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Letter to the Editor

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Usefulness of chromatographic methods for the determination of drug–protein binding parameters

Sir,

Dr. Parsons' analysis[7] of our paper raises interesting suggestions on which we want to comment. The most important point involves the influence of the HSA (human serum albumin) concentration in the column used for applying Hummel and Dreyer's method[1].

From a theoretical point of view, multiple equilibria theory[2] gives:

$$\bar{r} = \sum_1^m \frac{n_i\, k_i\, |A|}{1 + k_i\, |A|} \qquad (1)$$

where $\bar{r}$ is the mean number of moles of bound ligand per mole of macromolecule (*i.e.*, per HSA), n_i the number of binding sites, k_i the corresponding association constant, $|A|$ the concentration of free ligand and m the number of independent classes of binding sites. It is clear that $\bar{r}$ is independent of the HSA [or polymer (P)] concentration.

However, this assumption is based on the fact that only combinations of 1 mole of P with 1 or several moles of A, PA, PA$_2$, PA$_3$, ..., PA$_n$, exist in the solution. If some polymerization occurs with P, P$_2$, P$_3$, ..., P$_a$ and if the resulting macromolecule can bind A, eqn. 1 can no longer be used and $\bar{r}$ depends on the P concentration.

Cann and Hinman[3] considered this possibility and concluded that if this event occurs, then the chromatogram will show a third peak, between the ligand HSA peak and the negative peak that represents the ligand trapped by the protein. In our experiments, we have never observed such a third peak and we conclude that such an interaction between HSA molecules is unlikely to occur.

In drug–protein binding studies at plasma levels, it is important to note that the association constants of such interactions are significantly different when measured under physiological conditions (HSA = 40 g/l) to those measured with HSA = 2 g/l[4,5]. In our previous studies, we never found any difference in the k_i values of warfarin–HSA interactions with HSA concentrations varying from 1 to 10 g/l. However, we do not know what happens at higher concentrations.

As emphasized by Dr. Parsons, in the Hummel and Dreyer method the polymer concentration is not constant but decreases during the elution. We therefore checked a new method that holds constant the HSA concentration during the binding measurement[6]. The results in Fig. 1 indicate that $\bar{r}$ values vary only slightly, but it must be recognized that the range of HSA concentrations was not sufficiently wide.

The Hummel and Dreyer method requires a column able to separate the complex drug–polymer from the free ligand[6]. The area of the complex peak depends on the amount of protein injected and the concentration of the eluting ligand, but it
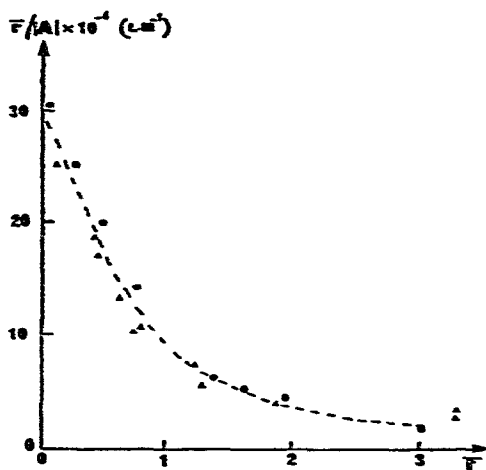
Fig. 1. Scatchard plot for HSA–warfarin interaction at 37°C. ●, Hummel and Dreyer method[1]; ▲, saturation method[6] (HSA concentration = 0.4 g/l); △, saturation method[6] (HSA concentration = 2.0 g/l).

is independent of ligand injected in excess. We have observed the latter property in all of our experimental determinations.

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1 J. P. Hummel and W. J. Dreyer, *Biochim. Biophys. Acta*, 63 (1962) 530.
2 I. M. Klotz, *Arch. Biochem.*, 9 (1946) 109.
3 J. R. Cann and N. D. Hinman, *Biochemistry*, 15 (1976) 4614.
4 C. J. Bowner and W. E. Lindup, *J. Pharm. Sci.*, 67 (1978) 1193.
5 S. W. Boobis and C. F. Chignell, *Biochem. Pharmacol.*, 28 (1979) 751.
6 B. Sebille, N. Thuaud and J. P. Tillement, *J. Chromatogr.*, 180 (1979) 103.
7 D. L. Parsons, *J. Chromatogr.*, 193 (1980) 520.