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Research Article

Evaluation of change in pH, Osmolarity upon change in concentration of mannitol and sodium acetate anhydrous at fixed concentration of gemcitabine hydrochloride

Sreedhar Bandari *[1], Seshasai Marella[2]

[1]Research & Development cell, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally-500085, Hyderabad, Andhra Pradesh, India
[2]Department of Formulation research & development, Hospira Healthcare India Pvt.Ltd, Plot No. B3, SIPCOT, Industrial Park, Irungattukottai, Sriperumbudur (T.K)-602105, Tamil Nadu, India

ABSTRACT

The experimental objective was to evaluate change in pH, Osmolarity upon change in concentration of Mannitol and Sodium acetate anhydrous at fixed concentration of Gemcitabine hydrochloride. So the experiment was designed by varying the concentrations of two excipients Mannitol and sodium acetate anhydrous. A model was generated using Quadratic modeling and CCF design with eight runs in design and three centre points. The innovator product/ reference list drug composition corresponds to 45.5408 mg/ml of Gemcitabine Hydrochloride equivalent to 40 mg/ml of Gemcitabine, 40.0 mg/ml of Mannitol and 2.5 mg/ml of Sodium Acetate anhydrous. The low and the high levels of each factor were specified in relation to the reference list drug composition. It was decided to vary Sodium Acetate anhydrous concentration from 0 to 2.5 mg/ml and Mannitol from 30 to 50 mg/ml keeping the concentration of Gemcitabine Hydrochloride constant. Then a standard experimental plan with eleven experiments was created. For each of the eleven experiments Osmolarity, pH of the solution was measured. As the drug product is intended for intravenous administration Osmolarity and pH of the solution play a vital role. So the drug product is designed to have comparable Osmolarity as that of RLD (Gemzar), in the pH range of 2.7 to 3.3. Based on the experimental results the Osmolarity of solution could be 510 mOsm/kg ±10% (Range 460 mOsm/kg to 560 mOsm/kg). Change in concentration of Mannitol did not result in significant change in pH of the solution. Whereas the increase in concentration of sodium acetate anhydrous resulted in higher pH of solution. The pH range of solution as specified in USP is 2.7 to 3.3. Increase in concentration of Mannitol or sodium acetate anhydrous or both results in the increase in Osmolarity of solution.

Keywords: Gemcitabine Hydrochloride; Mannitol; Sodium Acetate Anhydrous; Osmolarity; pH; Quadratic Modeling

INTRODUCTION

Buffers are added to a formulation to adjust the pH in order to optimize solubility & stability (Huber, 1979). For the parenteral preparations, it is desirable that the pH of the product be close to physiologic pH (Eremin, 1977). The selection of buffer concentration (ionic strength) and buffer species are important (Gazitua, 1979). Buffers have maximum buffer capacities near their pka (Simamora, 1995). For products, which may be subjected to excessive temperature fluctuations during processing (Sterilization or Lyophilization), it is important to select buffers with a small pKa (Trissel, 2001). The best buffers for a lyophilized product may be those which show the smallest pH change upon cooling, do not crystalline out, and can remain in the amorphous state protecting the drug. Acetates are good buffers at low pH (Kuwahara, 1998). Innovator formulation or reference list drug of Gemcitabine for injection USP (Gemzar) was manufactured in the lyophilized form due to its instability when in solution. On comparing the degradation profile of Drug substance during drug excipient compatibility, and in presence of Mannitol was found to be compatible (USP, 2011). Mannitol is used to modify the Osmolarity and as bulking or lyo/cryoprotectve agent. Mannitol is crystallized if the solution freezes, where Sorbitol &Lactose are remained in an amorphous state. Hence it is useful that the drug remains dispersed in the bulking agent upon freezing of the solution. Mannitol is crystalline and non-hygroscopic. Both before and after freezing, total moisture contents of 0.1 to 0.35 % w/w between 10 and 60% relative humidity.

The experimental objective was to evaluate change in pH, Osmolarity upon change in concentration of Mannitol and Sodium acetate anhydrous at fixed concen-

* Corresponding Author
Email: sreebandari@yahoo.co.in
Contact: +91-9963438195 Fax No: +914427141369
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tration of Gemcitabine hydrochloride (The Merck Index, 2006). So the experiment was designed by varying the concentrations of two excipients Mannitol and sodium acetate anhydrous. A model was generated using Quadratic modeling and CCF design with eight runs in design and three centre points. The reference list drug composition corresponds to 45.5408 mg/ml of Gemcitabine Hydrochloride equivalent to 40 mg/ml of Gemcitabine, 40.0 mg/ml of Mannitol and 2.5 mg/ml of Sodium Acetate anhydrous. The low and the high levels of each factor were specified in relation to the reference list drug composition. It was decided to vary Sodium Acetate anhydrous concentration from 0 to 2.5 mg/ml and Mannitol from 30 to 50 mg/ml keeping the concentration of Gemcitabine Hydrochloride constant. Then a standard experimental plan with eleven experiments was created (Kuwahara, 1998b). For each of the eleven experiments Osmolarity, pH of the solution was measured. As the drug product is intended for intravenous administration Osmolarity and pH of the solution play a vital role. So the drug product is designed to have comparable Osmolarity as that of reference list drug (Gemzar), in the pH range of 2.7 to 3.3 (Poole, 1999). The composition of solutions mentioned in experimental design given in Table 1. Such repeated testing is useful for determining the size of the experimental variation, known as the replicate error. Then the design space was generated based on the contour plots.

MATERIALS & METHODS

Gemcitabine Hydrochloride was generously supplied as a gift sample by Dr.Reddy's Laboratories Limited, (Hyderabad, India). Mannitol supplied as a gift sample by Dr.Reddy's Laboratories Limited, (Hyderabad, India. All other chemicals were of analytical reagent grade and were used as received.

In the present study, the Osmolarity was recorded on Osmometer instrument used model OSMOMAT 030 3 P – OSMOMETER.

EXPERIMENTAL DESIGN

The experimental objective was to evaluate change in pH, Osmolarity upon change in concentration of Mannitol and Sodium acetate anhydrous at fixed concentration of Gemcitabine hydrochloride. The experiment was designed by varying the concentrations of two excipients Mannitol and sodium acetate anhydrous. A model was generated using Quadratic modeling and CCF design given in Table 1, with eight runs in design and three centre points (Total of 11 runs).

The reference list drug composition corresponds to 45.5408 mg/ml of Gemcitabine Hydrochloride equivalent to 40 mg/ml of Gemcitabine, 40.0 mg/ml of Mannitol and 2.5 mg/ml of Sodium Acetate anhydrous. The low and the high levels of each factor were specified in relation to the reference list drug composition. It was decided to vary Sodium Acetate anhydrous concentration from 0 to 2.5 mg/ml and Mannitol from 30 to 50 mg/ml keeping the concentration of Gemcitabine Hydrochloride constant. Then a standard experimental plan with eleven experiments was created. For each of the eleven experiments Osmolarity, pH of the solution was measured. The experimental Design Matrix given in Table 2. As the drug product is intended for intravenous administration Osmolarity and pH of the solution play a vital role (Fonkalsrud, 1968). So the drug product is designed to have comparable Osmolarity as that of reference list drug, in the pH range of 2.7 to 3.3. The composition of solutions given in Table 3. which were mentioned in row 09-11 is same. Such repeated testing is useful for determining the size of the experimental variation, known as the replicate error.

Table 1: Design Model

Objective	Response surface modeling
Design	CCF
Process Model	Quadratic
Runs in design	8
Centre points	3
Total runs	11
Software used	MODDE 6.0

Table 2: Design Matrix

Experiment Number	Mannitol (mg/ml)	Sodium acetate anhydrous (mg/ml)
1	-1	-1
2	1	-1
3	-1	1
4	1	1
5	-1	0
6	1	0
7	0	-1
8	0	1
9	0	0
10	0	0
11	0	0

RESULTS & DISCUSSION

The standard experimental plan with eleven experiments was executed as per the experimental design matrix given in Table 2. For each of the eleven experiments Osmolarity, pH of the solution was measured by using pH meter and Osmometer given Table 3. Based on the results the drug product is designed to have comparable Osmolarity as that of reference list drug, in the pH range of 2.7 to 3.3. Then the design space was generated based on the contour plots as shown in Figure 1 and Figure 2. Increase in concentration of Mannitol or sodium acetate anhydrous or both results in the increase in Osmolarity of solution. Based on the reference list drug sample analysis data the Osmolarity of solution could be 510 mOsm/kg ±10% (Range 460 mOsm/kg to 560 mOsm/kg). Change in concentration of Mannitol did not result in significant change in pH of

Table 3: Study Design and Results

S. No.	Run Order	Gemcitabine hydrochloride (mg/ml)	Mannitol (mg/ml)	Sodium acetate anhydrous (mg/ml)	Osmolarity (mOsm/kg)	pH
1	9	45.5408	30	0	449	1.9
2	7	45.5408	50	0	571	1.98
3	2	45.5408	30	5	528	2.94
4	8	45.5408	50	5	613	2.96
5	4	45.5408	30	2.5	489	2.61
6	6	45.5408	50	2.5	575	2.67
7	5	45.5408	40	0	514	2.16
8	10	45.5408	40	5	538	3.08
9	3	45.5408	40	2.5	525	2.77
10	1	45.5408	40	2.5	527	2.78
11	11	45.5408	40	2.5	530	2.77

Table 4: Design space of Excipients used in the Gemcitabine for Injection

Parameters considered (Responses)	Ingredient	Design space	Concentration used
pH: 2.7 to 3.3 Osmolarity: 510 mOsm/kg ±10%	Gemcitabine HCl equivalent to Gemcitabine 40mg	45.5408 mg/mL	45.5408 mg/mL
	Mannitol (Non exceptional excipient)	40 mg/ml	40 mg/ml
	Sodium acetate anhydrous	2.5mg/ml to 5mg/ml.	2.5 mg/ml

the solution. Whereas the increase in concentration of sodium acetate anhydrous resulted in higher pH of solution. The pH range of solution as specified in USP is 2.7 to 3.3. The minimum concentration of sodium acetate required to get a pH of about 2.7 is 2.5mg/mL. The USP limit for pH of solution is 2.7 to 3.3. Increase in pH is obtained when sodium acetate concentrates is increased. Whereas, change in concentrate of Mannitol did not result in significant change in pH of solution. When the concentrate of sodium acetate is varied between 2.5mg/mL to 5mg/mL, the pH of solution varied between 2.7 to 3.1, which are within the USP specification limits of 2.7 to 3.3. Increase in concentration 2.5mg/mL to 5mg/mL of Osmolarity is observed when concentrations of Mannitol & sodium acetate are increased. From contour, plot showed in Figure 2, the Osmolarity range at different concentrates of buffer and Mannitol were summarized.

Evaluation of reference list drug showed that desired Osmolarity range for the product shall be 510 mOsm/kg ± 10% (460 mOsm / kg to 560 mOsm / kg), and Mannitol is a non exception excipient. The concentration of Mannitol should be identical to reference list drug, example 40mg/mL (200mg/5mL, 1000mg/25mL respectively).The The concentration of buffer that could be varied at fixed concentration of Mannitol (40mg/mL), and Gemcitabine (40mg/mL), shall be 2.5mg/mL to 5mg/mL so that solution shall have Osmolarity comparable to reference list drug (510 mOsm / kg ± 10%). Based on the experimental data the Design space of Excipients used in the Gemcitabine for Injection USP 200 mg & 1000 mg was given in Table 4.

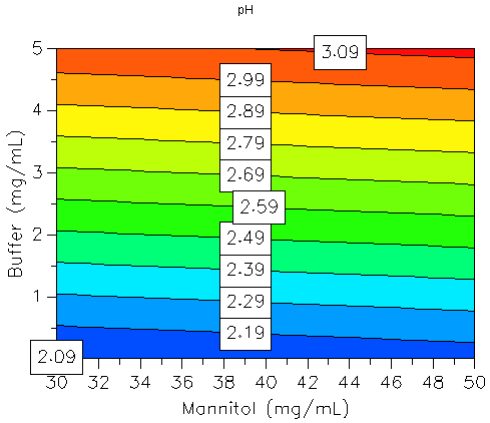

Figure 1: Effect of change in cons. of buffer and Mannitol on pH (Contour plot)

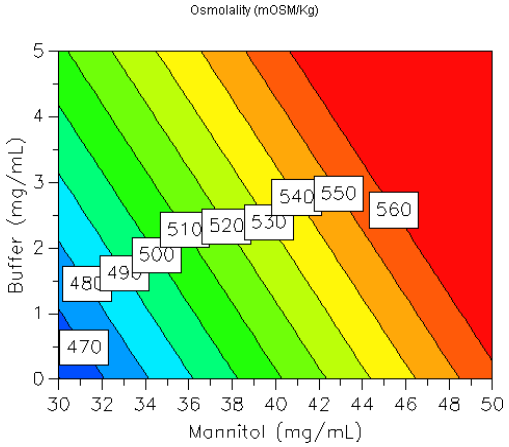

Figure 2: Effect of change in cons. of buffer and Mannitol on Osmolarity (Contour plot)

CONCLUSION

Increase in pH is obtained when sodium acetate concentrate is increased. Whereas, change in concentrate of Mannitol did not result in significant change in pH of solution. When the concentrate of sodium acetate is varied between 2.5mg/mL to 5mg/mL, the pH of solution varied between 2.7 to 3.1, which are within the USP specification limits of 2.7 to 3.3. Increase in concentration 2.5mg/mL to 5mg/mL of Osmolarity is observed when concentrations of Mannitol & sodium acetate are increased. The Osmolarity range at different concentrate of buffer, and Mannitol are summarized and design space established. The experimental data showed the significant change in Osmolarity upon change in concentration of Mannitol and Sodium acetate anhydrous and change in pH upon change in concentration of Sodium acetate anhydrous alone at fixed concentration of Gemcitabine Hydrochloride.

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