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Research

Formulation and Evaluation Topotecan Encapsulated Gum Ghatti Nanoparticles

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Abstract

Gum Ghatti (GG) nanoparticles containing Topotecan were the subject of this investigation to develop, optimize, and characterise them for use in cancer treatment. A solvent evaporation method involving aqueous and organic phases was used to make the nanoparticles. By adjusting various formulation and process parameters, the formulation was improved. Acetonitrile and phosphate buffer saline were used to develop the analytical method. To improve the nanoparticulate formulation, a variety of organic solvents and surfactants were evaluated. A Malvern zeta sizer was used to measure the size range and zeta potential. There were two distinct methods used for lyophilization, with a maximum drug entrapment rate of 35.2%. The dialysis method in phosphate buffer saline at a pH of 7.4 was used to evaluate the in vitro drug release of Topotecan nanoparticles. Over the course of 24 hours, the in vitro drug release demonstrated sustained drug release. As a result, GG nanoparticles loaded with Topotecan have the potential to serve as a drug delivery system. Due to their small size, they may also be able to reach extravascular target sites through the leaky endothelium of cancerous and inflamed regions, making them useful for site-specific drug delivery.

Keywords: Topotecan, Malvern zetasizer, phosphate buffer, Gum Ghatti, and the extravascular target endothelium are some of the ingredients.

INTRODUCTION

Nanoparticles are substances with sizes between one and one thousand nanometers. Oncology is the primary field in which these materials are utilized for the precise localization of cancer therapies and the early detection of malignancies with minimal adverse effects on somatic tissues. Drugs, vaccines, nutrients, and cosmetics are all protected by nanoparticles. By avoiding the reticuloendothelial system, utilizing enhanced permeability and retention effects, and specifically targeting tumours, they achieve site-specific drug delivery. Effective drug delivery depends on the formation of nanoparticles as well as their physicochemical parameters like pH, monomer concentration, ionic strength, surface charge, particle size, and molecular weight. Additionally, these nanoparticles have the potential to overcome multidrug resistance, a major issue in chemotherapy. They can be used to deliver therapeutic agents to a wide range of cellular targets thanks to their unique ability to access virtually

all cell types. Topotecan Hydrochloride Trihydrate works by stabilizing the cleavable complex in which topoisomerase I is covalently bound to DNA at a single-stranded break site. The anhydrous concentration of Topotecan Hydrochloride Trihydrate is at least 98.0 per cent. A DNA replication fork encounters these cleavable complexes, resulting in lethal DNA damage (fork collision model). Topotecan is a proton pump inhibitor that needs

MATERIALS AND METHODS

Zydus Research Centre in Ahmadabad provided the trihydrate topotecan chloride. Merck Specialities Pvt Ltd, Mumbai, supplied the acetonitrile and acetone. Direct Corporation, Birmingham Division, Pelham, supplied the poly(dl-lactide-co-glycolide) (50:50) material. Sigma Chemical Co., St. Louis, USA, supplied polyvinyl alcohol and polexamer 188. Canton Laboratories Pvt Ltd, located in Baroda, India, supplied sodium chloride and sulfate. The remaining chemicals were all analytical grade. The effects of stirring speeds of 400, 600, and 800 rpm on the preparation of nanoparticles were examined for various batches. The percentage of drug entrapment, the polydispersity index (PDI), and the particle size were evaluated.

RESULTS

Rate of Organic Phase Addition to the Aqueous Phase

The solvent's effect on the formulation will be shown by how quickly it is added. To accomplish this, the Effect of the Rate of Addition of Drug-Containing Solvent and Polymer to Aqueous Surfactant Solution: The aqueous surfactant solution was maintained at 0.25, 0.5, and 1 milliliters per minute for the solvent that contained the drug and polymer. Particle size, PDI, and the percentage of drug entrapment were examined concerning the solvent addition rate.

Formula Optimization and Solvent Selection (Organic Phase) The most frequently mentioned solvents for nanoparticle preparation are acetonitrile and acetone. In the beginning, the nanoparticulate system in the aqueous phase was used with both solvents in a 1:1 ratio while other parameters remained constant. Particle size, PDI, and the percentage of drug entrapment were evaluated by these solvents. The nanoparticles were produced in ACN after TUP was dissolved in the aqueous phase because it is insoluble in acetone.

Surfactant Selection PVA and poloxamer 188 concentrations ranging from 1% to 2% were used to prepare nanoparticles to maximize the concentration of the aqueous surfactant solution in TUP NP. All other parameters remained constant. Particle size, PDI, and the percentage of drug entrapment were examined.

Effect of Drug-to-Polymer Ratio on Formulation IRN NP was made with different drug-to-PLGA ratios (1:5, 1:10, and 1:20), while other parameters remained constant. While the amount of polymer varied, the amount of the drug remained constant. The percentage of drug entrapment, PDI, and particle size were evaluated.

Effect of Aqueous-to-Organic Phase Ratio on Formulation Three distinct formulation batches were used to optimize the aqueous-to-organic phase ratio for nanoparticle formation while maintaining other parameters constant. The optimal ratio was determined by evaluating the particle size, PDI, and percentage of drug entrapment.

Effect of Aqueous to Organic Phase Ratio on Formulation The ratio of aqueous to organic phase was optimized for nanoparticle formation by varying the amount of organic phase while maintaining the same amount of aqueous phase. For four distinct batches, the drug and PLGA were weighed and dissolved in 10 millilitres (1:1), 5 millilitres (1:2), 3.33 millilitres (1:3), and 2.5 milliliters (1:4) of acetonitrile, respectively. The percentage of drug entrapment, PDI, and particle size were evaluated.

Effects of Poloxamer 188 Concentration on the Formula TUP nanoparticles were made with Poloxamer 188 at various concentrations of 1%, 2%, 3%, and 4%, keeping other parameters constant, in order to maximize the concentration of the aqueous surfactant solution. Particle size, PDI, and the percentage of drug entrapment were studied in relation to Poloxamer 188.

Effects of Salt Addition on the Formula Two salts, sodium sulfate and sodium chloride, were used in varying amounts (2%, 1%, 0.5 percent, and 0.1 percent). Together with the surfactant, these salts were added to the aqueous phase. The effects of salt addition on the particle size, PDI, and percentage of drug entrapment of TUP nanoparticles were examined.

Characterization of TUP Nanoparticles A portion of TUP nanoparticle dispersion was added to CAN and thoroughly sonicated to completely dissolve the nanoparticles. Using a UV-visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan), the absorbance of the solution was measured at a maximum wavelength of 256 nm. The following formula was used to determine the percentage of drug entrapment: Measurement of Particle Size and Zeta Potential The Dynamic Light Scattering method was used to measure the mean particle size, polydispersity index, and zeta potential of the prepared TUP nanoparticles. Drug entrapment (%w/w) = Amount of drug in nanoparticlesAmount of drug in nanoparticlesAmount of drug in nanoparticlesAmount of drug in nanoparticlesAmount of drug in nanoparticlesAmount of drug in nano The cuvette containing the dispersion was then filled, and it was put into the Zetasizer (Nano 25, Malvern, UK). The analysis was carried out with a 90°

detection angle at 25°C. The sum of three measurements makes up each value that is reported. Particle size, PDI, and zeta potential were all measured in triplicate for each measurement.

The TUP Nanoparticles were lyophilized using sucrose and trehalose, two different cryoprotectants, in varying proportions of solid content to cryoprotectant. The ratios of total solid content to cryoprotectant were chosen as 1:3, 1:5, and 1:7 in weight-to-weight terms. The vials were lyophilized for approximately 36 hours with a lyophilizer (Vertis Advantage, USA) after the cryoprotectants were dissolved in the TUP nanoparticle dispersion according to the various ratios. The vials were immediately removed and sealed following lyophilization. Particle size and PDI were measured with the Zetasizer (Nano ZS, UK) after the lyophilized vials were reconstituted with 3 ml of DM water and bath sonicated for two minutes.

Description of the TUP Nanoparticles' In Vitro Drug Release Process The dialysis method was used in the TUP nanoparticles' in vitro drug release study. To put it succinctly, a dialysis tube with a molecular weight cutoff of 12,000 (Sigma Aldrich, Mumbai) contained 1.5 mg of TUP nanoparticle dispersion. A 500 ml beaker containing pH 7.4 phosphate-buffered saline (PBS) was used to seal the tube. The temperature of the buffer medium was kept at 37 2 °C while it was stirred at a speed of 100 rpm. Three milliliter (ml) samples were taken from the medium at predetermined intervals, and the medium was refilled with the same amount of new buffer. Using a UV-visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan), the calibration curve of TUP in PBS pH 7.4 was used to calculate the percent cumulative drug release.

DISCUSSIONS

Magnetic Stirrer's Speed

For the purpose of making TUP nanoparticles (NP), the mechanical stirrer's stirring speed was varied to 400, 600, and 800 rpm. Table 1 depicts the evaluation of the effects of stirring speed on particle size, PDI, and the percentage of drug entrapment. The water miscibility of the solvent is an important factor in the preparation of nanosuspension. Acetonitrile's high water solubility enables rapid diffusion into the aqueous phase from dispersed droplets at 600 rpm. As a result, during emulsion dilution, when the dispersed phase comes into contact with a significant amount of the aqueous phase, the organic solvent quickly diffuses, resulting in rapid drug precipitation and particle formation. The results confirm that there is less particle aggregation at higher speeds (800 rpm), but the observed PDI was higher than that of the batch prepared at 600 rpm, which had the same particle size but a lower PDI. Additionally, aggregation was observed at 400 rpm, resulting in a larger particle size. As a result, the stirring rate was increased to 600 rpm.

Effect of Organic Phase Addition Rate on Aqueous Phase The rates at which the drug and polymer-containing solvent were added to the aqueous surfactant solution were 0.25, 0.5, and 1 milliliters per minute, respectively. The results of a study on the effect of solvent addition speed on the preparation of nanoparticles are presented in Table 2. The rate at which the organic phase is added to the aqueous phase is what determines the formation of nanoparticles. The solvent's effect on the formulation is demonstrated by the rate at which it is added. The optimal particle size and lower PDI were achieved when the organic phase was added at a rate of 0.5 ml/min, as shown in Table 2. In addition, compared to other batches, the percentage of drug entrapment was higher. As a result, the speed at which the organic phase should be added to the aqueous phase was found to be 0.5 ml/min.

Solvent Selection The drug's solubility in various solvents is an essential step in developing a nanoparticle drug delivery system. Selecting a solvent in which the drug is maximally soluble before making nanoparticles is essential for the nanoparticle drug delivery system. Different solvents like CAN, DMSO, DMF, and ethyl acetic acid derivation were utilized for the planning of PLGA nanoparticles. For the formation of PLGA nanoparticles, acetone and acetonitrile, which are frequently mentioned in the literature, were utilized. Acetonitrile-prepared TUP nanoparticles had a higher percentage of drug entrapment and smaller particle sizes than acetone-prepared nanoparticles, as shown in Table 3. Acetone decreased drug entrapment because the drug dissolved in the aqueous phase during TUP nanoparticle preparation. As a result, acetonitrile was chosen as the preferred solvent for the preparation of nanoparticles.

Surfactant Selection PVA and Poloxamer 188 were used to make nanoparticles at concentrations ranging from one percent to two percent, with other parameters remaining unchanged, in order to maximize the concentration of the aqueous surfactant solution. Table 4 depicts the outcomes. It is essential for the development of a nanoparticle drug delivery system that the drug remains stable in a variety of surfactants. The right surfactant must be chosen before making nanoparticles. Molecules of surfactant keep emulsion nanodroplets stable and prevent them from joining together. Surfactant molecules must cover every droplet's organic/aqueous interfacial area for stabilization to work. In order to achieve a narrow size distribution and small particle size, a minimum number of surfactant molecules is required. Upon solvent evaporation, batches made with PVA exhibited aggregation, as shown in Table 4. Consequently, the preferred surfactant for the preparation of nanoparticles was 2 per cent of Poloxamer 188.

Drug: Polymer (D) Proportion

The solvent evaporation method was used to make (1:5, 1:10, and 1:20). The results are presented in Table 5, which show that the amount of polymer varied while the amount of the drug remained constant. Particle size increased and drug entrapment decreased as the drug-to-polymer ratio was decreased, according to observations. The ideal particle size and percentage of drug entrapment were observed at a polymer-to-polymer ratio of 1:10. As a result, the ratio of the drug to the polymer was chosen to be 1:10.

Aqueous

Organic Phase Ratio Particle size and drug entrapment percentage were measured by varying the aqueous to organic phase ratio. Table 6 depicts the outcomes. According to the findings, the particle size and PDI of TUP nanoparticles increased when the volume of the organic phase was decreased. Additionally, the percentage of drug entrapment decreased. However, changing the proportion of the organic phase to the aqueous phase did not affect the percentage of drug entrapment. Particle size decreased with low PDI at a ratio of 1:2. As a result, the ratio of aqueous to organic phase was chosen to be 1:2.

The concentration of Poloxamer 188 Nanoparticles was made using Poloxamer 188 at concentrations of 1%, 2%, 3%, and 4% while remaining constant in other parameters to maximize the concentration of the aqueous surfactant solution. Table 7 provides a summary of the findings. The mean diameter of the nanoparticles also increased as the concentration of Poloxamer 188 increased. As can be seen in Table 7, an increase in the concentration of Poloxamer 188 was observed... If you require more information about the effects of concentration, please let me know.)

The concentration of Poloxamer 188 Although increasing the concentration of Poloxamer 188 did not significantly alter the particle size, it did alter the percentage of drug entrapment and the PDI. When compared to Poloxamer 188 at a concentration of 2%, Poloxamer 188 at concentrations of 3% and 4% had no effect. Consequently, the concentration of Poloxamer 188 chosen was 2%.

Salt Addition Two salts—sodium sulfate and sodium chloride were used at varying concentrations of 2%, 1%, 0.5 per cent, and 0.1 per cent to increase the percentage of drug entrapment. Table 8 displays the obtained results. At concentrations of 0.1 per cent, both salts increased particle size and reduced drug entrapment; however, nanoprecipitation and the formation of TUP nanoparticles were not induced at concentrations of 1% and 2%. As a result, using NaCl and sodium sulfate to maintain a particle size close to 200 nm did not affect drug entrapment. As a result, no salts were incorporated into the formulation.

TUP NP's In Vitro Release Profile Table 9 and Figure 1 depict TUP NP's in vitro release pattern. Over the course of 24 hours, TUP NP's drug release profile revealed sustained drug release. At four hours, an initial burst release of 20.59 per cent was observed, followed by a sustained release. The outcomes were comparable to those of other drugs that dissolve in water and are entrapped in PLGA nanoparticles.

Lyophilization of TUP NP The following data were obtained after distilled water was used to reconstitute lyophilized TUP NP containing various ratios of cryoprotectants. After reconstitution, the particle size of trehalose as a cryoprotectant at a ratio of 1:3 was close to the initial size. Particle size and PDI were not significantly altered by trehalose at higher ratios (1:5 and 1:7). Compared to other ratios of trehalose and sucrose, it was discovered that trehalose at a ratio of 1:3 displayed a cryoprotective behaviour that was comparatively superior. Biomolecules appear to prefer trehalose as a cryoprotectant. It has lower hygroscopicity, no internal hydrogen bonds, which makes it easier to form hydrogen bonds with nanoparticles during freeze-drying, very low chemical reactivity, and a higher glass transition temperature (Tg) than other sugars.

Particle Size and Zeta Potential Measurement A zeta sizer (Nano ZS, Malvern, UK) was used to measure the particle size and zeta potential. The zeta potential indicates the potential physical stability of nanoparticle dispersions and the total charge that the particles acquire in a particular medium. The system is considered stable if all of the particles have a significant positive or negative zeta potential. As a result, they will repel one another. The system is more stable the higher the value. Particle size and zeta potential are depicted in Figures 2 and 3, respectively. The zeta potential that was obtained was -13.3 mV.

Table 1: characters of actual security

Appearance	Transparent quantifiable
Odor	odourless
Colour	White
Solubility	9.74±0.37mg/ml
Melting point	41-44ºC
Absorbance	207 nm

Table 2: Rotation per min

S. No	Rotation per min	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	600	215.9±10.8	0.16±0.03	32
2	800	220.4±12.6	0.11±0.03	33

Table 3: Rate of addition

S. No	Rate of addition	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	0.25 ml/min	309.6±10.8	0.27±0.20	28
2	0.50 ml/min	213.0±9.0	0.11±0.23	36.4
3	1 ml/min	246.9±1.0	0.19±0.24	23

Table 4: Effect of solvent

S. No	Effect of solvent	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	Acetone	337.1±9	0.21±0.01	11
2	Acetonitrile	224.2±1.3	0.11±0.01	34

Table 5: Surfactant

S. No	Surfactant	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	1% PVA	-	-	-
2	2% PVA	-	-	-
3	1% Poloxamer 188	445±11.4	0.343±0.010	25.5
4	2% Poloxamer 188	219±10.2	0.109±0.013	33

Table 5: Drug polymer ratio

S. No	Drug polymer ratio	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	1:5	191.3±8.5	0.27±0.013	8.46
2	1:10	224.1±8.3	0.07±0.018	39.2
3	1:20	324.2±9.7	0.11±0.010	6.2

Table 6: Polymer

polymer	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1%	292.4±9.2	0.372±0.014	16.62
2%	221.3±10.6	0.172±0.010	35.64
3%	239.4±13.7	0.297±0.016	27.56
1%	292.4±9.2	0.372±0.014	16.62

Table 7: Aqueous: organic

Aqueous: organic	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1:1	-	-	-
1:2	232.1±4.2	0.111±0.030	32.4
1:3	268.7±10.2	0.116±0.025	31.7
1:4	281.8±11.7	0.173±0.021	30.7

Table 2: Poloxamer 188 conc

Poloxamer 188 conc	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1%	292.4±9.2	0.372±0.014	16.62
2%	221.3±10.6	0.172±0.010	35.64
3%	239.4±13.7	0.297±0.016	27.56

Table 8: Salt

Salt	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
Sodium sulfate 2%	-	-	-
Sodium sulfate 1%	-	-	-
Sodium sulfate 0.1%	247.9±20.3	0.397±0.012	23.87
Sodium chloride 2%	-	-	-
Sodium chloride 0.1%	232.5±17.8	0.301±0.013	14.47
Sodium chloride 2%	-	-	-

Table 9: In vitro drug release

S. No	Time	Percentage
1	15 Mins	0±0.0
2	30 Mins	0.46±0.01
3	45 Mins	1.6±0.01
4	60 Mins	2.9±0.1
5	2 Hrs	8.7±0.2
6	4 Hrs	17.3±0.4
7	8 Hrs	35.8±0.4
8	12 Hrs	60.5±0.2
9	24 Hrs	84.8±0.7

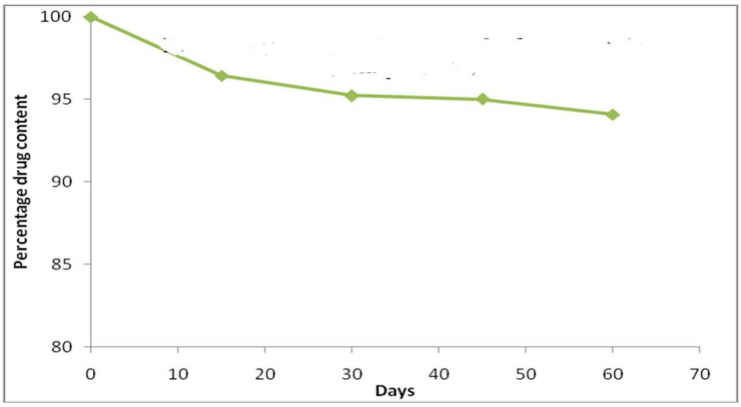

Fig 1: Prepared ITC 5 ºC ± 3 ºC (Long-term stability)

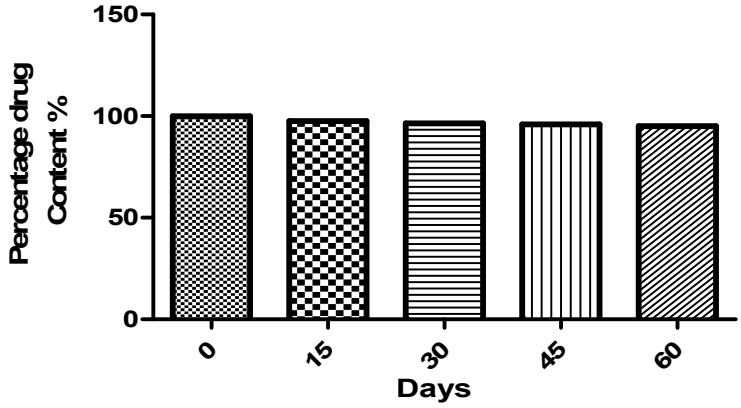

Fig 2: Proportion drug release prepared ITC 5ºC±3 ºC

SUMMARY AND CONCLUSION

The application of chemical and biological principles to control the temporal and spatial location of drug molecules in the body for therapeutic benefit is the definition of the science of drug delivery. Increasing drug efficacy and decreasing drug toxicity are typically the goals of targeted systems. By optimizing how much and how long the drug is delivered to the target cells, this can be accomplished. To get the most out of a drug's therapeutic effect, the right delivery system needs to be developed. For drug targeting, there are numerous options; However, there are still obstacles in the way of creating a complete target drug delivery system. Consequently, the industry is now concentrating on combining nanotechnology with therapeutic molecules for various diseases and miniaturizing drug delivery devices from the microscale to the nanoscale. By increasing the bioavailability, solubility, and retention time of therapeutic drugs and bioactive molecules, biodegradable nanoparticles are frequently utilized. In most cases, a hit-and-run approach to controlled release, targeted delivery, and therapeutic impact is used to develop desired nanomedicines. The development of novel drug delivery systems and potential therapeutic interests are the primary areas of focus for pharmaceutical companies' scientific efforts. They deal with new ways to put existing drug molecules into new ways that can make them work better and have less toxicity. Several conventional formulations are currently available for purchase. Innovative concepts in nanoscience and nanotechnology have emerged as a result of extensive scientific advancements, presenting several brand-new formulation development opportunities and addressing issues associated with conventional dosage forms. The purpose of this study was to investigate the potential of ITC in various nanoparticle types. Additionally, it investigated the formulation variables and physical properties that influence GGITC's stability and efficacy. Three distinct kinds of nanoparticles were prepared to accomplish this goal: ionic gelation for chitosan-based polymeric nanoparticles, emulsification-sonication-solvent evaporation for GG-based nanoparticles, and hot homogenization for solid lipid nanoparticles are all methods. The properties of nanoparticles that have not been studied can be investigated. To make the parameters suitable for biomedical applications, they need to be improved. Drug delivery system that works safely and effectively This work will be helpful in the development of additional nanoparticles for the treatment of cancer growth.

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