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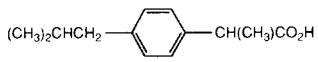
Letter to the Editor

Effect of ^2H_2O on the resolution of the optical isomers of ibuprofen on an α_1-acid glycoprotein column

Sir,

Recently we have been working with an α_1-Acid glycoprotein (α_1-AGP) column (purchased from Chrom Tech) for the chiral separation of a variety of molecules. We are interested in the mechanism of chiral recognition processes involved in the resolution of optical isomers as we feel that such an understanding will facilitate the prediction of the correct choice of analytical column for a particular separation. Unfortunately, as in the case of other immobilised protein systems the mechanism of chiral recognition by α_1-AGP is complex and not yet known in detail. Although hydrophobic and electrostatic interactions are thought [1] to affect chiral discrimination by protein columns, other interactions can also be of importance. To enhance our understanding of the mechanisms of interaction on α_1-AGP we have investigated the influence of replacing H_2O by ^2H_2O as the mobile phase on the separation of the enantiomers of a model carboxylic acid, namely ibuprofen.



Ibuprofen

We carried out the chiral resolution of the enantiomers of ibuprofen using phosphate buffer as the mobile phase. This consisted of 0.01 *M* KH_2PO_4 adjusted to the required pH with 0.01 *M* K_2HPO_4. The acidity (p^2H) of ^2H_2O solutions was measured with an ordinary glass electrode by adding 0.40 to the observed reading of a pH meter which was calibrated with standard buffers in aqueous solution [2]. The flow-rate of the mobile phase was maintained at 0.5 ml min^{-1} in all experiments. The temperature of the column was 25°C and UV detection was at a wavelength of 225 nm. Results are tabulated in Tables I and II.

It is known [3] that the retention of carboxylic acids is increased on the α_1-AGP column with a decrease in pH. This has been confirmed in our studies and may be due either to an increase in the unionised form of ibuprofen or to an increase in the number of positive charges on α_1-AGP or to a combination of both effects. Fig. 1 shows that the largest change in the capacity factors of the two enantiomers of ibuprofen (labelled k_A and k_B) occurs around a pH of 6.5. As the pK_a of ibuprofen is about 4.5, this molecule is expected to exist largely in the unionised form in the pH range of 5.6 to 7.5. Moreover, amine groups on the protein derived from lysine and

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TABLE I

RESOLUTION OF THE ENANTIOMERS OF IBUPROFEN USING H_2O IN THE MOBILE PHASE

k_A and k_B values are averages of two experiments.

pH	k_A	k_B	$k_B - k_A$	α
5.99	11.93	15.86	3.93	1.33
6.30	7.44	9.10	1.86	1.26
6.49	5.54	7.23	1.69	1.31
6.71	3.71	4.90	1.20	1.32
7.00	2.19	2.81	0.62	1.28
7.28	1.60	2.01	0.41	1.26
7.50	1.31	1.53	0.22	1.17

TABLE II

RESOLUTION OF THE ENANTIOMERS OF IBUPROFEN USING ^2H_2O

k_A and k_B values are averages of two experiments.

p^{2}H	k_A	k_B	$k_B - k_A$	α
6.17	21.63	28.51	6.89	1.32
6.50	12.58	17.12	4.54	1.36
6.80	8.47	11.36	2.89	1.34
7.30	4.37	5.46	1.09	1.25

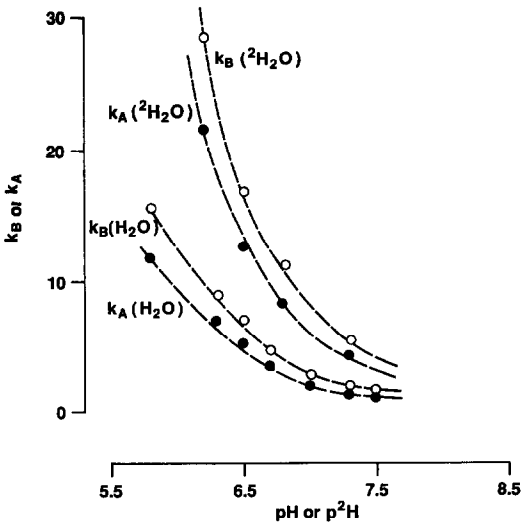

Fig. 1. Variation of k_A and k_B with pH using either H_2O or ^2H_2O as the mobile phase.

arginine residues have pK_a values of about 10.5 and over 12, respectively, so that in the pH region mentioned such amino groups are expected to be fully protonated. On the other hand, the basic site on a histidine residue will accept a proton to form a conjugate acid of $pK_a \approx 6.4$–7.0 and carrying a positive charge. A wider pH profile could have given a better indication on whether a histidine residue plays a role in the chiral separation of the antipodes of ibuprofen. Low pH values have an adverse effect on the stability of the α_1-acid glycoprotein column.

Fig. 1 also shows that both enantiomers of ibuprofen are retained more in ^2H_2O rather than in H_2O solutions of the same acidity. This isotope effect is further illustrated in the chromatograms shown in Fig. 2 for chiral resolutions carried out at pH or p^2H values close to 7.3; in this case almost baseline resolution of the enantiomers of ibuprofen is only obtained in ^2H_2O solution. The magnitude of the isotope effect is again demonstrated in Fig. 3 by plotting the difference in the capacity factors ($k_B - k_A$) either in ^2H_2O or H_2O solutions *versus* pH or p^2H.

As shown in Tables I and II the separation factor α does not change appreciably over a range of pH or p^2H values. In contrast the resolution factor R_s changes markedly with acidity and varies linearly as shown by other workers [4]. R_s values have been plotted *versus* pH or p^2H in Fig. 4. These were found to be generally higher in ^2H_2O compared to H_2O in the acidity region of 6–7, usually recommended for the α_1-AGP column. Moreover, the slope of R_s against p^2H is steeper than that of R_s against pH. We have performed repeated analyses of α_1-AGP using ^2H_2O with no apparent effect on the stability of the column although we have no knowledge about the effect of this solvent in altering the conformation of the protein via changes in hydrogen and hydrophobic bonds.

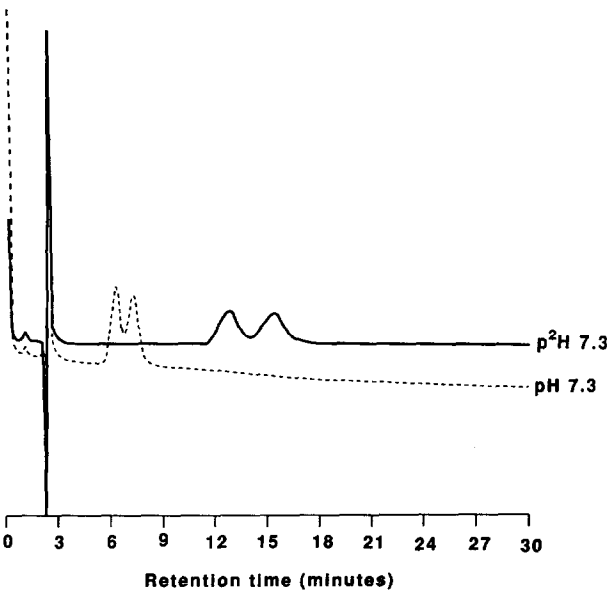

Fig. 2. Chromatograms of ibuprofen at a pH or p^2H around 7.3 (see Tables I and II for accurate acidity values).

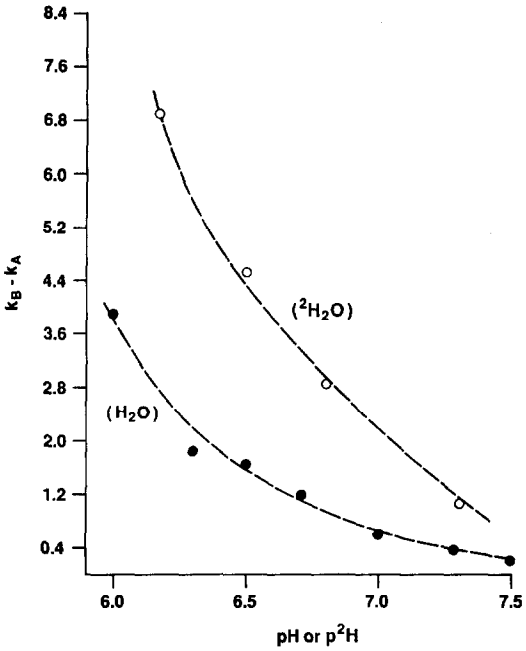

Fig. 3. A plot of $k_B - k_A$ *versus* pH or p^2H.

We feel that changes in retention characteristics of the enantiomers of a molecule observed on replacing H_2O with ^2H_2O as the mobile phase can have analytical advantages in that it can be used to improve chiral resolution, apparently by increasing the number of theoretical plates in a column, although the occurrence of such an effect is not universal with all compounds. In fact, preliminary studies carried out on Atenolol, which contains a secondary amine substituent, does not show any

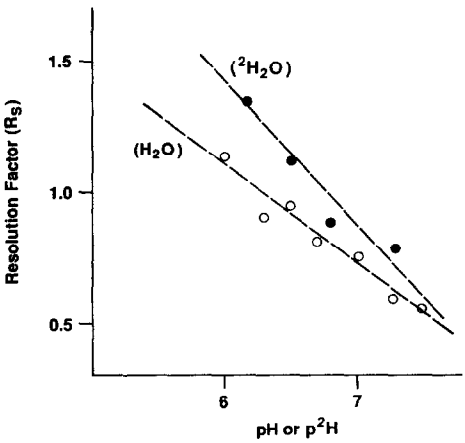

Fig. 4. Illustration of the increase of the resolution factor, R_s, with a decrease in acidity.

significant increase in resolution of the respective enantiomers using ^2H_2O as the mobile phase. Presently we are investigating the magnitude of a ^2H_2O effect on the chiral resolution of other acids and we shall report this data in due course.

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