Studies in Physico-chemical Properties of Caseins: Part VI. Selective Adsorption from Binary Solutions on Caseins

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Adsorption of water and methanol vapours on caseins isolated from milk by different methods is a physical and reversible process. There appears to be no evidence for chemisorption of any portion of the adsorbed vapours. The adsorption of these vapours takes place on the hydrophilic portion of the surface constituted by the presence of polar groups and, therefore, measures only the hydrophilic portion of the surface. The rest of the surface, constituted by the hydrophobic residues of the amino acids, escapes measurements. The composite adsorption isotherms of binary liquid solutions of methanol and benzene as well as methanol and carbon tetrachloride are V-shaped indicating strong preference for methanol in each case. The composite isotherms of binary solutions of benzene and *n*-heptane, in which both the components are non polar, are typical S-shaped indicating that there is no exclusive preference for any of the components. The extrapolation of the linear section of any one of the isotherms gives intercepts on the ordinates which define the composition of the adsorbed phase in the monolayer from which the total surface of the caseins including hydrophilic and hydrophobic portions, can be evaluated.

THE sorption isotherms of water[1] as well as methanol and ethanol vapours[2] on caseins isolated from milk by different methods were described in previous publications. The data were analysed using B.E.T. and Harkins and Jura equations. The values of specific surface area of casein calculated from these isotherms, by using either of the two equations, were fairly in good agreement with each other. Some workers[3,4], however, have questioned the applicability of B.E.T. equation to the sorption of water or methanol vapour on proteins on account of possibility of chemisorption of these vapours. The value for the net heat of sorption, $(E_1 - E_L)$, as obtained from B.E.T. constant, c, for the sorption of water[1] and methanol[2] vapours were sufficiently low to indicate any chemical interaction. It may be of interest, all the same, to adduce direct evidence for or against chemisorption in such systems.

There is another aspect of the problem. The close agreement between V_m and the amount of water or methanol required to be adsorbed per ionic group[1,2] indicates that, possibly the hydrophobic portion, which according to the calculations of Bull and Breese[5] constitutes, in certain proteins, nearly half of the total surface, escapes measurement. Schay and Nagy[6] have suggested the use of composite adsorption isotherms from binary solutions for estimating surface areas of a number of adsorbents including highly polar substances such as silica and alumina gels and non polar materials such as carbon blacks and charcoals. They have shown that the linear portion of the isotherm following the maxima represents constant composition of the monolayer in the absorbed phase from which the area of the surface can easily be calculated, provided there is no chemisorption of any of the constituents.

The estimation of hydrophobic/hydrophilic ratio of casein may have significant biological importance, and it is necessary, therefore, to estimate hydrophobic portion of the surface as well. The present work was, therefore, undertaken.

Experimental

Caseins were isolated from bovine milk by the methods described before[7]. The mass was washed in a blender first with water, then with acetone to remove free water and finally with ether. It was then dried over sulphuric acid in a vacuum desiccator.

Casein (2 g), taken in a small crucible, was wetted by water (or methanol) by adding the liquid drop by drop with continuous kneading, using a glass rod, so as to get an intimate agglomerate. This was allowed to stand in contact with the vapour of the liquid contained in a vacuum desiccator kept in an incubator maintained at 30° (±0·2°). After equilibration, as indicated by constant weight, the liquid was removed from the desiccator and the system evacuated to a pressure of 10^{-4} mm. for different intervals of time and the amount of the adsorbate retained by casein was determined gravimetrically at each step. In another set of experiments, the system was equilibrated at 30° as before but evacuation was done at 33°.

The effect of temperature on the amount of adsorption was studied by equilibrating the system at 40°, 45° and 50° also.

Selective adsorption was studied from the following binary solutions in the entire concentration range : (*a*) methanol-benzene, (*b*) methanol-carbon tetrachloride and (*c*) benzene-*n*-heptane. For this

purpose, casein (0·2 g.) was mixed with 5 ml of each mixture in a stoppered pyrex glass bulb which was then sealed and shaken mechanically in an incubator maintained at 30° for 24 hr. The change in composition was determined refractometrically using a highly sensitive interferrometer capable of measuring refractive index upto 7th place of decimal.

Results and Discussion

The amounts of water and methanol vapours adsorbed on two samples of casein at 30° and the amounts retained on evacuation at the same temperature, as well as at 33°, for different intervals of time are recorded in Table 1. It is seen that desorption sets in on evacuation and that almost the entire amount of each adsorbate escapes after continued evacuation for 12 hr. The small amount retained may be due to hysteresis generally observed at low

where Δx is the decrease in mole fraction of methanol due to adsorption on m g. for the adsorbent brought into contact with the binary mixture containing a

TABLE 2. AMOUNTS OF WATER AND METHANOL VAPOURS ADSORBED AT SATURATION VAPOUR PRESSURE BY CASEINS AT DIFFERENT TEMPERATURES

Temp. °C	Amount of water vapour adsorbed (mg./g)		Amount of methanol vapour adsorbed (mg /g.)	
	Hammarsten casein	Zoller casein	Hammarsten casein	Zoller casein
30	515.0	501.6	355.7	340.1
40	462.3	450.8	318.3	311.5
45	435.7	426.2	280.6	281.4
50	407.5	400.1	226.4	223 3

TABLE 1. AMOUNTS OF WATER AND METHANOL VAPOURS RETAINED BY CASEIN ON EVACUATION AT 30° AND 33° AFTER PRESATURATION AT 30°

Time for which evacuated (hrs.)	Amount of water vapour retained (mg./g.)				Amount of methanol vapour retained (mg./g.)			
	Hammarsten casein		Zoller casein		Hammarsten casein		Zoller casein	
	30°	33°	30°	33°	30°	33°	30°	33°
0	515.0*	515.0*	501.6*	501.6*	355.7*	355.7*	340.1*	340.1*
1	385.2	228.6	380.2	216.4	57.2	32.1	55.4	34 2
2	50.1	20.3	47.7	15.7	2.9	0.8	2.7	1 9
4	34.4	3.4	33.5	4.1	1 0	0.0	1.1	0.0
5	8.8	0.0	7 3	0.0	0.0	—	0.0	—
12	3.7	—	2.9	—				

* These are the amounts of vapours adsorbed on equilibrating the samples with water or methanol vapours at 30°.

relative vapour pressures. The rate of desorption on raising the temperature of the system from 30° to 33° becomes faster and the process gets completed within a shorter interval of 5 hr. These results show fairly convincingly that adsorption of water and methanol vapours by casein is a purely physical process and that there is no irreversible fixation or chemisorption of these vapours. Further, the amount of adsorption of each vapour is seen (Table 2) to fall on raising the temperature of the system to 40°, 45° and 50°, the higher the temperature. the greater is the fall. Since the magnitude of physisorption decreases while that of chemisorption increases with rise of temperature, there seems to be no evidence for chemisorption of these vapours.

Composite Adsorption Isotherms : The composite adsorption isotherms of methanol-benzene solutions of various samples of casein are shown in Fig. 1. The concentration axis (abscissa) represents x, the mole fraction of methanol in the liquid phase at equilibrium, while the ordinate represents $n_0 \Delta x/m$

total of n_0 millimoles of both the liquids. It is seen that Δx increases with increase in x, becomes maximum when x is around 0·15 and shows a continuous fall thereafter, but at no stage it attains a negative value. This shows that methanol is preferred to benzene in almost the entire concentration range.

The various samples of casein show small variations among themselves. The sample isolated by the ultracentrifuge method, which is not chemically altered at all, shows the maximum while that isolated by the rennet method, which probably undergoes structural changes as a result of enzymatic reactions, shows the lowest preference for methanol. The remaining three caseins (acid caseins) lie in between with regard to their preference for methanol.

The composite adsorption isotherms of methanol-carbon tetrachloride mixtures (Fig. 2) show similar trends although methanol is seen to be preferred now to a somewhat larger extent. This may be due to the fact that benzene is less non polar (due to the presence of π electrons) than carbon tetrachloride.
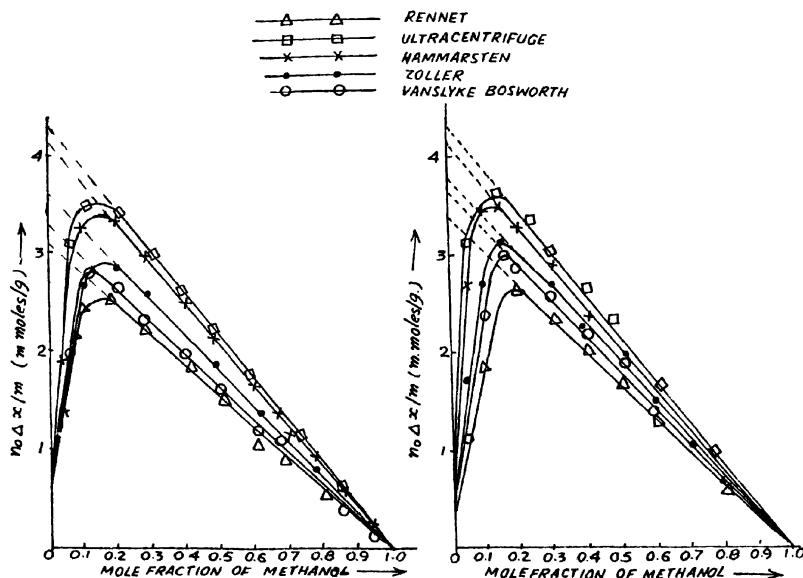
Fig. 1. Composite adsorption isotherms at 30° of Methanol-Benzene mixtures on the various samples of casein.
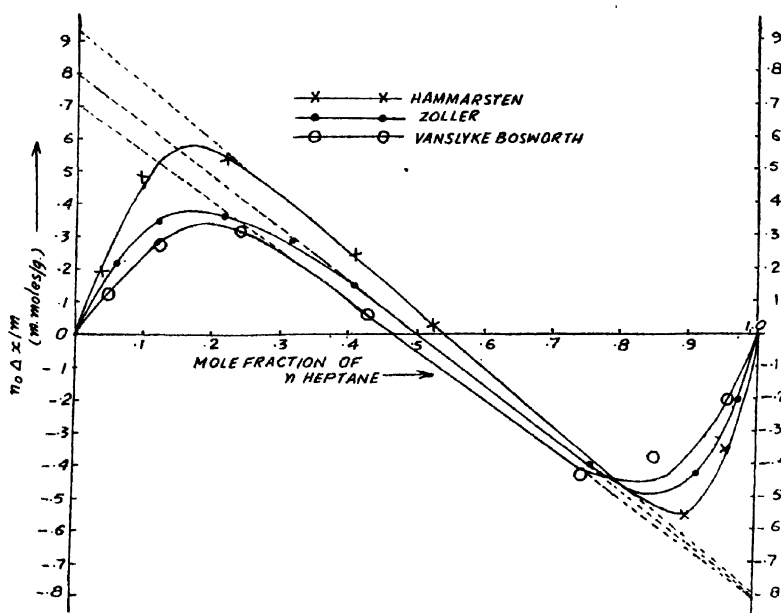

Fig. 2. Composite adsorption isotherms at 30° of Methanol-Carbon tetrachloride
mixtures on the various samples of casein.

The composite isotherms plotted in Figs. 1 and 2 are of type II of Schay and Nagy classification. In such cases adsorption of the preferred component is so strong that at concentrations higher than the one corresponding to the maximum of the isotherm, the second component is expelled almost completely from the surface. Further, since the composition of the adsorbed phase remains constant along the linear portion of the isotherm, the extrapolation of the linear section to $x = 0$ gives intercept on the ordinate which corresponds to the number of moles of methanol in the monolayer covering the entire surface.

The specific surface areas of various samples of casein, thus obtained from methanol-benzene as well

as methanol-carbon tetrachloride isotherms, are recorded in Table 3. These values are seen to be in fair agreement with each other for a given sample of casein but nearly twice as high as the B.E.T. values obtained from adsorption of water or methanol from vapour phase reported earlier[1,2] and now reproduced in Table 3 for ready reference. Since the

placed directly in contact with the appropriate solution and under these conditions each site, whether hydrophilic or hydrophobic (i.e., polar or non polar), gets covered by methanol. Similar findings have been reported[8] in the case of strongly polar adsorbents like silica and alumina gels, as well as non polar adsorbents, like charcoals and carbon blacks.

TABLE 3. SPECIFIC SURFACE AREAS OF VARIOUS CASEINS

Specific Surface Areas as obtained from

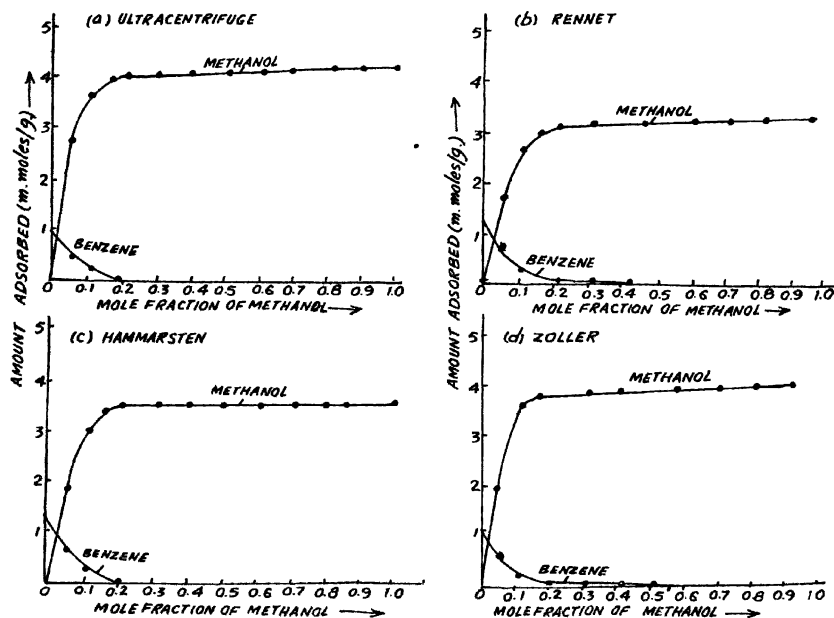| Description of the Sample	Composite Adsorption Isotherms			B.E.T. Equation	
	Methanol-benzene mixtures	Methanol-carbon tetrachloride mixtures	Benzene-n heptane mixtures	Methanol-Isotherms (2)	Water Isotherms (1)
Rennet	320	340	—	177	187
Ultracentrifuge	420	428	—	—	193
Hammarsten	405	410	430	175	170
Zoller	360	380	400	170	176
Vanslyke-Bosworth	330	360	380	187	169

Fig. 3. Composite adsorption isotherm at 30° of Benzene-n heptane mixtures on the various samples of casein.

B.E.T. values were found to correspond to sorption of one molecule of water (or methanol) per ionic (hydrophilic) group, the hydrophobic portion of the surface must have remained unaffected by the sorption of water or methanol from vapour phase. However, in the present experiments, casein was

The composite adsorption isotherms from benzene-n-heptane mixtures, in which both the components are non polar, on three of the samples of casein are plotted in Fig. 3. The curves are typical S-shaped (type III) indicating that there is no exclusive preference for any one of the constituents. The pre-

ference for benzene is greater in the earlier portion and that for *n*-heptane in the latter portion of the isotherm. In such cases, extrapolation of the linear section of the isotherm gives intercepts on the ordinates through $x = 0$ and $x = 1$ and these define the composition of the adsorbed phase in the mono-layer. Taking molecular areas of benzene and *n*-heptane as [9] $40 \cdot 0°$ and $44 \cdot 0$ Å [10] respectively, the specific surface areas of the various caseins are obtained. These are included in Table 3 and are seen to be in fair agreement with those obtained from methanol-benzene and methanol-carbon tetrachloride isotherms.

The observations recorded above suggest that composite adsorption isotherms from binary liquid mixtures place at our disposal a convenient method for estimating total specific surface areas of caseins and may be of other proteins as well.

Adsorption isotherms of individual components can be calculated by combining the following two equations as has been done by Gasser and Kipling[11] :

$$n_0 \, \Delta x/m = n_1{}^s(1-x) - n_2{}^s x \qquad \ldots \text{(i)}$$

$$n_1{}^s/(n_1{}^s)_\mathrm{m} + n_2{}^s/(n_2{}^s)_\mathrm{m} = 1 \qquad \ldots \text{(ii)}$$

where $n_1{}^s$ and $n_2{}^s$ are the number of moles of components 1 and 2 respectively, adsorbed on 1 g. of solid in equilibrium with liquid solution of mole fraction x, and $(n_1{}^s)_\mathrm{m}$ and $(n_2{}^s)_\mathrm{m}$ are the monolayer values of the two components when adsorbed on the solid under examination and can be calculated from the surface area of the solid and the respective molecular areas of the adsorbates concerned. The
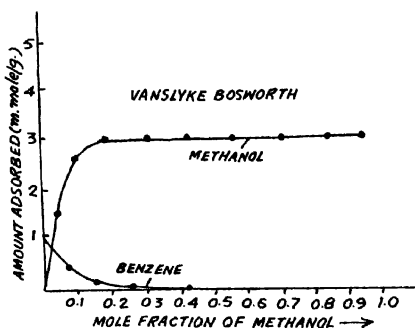

Fig. 4. Individual adsorption isotherms of Methanol and Benzene from their mixtures on various caseins.

thickness of the adsorbed layer is taken as 1. The individual isotherms of methanol and benzene from their mutual solutions are plotted in Fig. 4. It is seen that the sorption of methanol increases with increase in its mole fraction x till x approaches $0 \cdot 15 – 0 \cdot 18$ after which there is no more sorption of any one of the components, monolayer having been completed. The sorption of benzene, on the other hand, is seen to take place only when the mole fraction of methanol is less than $0 \cdot 15 – 0 \cdot 18$ i.e. mole fraction of benzene, $(1 - x)$ is $0 \cdot 82 – 0 \cdot 85$. The individual isotherms of methanol and carbon tetrachloride from their mutual solutions had similar features. The individual adsorption isotherms of benzene and *n*-heptane from their mutual solutions showed that the constituent which is present in greater proportion in the mixture is taken up to a larger extent. This is as expected from the trends of the composite adsorption isotherms (Fig. 3). These curves are not given here for reasons of space.

Acknowledgement

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