4P

A DOUBLE EQUILIBRIUM GEL SEPARATION TECHNIQUE FOR DETERMINATION OF BILE SALT MONOMERS

C.J. Olliff, P.M. Tang, Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ

Bile salts are known to be involved in several biologically important physicochemical reactions such as gallstone dissolution and membrane interaction (Barbara et al 1983) and a knowledge of the concentration of bile salt monomer could help in understanding some of the mechanisms involved. Several methods have been reported in the literature for obtaining monomer concentration including that of Amman and Walter (1982). Their technique uses Sephadex G10, ^{14}C labelled bile salt and ^{3}H labelled aqueous phase marker, raffinose. The object of this work was to develop a method without the use of radiochemicals or an aqueous phase marker.

Sephadex G10 beads have an exclusion limit of 700 daltons which prevents dimers and higher aggregates of bile salts entering the gel. The following equilibria could exist in a solution containing Sephadex G10 beads and bile salt: $B_{MB} \rightleftharpoons B_{MA}$ and $xB_{MA} \rightleftharpoons (B_{MA})_x$ where B_{MB} and B_{MA} are bile salt monomer in Sephadex and in supernatant respectively and $(B_{MB})_x$ are bile salt aggregates $x \geqslant 2$. If aggregation does not occur then total bile concentration $[B_T]$ equals monomer concentration $[B_M] = [B_{MB}] + [B_{MA}]$. If the beads are separated and allowed to reestablish equilibria in a fresh buffer solution then $[B_{MB}] = [B''_{MB}] + [B''_{MA}]$ since only monomer is present in the beads (superscript refers to second equilibrium between gel and buffer). Assuming that distribution coefficients are constant then for a micellar solution the monomer concentration $[B_M] = [B''_{MA}]/K$ where the constant K is the slope of plot of $[B''_{MA}]$ against $[B_T]$ when $[B_T]$ < critical micelle concentration (CMC). Bile salts can be assayed by reverse phase high performance liquid chromatography (HPLC) using C_{18} columns with an acetonitrile water mobile phase and a UV detector operating at 200 nm (Linnet 1982). The procedure adopted used 200 mg Sephadex G10 beads which were incubated for three hours with shaking at room temperature with 10 cm^3 of 0.02 M phosphate buffer pH 7.4 containing taurodeoxycholate (TDC) concentrations up to 20 mM. Further experiments were carried out in which sodium chloride (0.158 M) and a range of egg lecithin concentrations were added to the incubation mixtures. The Sephadex beads were separated by centrifuging at 2000 rpm for ten minutes, allowed to drain by inversion and then resuspended in buffer. Equilibrium was allowed to be established and the beads recentrifuged. The supernatant was then analysed by HPLC. All experiments were repeated at least in duplicate. K used in the calculations was determined in each experiment using TDC concentrations below its CMC and gave a value of 0.420 $\pm$ S.D. 0.008 for buffer and 0.382 $\pm$ 0.012 for 0.158 M NaCl. The results show that the distribution is constant and independent of concentration up to the CMC. From a graph of monomer concentration plotted as a function of bile salt concentration a CMC of 1.9 mM was obtained. The number of monomers present was reduced by the order of 16% when the solution contained 0.158 M sodium chloride. In solutions containing equimolar concentrations of TDC and egg phosphatidylcholine in phosphate buffer the CMC was lowered to 1 mM TDC and the monomer concentration at higher TDC values all decreased by approximately the same amount (38% $\pm$ S.D. 0.8). These results are in good agreement with those found by Amman and Walter (1982) and Kratohvil (1984). The method developed can be used for the determination of bile salt monomers in the presence of other biochemically important compounds which will enable the relevance of the monomer concentration in bile salt interactions to be evaluated.

Barbara, L. et al (Ed) (1983) Bile Acids in Gastroenterology, Pub. MTP Press
Amman, H.V. & Walter, Z.G. (1982) Anal. Chem. 54: 2079-2082
Linnet, K. (1982) Scand. J. Clin. Lab. Invest. 42: 455-460
Kratohvil, J.P. (1984) Hepatology 4: 855-915