polar environment and whether internal hydrogen bonding is stabilized or whether hydrogen bonding to a solvent or to other biochemical entities has occurred. Hydroxyurea appears to possess the same type of carbonyl nitrogen character before and after equilibration with water. Therefore, no conformational preference may exist in hydroxyurea in solutions where conformational preference may be conferred to substituted hydroxyureas by their bulk or steric hindrance or to some electronic stabilization factor. The crystal structure of hydroxyurea indicates that intermolecular hydrogen bonding exists between the hydroxyl group of one molecule and the carbonyl oxygen of another molecule rather than intramolecular hydrogen bonding (13), and internal hydrogen bonding may not be preferred in solution.

The *in vitro* inhibition of ribonucleotide reductase by selected hydroxyureas (Table II) relative to hydroxyurea seems to indicate a solvent dependency. Although the enzyme system is quite complex and its activity is dependent on many variables (14), its inhibition by hydroxyureas followed a general trend, except for the 3-*n*-propyl analog (V) in the first determination. The solvent for hydroxyurea and the 1-analogs (II and III) was water for all determinations, and water was used for V in the first determination. The solvent for all other determinations for V and VII was 1% dimethyl sulfoxide.

The inhibitory concentration required to obtain 50% enzyme activity with V was much higher in the first determination than in the other determinations (2–4 in Table II), and this result was due to some error in the enzyme determination or to the different solvent. To verify which factor was the determinant in the inhibition study, another analysis was performed (5 in Table II) in which water was the solvent for all compounds. Again the concentration required for 50% enzyme inhibition for V was appreciably higher than in determinations where dimethyl sulfoxide was the solvent. This result implicates the solvent as a potential variable in the ability of hydroxyureas to function in the *in vitro* inhibition of ribonucleoside diphosphate reductase, and this property should be considered in the elucidation of the biodynamics of drugs in the hydroxyurea class.

Although it is not possible to determine whether the solvent-dependent conformational system observed in substituted hydroxyureas is the actual cause of the poor inhibition of the enzyme, with V the conformation and solvent have an interdependence that may affect the biological action of the drug. The transport and partitioning properties of hydroxyureas in octanol–water are currently being investigated to determine whether predicted and actual partition coefficients support the solvent-dependent conformation and the importance of this property in drug transport *in vivo*.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 14, 1976, from the *Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, TX 77004.*

Accepted for publication September 16, 1976.

Presented in part at the 3rd Annual Southcentral Medicinal Chemistry Meeting in Miniature (ALTO), Monroe, La., May 1976.

Supported in part by National Institutes of Health Grant CAO-4464.

The authors thank Miss Barbara Stearns, Squibb Institute of Medical Research, for supplying hydroxyurea and the 1-substituted analogs.

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NOTES

Improved Recovery of Morphine from Biological Tissues Using Siliconized Glassware

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Abstract □ The adsorption of morphine onto glassware during analysis in aqueous solutions and biological tissues is a common problem, resulting in lower recoveries than expected. The problem can be remedied by siliconization of all glassware involved. The same glassware cannot be used repeatedly without incurring a significant loss of morphine.

Keyphrases □ Morphine—adsorption onto glassware during fluorometric analysis, effect of siliconization □ Adsorption—morphine onto glassware during fluorometric analysis, effect of siliconization □ Siliconization—of glassware, effect on adsorption of morphine during fluorometric analysis □ Narcotic analgesics—morphine, adsorption onto glassware during fluorometric analysis, effect of siliconization

Several methods including fluorometric (1, 2), flame-ionization (3) and electron-capture (4) GLC, and radioimmunoassay (5) have been used to measure morphine levels in biological tissues. The fluorometric procedure is still the most widely used method because of its simplicity and rapidity.

A major problem in the extraction of morphine from aqueous solutions and biological tissues has been adsorption of the drug onto detergent-cleaned glassware. Wilkinson and Way (3) were unsuccessful in circumventing this problem since they siliconized only the centrifuge tubes. The present paper reports that siliconization of all glassware that comes in contact with aqueous solutions of morphine reduces the loss of morphine by adsorption and thus significantly increases its recovery from aqueous solutions and from biological tissues.

EXPERIMENTAL

Chemicals—1-Butanol[1], chloroform[2], sodium bicarbonate[3], monobasic sodium phosphate[3], dibasic sodium phosphate[3], potassium ferricyanide[3], and potassium ferrocyanide[3] were the highest quality commercially available.

Siliconization of Glassware—The glassware was siliconized by using a water-soluble silicone concentrate[4] and was dried at 100° for 10 min according to the directions on the container.

Procedures—*Recovery of Morphine from Rat Brain*—The procedure of Kupferberg *et al.* (2), slightly modified, was used. Individual rat brain samples were homogenized[5] for 30 sec with 1.5 ml of isotonic saline in a 50-ml polyethylene tube. The shaft of the homogenizer was then washed with 0.5 ml of saline into the centrifuge tube. Different amounts (0.125–0.5 μg) of morphine sulfate contained in equal volumes (50 μl) were added to the homogenate. Each concentration was run in duplicate.

Sodium bicarbonate (0.5 g) and 10% 1-butanol in chloroform (10 ml) were added to the homogenate. The mixture was shaken on a mechanical shaker for 10 min at 200 oscillations/min and then centrifuged at 28,000×*g* for 30 min at 4°. The aqueous layer was aspirated, and the matted tissue was removed with a spatula. A 7-ml aliquot of the organic phase (butanol–chloroform) was transferred to a 15-ml glass-stoppered centrifuge tube containing 1.2 ml of 0.01 *M* hydrochloric acid; the tube was gently inverted 30 times and then centrifuged for 10 min at 2000 rpm at 4°.

One milliliter of the acid phase was removed and added to 1 ml of phosphate buffer (pH 8.5), and this mixture was oxidized with 0.1 ml of potassium ferricyanide–ferrocyanide reagent (2). The fluorescence of the solution was measured at 440 nm after excitation at 250 nm on a spectrophotofluorometer[6].

Recovery of Morphine from Aqueous Solution—The assay procedure used for the brain tissue was also used to determine recovery of morphine sulfate (0.125–0.5 μg) from aqueous solutions.

RESULTS AND DISCUSSION

A linear relationship existed between the amount (0.125–0.5 μg in the original sample) of morphine sulfate used and the relative fluorescence intensity. A similar relationship existed after morphine was extracted from aqueous solutions or rat brain homogenates to which morphine sulfate had been added. When all glassware was siliconized, the average recoveries of morphine from the water standards and brain tissues, excluding aliquot losses, were 52.6 ± 1.9 (n = 5) and 46.5 ± 2.0% (SE) (n = 4), respectively (Table I). Similarly, the recoveries of morphine, including aliquot losses, were 90.0 and 79.4%, respectively. When none of the glassware was siliconized, the recovery from the rat brain, including aliquot losses, was only 26.3%.

The major loss of morphine occurred when the volumetric flasks used to prepare stock solutions and dilutions of morphine were not siliconized.

[1] Merck and Co., Rahway, N.J.
[2] Mallinckrodt Chemical Co., St. Louis, Mo.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Siliclad, Clay Adams, Division of Becton, Dickinson and Co., Parsippany, N.J.
[5] Polytron homogenizer, Brinkmann Instruments.
[6] Model 8202G, American Instrument Co.

Table I—Effect of Siliconization on Recovery of Morphine[a]

Number of Times Glassware Was Used	Fluorescence Intensity for 0.5 μg of Morphine		Recovery from Water, %	Recovery from Rat Brain, %
	Absolute	%		
1	140	100.0	47.0	39.5
2	125	89.2	47.3	38.6
3	110	78.4	46.2	36.5
4	82	58.4	46.9	32.9
0[b]	225	—	52.6	46.5

[a] All glassware was siliconized except for the volumetric flasks used for making stock solutions of morphine. [b] Represents values when all glassware was siliconized.

The absolute fluorescence units for 0.5 μg of morphine fell from 225 to 140, a decrease of 38% (Table I).

Repeated use of glassware also decreased the absolute fluorescence intensity of morphine. The silicone coating is probably lost during the cleaning procedure, resulting in a loss of morphine due to its adsorption to the glass. Table I shows the fluorescence intensities for morphine recovered from aqueous solutions and from brain homogenates when glassware was used four consecutive times after siliconization. The glassware was cleaned with laboratory detergents and deionized water prior to each experimental run.

The recovery of morphine from the water standard or aqueous solution was not affected significantly by repeated use of glassware, but it was lower than when all glassware was siliconized before use. The recovery of morphine from rat brain samples decreased with each successive use of the glassware. Thus, both the absolute recovery and the extraction recovery were reduced when all glassware was not siliconized. This result could be seen by the decrease in the slope of the calibration curves (Table I). A net loss of 10, 20, and 40% of morphine was observed during the second, third, and fourth use, respectively, of the same glassware. No loss of morphine was observed when nonsiliconized pipets were used to transfer butanol–chloroform extract containing morphine, indicating that adsorption losses occurred only when morphine was present in the aqueous solution.

In conclusion, the present study shows that significant loss of morphine onto glassware can occur. This adsorption loss can be minimized by siliconization of all glassware that comes in contact with aqueous solutions of morphine. Since the slope of the calibration curve declines with successive use of the glassware, the sensitivity of the method decreases. Therefore, to minimize adsorption losses, all glassware should be siliconized each time morphine is to be analyzed in aqueous solutions and tissue samples.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 6, 1976, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.*

Accepted for publication August 18, 1976.

Supported in part by a grant from the Graduate College Research Board, University of Illinois at the Medical Center, Chicago, IL 60612.

The author thanks Dr. Norman R. Farnsworth for interest and encouragement and Mr. George Matwyshyn for technical assistance.