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Abstract

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Keywords: NMR, Low-resolution NMR, Food, Exchangeable hydrogen, Water binding, Durum wheat, Semolina

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Low-Resolution, Pulsed NMR Studies of Water Binding and of Exchangeable Hydrogen Content in Solids

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Solids with exchangeable hydrogens are treated with D_2O. The free-induction decay in a pulsed hydrogen NMR experiment is then interpreted in terms of the fraction of hydrogens in the sample which have been made mobile by this treatment. Data are given for the hydration of starch in durum wheat semolina.

The difference in decay rates of the free-induction signals of solids and liquids has been extensively exploited for determining solid/liquid ratios in multiphase systems (*1–9*). The method is applicable whenever the sample contains two types, S and L, of protons such that the T_2 values of all protons of type S are much shorter (an order of magnitude or more) than the T_2 values of all protons of type L. It is then both easy and fast to determine the relative content of the two types of protons in the sample directly from the free-induction decay (FID). In most applications, S and L stand for "solid" and "liquid"; other criteria may, however, apply (e.g., crystalline and amorphous phases of a polymer, adsorbed and free water, etc.).

In this paper we present a rather novel application of this technique based on the exchange of hydrogen between a solid phase and an added amount of D_2O. The exchangeable hydrogens generally belong to functional groups such as –OH, –COOH, $-NH_2$, etc., or from strongly bound (crystal) water. In both cases, the idea is to make such hydrogens appear "mobile" (i.e. contributing to the slowly decaying part of the FID) by exchanging them with deuterium.

An obvious application is a rapid determination of the total fraction of exchangeable hydrogen in the sample. This parameter by itself is an important characteristic of many substances such as proteins, polysaccharides, certain kinds of polymers, etc. The percentage of "mobile" hydrogen (X) is a function of the relative amount (W) of D_2O added to the dry matter. This dependence is quite sensitive to the way the added water interacts with the solid. Analysis of the function $X(W)$ enables one to distinguish between different types of exchangeable hydrogens as well as to elucidate the hydration mechanisms.

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As an example we report the data obtained by this method on durum wheat semolina which consists predominantly of starch, (starch: 77.3%; proteins: 14.0%, D.B.). Durum wheat semolina was dried for 90 min at 130°C (an official norm for humidity determination in flours). After this treatment no mobile hydrogen was apparent in the FID of the samples. Varying amounts of D_2O were then added to the samples, and the resulting percentage of mobile hydrogen was determined from the FID following a procedure developed in a preceding study[1] (*9*). The results are given in Fig. 1.

It is apparent that the total percentage of exchangeable hydrogen is about $38.5 \pm 1.0\%$, which is considerably more than the 30% one would expect on the basis of the molecular formula, $(C_6H_{10}O_5)n$, of anhydrous starch (10 hydrogens per monomeric unit, 3 of which belong to –OH groups). The form of the $X(W)$ dependence is also rather surprising and can be explained only on the basis of a rather complex model. Suppose that the added water forms a separate phase, and that 1 g of dry matter (DM) contains a total of T gram atoms of hydrogen and E gram atoms of exchangeable hydrogen. If W moles of D_2O are now added, then $[2W/(E+2W)] \cdot E$ moles of the exchangeable hydrogen will eventually appear in the mobile water phase, while $[E/(E+2W)] \cdot E$ moles will remain in the solid matrix. Since the T_2 values of the water phase are of the order of 1 msec (Ref (*10*) and our own data) it is unlikely that the exchange of hydrogen between the water and solid phases can be fast enough to make all the exchangeable hydrogens appear as mobile. In this case the expected percentage of mobile hydrogen is simply

$$X = \frac{E}{T}\frac{2W}{E+2W}. \qquad [1]$$

According to the molecular formula, $T = 0.0617$ for anhydrous starch and $T = 0.0667$ for starch with one H_2O molecule per monomeric unit. Choosing E in such a way as to make the limit of X for very large W coincide with the experimental value of 0.385, Eq. [1] leads for the two values of T to curves 1a and 1b in Fig. 1, respectively.

Clearly, there is complete disagreement. Even if the exchange were rapid with respect to T_2, the experimental data could not be fitted, since in this case even a small addition of D_2O would make *all* the exchangeable hydrogens mobile and the curve $X(W)$ would be extremely steep at the beginning. A detailed evaluation of the rather complex intermediate stages between slow and fast exchange shows, in fact, that none of these models can fit the experimental data.

In order to fit the data one must introduce the concept of hydration sites. The assumption is that an exchangeable hydrogen atom remains completely fixed within its solid matrix until the functional group to which it belongs is hydrated. The apparent hydrogen mobility is limited to the hydrated sites, i.e., the sites to which a water molecule has been coordinated. In order to make our model as general as possible let us suppose that per monomeric unit of starch there are:

(a) A groups C–O···C originating from a condensation of two hydroxyl groups (the dotted bond indicates a link between two different monomeric units);

[1] FID values were measured on a pulsed low-resolution NMR spectrometer (20 MHz, model Minispec P20, Bruker Spectrospin).

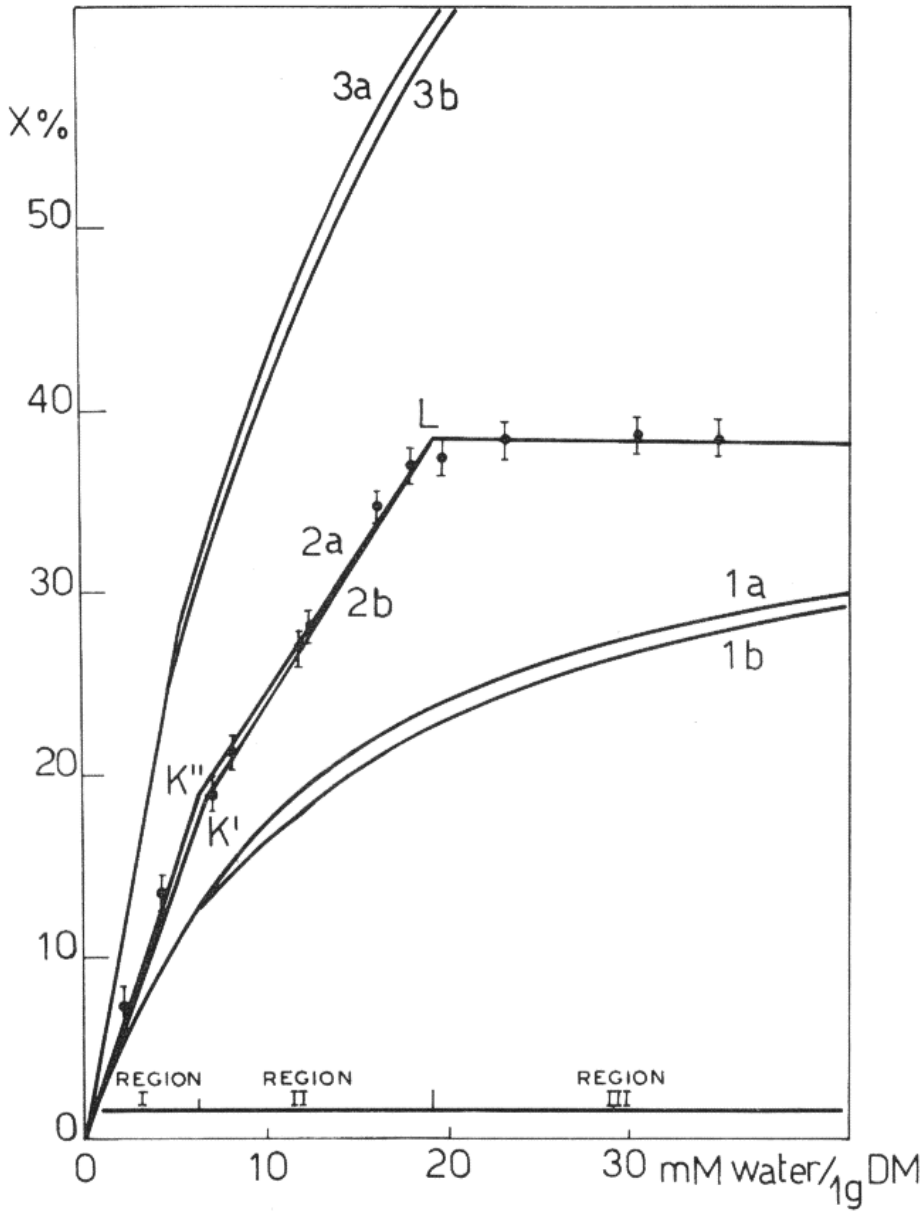

FIG. 1. Dependence of the relative content of mobile hydrogen (X) on the D_2O content (W) of durum wheat semolina. The dots represent experimental points. Curves 1a and 1b are calculated for the exchanging phase model; 1a corresponds to anhydrous starch and 1b corresponds to starch with one molecule of crystal water per monomeric unit. Curves 2a and 2b were calculated for the hydration site model described in the text; the two curves correspond to the extreme sets of parameters compatible with point L. Curves 3a and 3b, corresponding to the same sets of parameters, are calculated for varying additions of H_2O rather than D_2O.

(b) B hydroxyl groups having a partner –OH site located in such a position that a water molecule can coordinate with both of them (C–OH···W···HO–C, where W stands for water);

(c) C molecules of crystal water; and

(d) D hydroxyl groups without a partner –OH site available for coordination with the same water molecule.

The bend in the initial part of the experimental $X(W)$ curve can be accounted for only if water molecules coordinate preferentially to those sites at which two hydrogen bonds are formed (i.e., sites (b) and (c)). Then, until all sites (b) and (c) are hydrated, every molecule of D_2O will make exactly two hydrogens appear as mobile. Let us denote this region as I.

Consider that

(i) the average molecular weight of one monomeric unit is

$$M = 162 + 18C - 18A; \qquad [2]$$

(ii) the total amount of hydrogen in gram atoms per monomeric unit is

$$H_t = 10 + 2C - 2A; \qquad [3]$$

(iii) the amount of added D_2O in gram moles per monomeric unit is WM, where W is in moles per 1 g of dry matter. This means that in region I the amount of mobile hydrogen H_m will be

$$H_m = 2WM, \qquad [4]$$

and therefore

$$X = H_m/H_t = (2M/H_t)\ W = \text{const} \cdot W. \qquad [5]$$

The linear relationship holds until $WM = C + B/2$, that is, until all sites (b) and (c) are hydrated. At this point, which will be denoted by the letter K,

$$W_K = (C + B/2)/M, \qquad X_K = (2C + B)/H_t. \qquad [6]$$

Beyond the point K, any further molecule of D_2O added hydrates just one –OH group (sites (d)) and therefore makes mobile just one hydrogen. This is true until all sites of type (d) are hydrated; the corresponding region will be denoted as II. Within region II obviously

$$H_m = 2W_K M + (W - W_K)M, \qquad [7]$$

so that

$$X = X_K/2 + (M/H_t)W. \qquad [8]$$

The relationship is still linear and holds until $WM = C + B/2 + D$, that is, until all available sites are hydrated. This point is denoted as L. Considering that for stoichiometric reasons $D = 3 - 2A - B$, the coordinates of the point L are

$$W_L = (3 - 2A - B/2 + C)/M, \qquad X_L = [3 + 2(C - A)]/H_t. \qquad [9]$$

Beyond the point L the additional water cannot coordinate to any sites on the solid matrix and starts therefore forming a genuine separate phase. If D_2O is added, this means that X_L will remain constant for $W > W_L$ (region III). The coordinates of the point L estimated from the experimental data are

$$W_L = 0.019 \text{ [moles/g dry matter]}, \qquad X_L = 0.385.$$

Comparing these values with Eqs. [9] and taking into account that A, B, and C must *all* be nonnegative, one gets $0.69 \leq C \leq 1.07$. For any value of C within these limits there corresponds a unique set of parameters A, B, and D, the limit cases being

(i) $C = 1.07$, $A = 0.38$, $B = 0$, $D = 2.24$;
(ii) $C = 0.69$, $A = 0$, $B = 0.75$, $D = 2.25$.

The point K can now be calculated for each of the above sets of parameters. The respective points K′ and K″ calculated in this way are given in Fig. 1. The data are fitted very well with both sets of parameters. Despite this uncertainty, the information we have obtained about the hydration process is surprisingly detailed.

One can show that the above results are not overly affected by the fact that durum wheat semolina is not a pure starch but contains about 14% of protein.

Let us point out that data on echo decay in wet corn starch (*10*) give strong support to our theory. The authors find that nonexponentiality of the echo decay begins only for water concentrations higher than 0.45 g H_2O/g dry matter (i.e., 0.025 moles H_2O/g dry matter). Our own unpublished data on wheat flour indicate that this limit is in fact somewhat lower (about 0.4 g H_2O/g dry matter) coinciding well with the appearance of two separate water phases only beyond point L.

In principle, a similar study could be done by adding H_2O rather than D_2O. Using the hydration site model, the dependence of X on W can be calculated (curves 3a, 3b in Fig. 1). Clearly, such curves are difficult to evaluate; the use of D_2O is therefore essential for this kind of study.

Finally, we would like to point out an additional possibility which this new technique offers of following the kinetics of the hydrogen exchange in those cases where it is slow (characteristic times greater than a few seconds). Such studies might be of interest in connection with water absorption and/or diffusion through many solid substances such as wood, silica, minerals, seeds, etc.

REFERENCES

1. K. VAN PUTTE AND J. VAN DEN ENDEN, *J. Sci. Instrum.* **6,** 910 (1973).
2. K. VAN PUTTE AND J. VAN DEN ENDEN, *J. Amer. Oil Chem. Soc.* **51,** 316 (1974).
3. P. N. TIWARI, P. N. GAMBHIR, AND T. S. RAJAN, *J. Amer. Chem. Soc.* **51,** 104 (1974).
4. E. SAMBUC, *Rev. Fr. Corps Gras* **21,** 689 (1974).
5. V. L. MAKARENKO, A. D. GRISHERENKO, A. K. AWAKUMOV, AND A. F. BABKIN, *Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol.* **1,** 72 (1975).
6. K. VAN PUTTE, L. VERMAAS, J. VAN DEN ENDEN, AND C. DEN HOLLANDER, *J. Amer. Oil Chem. Soc.* **52,** 179 (1975).
7. E. SAMBUC AND M. NANDET, *Rev. Fr. Corps Gras* **22,** 261, 317 (1975).
8. R. E. HESTER AND D. E. C. QUINE, *J. Food Technol.* **11,** 331 (1976).
9. E. BROSIO, F. CONTI, C. LINTAS, AND S. SYKORA, *J. Food Technol.* **13,** 107 (1978).
10. H. K. LEUNG, M. P. STEINBERG, L. S. WEI, AND A. I. NELSON, *J. Food Sci.* **41,** 297 (1976).