acetylcholinesterase. Although VI was prepared as an inhibitor of pseudocholinesterase and may be an inhibitor of acetylcholinesterase, there is no evidence that the uptake of VI by the heart is related to binding to either enzyme. *In vitro* studies are in progress to investigate the specific mechanism responsible for the accumulation of VI in heart muscle.

The simple, rapid synthetic technique employed in the preparation of VI would easily allow the 13-hr half-life of iodine 123, which has excellent imaging characteristics, to be substituted for the stable iodine in the molecule. The apparent *in vivo* stability and the high heart-to-blood and heart-to-lung ratios obtained in mice with VI suggest that iodine 123-labeled VI may be useful for imaging the heart in humans and warrants further study as a myocardial-imaging agent.

REFERENCES

(1) D. M. Wieland, R. D. Ice, and W. H. Beierwaltes, *J. Nucl. Med.*, **17**, 525 (1976).

(2) H. D. Burns, L. G. Marzilli, R. F. Dannals, T. E. Dannals, T. C. Trageser, P. Conti, and H. N. Wagner, Jr., *ibid.*, **21**, 875 (1980).

(3) G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, **133**, 375 (1940).

(4) K.-B. Augustinsson, *Acta Physiol. Scand.*, **15** (Suppl. 52), 1 (1948).

(5) K.-B. Augustinsson, in "Handbook of Experimental Pharmacology," vol. 15, G. B. Koelle, Ed., Springer-Verlag, Berlin, Germany, 1963, pp. 89–128.

(6) B. Mendel, D. B. Mundell, and H. Rudney, *Biochem. J.*, **37**, 473 (1943).

(7) B. Mendel and H. Rudney, *ibid.*, **37**, 59 (1943).

(8) D. Nachmansohn and M. A. Rothenburg, *J. Biol. Chem.*, **158**, 653 (1945).

(9) G. B. Koelle, in "Handbook of Experimental Pharmacology," vol. 15, G. B. Koelle, Ed., Springer-Verlag, Berlin, Germany, 1963, pp. 187–298.

(10) M. G. Ord and R. H. S. Thompson, *Biochem. J.*, **46**, 346 (1950).

(11) R. E. Counsell and R. D. Ice, in "Drug Design," vol. VI, E. J. Ariens, Ed., Academic, New York, N.Y., 1975, p. 187.

(12) H. G. Gray, B. G. Humpris, W. V. Thorpe, K. White, and P. B. Wood, *Biochem. J.*, **59**, 162 (1955).

(13) A. R. Main, in "Biology of Cholinergic Function," A. M. Goldberg and I. Hanin, Eds., Raven, New York, N.Y., 1976, p. 281.

ACKNOWLEDGMENTS

Supported in part by U.S. Public Health Service Grants GM-10548 and HL-17655.

Effect of Macromolecules on Aqueous Solubility of Cholesterol and Hormone Drugs

DONALD E. CADWALLADER ˣ and DEVENDRA K. MADAN

Received August 6, 1980, from the *Department of Pharmaceutics, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication October 1, 1980.

Abstract □ The solubility of cholesterol and some hormone drugs in aqueous macromolecule solutions was investigated. Polyvinylpyrrolidone, dextrans, and heparin increased the solubilities of progesterone, testosterone, and diethylstilbestrol, while acacia, pectin, and carrageenans decreased their solubilities. Dextrans increased the solubilities of cholesterol and the three hormone drugs. Acacia and pectin greatly increased cholesterol solubility; however, these macromolecules significantly decreased progesterone and diethylstilbestrol solubilities and slightly decreased testosterone solubility. Heparin decreased cholesterol solubility and increased progesterone, testosterone, and diethylstilbestrol solubilities. Carrageenans significantly decreased cholesterol, progesterone, and testosterone solubilities and had little effect on diethylstilbestrol solubility. A strong bathochromic shift in the absorption spectra of progesterone and testosterone in polyvinylpyrrolidone solutions indicated an attachment between the polymer and the C=O group of the steroids.

Keyphrases □ Cholesterol—effect of macromolecules on aqueous solubility □ Hormone drugs—effect of macromolecules on aqueous solubility □ Solubility—cholesterol and hormone drugs, effect of macromolecules □ Macromolecules—effect on aqueous solubility of cholesterol and hormone drugs

Several macromolecular compounds have been reported to have hypercholesterolemic effects. Acacia and pectin (1–6) and dextran (6–11) have been reported to lower serum cholesterol in humans and animals. Heparin reportedly retards atherosclerosis development and minimizes the degree of hypercholesterolemia (12–14). Carrageenans have been found effective in suppressing hyperlipidemia and atherosclerosis in cholesterol-fed rabbits (15, 16). Polyvinylpyrrolidone was reported to have hypocholesterolemia activity (17).

These reports contain conflicting statements concerning the *in vivo* effect and mechanism of action of these macromolecules for lowering serum cholesterol. A previous article from this laboratory (18) reported on the interesting *in vitro* effects of carbohydrate macromolecules on the aqueous solubility of cholesterol. The large increases in the apparent solubility of cholesterol in 0.5% pectin and acacia solutions provided a possible explanation for the serum cholesterol-lowering ability of these hydrocolloids and prompted further solubility studies involving other macromolecules. This report concerns the effect of various macromolecules on the solubility behavior of cholesterol and the related hormone drugs, testosterone, progesterone, and diethylstilbestrol.

EXPERIMENTAL

Materials—Diethylstilbestrol[1], progesterone[2], and testosterone[3] were recrystallized from ethanol. Cholesterol[4] was purified by recrystallization from acetic acid, and the crystals were dried at 90°. The sample was then recrystallized from 70% alcohol–water and dried in a vacuum desiccator. The melting points and IR spectra of the compounds were in good agreement with literature values. Dextrans[5], pectin (citrus)[6], acacia[1], polyvinylpyrrolidone[7], heparin sodium[2], and carrageenans[8] were used as received.

Solubility Experiments—A stock solution of cholesterol in benzene was prepared containing 1.0 mg of cholesterol and 10 μCi of [26-^{14}C]-cholesterol[9]. Stock solutions of testosterone and progesterone were

[1] Matheson, Coleman and Bell.
[2] Sigma Chemical Co.
[3] Supplied by Schering Laboratories.
[4] Fisher Scientific.
[5] Dextran T_{40} and T_{70}, Pharmacia Laboratories.
[6] Eastman Kodak Co.
[7] NP-K30, GAF Corp.
[8] Seakem 11 and 21, Marine Colloids.
[9] Nuclear Chicago.

0022-3549/81/0400-0442$01.00/0

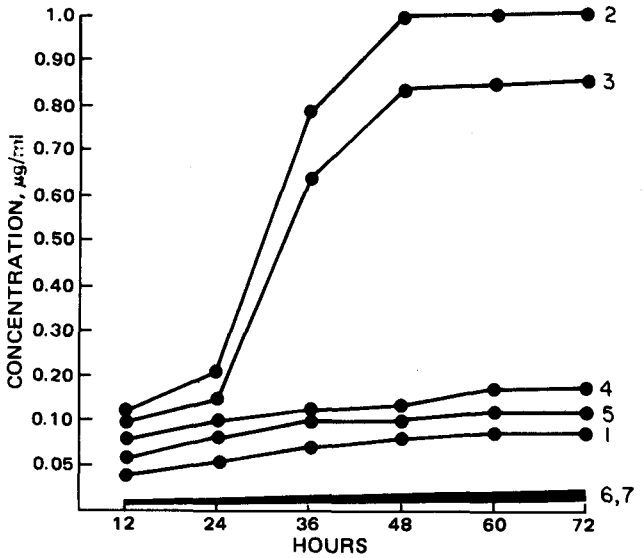


Figure 1—*Effect of various macromolecules on the aqueous solubility of cholesterol at 30°. Key: 1, water; 2, 0.5% pectin; 3, 0.5% acacia; 4, 10% dextran; 5, 6% dextran; 6, 1% heparin; and 7, 0.1% carrageenans.*

prepared to contain 200 mg of untagged compound and 5 μCi of labeled compound ([26-^{14}C]progesterone and [26-^{14}C]testosterone)[9] in 100 ml of benzene. A similar stock solution of diethylstilbestrol (using [*monoethyl*-1-^{14}C]diethylstilbestrol)[9] was prepared in methanol.

A known amount of a particular stock solution was transferred into a 125-ml iodine flask, and the solvent was evaporated under a mild nitrogen stream with constant shaking. A 50-ml volume of dissolution medium kept at 30° was added to the flask. Dissolution studies were carried out at 30 ± 0.5° using a controlled environment unit[10] to maintain the temperature. The solution was stirred at ~300 rpm using a magnetic stirrer and a 2.7 × 1-cm polytef[11]-coated spin bar. A 100-ml volume of dissolution medium in a 250-ml iodine flask was used for testosterone experiments.

For each dissolution study, the amount of compound used was in ~10-fold excess of its previously determined aqueous solubility. At predetermined intervals, samples were withdrawn from the system and filtered immediately and rapidly through a filtration assembly[12] containing a 0.45-μm filter. Because filtration adsorption losses may cause errors (19), stock solutions containing known amounts of the compounds were filtered and compared. No significant adsorption was detected with the rapid filtration technique.

Radioactive Assay—A 0.20-ml volume of the particle-free filtrate was pipetted into a liquid scintillation vial. To this vial was transferred 15 ml of dioxane–naphthalene scintillation cocktail (20). The scintillation vial was tightly capped and shaken for about 30 sec to ensure thorough mixing of the experimental solution and the cocktail. At the same time, a blank was prepared in the same manner except that the compound was omitted from the solution. The standard was prepared by pipetting 0.10 ml of the standard solution of the experimental compound into a scintillation vial. After the solvent (benzene or methanol) was evaporated, 0.20 ml of water and 15 ml of dioxane–naphthalene scintillation cocktail were added and the vial was shaken.

Duplicate samples along with appropriate standards and blanks were counted directly using a liquid scintillation system[13]. Sufficient time was allowed for the samples to cool to 4.0° to minimize quenching, and the samples were counted for 10 min. The solubility values were obtained from the ratio of the counts per minute of the internal standard and the sample.

UV Absorption Studies—Progesterone and testosterone in water both absorb a maximum of radiated energy at 249 nm. With a spectrophotometer[14], UV scans were carried out for each of these two hormones in aqueous solutions containing various concentrations of the macromolecules. Macromolecule solutions containing no hormone drugs were used as blanks in comparison cells.

[10] Hot Pack model 1278-8.
[11] Teflon.
[12] Millipore.
[13] Unilux II, Nuclear Chicago.
[14] Perkin-Elmer model 202.

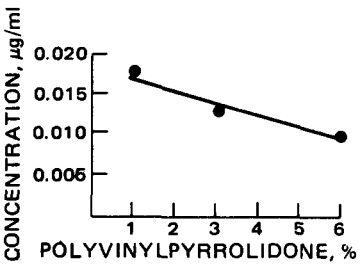


Figure 2—*Effect of polyvinylpyrrolidone solutions on the aqueous solubility of cholesterol after 72 hr at 30°.*

RESULTS AND DISCUSSION

The aqueous solubilities of cholesterol, progesterone, testosterone, and diethylstilbestrol were reported previously (21). Figures 1–7 illustrate the effect of various macromolecules on the solubility behavior of these compounds. Each point on the solubility curves represents an average of at least six or eight determinations. Statistical analyses of the data with *t* and *F* tests at 95% confidence levels indicated the differences in solubilities between various test solutions to be significant.

Figure 1 illustrates the effect of various macromolecules on cholesterol. The large increases in 0.5% pectin and acacia solutions and the slight increase in dextran solutions were discussed previously (21). Heparin and both carrageenans[8] decreased the aqueous solubility (at equilibrium) of cholesterol to ~0.01 and 0.02 μg/ml, respectively. Polyvinylpyrrolidone decreased the aqueous solubility of cholesterol, and the decrease was proportional to the polyvinylpyrrolidone concentration (Fig. 2).

The solution behavior of testosterone (Figs. 3 and 4) showed that heparin and 10% dextran slightly increased the solubility; 6% dextran (both high and low molecular weights), pectin, and acacia had no significant effect; and the carrageenans decreased solubility. Testosterone solubility was significantly increased in polyvinylpyrrolidone solutions, and the increase was proportional to the polyvinylpyrrolidone concentration (Fig. 7). The equilibrium solubility of testosterone in water and macromolecule solutions, with the exception of heparin, was approached from supersaturation and not from undersaturation. The portion of the solution behavior curve that depicts the solubility decrease after the peak was reached is apparently indicative of the rate at which the less soluble hydrated form of testosterone is crystallized out of solution (22–24). Heparin appears to prevent crystallization.

Polyvinylpyrrolidone, dextran (both high and low molecular weights), and heparin significantly increased progesterone solubility (Fig. 5). Pectin, acacia, and carrageenans[8] all decreased progesterone solubility to approximately the same level.

Polyvinylpyrrolidone, dextran (10%), and heparin increased diethylstilbestrol solubility, whereas dextran (at the 6 and 2% levels) and carrageenans had no significant effect (Fig. 6). Acacia and pectin decreased the aqueous solubility.

In general, polyvinylpyrrolidone, dextran, and heparin increased the solubilities of the hormone drugs while acacia, pectin, and the carrageenans decreased their solubilities. In this study, dextran was the only macromolecule that increased the solubilities of cholesterol and the three hormone drugs.

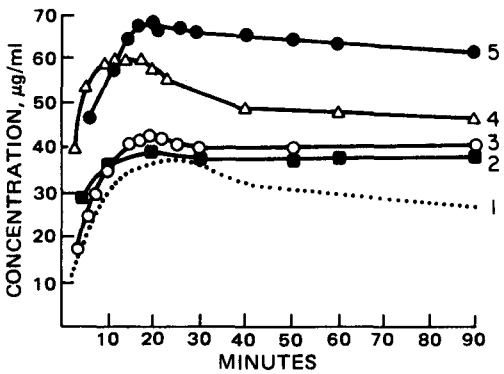


Figure 3—*Effect of various macromolecules on the aqueous solubility of testosterone at 30°. Key: 1, water; 2, 1% heparin; 3, 1% polyvinylpyrrolidone; 4, 3% polyvinylpyrrolidone; and 5, 6% polyvinylpyrrolidone.*

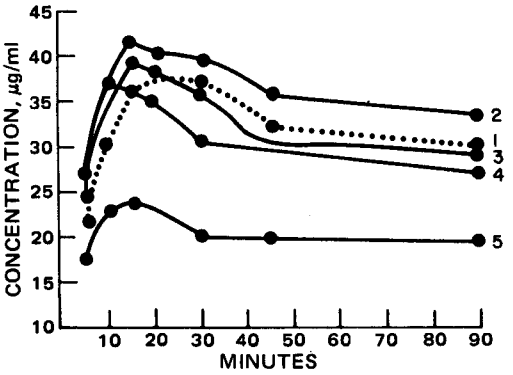


Figure 4—*Effect of various macromolecules on the aqueous solubility of testosterone at 30°. Key: 1, water; 2, 10% dextran; 3, 6% dextran; 4, 0.5% pectin or 0.5% acacia; and 5, 0.1% carrageenans.*

A 10% dextran solution almost doubled diethylstilbestrol and cholesterol solubilities, caused an appreciable increase in progesterone solubility, and produced a slight increase in testosterone solubility. Dextran at the 6% level increased cholesterol and progesterone solubilities; however, there was little effect on testosterone and diethylstilbestrol solubilities. Although the solubilities of cholesterol and the hormone drugs were dependent on the dextran concentration, the degree of solubility of each compound was inconsistent. For example, the solubilities of progesterone in 10, 6, or 2% dextran solution were nearly the same (17–16 μg/ml), while the solubilities for diethylstilbestrol in 10 and 6% dextran were 46.1 and 26.6 μg/ml, respectively. Solubility experiments were carried out with both high and low molecular weight dextrans[5]; in all instances, the solubility data were essentially identical for each test compound, and their curves were superimposable. The dextran solubility curves in Figs. 1 and 4–6 represent the behavior of both high and low molecular weight dextrans and indicate that the molecular weight of dextran was not a factor in these solubility phenomena. Dextran, a linear glucose polymer, forms colloidal solutions in water, and it is possible that drug molecules may be dispersed or adsorbed on the colloidal particles. Starches rich in amylose (25), cyclodextrins, and modified dextrans (26) form inclusion compounds and are capable of complexing with various drug molecules.

Acacia and pectin greatly increased cholesterol solubility; however, these carboxylic acid-type polysaccharides significantly decreased progesterone and diethylstilbestrol solubilities and slightly decreased testosterone solubility. On the other hand, heparin, a highly sulfated mucopolysaccharide, decreased cholesterol solubility and increased progesterone, testosterone, and diethylstilbestrol solubilities. Carrageenans[10]

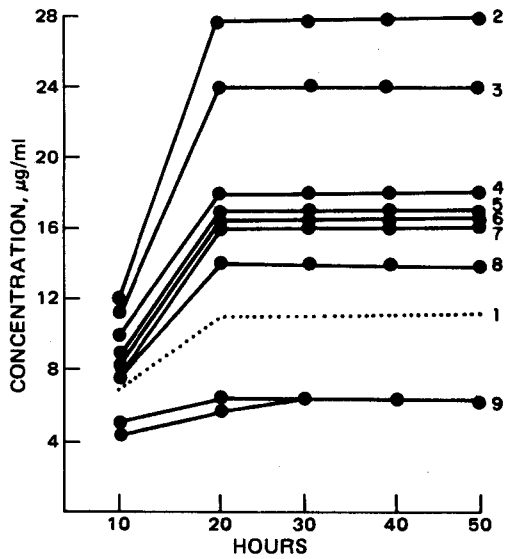


Figure 5—*Effect of various macromolecules on the aqueous solubility of progesterone at 30°. Key: 1, water; 2, 6% polyvinylpyrrolidone; 3, 3% polyvinylpyrrolidone; 4, 1% polyvinylpyrrolidone; 5, 10% dextran; 6, 6% dextran; 7, 2% dextran; 8, 1% heparin; and 9, 0.5% pectin, 0.5% acacia, or 0.1% carrageenans.*

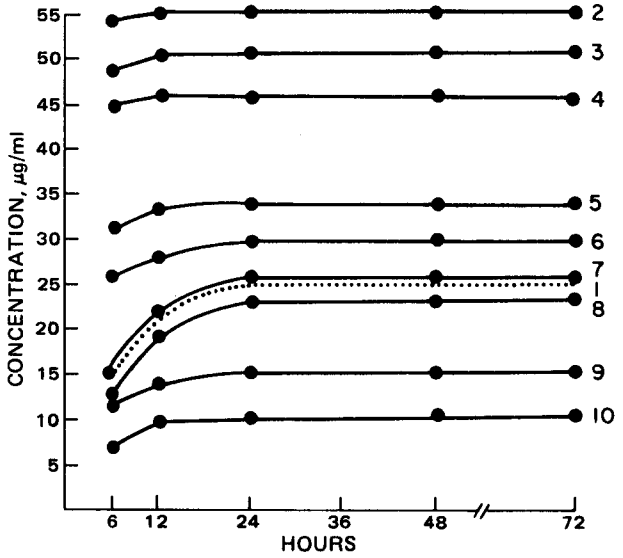


Figure 6—*Effect of various macromolecules on the aqueous solubility of diethylstilbestrol at 30°. Key: 1, water; 2, 6% polyvinylpyrrolidone; 3, 3% polyvinylpyrrolidone; 4, 10% dextran; 5, 1% polyvinylpyrrolidone; 6, 1% heparin; 7, 6% dextran; 8, 0.1% carrageenans; 9, 0.5% acacia; and 10, 0.5% pectin.*

(sulfated polysaccharides) at the 0.1% level significantly decreased the solubilities of cholesterol, progesterone, and testosterone and had little effect on diethylstilbestrol solubility. A pattern emerges that indicates that acid-type polysaccharides will interact and solubilize a very nonpolar, lipophilic molecule such as cholesterol and interact and reduce the solubilities of other more polar hormone compounds, whereas the opposite behavior occurs for the sulfated polysaccharides. This pattern holds for acacia, pectin, and heparin; however, the consistency appears to break down for the carrageenans, which decreased the solubility of cholesterol and other hormone compounds. This finding is not surprising since the carrageenans are strongly charged anionic polyelectrolytes of large size and can probably enter into reactions with other substances through several mechanisms involving ionic bonding, hydrogen bonding, or van der Waals forces.

Graham *et al.* (27) studied the complex formation between hydrocolloids and tranquilizers and hypotensive agents and found that the sulfated polysaccharides were the most reactive and that the carrageenans formed insoluble complexes with reserpine, chlorpromazine hydrochlo-

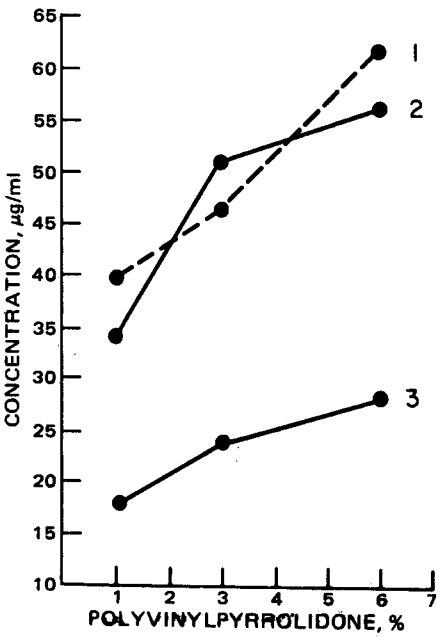


Figure 7—*Effect of polyvinylpyrrolidone on the aqueous solubility of testosterone (1), diethylstilbestrol (2), and progesterone (3) at equilibrium at 30°.*

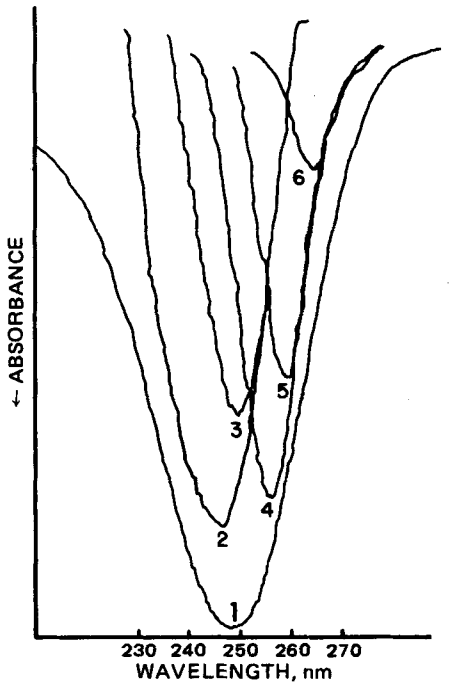


Figure 8—*Effect of polyvinylpyrrolidone on the absorption spectra of testosterone and progesterone. Key: 1, water; 2, 0.5%; 3, 1%; 4, 5%; 5, 10%; and 6, 20%.*

ride, and promazine hydrochloride. They reported the relative reactivities of four types of carrageenans with these drugs to be from 16 to 10 while the relative reactivities of acacia and pectin with the same drugs were 0.15 to 0.01. Pectin also formed precipitates with promazine and chlorpromazine hydrochlorides but not with reserpine; acacia did not precipitate any of these drugs (27). In the current study, the processes that bring about solubilization or decreased solubility phenomena of cholesterol and hormone drugs by various macromolecules are very difficult to describe qualitatively and quantitatively because of the complexities of both the hormone drugs and macromolecules (especially the natural hydrocolloids).

Polyvinylpyrrolidone significantly increased the testosterone, progesterone, and diethylstilbestrol solubilities (Fig. 7) and decreased cholesterol solubility (Fig. 2). Both increases and decreases in solubility were dependent on the polyvinylpyrrolidone concentration. Polyvinylpyrrolidone has the ability to complex with drugs, dyes, toxins, and other substances, and these complexes may be water soluble or water insoluble (28). Numerous investigations have been carried out on and mechanisms advanced for the binding of polyvinylpyrrolidone with drugs, preservatives, and organic ions such as dyes (29–40). Jurgensen Eide and Speiser (29, 30) reported that ionic compounds having an —OH, —NH_2, or —COOH group showed a higher degree of complexation than compounds without such groups and suggested that the complexing tendency was due to hydrogen bonding. Frank (26) suggested that van der Waals forces between the aromatic system of erythrosine ions and the paraffinic chain of the polyvinylpyrrolidone molecule are responsible for the binding of this dye. Higuchi and Kuramoto (31, 32) also suggested that the polyvinylpyrrolidone molecule could associate through ion dipole and van der Waals interactions. The polyvinylpyrrolidone molecule does not have an ionizable group; however, the lactam bond in the pyrrolidone ring represents a dipole that may undergo ion–dipole interaction with anions and aid in their binding by supplying the necessary attraction force to bring the two components into close contact (37).

Figures 8 and 9 show the effect of polyvinylpyrrolidone on the absorption spectra of testosterone and progesterone. As the polyvinylpyrrolidone concentration increased, the absorbance also increased. This bathochromic effect indicated an attachment between the polymer and the C=O group of the hormones. Anderson and Boyce (38) observed similar behavior in the interaction between erythrosine and polyvinylpyrrolidone, and other investigators (36, 37) attributed the bathochromic shifts observed between polyvinylpyrrolidone and erythrosine sodium dye to charge transfer or van der Waals forces, and Anderson and Boyce supported this suggestion (38). The decrease in the λ_{max} with the low (0.5%) concentration of polyvinylpyrrolidone might indicate micellar solubilization (41).

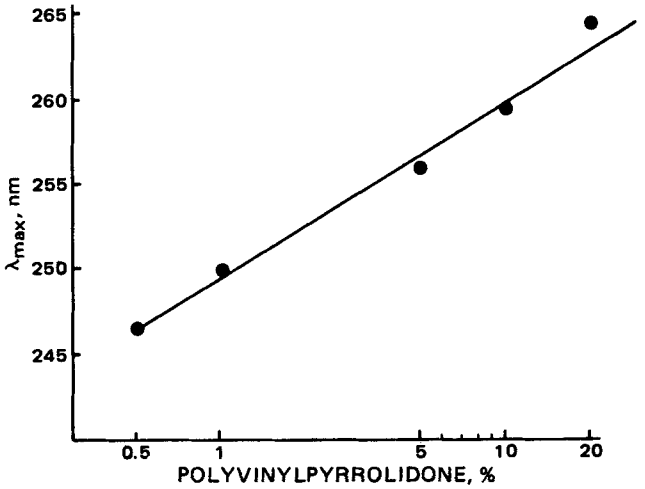


Figure 9—*Effect of polyvinylpyrrolidone solutions on the λ_{max} of testosterone and progesterone.*

With the carrageenans (0.25 and 0.5%), a slight shift toward longer wavelength was observed (from 249 to 252.5 nm), indicating direct participation with the carbonyl groups of progesterone and testosterone. No change was observed in the λ_{max} of testosterone or progesterone in aqueous solutions containing various concentrations (0.25–20%) of dextran, acacia, and pectin. That the shifts were not due to changes in pH or ionic strength of the system was confirmed by the absence of changes in the λ_{max} after the addition of acid, base, or 0.1 *M* NaCl.

REFERENCES

(1) A. F. Wells and B. J. Ershoff, *J. Nutr.*, **74**, 87 (1961).

(2) H. Fisher, P. Griminger, and W. Siller, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **24**, 263 (1965).

(3) A. Keys, F. Grande, and J. T. Anderson, *Proc. Soc. Exp. Biol. Med.*, **106**, 555 (1961).

(4) R. M. Kay, P. A. Judd, and A. S. Truswell, *Am. J. Clin. Nutr.*, **31**, 562 (1978).

(5) H. Fisher, G. W. Van der Woot, W. S. McGrath, and P. Griminger, *J. Atheroscler. Res.*, **6**, 190 (1966).

(6) T. M. Lin, K. S. Kim, E. Karvinen, and A. C. Ivy, *Am. J. Physiol.*, **188**, 66 (1957).

(7) A. W. Mollison and J. B. Rennie, *Br. Med. J.*, **1**, 893 (1954).

(8) G. Wallneius, *Nord. Med.*, **57**, 283 (1957).

(9) C. T. Flotte and R. W. Buxton, *Circulation (Suppl.)*, **28**, 721 (1963). *Ibid.*, **32**, 85 (1965).

(10) D. M. Brahmankar and W. E. Connor, *Circ. Res.*, **21**, 817 (1967).

(11) J. Ditzel and J. Dyerberg, *J. Atheroscler. Res.*, **10**, 5 (1969).

(12) H. Engelberg, R. Kuhn, and M. Steiman, *Circulation*, **23**, 489 (1956).

(13) L. Horlick and G. Duff, *Arch. Pathol.*, **57**, 417, 495 (1954).

(14) G. Szag and F. Harder, *ibid.*, **56**, 36 (1953).

(15) B. Ershoff and A. F. Wells, *Proc. Soc. Exp. Biol. Med.*, **110**, 580 (1962).

(16) K. Murata, *Nature*, **191**, 189 (1961).

(17) J. C. Allen, J. H. Baxter, and H. Goodman, *J. Clin. Invest.*, **40**, 499 (1961).

(18) D. K. Madan and D. E. Cadwallader, *J. Pharm. Sci.*, **59**, 1363 (1970).

(19) R. Hahnel, *J. Steroid Biochem.*, **2**, 61 (1971).

(20) G. A. Bray, *Anal. Biochem.*, **1**, 279 (1960).

(21) D. K. Madan and D. E. Cadwallader, *J. Pharm. Sci.*, **62**, 1567 (1973).

(22) A. L. Thakker and N. A. Hall, *ibid.*, **56**, 1121 (1967).

(23) *Ibid.*, **57**, 1394 (1968).

(24) *Ibid.*, **58**, 68 (1969).

(25) E. Guth and Z. Mansour, *J. Pharm. Sci.*, **57**, 404 (1968).

(26) S. G. Frank, *ibid.*, **64**, 1585 (1975).

(27) H. Graham, Y. M. Baker, and A. N. Njoku-obi, *ibid.*, **52**, 192 (1963).

(28) "PVP—An Annotated Bibliography, 1951–1966," vol. 1, General Aniline & Film Corp., New York, N.Y., 1967, pp. 13–20.

(29) G. Jurgensen Eide and P. Speiser, *Acta Pharm. Suec.*, **4**, 185

(1967).
(30) *Ibid.*, **4**, 20 (1967).
(31) T. Higuchi and R. Kuramoto, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 393 (1954).
(32) *Ibid.*, **43**, 398 (1954).
(33) G. M. Miyawaki, N. K. Patel, and H. B. Kostenbauder, *ibid.*, **48**, 315 (1959).
(34) D. Guttman and T. Higuchi, *ibid.*, **45**, 659 (1956).
(35) P. Molyneux and H. P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).
(36) H. P. Frank, S. Barkin, and T. R. Eirich, *J. Phys. Chem.*, **61**, 1375 (1957).
(37) R. E. Phares, Jr., *J. Pharm. Sci.*, **57**, 53 (1968).
(38) J. C. Anderson and G. M. Boyce, *ibid.*, **58**, 1425 (1969).
(39) A. P. Simonelli, S. C. Mehta, and W. I. Higuchi, *ibid.*, **59**, 633 (1970).
(40) M. J. Cho, A. G. Mitchell, and M. Pernarowski, *ibid.*, **60**, 720 (1971).
(41) S. Riegelman, N. A. Allawalla, M. K. Hrenoff, and L. A. Strait, *J. Colloid Sci.*, **13**, 208 (1958).

Analysis of Pilocarpine and Isopilocarpine in Ophthalmic Solutions by Normal-Phase High-Performance Liquid Chromatography

DANNY L. DUNN ˣ, BRITT S. SCOTT, and EDWIN D. DORSEY

Received June 26, 1980, from *New Product Research/Analytical Chemistry, Alcon Laboratories, Inc., Fort Worth, TX 76101.* Accepted for publication October 2, 1980.

Abstract □ A normal-phase high-performance liquid chromatographic separation for pilocarpine and isopilocarpine was developed which is suitable for the routine analysis of ophthalmic preparations. The method utilizes a column packed with 5-μm silica with a mobile phase of hexane–2% ammonium hydroxide in 2-propanol (70:30). Peak detection is by UV at 220 nm. It is suggested that a partition separation mechanism is involved rather than adsorption. A separation factor (α) of 1.17 was obtained with a relative separation (R_s) of 2.13. Column lifetimes were typically 6–8 months with daily use. Several standard pilocarpine–isopilocarpine mixtures and five commercially available ophthalmic solutions from different manufacturers were analyzed. The method was specific for pilocarpine and isopilocarpine in the presence of each other and pilocarpic acid and is a significant improvement over the nonspecific colorimetric methods of analysis.

Keyphrases □ Pilocarpine—high-performance liquid chromatographic analysis in the presence of isopilocarpine, ophthalmic solutions □ Isopilocarpine—high-performance liquid chromatographic analysis in the presence of pilocarpine, ophthalmic solutions □ High-performance liquid chromatography—separation of pilocarpine and isopilocarpine, ophthalmic solutions

Pilocarpine, a widely used alkaloid, possesses several important pharmacological properties. For example, it is a miotic and lowers intraocular pressure. The major ophthalmic application of pilocarpine has been for the treatment of glaucoma. The structure of pilocarpine is well established and contains both an imidazole and a lactone.

In aqueous solution, pilocarpine (I) decomposes through two major pathways, which are both base catalyzed (Scheme I). The lactone ring can undergo hydrolysis, resulting in the formation of pilocarpic acid (II), or epimerization can occur at the α-carbon to form isopilocarpine (III). Both pilocarpic acid and isopilocarpine are essentially pharmacologically inactive (1, 2).

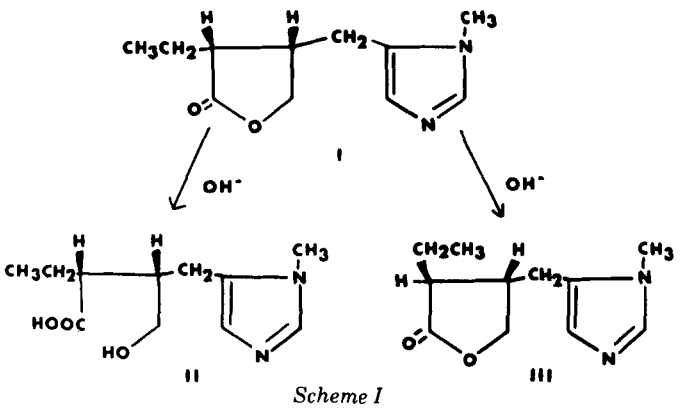
BACKGROUND

Analytical methods to measure the extent of pilocarpine degradation in ophthalmic medications have been published, but most cannot differentiate between pilocarpine and isopilocarpine. For instance, the USP method for determining pilocarpine is a colorimetric assay based on the formation of hydroxamic acid (3). Since the moiety responsible for producing color is an intact lactone ring, the assay can distinguish between pilocarpine and pilocarpic acid but not between pilocarpine and isopilocarpine (4). This flaw is serious since recent studies indicated that the primary degradation product of pilocarpine is not pilocarpic acid but its diastereomer, isopilocarpine (4).

Several techniques have been investigated to produce an analytical separation of pilocarpine and isopilocarpine. Some of the published methods include TLC (5), NMR (6–9), IR spectroscopy (10), GLC (8, 11), and polarimetry (12, 13). However, most of these separations are not suitable for development into a routine analysis. The reported TLC separation is questionable and could not be duplicated by one investigator (10); the NMR analysis requires an expensive 100-MHz instrument (10); IR analysis gives poor results when one diastereomer predominates (10), which is usually the case in ophthalmic solutions; and GLC requires derivatization prior to analysis, which makes multiple-sample runs too time consuming. Of these techniques, only polarimetry seems to have practical value. In fact, a UV–optical rotation analysis has been used successfully by this laboratory for several years[1].

The UV–optical rotation method requires elaborate sample preparation and is tedious in actual practice. An analytical procedure such as high-performance liquid chromatography (HPLC), which minimizes sample preparation and retains a high separation capability, would be highly desirable, and several HPLC separations of pilocarpine and isopilocarpine have been reported. Initially, ion-exchange columns packed with cation-exchange resins[2] were used with UV peak detection at 217 nm (4). Unfortunately, these HPLC systems gave variable peak retention times and generally produced erratic results (10, 14).

Khalil (15) reported an HPLC analysis for pilocarpine using an octa-



Scheme I

[1] See B. S. Scott, D. L. Dunn, and E. D. Dorsey, *J. Pharm. Sci.*, in press.
[2] Aminex A-7 (7–11 μm), Bio-Rad Laboratories.

0022-3549/81/0400-0446$01.00/0