Microscopic Trends in Methanol/Water and Acetonitrile/Water Systems

Muhammed Alzweiri[1]✉, John A. Parkinson[2], David W. Watson[3] and Steve Steer[3].

[1] Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman-11942, Jordan
[2] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, U.K.
[3] Strathclyde Institute for Pharmaceutical and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, U.K.

ABSTRACT

Examination of the some trends and differences between acetonitrile/water and methanol/water mixtures has been carried out. These systems are in interest for chromatographers since they are common mobile constituents. Some differences in the molecular arrangement of theses solvents were observed from NMR and IR results. These observations might be in use in understanding and developing chromatographic separations.

IR studies revealed C-H bond compression in methanol compared with acetonitrile which indicates different molecular arrangement in their aqueous mixtures. NMR and IR results show a clear non-linear behavior in molecular arrangement. This justifies the deviation of methanol and acetonitrile aqueous solution from the norm. However, both solvents showed similar ionization suppression effects on examples of weak acids and bases.

Keywords: Chemical Shift, Infra-red, Nuclear Magnetic Resonance, Physical Properties, Methanol, Acetonitrile, Formic Acid and Triethylamine.

INTRODUCTION

Methanol/water and acetonitrile/water mixtures are commonly used in chromatographic mobile phases. Several physical properties were used to understand solvent interactions within these mixtures, such as excess molar volume and enthalpy change, have been reported intensively in the literature [1-5]. These physical properties are sometimes sensitive enough to show differentiation between close molecular compounds like methanol and ethanol [6]. Two important conclusions may be drawn from the literature concerning solvent mixture interaction behaviour. The first conclusion is that the solvent mixtures exhibit non-linear behaviour of most physical properties, which might indicate different possible structural arrangements of the solvent molecules. The second conclusion is the common existence of critical solvent ratios at which departure of the physical properties occurs from the norm. For example, the change of molar volume of a methanol-water system at 137 bar and 588 K reaches a maximum when the methanol mole fraction equals 0.3 (about 55% v/v) [2]. This indicates a significant change in the interactions of solvent molecules at that point.

An alternative approach to studying solvent interaction behaviour is to focus on the mixtures at the molecular level. For example, Fortes described a chain structure of methanol monohydrate at 160 K by using Powder neutron diffraction[7]. Nuclear Magnetic Resonance (NMR) and Infra-Red (IR) are also important in obtaining structural information of solvent arrangements. For instance, NMR chemical shift was used to study proton exchange reactions of some protic solvents in the supercritical state and IR was used in studying the effect of acetonitrile on the water of hydration of serum albumin [8,9].

The spectroscopic studies of the solvent mixtures may be used in building theoretical understandings of solvent interactions, for example during chromatographic separations [10-15]. In this article, molecular analytical approach is utilized to study the molecular arrangement

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✉ E-mail: Muhammed_h_Z@yahoo.com

behaviour in methanol and acetonitrile aqueous mixtures in order to give help in theoretical decisions on their selection as common alternative solvents.

In this study methanol, acetonitrile and water are used because of their wide application in chromatography. In addition, formic acid, triethylamine and pyridine are used as examples of commonly used acidic and basic analytes.

1. Experimental

2.1 Materials

Acetonitrile and water were HPLC grade (VWR International Ltd., Lutterworth, U.K.). Formic acid 98%, 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt 98 atom %*d* (TSP), d_4-methanol 99.8% and d_3-acetonitrile, deuterium oxide 99.9 atom % *d*, were purchased from Sigma-Aldrich (Germany) and pyridine from Fluka (U. K.). NMR capillary tube assembly (516-CC-5) were purchased from Wilmad.

2.2 Instrumentation

NMR data were acquired on a Bruker 9.4 T Avance/DPX NMR spectrometer operating at a proton resonance frequency of 400.13 MHz controlled with XWIN-NMR 3.5 software, while IR analyses were carried out using a JASCO FT/IR-4200 instrument equipped with an ATR (Attenuated Total Reflectance) unit. The instrument was controlled by Spectra Manager version 2.0 (Copyright © 2002-2005, JASCO corporation).

2.3 Procedures

2.3.1 Solvent Interactions:

Methanol or acetonitrile were mixed with water at different ratios and 0.07 mL from each ratio mixture was subsequently introduced into the external tube of the capillary NMR tube assembly. 0.6 mL of a 1 mg/mL solution of TSP in D_2O was added into the internal tube of the assembly to act as an independent external chemical shift calibration standard.

In addition, the ATR unit of the Infra-Red spectrometer (IR) was used to study methanol-water and acetonitrile-water mixtures at different ratios.

2.3.2 Analyte-Solvent Interactions

The pH of aqueous pyridine solution was adjusted with HCl to 50% ionized form of pyridine. 1 mL of this solution was diluted to 100 mL with water, methanol or acetonitrile. The test was repeated for triethylamine instead of pyridine. Furthermore, the pH of aqueous formic acid was adjusted with NaOH to 50% ionization then 1 mL diluted to 100 mL with water, methanol or acetonitrile.

2. Results and Discussion

3.1 NMR Study of Solvent Interaction

An NMR capillary assembly was used to study methanol/water and acetonitrile/water mixtures. Each capillary tube assembly was composed of internal and external NMR tubes. The deuterated calibration solvent was located in the internal tube to avoid any interaction of the calibrant with the water/acetonitrile or water/methanol mixtures in order to avoid solvent-induced calibrant resonance chemical shift changes. A thin layer of acetonitrile-water or methanol-water mixture was held between the internal and external tube walls through capillary attraction. Significant change in the shift of the acetonitrile methyl group, which becomes more shielded with increasing water content, was observed (Figure 1). The same effect was observed for the methanol methyl group with increasing percentage of water (Figure 2). The degree of increased shielding of the methyl group with increasing water content was larger for the methyl group of acetonitrile ($Shift_{max}$=1.5 ppm) compared with the methyl group of methanol ($Shift_{max}$= 1.0 ppm). Additionally, acetonitrile induced an increase in the shielding of the proton of the water hydroxyl group. This would be consistent with a dipole-dipole interaction where the HO bond is enriched with some of the electron density from the CN group of the acetonitrile. The OH proton in methanol shifted to high ppm compared with the proton resonance of pure water. This was despite the fact that the methanol methyl group is electron donating, an effect that would theoretically cause the methanol OH proton to resonate at lower ppm compared with the water OH proton. Hoffman *et al.* [8] noted that the methanol OH proton resonance appears at lower ppm compared with water protons at high temperatures under conditions where intermolecular H-bonding associations could be considered to be minimized. This indicates that the natural resonance of

the monomeric methanol OH would appear at lower ppm compared with the water OH proton. Extension of this idea leads to the explanation that the observed OH resonance position under normal room temperature conditions is higher than water because of the presence of other physical effects including intermolecular structures such as clathrate formation and intermolecular hydrogen bonding interactions.

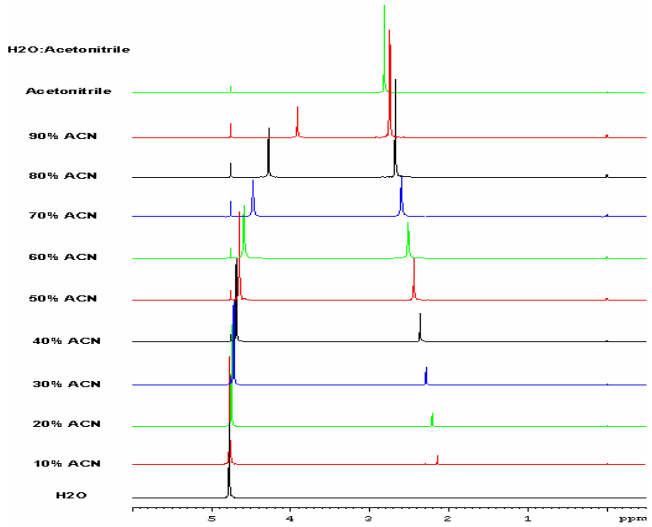

Figure 1. NMR spectra of acetonitrile-water mixtures using NMR capillary assembly tubes. Internal tube contains TSP/D2O calibration solution, while the external tube has solvent mixtures.

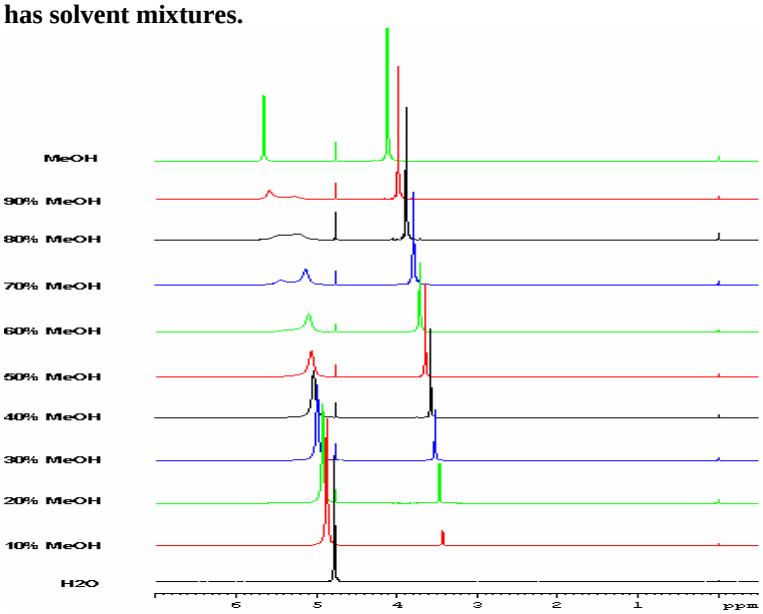

Figure 2. NMR spectra of methanol-water mixtures using NMR capillary assembly tubes. Internal tube contains TSP/D2O calibration solution, while the external tube has solvent mixtures.

Where water was only 10-40% v/v of the mixture, the exchange rate between OH protons of methanol and protons of water was slow resulting in the observation of two broad OH signals (Figure 2). Under these circumstances, the dielectric constant of the medium is less than that of water

(methanol = 33; water = 80). At 50% v/v water a single broad signal is visible reflecting a more rapid exchange rate between the two OH groups giving an average signal. Between 50 and 90% water content, in which the dielectric constant of the medium becomes close to that of water, the OH proton signal becomes sharper indicating an increasingly rapid proton exchange rate between the OH group of methanol and the OH group of water.

3.2 Infra-Red Study of Solvent Interaction

The effects of mixing the organic solvents with water were reflected in the behaviour of the CN and CO bond vibrations in the IR spectra of the mixtures (Figures 3 & 4). Interestingly, the acetonitrile CN bond strength increases gradually as the percentage of water increases while the methanol CO bond strength decreases slightly with the increasing percentage of water. This could be justified by the different arrangements of water molecules around these two solvent molecules, which results in diverse effects despite the close similarity of their physiochemical properties. Also, the C-H symmetric and asymmetric stretching vibrations in methanol showed increasing energy as the percentage of water increased in the methanol water mixtures (Figure 5), suggesting that the methyl group becomes compressed by the surrounding water molecules. In contrast, little effect was observed for the vibrational energy of the C-H bonds of acetonitrile upon the addition of different water/acetonitrile ratios (Figure 6) although both methyl groups showed increased shielding in the NMR data (Figures 1 & 2). This depicts potentially valuable structural differences between the two common HPLC mobile phase components methanol and acetonitrile.

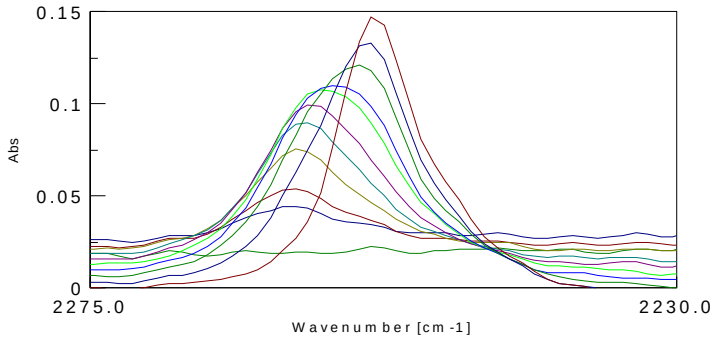

Figure 3. Infra red analysis of carbon-nitrogen bond in acetonitrile. Water was added to acetonitrile at 10% ratios starting from zero to 100%. Water causes upper field shift to C-N bond

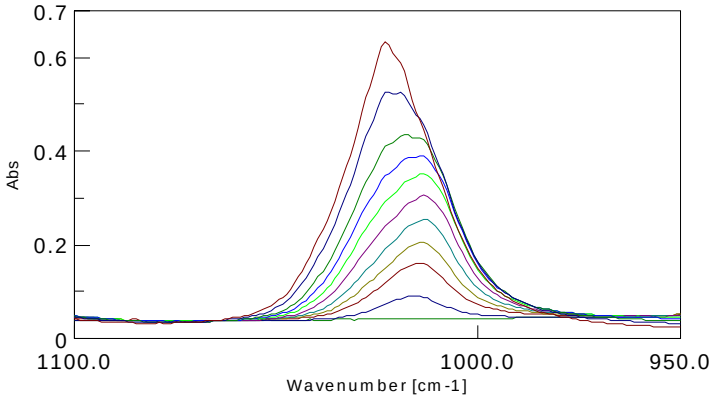

Figure 4. Infra red analysis of carbon-oxygen bond in methanol. Water was added to methanol at 10% ratios starting from zero to 100%. Water causes lower field shift to C-O bond more significantly at low ratios of water.

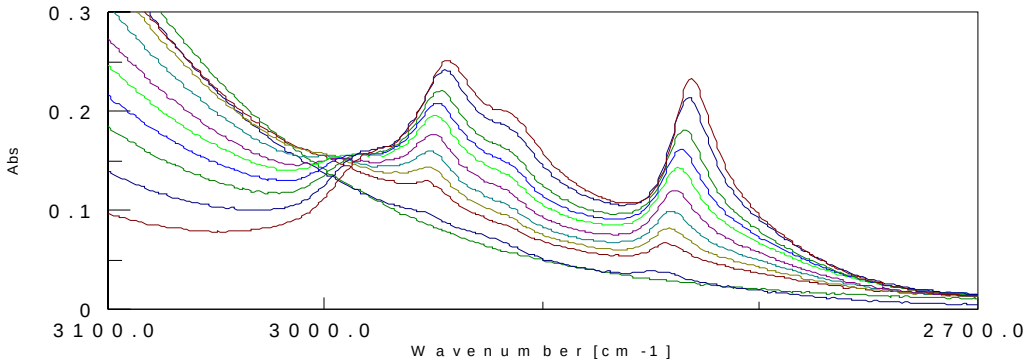

Figure 5. Infra red analysis of carbon-hydrogen bond in acetonitrile. Water was added to acetonitrile at 10% ratios starting from zero to 100%. Water causes upper field shift to C-H bond

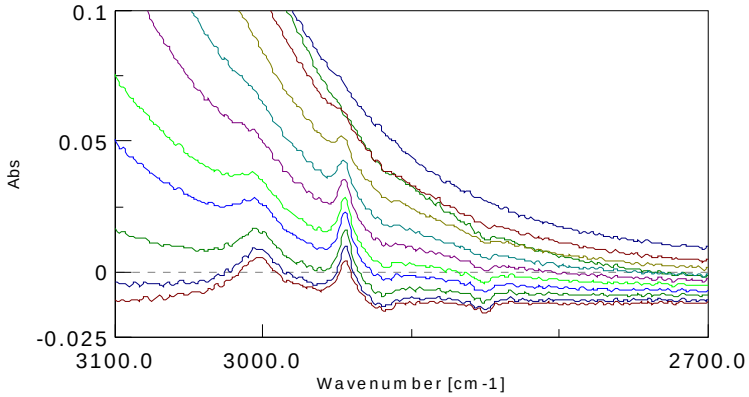

Figure 6. Infra red analysis of carbon-hydrogen bond in methanol. Water was added to methanol at 10% ratios starting from zero to 100%. Water causes no significant shift for C-H bond.

2.3 NMR study of Solvent-Analyte Interaction

An observation relates to the inclusion of methanol or acetonitrile on buffer solutions used in chromatographic separations. It can be readily observed if methanol or acetonitrile are mixed with a buffer based on a weak acid that the pH of the solution as measured by a glass electrode increases by about 1 pH unit with 50% methanol or acetonitrile. Correspondingly, the pH of a buffer based on a weak base falls upon the addition of methanol or acetonitrile. To study the effect of solvent arrangements on the buffer pH, an NMR study was carried out using formic acid in three different solvents namely water, methanol and acetonitrile. Figure 7 shows the NMR spectrum of the non-acidic proton of formic acid adjusted to a pH where it was 50% ionised and dissolved in water, methanol or acetonitrile. This signal reports indirectly on the protonation state of the carboxylic acid group and is sensitive therefore to pH. The signal in water occurs at 8.25 ppm. The chemical shifts for the same proton in methanol and acetonitrile are similar to one another (8.81 ppm and 8.86 ppm, respectively) and occur at higher ppm compared with the observation for water. In the cases of acetonitrile and methanol solvents, non-dissociated molecules form a high percentage of the formic acid present in solution. This causes an average deshielding of the formic acid non-acidic hydrogen relative to formic acid in water. In the case of water, a high percentage of dissociated formic acid molecules are present in solution, producing an average shielding effect on the non-acidic hydrogen of formic acid due to the increased electron density of the ionised carboxyl group. It can be concluded that formic acid in methanol has almost the same K_a value compared with formic acid in acetonitrile. This explains the rise in pH on mixing methanol or acetonitrile with buffers containing weak acids. On the other hand, triethylamine was used as an example of weak base. As expected, water promotes ionisation but no significant difference exists between methanol and acetonitrile solutions (Figure 8). The presence of acetonitrile or methanol promotes deprotonation of the triethylammonium ion causing an average shielding effect compared with water and resulting in increased acidity, thereby offecting a reduction in pH of weak base buffers on organic solvent addition as observed.

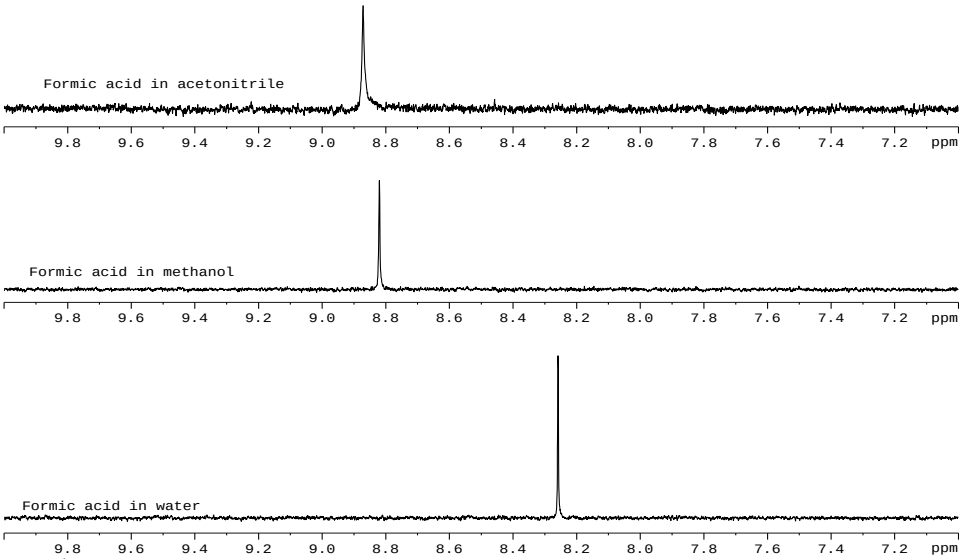

Figure 7. H^1 NMR of 50% ionized formic acid dissolved in acetonitrile (A), in methanol (M) and in water (W).

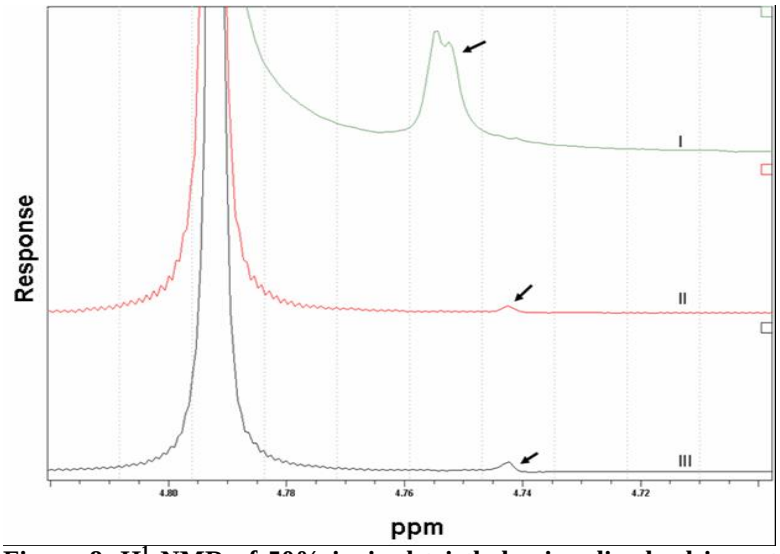

Figure 8. H^1 NMR of 50% ionized triethylamine dissolved in water, (I). Acetonitrile: (II) and methanol (III).

3. Conclusion

The method used revealed critical differences in the behaviour of two chromatographically significant solvents, namely acetonitrile and methanol. Despite their close physicochemical properties, differences were observed in their behaviour and response. IR measurements showed that C-H bonds in methanol are more compressed than the corresponding bonds in acetonitrile, which may be indicative of entropic changes that occur in a methanol/water matrix, the latter observed as a temperature variation. Formic acid and triethylamine were used as examples of simple analytes and probes of ionisation effects caused by different solvents. Both methanol and acetonitrile showed virtually equivalent ionisation suppression effects for these molecules.

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الطرائق الدقيقة لنظم محاليل الأسيتونيتريل في الماء وكذلك محاليل الميثانول في الماء

محمد الزويري[1]، جون باركنسون[2]، دافيد ولنسون[3] وستيف ستير[3]

[1] قسم العلوم الصيدلانية، كلية الصيدلة، الجامعة الأردنية، عمان، الأردن.
[2] قسم الكيمياء التطبيقية، جامعة ستراثكلايد، جلاسكو، المملكة المتحدة.
[3] معهد ستراثكلايد للعلوم الصيدلانية والحيوية الطبية، جامعة ستراثكلايد، جلاسكو، المملكة المتحدة.

ملخـص

لقد أجريت التجارب لتبيان الاتجاهات والاختلافات لمحاليل الأسيتونيتريل / الماء، وكذلك الميثانول /الماء. وتتجلى اهمية هذه المحاليل باستخدامها في أطوار الكروماتوغرافيا المتحركة. ولقد لوحظ بعض الاختلافات في الترتيب الجزيئي للمذيبات من خلال نتائج الرنين المغناطيسي والأشعة تحت الحمراء. ويمكن لهذه الاختلافات أن تكون قيد الاستخدام في فهم وتطوير الفصل الكروماتوغرافي .

وكشفت دراسات الأشعة تحت الحمراء انضغاط روابط (C-H) في الميثانول مقارنة مع الأسيتونيتريل، وهذا يشير إلى ترتيب جزيئي مختلف لمخاليطها المائية. وتظهر نتائج الرنين المغناطيسي والأشعة تحت الحمراء سلوكا غير خطي في ترتيب الجزيئات. و يمكن لهذا أن يبرر انحراف هذه المحاليل عن المتوقع. ومع ذلك، أظهرت كل من هذه المذيبات آثار تثبيط مماثلة لتأين أمثلة من الأحماض والقواعد الضعيفة.

الكلمات الدالة: التحول الكيميائي، الأشعة تحت الحمراء، الرنين المغناطيسي، الخصائص المادية، الميثانول، الأسيتونيتريل، حمض الفورميك، ثلاثي الإيثيلامين.

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