

Sorption of Water & Some Straight- & Branched-chain Aliphatic Alcohols by Casein

R. K. SETHI & S. L. CHOPRA

Department of Chemistry & Biochemistry, Punjab Agricultural University, Ludhiana

Received 24 August 1974; accepted 4 April 1975

Sorption isotherms of water, methanol, ethanol, *n*-propanol, *n*-butanol, *n*-pentanol, isopropanol, isobutanol, *s*-butanol, *t*-butanol and isopentanol on casein have been determined at 35°, using quartz fibre spring technique. The sorption capacity of the protein is greater for branched chain alcohols than that for normal alcohols and the amount of sorbate taken up decreases with increase in the length of the hydrocarbon chain. The specific surface area of casein has been calculated by the application of the BET theory. The values of the thermodynamic functions indicate the absence of chemical interactions between the adsorbate and adsorbent.

SORPTION of water vapour by a variety of proteins¹⁻⁶ including caseins⁷⁻⁹ has been extensively studied. However, little work has been done on the sorption of alcohols and related compounds by these materials. The importance of water sorption as a possible tool for the estimation of surface area of proteins, in elucidating varietal differences and for indicating possible interaction with the ionic groups in these substances has rightly been emphasized by several investigators^{5,8,9,10}. A systematic study of the sorption behaviour of a series of polar sorbates of equal capacity for binding but of varying size has now been undertaken. This is expected to throw some light on the variation of sorptive behaviour of proteins with change in the hydrocarbon chain of the alcohol molecules. Of particular importance are the studies on the sorption of alcohols of low molecular weight on casein because unlike water these alcohols which are similar to water in certain chemical properties do not cause swelling of the protein network.

Materials and Methods

The vitamin-free, crystallized and lyophilized sample of casein (purity >99%) was obtained from M/s Calbiochem, Los Angeles, USA. The particle size was that of 100 mesh British standard sieve. The sorbates used were water, methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, *s*-butanol, isobutanol, *t*-butanol, *n*-pentanol and isopentanol. Alcohols were of AR grade (BDH).

The quartz fibre spring technique described by Rao¹¹ was employed in the present work. The sorption apparatus was kept at 35° inside an air thermostat. The apparatus was evacuated to 10⁻² mm. A cathetometer reading up to 10⁻² mm was employed for measuring the length of the spring. The rate of sorption of water and alcohols on casein was slow and the equilibrium was established generally after 4 to 5 hr.

Results and Discussion

Sorption process—The adsorption isotherms for water and normal alcohols are shown in Fig. 1a

while those for isomeric alcohols are plotted in Fig. 1b. The isotherms for both types of alcohols are similar except that the sorption values for branched-chain alcohols are slightly higher in comparison to those for the normal alcohols. The amounts of sorbates taken by casein at the saturation pressure of water, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *s*-butyl, *t*-butyl, *n*-pentyl and isopentyl alcohols are 35.6, 30.2, 27.0, 25.0, 32.6, 17.5, 24.5, 26.2, 28.8, 12.2 and 12.5% respectively.

It is observed that the amount of sorbate taken up by casein decreases with the increase in the length of the hydrocarbon chain. The amount sorbed starts falling off much faster as soon as the length

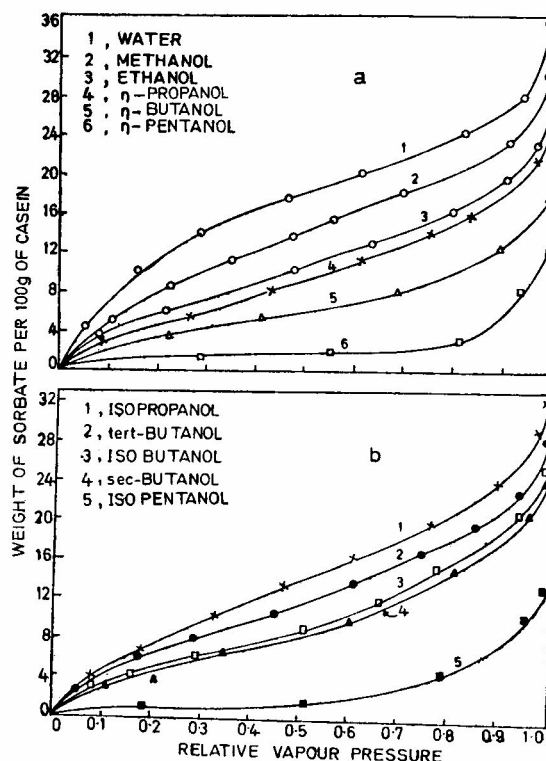


Fig. 1—Adsorption isotherms of water and different alcohols

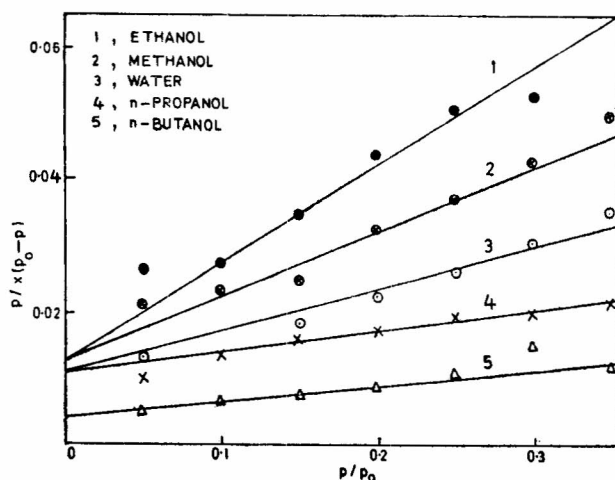


Fig. 2 — Application of BET equation

of the alkyl groups increases beyond three carbon atoms. The alcohols under study have essentially the same polarity (for example, the dipole moment values of methanol, ethanol, *n*-propanol and *n*-butanol are 1.70, 1.69, 1.66 and 1.66 respectively), and therefore the decrease in the extent of sorption is mainly due to the increase in the molecular size. The amount of branched-chain alcohols sorbed is comparatively more than that of normal alcohols. This is due essentially to shorter chain lengths of the former. The adsorption is speculated³ to be on the freely exposed surface, followed by penetration and is promoted by swelling to some extent. Therefore, the longer the chain, higher will be the energy barrier for the penetration. This may be due to a rapid increase in steric effects at this length. Our observations on the mechanism of sorption and diffusion of water vapour by albumins strongly support the above hypothesis.

Surface area of casein—The sorption isotherms (Fig. 1, a and b) are sigmoid in shape and belong to type-II of the BET classification, indicating that the sorption of water and aliphatic alcohols by casein is multimolecular. The BET plots are shown in Fig. 2. The plots for *n*-pentanol and branched-chain alcohols are not given for reasons of space. From the slope and intercept of each plot, the values of monolayer capacity have been calculated¹². These values along with the cross-sectional area of the sorbate molecule^{13,14} have been employed to calculate the surface area¹² of casein. The results are recorded in Table 1.

The value of the specific surface of casein determined using liquids of various vapours are not coincident. This casts a doubt on the application of BET equation to systems in which there is swelling of the sorbent. Casein certainly swells better with water and it may swell to different extents with various alcohols. However, it does not seem likely that the variation in the surface area due to swelling would be large enough to account for all these differences.

Pore-size distribution—Following the procedure of Foster^{12,15}, the pore-size distribution has been determined from the isotherms. No allowance is

made for the changing thickness of the adsorbed layer on the walls of the pores. The isotherms of water, methanol and ethanol have been chosen for these calculations as the contact angle is assumed to be zero for these sorbates. The predominant pore size for casein has been found to be 4.2, 5.8 and 5.4 Å respectively for these systems. It is interesting to note that there are micropores in the protein according to the classification of adsorption isotherms by Dubinin¹⁶.

Thermodynamic constants—The values of the net heat of adsorption in the BET equation has been calculated from Eq. (1)¹³,

$$c = e^{(E_1 - E_L)/RT} \quad \dots(1)$$

where E_1 is the heat of adsorption in the first layer and E_L is the heat of liquefaction of the adsorbate (Table 2). The low magnitude of the heat of adsorption is indicative of the weaker interaction of alcohols with casein and rules out the possibility of any chemical interaction. Moreover, the values are of the same order for all the alcohols showing that the interacting forces are of the same kind. There appears to be no precise relation, however, between the energy liberated and the length of the carbon chain.

Further, the free energy change accompanying the sorption of vapour by casein has been evaluated using Eq. (2),

$$\Delta G = \frac{RT}{M} \int_0^f a \cdot \frac{df}{f} \quad \dots(2)$$

where a is the weight of the liquid sorbed at a relative vapour pressure f and other parameters have their

 TABLE 1 — VALUES OF MONOLAYER CAPACITY (X_m), MOLECULAR CROSS-SECTION (A_m) AND SPECIFIC SURFACE (S) FOR VARIOUS SORBATES

Sorbate	X_m (g/g of adsorbent)	A_m (Å ²)	S (m ² /g)
Water	0.1393	10.6	487.9
Methanol	0.0898	21.2	358.4
Ethanol	0.0726	27.0	256.5
<i>n</i> -Propanol	0.1592	31.4	501.5
<i>n</i> -Butanol	0.2272	36.0	631.8
<i>n</i> -Pentanol	0.0606	40.2	166.7
Isopropanol	0.0896	14.5	130.4
Isobutanol	0.0519	25.0	105.8
<i>s</i> -Butanol	0.0500	17.4	70.8
<i>t</i> -Butanol	0.0723	55.5	326.4
Isopentanol	0.0947	28.8	186.6

TABLE 2 — THERMODYNAMIC PARAMETERS AT 35°

Sorbate	Heat of adsorption ($E_1 - E_L$) (cal/mole)	$-\Delta G$ (cal/mole)
Water	545	1197
Methanol	579	288
Ethanol	636	302
<i>n</i> -Propanol	471	203
<i>n</i> -Butanol	473	107
<i>n</i> -Pentanol	345	32
Isopropanol	654	323
Isobutanol	636	166
<i>s</i> -Butanol	644	156
<i>t</i> -Butanol	637	212
Isopentanol	750	36

usual significance. The values of free energy (Table 2) are again quite low and indicate the involvement of physical interactions. In a series of analogous reactions, the rates of reactions are often roughly in the order of free energy change. Thus a comparison between the straight-chain and the branched-chain alcohols reveals that the latter have a comparatively greater affinity for the casein surface.

Contact angle and the sorption isotherms — According to Gregg¹², the contact angle is assumed to be zero when the surface is pure and the liquid wets the surface; as a result the isotherm is asymptotic to the saturation pressure ordinate. But with liquids which have a definite contact angle, the isotherms intersect the ordinate at an angle. In the present experiment, the asymptotic nature of water, methanol, ethanol and *n*-propanol isotherms leads to the conclusion that the contact angle is zero. Fox and Zisman¹⁷ have shown that for many liquids on solids, the contact angle decreases with the decreasing surface tension of the liquid. The values of the surface tension¹⁸ of *n*-butanol and *n*-pentanol are 23.35 and 24.30 dynes/cm respectively (at 35°) showing thereby that contact angle increases from *n*-butanol to *n*-pentanol. As regards branched-chain alcohols, the values of surface tension^{18,19} of *t*-butyl, isopropyl, isobutyl, *s*-butyl and isoamyl alcohols are 18.8, 20.5, 21.8, 22.2 and 22.7 dynes/cm respectively (at 35°). In the light of Fox and Zisman's conclusion, the contact angle of these alcohols should be in the order: *t*-butyl < isopropyl < isobutyl < *s*-butyl and < isopentyl alcohol. The shapes of the adsorption isotherms (Fig. 1) support these observations.

Acknowledgement

One of the authors (R.K.S.) thanks the CSIR, New Delhi, for the grant of a research fellowship.

References

1. ELEY, D. D. & LESILE, R. B., *Trans. Faraday Soc.*, **62** (1966), 1002.
2. RAO, K. S. & DAS, B., *J. phys. Chem.*, **72** (1968), 1223.
3. DAS, B., SETHI, R. K. & CHOPRA, S. L., *J. Indian chem. Soc.*, **50** (1973), 119.
4. SEEHOF, J. M., KOILIN, B. & BENSON, S. W., *J. Am. chem. Soc.*, **75** (1953), 2427.
5. MCLAREN, A. D. & ROWEN, J. W., *J. polymer Sci.*, **7** (1951), 289.
6. BULL, H. B., *J. Am. chem. Soc.*, **66** (1944), 1499.
7. BERLIN, E., ANDERSON, B. A. & PALLANSCH, M. J., *J. phys. Chem.*, **73** (1969), 303.
8. PURI, B. R., TOTEJA, K. K. & MALIK, R. C., *J. Indian chem. Soc.*, **46** (1969), 554.
9. GREEN, R. W., *Proc. R. Soc. (New Zealand)*, **77** (1949), 313.
10. MELLON, E. F., KORN, A. H. & HOOVER, S. R., *J. Am. chem. Soc.*, **70** (1948), 3040.
11. RAO, K. S., *J. phys. Chem.*, **45** (1941), 500.
12. GREGG, S. J. & SING, K. S. W., *Adsorption, surface area and porosity* (Academic Press, London and New York), 1967.
13. RAO, K. S. & DAS, B., *J. Colloid & Interface Sci.*, **32** (1970), 24.
14. RAO, K. S. & DAS, B., *Proc. Indian Acad. Sci.*, **70** (1969), 18.
15. FOSTER, A. G., *J. chem. Soc.*, (1952), 1806.
16. DUBININ, M. M., *Q. Rev. chem. Soc.*, **9** (1959), 101; *Chem. Rev.*, **60** (1960), 235.
17. FOX, H. W. & ZISMAN, W. A., *J. Colloid Sci.*, **5** (1950), 514; **7** (1952), 109.
18. TIMMERMANS, J., *Physico-chemical constants of pure organic compounds* (Elsevier, Amsterdam), 1950.
19. *International critical tables*, edited by W. Washburn, Vol. 4 (McGraw-Hill, New York), 1928, 453.