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THE EFFECT OF SOLVENT COMPOSITION ON THE MOBILITY OF
(±)-CATECHIN ON CELLULOSE

G. J. HART

Department of Pharmaceutical Sciences, Rhodes University, Grahamstown (South Africa)

AND

A. E. RUSSELL AND D. R. COOPER

Leather Industries Research Institute, Rhodes University, Grahamstown (South Africa)

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SUMMARY

Organic solvents were used as a means of investigating the non-covalent interactions that affect the mobility of (±)-catechin on cellulose. The effect on catechin mobility of changing the solvent-water ratios is interpreted in terms of the polar bonding capacities of the solvent mixtures and the influence of water 'structure' on these capacities.

INTRODUCTION

The presence of hydroxyl groups in cellulose polymer is considered to be the main factor governing the chemical reactivity of paper surfaces¹. Cellulose is capable of entering into strong polar association with environmental molecules of a sufficiently electronegative character. CONSDEN *et al.*², however, have contended that the paper serves merely as a support for the aqueous stationary phase and does not actively participate in polar interactions with the constituent molecules of the mobile phase or with the sample itself. In these terms the stationary phase is regarded as a relatively immobile film of water molecules that are hydrogen-bonded to the cellulose matrix. A major objection to this approach arises out of the use of water-miscible solvents to effect separations³⁻⁶. This would not be possible if the separation was due to a simple partitioning process. HANES AND ISHERWOOD⁴ postulated the existence of a water-cellulose complex, with partitioning between this complex and the mobile phase being the effective mechanism. It seems unrealistic to ignore the possibility of direct polar interactions between cellulose and environmental molecules other than water, however, and the fact that many dyes and other substances are known to adsorb is of considerable relevance in this connection⁷.

On the basis of the above arguments, and as the polyhydroxy catechin molecule presents a relatively large number of polar groups to the external environment, it is assumed that direct polar interactions between catechin and cellulose do occur, and that any interference with these interactions will affect the chromato-

graphic mobility of catechin on a cellulose substrate. The results given in this paper are therefore interpreted in terms of the polar atom content of organic solvents, the steric accessibility of functional groups and changes in the dielectric properties of the medium.

EXPERIMENTAL

Materials

Solvents were all AnalaR grade or laboratory-reagent grade. The (\pm)-catechin used as the chromatographic reference substance was supplied by Koch-Light Laboratories, Colnbrook, Great Britain. Catechin spots were visualized by spraying with a 14% solution of ammoniacal silver nitrate. Whatman No. 1 chromatography paper was used throughout.

Methods

A simple ascending technique was used. The air temperature in the chromatography room was maintained at $22 \pm 1^\circ$. Chromatography paper was cut into strips 18×2 cm. The solvent flow was in the machine direction in all instances. Pencil lines 2 cm and 12 cm from the lower ends of the strips served to mark the origins and 10-cm flow limits, respectively. An approximately 5% solution of catechin in methanol was spotted at the origins, by using a fine glass capillary, and the spots were allowed to air-dry for 2-3 min. The strips were then suspended in glass-stoppered reagent jars and allowed to equilibrate for 20-30 min with the lower ends of the strips clear of the solvent mixtures (20 ml) in the jars. The strips were fitted into lower positions in the jars by raising and lowering the closures slightly, until contact was made with the solvent mixtures. In most instances development was allowed to proceed until the solvent fronts reached the pencil lines 10 cm above the origins, and the strips were then removed and oven-dried at $\pm 100^\circ$. When using high concentrations of very viscous solvents, however, the rates of solvent ascent were extremely slow, and the strips were therefore removed and dried before the 10-cm marks had been reached. In such circumstances R_F values were calculated on the basis of the shorter ascent distances.

When the strips were dry, they were sprayed with ammoniacal silver nitrate solution to effect visualization of the catechin spots. The strips were subsequently rinsed in two changes of distilled water and then washed in running tap-water for 20-30 min. The addition of a few crystals of sodium thiosulphate to the final rinse water helped to diminish strip discoloration.

RESULTS

Ethylene glycol and its derivatives

Fig. 1 shows the original form of a typical mobility profile. The R_F values from such profiles obtained by using ethylene glycol and its monoalkyl ethers are shown in Fig. 2 as plots of functions of the molarity of the organic component in water. It can be seen from Fig. 2 that ethylene glycol caused a progressive increase in the mobility of catechin with increasing concentration of the organic component. No maximum was observed at intermediate concentrations. In contrast, the alkyl derivatives produced mobility maxima at intermediate concentrations, this effect

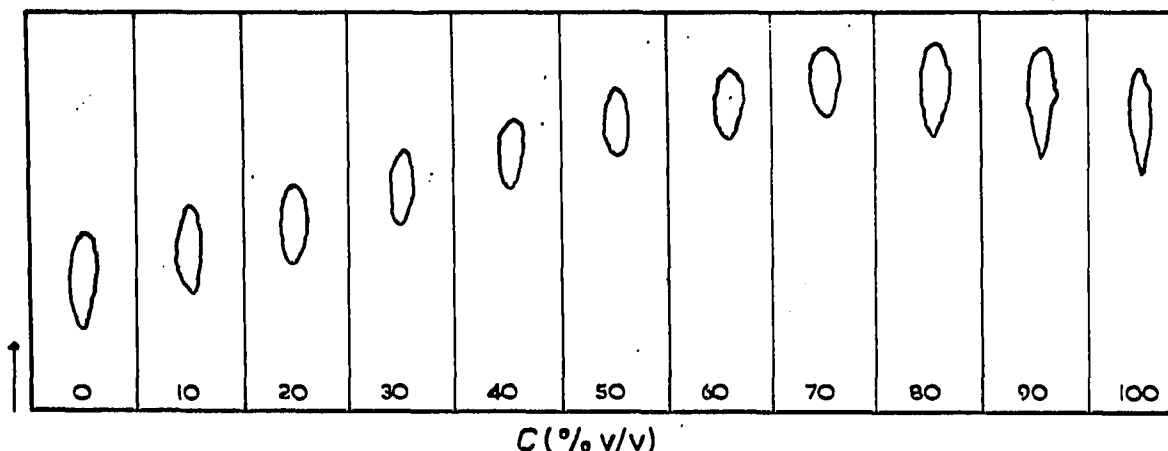


Fig. 1. Example of chromatographic profile produced by catechin on paper strips at various concentrations of methanol in water. Methanol concentrations (C , % v/v) are given on the strips. Temperature, 21.5°.

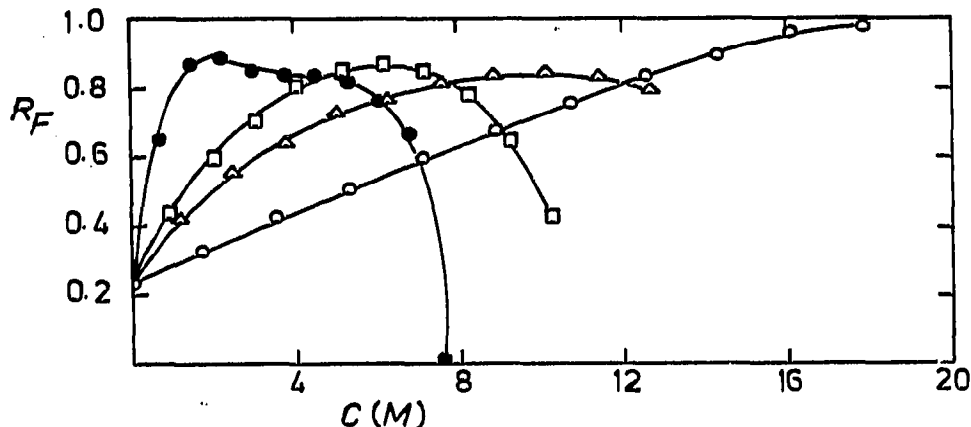


Fig. 2. Effect of monoalkyl substitution of ethylene glycol on the chromatographic mobility (at 21.5°) of catechin on cellulose in solvent-water systems at various concentrations, C (M), of \circ , ethylene glycol; Δ , 2-methoxyethanol; \square , 2-ethoxyethanol; and \bullet , 2-butoxyethanol.

becoming more marked with increasing carbon chain-length in the alkyl substituent. Simultaneously, there was a sharper decline in the mobility of catechin at the high concentration limit as the hydrocarbon content of the organic solvent molecules increased. The peculiar double peak of the 2-butoxyethanol profile was confirmed in replicate experiments.

Propanediol isomers

The effects of ethylene glycol and the propanediol isomers are compared in Fig. 3. The additional methyl group of propane-1,2-diol and the methylene group of propane-1,3-diol shift the mobility maxima to lower concentrations of the organic component, and cause a sharper decline in mobility after the maxima have been passed, than does ethylene glycol itself. The 1,2-isomer appears to enhance the mobility to a lesser extent than does propane-1,3-diol.

Isomeric butanediols and diethylene glycol

The effects of the isomeric butanediols on the mobility of catechin are shown in Fig. 4. At low and intermediate concentrations, their effects were similar. However, some differences were observed as the upper limit of the organic solvent concentration was approached.

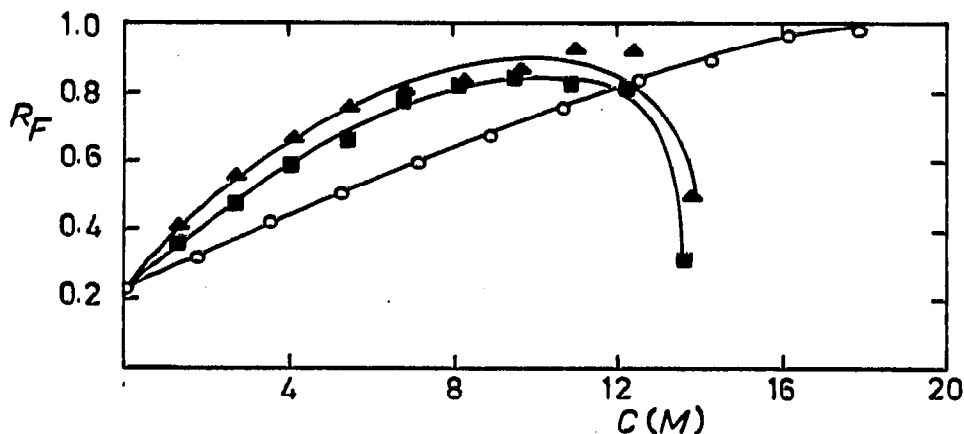


Fig. 3. Effect of concentration, (M), of \circ , ethylene glycol; \blacktriangle , propane-1,3-diol and \blacksquare , propane-1,2-diol on the chromatographic mobility (at 21.5°) of catechin on cellulose in solvent-water systems.

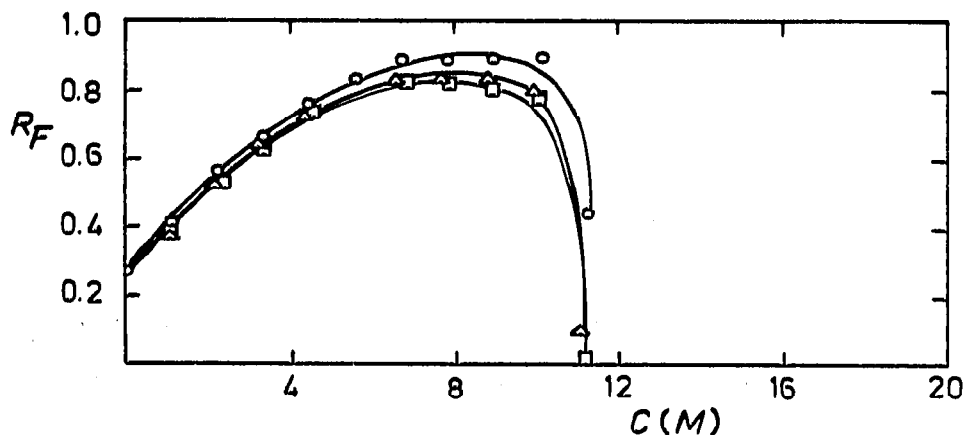


Fig. 4. Effect of concentration (M), of \circ , butane-1,4-diol; \triangle , butane-1,3-diol and \square , butane-2,3-diol on the chromatographic mobility (at 21.5°) of catechin on cellulose in solvent-water systems.

Fig. 5 shows that diethylene glycol enhanced the mobility of catechin to a greater extent than did butane-1,4-diol. In addition, there was no decline in mobility at high concentrations of diethylene glycol. Diethylene glycol therefore produced similar effects to the other solvents that have high polar atom: carbon atom ratios (*i.e.*, relatively low hydrocarbon contents), namely ethylene glycol, glycerol and methanol.

Ethylene glycol homologues

Mobility patterns obtained by using ethylene glycol and its higher homologues, propane-1,3-diol, butane-1,4-diol, pentane-1,5-diol and hexane-1,6-diol, are compared in Fig. 6. The following relationship is evident at low and intermediate

concentrations of the organic components: the enhancement of the mobility of catechin is in the order hexane-1,6-diol > pentane-1,5-diol > butane-1,4-diol > propane-1,3-diol > ethylene glycol. This is the inverse of the order of the polar atom: carbon atom ratios of the solvents.

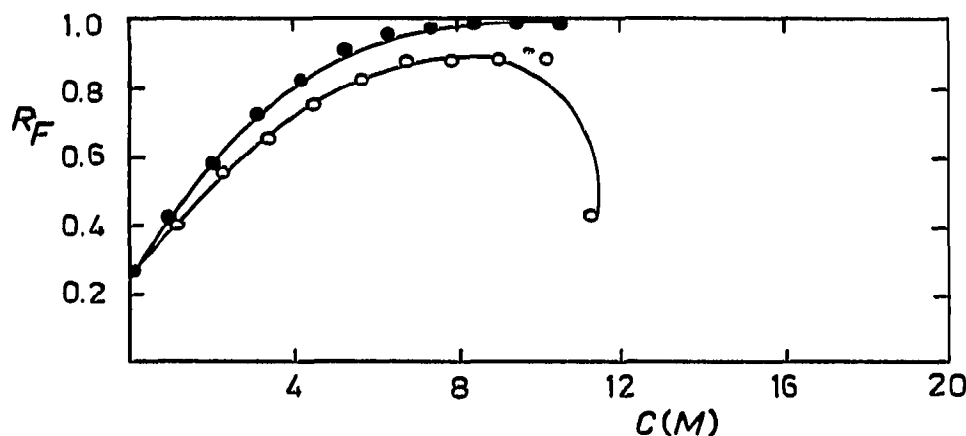


Fig. 5. Comparison of effects of concentration, C (M) of ●, diethylene glycol and ○, butane-1,4-diol on the chromatographic mobility (at 21.5°) of catechin on cellulose in solvent-water systems.

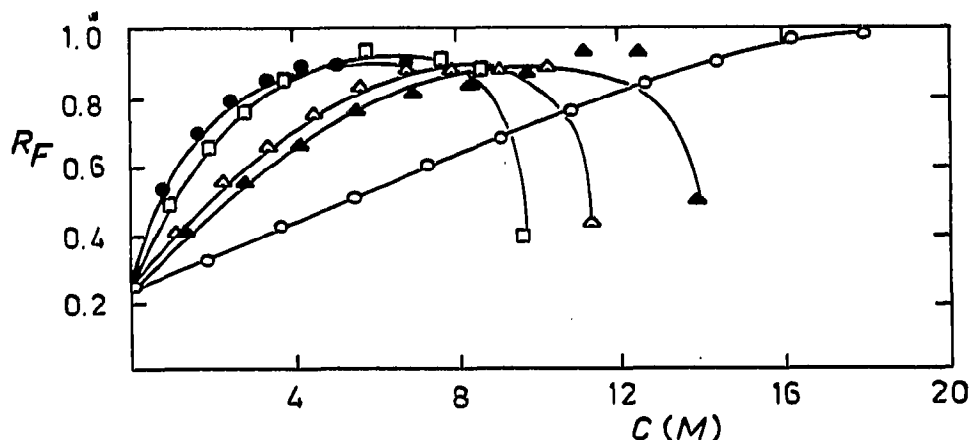


Fig. 6. Effect of concentration, C (M) of homologous diols on the chromatographic mobility (at 21.5°) of catechin on cellulose in solvent-water systems. ○, Ethylene glycol; ▲, propane-1,3-diol; △, butane-1,4-diol; □, pentane-1,5-diol; ●, hexane-1,6-diol.

It therefore appears that organic solvents that have the highest hydrocarbon content, *i.e.*, the lowest polar atom:carbon atom ratios, enhance the mobility of catechin to a greater extent (at low concentrations) than do solvents that have higher polar atom:carbon ratios. This suggests that some factor other than, or in addition to, simple polar interaction between the organic solvent molecules and the substrates may be involved in masking the polar interactions between catechin and cellulose.

Polar atom: carbon atom ratios and profile shape

Fig. 7 shows the distinct similarity, with respect to curve shape, of the profiles produced by solvents that have the same polar atom:carbon atom ratios. Methanol, ethylene glycol and glycerol all gave rise to a long shallow climb to a

maximum R_F at high molarities, followed by curve flattening or a slight decrease in R_F as the limiting condition was approached. In contrast, Fig. 8 shows that alteration of the polar atom:carbon atom ratio gives rise to curves that are considerably different in shape. It can therefore be seen from Fig. 8 that the effect of adding polar groups in the series *n*-propanol, propane-1,2-diol, glycerol was to decrease the mobility of catechin at low solvent concentrations while increasing the mobility at high proportions of the organic component.

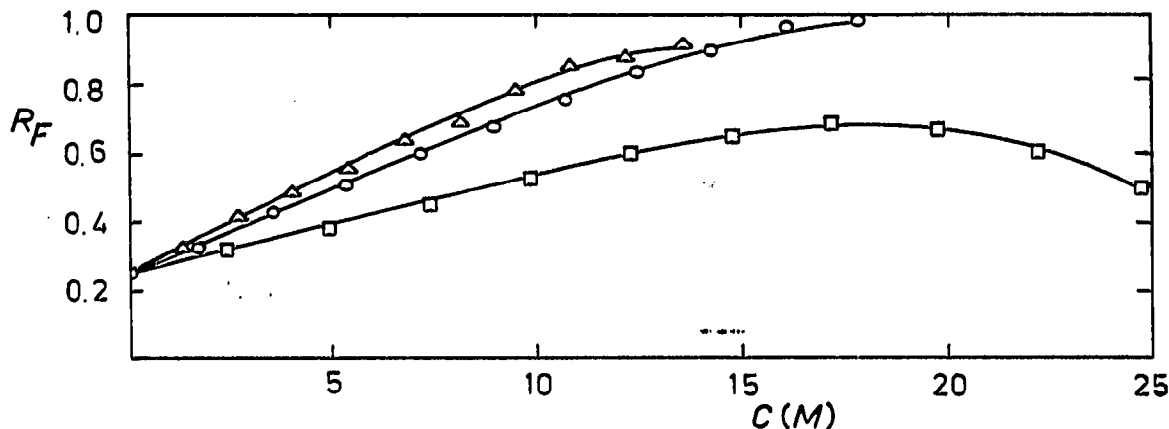


Fig. 7. Comparison of effects of solvent concentration, C (M), on the shapes of the chromatographic mobility profiles of catechin on cellulose (at 21.5°) in solvent-water systems for solvents that have identical polar atom:carbon atom ratios. \circ , Ethylene glycol; \triangle , glycerol; \square , methanol.

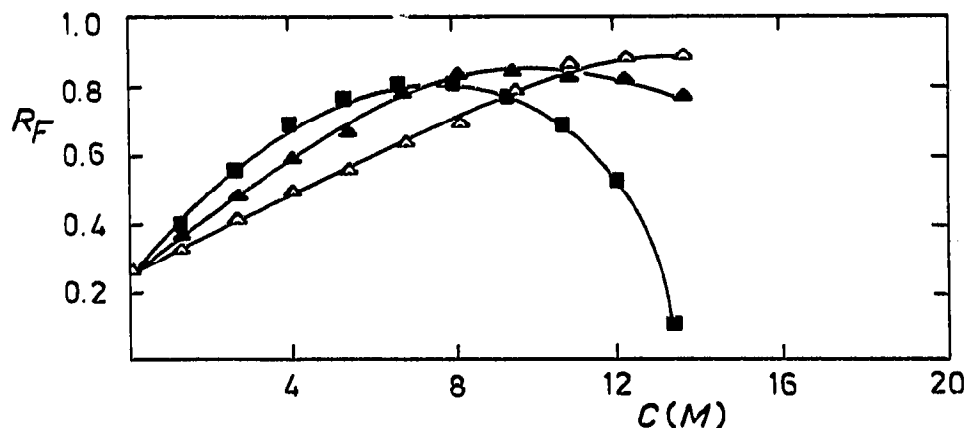


Fig. 8. Comparison of effects of solvent concentration, C (M) on the shapes of the chromatographic mobility profiles of catechin on cellulose (at 21.5°) in solvent-water systems for solvents that have different polar atom:carbon atom ratios. \triangle , Glycerol; \blacktriangle , propane-1,2-diol; \blacksquare , *n*-propanol.

DISCUSSION

In all the organic solvent-water systems examined, an increase in the hydrocarbon content of the solvent systems caused an initial increase in the mobility of catechin. This increase was gradual and maintained, with those solvents that have high polar atom:carbon atom ratios, whereas solvents that have low polar atom:carbon atom ratios produced sharp maxima at low solvent:water ratios, followed by a marked decrease in the mobility of catechin at high concentrations of the organic component. Any mechanistic interpretation of these results must

account both for the progressive enhancement of the mobility of catechin at low solvent proportions and for the decline in the mobility at high solvent concentration. The apparent weakening of polar interactions between catechin and cellulose at low concentrations of organic component must therefore be reconciled with the enhancement of these interactions when the hydrocarbon content of the system is high. The model must also account for the effect of the carbon chain length, namely, the observation that longer apolar chains caused sharper maxima at low concentrations as well as a sharper decline in the mobility at high concentrations than did shorter chains or those interrupted by polar groups.

Recent work⁸⁻¹³ on mobility patterns produced by vegetable tannins on cellulose and matrix collagen substrates is relevant to the present discussion. Use was made of an ascending chromatographic technique to study the essentially reversible association between tannin and substrate and the effects on such interactions of changing the constituents and compositions of the solvent systems. These workers observed an initial rise in the mobility of tannin, which reached a maximum at intermediate organic solvent:water ratios, followed by a decrease in the mobility as the pure organic solvent limit was approached. These results are qualitatively very similar to those reported here.

The interpretative approach that we suggest represents an attempt to account for the effects recorded both in this study and in the earlier work mentioned above. The model consists of a development of suggestions made by RUSSELL *et al.*⁸⁻¹² and HART¹⁴, and incorporates the concept of water-structure formation¹⁵⁻¹⁸ to facilitate the explanation of the influence of hydrocarbon chain length and isomerism. Thus, we suggest that structuring of water in regions immediately adjacent to the non-polar part of an organic solvent molecule, hydrogen-bonded through its polar group to catechin or cellulose, could be expected to inhibit the freedom of approach of all other molecules in the immediate vicinity. This would serve to prolong the lifetime of the transient polar bond between the organic solvent molecule and the catechin or cellulose. Furthermore, if such water structures have appreciable lifetimes relative to the frequency of thermal oscillation of the hydrocarbon chain, then a considerable build-up of structured water seems to be feasible. The greater mobility and extended sweep-out volumes of linear isomers, higher homologues and alkyl-substituted derivatives could therefore be expected to produce larger volume elements of ordered vicinal water than would their more compact branched-chain counterparts, lower homologues or parent molecules, respectively. Similarly, organic solvent molecules in which the hydrophobic groups are sterically more accessible to the aqueous environment would be structure-promoting to a greater extent than would isomers in which the accessibility of the hydrocarbon chain is decreased as a result of interference by polar groups. A theoretical model of this nature should be widely applicable to organic solvent-water systems. The perturbation of the triple helical structure of the collagen macromolecule in solution, for example, can be readily interpreted in terms of the enhancement of the strength of the polar bond by the formation of water structure¹⁹⁻²¹.

The decrease in the mobility of catechin when the concentration of the organic component in the solvent system is high can also be explained in terms of the above hypothesis. In such systems, the concentration of water would become the limiting factor as the formation of structured water 'clusters' around hydro-

carbon chains must progressively diminish as the water content of the total environment is decreased. Thus the *raison d'être* for the enhancement of the mobility of catechin is removed and the R_F decreases sharply. An effect due to the decrease in the dielectric constant with increasing concentration of organic solvent must also contribute to the decrease in mobility. Significantly, organic solvents that have high polar atom:carbon atom ratios, e.g., ethylene glycol, glycerol, methanol and diethylene glycol, did not produce mobility maxima at intermediate concentrations. This is in accordance with the proposed mechanism for the enhancement of mobility as these molecules do not carry pendant hydrocarbon chains of any significant size and therefore would not be expected to promote water structure formation to an effective extent. These solvents also have higher dielectric constants than those solvents that have higher hydrocarbon contents, and therefore do not give rise to the decrease in mobility at the upper limit of the organic component.

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