ferent hydrogen-bonding glasses also yields emission spectra consistent with the absence of the hydrogen bond in the triplet state. Thus the hydrogen bond is found to be very weak in the excited state for two cases of different pK_a (pyrazine 0.6 and pyrimidine 1.30).[9]

The discussion in I, which was entirely in terms of Franck–Condon destabilization effects, now needs modification. Consistent with the view that the hydrogen bond breaks or becomes very weak on excitation to the singlet state (*i.e.*, $W_1 = 0$), eq. 1 of I becomes (see Fig. 7 of I)

$$\Delta\nu_a = W_0 + \omega_1 \quad (1)$$

Inasmuch as the emission spectra results show that the hydrogen bond is very weak in both the initial and final state (*i.e.*, W_1, $W_0 = 0$), eq. 2 of I becomes

$$\Delta\nu_e = -\omega_0 \quad (2)$$

where ω_0 and ω_1 are the analogs of the same quantities in I but now for a repulsive potential. From eq. 1 we conclude that the $n \rightarrow \pi^*$ absorption blue shift should be greater than the energy of the N—H–O hydrogen bond. For the N-heterocyclics where protonation effects have been studied in detail[2,3a,7] the observed absorption blue shifts [are 2285 (pyrazine), 3340 (pyrimidine) and 3890 cm.$^{-1}$ (pyridazine)]. Inasmuch as the N—H (water) bond energy for the strongest base, pyridazine (as determined by McGowan equation), is only 5.24 kcal.[10] (1880 cm.$^{-1}$)—a value less than the smallest blue shift—the absorption blue shifts are in full agreement with the above prediction. The blue shifts are in addition related linearly to H-bond energies as determined by McGowan equation[10] (4.38, 4.84 and 5.24 for pyrazine, pyrimidine and pyridazine, respectively).[11]

From eq. 2 it follows that $n \rightarrow \pi^*$ emissions (fluorescence or phosphorescence) should not undergo the large blue shifts inherent in absorption but should undergo small red shifts corresponding to the quantity ω_0. For pyrazine $\Delta\nu_1 = -60$ cm.$^{-1}$ $\pm$ 30 cm.$^{-1}$ for pyrimidine $\Delta\nu_e = -100 \pm 30$ cm.$^{-1}$.

These conclusions are in agreement, in the main, with Kasha's original suggestion[12] that the absorption blue shift of $n \rightarrow \pi^*$ transitions on protonation can be attributed to the hydrogen bonding of the solvent with the solute. For hydrogen bonding solvents (including direct protonation) both formation of a hydrogen bonding species in the ground state, and the Franck–Condon destabilization, as discussed in I, are seen to be operative in the absorption solvent shift but only the latter effect in the emission shift.

Acknowledgment.—The authors thank Professor Norman C. Deno for helpful suggestions and criticism of the manuscript.

pyrazine both in EPA and hydrocarbon solvents, the mirror image relation of the emission spectrum with the non-hydrogen bonded species was not apparent.

(9) In two recent papers (*Spectrochim. Acta*, **17**, 14, 30 (1961)) Shimada reports that the pyrazine (10^{-2} *M*) phosphorescence at 77°K. disappears in 10^{-2} *N* HCl (glass unspecified). Our experiments on pyrazine emission were repeated using high concentrations of acid up to 1.0 *N* in ether–ethanol (1:2) glass *without an appreciable reduction in the pyrazine phosphorescence intensity.* Shimada proposes solvent effects as a means of differentiating n $\leftarrow$ π^* from $\pi \leftarrow \pi^*$ emissions and suggests the persistence of pyrimidine emission in acidic media as an indication for a $\pi \leftarrow \pi^*$ emission. The work on pyrazine and pyrimidine reported here shows that the basicity for these molecules in the triplet states is very low and hence n $\leftarrow$ π^* emissions cannot be differentiated from $\pi \leftarrow \pi^*$ emissions by solvent effects.

(10) N. C. Deno, private communication; see *J. Chem. Eng. Data*, **5**, 1 (1960) for the method of determining hydrogen bond energies. E. M. Kosower, *J. Am. Chem. Soc.*, **80**, 3261 (1958), has shown that the $n \rightarrow \pi^*$ blue shift is greater than the hydrogen bonding energy in the case of carbonyl compounds.

(11) Attention is called to A. Weller's work (*Z. physik. Chem. N. F.*, **18**, 163 (1958), on *p*Ka values for both excited and ground states. In addition Kosower's correlation of spectroscopic absorption shifts with *Z* values (ref. 10) has indicated for several classes of compounds an empirical linearity of transition energy with hydrogen bond energy.

(12) M. Kasha, *Discussions Faraday Soc.*, **9**, 14 (1950).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, EMORY UNIVERSITY, ATLANTA 22, GEORGIA]

Methyl Group Substituent Effects on Chemical Shifts in the Nuclear Magnetic Resonance Spectra of Ethylenic Systems

BY G. S. REDDY AND J. H. GOLDSTEIN

RECEIVED NOVEMBER 14, 1960

It has been found that in a series of ten ethylenic derivatives of the form $H_2C{=}CHX$, with X = CN, Cl, Br and CH_3, the effect of methyl substitution upon the chemical shifts of the ethylenic protons is approximately constant for each proton position relative to the methyl group. These substituent effects vary somewhat with the nature of X, but for each X this variation is nearly the same for all protons. Hence the effect of methyl substitution on the internal shifts can be rather reliably predicted. In the butenes it is found that the methyl group effects also appear to be additive. Examples are given to illustrate the utility of these generalizations in assigning and analyzing n.m.r. spectra.

Introduction

The accumulation of the data of nuclear magnetic resonance spectroscopy has now reached such a point as to permit the establishment of a number of reasonably reliable correlations and generalizations with at least a semi-theoretical justification. These results can be important for two reasons. In the first place, they are useful in the prediction and interpretation of n.m.r. spectra as related to conventional chemical structures, and, in the second place, they may ultimately provide new routes to the understanding and evaluation of some of the working concepts of valence theory, *e.g.*, hyperconjugation.

In this investigation we have attempted to arrive at some new generalizations concerning the effect of methyl substitution on chemical shifts in n.m.r. spectra of various ethylenic systems, of the general structure $H_2C{=}CHX$, where X = CH_3, Cl, Br, CN, etc., and with the CH_3 group replacing one of the protons.

In certain cases the data employed were obtained

in this Laboratory, but in others the results of previously published studies were adopted. It is advisable to summarize at the outset the precautions taken to assure that all data used were reliable enough for our particular purposes. Tetramethylsilane was used as the solvent throughout to minimize solvent–solute interactions, to eliminate the need for a variety of bulk susceptibility corrections and to provide a common internal reference. Elimination of concentration shifts due to the interaction of solute with itself may be effected, as usual, by extrapolation to infinite dilution, but this is tedious and time-consuming when applied to a large number of compounds. As a practical matter we have ascertained that concentration shifts were usually quite small below about 5%, and in some cases we have established this limit as assuring data acceptable for our purposes. For those molecules for which accurate chemical shifts with respect to the common reference were not available, the comparisons were based upon relative shifts internal to the molecule itself.

For the series of compounds discussed here, ethylene and propylene will serve as the two basic reference compounds. The proton shift for ethylene in TMS, as obtained in these Laboratories, is −211.5 c.p.s. In addition we have adopted the results of Bothner-By for propylene,[1] in 50% solution in TMS, since we have found no appreciable concentration effect for this system. On this basis the α-proton in propylene is 17.8 c.p.s. lower than the ethylene proton, while the β-protons are both at higher field, the *trans* by 16.5 c.p.s. and the *cis* by 13.0 c.p.s. An analogous low-field shift for protons at the α-position occurs with methyl substitution in alkanes,[2] as is shown by the data in Table I.

TABLE I

CHEMICAL SHIFTS IN METHANE AND METHYL SUBSTITUTED PRODUCTS[a]

Compound	Chemical shift[b]
CH_4	250.0
CH_3–CH_3	224.9
CH_3–CH_2–CH_3	223.4 (methyl)
	206.0 (methylene)
CH_3—CH(—CH_3)—	223.4 (methyl)
	198.0 (tertiary proton)

[a] J. R. Cavanaugh and B. P. Dailey, private communication. [b] Here, and in the succeeding tables, all values are in cycles per second (c.p.s.).

It is not likely that this low-field shift at the α-position is due to an inductive or hyperconjugative withdrawal of charge from the methyl group, since such a mechanism would produce the opposite shift. It has been suggested, however, that the effect in the alkanes (see Table I) might be accounted for by magnetic anisotropy of the C–C and C–H bonds.[3,4] Qualitatively, at least, a similar explanation might be applied to the low-field shift of the α-proton in propylene relative to ethylene.

(1) A. A. Bothner-By, private communication.

(2) J. R. Cavanaugh and B. P. Dailey, private communication.

(3) J. Tillieu, *Ann. Phys.*, **2**, 471, 631 (1957).

(4) P. T. Narasimhan and M. T. Rogers, *J. Chem. Phys.*, **31**, 1302 (1959).

The shift to high field of the β-protons as compared to ethylene is most plausibly explained by a hyperconjugative mechanism. Inductive effects are presumably too short-ranged to produce so large a shift of the β-protons, and it is unlikely that anisotropic shielding by the methyl substituent would amount to as much as 15 c.p.s. at the β-position, since this is just the order of magnitude of the shift of the nearby α-proton.

This paper reports our investigation of the extent to which methyl substitution produces characteristic and approximately constant shifts at the various positions in a series of ethylene derivatives and compares these results with that for ethylene itself. In addition, the effects due to substitution of two methyl groups in ethylene have been considered.

Results

I. Vinyl Cyanide and Cyanopropenes.—Complete analyses of vinyl cyanide and the cyanopropenes have been carried out in this Laboratory. TMS was used as the solvent and internal reference, and the spectral parameters were obtained by extrapolation to infinite dilution. Complete details of this work will appear elsewhere.[5] A feature of these analyses was the correction of all proton shifts for effects due to the diamagnetic anisotropy of the nitrile group. For our present purposes, however, it is not necessary to take such corrections into account, since we may reasonably assume that the nitrile group anisotropy is unaffected by methyl substitution. The chemical shifts for the nitrile series, uncorrected for anisotropy, are given in Table II.

The effects of substitution in the nitriles will be discussed in some detail here to serve as a model for the treatment of the three series described later.

In propylene the *trans*-proton is 3.5 c.p.s. higher than the *cis*-proton, while in vinyl cyanide the *cis*-proton (*cis* to nitrile) is 7.1 c.p.s. lower than the *trans*. Assuming these two effects to be additive, we would predict that in 2-cyanopropene the proton *cis* to nitrile would be 3.6 c.p.s. lower than the *trans*-proton. The experimental difference is 4.2 c.p.s. in the same direction (see Table II), in good agreement with expectations in view of the possible uncertainty of about 1.0 c.p.s. in the observed shifts.

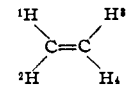
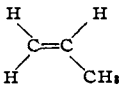
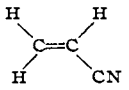
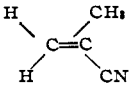
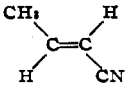
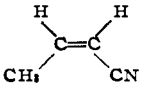
Similarly, in propylene the α-proton is 30.8 c.p.s. lower than the *cis* and in vinyl cyanide the *cis*-proton is 19.7 c.p.s. lower than the α-proton. If additivity holds here, the β-proton in *trans*-crotonitrile should be 50.5 c.p.s. lower than the α-proton. Again, the observed difference of 52.5 c.p.s. is considered to be in excellent agreement with the predicted value. By a similar line of reasoning we expect that in *cis*-crotonitrile the β-proton will occur 46.9 c.p.s. lower than the α-proton, and the observed difference is 44.5 c.p.s.

The above line of reasoning leads to the conclusion that the effect of methyl substitution is nearly constant for all the protons in the nitrile series. For most purposes it is preferable to apply this result not to the internal differences

(5) G. S. Reddy, J. H. Goldstein and L. Mandell, *J. Am. Chem. Soc.*, **83**, 1300 (1961).

TABLE II

CHEMICAL SHIFTS IN ETHYLENE, PROPYLENE,[a] VINYL CYANIDE AND CYANO PROPENES

						
ω_1	−211.5	−198.5	−231.6	−221.6	.....	−251.0
ω_2	−211.5	−195.0	−238.7	−225.8	−261.3	.....
ω_3	−211.5	.....	−219.0	.....	−208.7	−206.6
ω_4	−211.5	−229.3	.....	.....	.....	.....

[a] The propylene values are those reported by Bothner-By, ref. 1.

but rather to the chemical shift values. If we designate by Δ_i the methyl substituent effect on the chemical shift of the i-th proton and by $\Delta_i(Pr)$ the shift for the proton in the same position relative to CH_3 in propylene, then we may write

$$\Delta_i = \Delta_i(Pr) - 211.5 \text{ c.p.s.} \quad (1)$$

(the numerical value, −211.5 c.p.s. is the chemical shift of ethylene.) In this way one obtains the following values for the methyl substituent effect: − 17.8 c.p.s. for α, + 16.5 c.p.s. for β (*trans* to CH_3) and + 13.0 c.p.s. for β (*cis* to CH_3). These results were applied to the cyanopropenes by adding the appropriate substituent term to the chemical shift of the corresponding proton in acrylonitrile. In this way we obtained the values shown in the third column of Table III. All six predicted values are high by a relatively small amount, the average difference being 3.6 ± 0.8 c.p.s. If we choose to regard this discrepancy as reflecting a constitutive effect, we may modify eq. 1 by subtracting the constant value, 3.0 c.p.s., from each Δ_i, thus giving

$$\Delta_i' = \Delta_i(Pr) - 214.5 \text{ c.p.s.} \quad (2)$$

These modified predictions are given in the fourth column of Table III.

TABLE III

PREDICTED AND OBSERVED CHEMICAL SHIFTS IN CYANOPROPENES

Compound	Proton position with respect to the nitrile group	Chemical shifts in c.p.s. from TMS internal reference Predicted[a]	Predicted[b]	Obsd.
α-Methacrylonitrile	*cis*	−222.2	−225.2	−225.8
	trans	−218.6	−221.6	−221.6
trans-Crotonitrile	α	−206.0	−209.0	−208.7
	β	−256.5	−259.5	−261.3
cis-Crotonitrile	α	−202.5	−205.5	−206.6
	β	−249.4	−252.4	−251.0

[a] Without the additional constitutive correction of −3.0 c.p.s. [b] With the additional constitutive correction of −3.0 c.p.s.

II. Vinyl Chloride and Chloropropenes.—Complete analyses of vinyl chloride and the chloropropenes have been carried out in these Laboratories using dilute solutions in cyclohexane and TMS, the solvent serving as the internal reference. Concentration effects are approximately the same for both of these solvents, hence the shifts in cyclohexane were converted to TMS reference using the value of the cyclohexane shift in 50% TMS solution. The shifts converted in this manner have been checked by comparison with the shifts obtained for various deuterated vinyl chlorides in TMS.

By comparison of vinyl chloride and propylene, and assuming additivity as in the case of the nitriles, we would predict that in 2-chloropropene the proton *trans* to chlorine will be 1.6 c.p.s. higher than the *cis*-proton. There are some indications from the study of the spectrum of this molecule that the proton *trans* to chlorine is at higher field than the *cis*, so that the experimental *trans–cis* difference will be +1.2 c.p.s., in good agreement with the prediction.

By using eq. 1 and the chemical shifts obtained for vinyl chloride, we have predicted all the chemical shifts for the three different chloropropenes as shown in Table IV. Agreement with observation is quite satisfactory, the deviation being about one cycle per second (data are not yet available for *cis*-1-chloropropene-1). Because the discrepancy is so small, no constitutive correction has been applied in this series.

TABLE IV

PREDICTED AND OBSERVED CHEMICAL SHIFTS IN CHLOROPROPENES

Compound	Proton position with respect to chlorine	Chemical shifts in c.p.s. from TMS internal reference Predicted[a]	Obsd.
2-Chloropropene	*cis*	−200.0	−201.0
	trans	−198.5	−199.8
trans-1-Chloropropene-1	α	−234.8	−235.3
	β	−234.3	−232.8
cis-1-Chloropropene-1	α	−231.3	.....
	β	−229.3	.....

[a] No additional constitutive correction was necessary for these compounds.

III. Vinyl Bromide and Bromopropenes.—Complete analyses of the n.m.r. spectra of vinyl bromide have been reported by several workers.[6-8] Since these studies used different solvents and references and, moreover, showed several points of disagreement, the analysis was repeated here, under conditions exactly comparable to those employed for the other series studied. The complete n.m.r. studies and analyses of 2-bromopropene and *trans*-1-bromopropene-1 as well as a partial analysis for *cis*-1-bromopropene-1 have been carried out here.

From comparisons analogous to those used above, we can predict that in 2-bromopropene the proton *cis* to bromine is 7.3 c.p.s. higher than the *trans*-proton. The analysis of the 2-bromopropene spectrum shows that the two β-proton shifts are separated by 7.2 c.p.s., with the proton *trans* to

(6) H. S. Gutowsky, M. Karplus and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).

(7) E. O. Bishop and R. E. Richards, *Mol. Phys.*, **3**, 114 (1960).

(8) C. N. Banwell, N. Sheppard and J. J. Turner, *Spectrochim. Acta*, **16**, 794 (1960).

bromine at lower field as inferred from the coupling constants obtained by Alexander for *cis*- and *trans*-dibromopropenes.[9] The observed internal difference is thus in excellent agreement with prediction.

Observed and predicted values for the chemical shifts in the bromopropenes are given in Table V. For the four cases where experimental values are available, the predictions agree quite well, but are consistently low, the average discrepancy being -3.5 ± 0.7 c.p.s. Application of a constitutive correction of + 3.0 c.p.s. gives the values shown in the fourth column of Table V.

TABLE V

PREDICTED AND OBSERVED CHEMICAL SHIFTS IN BROMOPROPENES

Compound	Proton position with respect to bromine	Chemical shifts in c.p.s. from TMS internal reference Predicted[a]	Predicted[b]	Obsd.
2-Bromopropene	*trans*	−221.0	−218.0	−218.3
	cis	−213.7	−210.7	−211.1
trans-1-Bromopropene-1	α	−241.5	−238.5	−238.1
	β	−248.0	−245.0	−243.3
cis-1-Bromopropene-1	α	−238.0	−235.0	.....
	β	−251.8	−248.8	.....

[a] Predicted chemical shifts without the constitutive correction of + 3.0 c.p.s. [b] Predicted chemical shifts after correction.

IV. Propylene and Methylpropenes.—The chemical shifts in isobutylene, which is the α-methyl derivative of propylene, have been obtained here by Whipple,[10] using TMS as solvent. The data for *cis*- and *trans*-butenes are the published values[11] obtained relative to an external water reference at an unspecified temperature. These values were converted to the TMS reference by subtracting 211.5 c.p.s., which is the shift of TMS from an external water reference at 21°, as measured here.

Assuming additivity for two methyl groups, regardless of their location in the ethylene skeleton, the chemical shifts for this series have been calculated and are compared with the observed values in Table VI. The agreement is excellent for isobutylene and *cis*-butene, the average deviation being about 1.0 c.p.s. and in the same direction for both protons. However, in *trans*-butene there is a discrepancy of about −6 c.p.s. The predicted value of Δ is −4.8 c.p.s., but the published results indicate that Δ is + 1.5 c.p.s. It should be pointed out that the conditions under which the butene-2 spectra were obtained are not known, and no great stress should be placed upon the above deviation until the spectra have been re-examined under conditions comparable to those employed here.

TABLE VI

PREDICTED AND OBSERVED CHEMICAL SHIFTS IN BUTENES

Compound	Chemical shift in c.p.s. from TMS Predicted	Obsd.
Isobutylene	−182.0	−183.5
cis-Butene-2	−212.8	−213.4[a]
trans-Butene-2	−216.3	−210.0[a]

[a] These are the converted values from H_2O external reference to TMS internal reference by subtraction of 211.5 c.p.s.

Discussion

It has been shown that, for the series of ethylenic derivatives studied here, the effect of methyl substitution upon the chemical shifts of the ethylenic protons is approximately constant and that the internal shifts can be predicted quite reliably. The only anomaly occurred in *trans*-butene-2, and in this case a reinvestigation of the spectrum in TMS appears to be warranted.

The methyl substituent effect shows a minor but appreciable dependence upon the nature of the second substituent. Thus for X = CN, Cl and Br, protons *trans* to CH_3 are shifted an average of 13.5, 15.5 and 19.0 c.p.s., respectively, while the *cis*-protons experienced shifts of 10, 12.5 and 15.5 c.p.s., respectively. It is only possible at this time to speculate on the reason for this variation, and it would appear desirable to obtain similar data for a greatly extended series of methyl-substituted compounds to provide a basis for rationalizing and/or correlating the observed trend. In any case, as mentioned above, it is easily seen that the constitutive effects do not affect greatly the internal shifts of the β-protons.

A total of ten methyl-substituted compounds have been studied. In some cases, the applicability of the above generalizations was assumed in order to provide a starting point for analyzing the n.m.r. spectra of the compounds. The justification for this procedure rests upon the over-all consistency of the results obtained for the entire series, as well as upon the satisfactory character of the spectral analyses obtained with this approach.

As a consequence, we are inclined to adopt the approximate value of the substituent shifts, and the even more reliable internal shifts predicted here, as a very useful guide for assignments and a source of initial values of spectral parameters for the purpose of fitting n.m.r. spectra.

We have, for example, used the methyl substituent-effect values to aid in fixing the assignments in 2-methylacrolein. With *trans*-crotonaldehyde, for which there is no ambiguity in proton assignments, the two β-protons in α-methylacrolein were quickly assigned by a calculation of the net change of substituent effect on transferring the CH_3 group from the α- to the β-position. The computed shift for the proton *cis* to aldehyde in α-methylacrolein was within 1.5 c.p.s. of one of the observed shifts. The other observed value was about 13 c.p.s. lower and was assigned to the proton *trans* to the aldehyde group.

The reported assignments of β-proton shifts in the 2-substituted propenes have sometimes been ambiguous and contradictory. One of the principal criteria used in making such assignments is the relative values of the two long-range H–CH_3 coupling constants in these structures, about which there is no general agreement. In such cases, discriminating use of the substituent effect values should resolve the problem quite easily.

(9) S. Alexander, *J. Chem. Phys.*, **28**, 358 (1958).

(10) E. B. Whipple, Doctoral Dissertation, Emory University, 1959.

(11) J. A. Pople, W. G. Schneider and J. H. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 238–236.

The substituent effect values may also be used to provide reasonably good initial values of the shifts in ABC systems ($H_2C{=}CHX$). For example, the case of vinyl bromide, although cited above in support of these generalizations, was actually solved by using the chemical shifts of *trans*-bromopropene and 2-bromopropene and computing the effect of removal of the methyl group. The initial shifts so obtained for vinyl bromide were all within 3–4 c.p.s. of the final values, the discrepancy being in the same direction for all protons. Hence the relative positions of all shifts turned out to be correctly predicted to within less than one c.p.s. (see Table VII). This constant deviation of about 3 c.p.s. is what we have termed the "constitutive" effect.

Finally, it may be remarked that since the observed substituent effect of methyl on β-proton shifts is a long-range effect and is roughly independent of wide variations in the polarity of other substituents, it seems reasonable to ascribe its origin to hyperconjugation. It is possible that the constitutive effect arises from variations in the excitation energy, ΔE, of Ramsay's equation.[12] This is, at present, only a speculation, however.

(12) N. F. Ramsay, *Phys. Rev.*, **78**, 699 (1950); **86**, 243 (1952).

TABLE VII

PREDICTED[a] AND OBSERVED CHEMICAL SHIFTS IN VINYL BROMIDE

Proton position	Chemical shift in c.p.s. from TMS internal reference: Predicted[a]	Obsd.
α	−254.1	−254.5
β (*cis*)	−230.6	−230.2
β (*trans*)	−234.3	−234.0

[a] After making the "constitutive" correction of −3.0 c.p.s.

The interpretation of the effect of methyl on α-protons is somewhat more complex because of complications from possible inductive and anisotropy effects.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES 7, CALIFORNIA]

Tracer Electrophoresis. V. The Mobility and Charge of Human Serum Albumin at Low Concentrations and Low Ionic Strengths[1]

BY ESTELLA K. MYSELS AND KAROL J. MYSELS

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It is shown that the open tube tracer electrophoresis method yields accurate and precise values for the mobility of human serum albumin if radio-iodine tagged albumin is used as the tracer. The method may be used over a very wide range of protein and buffer concentrations. ζ potentials and charges are calculated from the data and are found to agree well with titration charges (pH = 8.6, veronal buffer) if a radius of 34 Å. is assumed for the hydrodynamically equivalent sphere, provided a moderate expansion of the sphere at very low ionic strength is allowed for. Evidence for such expansion from viscosity data is presented. In the more concentrated buffer solutions the electrophoretic charges agree very closely with the titration charge. However, it is much lower than the net charge of the protein if one takes into account the anion adsorption. The possible role of sodium ion adsorption in this compensation of charge is discussed briefly.

Introduction

The electrophoretic mobilities of proteins have been studied widely over the years and the results have been used extensively for analytical and also for preparative purposes.[2] On the other hand, some difficulty has been experienced in their theoretical interpretation.[3] In particular it has been difficult to correlate the charge of the protein as obtained by titration and ion binding studies with the values calculated from electrophoretic mobilities. A serious stumbling block has been the dearth of data in sufficiently dilute solutions, both with respect to protein and to buffer concentrations.[3] The required data are difficult or even impossible to obtain by the conventional methods. However, the tracer method first developed by Hoyer, *et al.*,[4] readily permits determination of electrophoretic mobilities over a very wide range of conditions, provided only that the protein may be suitably tagged. Human serum albumin is available tagged with iodine-131 and this material behaves essentially as the non-iodinated one with respect to its electrophoretic properties. It was therefore decided to study the electrophoretic behavior of this protein in some detail. Veronal buffer at pH 8.6 was chosen as the medium for most experiments.

Experimental

The experimental method was essentially that described in detail previously.[4,5] Briefly, a horizontal capillary tube (central compartment) was filled with the protein solution of known radioactivity and the anode and cathode compartments were filled with an identical but untagged solution

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(2) See for example, M. Bier, "Electrophoresis," Academic Press, Inc., New York, N. Y., 1959.

(3) J. Th. G. Overbeek, "Advances in Colloid Science," **3**, 97 (1950); see also ref. 2, chapters 1 and 8.

(4) H. W. Hoyer, K. J. Mysels and D. Stigter, *J. Phys. Chem.*, **58**, 385 (1954).

(5) K. J. Mysels and C. I. Dulin, *J. Colloid Sci.*, **10**, 461 (1955).