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Stabilization and preformulation of anticancer drug—SarCNU

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Abstract

The stability of SarCNU (NSC364432), 1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea in several pharmaceutically acceptable solvents was investigated by high pressure liquid chromatography (HPLC). The influences of light, ionic strength, pH, buffer concentration, and the following excipients: benzyl alcohol, ascorbic acid, sodium bisulfite, and disodium EDTA were studied at room temperature. The stability of the drug was also determined in water, EtOH, PG, Capmul PG, DMSO, and in different combinations of these cosolvents at four different temperatures. The degradation of the drug, which is catalyzed not only by general but also by specific acid and base, follows first order kinetics. Antioxidants, EDTA, and light have no effect on the degradation rate, suggesting oxidation is not a major degradation pathway. The t_{90} in pure cosolvent is 25–50 times higher than that in water or semi-aqueous vehicles. Neat EtOH can be used to store the drug in a nonaqueous concentrate that is diluted with aqueous solvent prior to injection.

Keywords: Stability; 1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea; Double syringe

1. Introduction

1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea (SarCNU) (Fig. 1) was selected for formulation by the National Cancer Institute because of its therapeutic advantage in the treatment of malignant glioma. SarCNU is a chloroethylnitrosourea that is methylated in the N-3 position to make it more stable compared to the other nitrosoureas (Bosanquet, 1985). In spite of its being more stable than other nitrosoureas, it still degrades very rapidly in aqueous media. Its t_{90} in aqueous solution at room temperature is less than 6 h (Ni et al., 2001).

SarCNU is a white odorless powder with a molecular weight of 222.66. It is soluble in water and water miscible organic solvents such as ethanol (EtOH), propylene glycol (PG), Capmul

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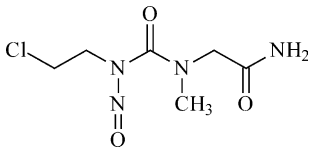

Fig. 1. Sturcture of SarCNU (1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea).

PG, and dimethyl sulfoxide (DMSO). Although it is soluble in aqueous media, the high instability of SarCNU prevents its formulation in an aqueous or a semi-aqueous vehicle. A freeze-dried formulation of SarCNU was investigated in our previous study (Ni et al., 2001). Since it is inherently expensive, freeze-drying is not a first choice for formulation. Therefore, a liquid formulation of SarCNU is investigated in this study.

Water is an active participant in many chemical degradation processes because of its role as a medium for the movement of molecules, as an environment fostering conformational changes, and as a reactant for hydrolysis (Martin, 1993). Therefore, many degradation reactions of drugs can be either suppressed or completely eliminated by simply removing water.

Cosolvents can not only increase the solubility of drugs but also increase the stability of drugs (Yalkowsky et al., 1993; Yalkowsky, 1999). The addition of cosolvent reduces the collision probability between a water molecule and a drug molecule which is necessary for hydrolysis. Also decreasing the polarity of the reaction medium by the addition of cosolvent will unfavor the formation of the charged species. It will stabilize a solute against any reaction that produces charged products or proceeds through a charged transition state (Yalkowsky, 1999; Zhao and Yalkowsky, 2001).

The double syringe is an injection apparatus that can mix two solutions completely before the mixture is injected into the body. Using the double syringe, we can formulate the drug in pure organic solvent and eliminate hydrolysis while administering the cosolvent in a pharmaceutically acceptable concentration. In order to find a suitable solvent to formulate SarCNU, the stability of the drug was determined in various aqueous and non aqueous solutions.

2. Materials and methods

2.1. Materials

SarCNU (NSC-364432) was provided by the Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Capmul PG-8, a propylene glycol monoester of medium chain fatty acids, was obtained from Abitec (Janesville, WI). All other chemicals were analytical or high pressure liquid chromatographic assay (HPLC) grade.

2.2. High pressure liquid chromatographic assay (HPLC)

The gradient high pressure liquid chromatography assay of Peninsula Laboratories has been modified for SarCNU as described below: A Beckman System Gold (Beckman Instruments, Fullerton, CA) equipped with a model no. 167 detector at 254 nm was used for HPLC analysis. The injection volume was 20 μl. Separations were achieved on a Restek Pinnacle ODS Amine column (5 U, 4.6×250 mm^2) (Restek, Bellefonte, PA) at room temperature with a flow rate of 1.0 ml/min. The initial mobile phase was 9 parts of 1% acetic acid and 1 part acetonitrile. This was changed over a period of ten minutes to the final composition of 6 parts of 1% acetic acid and 4 parts of acetonitrile. Samples were dissolved in methanol. The observed relative retention time of SarCNU was 9 min as shown in Fig. 2.

2.3. Stability studies

All kinetic studies were conducted at 25 °C under ambient light except for the experiments where effects of temperature and light were tested. The stock solution in each solvent was 1 mg/ml. All stock solutions were sealed in glass ampoules. The reaction samples were withdrawn at suitable time intervals and diluted with methanol before they were immediately assayed by HPLC.

The influence of pH on the solubility of SarCNU was tested by using 0.1 M phosphate buffer solutions at pH 2.0, 3.0, 4.0, 5.0, and 6.0, as

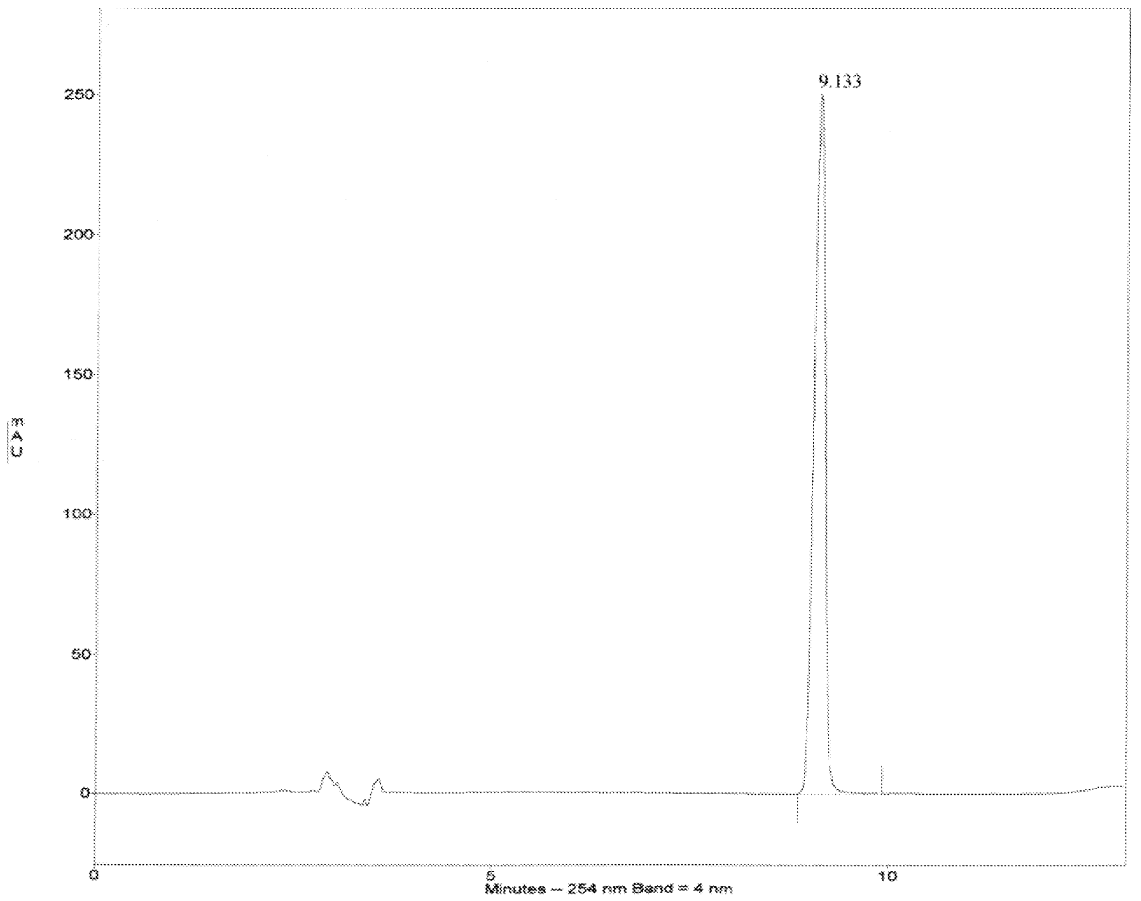

Fig. 2. Representative chromatography of SarCNU.

well as using sodium borate/HCl buffer solution at pH 7.0 and glycine/NaOH buffer solution at pH 8.5. Both sodium borate/HCl and glycine/NaOH buffer solutions were selected instead of phosphate buffer at pH 7.0 and 8.5 since phosphate buffer precipitated on dilution with methanol.

The influence of buffer concentration on the stability of SarCNU was tested by using 0.01, 0.05, and 0.1 M phosphate buffer solutions at pH 2.0 and glycine/NaOH buffer solutions at pH 8.5. The effect of the ionic strength on the stability of SarCNU was investigated by adding 1% (W/V) or 0.17 M NaCl to the 0.01M buffer solutions at pH 2.0, 4.0, 6.0, and 8.5. The effect of the antimicrobial agent on the stability of SarCNU was also investigated by using 0.75% benzyl alcohol (Nema et al., 1997) at 37 °C. The effects of antioxidants, ascorbic acid (AA) and sodium bisulfite (SB), as well as a chelating agent, disodium EDTA on the stability of SarCNU were determined in the 0.01 M phosphate buffer solutions at pH 2.0 and 6.0 with and without 0.005% of AA, SB or disodium EDTA at 25 °C.

Stock solutions of 1 mg/ml were prepared in different pure cosolvents (EtOH, PG, Capmul PG, and DMSO) and two combinations of solvents (40% PG: 10% EtOH: 50% water and 80% PG: 20% EtOH). Accelerated stability testing was conducted for all the samples at four different temperatures (25, 37, 50, and 60 °C).

3. Results and discussions

3.1. Degradation kinetic order

The degradation of SarCNU follows apparent first-order kinetics under all conditions. The degradation rate constants were calculated from linear relationship between the logarithm of the remaining drug concentration and time as shown in Figs. 3 and 4.

3.2. pH rate profile

Fig. 5 shows the pH rate profiles for SarCNU at 25 °C with and without light. The U-shaped profiles show that maximum stability is obtained in the 4–6 pH range, which is consistent with the literature report (Supko et al., 1996). The U-shaped pH rate profiles suggest that the degradation of SarCNU is catalyzed by specific acid (hydronium ion) and specific base (hydroxide ion) (Martin, 1993). Fig. 5 also shows that there is no effect of light on the degradation of SarCNU over the whole pH range, inferring light oxidation is not a major degradation mechanism for SarCNU.

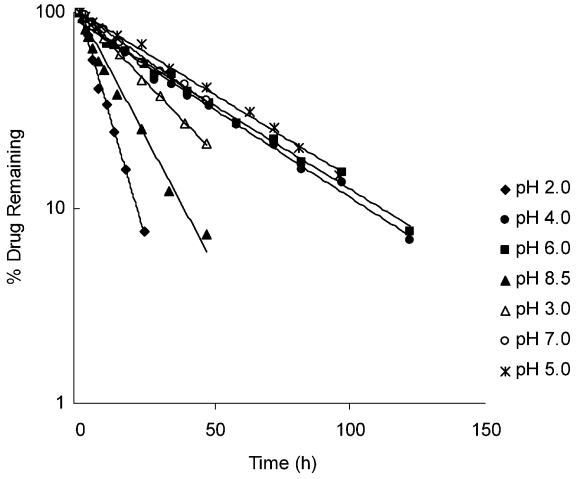

Fig. 3. Apparent first-order reaction of SarCNU in 0.1 M buffer solutions at various pH values without light at 25 °C.

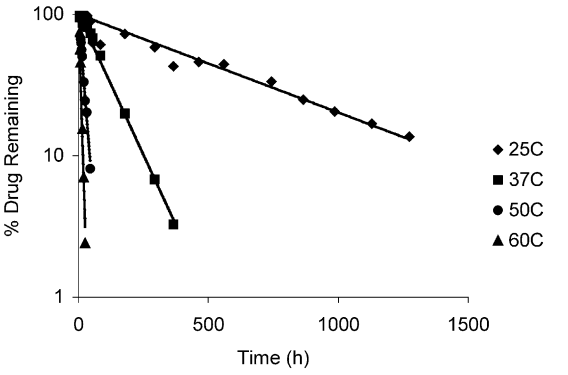

Fig. 4. Apparent first-order reaction of SarCNU in PG without light at four different temperatures.

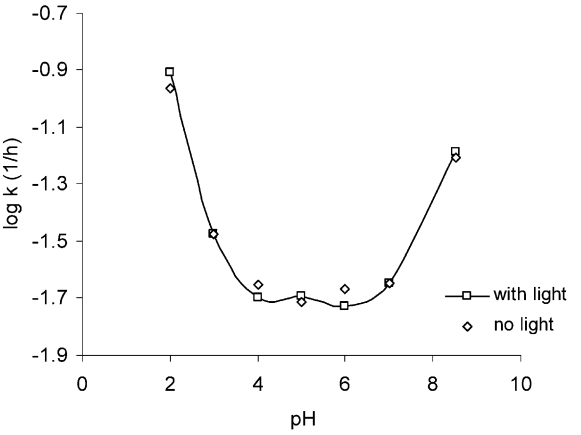

Fig. 5. pH-rate profile of SarCNU with/without light (0.1 M phosphate buffer).

3.3. Ionic strength effect

The effect of ionic strength in the 2.0–8.5 pH range on the rate of degradation of SarCNU is shown in Table 1. We can conclude from the table that the ionic strength has no significant effect on the degradation rate constant of SarCNU.

3.4. General acid/base catalysis

The contribution of the buffer at a given pH to the degradation rate constant can be calculated from a series of measurements at constant pH, solvent composition, and ionic strength but with a different buffer concentration by using equation $k_{obs} = k_0 + k^*_{buf} C_{buf}$. According to the equation, the observed degradation rate, k_{obs} plotted against the buffer concentration, C_{buf}, yields a straight line with a slope (catalytic coefficient) equal to the

Table 1
Effect of ionic strength on the degradation rate constant of SarCNU at different pH values

	k (1/h)			
	pH 2.0	pH 4.0	pH 6.0	pH 8.5
0.01 M phosphate buffer	0.0459	0.0223	0.0219	0.0263
0.01 M phosphate buffer+1% (0.17 M) NaCl	0.0400	0.0211	0.0215	0.0256

contribution of the buffer-catalyzed reaction, k_{buf}. The intercept corresponds to the degradation rate in the absence of buffer species.

The linear relationship between the buffer concentration and degradation rate constant of SarCNU at pH 2.0 and 8.5, which is shown in Fig. 6, suggests that the degradation of SarCNU is catalyzed by buffer, i.e. general acid and base species. The buffer catalytic coefficient, k_{buf}, equals 0.8642 and 0.4330 at pH 2.0 and 8.5, respectively. The degradation rate of SarCNU in the absence of buffer species, k_0, is equal to 0.0329 and 0.0206 (1/h) at pH 2.0 and 8.5, respectively. The ionic strength was not controlled in this study because it has no significant effect on the degradation rate of SarCNU as discussed in Section 3.3.

3.5. Effect of additives

To further confirm that oxidation is not involved in the degradation of SarCNU, the effect of antioxidants, AA and SB were investigated. Additionally, the effect of a chelating agent, disodium EDTA was studied due to its complexation with heavy metal ions which may catalyze the oxidative degradation of SarCNU. In all cases, additives have no effect on the stability of SarCNU which is shown in Table 2.

Similarly Table 3 indicates that ionic strength and benzyl alcohol which is often used as antimicrobial agent in parental formulations (Nema et al., 1997) have no significant effect on the stability of SarCNU.

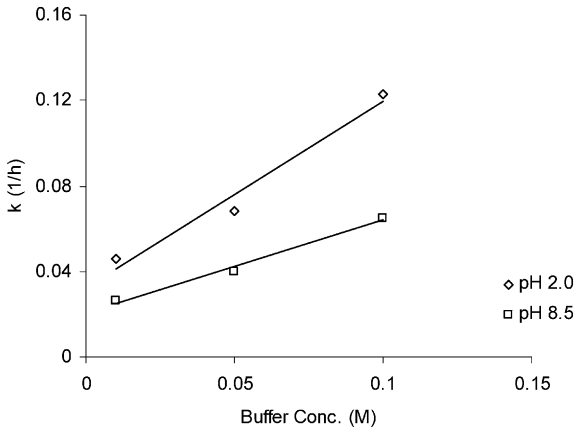

Fig. 6. Effect of buffer concentration on the degradation rate constant of SarCNU at pH 2.0 and 8.5.

3.6. Stability study of SarCNU in different solvents

The stability of SarCNU was investigated over the temperature range of 25–60 °C in water,

Table 2
Effect of additives on the degradation rate constant of SarCNU at pH 2.0 and 6.0 phosphate buffer (0.01 M) without light

	k (1/h)	
	pH 2.0	pH 6.0
None	0.0459	0.0219
AA	0.0466	0.0200
SB	0.0428	0.0206
EDTA	0.0428	0.0226

Table 3
Effect of ionic strength and benzyl alcohol on the degradation rate constant of SarCNU at 37 °C

	k (1/h)
Water	0.0807
1% NaCl solution	0.0847
0.75% Benzyl alcohol	0.0791

DMSO, EtOH, PG, Capmul PG, an 80% PG:20% EtOH mixture (PE), and in a semi-aqueous vehicle (WPE) containing 50% water:40% PG:10% EtOH. This vehicle (WPE) is used in a number of marketed products, including: digoxin, phenytoin, pentobarbital, and diazepam (Sweetana and Akers, 1996).

Table 4 shows that SarCNU degrades very quickly in water and WPE. Its t_{90} values at room temperature in these vehicles are only 0.25 and 0.50 days, respectively. The t_{90} is only 9 days in WPE even at 4 °C. Therefore, a reasonable shelf life cannot likely be reached when SarCNU was formulated in an aqueous vehicle.

In Fig. 7, the observed rate constants in water, WPE, EtOH, PG, PE, Capmul PG, and DMSO are plotted versus inverse of temperature according to the Arrhenius equation (Connors et al., 1986; Carstensen, 1995). The figure shows that there is no significant difference in the slopes for the different solvents, suggesting the similar degradation mechanism of SarCNU in all solvents. Furthermore, the order of stabilization by these vehicles is Capmul PG > EtOH > PE > PG > WPE > water, which is in agreement with decreasing the polarities of the vehicles (Etman and Nada, 1999).

Table 4 shows the degradation rate constant and t_{90} of SarCNU in each solvent at −4, 4, and 25 °C. The greatest stability was observed with Capmul PG. Table 4 also shows that both Capmul PG and EtOH formulations can be stored at −4 °C for over 2 years.

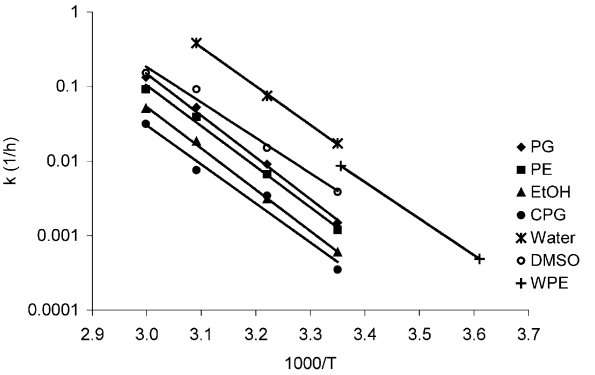

Fig. 7. Arrhenius plots for the apparent first order rate constant of SarCNU in different solvents.

3.7. Double syringe

Fig. 8 shows double syringe which is proposed for administering a drug with a very short shelf life in water. It consists of two syringes that are connected to a single needle. One syringe contains the aqueous diluent, while the other contains the drug concentrate. The drug concentrate can be a buffered solution at low pH or containing a high concentration of surfactant or complexing agent or a semi-aqueous or non-aqueous solution in which the drug has desired stability. The drug is stored as a concentrate and mixed with an aqueous diluent immediately prior to injection. The mixing ratio of concentrate and diluent is controlled by the radii of the syringes.

The stability profile suggests that we can increase the shelf life of SarCNU by stabilizing the drug in Capmul PG and EtOH. This approach

Table 4
Observed degradation rate constant and T_{90} of SarCNU in different solvents at 25, 4 and −4 °C

	Room (25 °C)		Refrigerator (4 °C)		Freezer (−4 °C)	
	$k \times 10^{-3}$ (1/day)	T_{90} (days)	$k \times 10^{-3}$ (1/day)	T_{90} (days)	$k \times 10^{-3}$ (1/day)	T_{90} (days)
Water	414.0	0.25	17.78	5.90	4.896	21.40
WPE	206.4	0.50	11.71	8.96	N/A	N/A
DMSO	92.4	1.14	5.52	19.03	1.704	61.65
PG	36.0	2.92	1.35	77.78	0.338	311.11
PE	28.8	3.64	1.17	89.50	0.305	344.42
EtOH	14.4	7.29	0.53	199.52	0.133	786.49
Capmul PG	8.4	12.50	0.43	242.57	0.115	912.36

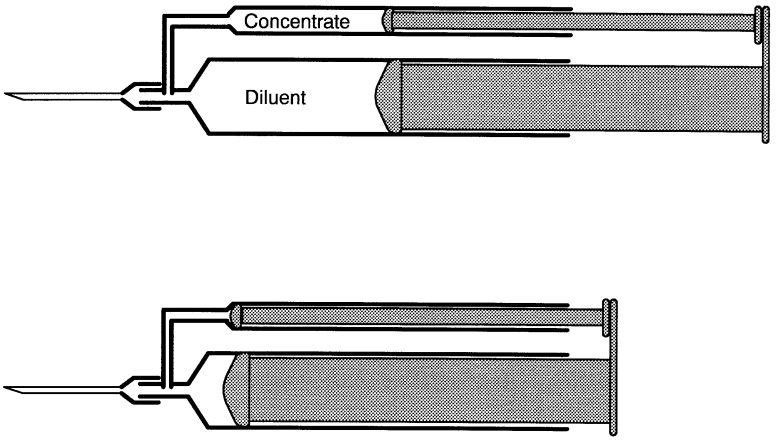

Fig. 8. Instrument of double syringe.

Table 5
Solubility of SarCNU in different solvents at 25 °C

	Water	EtOH	PG	Capmul PG	DMSO
Solubility (mg/ml)	> 18	> 28	> 13	> 10	> 60

can be used to store drug so that it can be diluted with aqueous solvent prior to injection with the aid of a double syringe. But only EtOH was chosen as a solvent to formulate SarCNU as EtOH mixes simultaneously with water to form homogeneous solution. Therefore, phase separation will not occur before the drug is injected into the human body.

The solubilities of SarCNU in different solvents as determined by visual observation are summarized in Table 5. The high aqueous solubility of SarCNU ensures that precipitation will not occur when the formulation is diluted with aqueous solvent prior to injection.

4. Conclusion

This study shows that the degradation of SarCNU follows first order kinetics. It is catalyzed by H^+, OH^-, and buffer species, but oxidation is minimal. The stability of SarCNU can be increased by simply reducing water in the formulation vehicle. EtOH is a potential vehicle to formulate SarCNU. The formulation can be stored at −4 °C over 2 years. Before the drug is delivered to patient, it can be mixed with water in any ratio through double syringe.

Acknowledgements

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