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FORMULATION AND EVALUATION OF BUSULFAN POLYMERIC NANOPARTICLES

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Abstract

In present work Nano particulate drug delivery system was developed for Busulfan an anticancer drug by using by ionic gelation method. Busulfan nanoparticles were prepared by ionic cross linking of Chitosan solution in various concentrations with Tri poly phosphate (TPP) anions. The pre-formulation studies and drug-excipient compatibility studies were determined and the results were found to be satisfactory. The Busulfan nanoparticles were evaluated for its drug content, entrapment efficiency, particle size, zeta potential and *in vitro* drug release. From the evaluation results, BNP5 was selected as best formulation due to its ideal particle size (405.71nm), Zeta potential (23.1mV), high entrapment efficiency (85.28%) and desirable drug release 99.82% at the end of 24 h. Hence it may be concluded that the newly formulated Busulfan nanoparticles may be ideal and effective in the management of Cancer by allowing the drug to release continuously for 24 hrs.

Keywords: Chitosan, *In-Vitro* drug release, TPP, Tween80

Introduction

Nano particulate drug delivery systems have been widely investigated as ideal drug delivery systems to increase the potency and therapeutic efficacy of drugs and reduce their side effects. As the Nanoparticles have a large surface area to volume ratio, they could encapsulate high volume of drugs and increase drug delivery to cancerous cells [1]. These nanoparticles are also considered as excellent drug delivery systems, due to isolating the chemotherapeutic compounds from the systemic environment and targeted accumulation of the compounds in solid tumours with leaky vasculature and impaired lymphatic's, resulting in improved cellular uptake. In addition, nanoparticles are able to increase the water solubility of small insoluble molecules, leading to improving their solubility and bioavailability. Chitosan polymeric nanoparticles have been broadly used for drug delivery purposes which are biodegradable particles and employed a vital role in the alteration of pharmacokinetics of therapeutic agents in the blood. In addition to this, Chitosan polymeric nanoparticles take advantage of easy method of preparation and purification processes [2]. These features make the Chitosan polymeric nanoparticles as a suitable carrier for controlled drug delivery of many drugs. The present study aimed to use Chitosan polymeric nanoparticles as carrier for Busulfan delivery [3]. For this purpose, the ideal concentration of Chitosan as the Busulfan carrier was evaluated for its ideal particle size, zeta-potential, entrapment efficiency and in vitro drug release which may be very helpful in the treatment and management of cancer [4].

Materials and methods

Materials

Busulfan, Chitosan and tween80 were purchased from Sigma Aldrich Pvt. Ltd and tween 80, acetic acid and Tri-polyphosphate from Hi-media Laboratories Pvt. Ltd. All the other chemicals used for this study are of analytical grade.

Pre-formulation studies

Preparation of Busulfan standard calibration curve in pH 1.2, pH 7.4 and pH 6.8 buffer solutions.

An accurately weighed amount of Busulfan 100mg was dissolved in small volume of buffer solutions in each of three 100ml volumetric flask and the volume was adjusted to 100ml with buffer solutions [5]. A series of

standard solution containing in the concentration range from 10 to 50 µg/ml of Busulfan were prepared for pH 1.2, pH 7.4 and pH 6.8 buffer solutions separately [6]. The absorbance was measured at 260 nm and calibration graph was plotted using concentration versus absorbance [7,8].

Drug-excipient compatibility study by Differential scanning calorimetry (DSC)

Samples of individual compounds as well as each drug-excipient were weighed (Mettle Electronic Balance) directly in pierced aluminum crucible pans (5-10mg) and scanned in the 50-300˚C temperature range under static air, with heating rate of 10^0C/min, using shimadzu DSC-60 equipment[9,10].

Method of preparation

Preparation of Busulfan nanoparticles by ionic gelation method

Busulfan nanoparticles were prepared by ionic cross linking of Chitosan solution with Tri poly phosphate (TPP) anions. Chitosan at various concentrations such as **50-250 mg (BNP1-BNP5)** were dissolved in 6% v/v solution of acetic acid in water and subjected to magnetic stirring at room temperature.5ml of 0.25% w/v TPP aqueous solution was added drop wise into 10ml Chitosan solution containing 10mg of Busulfan dissolved in tween 80.

The solution was stirred for about 20 min which results in the formation of suspension of Busulfan nanoparticles. The resultant Busulfan nanoparticle suspensions were centrifuged to separate the Busulfan nanoparticles.

Table1. Formula used for the preparation of Busulfan nanoparticles

S.NO	FORMULATION	DRUG (mg)	CHITOSAN (mg)	Drug/Polymer ratio
1.	BNP1	10	50	1:5
2.	BNP2	10	100	1:10
3.	BNP3	10	150	1:15
4.	BNP4	10	200	1:20
5.	BNP5	10	250	1:25

Characterization studies

Drug content

1gm of Busulfan nanoparticles were accurately weighed and transferred into a 25ml volumetric standard flask. The sample was dissolved with pH 6.8 phosphate buffer solution. 1ml of this solution was diluted to 25ml with the same buffer solution [11].

The standard Busulfan was dissolved and diluted with same buffer solution. Then the standard and sample absorbance was measured at 260 nm using UV-Visible spectrophotometer [12]. The percentage of drug contact was calculated.

Particle size and Surface charge:

The prepared Busulfan nanoparticles were evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS) using Zeta seizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per seconds (Kcps). Analysis was carried out at 25˚C with an angle of detection of 90˚.

Entrapment efficiency

The Busulfan loaded chitosan nanoparticles in buffer solutions were subjected to centrifugation at 15000 rpm for 30 minutes. The supernatant liquid was separated and 1ml of this solution was diluted with buffer solution and the absorbance was measured at 260 nm.

The amount of Busulfan unentrapped in the supernatant was calculated. The amount of Busulfan entrapped was determined by subtracting amount of free unentrapped Busulfan from the total amount of Busulfan taken for preparation [13].

The formula used to calculate entrapment efficiency was given below

$$\text{Drug entrapment}(\%) = \frac{\text{mass of drug in nanoparticles x100}}{\text{mass of drug used in formulation}}$$

In Vitro release

In vitro release studies were performed for 24h using dialysis membrane by using the Franz diffusion cell. The prepared Busulfan nanoparticles (equivalent to 2mg of Busulfan) were placed inside a dialysis membrane and immersed in pH 6.8 phosphate buffer.

At predetermined time intervals the sample was withdrawn and the amount of Busulfan release was determined by measuring the absorbance at 260 nm using a UV visible spectrophotometer [14,15]. From the absorbance values the cumulative percentage drug release was calculated.

Results and discussion

Pre-formulation studies

Preparation of calibration graph for Busulfan [16-18]

Standard calibration data of Busulfan in pH 1.2, 7.2 and 6.8 buffers at 260 nm . Standard calibration curve of Busulfan was carried out in 1.2 pH, 7.2 pH and 6.8 pH buffer at 260 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

Table 2. Absorbance of Busulfan in buffer solutions

S.No	Concentration	Absorbance		
		pH 1.2	pH 7.2	pH 6.8
1	0	0	0	0
2	10	0.020	0.049	0.100
3	20	0.040	0.100	0.201
4	30	0.060	0.149	0.302
5	40	0.082	0.201	0.402
6	50	0.100	0.250	0.503

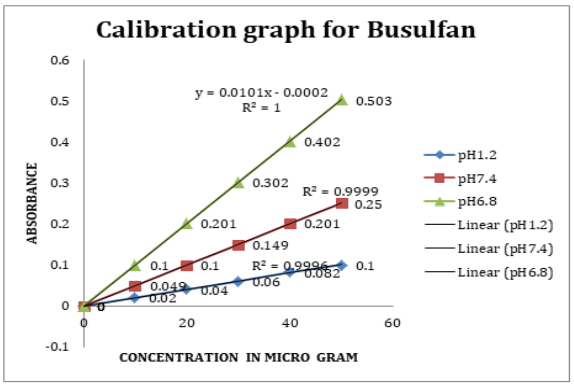

Fig.1. Calibration curve of Busulfan in pH 1.2, 7.2 and 6.8 buffers

DSC analysis

DSC of Busulfan showed a sharp endothermic peak at about 117.13^{0}C (melting point). The physical mixture of Busulfan with other excipients also showed the same thermal behavior (116^{0}C) as the individual component. DSC results also revealed that the physical mixture of Busulfan with excipients showed superimposition of the thermo-gram [19]. There was no significant change observed in melting endotherm of physical mixture of Busulfan and excipients. Hence from the DSC study, it was found that there was no interaction between Busulfan and other excipients used in the formulation.

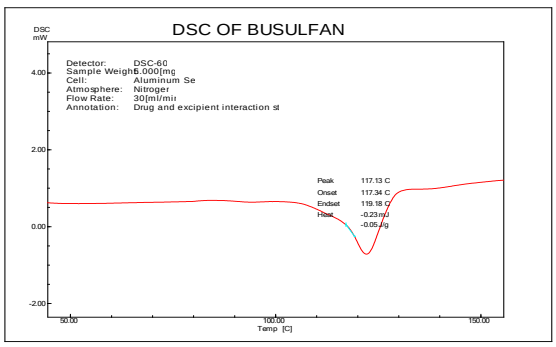

Fig.2

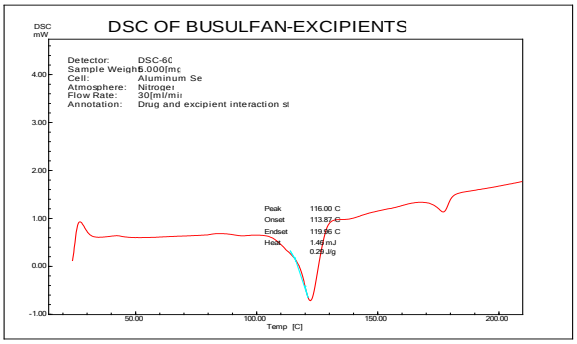

Fig.3

Drug –Excipients accelerated compatibility study - Physical observation and assay

Upon analysis of the drug excipient mixture for their physical characteristics no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients.

Table 3. Physical characteristics of Busulfan

S.No	Physical parameters	Results
1	Description	White crystalline powder
2	Melting point	117°C
3	Loss on drying	0.08%
4	Assay	99.82%

Table 4. Physical characteristics of individual drug and excipients

S.No	Sample ID	Initial description	Final description
1.	Busulfan	White crystalline powder	No change
2.	Chitosan	Off white powder	No change

Table 5. Physical characteristics of drug-excipient mixture

S.No	Sample ID	Initial description	Final description
1	Busulfan	White crystalline powder	No change
2	Busulfan+ Chitosan	Almost white coloured powder	No change

Table 6.Drug content and entrapment efficiency Particle size and zeta potential of Busulfan nanoparticles.

Trials	Zeta potential (mV)	Particle size (nm)	Entrapment Efficiency (%)	Drug Content (%)
BNP1	16.8	400.12	45.71	99.81
BNP2	17.4	401.27	52.37	99.82
BNP3	20.6	403.32	61.35	99.81
BNP4	21.4	404.18	65.86	99.81
BNP5	**23.1**	**405.71**	**85.28**	**99.82**

Particle size, Zeta potential and entrapment efficiency of the Busulfan nanoparticles (BNP1- BNP5) were increased with increasing Chitosan concentration (50-250mg). There were no significant differences in the particle size of all five formulations. Formulation which contains 250mg of chitosan (BNP5) shows excellent entrapment efficiency (85.28%) when compared other trials (BNP1- BNP4).This may be due to high amount of availability of Chitosan to encapsulate the drug, upon increasing the Chitosan concentration, number of layers coated the drug was increased, this resulted in increased particle size and entrapment efficiency.

In- vitro drug release

Table 7. *In vitro* release studies of Busulfan nanoparticles

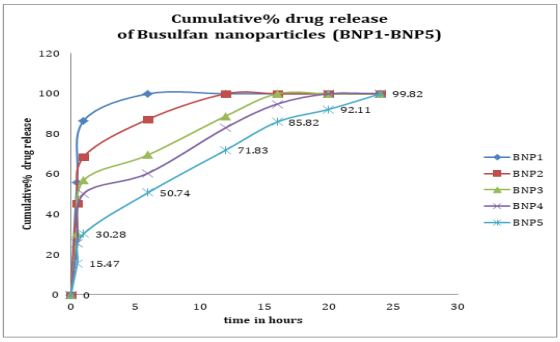

Fig.4

From the *in vitro* drug release study results, the maximum percentage drug release 99.82% at the end of 24hwas observed with trial BNP5 which contains 250mg of Chitosan. Below 250mg of Chitosan as in the case of trials BNP1-BNP4, the drug release were fast and the maximum percentage drug release were obtained within 6,12,16 and 20hrs which may be due to the insufficient concentration of Chitosan to control the release of drug from the prepared nanoparticles. Hence these (BNP1-BNP4) formulations were considered as undesirable for controlled drug release of Busulfan. Above 200mg concentration of Chitosan, the drug release was found to be controlled due to the availability of sufficient concentration of polymer Chitosan to control the drug release [20]. The maximum and ideal controlled drug release was obtained with BNP5 and it was found to be 99.82% at the end of 24h which contains 250mg of Chitosan. Hence 250mg of Chitosan was selected as optimized concentration for the preparation of Busulfan nanoparticles by ionic gelation method. From the *in vitro* drug release data for BNP1-BNP5, it was observed that increase in Chitosan concentration delays the drug release due to increased particle size and reduced surface area of the prepared nanoparticles. From all the formulations, **BNP5** was selected as best formulation due to its ideal particle size **(405.71nm)**, Zeta potential (**23.1mV**), high entrapment efficiency (**85.28%**) and desirable drug release **99.82%** at the end of 24 h.

SUMMARY AND CONCLUSIONS

In the present study Chitosan nanoparticles containing Busulfan were prepared. Chitosan concentrations were optimized by conducting various trials. The effect of increase or decrease in Chitosan concentration on various parameters like Particle size, Zeta potential, Entrapment efficiency, Drug content and *In-vitro* release profile were studied. Based on the results of drug content, entrapment efficiency, particle size, zeta potential and desired *in vitro* drug release profile of prepared Busulfan nanoparticles **(BNP1- BNP5)**, formulation **BNP5** was selected as the best formulation. The *in vitro* % drug release of **BNP5** formulation was **99.82%** and it was found to be suitable formulation to manage the condition of Cancer. Hence it can be concluded that the newly formulated Busulfan nanoparticles may be ideal and effective in the management of Cancer and antiplatelet action [21] by allowing the drug to release continuously for 24 hrs.

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