Solubilization and Stability of Phenobarbital by Some Aminoalcohols

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Four well-known surface-active aminoalcohols—namely, monoethanolamine, triethanolamine, *N,N*-dimethylethanolamine and *N*-methylglucamine—were found to be effective solubilizers for phenobarbital in water. Equimolar solubilization was possible only at extremely low concentrations. The relative stabilities of the resultant phenobarbital solutions at both autoclaving and room temperatures were generally comparable and in several cases even superior to aqueous solutions of the sodium salt of phenobarbital as stabilized by the inclusion of some polyhydric alcohols.

BOTH AQUEOUS and nonaqueous solutions of phenobarbital sodium have been stabilized by polyhydric alcohols, such as propylene glycol and the polyethylene glycols. The enhanced stability of these solutions has been ascribed to a possible complex formation (1). An attempt therefore was made to investigate further the possibilities for the successful use of some other nontoxic aminoalcohols for obtaining more stable phenobarbital solutions in addition to diethanolamine which previously had been used in conjunction with the solubilization and consequent stabilization of one of the derivatives of barbituric acid (2). In this study, the aminoalcohols selected were monoethanolamine, triethanolamine, *N,N*-dimethylethanolamine, *d*(+)glucosamine, and *N*-methylglucamine. However, glucosamine was discarded early during preliminary tests because of its poor solubilizing performance.

EXPERIMENTAL

Equipment.—A Beckman model DU spectrophotometer, Beckman model N pH meter, Fisher motor stirrers, Fisher mercury thermoregulators, Fisher magnetic stirrers (glass-covered), and Bausch & Lomb 100-w. illuminator were used.

Materials.—Phenobarbital U.S.P. and phenobarbital sodium U.S.P., Merck; triethanolamine and *N,N*-dimethylethanolamine, Eastman Kodak; monoethanolamine, Matheson; *N*-methylglucamine, Du Pont; *d*(+)glucosamine, Nutritional Biochemicals; and propylene glycol U.S.P., Fisher Scientific Co., were utilized.

Method of Analysis.—The ultraviolet spectrophotometric method of quantitative analysis for phenobarbital, employed successfully by a number of workers (3–5), was used wherever possible throughout this study. It was an accurate, simple, and rapid method of analysis and conformed to the Beer-Lambert law when spectrophotometric absorbance was plotted against phenobarbital concentrations within the concentration range of 2.5 to 30.0 mcg./ml. and at the wavelength range of 225–250 mμ. Maximum absorbance was maintained within the pH range 9.0 to 10.0 at wavelength 240 mμ (Fig. 1).

However, an argentometric method, similar to Budde's (6), was substituted in those cases where phenobarbital sodium solutions had stood in prolonged contact with aqueous solutions of polyhydric compounds. Briefly, this involved an accurate volumetric titration of the analytical samples with 0.1 *N* silver nitrate solution delivered from a microburet. Each sample which contained the equivalent of 100–200 mg. of phenobarbital, 50 ml. of freshly prepared 3% sodium carbonate solution, and 10 ml. of 95% ethyl alcohol was mixed continuously with the aid of a glass-covered magnetic stirrer throughout the process of each titration. All titrations were performed in a darkened room to facilitate the end-point determinations. The first permanent cloudiness, viewed at a right angle to the strong and narrow beam of light from an illuminator which was projected through the solution, marked the end point.

Procedure for Solubilization Study.—The various amounts of the aminoalcohols which were needed to prepare up to 10% (w/v) or approximately 0.4 *M* phenobarbital solutions were first computed, em-

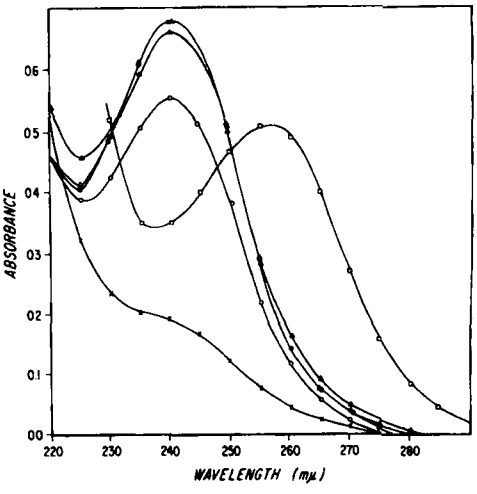

Fig. 1.—The ultraviolet spectrophotometric absorbance of phenobarbital as influenced by pH within the wavelength range of 220–290 mμ. Key: ×, pH 7.00; ○, pH 8.15; ●, pH 9.30; △, pH 9.85 ▲, pH 10.95; □, pH 12.00.

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TABLE I.—RELATIVE PROPORTIONS OF PHENOBARBITAL AND AMINOALCOHOLS FOR SOLUBILIZATION[a]

	Aminoalcohol		Phenobarbital		pH of Soln.
	Gm./100 ml.	Moles/L.	Gm./100 ml.	Moles/L.	
Monoethanolamine	0.40	0.0655	1.57	0.0676	8.10
	0.80	0.1310	2.93	0.1262	8.20
	1.20	0.1964	4.12	0.1774	8.20
	1.60	0.2619	5.21	0.2244	8.25
	1.80	0.2947	5.91	0.2545	8.25
	2.00	0.3274	6.46	0.2782	8.35
N,N-Dimethylethanolamine	1.00	0.1121	2.23	0.0960	8.40
	2.00	0.2244	3.95	0.1701	8.60
	3.00	0.3365	5.43	0.2338	8.70
	4.00	0.4487	6.94	0.2988	8.75
	5.00	0.5610	8.52	0.3668	8.90
	6.00	0.6732	9.61	0.4138	8.90
N-Methylglucamine	2.00	0.1024	2.22	0.0956	8.10
	3.00	0.1537	3.18	0.1370	8.20
	4.00	0.2049	4.17	0.1796	8.20
	5.00	0.2563	5.06	0.2179	8.30
	6.00	0.3075	6.03	0.2596	8.30
	7.00	0.3587	6.84	0.2950	8.45
Triethanolamine	3.00	0.2010	1.31	0.0564	8.30
	6.00	0.4022	2.06	0.0887	8.40
	12.00	0.8042	3.31	0.1425	8.60
	18.00	1.2060	4.44	0.1912	8.70
	24.00	1.6460	5.81	0.2502	8.80
	30.00	2.0100	6.45	0.2777	8.82

[a] Solubilization study was performed at room temperature of 25 ± 0.1°.

ploying the solubilization data from preliminary tests. Solutions of six different strengths of each of the aminoalcohols were then accurately prepared and placed in suitable conical flasks to be immersed in a constant-temperature water bath. They were allowed to attain equilibration of temperature at 25 ± 0.1° before the addition of an excess of phenobarbital. The solutions were subjected to gentle mechanical stirring and at regular intervals of 15 to 30 minutes, samples were withdrawn, filtered, suitably diluted, and their pH's adjusted to 9.0 to 10.5 with dilute sodium hydroxide solution for spectrophotometric analysis at 240 mμ. The concentrations of phenobarbital solubilized (Table I), as found at the point of equilibration of solution, were computed from the per cent transmittance readings when no further change in absorbance was noted. These values were plotted against the corresponding amounts of the aminoalcohols needed for the solubilization (Fig. 2) as well as against the respective pH's of the resultant solutions (Fig. 3). Interferences in analysis owing to absorbance by the solubilizing agents themselves were negligible, except for triethanolamine, in which case a correction factor was applied.

Procedure for Stability Study.—(*a*) *At Autoclaving Temperatures.*—Exactly 5% phenobarbital solutions were prepared under ordinary room temperature conditions by manual agitation of phenobarbital in aqueous solutions of 1.6% monoethanolamine, 3.0% *N,N*-dimethylethanolamine, 5.0% *N*-methylglucamine, and 24.0% triethanolamine. These were placed in air-tight containers and subjected to autoclaving at 10 p.s.i. pressure for 30 minutes. The solutions were gradually allowed to cool to room temperature and stored overnight. Any precipitate was filtered off. The calculated volumes for dilution and subsequent spectrophotometric analysis were pipeted from these filtrates as well as from the corresponding solutions before they were subjected to autoclaving. A 5.0% concentration of phenobarbital sodium in 10% dextrose solution and in 60% propylene glycol solution were studied under similar experimental conditions for purposes of comparison of stability.

(*b*) *At Room Temperatures.*—To simulate closely official elixir strength of 0.44% of phenobarbital sodium, exact 0.5% solutions were prepared employing proportionately lower concentrations of the aminoalcohols. These solutions together with a similar set of the above 5.0% phenobarbital solutions were stored in 120-ml. dispensing bottles

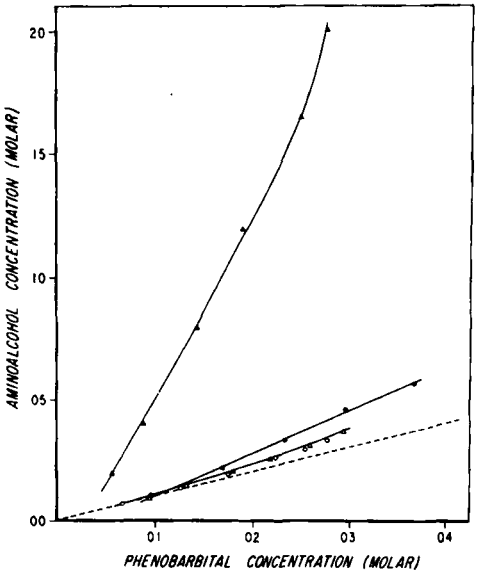


Fig. 2.—Curves for solubilization of less than 0.4 *M* phenobarbital by the following aminoalcohols at 25°. Key: - - -, hypothetical plot for equimolar solubilization; ▲, triethanolamine; ●, *N,N*-dimethylethanolamine; Δ, *N*-methylglucamine; O, monoethanolamine.

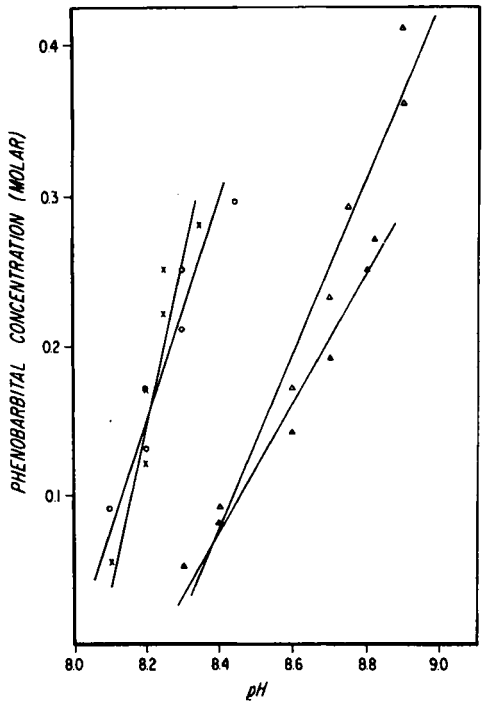


Fig. 3.—The effect of pH on the solubilization of phenobarbital by the following aminoalcohols. Key: ×, monoethanolamine; O, *N*-methylglucamine; △, *N*,*N*-dimethylethanolamine; ▲, triethanolamine.

away from direct light and at room temperatures of 23–25° for a total period of 2 weeks. At intervals of 5 days, samples were withdrawn and assayed spectrophotometrically as in (*a*), except for those aqueous solutions of phenobarbital sodium in pure distilled water, 10% dextrose, and 60% propylene glycol solutions. These aqueous solutions of phenobarbital sodium exhibited increased absorbance at 240 mμ on prolonged standing analogous to studies carried out by Kubota for the enhanced spectrophotometric absorbance of polyhydric alcohols or hydroxy acids in aqueous solutions owing to the effect of alkali metals. Kubota hypothesized this phenomenon to a possible metal-complex formation involving static electricity of the metal ion with hydroxyl groups of the polyhydric compounds (7, 8). The usual spectrophotometric method of analysis therefore was replaced by the argentometric titration method for the quantitative determination of phenobarbital in such solutions. Any attempt at spectrophotometric analysis of these solutions resulted in erratic and erroneous determinations (Table II), possibly because of the simultaneous effects of varying rates of increased spectrophotometric absorbance, due to metal-complex formation and the actual degradation of phenobarbital. The argentometric titration was considered unnecessary for the assay of those phenobarbital solutions where alkali metal ions were absent or where solutions of the phenobarbital salt had been in contact with some polyhydric compounds for only a relatively short period.

RESULTS AND DISCUSSION

The increased solubility of phenobarbital by the aminoalcohols may be attributed to the formation of either a salt or a "complex." The solubilization of phenobarbital caused by a slight increase in pH of the medium as a result of higher concentrations of the aminoalcohol followed a linear relationship for each series (Fig. 3) and indicated favorably a simple acid-base salt formation. In the case for the possible formation of a weak "complex" or an association compound, it is assumed that this occurs between the electronegative nitrogen of the

Table II.—Stability of 5.0 and 0.5% Phenobarbital Solutions on Storage at Room Temperature

Phenobarbital Soln.	pH	% Undecomposed Phenobarbital			
		5 Days[a]	10 Days[a]	15 Days[a]	15 Days[b]
Phenobarbital sodium in distilled water					
5.0% Concn.	8.80	100.00	99.65	98.15	92.41
0.5%	8.80	99.17	99.08	98.45	95.35
Phenobarbital sodium in 60% propylene glycol soln.					
5.0% Concn.	9.50	100.80	101.30	102.50	96.94
0.5%	9.25	102.10	103.70	100.80	98.15
Phenobarbital sodium in 10% dextrose soln.					
5.0% Concn.	8.00	100.20	99.38	92.51	91.49
0.5%	8.60	102.30	102.00	101.50	96.79
Phenobarbital with monoethanolamine					
5.0% Concn.	8.70	99.88	98.24	97.83	...
0.5%	8.50	99.93	99.51	98.97	...
Phenobarbital with *N*,*N*-dimethylethanolamine					
5.0% Concn.	8.80	99.42	98.63	97.90	...
0.5%	8.80	99.97	99.97	98.49	...
Phenobarbital with *N*-methylglucamine					
5.0% Concn.	8.75	99.19	98.63	97.16	...
0.5%	8.70	99.93	99.10	99.26	...

[a] Values for per cent undecomposed phenobarbital were obtained from spectrophotometric analysis at 240 mμ. [b] Corrected values for per cent undecomposed phenobarbital as obtained by argentometric titration.

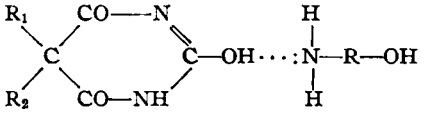
TABLE III.—RELATIVE PROPORTIONS OF PHENOBARBITAL AND AMINOALCOHOLS FOR APPROXIMATE EQUIMOLAR SOLUBILIZATION[a]

	Aminoalcohol		Phenobarbital	
	mg./100 ml.	moles/L. (moles × 10^{-3})	mg./100 ml.	moles/L. (moles × 10^{-3})
Monoethanolamine	10.0	1.6370	36.0	1.5500
	12.0	1.9640	44.2	1.9030
	14.0	2.2920	52.2	2.2480
	16.0	2.6190	57.8	2.4890
(Distilled water as blank)	18.0	2.9470	61.6	2.6530
	...	...	124.0	...
N,N-Dimethylethanolamine	5.0	0.5610	9.1	0.3876
	10.0	1.1220	21.7	0.9346
	15.0	1.6830	31.6	1.3610
	20.0	2.2440	42.3	1.8220
	25.0	2.8040	52.2	2.2480
	30.0	3.3660	65.4	2.8160
(Distilled water)	...	...	124.0	...
N-Methylglucamine	10.0	0.5124	10.7	0.6635
	20.0	1.0240	23.2	0.9990
	30.0	1.5370	35.1	1.5110
	40.0	2.0490	45.7	1.9680
	50.0	2.5630	56.8	2.4450
	60.0	3.0750	67.0	2.8850
	70.0	3.5870	72.8	3.1340
	80.0	4.1000	73.2	3.1520
	90.0	4.1000	73.2	3.1520
(Distilled water)	...	...	126.8	...
Triethanolamine	7.5	0.5030	9.7	0.4178
	15.0	1.0060	20.4	0.8784
	22.5	1.5090	30.9	1.3300
	30.0	2.0120	38.4	1.6540
	37.5	2.5150	45.9	1.9760
	45.0	3.0180	53.4	2.2990
(Distilled water)	...	...	127.8	...

[a] Solubilization study was carried out at room temperature of 25 ± 0.1°.

amine and the enolic hydrogen of phenobarbital, in analogy to the complex formed between the electron-donating oxygen of polyethylene glycol and the acidic hydrogen of phenobarbital described by Higuchi (1).

"COMPLEX" OF PHENOBARBITAL WITH AN AMINOALCOHOL



where $R_1 = C_6H_5$, $R_2 = C_2H_5$.

Such a complex will form hydrogen bonding with water molecules through the hydrophilic hydroxyl groups of the alcohol moiety to achieve water solubility. The inability to isolate these weak "complexes" from their aqueous solutions provided some indication toward their extreme instability.

The solubilization of phenobarbital by the aminoalcohols in approximately equimolar proportions was possible only with very low concentrations (Table III, Fig. 4); with increasing concentrations, however, especially with triethanolamine, more than the equimolar ratios were required. Results above indicate that a relatively much greater proportion of the aminoalcohols was required to solubilize and obtain the more concentrated phenobarbital solutions. This phenomenon can be attributed to a diminished activity of "solvent" where its rational

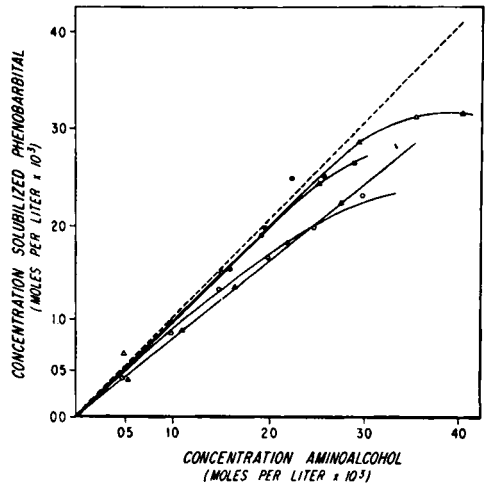


Fig. 4.—Curves for solubilization of less than 0.003 *M* phenobarbital by the following aminoalcohols at 25°. Key: - - -, hypothetical plot for equimolar solubilization; Δ, *N*-methylglucamine; ●, monoethanolamine; ▲, *N,N*-dimethylethanolamine; O, triethanolamine.

TABLE IV.—RELATIVE STABILITIES OF PHENOBARBITAL SOLUTIONS BASED ON ΔH_a OF THE ARRHENIUS EQUATION

Phenobarbital Soln.	pH	$k \times 10^4$ per Hr. 59.6°	69.6°	ΔH_a, Kcal./mole
0.10% Phenobarbital solution with monoethanolamine	8.25	17.08	28.77	11.80
0.44% Phenobarbital sodium in 60% propylene glycol	9.10	14.40	27.21	14.41
0.44% Phenobarbital sodium in 10% dextrose solution	8.45	11.90	28.78	20.00
0.10% Phenobarbital solution with triethanolamine	7.55	14.40	37.01	21.37
0.10% Phenobarbital solution with *N,N*-dimethylethanolamine	8.00	16.12	51.42	26.26
0.44% Phenobarbital sodium in distilled water	8.75	18.23	74.85	31.98
0.10% Phenobarbital solution with *N*-methylglucamine	8.40	12.21	59.06	35.69

activity coefficient becomes progressively less than 1 with increasing concentration of solution (9).

A prediction of the relative stabilities for the various phenobarbital solutions which was based upon the study of the energy of activation ΔH_a, for the decomposition of phenobarbital (Table IV), as directly derived from the Arrhenius equation $\log(k_2/k_1) = \Delta H_a/2.303\ R \cdot (T_2 - T_1)/T_2T_1$, at temperatures of 59.6 and 69.6° was unreliable for general application. Any evaluation of the relative stabilities from such a prediction differed significantly from the actual stabilities of phenobarbital solutions as determined on the basis of

per cent undecomposed drug by degradation at ordinary room temperature storage conditions and autoclaving temperatures (Tables II and V). This presumably may be due to several involved factors such as pH, dielectric constant of medium, and ionic strength of solution (10); each of these may considerably contribute toward the degradation of phenobarbital at varying levels of activity, depending upon temperature. Using data from Tables II and V, a final classification for the relative stabilities of phenobarbital solutions exposed to the above-mentioned experimental conditions is presented in Table VI.

SUMMARY AND CONCLUSIONS

1. A solubilization study for phenobarbital in water at 25° by means of mechanical stirring showed the following aminoalcohols as potential solubilizers for the drug; they are in decreasing order of solubilizing performance: monoethanolamine, *N,N*-dimethylethanolamine, and *N*-methylglucamine.

2. The formation of either a salt or a "complex" is postulated as a possible mechanism for the increased solubility of phenobarbital in water by the aminoalcohols.

3. The stability of aqueous solutions of phenobarbital as solubilized by these three aminoalcohols at both room and autoclaving temperatures compared favorably with that provided by 60% propylene glycol solution to phenobarbital sodium.

4. Optimum stability on a relative basis was indicated by the following solutions when studied under these conditions:

(*a*) At autoclaving temperatures—the 5.0% phenobarbital sodium in 60% propylene glycol closely followed by 5.0% phenobarbital as solubilized by *N*-methylglucamine.

(*b*) At room temperature—the 5.0 and 0.5% phenobarbital solutions as solubilized by *N,N* - dimethylethanolamine and *N*-methylglucamine, respectively.

5. The argentometric titration method was utilized as an alternative for the assay of aqueous solutions of phenobarbital sodium which had stood in prolonged contact with polyhydric compounds. Such solutions produced an increased spectrophotometric absorbance.

6. In conclusion, it is proposed that further investigations be performed on these solutions of phenobarbital which contain the aminoalcohols for their toxicological and pharmacological effects through biological testing.

TABLE V.—STABILITY OF 5% PHENOBARBITAL SOLUTIONS AT AUTOCLAVING TEMPERATURES

Phenobarbital Soln.	pH	Dec., %	Remarks: Precipitate	Remarks: Color
Phenobarbital sodium in 60% propylene glycol	9.50	7.83	...	...
Phenobarbital solution with 5.0% *N*-methylglucamine	8.80	8.83	*[a]	Straw-colored
Phenobarbital solution with 1.6% monoethanolamine	8.80	9.45	**	Yellowish tinge
Phenobarbital solution with 3.0% *N,N*-dimethylethanolamine	8.60	10.05	*	...
Phenobarbital sodium in distilled water	8.80	11.22	***	...
Phenobarbital sodium in 10% dextrose solution	8.80	18.69	***	Color and odor of caramel

[a] Quantitative representation of precipitate as observed visually.

TABLE VI.—STABILITY CLASSIFICATION FOR 5.0 AND 0.5% PHENOBARBITAL SOLUTIONS AT AUTOCLAVING AND ROOM TEMPERATURES

	Degradative Conditions: Autoclave Sterilization	Degradative Conditions: Room Temperature Storage
5.0% Phenobarbital Soln.		
Phenobarbital sodium in distilled water	V[a]	V
Phenobarbital sodium in 60% propylene glycol	I	IV
Phenobarbital sodium in 10% dextrose solution	VI	VI
Phenobarbital solution with monoethanolamine	III	III
Phenobarbital solution with *N,N*-dimethylethanolamine	IV	I
Phenobarbital solution with *N*-methylglucamine	II	III
5.0% Phenobarbital Soln		
Phenobarbital sodium in distilled water	...	VI
Phenobarbital sodium in 60% propylene glycol	...	IV
Phenobarbital sodium in 10% dextrose solution	...	V
Phenobarbital solution with monoethanolamine	...	II
Phenobarbital solution with *N,N*-dimethylethanolamine	...	III
Phenobarbital solution with *N*-methylglucamine	...	I

[a] Roman numericals I to VI show order of decreasing stability.

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