Solubilization and Evaluation of Poly-*N*-vinyl-5-methyl-2-oxazolidinone Barbiturate Systems

By BERNARD B. WOLFSON* and GILBERT S. BANKER

A study was undertaken to investigate the solubilization (complexation) of phenobarbital and several other barbituric acid derivatives by a nonionic macromolecule, poly-*N*-vinyl-5-methyl-2-oxazolidinone (PVOM). A method was demonstrated whereby the acidification of an aqueous solution of PVOM and sodium phenobarbital proved to enhance significantly the extent of the interaction of polymer and drug over other methods studied. A comparison of several barbiturates has shown that the greater the aliphatic character of the disubstitution at the 5-position of barbituric acid, the lesser the affinity of the barbiturate for the polymer. PVOM was also shown to interact with phenobarbital to a greater degree than polyvinylpyrrolidone. Dialytic release rates and *in vivo* evaluation of polymer-drug solutions demonstrated no appreciable difference in the availability of the drug from the drug complex with respect to the free drug. The nature of the polymer-drug interaction was elucidated by various chemical and analytical techniques.

WEAK ORGANIC acids or bases currently are employed to a great extent as effective therapeutic agents by the medical profession. Since these medicaments are relatively insoluble in water, they are usually administered or applied as solid or semisolid dosage forms. Frequently, aqueous solutions of these agents are desired; when this is so, the soluble salts are utilized. Many times, however, soluble salts of a drug or class of drugs may not be stable in aqueous solution. One such class of drugs is the barbiturates.

The degradation of barbiturates in alkaline solutions is well known to pharmaceutical practitioners (1, 2). The stability may be increased by selection of a suitable cosolvent or solubilizing agent, *e.g.*, alcohol (3), propylene glycol (4), polysorbate 80 (5), etc., and/or by a lowering of the pH (6). If one desires to obtain maximum stability of a barbiturate by lowering the pH, precipitation of the free acid occurs when the pH of the solution reaches the pKa′ of the respective barbiturate [pKa′ of commonly used barbiturates is between 7–8 (7)]. At this point, the concentration of the free acid formed is greater than its aqueous solubility. This "good stability" and "poor solubility" (8) situation has encouraged researchers to seek new and better methods for solubilizing the acidic forms of the barbiturate drugs.

Complexation may provide one answer to the good stability–poor solubility problem. Martin (9) has defined an organic coordination compound or molecular complex as "one held together by weak forces of the donor-acceptor type or by hydrogen bonds." Essentially inert, nonionic macromolecules, such as polyvinylpyrrolidone (10), polyglycols (11), and methylcellulose (12), have been shown to form both soluble and insoluble molecular complexes with a large number of acidic compounds in aqueous solution. Recently, Rich (13) has demonstrated that a new nonionic macromolecule, poly-*N*-vinyl-5-methyl-2-oxazolidinone, exhibited weaker complexing tendencies than was shown by the structurally similar polyvinylpyrrolidone, for the same compounds in aqueous solution. The authors (10–13) attributed complex formation primarily to a dipole–dipole interaction or to hydrogen bonding between the polymer and the drug. However, the degree of complexation or the extent to which the drugs were solubilized rarely attained a concentration that would be of any practical significance to the pharmaceutical industry, especially in the case of the barbiturate drugs. It was for this reason that this study was undertaken in an attempt to solubilize enough of a barbiturate or barbiturates by complexation phenomenon, so that a stable aqueous dosage form might be produced, containing at least as much of the acidic barbiturate moiety as the official elixirs.

EXPERIMENTAL

Materials and Equipment.—Poly-*N*-vinyl-5-methyl-2-oxazolidinone,[1] (PVOM) (14) is a white free-flowing polymeric powder whose structural

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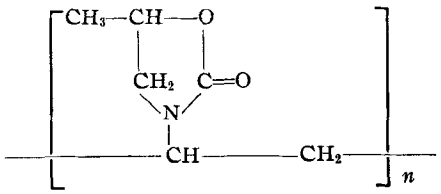
* Present address: Ayerst Laboratories, Inc., Rouses Point, N. Y.

[1] Devlex 130. Supplied through the courtesy of the Dow Chemical Co., Midland, Mich.

formula may be represented as in Scheme I. The



Scheme I

properties of PVOM (15) are in some respects similar to those of naturally occurring proteins. The solubility properties of PVOM are suggestive of the albumin group of proteins, which are soluble readily in pure water but are salted out by neutral salts. Although the polymer is extremely stable to hydrolytic cleavage, it displays other properties typical of proteins, such as denaturation-insolubilization at its isoelectric point (highly alkaline solutions) and heat coagulation. Heat coagulation of PVOM is reversible and occurs at about 40°. No permanent chemical or physical property effects have been observed following repeated phase transitions. Aqueous solutions of the polymer exhibit viscosities markedly lower than expected for a polymer of its molecular weight (approximately 165,000). The relative viscosity of a 1% solution of PVOM in water, based on the *K*-value of Fikentscher (16), is 31.8. Aqueous solutions of this polymer are essentially neutral.

Polyvinylpyrrolidone (PVP),[2] a polymer structurally similar to PVOM, was studied for its relative complexing tendencies for phenobarbital.

Phenobarbital, either as the recrystallized U.S.P. free acid or the sodium salt U.S.P., was chosen as the model barbiturate for the major portion of this research. The selection of phenobarbital over the other barbituric acid derivatives was based on (*a*) a chemical structure which suggested a greater possibility for interaction with PVOM, (*b*) solubility and other physical and chemical characteristics indicative of the behavior of the commonly used barbiturates, and (*c*) well-defined chemical, physical, and pharmacological properties, as described in many literature references. Six other U.S.P. and N.F. barbiturates were studied for extent of solubilization by interaction with PVOM.

Assay of Barbituric Acid Derivatives.—All barbiturates were assayed at 241 mμ with a Beckman DU spectrophotometer by the differential ultraviolet spectrophotometric procedure of Walker and co-workers (17), which employed the difference maxima obtained from the nonionized and the first ionized species of the barbiturate. The assay procedure was not only insensitive to the presence of the decomposition products of the barbituric acid derivative (18) but also to the polymer.

Standard Solubility Method.—A standard solubility method (11) was employed to determine the extent of the complexing tendency of PVOM for phenobarbital. An excess quantity, 400 mg. of phenobarbital, was placed in each of twelve 50-ml. portions of a U.S.P. phosphate buffer solution (pH 5.8) containing 0 to 10% PVOM. The samples were equilibrated for 48 hours in a constant temperature rotating water bath at 25 ±1° and subsequently were assayed for phenobarbital content.

Stability Studies.—The effect of PVOM on the hydrolytic rate of phenobarbital was ascertained by conducting stability studies of PVOM–phenobarbital solutions (0.20–0.80% PVOM–0.18% phenobarbital at pH 8.05, 9.00, and 9.50). The ionic strength of the buffer solutions was adjusted to 1.0 with potassium chloride. The solutions were initially assayed for phenobarbital content, after which they were stored in 4-oz. clear glass bottles in a water bath at 30 ± 0.1°. The solutions were then assayed periodically for phenobarbital content.

Acidification Solubility Method.—A method was developed whereby the acidification of an aqueous solution of PVOM and sodium phenobarbital to below the pH of 7.41 (pKa′ phenobarbital is 7.41) proved to enhance significantly the extent of interaction of polymer and drug with respect to the results of the previously mentioned standard solubility method. Upon acidification of a 0.50% aqueous solution of sodium phenobarbital in the presence of 0.2% or more of PVOM, phenobarbital did not immediately precipitate from solution as the phenobarbital in the absence of polymer did. However, precipitation of the free acid did occur slowly in the presence of the lower concentrations of polymer and was detected visibly after a few days, while solutions containing higher concentrations of polymer appeared to inhibit precipitation of phenobarbital.

The following procedure exemplifies the preparation of a PVOM–phenobarbital solution.

A specified amount of polymer, corresponding to 0 to 12% polymer, was dissolved in about 80 ml. of a solution containing the sodium propionate fraction of a propionic acid–sodium propionate combination preservative-buffer system. One-half gram of sodium phenobarbital (corresponding to 0.46 Gm. of phenobarbital) was dissolved in this solution. Ten milliliters of a propionic acid stock solution, containing enough of the acid to produce 100 ml. of a buffer solution of the desired pH (4.2, 5.1, or 5.8), was pipeted into the sample. The sample was brought to a volume of 100.0 ml. with the sodium propionate solution and was assayed for initial phenobarbital content. Each solution thus prepared was then placed in a 3-oz. amber square bottle and was assayed for phenobarbital content at various time intervals to determine the equilibrium (solubility) of these systems. The three pH levels used in this part of the study were selected to (*a*) assure adequate stability of the barbiturate without the solution being too acid (commercial aspect) and (*b*) determine what effect a slight change of pH would have on the extent of complexation between polymer and drug.

The interaction of polymer and drug at a pH of 1.7, which was more acid than could be obtained with the propionic acid buffer system, was studied in 0.06 *N* HCl using the acidification solubility method of addition.

Other methods of producing the PVOM–phenobarbital interacted systems in acid media were investigated, involving the addition of sodium phenobarbital or PVOM and sodium phenobarbital to the acid buffer solutions. Gelatinous precipitates, which slowly redissolved, formed when these other methods were employed. Consequently, the acidification solubility method was employed for the preparation of the PVOM–barbiturate systems.

[2] Plasdone, type NP-K30. Supplied through the courtesy of General Aniline and Film Corp., New York, N. Y.

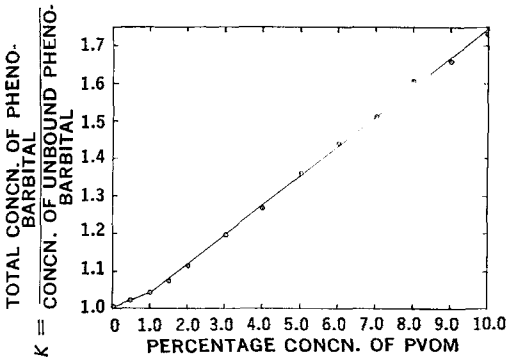


Fig. 1.—Standard solubility method. Interaction of PVOM and phenobarbital (total drug content, 3.44×10^{-2} *M*) at 25°, pH 5.8.

Solubilization of Various Barbiturates in a PVOM Buffered System by the Acidification Solubility Method.—A comparative study of the solubility of other PVOM–barbiturate systems and the PVOM–phenobarbital system was undertaken to determine what effect the disubstitution at the 5-position of barbituric acid would have on the solubility (complexation) phenomenon. Solutions were made of the sodium salts of amobarbital, butabarbital, pentobarbital, and secobarbital and of calcium cyclobarbital, with varying concentrations of PVOM. These solutions were acidified to pH 4.2 utilizing the appropriate propionate buffer. The pH 4.2 was well below the pKa′ of the various barbiturates (7) and was also an effective pH for the propionate preservative system. These solutions were assayed periodically for barbiturate content.

Miscellaneous Systems.—Other polymer–barbiturate solutions prepared by the acidification solubility method included: (*a*) 0.46% of phenobarbital and varying amounts of polyvinylpyrrolidone in propionate buffer (pH of system, 4.2) to compare the complexing tendencies of PVP and PVOM, (*b*) 2.23% of barbital and varying amounts of PVOM in propionate buffer (pH of system, 5.4) to determine the effect of both the relatively high solubility of the free acid barbital and its short diethyl substitution at the 5,5-position of barbituric acid on the degree of complexation, and (*c*) 0.73, 0.91, and 1.10% of phenobarbital and varying amounts of PVOM in propionate buffer (pH of systems, 4.3, 4.4, and 4.5, respectively) to determine the maximum amount of phenobarbital that may be solubilized within certain limits of polymer concentrations.

Dialysis Studies.—Dialysis studies were conducted to determine if the dialytic rate or dialyzate equilibrium of the phenobarbital in the polymer–drug interacted system varied greatly from the rate and equilibrium of the unbound drug in artificial gastrointestinal fluids. It was thought that this test might give some preliminary indication concerning whether an alteration in the rate of absorption or availability of the drug from the complex might be expected. The dialysis membrane[3] was verified to be impermeable to the macromolecule but allowed the low molecular weight drug to traverse the membrane freely.

Dialysis sacs containing solutions of either phenobarbital or PVOM and phenobarbital were prepared in artificial gastrointestinal fluids (without enzymes) at pH 1.4, 5.1, and 7.5, and these sacs were rotated for preselected time periods of 0.5, 2.5, 4.5, 6.5, and 8 hours at 37 ± 2°. The initial concentration of phenobarbital in the 20-ml. sac was 1.10 mg./ml. The contents inside the sac as well as the surrounding medium (70 ml.) were assayed for phenobarbital.

The effect of concentration of PVOM–phenobarbital on the dialysis of the system was determined by conducting dialysis studies of solutions of PVOM–phenobarbital in 0.001 *N* HCl at 25 ± 1° in such a concentration to exceed greatly the solubility of the free drug in this medium (approximate solubility of phenobarbital, 1.2 mg./ml.). The initial concentration of PVOM–phenobarbital in the 20-ml. sac was 4.56 mg. phenobarbital per milliliter with varying concentrations of PVOM (0.5–10.0%).

In Vivo Procedures.—The onset, extent, and duration of sedation of PVOM–phenobarbital *versus* sodium phenobarbital were determined with Williamson activity cages (19) using previously nondrugged female Holtzman strain albino rats having a weight range of 150 to 240 Gm.

Animals were orally dosed using a rubber catheter and syringe with either (*a*) an aqueous solution of sodium phenobarbital (50 mg./Kg.), (*b*) an aqueous solution of PVOM–phenobarbital complex (46 mg. drug/Kg. biologically equivalent dose), (*c*) distilled water (2.5 ml.), or (*d*) an aqueous solution of PVOM (2.5 ml., 6.0%). The animals were dosed between 3:45 and 4:45 p.m. to eliminate diurnal activity variation (rats more active at night) and were placed in the activity cages located in a dark isolated room. Animal activity was recorded for a period of 12 hours.

Acute toxicity studies were undertaken at a dose level of 170 mg./Kg. of sodium phenobarbital and an equivalent dose of the PVOM–phenobarbital interacted system.

Elucidation of Drug Complex.—The nature of the polymer–drug interaction was elucidated by subjecting the precipitates obtained from both acid and alkaline solutions containing approximately equal amounts of PVOM and barbiturate to ultraviolet and infrared spectral analyses, melting point determinations, and solubility studies.

RESULTS AND DISCUSSION

Solubilization of Phenobarbital by the Standard Solubility Method.—Figure 1 depicts the effect of increasing quantities of PVOM on the solubility of phenobarbital following the addition of phenobarbital to the buffered PVOM solutions. The amount of phenobarbital in equilibrium with the solid phase increased linearly with polymer concentration, owing to the complex formed between polymer and drug. Due to the linear relationship of the reaction products, the stoichiometric ratio and hence the true stability constant of the complex formed could not be determined from the phase diagram. The *K* values of Fig. 1 were obtained by dividing the total concentration of barbiturate present in solution (both bound and unbound) by the concentration of unbound drug in solution.

Stability Studies.—PVOM was shown to exhibit no stabilizing effect on the hydrolytic rates of aqueous alkaline solutions of phenobarbital at 30°. As expected, the hydrolytic reactions of pheno-

[3] NoJax Casing, size 30, Visking Co., Chicago, Ill.

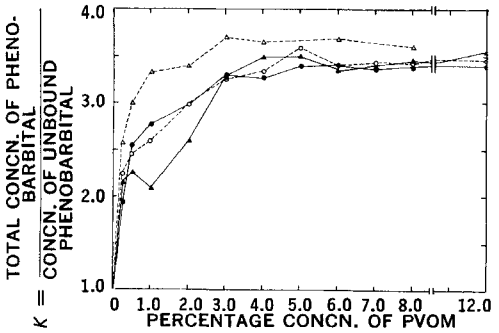


Fig. 2.—Acidification solubility method. Solubilization of phenobarbital (total drug content, $1.97 \times 10^{-2} M$) by PVOM at various acid pH levels. Key: △, pH 1.7; ●, pH 4.2; O, pH 5.1; ▲, pH 5.8.

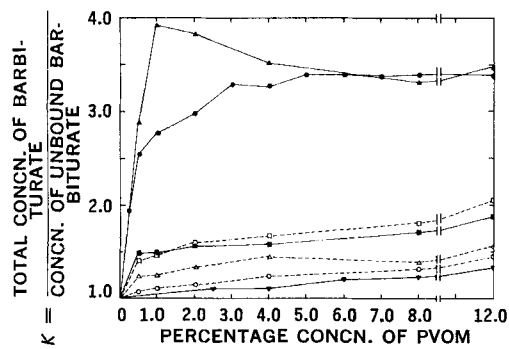


Fig. 3.—Acidification solubility method. Comparison of the solubilization of certain barbituric acid derivatives by PVOM. Key: ▲, butabarbital ($2.13 \times 10^{-2} M$) at pH 4.2; ●, phenobarbital ($1.97 \times 10^{-2} M$) at pH 4.2; □, cyclobarbital ($1.96 \times 10^{-2} M$) at pH 4.2; ■, amobarbital ($2.01 \times 10^{-2} M$) at pH 4.2; △, pentobarbital ($2.01 \times 10^{-2} M$) at pH 4.2; O, secobarbital ($1.92 \times 10^{-2} M$) at pH 4.2; ▼, barbital ($1.21 \times 10^{-1} M$) at pH 5.4.

barbital were of pseudo-first-order nature (19) since a linear relationship existed between the log of absorbance and time, and the rate of hydrolysis was accelerated greatly by the catalytic action of hydroxyl ion concentration.

PVOM–Phenobarbital Solutions Prepared by the Acidification Solubility Method.—Periodic assays of the PVOM–phenobarbital solutions verified that equilibrium existed at approximately 60 days. The spectral readings of the PVOM–phenobarbital propionate buffer solutions (pH 4.2, 5.1, and 5.8) and of the PVOM–phenobarbital 0.06 *N* HCl solutions (pH 1.7) for phenobarbital content are plotted in Fig. 2.

The results indicated that no appreciable difference exists in the complexing tendencies of PVOM for phenobarbital at pH 4.2, 5.1, and 5.8. These curves appear to exhibit the same general slope and essentially to overlap one another. A slight discrepancy in the curve at pH 5.8 was observed in a few solutions of lower polymeric concentration. Visual examination and pH determinations of these solutions indicated that the preservative activity and buffer capacity of the propionate preservative–buffer system was inadequate at pH 5.8.

The curve obtained for the solutions at pH 1.7 denotes slightly greater solubilization ratios (*K* values) than the ratios obtained for the solutions at the pH range of 4.2 to 5.8. These somewhat higher *K* values at pH 1.7 may be attributed to a slightly lower solubility of the free phenobarbital at pH 1.7 than at pH 4.2 to 5.8. However, it is safe to conclude from Fig. 2 that the degree of acidity (< pH 5.8) has no adverse effect on the interaction of PVOM and phenobarbital in these particular systems.

Temperature changes from 0 to 35° were shown to have no apparent effect on solutions of the PVOM–phenobarbital complex. Heat coagulation of the systems occurred at 40°. However, dissolution of these coagulates readily took place at temperatures below 40°.

The solubility data (Fig. 2) show that approximately 5 to 6% PVOM is needed to assure the solubilization of phenobarbital (0.46%) at pH 1.7, 4.2, 5.1, and 5.8. The great increase in the solubilization of phenobarbital by PVOM achieved with the acidification solubility method, compared with the solubilization achieved with the standard solubility method, is indicated by comparing the *K* values of Figs. 1 and 2. The acidification solubility method, by promoting the transition of the essentially ionized sodium phenobarbital species to the essentially nonionized phenobarbital species in the presence of PVOM, apparently enhances the extent of interaction of PVOM and phenobarbital. It appears that both the solute and complexing agent must be brought into intimate contact with each other to gain the maximum effect of the complexing reaction.

Comparative Study of Various PVOM–Barbiturate Solutions Prepared by the Acidification Solubility Method.—The solubility phase diagrams (Fig. 3) show that PVOM exhibits a strong attraction for the phenobarbital molecule, but a weaker relative attraction is observed for the other barbiturates studied, except for butabarbital.

The relatively great reactivity of phenobarbital can probably be ascribed to the aromatic phenyl

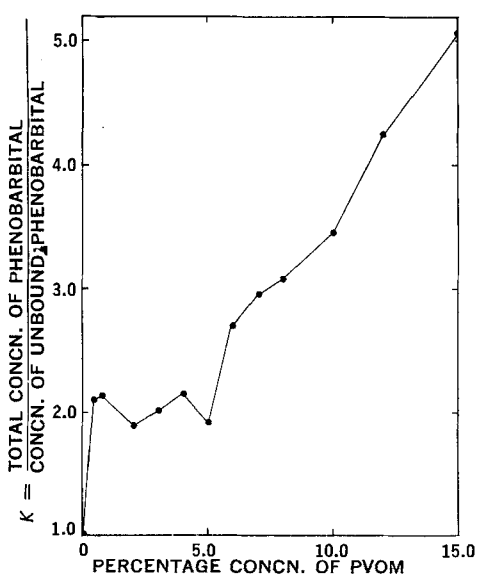


Fig. 4.—Acidification solubility method. Solubilization of phenobarbital (total drug content, $3.14 \times 10^{-2} M$) by PVOM at pH 4.3.

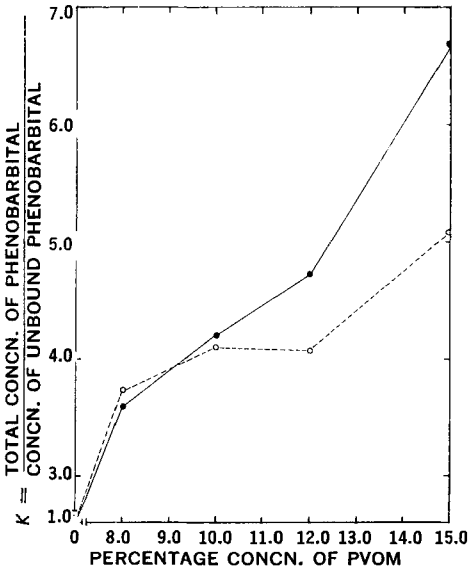


Fig. 5.—Acidification solubility method. Solubilization of phenobarbital (total drug content: ●, $3.92 \times 10^{-2}M$ at pH 4.4; O, $4.74 \times 10^{-2}M$ at pH 4.5) by PVOM.

group at the 5-position. This group introduces a greater polarity to the phenobarbital molecule than is imparted by the lesser aromatic, more aliphatic groups, at the corresponding 5-position of the other barbituric acid derivatives studied. For example, cyclobarbital exhibits less affinity for PVOM than shown by phenobarbital, probably due to the less aromatic nature of the cyclohexenyl group of cyclobarbital with respect to the phenyl group of phenobarbital.

Butabarbital exhibits a considerable increase in solubility in the presence of lower concentrations of PVOM, reaching a maximum increase at about 1% polymer. The curve tapers off somewhat as the concentration of polymer is increased until a plateau is reached at approximately 5% polymer. Relatively high K values exist throughout, even at the plateau region. Upon the introduction of just one methyl group to the 1-methyl-propyl group at the 5-position of butabarbital (amobarbital, pentobarbital), a considerable decrease in the solubilization of the barbiturate by PVOM is observed. Since secobarbital contains the largest aliphatic disubstitution of the barbiturates studied, it is not surprising that this molecule shows the least affinity for the polymer.

Since relatively short diethyl groups are the 5,5-substitutions in the barbital molecule, large K values were expected. The phase diagram indicates quite the opposite effect. Low K values were obtained throughout the polymer concentration range of 0 to 12.0%. These low complexation ratios are probably due to the relatively high hydrophilic nature of barbital (solubility approximately tenfold that of the other barbiturates studied).

It is evident that the disubstitution at the 5-position of barbituric acid exerts a definite influence on the interaction of PVOM and the several barbiturates studied. Apparently the greater the aliphatic nature of the disubstitution, the weaker the affinity of the barbiturate for the polymer. This decreased affinity may be due in part to a decrease in polarity of the molecule and/or to steric factors.

Determination of the Solubility Limit of Phenobarbital with PVOM by the Acidification Solubility Method.—The solubilization of systems initially containing 0.73% phenobarbital and 0–15.0% PVOM is depicted by Fig. 4. A plateau region is exhibited by solutions containing approximately 0.4 to 5% polymer, which gives lower K values than the respective K values obtained for the 0.46% solutions of phenobarbital (Fig. 2). However, as the concentration of PVOM exceeds 5%, the solubility of phenobarbital increases progressively until a 15% polymer solution shows a K value of about 5.

The solubility of systems initially containing 0.91 and 1.10% phenobarbital and 8.0 to 15.0% PVOM is illustrated by Fig. 5. The samples containing 1.10% phenobarbital exhibited a plateau region at approximately 10 to 12% polymer. The K values of this region and of the 15% polymeric solutions are smaller than the respective K values of the 0.91% phenobarbital solutions.

These solubility results seem to indicate that the effect of the initial concentration of sodium phenobarbital in solution has some definite unexplained bearing on the solubility (complexation) phenomenon of the PVOM–phenobarbital systems.

Comparison of PVOM–PVP Phenobarbital Solutions Prepared by the Acidification Solubility Method.—Figure 6 represents a comparative study conducted with 0.46% solutions of phenobarbital containing varying concentrations (0–12.0%) of the respective polymer. Contrary to the findings of Rich (13), these phase diagrams show PVOM to have a greater affinity for phenobarbital than PVP.

Drug Availability from Polymer–Drug Solution.—Dialysis studies in artificial gastrointestinal fluids at pH 1.4, 5.1, and 7.5 have shown the traversal rate of phenobarbital of a PVOM–phenobarbital solution to be slightly slower than the traversal rate of phenobarbital of a respective free phenobarbital solution (Table I). The dialytic data were reported as

$$\frac{\text{inside concn. (mg./ml.)} - \text{outside concn. (mg./ml.)} \times 100}{\text{total concn. (mg./2.0 ml.)}} = \text{excess \% drug in sac at a specific time}$$

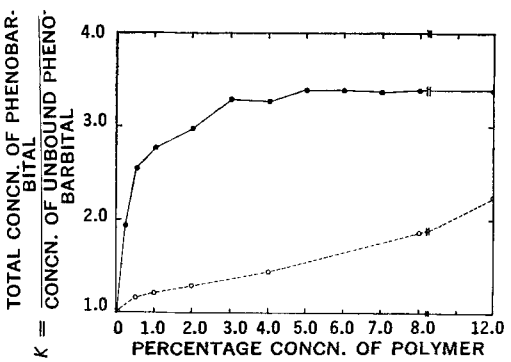


Fig. 6.—Acidification solubility method. Comparison of the solubilization of phenobarbital (total drug content, $1.97 \times 10^{-2}M$) by PVOM and PVP at pH 4.2. Key: ●, PVOM; O, PVP.

TABLE I.—COMPARISON OF THE DIALYTIC RATES OF FREE PHENOBARBITAL AND OF PVOM–PHENOBARBITAL SOLUTIONS

pH of Fluid	Excess % of Drug in Sac[a] 0.5 Hr.	1.5 Hr.	2.5 Hr.	4.5 Hr.	6.5 Hr.	8 Hr.
Free Drug Soln.						
1.4	73.27	31.04	15.38	2.49	0.99	0.27
5.1	72.76	31.14	8.88	1.70	0.00	1.08
7.5	77.89	41.93	16.81	2.44	0.97	0.05
Polymer-Drug Soln.						
1.4	76.12	34.24	19.00	6.25	5.06	4.41
5.1	73.10	34.52	16.86	6.97	2.52	4.51
7.5	78.61	41.19	22.59	7.47	6.69	5.57

[a] These per cent figures represent the average of duplicate sets of solutions at pH 1.4 and 7.5. Only one set of solutions was conducted at pH 5.1.

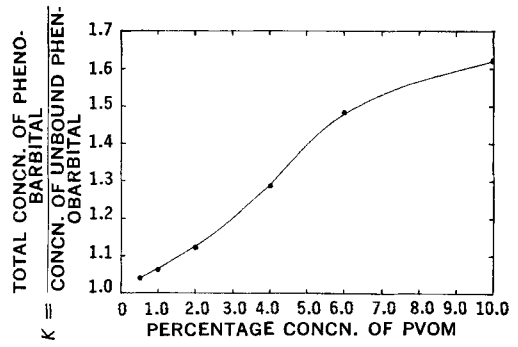


Fig. 7.—Effect of polymer concentration on the dialysis of phenobarbital (initial drug concentration in 20-ml. sac, 4.56 mg./ml.) at 25°, pH 2.8.

Effect of Concentration of PVOM–Phenobarbital Solutions on the Dialysis of the System.—Figure 7 depicts the effect of concentration of PVOM on the dialyzate equilibrium of phenobarbital (4.56 mg./ml. initially in 20-ml. sac) after 48 hours, illustrated by the K values (ratio of total drug concentration in the internal solution to concentration of unbound drug in the external solution). The K values of the drug are shown to increase progressively with higher concentrations of polymer. The results appear to substantiate the water replacement hypothesis (11), because the larger the concentration of both the polymer and the drug, the greater the opportunity for the acidic hydrogens of the solute to coordinate with the negative centers of the polymer to replace water from these negative groupings.

In Vivo Procedures.—The results obtained from the *in vivo* drug response of phenobarbital and of a PVOM–phenobarbital interacted system are illustrated by Fig. 8. Each point represents an average value for five animals. These preliminary results indicate no difference in the onset, extent, and duration of animal sedation for the free drug and the polymer-drug samples. These data substantiated the results of the dialytic rate studies previously conducted which showed only a slightly slower traversal rate of the drug of a polymer-drug solution than that of a free drug solution.

In a preliminary acute toxicity study, eight out of 11 female rats died within 24 hours when orally dosed with 170 mg./Kg. of free sodium phenobarbital in aqueous solution, while an equivalent dose of 155 mg./Kg. of phenobarbital when administered in solution as the polymer-drug interacted system killed three out of the 11 rats.

This preliminary acute toxicity study suggested that the toxicity of the drug is not augmented by the presence of polymer and may even be somewhat reduced due to an apparently slightly slower absorption of the drug.

Chronic toxicity studies of PVOM performed on both male and female rats (15) showed no evidence of adverse effects.

Elucidation of Complex.—Gelatinous precipitates were observed upon the acidification of solutions containing approximately equal concentrations of PVOM and sodium phenobarbital (> 1.5%). However, as the concentration of polymer was increased with respect to the drug, precipitation of the complex decreased. The analyses of the gelatinous precipitates (Table II) were indicative of some kind of interaction occurring between polymer and drug, for polymer alone was not precipitated by the addition of acid.

Standard solubility studies (11) of the acidic precipitated PVOM–phenobarbital products were conducted in both distilled water and absolute ethanol.

Analysis of the aqueous solution for phenobarbital content showed a K value of about 3.2. This appreciable increase in the solubility of phenobarbital was clearly an indication of the existence of an association of polymer and drug.

Analysis of the alcoholic solution revealed the recovery of most of the phenobarbital initially present in the complex. Since PVOM is essentially insoluble in absolute ethanol, the presence of phenobarbital in the alcohol was an indication that the

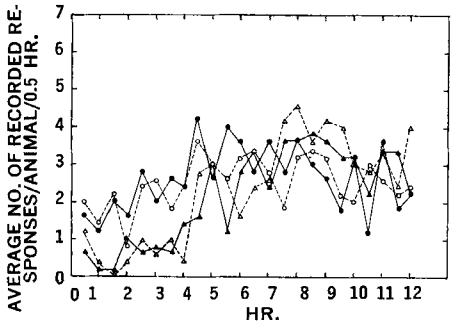


Fig. 8.—*In vivo* comparison of PVOM–phenobarbital and sodium phenobarbital solutions. Key: ▲, NaPb group, 50 mg./Kg.; △, PVOM–Pb group, 46 mg./Kg. (biol. equiv.); ●, H_2O control group; O, PVOM control group.

TABLE II.—DRUG CONCENTRATION AND MELTING POINTS OF PRECIPITATED PRODUCTS

Initial Amt. of Drug, Gm.	Initial Amt. of Polymer, Gm.	pH of Final Mixture	% Drug Concn.	M. p. of Product, °C.
2.0	1.0	2.5	65.55	165[a]
2.0	2.0	2.5	44.83	180
2.0	4.0	2.5	31.87	183
2.0	1.0	9.0	16.57	>275[b]
2.0	2.0	9.0	15.21	>275
2.0	4.0	9.0	9.01	>275

[a] Uncorrected melting points obtained from Fisher-Johns melting point apparatus. [b] Temperature at which the material appeared to soften.

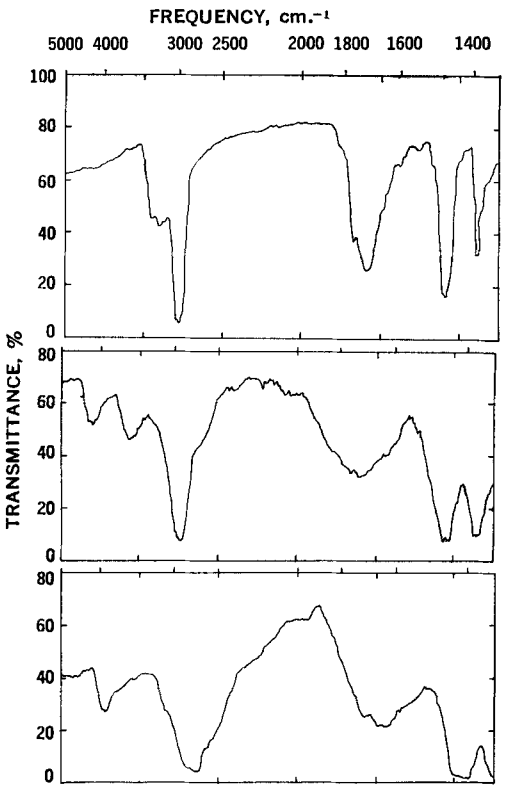


Fig. 9.—Infrared spectra of phenobarbital (top), PVOM (middle), and PVOM–phenobarbital (bottom) in Nujol. Perkin-Elmer model 21 spectrophotometer; sodium chloride prism.

absolute ethanol was breaking the complex by dissolving the phenobarbital.

Acidic precipitation studies were conducted similarly with sodium pentobarbital and sodium amobarbital, shown by previous solubility studies essentially to be inactive toward PVOM. Finely precipitated products were observed upon acidification of both polymer–barbiturate solutions rather than the characteristic gelatinous precipitates exhibited by the PVOM–phenobarbital solutions. Analyses of these precipitated products showed the presence of 96.4% amobarbital and 99.9% pentobarbital. These results substantiated the previously conducted acidification solubility studies in which phenobarbital was shown to exhibit a far greater attractive tendency for PVOM than either pentobarbital or amobarbital.

An attempt was made to isolate the complex in basic solution by salting out the PVOM with sufficient sodium chloride. A relatively small amount of sodium phenobarbital was complexed out of solution by the polymer (Table II). This effect was probably due to a physical entrapment of the drug molecules by the polymer rather than to any chemical binding of polymer to drug.

To obtain better insight of the force or forces responsible for the interaction of PVOM and phenobarbital, the product isolated in the acidic precipitation studies containing 44.8% of phenobarbital (Table II) was subjected to infrared analyses (Nujol mulls) using a Perkin-Elmer model 21 spectrophotometer.

Figure 9 depicts the absence of the characteristic NH stretching absorption band of phenobarbital in the PVOM–phenobarbital spectrum at 3200–3000 cm.$^{-1}$. This absence of absorption may be ascribed to shifting to a lower frequency of the NH group due to hydrogen bonding or to the NH group not being strong enough to exhibit good resolution of its band. More conclusive for the presence of hydrogen bonding was the apparent shifting of the distinctive carbonyl band at 1770–1710 cm.$^{-1}$, exhibited by both the free drug and the polymer, to a lower frequency of 1670–1630 cm.$^{-1}$ in the case of the complex. This shifting to a lower frequency could be due to induced polarization of the carbonyl groupings.

SUMMARY AND CONCLUSIONS

1. Standard solubility studies were conducted consisting of the equilibration of excess quantities of phenobarbital with varying amounts of poly-*N*-vinyl-5-methyl-2-oxazolidinone (PVOM) in buffer solution (pH 5.8) at 25°. The amount of phenobarbital in equilibrium with its solid phase increased linearly with polymer concentration; therefore, the stoichiometric ratio and the true stability constant of the complex could not be determined.

2. Aqueous stability studies of phenobarbital with varying amounts of PVOM in alkaline media have revealed that no dissimilarity exists between the hydrolytic rates of solutions of phenobarbital and their respective polymer-drug solutions.

3. A method was demonstrated whereby the acidification of an aqueous solution of PVOM and sodium phenobarbital to below the pH of 7.41 (phenobarbital, pKa′ 7.41) proved to enhance significantly the extent of interaction of polymer and drug with respect to the results of the previously mentioned standard solubility study. Approximately 5 to 6% PVOM was needed to assure the solubilization of 0.46% phenobarbital. This work suggested that both polymer and drug should be brought into intimate contact with each other to gain the maximum effect of the complexing reaction.

4. A comparison of several barbiturates has shown that the greater the aliphatic character of the disubstitution at the 5-position of barbituric acid, the lesser is the affinity of the barbiturate for the polymer. This may be due in part to a decrease in polarity of the molecule or to steric factors.

5. A comparison of the complexing tendencies of structurally similar PVOM and polyvinylpyrrolidone (PVP) for phenobarbital has demonstrated that PVOM has an appreciably greater affinity for the phenobarbital molecule than PVP.

6. Dialysis studies in artificial gastrointestinal fluids have shown the traversal rate of phenobarbital of a PVOM–phenobarbital solution to be slightly slower than the traversal rate of phenobarbital of a respective free phenobarbital solution. These results suggest that the availability of the barbiturate would be approximately the same for solutions of both free drug and drug-complex.

7. Dialysis studies at pH 2.8 of PVOM–phenobarbital having a concentration of phenobarbital so as to exceed the solubility of the free drug in this medium have demonstrated a relatively greater complexing tendency of the polymer for the drug than shown by less concentrated drug-complex solu-

tions. This greater complexing tendency probably can be ascribed to the proximity of the polymer and drug molecules in the solutions.

8. *In vivo* evaluation in rats, employing Williamson activity cages, substantiated the results of the dialytic rate studies by showing no appreciable difference in the onset, extent, and duration of sedation of the drug complex with respect to the free drug. *In vivo* studies also demonstrated that the interacted system had a somewhat reduced oral toxicity.

9. The nature of the polymer-drug interaction was elucidated by subjecting the precipitates obtained from solutions containing approximately equal amounts of PVOM and barbiturate to ultraviolet and infrared spectral analyses, melting point determinations, and solubility studies.

10. Results of this research strongly suggest the existence of an association between PVOM and certain barbiturates. It is felt that the association can be ascribed primarily to a dipole–dipole interaction or hydrogen bonding between the polymer and the drug, abetted by secondary attractive forces such as Van der Waals' bonds, spatial arrangement, etc.

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Effects of Heavy Water on *Atropa belladonna*

By R. A. UPHAUS*, F. A. CRANE, M. I. BLAKE, and J. J. KATZ*

The growth of belladonna is strongly inhibited by D_2O concentrations greater than 50 per cent. The alkaloid content decreases progressively as the deuterium level in the plant increases. Germinative ability of seeds containing up to 40 per cent deuterium is unimpaired.

THE EFFECTS of deuterium on algae and other microorganisms have been the subject of numerous investigations (1, 2), but studies of the effects of this isotope on higher plants have been relatively few. The long growth cycles, the relatively large amounts of water lost through transpiration, and the fact that only a few individual plants may be cultured at a time are probably the chief reasons for this situation. Early work concerning deuterium isotope effects on higher plants was concerned often with either short-termed effects or possible effects at very low concentrations of D_2O or effects on some special aspect of plant development, such as germination. Early work has been summarized by Morowitz and Brown (3) and more recently by Thomson (4), while the cytological aspects of deuterium substitution in higher plants have been reviewed by Flaumenhaft *et al.* (5). Recent detailed studies in these laboratories on the growth of the higher plant, *Mentha piperita* L., in heavy water included scrutiny of the morphological (6) and histological (7) changes evoked by various concentrations of D_2O as well as the concurrent effects of various growth regulators (8). These studies provided the first detailed descriptions of the effects of deuterium when present throughout the lifetime of the plant and have encouraged further research with higher plants of pharmaceutical importance. The present communication describes some effects of heavy water on *Atropa belladonna* and includes data on growth, morphology, flowering, and alkaloid content of belladonna plants grown with various concentrations of heavy water in the nutrient medium.

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* Chemistry Division, Argonne National Laboratory, Argonne, Ill.