Table V—Dissolution Rates of 0.5- and 1.0-mg Dihydroergotoxine Tablets in 500 ml of Distilled Water at 25° Using Rotating-Paddle Method

Minutes	0.5 mg					1.0 mg,
	Product M2[a]	Product P	Product B1	Product R	Product S1	Product M4
2	2.5, 5.5[b]	0.0, 2.0	2.0, 0.6	27.3, 38.8	0.6, 3.0	8.7, 8.3
5	11.0, 13.0	4.0, 7.5	9.5, 5.8	66.8, 67.8	6.3, 6.0	20.2, 20.3
10	23.5, 25.5	16.0, 13.5	22.0, 23.3	75.3, 84.8	8.3, 10.6	37.2, 39.8
15	29.5, 39.0	22.0, 37.5	30.5, 37.8	79.8, 87.8	16.3, 16.0	48.7, 60.8
20	45.5, 56.0	33.5, 57.0	50.5, 59.8	85.3, 82.8	19.3, 16.5	73.2, 73.3
30	74.5, 81.2	41.5, 54.5	71.5, 70.3	75.8, 87.8	27.3, 23.0	94.2, 97.8
60	89.0, 90.5	61.0, 55.5	75.0, 75.3	82.8, 84.3	34.8, 42.0	106, 101

[a] Letter represents manufacturer, and number represents a different production lot. Refer to Tables II and III for explanation of symbols. [b] Values for two tablets.

variations. Other studies, under slightly modified test conditions, also helped to establish that dissolution differences may be due to formulation differences. Two brands, P and B2, failed to achieve total dissolution (Fig. 1). With both preparations, insoluble material in the dissolution medium and a film on the stirring shafts and paddles indicated the possibility of an insoluble complex formation. Total dissolution could be achieved only by further dilution to 900 ml of medium.

The extent of dissolution as a function of temperature was not clear cut and predictable because of the differences in formulations (Table V). For example, Products M2, P, B1, and M4 initially dissolved slowly at 25°; however, after 60 min, the extent of dissolution was comparable to that at 37°. On the other hand, temperature had no influence on Product R (the dissolution profiles at 25 and 37° were identical). However, the rate and extent of dissolution of Product S1 were decreased significantly at 25° when compared to the results obtained at 37° (Fig. 2).

Based on the *in vitro* dissolution results, the FDA implemented a dissolution specification for 0.5- and 1.0-mg sublingual tablets using the paddle method (4) where the dissolution medium is 500 ml of distilled water at 37°. The paddle height is 4.5 cm, and the stirring speed is 50 rpm. The dissolution specimens are filtered with either glass or polytef filters prior to drug content analysis.

In vivo bioavailability data for *in vitro–in vivo* correlations are lacking. Therefore, based on the *in vitro* performance of eight batches representing 0.5- and 1.0-mg tablets of the innovator product[15], a dissolution specification was set. For tablets to be considered acceptable, they must dissolve not less than 55% in 15 min and not less than 85% in 30 min for a mean of 12 tablets. However, no tablet should fall below 45 and 75% at those respective times.

The described *in vitro* dissolution method and its specifications should help ensure both product-to-product and lot-to-lot uniformity and consistency for dihydroergotoxine sublingual tablet dosage forms.

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(3) "SAS User's Guide, 1979 Edition," SAS Institute, Raleigh, N.C., 1979, p. 313.

(4) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 959.

[15] Sandoz.

NOTES

Loss of Nitroglycerin from Solutions to Intravenous Plastic Containers: A Theoretical Treatment

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Abstract □ The physical instability of nitroglycerin solutions in plastic containers has been reported extensively. A systematic study of potency loss in plastic infusion bags was reported recently. This paper presents a theoretical treatment of the data and a proposed model consisting of adsorption onto the surface followed by partitioning into the plastic.

Keyphrases □ Nitroglycerin—loss from solution to plastic intravenous containers, a theoretical treatment □ Plastic containers—loss of nitroglycerin from intravenous plastic containers, a theoretical treatment □ Adsorption, potential—nitroglycerin loss from solution in plastic intravenous containers, a theoretical treatment

Numerous studies (1–10) have reported stability problems associated with nitroglycerin solutions in plastic containers meant for intravenous infusions. Adsorption of the drug by plastic containers and infusion sets was suggested as the cause. Yuen *et al.* (8) studied the loss of nitroglycerin from aqueous solutions in immersed strips of plastic. Based on equilibrium and kinetic studies, these workers proposed an adsorption–absorption mechanism in which adsorption plays a minor role. Although their report provided some insight into the phenomenon, it did not deal with the system as a whole, *i.e.*, nitroglycerin solutions contained in intravenous bags.

The loss of nitroglycerin from solutions stored in commercial plastic intravenous bags was reported recently (10). Immediate significant losses were followed by a gradual decrease in concentration. No chemical degradation was observed. In this report, a theoretical treatment of the data and a proposed model are presented.

EXPERIMENTAL

The applicability of the proposed model was tested using recently reported data of nitroglycerin compatibility with intravenous admixture aids (10). In brief, the procedures for obtaining the kinetic data entailed: (*a*) the addition by syringe of an aqueous nitroglycerin preparation to

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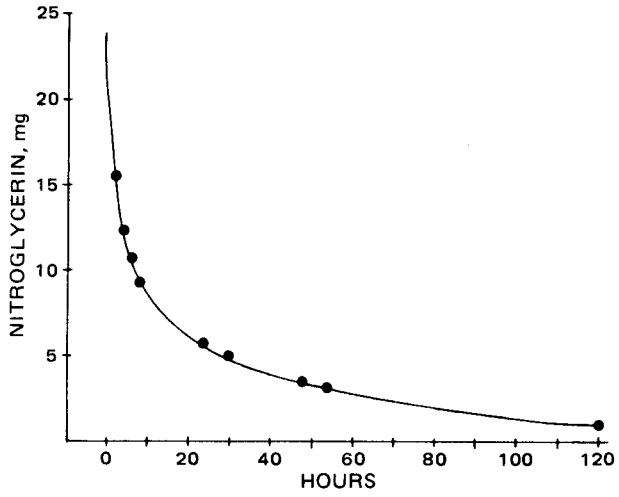

Figure 1—*Amount of nitroglycerin in a plastic bag as a function of time. The symbols (●) are experimental points. The solid line is the theoretically computed curve according to Eq. 10.*

a 100-ml polyvinyl chloride[1] plastic bag to yield a final nominal concentration of 50 μg/ml, and (*b*) mixing the solution and sampling at given intervals for potency determination by high-performance liquid chromatography (HPLC). Nitroglycerin potency assays were performed according to the method of Baaske *et al.* (10). Figure 1 summarizes the results.

THEORETICAL

The loss of nitroglycerin from solution in plastic bags can be described by two processes: rapid adsorption of nitroglycerin by the surface of the plastic bag, followed by dissolution of nitroglycerin into the plastic. This loss can be represented diagramatically as in Scheme I.

$$\text{nitroglycerin in aqueous solution} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{adsorbed nitroglycerin on plastic surface} \xrightarrow{k_3} \text{nitroglycerin dissolved in plastic matrix}$$

Scheme I

The process may be considered to be dependent on the following factors: (*a*) the amount of nitroglycerin in solution, (*b*) the ratio of nitroglycerin solution volume to the area of the plastic surface in contact with the solution, (*c*) the rate at which fresh nitroglycerin molecules from the solution are adsorbed at the plastic surface following the sorption of nitroglycerin by the plastic matrix, and (*d*) the solubility of nitroglycerin in the plastic matrix and the partition coefficient of nitroglycerin between plastic and the aqueous phase.

Scheme I can be expressed in terms of the instantaneous amounts of nitroglycerin in solution (A), nitroglycerin adsorbed to the plastic surface (B), and nitroglycerin dissolved in the plastic matrix (C) (Scheme II).

$$A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_3} C$$

Scheme II

The differential equations describing the rate of change of A, B, and C are:

$$\frac{dA}{dt} = -k_1A + k_2B \qquad \text{(Eq. 1)}$$

$$\frac{dB}{dt} = k_1A - k_2B - k_3B \qquad \text{(Eq. 2)}$$

$$\frac{dC}{dt} = k_3B \qquad \text{(Eq. 3)}$$

Experimental data show a rapid initial drop of nitroglycerin in solution followed by a relatively slower rate of loss. This finding suggests that the rate of nitroglycerin adsorption to the surface is much greater than the rate of sorption by the plastic matrix. The adsorbed layer of nitroglycerin onto the plastic surface can be pictured as a few molecules thick at most.

[1] Viaflex container, Travenol Laboratories, Morton Grove, IL 60053.

The molecules of the adsorbed layer of nitroglycerin that are removed by sorption into the plastic are expected to be replaced rapidly by fresh molecules from the solution. Since nitroglycerin is being adsorbed continually at the surface and removed by dissolution into the matrix, B should approach steady state:

$$\frac{dB}{dt} = 0 = k_1A - k_2B - k_3B \qquad \text{(Eq. 4)}$$

By introducing the steady-state approximation and the initial conditions (at $t = 0$, $A = A_0$, $B = 0$, and $C = 0$), the following solution for A is obtained:

$$A = \alpha e^{-[k_1k_3/(k_2+k_3)]t} + \beta e^{-(k_2+k_3)t} - \alpha e^{-k_1t} - \beta e^{-k_1t} + A_0e^{-k_1t} \qquad \text{(Eq. 5)}$$

where α and β are constants.

A comparison of the processes controlled by k_2 and k_3 in the scheme suggests that the magnitude of k_2 is smaller than k_3. The loss of nitroglycerin from the adsorbed layer to the relatively polar aqueous phase is described by k_2, whereas k_3 describes the sorption of the adsorbed nitroglycerin molecules by the nonpolar plastic matrix. Since the affinity of nitroglycerin is expected to be much greater for the nonpolar plastic matrix than the aqueous phase, it is expected that $k_3 \gg k_2$.

Following this assumption, Eq. 5 reduces to:

$$A = \beta e^{-k_3t} + (A_0 - \beta)e^{-k_1t} \qquad \text{(Eq. 6)}$$

where:

$$\beta = \frac{A_0}{\frac{k_3^2}{k_1k_2} + \frac{k_1^2}{k_2} - \frac{2k_3}{k_2}} \qquad \text{(Eq. 7)}$$

and k_1 is a function of the amount of drug in solution, the surface area available for adsorption, and the formulation differences between solutions and plastic matrixes, and k_3 is a function of the volume of the plastic matrix and the solubility of nitroglycerin in the plastic matrix.

The k_3/k_2 ratio describes the overall partitioning behavior of nitroglycerin between the plastic and the aqueous phase. The relationship of the true partition coefficient (PC) and k_3/k_2 can be described by:

$$\frac{k_3}{k_2} = aPC \qquad \text{(Eq. 8)}$$

where a is a proportionality constant related to, among other factors, the mass of the plastic, the volume of aqueous nitroglycerin solution, and the formulation differences between the solution and the plastic bag.

Similarly, the k_1/k_2 ratio can be related to the affinity constant (k_{Lang}) in a Langmuir type of adsorption (11):

$$\frac{k_1}{k_2} = \rho k_{Lang} \qquad \text{(Eq. 9)}$$

where ρ is a proportionality constant that is also related to the surface area of the plastic, the volume of the aqueous nitroglycerin solution, and the formulation differences between the solution and the plastic bag.

Langmuir adsorption treatment cannot be applied rigorously to this model since an equilibrium situation between the adsorbate (nitroglycerin) and the adsorbent (plastic) does not exist during the experiment. The adsorbed molecules, instead of reaching an equilibrium with the nonadsorbed molecules in the aqueous solution, are removed continually from the surface by the plastic matrix.

RESULTS AND DISCUSSION

Figure 1 shows a plot (at room temperature) of the amount of nitroglycerin remaining in the plastic bag as a function of time. As predicted by the model, a biexponential loss of nitroglycerin with time is seen. The rate constants for adsorption (k_1) and sorption by the plastic matrix (k_3), together with the constants β and $A_0 - \beta$, were obtained by using a back-projection (stripping) technique (12). Figure 1 shows the actual data points and the curve obtained by theoretical computation. The solid theoretical curve was generated by using the equation derived for the proposed model (Eq. 6) and substituting the values of the constants obtained from the experimental data. The final form of the equation is:

$$A = 8.957e^{-0.028t} + 14.943e^{-0.235t} \qquad \text{(Eq. 10)}$$

The experimental data points show an excellent fit with the solid theoretical line. Thus, the proposed model describing (*a*) the loss of nitroglycerin from the solution in the plastic bags as a rapid adsorption to the

plastic surface, (*b*) followed by sorption of nitroglycerin to the plastic matrix by dissolution is in good agreement with the actual data. The model presented here should be useful for future investigations.

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High-Performance Liquid Chromatographic Determination of Verapamil in Plasma by Fluorescence Detection

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Abstract □ A rapid and highly sensitive method is reported for the quantitative determination of verapamil in plasma. Verapamil and its internal standard are extracted from alkalinized plasma with heptane and then back-extracted into dilute sulfuric acid. An aliquot is injected directly into a high-performance liquid chromatograph, separated by reversed-phase chromatography, and quantified by a fluorescence detector. The procedure is suitable for the routine determination of verapamil in plasma in concentrations as low as 1 ng/ml.

Keyphrases □ Verapamil—high-performance liquid chromatography, fluorescence detection, canine plasma □ High-performance liquid chromatography—verapamil, canine plasma, fluorescence detection □ Antiarrhythmic agents—high-performance liquid chromatography, verapamil, canine plasma, fluorescence detection

Verapamil, 5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (I), has been used for several years in Europe as an effective antiarrhythmic drug and is now undergoing clinical evaluation in the United States. Studies are underway in this laboratory to show the efficacy of verapamil in suppressing halothane–epinephrine arrhythmias (1).

Several analytical procedures have been reported for determining verapamil in plasma, including spectrophotometry (2), GLC with mass fragmentography (3), GLC (4), and high-performance liquid chromatography (HPLC) (5). None of these methods was considered completely satisfactory for reasons of simplicity, speed, and the ability to determine metabolites quantitatively. Therefore, an HPLC procedure was developed, and its application for the determination of verapamil in canine plasma following intravenous injection is reported here.

EXPERIMENTAL

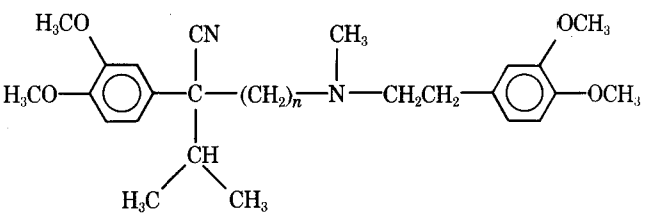
Chemicals and Reagents—Verapamil[1] (I) and its internal standard, 4-[(3,4-dimethoxyphenethyl)methylamino] -2- (3,4-dimethoxyphenyl)-2-isopropylbutyronitrile[2] (II), were obtained as hydrochloride salts. Glass-distilled heptane[3] and HPLC grade acetonitrile[4] were used. Sodium hydroxide (2 *N*), sulfuric acid (0.2 *N*), and pH 3.0 phosphate buffer (1 *M*) were prepared with deionized water.

HPLC—A liquid chromatograph[5] was used with a fluorescence detector[6]. The column[7] was C_{18} reversed phase, and the eluting solvent was 45% acetonitrile–55% pH 3.0 potassium phosphate (0.1 *M*). The solvent was degassed prior to use by applying a vacuum. The flow rate was set at 1 ml/min. The detector settings were 203 nm for excitation with a 320-nm emission filter.

Procedure—To 0.5 ml of plasma were added 50 ng of II, 0.25 ml of 2 *N* NaOH, and 3 ml of heptane. The mixture was shaken mechanically for 15 min and then centrifuged. The organic layer was transferred to a 5-ml conical tube, and 50 μl of 0.2 *N* H_2SO_4 was added. The contents were mixed in a vortex mixer for 1 min and centrifuged, and a 10-μl sample was injected into the chromatograph.

RESULTS AND DISCUSSION

Standard curves were prepared routinely from spiked plasma samples containing 40, 100, and 250 ng of verapamil/ml. Peak height ratios were plotted as a function of the verapamil concentration added and were linear within this range. Intercepts usually passed through the origin. The lower limit of sensitivity of the assay was ~1 ng/ml, and this level was completely adequate for analyzing the samples in this study. Assay reproducibility and precision were evaluated by the repeated analyses of spiked plasma samples containing 100 ng of verapamil/ml. The coefficient of variation at this level was 4.6% (n = 12). The slopes of the standard curves from water or biological fluids were similar, indicating insignificant



I: n = 3
II: n = 2

[1] Knoll Pharmaceuticals, Whippany, N.J.
[2] D517, Knoll AG, Ludwigshafen, West Germany.
[3] Mallinckrodt Chemical Co., St. Louis, Mo.
[4] MCB, Cincinnati, Ohio.
[5] Model 330, Altex, Berkeley, Calif.
[6] Model 970, Schoeffel Instruments, Westwood, N.J.
[7] Ultrasphere ODS, Altex, Berkeley, Calif.

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