sum of the rate constants for parallel first-order loss from the depot, a loss which amounts to 23% of the dose.

It would thus appear that the fraction of dose absorbed should be routinely calculated in studies employing the Wagner–Nelson (1) or Loo–Riegelman (2) method to determine whether or not additional data might be needed to assign a physical meaning to the calculated value for the absorption rate constant.

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Effects of Protein Binding of Drugs on Areas under Plasma Concentration–Time Curves

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Abstract □ By using a conservation-of-mass treatment, it can be shown that the area under a free drug concentration–time curve is determined by the rate constant of elimination of free drug, regardless of the extent of protein binding. In the absence of information on the free drug elimination constant, the area under the free drug curve can be calculated from the limiting value of the apparent total drug elimination constant and the binding parameters. The effect of competitive binding inhibitors depends strongly on the pharmacokinetics of the inhibitor; and, if the inhibitor is eliminated much more rapidly than drug, it is without effect on the area under the free drug curve.

Keyphrases □ Protein binding—effects on area under plasma concentration–time curve, conservation-of-mass treatment, equations □ Plasma concentration–time curves—effects of protein binding of drugs on area under curve, pharmacokinetics of inhibitor, equations

In the course of recent investigations into the quantitative aspects of nonlinear plasma protein binding effects on pharmacokinetics (1), some interesting relationships between binding parameters and the areas under plasma concentration–time curves emerged. First, if the elimination rate constant for free drug is known, the standard relationship yields the area under the free drug concentration curve, regardless of the extent of the binding. Second, the effect of competitive inhibition of binding on the area under a concentration–time curve depends strongly on the pharmacokinetics of the inhibitor and, in some cases, inhibition of binding may have no effect on this parameter.

THEORETICAL

The notations are identical to those used in a previous paper (1): C_f = concentration of free drug; C_b = concentration of bound drug; C_t = total concentration of drug, *i.e.*, $C_b + C_f$; P = concentration of protein–drug binding sites; and K_d = dissociation constant of the drug–protein complex.

If V_1 is the volume of the plasma compartment, then, regardless of the number of compartments in the system, the amount of drug eliminated from the plasma in the time interval, dt, is given by:

$$dA_{out} = V_1 k_{app} C_t \, dt \qquad \text{(Eq. 1)}$$

where k_{app} is an apparent constant of elimination. In systems that include binding of drugs to plasma proteins, k_{app} is not constant but is a function of time.

The total amount of drug eliminated is then:

$$A_{out} = V_1 \int_0^\infty k_{app} C_t \, dt \qquad \text{(Eq. 2)}$$

By applying the conservation-of-mass treatment of Wagner (2) and letting D equal the total amount of drug absorbed or injected:

$$\frac{D}{V_1} = \int_0^\infty k_{app} C_t \, dt \qquad \text{(Eq. 3)}$$

However, k_{app} is simply the elimination rate constant for free drug multiplied by the fraction of drug that is free:

$$k_{app} = k_2 \frac{C_f}{C_t} \qquad \text{(Eq. 4)}$$

where k_2 is the free drug elimination rate constant. Substituting in Eq. 3:

$$\frac{D}{V_1 k_2} = \int_0^\infty C_f \, dt \qquad \text{(Eq. 5)}$$

Thus, if the elimination rate constant for free drug is used, the standard expression (2) for the area under the concentration–time curve always relates to free drug, regardless of the amount of drug bound.

However, it is not always necessary to know k_2. If the concentration–time curve is extended to sufficiently low concentrations, the right-hand side of the binding relationship:

$$C_b = \frac{PC_f}{K_d + C_f} \qquad \text{(Eq. 6)}$$

reduces approximately to PC_f/K_d, and C_t/C_f becomes constant:

$$\frac{C_t}{C_f} = \frac{C_b + C_f}{C_f} = 1 + \frac{P}{K_d} \qquad \text{(Eq. 7)}$$

The value of k_{app} then becomes constant at sufficiently long times;

and, from Eqs. 4 and 7:

$$\lim (k_{app}) = \frac{k_2}{B} \qquad \text{(Eq. 8)}$$

where $B = 1 + (P/K_d)$.

Substituting in Eq. 5:

$$\int_0^\infty C_f\, dt = \frac{D}{BV_1 \lim (k_{app})} \qquad \text{(Eq. 9)}$$

and, again, the area under the free drug concentration–time curve may be related to measurable pharmacokinetic parameters.

Equation 9 gives rise to an interesting observation on the effect of competitive binding inhibition. If a competitive inhibitor of drug–protein binding is present, C_t/C_f is decreased since more free drug is present. Thus, values of k_{app} increase as long as the inhibitor is present. However, if the inhibitor is itself eliminated rapidly enough that measurements of total drug concentration can be made after the inhibitor has disappeared, the observed value of lim (k_{app}) is obtained in the absence of inhibitor. Since the value of B refers to the binding parameters effective at the time the measurement of lim (k_{app}) is made, this value is also unchanged. Therefore, according to Eq. 9, the area under the free drug curve is unchanged.

Simply stated, the effect of displacement of bound drug on the area under the plasma free drug concentration–time curve depends on the pharmacokinetics of the displacing agent. In particular, if the displacing agent is eliminated much more rapidly than the drug, there is no effect on the area under the free drug curve.

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Solvation of Montmorillonite

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Abstract □ It is known that polar solvents penetrate the montmorillonite lattice and that multiple layers of solvent can exist between the silicate layers. It is shown here that the number of layers in the interlaminar spacing is different when the clay is equilibrated with liquid than when it is equilibrated with vapor. A method for calculating the number is presented, and a generally usable experimental approach is suggested. Acetonitrile, ethanol, and methanol form 11, 5, and 7 layers, respectively, when equilibrated with montmorillonite as liquids but form only 3, 2, and 2 layers, respectively, when equilibrated with vapors.

Keyphrases □ Montmorillonite—solvation, comparison of liquid and vapor, number of layers in interlaminar spacing □ Solvation—montmorillonite, comparison of number of layers in interlaminar spacing when equilibrated with liquid and vapor

The degree of solvation of montmorillonite has received some attention in the past. Norrish (1) showed that the hydration occurs in two stages: (*a*) adsorption, and (*b*) penetration of water molecules into the crystal lattice. Packter (2) showed that the rate of gelation increases with the sixth power of the montmorillonite concentration. Mering (3) reported that the solvation of montmorillonite is one by which solvent molecules penetrate the clay lattice and that one or more solvent layers are present between the silicate layers, and MacEwan (4) and MacEwan and Talib-Uddin (5) reported the increase in lattice spacing per layer added in between the silicate layers.

These latter studies were conducted by equilibrating the clay with solvent vapor. Quite different phenomena might occur in the (pharmaceutically) more applicable situation of solid–liquid equilibria.

EXPERIMENTAL

Since the solvents with high dielectric constants are the ones liable to form multilayers between the silicate layers (4, 5), the following solvents were checked: acetonitrile, ethanol, and methanol; mixed solvents were avoided. The solute (adsorbate) employed was diazepam, since the diazepam–montmorillonite system has been well elucidated (6). Due to the poor water solubility of diazepam, water could not be included as a solvent in this study. The montmorillonite used was the hydrogen form of the clay and was a micropulverized grade[1]; it was dehydrated under high vacuum

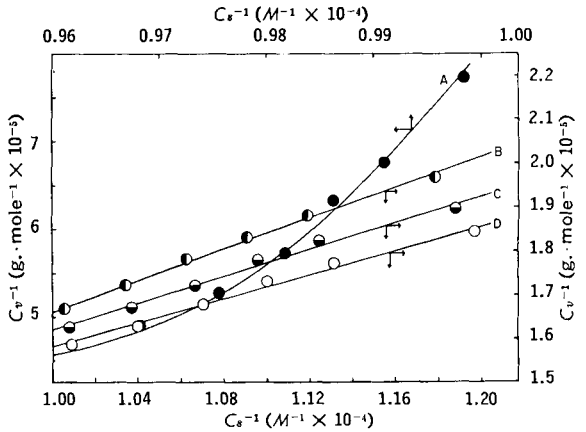

Figure 1—*Adsorption isotherms of diazepam on montmorillonite from acetonitrile. Curve A is the conventionally treated curve, where no allowance is made for solvent intercalation. Curves B, C, and D are curves where allowance is made for 12, 12.5, and 13 layers, respectively, of acetonitrile between silicate layers.*

[1] Veegum neutral micronized, R. T. Vanderbilt Co., Inc., New York, NY 10017.