70±0.05	+6±6
F_7	4	0.4	0.4	1890±9	0.74±0.06	+3±5
F_8	4	0.6	0.6	2654±3	0.80±0.05	+4±7
F_9	4	0.8	0.8	2783±8	0.85±0.08	+6±3

4.1.2 Influence of sonication on the size of particles: Optimising the period of sonication is crucial in the creation of chitosan nanoparticles, as it is necessary for reducing the size of the particles. The highest quality nanoparticles, with a measured size of 229±7 nm, were achieved via 90 seconds of sonication. The chitosan molecules undergo fragmentation into smaller particles due to the significant shear stresses produced by the acoustic cavitations created during ultrasonication. As a result, the size of the particles is decreased. As the time of sonication extended from 30 to 90 seconds, the particle sizes were seen to decrease progressively. However, it was observed that the sonication time did not decrease beyond 90 seconds. This indicates that the ideal length for sonication to get the smallest nanoparticles is 60 seconds (Kalyane et al., 2022). The relationship between sonication duration and particle size is illustrated in Figure 1 using a bar graph.

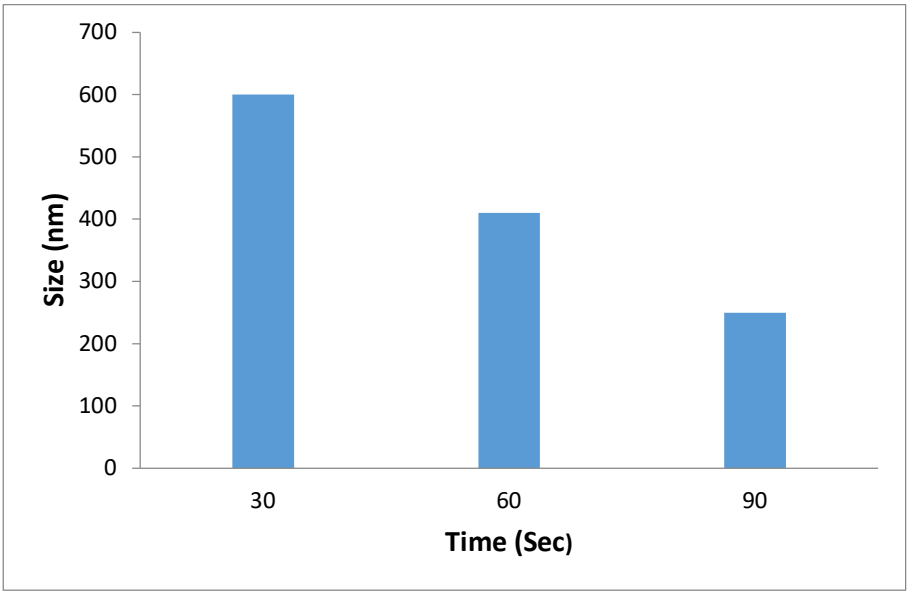

Figure 1: The impact of sonication on the particle size of nanoparticle loaded with Sacituzumab.

4.1.3 Characteristics of particles and their electric charge: Nine distinct formulations were generated by using varying amounts of tripoly phosphate and chitosan in a study aimed at producing chitosan nanoparticles. The particle sizes of the formulations ranged from 232 ± 4 to 2857 ± 6 nm. Increasing the concentration of chitosan resulted in a higher level of protonation of its amino groups, leading to a decrease in atom dimension and an elevation in zeta potential. This is observed. The optimal cross-linking and particle structural integrity were achieved by combining 0.8% chitosan with 0.4% tripoly phosphate, resulting in a commendable net charge. The heightened neutralisation of charged amino acids was the

underlying cause of this phenomenon. The Sacituzumab-loaded chitosan nanoparticles had excellent colloidal stability, as seen by Figures 2. The particle size in this optimal formulation (F3) was measured to be 229 ± 7 nm, with a zeta potential of 6 ± 2 mV (Tantra et al., 2010).

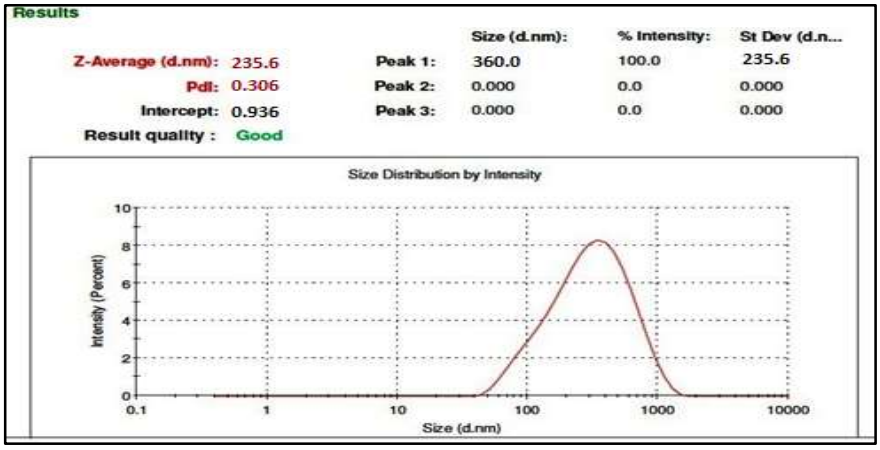

Figure 2: Particle size of Sacituzumab loaded nanoparticles

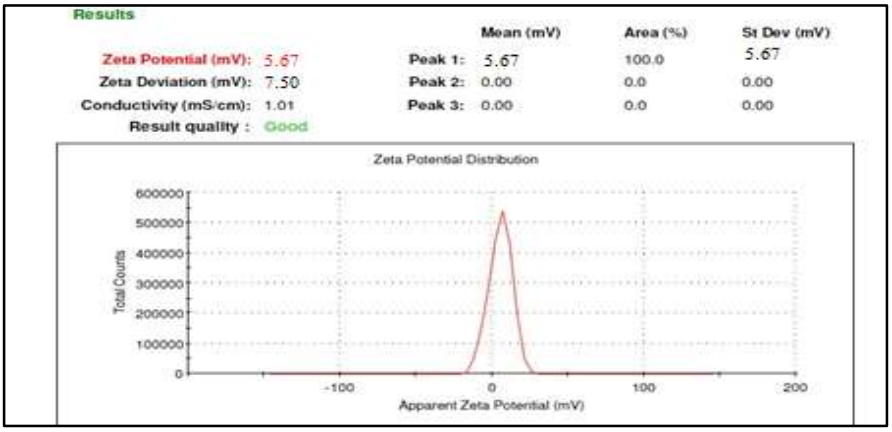

Figure 3: Zeta potential of Sacituzumab loaded nanoparticles

4.1.4 Field emission scanning electron microscopic (FESEM): On average, the nanoparticles containing Sacituzumab were around 100 nm in size and had a spherical or sub-spherical shape. The sacituzumab-loaded chitosan nanoparticles (F3) were obviously spherical in form and around 100 nm in size, as seen by the Field Emission Scanning Electron Microscopy (FESEM) pictures (Caldorera-Moore et al., 2010).

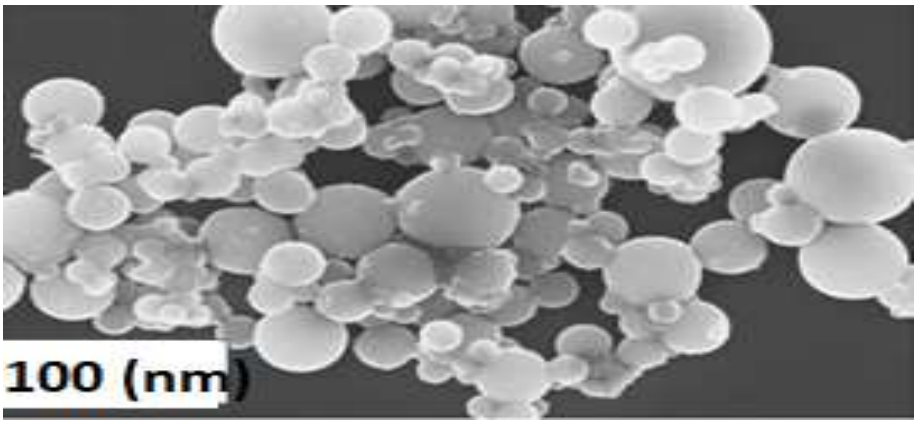

Figure 4: Field emission scanning electron microscopic of Sacituzumab loaded nanoparticles

4.1.5 Assessment of Transmission electron microscopy (TEM): The chitosan nanoparticles loaded with Sacituzumab had a mostly orbicular and sub-spherical exterior structure, through a particle dimension of about 100 nm. The shape of the optimised formulation (F3) was confirmed by the use of Transmission Electron Microscopy (TEM) pictures, which revealed the presence of nanoparticles measuring 100 nm in size, as seen in the accompanying figure 5.

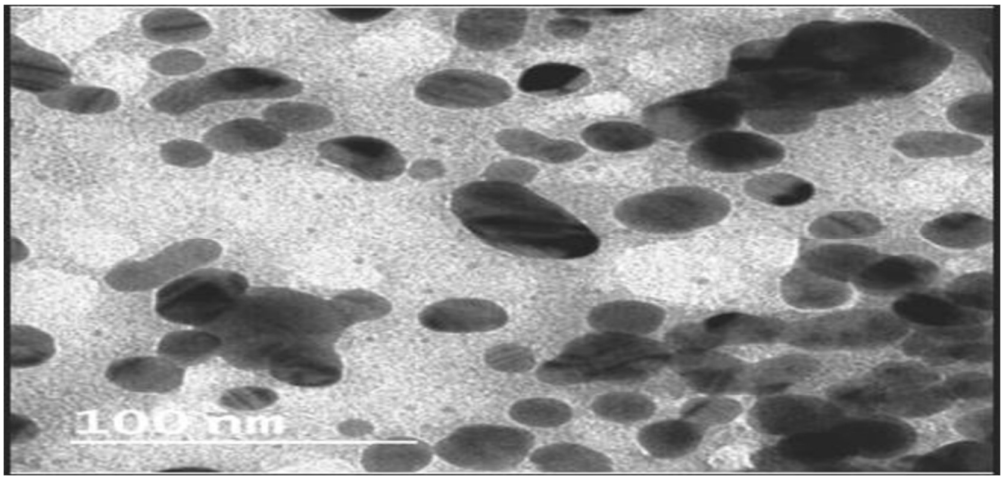

Figure 5: Transmission electron microscopy (TEM) of Sacituzumab loaded nanoparticles

4.1.6 Assessment of Autonomic force microscopy (AFM): An Atomic Force Microscope (AFM) will be used to examine the 3D surface characteristics and form of nanoparticles loaded with Sacituzumab. The 3D AFM images clearly demonstrate that the particles have a sub-spherical shape and are densely arranged, providing a comprehensive view of their surface structure (Jaiswal et al., 2015) (Rajabi et al., 2013).

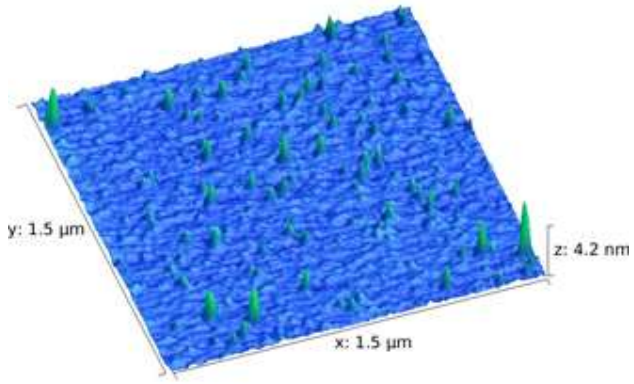

Figure 6: 3D Force microscopy of Sacituzumab loaded nanoparticles

4.1.7 Entrapment efficacy (EE) and Drug loaded content of Sacituzumab loaded nanoparticles: The sacituzumab-loaded chitosan nanoparticles demonstrated a trapping effectiveness ranging from 53.2 ± 1.5% to 68.5 ± 0.3%. The effectiveness of capture was enhanced by adjusting the amount of chitosan within the range of 0.4% to 0.8%, while keeping the content of tripoly phosphate fixed at 0.4%. Formulations F3 was selected as the optimal formulation for future studies based on its outstanding entrapment efficiency, favorable zeta potential, and appropriate particle size. The table 2 above displays the entrapment efficiency and drug loading content of Sacituzumab nanoparticles, along with the optimization parameters (Song et al., 2020).

Table 2: Entrapment efficacy (EE) and Drug loaded content of Sacituzumab loaded nanoparticles

Sr. No.	Run	Entrapment efficacy (EE)	Drug loaded content
1	F_1	64.9±1.7	60.3±1.8
2	F_2	66.4 ±1.4	62.7±0.9
3	F_3	68.5±0.3	78.3±1.4
4	F_4	62.3±0.6	64.9±1.6
5	F_5	61.7±1.4	68.3±1.8
6	F_6	59.1±0,6	71.4±1.3
7	F_7	53.2±1.5	72.8±1.5
8	F_8	54.1±2.1	76.6±0.6
9	F_9	53.9±0.6	79.4±0.5

4.1.8: DSC Examination: The DSC thermograms of Sacituzumab, chitosan, and their nanoparticles were acquired within the temperature range of 10-300°C. Chitosan had a glass transition at a temperature of 241.4°C, while sacituzumab showed two sharp increases in heat absorption at a temperature of 170°C. Following the freeze-drying process, the Sacituzumab endotherm exhibited a wide and diminutive profile in the final formulation, suggesting a shift from its crystalline structure to an amorphous state inside the chitosan matrix. In addition, the DSC thermogram of Sacituzumab showed a sharp increase in temperature at 215.56°C, which gradually dropped in strength and grew wider in the formulation. The transition from a crystalline to an amorphous or disordered crystalline phase is most likely caused by the assimilation and manipulation of the chitosan nanoparticles. The variations in the thermal

characteristics highlight the influence of nanoparticle formulation on the physical state and stability of medications.

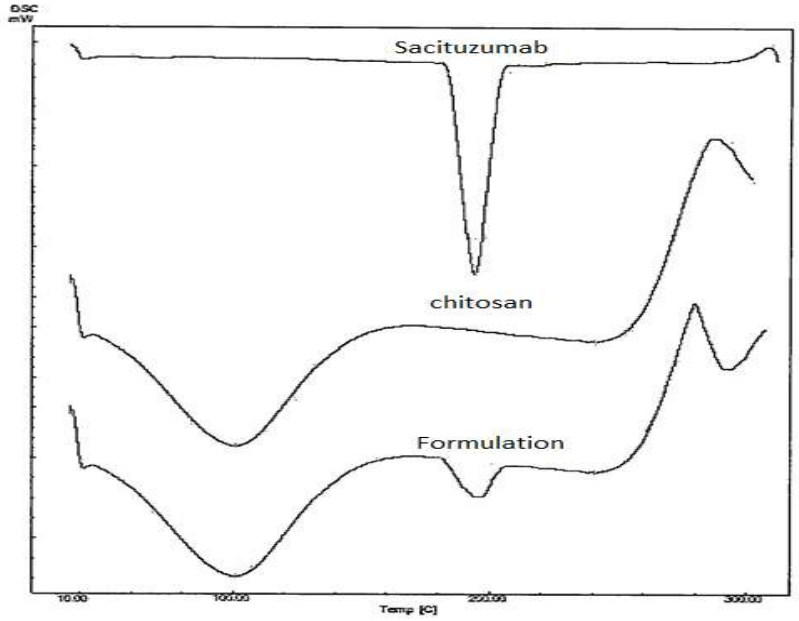

Figure 7: DSC Examination Sacituzumab loaded nanoparticles

4.1.9 An in-vitro investigation was conducted to analyse the release of Sacituzumab from nanoparticles: This study investigated the release profile of Sacituzumab-loaded chitosan NPs in a phosphate buffer with a pH of 7.4. When evaluating the percentage of medication discharge over time, all formulations (F1 to F9) exhibited a rapid initial release during the first two hours. Subsequently, there was a consistent and uninterrupted discharge that persisted for a whole day. Within a 24-hour period, about 98.2 ± 1.4% of sacituzumab from formulation F3 was eliminated. Like the chitosan nanoparticles, the release profile showed an initial rapid dissolution of twenty-five percent in the first hour, subsequent to a sustained release of 98% over the following 24 hours. The first explosion was caused by the detachment of drugs that had been attached loosely to the nanoparticle's surface, as seen in Table 3 and the figure that follows. The following continuous release, on the other hand, was a result of the progressive release of Sacituzumab that was encapsulated inside the chitosan nanoparticles (Hashemi & Kaykhaii, 2021) .

Table 3: Cumulative % drug release of Sacituzumab loaded nanoparticles

Time (hrs)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
0	0	0	0	0	0	0	0	0	0
1	19.5±0.6	26.8±1.6	21.3±0.7	20.7±1.6	27.9±1.8	26.2±0.4	22.6±0.5	27.2±1.1	23.6±1.9
2	26.7±1.5	32.9±1.0	32.8±1.9	29.2±0.9	36.8±0.5	33.3±1.3	30.1±0.4	38.4±`1.7	31.9±1.6
4	32.1±1.4	45.6±0.8	37.9±1.3	47.0±2.7	41.2±1.4	36.7±0.1	42.3±1.7	44.0±0.1	40.8±0.7
6	46.7±0.3	55.7±0.6	42.3±1.7	43.0±2.8	51.9±2.1	54.5±1.5	41.3±0.9	50.0±0.6	44.5±1.7
8	54.1±0.5	60.8±1.7	49.6±1.9	50.8±1.7	61.2±1.9	61.8±1.5	50.6±0.4	54.3±0.4	58.9±2.5
10	66.7±1.8	66.7±1.7	64.8±1.9	62.9±2.1	67.9±0.9	67.6±2.9	59.5±1.7	62.4±2.8	65.8±1.0
12	75.4±1.6	72.7±2.7	71.2±2.8	70.9±1.3	73.3±2.1	71.8±2.5	68.9±2.9	73.8±2.5	71.1±1.6

16	80.9±2.1	79.9±0.8	78.9±0.7	75.7±0.3	80.7±1.7	79.0±1.9	66.7±2.1	80.6±0.2	78.9±1.3
20	85.3±1.5	86.9±0.7	84.6±0.4	83.7±1.6	84.3±1.7	85.1±0.4	82.9±1.8	83.8±1.4	84.6±0.9
24	89.9±2.5	90.8±2.8	97.6±0.8	88.7±1.7	87.6±0.6	90.7±1.7	87.4±1.6	88.9±0.4	90.7±0.8

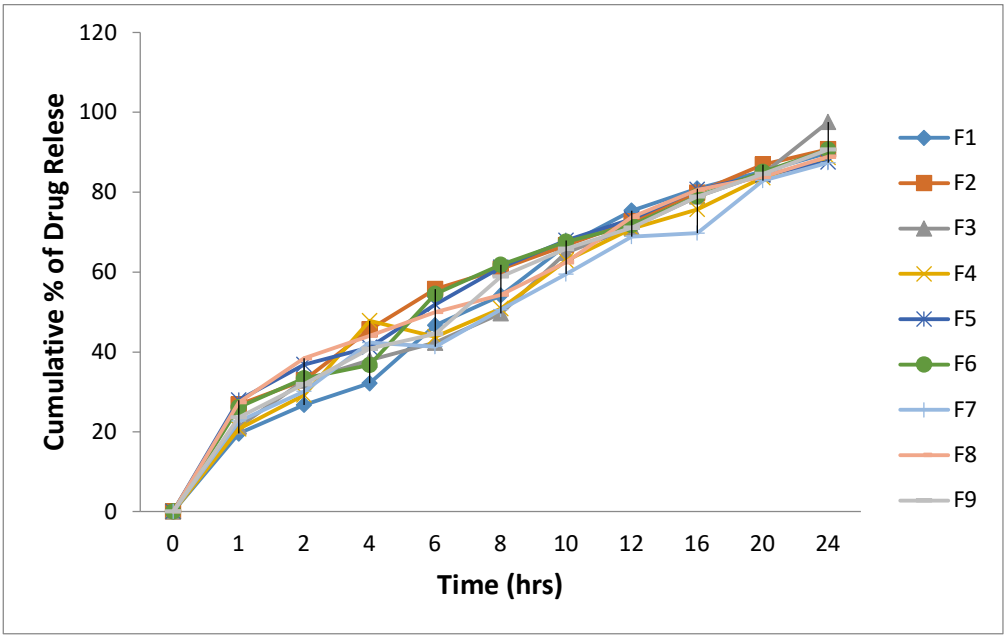

Figure 8: Cumulative % drug release of Sacituzumab loaded nanoparticles

5. Stability Study Sacituzumab loaded nanoparticles:

Over time, the formulation retained a nice physical look. Particle size was regularly evaluated during the 0-3 month storage period. The original particle size at the start of the experiment was 229±7, and after 3 months, the particle size increased to 248±6. The little rise in particle size may be ascribed to the feeble van der Waals force that holds the particles together, leading to the creation of soft agglomerates. The medication concentration remained constant at the six-month mark, indicating the absence of any drug leakage (Sallal et al., 2020). The assessment criteria are outlined in the following table 4:

Table 4: Stability Study of Sacituzumab loaded nanoparticles

Temperature	Evaluation parameters	Observation in Months			
		0	1	2	3
35±2^{0}C RH = 75±5%	Physical Appearance	White Colour	White Colour	White Colour	White Colour
	Particle size (nm)	229±7	233±5	238±4	248±6
	Drug Loading %	78.3±1.4	78.5±1.7	78.7±1.8	78.9±1.9

6. Discussion:

A thorough investigation of the production and characterization of Sacituzumab-loaded chitosan nanoparticles revealed significant insights into how formulation parameters affect

particle properties and drug delivery effectiveness. The findings indicate that a chitosan concentration of 0.8% was necessary to get the desired physicochemical characteristics and an ideal nanoparticle size (<250 nm). Formulation F3 exhibited the smallest particle size (229 ± 7 nm) and a stable zeta potential (+6 ± 2 mV).

The DSC thermal study revealed that Sacituzumab underwent a significant transition from a crystalline to an amorphous state when freeze-dried inside the chitosan matrix. This alteration may have improved the drug's capacity to be absorbed by the body and dissolved in a liquid. The nanoparticles' size was further decreased by the ideal sonication time of 90 seconds. The morphological examinations done with FESEM and TEM validated the nanoparticles' uniform size distribution and spherical shape, while AFM provided extensive information about their surface characteristics.

The in-vitro drug release trials exhibited a biphasic release pattern, typified by an early spike followed by a continual release. This is beneficial for maintaining optimal levels of medicinal drugs. Formulation F3 is the most favorable option for further investigation since it has a better ability to capture substances (68.5 ± 0.3%) and a large capacity to load drugs (78.3 ± 1.4%). Based on stability trials, the nanoparticles demonstrated consistent size, appearance, and drug loading efficacy over duration of three months under the specified testing circumstances.

The study highlights the crucial significance of adjusting formulation parameters to improve the effectiveness of medication administration and the properties of nanoparticles. This will enhance the use of sacituzumab-loaded chitosan nanoparticles in clinical settings with the specific aim of targeted cancer therapy.

Conclusion:

The ionotropic gelation method was used to create and refine chitosan nanoparticles containing Sacituzumab. The research achieved success in this aim. The use of the electrostatic interactions between tripoly phosphate and chitosan in this method resulted in the production of nanoparticles that had remarkable colloidal stability, efficient drug encapsulation, and regulated dimensions. The F3 formulation demonstrated the maximum entrapment effectiveness of 68.5±0.3%, a zeta potential of +6±2 mV, and a particle size of 229±7 nm. The most effective period for sonication was 90 seconds, which played a major role in decreasing the size of the particles. The uniform distribution and spherical shape of the nanoparticles were confirmed by morphological evaluations carried out using FESEM, TEM, and AFM. These assessments also provided additional evidence of the nanoparticles' structural integrity. The in-vitro drug release investigation showed that Sacituzumab had a sustained release pattern, characterised by an early spike followed by a constant release, culminating in a high of 98.2±1.4% over a 24-hour period. The nanoparticles' capability for long-term storage was shown by their ability to preserve their morphological appearance, particle size, and drug loading capacity over a three-month period, as evidenced by stability experiments. The Sacituzumab-loaded chitosan nanoparticles developed in this work exhibit favorable attributes for further investigation and prospective therapeutic use, highlighting their effectiveness in controlled drug delivery systems.

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