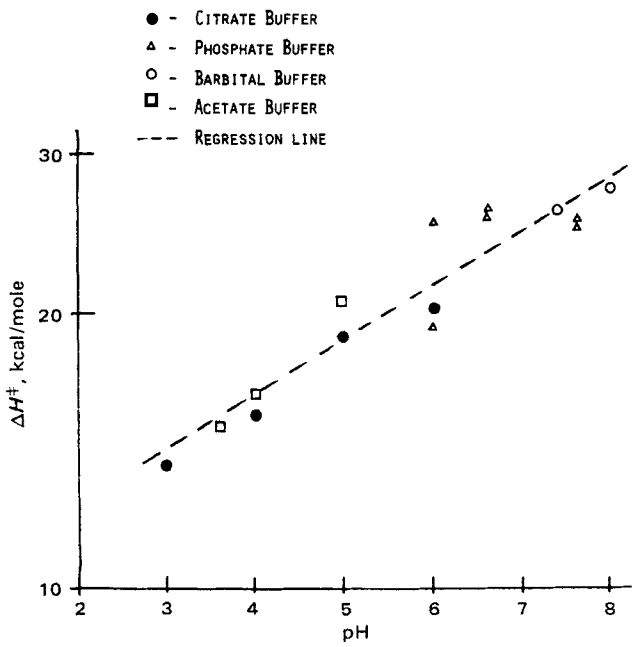

Figure 4—*Logarithm activation energy–pH profile for buffered aqueous systems.*

the accelerated stability analysis studies (Table I) indicates that I would have a short useful life at room temperature in the buffer systems studied. Ten percent degradation would occur in about 14 hr in the citrate buffer at pH 5.0. However, storage in the refrigerator (~5°) would prolong the time for 10% degradation to about 5.5 days.

These preliminary results indicate that it may be feasible to reconstitute I in some intravenous solutions at pH 5.2–5.5, to store the mixture at 5° for a few days, if necessary, and then to administer I parenterally. Further work is underway to determine the effect of certain electrolytes, commonly found in intravenous solutions, on the stability of I.

REFERENCES

(1) F. M. Schabel, Jr., T. P. Johnston, G. S. McCaleb, J. A. Montgomery, W. R. Laster, and H. E. Skipper, *Cancer Res.*, **23**, 725 (1963).

(2) D. P. Rall, D. M. McCarthy, and M. Ben, *Proc. Am. Assoc. Cancer Res.*, **4**, 55 (1963).

(3) M. D. Walker and B. S. Hurwitz, *Cancer Chemother. Rep.*, **54**, 263 (1970).

(4) T. L. Loo, R. L. Dion, R. L. Dixon, and D. P. Rall, *J. Pharm. Sci.*, **55**, 492 (1966).

(5) J. L. Boivin and P. A. Boivin, *Can. J. Chem.*, **29**, 478 (1954).

(6) E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 767 (1960).

(7) J. A. Montgomery, R. James, G. S. McCaleb, and T. P. Johnston, *J. Med. Chem.*, **10**, 668 (1967).

(8) V. T. DeVita, P. D. Carbone, A. Owens, Jr., G. L. Gold, M. J. Krant, and J. Edmonson, *Cancer Res.*, **25**, 1876 (1965).

(9) S. K. Carter, F. M. Schabel, Jr., L. E. Broder, and T. P. Johnston, *Adv. Cancer Res.*, **16**, 273 (1972).

(10) J. P. Davignon, K. W. Yang, H. B. Wood, Jr., and J. C. Cradock, *Cancer Chemother. Rep., Part 3*, **4**, 7 (1973).

(11) D. W. Flamberg, D. L. Francis, S. L. Morgan, and G. F. Wickes, *Bull. Parenter. Drug Assoc.*, **24**, 209 (1970).

(12) J. P. Davigon, *ibid.*, **23**, 83 (1969).

(13) "Biochemists Handbook," C. Long, Ed., Van Nostrand, Princeton, N.J. 1961, p. 30ff.

(14) "Documenta Geigy Scientific Tables," 7th ed., K. Diem, Ed., Geigy Pharmaceuticals, Ardsley, N.Y., 1970, p. 280ff.

(15) T. L. Loo and R. L. Dion, *J. Pharm. Sci.*, **54**, 809 (1965).

(16) E. R. Garrett, *ibid.*, **51**, 811 (1962).

(17) E. R. Garrett, S. Goto, and J. F. Stubbins, *ibid.*, **54**, 119 (1965).

(18) E. R. Garrett and S. Goto, *Chem. Pharm. Bull.*, **182**, 973 (1973).

(19) R. R. Sokal and F. J. Rohlf, "Biometry," Freeman, San Francisco, Calif., 1969, p. 468ff.

(20) J. T. Carstensen, "Theory of Pharmaceutical Systems," vol. I, Academic, New York, N.Y., 1972, p. 181.

ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1975, from the *Department of Pharmaceutical Science, School of Pharmacy, Oregon State University, Corvallis, OR 97331.*

Accepted for publication August 23, 1976.

Abstracted in part from a thesis submitted by P. A. Laskar to the Graduate School, Oregon State University, in partial fulfillment of the Doctor of Philosophy degree requirements.

[x] To whom inquiries should be directed. Present address: Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.

Degradation of Carmustine in Mixed Solvent and Nonaqueous Media

PAUL A. LASKAR [x] and JAMES W. AYRES

Abstract □ The degradation rate of carmustine in several solvent mixtures and in mannitol solution was investigated at 5, 22, and 37°. The solvents chosen were those utilized as parenteral diluents. The apparent first-order degradation rate constants were computed using a linear regression procedure. The most nonaqueous solvent mixtures demonstrated minimum apparent degradation rates. The apparent degradation rate constant decreased with a decrease in the macroscopic dielectric constant. From the data at several temperatures, the apparent activation energies for carmustine degradation in the several solvent mixtures were calculated. There was no evidence for a relationship between the apparent activation energy and the dielectric constant.

Keyphrases □ Carmustine—degradation rate in mixed solvents and nonaqueous media, effect of temperature □ Degradation rate—carmustine in mixed solvents and nonaqueous media, effect of temperature □ Antineoplastic agents—carmustine, degradation rate in mixed solvents and nonaqueous media, effect of temperature □ Stability—carmustine in mixed solvents and nonaqueous media, effect of temperature

Numerous chemotherapeutic agents, including the nitrosoureas, show promise in the treatment of neoplastic diseases. The formulation of these agents into clinically useful dosage forms has, however, been slow. Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; NSC 409962] (I), like many other nitrosoureas, has relatively poor aqueous sol-

Table I—Degradation Rate Constants of Carmustine in Mixed Solvents

Solvent in Water	Concentration, %	Rate Constant, 10^4 hr^{-1} (*SE*) 5°	22°	37°
Ethanol	2	13.9 (0.43)	220.4 (12.80)	707.3 (29.30)
	25	7.9 (0.13)	192.6 (4.54)	406.0 (13.36)
	50	2.4 (0.12)	25.6 (0.56)	149.1 (5.57)
	95	0.6 (0.18)	2.2 (0.34)	10.9 (0.33)
Propylene glycol	25	9.709 (0.20)	194.9 (6.01)	151.9 (11.50)
	50	4.572 (0.16)	89.7 (3.80)	275.1 (5.72)
	100	1.314 (0.22)	8.5 (0.46)	49.2 (1.80)
Dimethyl sulfoxide	2	13.7 (0.45)	389.1 (16.49)	1864.9 (46.30)
	25	7.5 (0.16)	240.4 (15.69)	1551.2 (110.70)
	50	5.1 (0.16)	74.5 (1.90)	366.8 (10,92)
	100		12.2 (0.28)	74.1 (1.83)
Mannitol	5		147.3 (1.92)	
	10		130.3 (1.22)	
	20		124.4 (2.49)	

ubility and is quite unstable in aqueous media (1–4). Therefore, in efforts to obtain a desirable dosage form, preformulation studies with I in aqueous, mixed solvent, and nonaqueous media are appropriate. Studies with I in aqueous media were reported previously (5). The mixed solvent and nonaqueous media chosen are those generally utilized in the parenteral administration of various drug agents including nitrosoureas (3).

Little work has been reported on optimizing a parenteral dosage form of I. To date, studies have emphasized technological considerations in the delivery of I as well as other antineoplastic nitrosoureas (3, 6–8).

The present investigation was undertaken at several temperatures to obtain useful shelflife and storage data *via* accelerated stability studies.

EXPERIMENTAL

Materials and Methods—All chemicals and solvents were reagent or USP grade except where noted. Solutions of I were prepared and analyzed as described previously (5).

Degradation Experiments—For ambient (22 ± 1°) and physiological (37.0 ± 0.5°, forced-air oven) temperature experiments, the media were first allowed to equilibrate for at least 16 hr. For experiments at reduced temperature, the media were first chilled in an ice bath (1.0 ± 0.5°) for at least 15 min.

To a volume (~40–45 ml) of temperature-equilibrated media was added 1.0 ml of I stock solution (1 μg/ml). Then the volume was brought rapidly to 50 ml with the appropriate medium, the mixture was shaken, and the time was recorded. A zero-time sample (0.2 ml) was taken for analysis, and the experimental solution was stored at the appropriate temperature. At designated time intervals, a sample volume (0.2, 0.5, 1.0, or 2.0 ml) was removed for analysis.

The criterion used to determine the sample size was that the apparent concentration should not fall below 1.0 μg/ml or exceed 5.0 μg/ml; the optimum range was 2–4 μg/ml. The sampling time interval depended in part on a previously observed or expected degradation rate.

Table II—Dielectric Constants for Solvents Utilized

Solvent	Concentration, %	Dielectric Constant 5°	22°	37°
Ethanol	25	71.10	65.20	60.50
	50	54.50	49.80	41.00
	90	29.75	26.75	23.40
Propylene glycol	25	75.09	67.56	61.54
	50	61.91	58.33	50.74
	100	34.80	31.31	28.52
Dimethyl sulfoxide	2	81.53	80.90	79.13
	25	81.13	80.50	78.73
	50	79.03	78.40	76.63
	100	—	48.75	47.63
Water		86.04	79.60	74.20
Mannitol	5	—	78.80	—
	10	—	78.50	—
	20	—	71.80	—

RESULTS AND DISCUSSION

The applicability of this analytical method to I was reported previously (5). Several nitrosoureas and compounds containing nitroso groups exhibit an apparent first-order degradation dependency (1, 4, 9, 10). Therefore, degradation exhibiting apparent first-order kinetics was hypothesized, and the collected data were tested for adherence to this model.

The suitability of the apparent first-order model was initially estimated visually from the graph of the logarithm of the concentration of I remaining (micrograms per milliliter) *versus* time. Since the data did not negate the assumption of apparent first-order dependence, a least-squares regression of the natural logarithm of I concentration on time was calculated and the linear parameters were estimated using a standard computer program (11). The standard error of the linear parameters, the slope, and the intercept were also calculated.

Curvilinearity was estimated qualitatively by examining residuals as well as more quantitatively by utilizing a multiple regression of the natural logarithm of I concentration on a third-degree polynomial in time (12, 13). The multiple regression was performed in a forward stepwise manner, and an analysis of variance (ANOVA) was calculated at each step to enable visualization of the individual variance contribution of the linear, quadratic, and cubic components (13). The results did not negate the assumption of an apparent first-order degradation of I in the several solvent mixtures.

The dependency of the degradation rate constant on the reciprocal dielectric constant and temperature was determined using a least-squares regression of the natural logarithm of the rate constant on the appropriate independent variable, and the regression coefficient(s) were computed. Homogeneity of the activation energy in buffered aqueous systems and solvent systems was evaluated using covariance analysis (14).

From the data calculated for I in the solvent mixtures, the apparent stability increased with an increasing nonaqueous solvent concentration (Table I). The apparent degradation rate constant in optimally buffered aqueous media was about 8×10^{-4} hr^{-1} at 5° (5). The apparent rate constants in solvent mixtures were of this magnitude or less in ethanol, propylene glycol, or dimethyl sulfoxide concentrations of 25% or greater at 5°. The solvent mixture demonstrating minimum degradation (95% ethanol at 5°) had a rate constant more than an order of magnitude less than the buffered aqueous system, thereby providing a t_{90} of about 78 days [*cf.*, about 5.5 days in the buffered aqueous media (5)].

A disadvantage of 95% ethanol as a solvent for direct parenteral administration of I is its irritation potential; this problem would necessitate dilution to a concentration at which irritation is sufficiently reduced. Propylene glycol does not have this irritation potential, although the minimum apparent degradation rate is about twice that for ethanol.

Table III—Regression of Logarithm of Apparent Rate Constant for Degradation of Carmustine on Reciprocal Dielectric Constant of Solvent

Solvent	Regression Coefficient (*SE*) 5°	22°	37°
Ethanol	−180.7 (84.8)	−193.2 (31.0)	−141.0 (2.8)
Propylene glycol	−130.6 (19.9)	−176.4 (12.4)	−101.6 (39.3)
Dimethyl sulfoxide	−355.8 (2740.3)	−350.6 (112.1)	−326.3 (115.2)
Mannitol	—	−180.2 (263.4)	—

Table IV—Activation Energies of Carmustine Degradation in Solvent Mixtures

Solvent	Concentration, %	Activation Energy, kcal/mole (*SE*)
Ethanol	2	21.43 (1.61)
	25	21.53 (3.22)
	50	22.08 (0.23)
	95	15.76 (0.94)
Propylene glycol	25	15.32 (4.73)
	50	22.21 (2.23)
	100	19.33 (0.50)
Dimethyl sulfoxide	2	26.56 (1.95)
	25	28.77 (1.59)
	50	22.97 (0.94)
	100	21.91 (——)

However, due to its high viscosity, propylene glycol presents potential difficulty for use in a syringe.

Dimethyl sulfoxide is not currently approved for use as a parenteral solvent, although it has been used in some animal studies. The results in this solvent and its mixtures were generally similar to those found for ethanol and propylene glycol. Degradation of I in 100% dimethyl sulfoxide was not followed at 5° since the mixture is solid at this temperature, presenting sampling difficulties. More important, interpretation of results might be compromised due to the "freezing out" of I, thus considerably altering the microscopic solvent environment for the degradation of I. While this occurrence has not been demonstrated for the nitrosoureas, it has been shown for other substances (15).

The results obtained for the solvent mixtures suggest that a proportionality exists between the apparent degradation rate constant and the macroscopic dielectric constant. The dielectric constants for the various solvent mixtures were obtained from the literature and adjusted for temperature (16–18) (Table II). The apparent degradation rate constant of I in a given solvent was regressed on the reciprocal dielectric constant of the solvent, and the results were tabulated (Table III). These computed data indicate a significant decrease in the rate constant with a decrease in the dielectric constant. There is likely to be disparity between the macroscopic tabulated dielectric constants and the microscopic dielectric constant existing in the immediate vicinity of the reacting species (19, 20), particularly in mixtures of solvents with disparate dielectric constants as a result of microscopic dielectric constants reflecting selective solvation (21). Therefore, these regression coefficients computed for I should be considered as qualitative rather than quantitative; they reflect the dielectric constant effect on the degradation rate constant for I.

Mannitol is used as a soluble diluent for I in dry filling vials for reconstitution. Several concentrations of mannitol were utilized to examine the effect of an altered dielectric constant on the apparent degradation rate constant of I. The effect of an increasing mannitol concentration, and thus a decreasing dielectric constant, was comparable to that for the solvent mixtures.

The temperature dependence of the apparent degradation rate constant for I in the solvent mixtures was examined using the Arrhenius equation. The activation energies were computed from the parameters estimated from the least-squares regression equation (Table IV). The regression coefficients were tested for common slope using ANOVA. The results (Table V) do not cause rejection of the hypothesis of a common slope for the temperature dependence of the degradation rate constant in the several solvent mixtures. Therefore, the activation energies may be regarded as constant and parallel. Since the degradation rate constants vary significantly for the solvent mixtures, the frequency factor term in the Arrhenius equation must vary.

Such variation in the frequency factor, and thus the degradation rates, results from the extent of solvation of the reactants and the transition state and affects the entropy of activation (20, 21). Following this reasoning, these data suggest that the differences in the degradation rate constants for I in the solvent mixtures are attributable to these factors relating to solvation.

Table V—Examination of Homogeneity of Slope of Activation Energy of Carmustine Degradation at Several Dielectric Constants

Source of Variation	*df*	*SS*	*MS*
Among regression coefficients	9	3.80	0.42
Within regression (weighted mean of deviations)	12	48.5	3.46
Common slope = 23.29 kcal/mole			

Solutions of I in concentrated ethanol or propylene glycol possessed t_{90} values at room temperature of about 2.5 and 1 week, respectively. Under refrigerated temperatures, the t_{90} values increased to about 2.5 and 1 month, respectively. The useful lifespans in these solvent mixtures permit reconstitution and refrigerated storage for reasonable time periods. Prior to parenteral administration, the reconstituted ethanol or propylene glycol solution of I would be diluted in a suitable parenteral vehicle.

Mannitol in solutions of I also stabilized I to some extent when compared to an approximately pure aqueous system. This finding may be attributable to the effect on the dielectric constant and to solvation effects. The extended timespans in solvent mixtures were significantly longer than those in buffered aqueous media, possibly attributable, at least in part, to the altered solvation of reactants and intermediates.

REFERENCES

(1) E. R. Garrett, S. Goto, and J. F. Stubbins, *J. Pharm. Sci.*, **54**, 119 (1965).

(2) T. L. Loo, R. L. Dion, R. L. Dixon, and D. P. Rall, *ibid.*, **55**, 492 (1966).

(3) J. P. Davignon, K. W. Yang, H. B. Wood, Jr., and J. C. Cradock, *Cancer Chemother. Rep., Part 3*, **4**, 7 (1973).

(4) J. A. Montgomery, R. James, G. S. McCaleb, and T. P. Johnson, *J. Med. Chem.*, **10**, 668 (1967).

(5) P. A. Laskar and J. W. Ayres, *J. Pharm. Sci.*, **66**, 1073 (1977).

(6) D. W. Flamberg, D. L. Francis, S. L. Morgan, and G. F. Wickes, *Bull. Parenter. Drug Assoc.*, **24**, 209 (1970).

(7) J. P. Davignon, *ibid.*, **24**, 88 (1969).

(8) C. L. Fortner, W. R. Grove, D. Bowie, and M. D. Walker, *Am. J. Hosp. Pharm.*, **32**, 582 (1975).

(9) E. R. Garrett, *J. Amer. Pharm. Assoc., Sci. Ed.*, **49**, 767 (1960).

(10) E. R. Garrett and S. Goto, *Chem. Pharm. Bull.*, **21**, 1811 (1973).

(11) D. Guthrie, C. A. Avery, and K. R. Avery, "Statistical Interactive Programming System: Preliminary Users' Guide," Department of Statistics, Oregon State University, Corvallis, Oreg., Sept. 1972.

(12) N. R. Draper and H. Smith, "Applied Regression Analysis," Wiley, New York, N.Y., 1966, p. 62.

(13) R. R. Sokal and F. J. Rohlf, "Biometry," Freeman, San Francisco, Calif., 1969, pp. 468ff.

(14) *Ibid.*, p. 450.

(15) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969, pp. 35, 36.

(16) G. Akerlöf, *J. Am. Chem. Soc.*, **54**, 4125 (1932).

(17) "Handbook of Chemistry and Physics," 54th ed., R. C. Weast, Ed., CRC Press, Cleveland, Ohio, 1973, p. E-45.

(18) Y. Doucet, F. Calmes-Perreault, and M. T. Durand, *C.R.*, **260**, 1878 (1965).

(19) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969, p. 585.

(20) K. J. Laidler, "Chemical Kinetics," 2nd ed., McGraw-Hill, New York, N.Y., 1965, p. 228.

(21) E. S. Amis, "Solvent Effects on Reaction Rates and Mechanisms," Academic, New York, N.Y., 1966, pp. 20, 21.

ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1975, from the *Department of Pharmaceutical Science, School of Pharmacy, Oregon State University, Corvallis, OR 97331.*

Accepted for publication August 23, 1976.

Abstracted in part from a thesis submitted by P. A. Laskar to the Graduate School, Oregon State University, in partial fulfillment of the Doctor of Philosophy degree requirements.

x To whom inquiries should be directed. Present address: Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.