Influence of Volume Shift in Equilibrium Dialysis to Estimate Plasma Protein Binding of Drugs

Joseph J. H. M. Lohman[1,3], Piet M. Hooymans[1], Maurice T. J. M. Verhey[2], Marli L. P. Koten[2], Frans W. H. M. Merkus[1,2]

Received: March 2, 1984; accepted March 17, 1984.

Osmotic volume shifts across the dialysis membrane can occur using equilibrium dialysis in the determination of plasma protein binding of drugs (1, 2). As a result of this dilution of serum or plasma with buffer the drug concentration in the serum or plasma compartment is lowered. If the free fraction of a drug is calculated as the ratio of the equilibrium concentrations in both compartments, this leads to an overestimation of the free fraction.

At equilibrium the serum can be considered as being diluted with buffer, containing the drug at the unbound concentration (2). Therefore the total (bound plus unbound) concentration C_t' in the serum after dialysis can be represented as:

$$C_t' = \frac{C_t \cdot V_s + C_f \cdot \Delta V_s}{V_s + \Delta V_s} \quad \text{(Eq. 1)}$$

where C_t indicates the total drug concentration in serum at equilibrium, assuming no volume shift had occurred, V_s the serum volume before dialysis, ΔV_s the increase of the serum volume and C_f the drug concentration in buffer at equilibrium. Thus the total equilibrium serum concentration, corrected for the volume shift, can be obtained from:

$$C_t = \frac{C_t'(V_s + \Delta V_s) - C_f \cdot \Delta V_s}{V_s} \quad \text{(Eq. 2)}$$

In cases of linear protein binding the free fraction of the drug is independent of the total concentration. Then the free drug concentration in the original serum (C_u) is determined from the relationship:

$$C_u = \frac{C_f}{C_t} \cdot C_s \quad \text{(Eq. 3)}$$

where C_s is the total drug concentration in the original serum. Substitution of eq. (2) in (3) gives:

$$C_u = \frac{C_f \cdot V_s}{C_t'(V_s + \Delta V_s) - C_f \cdot \Delta V_s} \cdot C_s \quad \text{(Eq. 4)}$$

Therefore, the following parameters must be known to calculate the free drug concentration: the serum volume before and after dialysis, the drug concentration in the original serum, and the drug concentrations in serum and buffer after dialysis.

The increase of serum volume after dialysis of 1 ml serum against 5 ml isotonic phosphate buffer was measured over a period of 5 to 16 hours. A Dianorm equilibrium dialysis apparatus (Diachema, Rüschlikon, Switzerland) in a 37 °C waterbath was used. The velocity of rotation was 12 r.p.m. Both halves of the teflon dialysis cells (type Makro 1S and Makro 5 respectively) were separated by a cellulose membrane (Diachema) with a molecular weight cut off of 5000. The results are presented in Table I. After dialyzing overnight (16 hours) the volume in the serum compartment was 1.43 ± 0.07 ml (mean ± SD, n = 194).

Fig. 1 shows the relationship between the volume shift and the relative overestimation of the free fraction as calculated using equations (1) and (2) for three hypothetical drugs with true free fractions of 0.50, 0.10 and 0.01 respectively. In case of an increase of the serum volume by 43 % the free fraction of highly bound drugs would be overestimated by approximately 40 %.

Table I. Post-dialysis serum volume after equilibrium dialysis of 1 ml serum against 5 ml isotonic phosphate buffer.

Time (h)	Volume (ml)	n
0	0.96±0.03[a]	30
5	1.06–1.13	3
6	1.16–1.18	3
7	1.16–1.21	3
8	1.12–1.21	3
9	1.17–1.30	3
10	1.24	3
11	1.22–1.31	3
12	1.25–1.34	3
13	1.30–1.35	3
14	1.22–1.37	3
16	1.43±0.07[a]	194

[a] mean ± SD

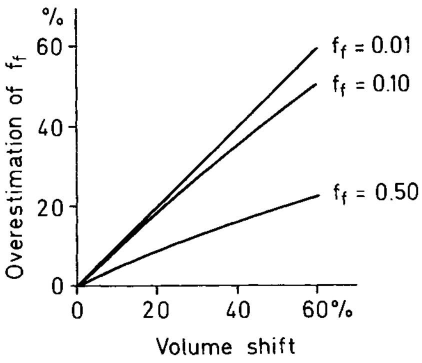

Fig. 1 Relationship between the volume shift and the relative overestimation of the free fraction as calculated using equations (1) and (2) for three hypothetical drugs with true free fractions (f_f) of 0.5, 0.1, and 0.01, respectively.

Most authors applying equilibrium dialysis do not mention any volume change. However, when using dialysis tubings, a considerable increase (30–60 %) of the serum volume has been reported (1). Recently a mean volume shift of 31 % was calculated in dialysis cells with equal volumes (0.5 ml) on both sides of the membrane (2). The correction method described here corresponds with procedures used earlier (1, 3). According to a recent method (2), measurement of the postdialysis volume is not necessary. However, with this approach significant binding to cell walls

---

[1] Department of Clinical Pharmacy, Hospital Sittard, Sittard, The Netherlands

[2] Department of Biopharmaceutics, University of Amsterdam, Amsterdam, The Netherlands

[3] Address Correspondence to: Dr. J. J. H. M. Lohman, Department of Clinical Pharmacy, Hospital Sittard, Walramstraat 23, 6131 BK Sittard, The Netherlands

or membrane has to be excluded first. Our method accounts for potential errors caused by volume shifts, binding to membrane or cell wall and transfer of drug from the serum side to the buffer side. In case of non-linear protein binding, other more complicated correction methods have to be applied (2, 4).

It can be concluded that failure to correct for volume shifts in protein binding experiments using equilibrium dialysis can lead to considerable errors, particularly with highly bound drugs. We agree with Tozer et al. (2) that many published data on protein binding may be in error.

References

(1) Ramirez-Ronda, C. H., Holmes, R. K., Sanford, J. P. (1975) Antimicrob. Agents Chemother. 7, 239–245.
(2) Tozer, T. N., Gambertoglio, J. G., Furst, D. E., Avery, D. S., Holford, N. H. G. (1983) J. Pharm. Sci. 72, 1442–1446.
(3) Huang, J. D. (1983) J. Pharm. Sci. 72, 1368–1369.
(4) Behm, H. L., Wagner, J. G. (1979) Res. Commun. Chem. Path. Pharmacol. 26, 145–160.

CONGRESS ANNOUNCEMENTS

August 27–31, 1984
VIIIth International Symposium on Medicinal Chemistry, Biochemical Center, University of Uppsala, Uppsala Sweden.

Septembre 2–6, 1985
45e Congrès International des Sciences Pharmaceutiques de la Fédération Internationale Pharmaceutique. Votre hôte l'Association Québécoise des Pharmaciens Propriétaires. 5115 rue St-Denis, Montréal, Québec, Canada, H2J 2M1, (514) 273-1721.

September 15–19, 1986
IXth International Symposium on Medicinal Chemistry. This symposium will be held in Berlin-West.
Info: **Prof. Dr. E. Mutschler,** Pharmakologisches Institut für Naturwissenschaften der Universität, Theodor-Stern-Kay, Gebäude 75a, D-6000 Frankfurt/Main, F.R.G.