

Summer 8-9-2014

# Polyol induced extraction (PIE) of water from organic solvents

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# **Polyol induced extraction (PIE) of water from organic solvents**

By

Mithilesh Deshpande

Ph.D. DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the  
Department of Chemistry and Biochemistry of Seton Hall University

Aug, 2014

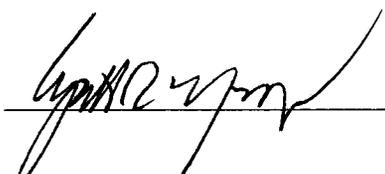
South Orange, New Jersey

We certify that we have read this thesis and that in our opinion it is sufficient in scientific scope and quality as a dissertation for the degree of Doctor of Philosophy

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**In loving memory of my late father Vilas K. Deshpande**

**Dedicated to my Parents, Wife and my Family**

## Abstract

Acetonitrile (ACN) is used in huge quantities for Reversed Phase High Performance Liquid Chromatography (RP-HPLC) experiments. For most of the part, these results in waste that is only contaminated with large amounts of water, buffer and trace organic impurities. It would seem worthwhile to purify the ACN from water using a mass separating agent (MSA). In a quest for an MSA, our investigation reveals polyols as a new class of MSAs. It is observed that when a polyol such as glycerol, sorbitol, erythritol, xylitol, Isomalt® or maltitol is added to a mixture of ACN-water, the ACN separates out of the mixture to form a new immiscible upper phase. The study reveals that glycerol, initiates a phase separation of 1:1 (v/v) ratio of an ACN-water mixture at 20 °C with a glycerol concentration of 17.5 wt/wt %. At 4 °C, only 5 wt/wt % is required to give separation. The results demonstrate that glycerol can improve purity of ACN up to 96 % at lower temperatures thus capable of breaking the ACN-water azeotrope. We also show that polyols as separating agents are equivalent or better than MSAs such as sugars and salts. Further, liquid polyols such as glycerol are far easier to remove, and are better suited to commercial processes.

Equilibrium constants for phase separation ( $K_{PS}$ ) have been measured by  $^1\text{H}$  NMR and GC-TCD techniques. For the glycerol/ACN/water and sorbitol/ACN/ water systems  $K_{PS}$  is  $< 1$  indicating that the free energy for the reaction is positive. For example, glycerol/ACN/water gives the following  $K_{PS}$  values at temperatures indicated in parentheses 0.38 (20 °C), 0.48 (10 °C), 0.60 (0 °C), 0.69 (-10 °C) and 0.81 (-20 °C). Thus,  $K_{PS}$  increases as temperature decreases. At -10 °C, glycerol ( $K_{PS}= 0.69$ ) is a more effective MSA than sorbitol ( $K_{PS}= 0.57$ ). The enthalpy of the phase separation process was investigated using isothermal calorimetry. At 20 °C,  $\Delta H^\circ$  is exothermic (-2.06 kJ/mol) for glycerol induced phase separation in ACN-water system and -7.07 kJ/mol at -10 °C. Considering

the glycerol/ACN/water system at  $-10\text{ }^{\circ}\text{C}$ ,  $\Delta G^{\circ}$  is  $0.83\text{ kJ}$  and  $\Delta H^{\circ}$  is  $-7.07\text{ kJ/mol}$ , this indicates that enthalpy is the dominant thermodynamic parameter for phase separation. A reasonable interpretation is that the polyol as a MSA forms such strong hydrogen bonds with water that this polyol displaces acetonitrile from its interaction with water to an extent that it forms a separate phase.

Our study also demonstrates that solvents in all of the major categories, non-polar (carbon tetrachloride, gasoline, kerosene, diesel, petroleum distillates, cyclohexane, hexanes), polar aprotic (acetonitrile, 2-butanone, acetone, cyclopentanone, dichloromethane, tetrahydrofuran, 2-methyl tetrahydrofuran, 1,2 dimethoxy ethane, methyl benzoate, propyl carbonate, ethyl acetate, chloroform, diethyl ether, mesitylene, p-xylene, benzene, toluene, t-butyl methyl ether, dimethylphthalate, dibutylphthalate) and polar protic (t-butanol, 1-butanol, isopentyl alcohol, cyclohexanol, 1-octanol) can be purified by the PIE process. Some solvents are not separated such as methanol/ $\text{H}_2\text{O}$ , ethanol/ $\text{H}_2\text{O}$ , dimethylsulfoxide/ $\text{H}_2\text{O}$ . In these cases, it is likely that the solvent-water interaction is stronger than the polyol-water interaction. Broader theory indicates that if we know the enthalpy of the solvent-water interaction and if it is less negative than the polyol-water interaction, then phase separation will occur.

These results extended the scope of applications in purification of organic solvents where presence of water as an impurity is of concern.

## **Acknowledgments**

First and foremost, I would like to express my sincere gratitude to my advisor Prof. John R. Sowa for the continuous support of my Ph.D. study and research. I will be forever grateful for his motivation, enthusiasm, and assistance both academically and emotionally. Dr Sowa believed in my ideas from the day one and was able to guide me positively throughout my journey at Seton Hall. I could not have imagined having a better advisor and mentor for my Ph.D study.

I am also very grateful to Dr Murphy, Dr Gorun and Dr Snow for their scientific advice, knowledge and many insightful suggestions.

A great deal of credit goes to my wife, Pooja for her sacrifice over past few years. I would like to specially thank her for encouraging me in all of my pursuits and inspiring me to follow my dreams.

Finally I would like to thanks my family especially my mother for her unconditional love and support.

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# CHAPTER 1:

## INTRODUCTION

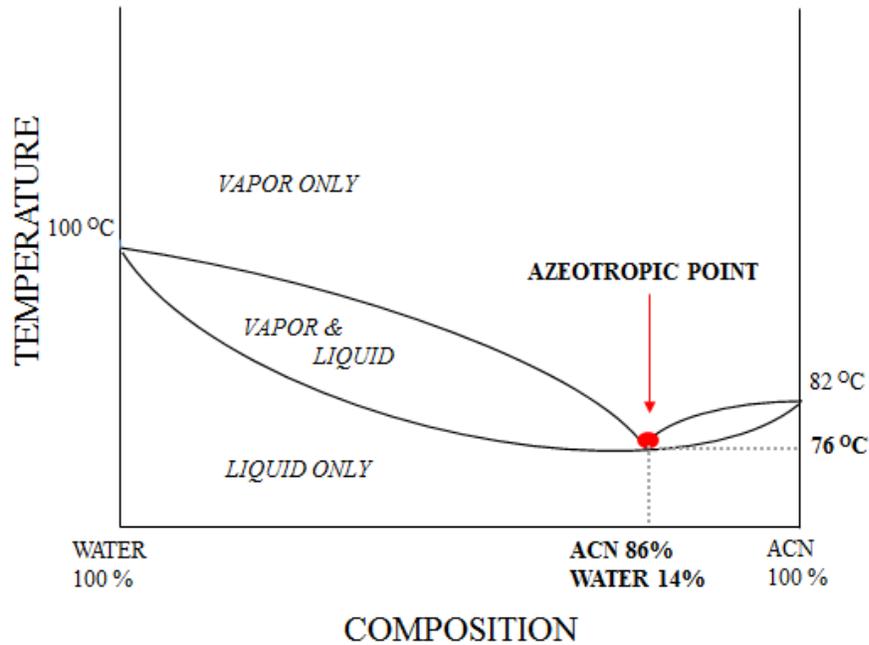
Solvents are indispensable to almost every process in fine chemicals, pharmaceuticals, petrochemicals and printing. Throughout the history of chemistry, a large number of methods have been devised to solve the problem of separating miscible liquids. Moreover, methods for purifying waste solvents as means of direct cost saving on solvent disposal along with increasing environmental legislation against emissions have stimulated interest of various chemical and pharmaceutical industries.

Acetonitrile is miscible with water in all proportions. Acetonitrile is an excellent organic solvent and has become widely used in organic synthesis and manufacturing, as well as for purity and quality control analysis of organic compounds used in pharmaceuticals, cosmetics, personal care products and many other applications in research and development. In the latter aspects, the miscibility of acetonitrile in water renders it one of the most useful solvents for reverse phase-high performance liquid chromatography (RP-HPLC). Several events have resulted in a shortage in acetonitrile causing prices of this solvent to rise dramatically.<sup>1</sup> Thus, there is considerable interest in the recovery of acetonitrile from research and manufacturing and RP-HPLC waste streams. However, like many other organic solvents, acetonitrile forms an azeotrope with water which cannot be fully separated from each other by distillation.

---

<sup>1</sup> (a)Majors, R.E. *LC-GC North America*, **2009**, 27,458-471; (b)Venkatasami, G.; Sowa, J.R. Jr.; A rapid, acetonitrile-free, HPLC method for determination of melamine in infant formula. *Analytica Chimica Acta*. **2010**. 665(2), 227-230.

**Figure 1: Acetonitrile water azeotrope**



According to the literature, since the azeotrope boils at 76 °C and is composed of 86% acetonitrile and 14% water, there is no possibility of obtaining acetonitrile of purity above 86% by means of simple distillation and more costly processes are required to purify acetonitrile to a level higher than the azeotrope.

Separating agents aid in separations of homogenous mixture of two or more liquids/solvents which transform the mixtures into their individual pure components. Separating agents can be essentially be classified into two main domains based on their nature:

*Energy separating agents (ESA) and Mass separating agents (MSA)*

A separation processes with aid of heat transfer to facilitate separation of mixture of solvents into their individual pure forms is an *Energy separating agent (ESA)*. Distillation is most common form of ESA. It is a method for separating the various components of a liquid solution depending

upon the distribution of these components between a vapor phase and a liquid phase.<sup>2</sup> Distillation has long been the standard technology for solvent recovery method to reduce hazardous waste and pollution,<sup>3</sup> and as a technology for purification in many areas of pharmaceutical and especially petrochemical industries.<sup>4</sup>

*Mass separating agents (MSA)* are chemical species added to a mixture of miscible solvent system to create an immiscible solvent system via phase separation where one of the solvent separates in its pure form.

## **1.1 Distillation**<sup>5</sup>

Distillation can be used to separate both dissolved solids and higher boiling components such as water. Distillation is uncomplicated as long as the solvents are easy to distill from water, and obey Raoult's law (The total vapor pressure of the mixture is equal to the sum of the individual partial pressures.) i.e., if they lend themselves to concentration of 'curve a' as shown in Figure 2. It becomes more difficult with low water content, if the difference between the equilibrium vapors and liquid concentration narrows (curve b). Once the equilibrium vapor and liquid phase concentrations are identical, i.e. the azeotropic point has been reached, further distillation serves no purpose because there can be no further concentration (curves c and d). The last traces of water can only be separated from a solvent with great effort (curve b, top right). The azeotropic point limits where distillation can be used (curves c and d).

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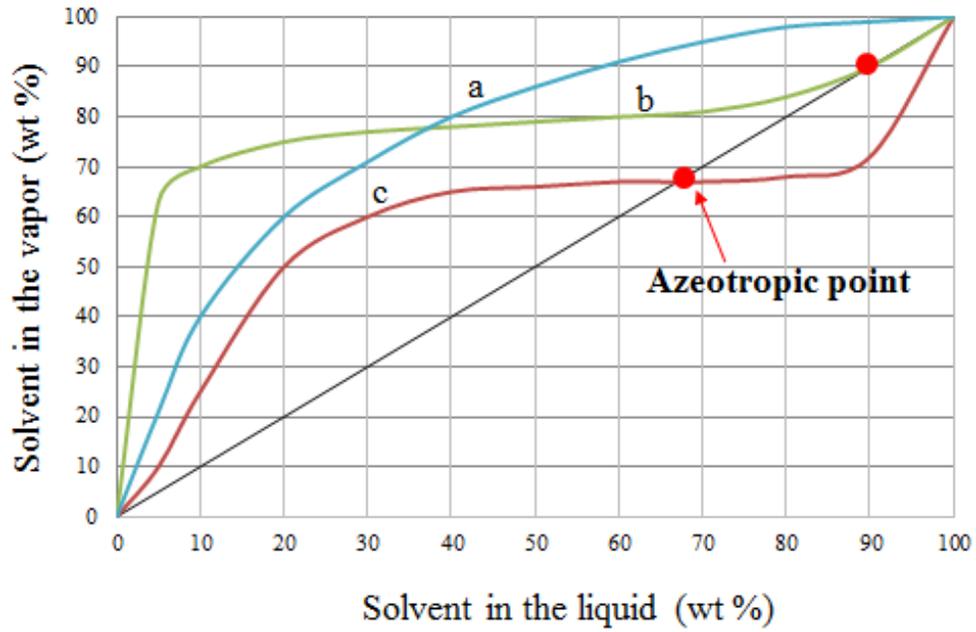
<sup>2</sup> Gmehling J. Group contribution methods- ideal tools for the synthesis and design of separation processes. *Pure Appl. Chem.* **1999**, 71, No. 6, pp. 939-949.

<sup>3</sup> Esquijarosa, J A; Rodríguez-Donis, I.; Jáuregui-Haza, U.; Nuevas-Paz, L.; Pardillo-Fontdevila, E., Recovery of acetonitrile from aqueous waste by a combined process: Solvent extraction and batch distillation *Separation and Purification Technology* November **2006**, Volume 52, Issue 1, pp 95-101.

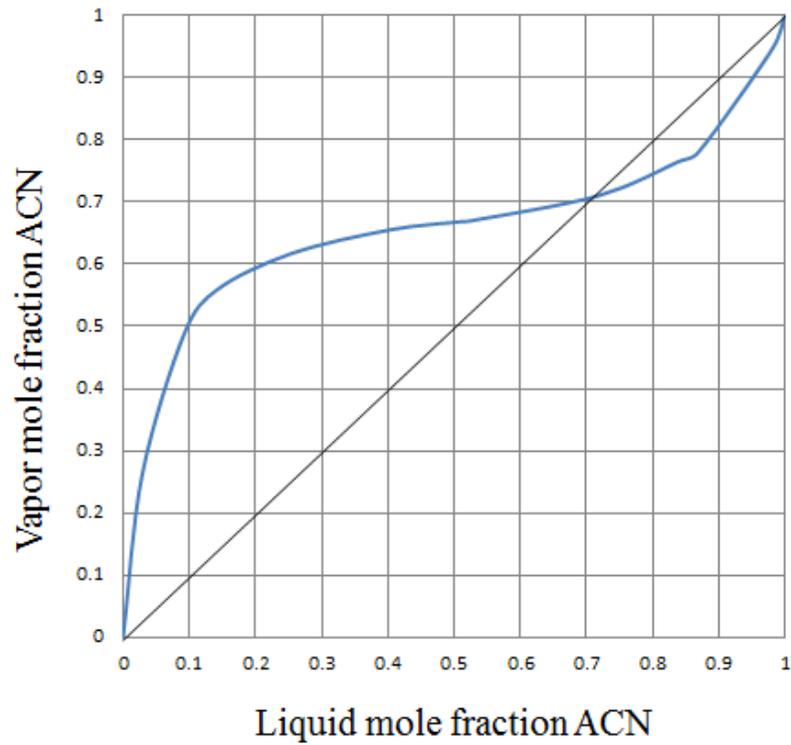
<sup>4</sup> Modla, G.;Lang, P. Removal and recovery of organic solvents from aqueous waste mixtures by extractive and pressure swing distillation. *nd. Eng. Chem. Res.* **2012**, 51 (35), pp 11473–11481.

<sup>5</sup> Martin N., Separating Azeotropic Mixtures *Sulzer technical reviews* **1998**, Volume 3, pp12-15

**Figure 2: Vapor-liquid equilibrium**



**Figure 3: ACN-water phase equilibrium**



## **1.2 ACN-water binary system and problems associated with its separation:**

The mixing of organic solvents with water is normally accompanied by a difference of enthalpy. This thermodynamic property is generally very different from that of an ideal binary mixture whose excess enthalpy of mixing is zero. The deviation from ideality is due to intermolecular interaction. When the mixing of two liquids, A and B, forms an ideal solution, the average A-B interactions must be the same as the average A-A and B-B interactions in the pure liquids; however, in real solutions A-A, B-B and A-B interactions are usually different.<sup>6</sup> For water-organic solvent mixtures, hydrogen-bonding, dispersive and dipole-dipole interactions, etc., lead to the formation of clusters preventing free movement of the solvent. The excess enthalpies of mixing of these two mixtures indicate that their thermodynamic properties are far from ideality, for example, endothermic mixing in case of ACN-water mixture.<sup>7</sup>

The separation of water from acetonitrile-water mixtures presents unusual difficulties for two reasons. In the first place, acetonitrile and water are miscible in all proportions and manifest a high degree of affinity for each other and secondly acetonitrile and water form a constant boiling mixture or azeotropic mixture, thereby precluding direct separation into pure constituents by means of distillation. Thus, solvent recovery typically involves purification of low-boiling organic solvents such as ACN that form minimum-boiling azeotropes with water. Therefore, the nonideal behavior of azeotropy during distillation complicates the recovery of acetonitrile from water.

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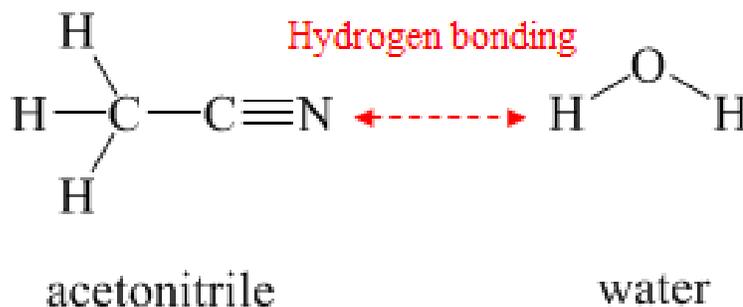
<sup>6</sup> P. W. Atkins, Physical Chemistry, Oxford University Press, Oxford, **1986**, p. 169.

<sup>7</sup> Wakisaka, A.; Abdoul-Carime, H.; Yamamoto, Y.; Kiyozumi, Y, Non-ideality of binary mixtures water-methanol and water-acetonitrile from the viewpoint of clustering structure. *J. Chem. Soc, Faraday Trans.* **1998**, Vol. 94.

### 1.3 Bonding between acetonitrile and water:<sup>8</sup>

The binary mixture of water (H<sub>2</sub>O) and acetonitrile (ACN) has been extensively studied. ACN is a symmetrical molecule with dipole moment of 3.9 D. Due to the presence of a partial negative charge on the nitrogen; ACN can form a hydrogen bond with H<sub>2</sub>O molecule through nitrogen. This determines excellent solubility of H<sub>2</sub>O in ACN. However this hydrogen bond is relatively weaker than hydrogen bond between pure H<sub>2</sub>O molecules. On addition of water into pure acetonitrile acetonitrile molecules are assembled to form three-dimensionally expanded clusters; the acetonitrile clusters are surrounded by water molecules through both hydrogen bonding and dipole–dipole interaction.

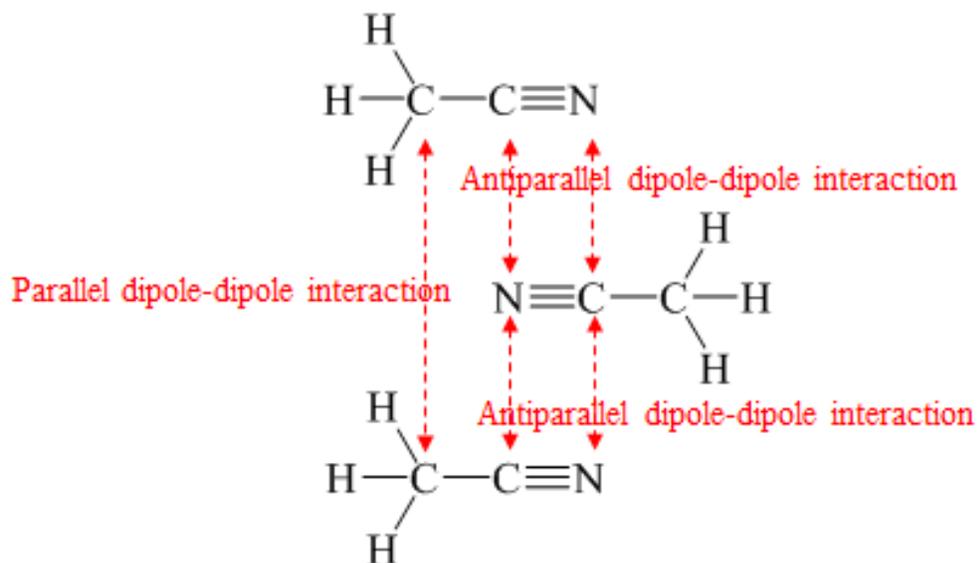
**Figure 4: Bonding between ACN-water**



X-ray diffraction studies of pure acetonitrile revealed that ACN molecules interact with two nearest neighbors by antiparallel and parallel dipole-dipole interaction. Thus, ACN molecules are alternately aligned to form a zig-zag structure.

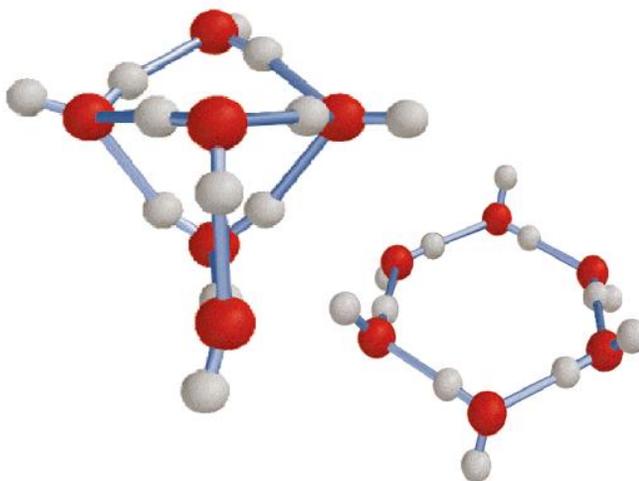
<sup>8</sup> Takamuku, T.; Tabata, M.; Yamaguchi, A.; Nishimoto, J.; Kumamoto, M.; Wakita, H.; Yamaguchi, T. Liquid structure of acetonitrile-water mixtures by X-ray diffraction and infrared spectroscopy. *J. Phys. Chem. B* **1998**, *102*, 8880-8888.

**Figure 5: Intermolecular bonding between ACN molecules**



In current understanding, water is a mixture of randomly hydrogen-bonded molecules and larger structures comprised of tetrahedral oxygen centers which, when hydrogen-bonded to each other, lead to five-membered and other rings that can aggregate to form three-dimensional structures.<sup>9</sup>

**Figure 6: Three dimensional clustering of water molecules through hydrogen bonding**



<sup>9</sup> Plumridge, T.; Waigh, R. Water structure theory and some implications for drug design. *J. Pharm. Pharmacol.* **2002**, 54(9):1155-1179.

Studies show that ACN and H<sub>2</sub>O react together with hydrogen-bonding and dipole-dipole interaction. Molecular dynamic simulation<sup>10</sup> and dynamic properties<sup>11</sup> have suggested that when the acetonitrile content is sufficiently high the structure is microheterogeneous, i.e., that it contains clusters of molecules of one type, either ACN or H<sub>2</sub>O, and that the order in the water structure increases at low acetonitrile content. Raman spectroscopy<sup>12</sup> and thermodynamic studies<sup>13</sup> have shown no such increase in the water structure at low acetonitrile content but have supported the formation of clusters at intermediate compositions.

## **1.4 Kosmotropes and chaotropes:**

The terms '*kosmotrope*' (order-maker) and '*chaotrope*' (disorder-maker) originally denoted solutes that stabilized, or destabilized, respectively, proteins and membranes; thus *chaotropes* unfold proteins, destabilize hydrophobic aggregates and increase the solubility of hydrophobes whereas *kosmotropes* stabilize proteins and hydrophobic aggregates in solution and reduce the solubility of hydrophobes. Later these terms referred to the apparent change in structuring of the water molecule.<sup>14</sup> Water is highly associated through hydrogen bonding and various solutes can change the distribution of hydrogen bonds by increasing or decreasing the number and strengths of the bonds.<sup>15</sup> Broadly speaking solutes are defined as *kosmotropic* if they contribute to the stability and structure of water-water interactions causing water molecules to favorably interact. On the other hand a *chaotropic* agent is a substance that disrupts the structure of water by destabilizing hydrogen bonding network.

---

<sup>10</sup> Kovacs, H.; Laaksonen, A. *J. Am. Chem. Soc.* **1991**, 113, 5596.

<sup>11</sup> Goldammer, E.; Herz, H. G. *J. Phys. Chem.* **1970**, 74, 3734.

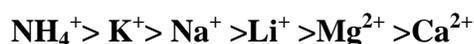
<sup>12</sup> Kabisch, G. *Z. Phys. Chem., Leipzig* **1982**, 263,48.

<sup>13</sup>(a)Moreau, C.; Douheret, G. *Thermochim. Acta* **1975**, 13, 385.; (b)Armitage, D. A.; Blandamer, M. J.; Foster, M. J.; Hidden, N. J.;Morcom, K. W.; Symons, M. C. R.; Wootten, M. J. *Trans. Faraday Soc.***1968**, 64, 1193.

<sup>14</sup> Marcus, Y, Effect of ions on the structure of water: Structure making and breaking. *Chem. Rev.* **2009**, 109, 1346-1370

<sup>15</sup> Desnoyers, J.H.; Structural effects in aqueous solutions: a thermodynamic approach. *Pure & Appl. Chem.* **1982**, Vol. 54, No. 8, pp.1469-1478.

Ionic *kosmotropes* should be treated differently from non-ionic *kosmotropes*, due mainly to directed and polarized arrangements of surrounding water molecules. Generally, ionic behavior parallels the *Hofmeister series*, where research by Franz Hofmeister in the early 20th century organized various anions and cations by their ability to salt-out proteins.<sup>16</sup> According to the *Hofmeister series* the cations are arranged as follows:



Likewise, the order for anions is:



*Chaotropes* are large singly charged ions, with low charge density ( $\text{SCN}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HSO}_4^-$ ,  $\text{HCO}_3^-$ ,  $\text{I}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{Cs}^+$ ,  $\text{K}^+$ ,  $(\text{NH}_2)_3\text{C}^+$  (guanidinium) and  $(\text{CH}_3)_4\text{N}^+$  (tetramethylammonium ions)) These exhibit weaker interactions with water than water with itself and thus interfere less in the hydrogen bonding of the surrounding water, whereas *kosmotropes* are small or multiply-charged ions, with high charge density ( $\text{SO}_4^{2-}$ ,  $\text{HPO}_4^{2-}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{H}^+$ ,  $\text{OH}^-$  and  $\text{HPO}_4^{2-}$ ) exhibiting stronger interactions with water molecules than water with itself and therefore are capable of breaking water-water hydrogen bonds.

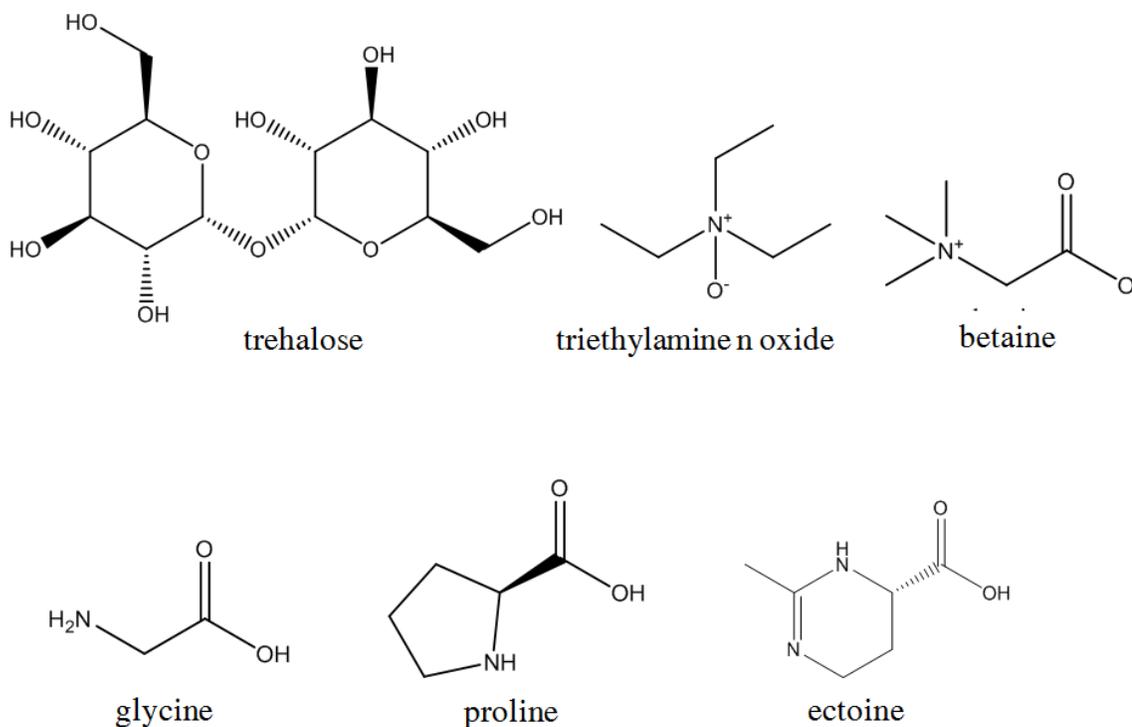
Non-ionic *kosmotropes* are very soluble well-hydrated molecules with little tendency to aggregate, having no net charge and strongly hydrogen bonding to water, that stabilize the structure of macromolecules in solution. They are preferentially solubilized within the bulk of the solution and are excluded from the solvation layers of macromolecular surfaces. In contrast *non-ionic chaotropes* break down the hydrogen-bonding network of water, allowing macromolecules more structural freedom. *Kosmotropes* are stabilizing solutes that increase the order of water (such as polyhydric

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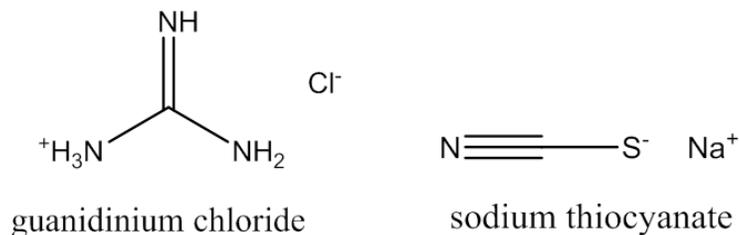
<sup>16</sup> Hofmeister, F. Arch. Exp. Pathol. Pharmacol. **1888**, 24, 247-260.

alcohols, trehalose, trimethylamine *N*-oxide, glycine betaine, ectoine, proline and various other zwitterions, Figure 7)<sup>17</sup> whereas chaotropes create weaker hydrogen bonding, decreasing the order of water, increasing its surface tension and destabilizing macromolecular structures (such as guanidinium chloride and sodium thiocyanate, Figure 8).

**Figure 7: Non-ionic kosmotropes**



**Figure 8: Non-ionic chaotropes**



<sup>17</sup> Uedaira, Hi and Uedaira, Ha, Role of hydration of polyhydroxy compounds in biological systems *Cell. Mol. Biol.* **2001** 47:823-829.

Saiellia *et al.*<sup>18</sup> in their research justified theory of preferential solvation especially of non-electrolytes such as glucose in ACN-water mixture. The structure of the solvation shell obtained by means of molecular dynamics simulation was analyzed using radial and spatial distribution functions. Their study concluded that in agreement with available experimental data, water is found to preferentially solvate the sugars. The micro-heterogeneity of the mixture, with clusters of hydrogen-bonded water molecules and clusters of dipole–dipole interacting acetonitrile molecules, favors the solvation of the carbohydrates by the water clusters.

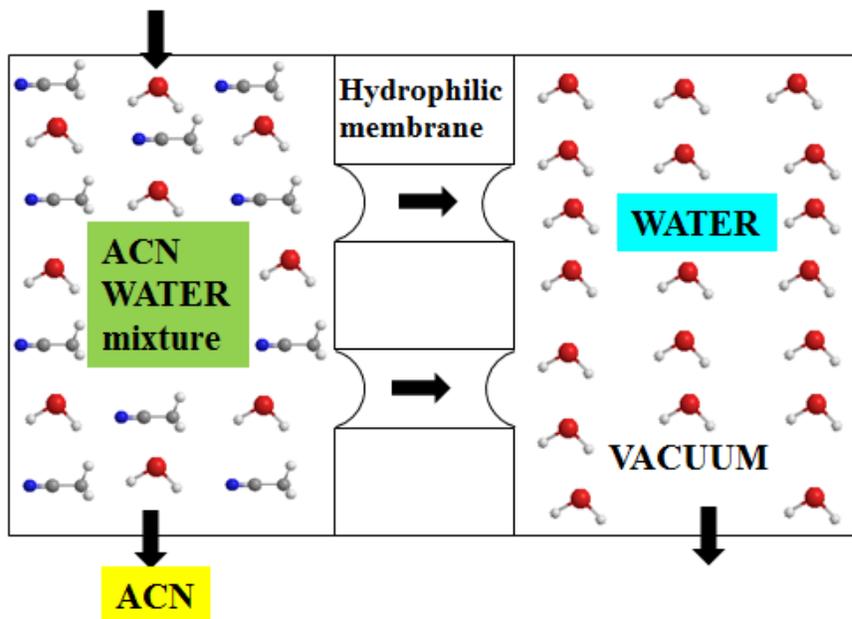
## **1.5 Pervaporation:**

Pervaporation (or pervaporative separation) is a processing method for the separation of mixtures of liquids by partial vaporization through a non-porous or porous membrane. Separation by pervaporation depends on the difference in partial vapor pressure between the two sides of a membrane and the selective sorption properties of the membrane with respect to the components in the mixture. The pressure difference is created by applying a lower pressure at the permeate side. Because the separation is not driven by the liquid-vapor equilibrium, separation of azeotropic mixtures is also feasible. The membranes used in pervaporation are able to absorb certain substances that have a chemical affinity for the membrane and reject substances that have less affinity. Hydrophilic membranes are available for separating water from solvents, and organophilic membranes are used to remove organics from water (Figure 8 and 9)

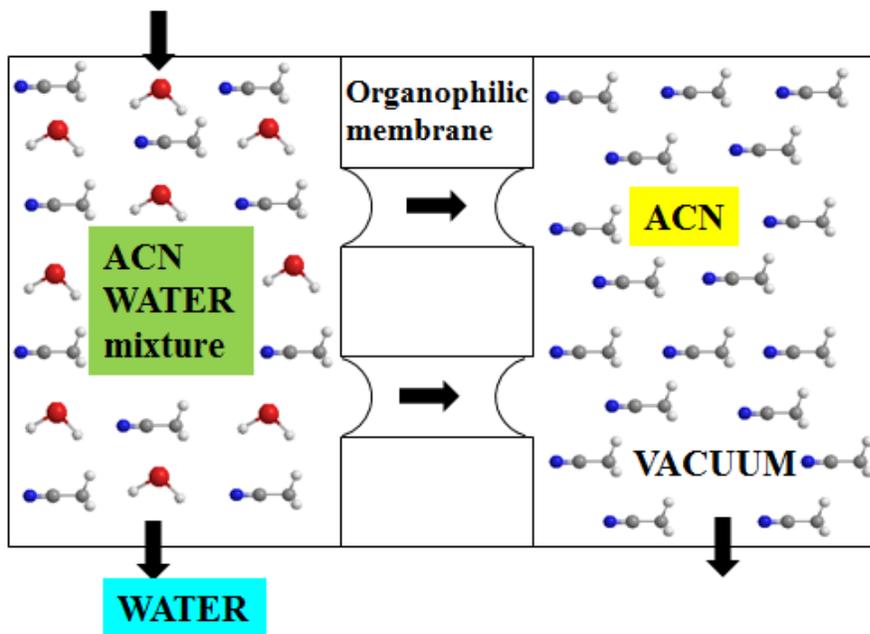
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<sup>18</sup> Saiellia, G.;Bagno,A. Preferential solvation of glucose and talose in water–acetonitrile mixtures: a molecular dynamics simulation study, *Physical. Chemistry Chemical Physics.*, **2010**, 12, 2981–2988

**Figure 9: Pervaporation using hydrophilic membrane**



**Figure 10: Pervaporation using organophilic membrane**



Khayet *et al.*<sup>19</sup> used the pervaporation process to successfully separate binary solutions of acetone–water, ACN–water and ethanol–water as well as multicomponent systems using Sulzer

<sup>19</sup> Khayet, M.; Cojocaru, C.; Zakrzewska-Trnadel, G. *Sep. Purif. Technol.* **2008**, 63, 303.

Pervap 4060 membranes. More recently, Mandal<sup>20</sup> examined the separation of ACN–water systems using Pervap 2202 and nanocomposite PVA-iron oxide membranes delivering up to 99% (wt.) water in the permeate. Both papers suggest that this is an efficient and cost-effective separation technique. However, these methods have yet to achieve commercial viability. Pervaporation is attractive because the energy required for the separation is strongly reduced due to the selective vaporization of the permeating components only. However, pervaporation involves higher capital cost than distillation processes.

## **1.6 Advanced distillation techniques for ACN recovery:**

Extractive and azeotropic distillations have been extensively used to achieve the recovery of ACN from aqueous waste. Acosta-Esquivarosa *et al.*<sup>21</sup> proposed a combined process of butyl-acetate solvent extraction followed by distillation. Using this technique the authors claimed an 83% recovery of ACN with a purity of 98%. Gomez *et al.*<sup>22</sup> proposed novel methods using hybrid pervaporation distillation units which are combination of distillation and membrane-based pervaporation technique for ACN recovery. In extractive distillation the azeotrope may also be broken via the use of an entrainer, i.e., an additive which shifts the thermodynamic equilibrium in such a way that the azeotrope is eliminated. Bernot *et al.*<sup>23</sup> and Dussel and Sticklmair<sup>24</sup> studied the feasibility of such operations by computer simulation. Later Rodriguez-Donis *et al.*<sup>25,26,27</sup> gave a

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<sup>20</sup> Mandal, M. K.; Sant, A. B.; Bhattacharya, P. K. *Colloids Surf., A* **2011**, 373, 11.

<sup>21</sup> Acosta-Esquivarosa, J.; Rodriguez-Donis, I.; Jauregui-Haza, U.; Nuevas-Paz, L.; Pardillo-Fontdevila, E. *Sep. Purif. Technol.* **2006**, 52, 95.

<sup>22</sup> Del Pozo Gomez, M. T.; Klein, A.; Repke, J.-U.; Wozny, G. *Desalination* **2008**, 224, 28.

<sup>23</sup> Bernot, C.; Doherty, M. F.; Malone, M. F. Patterns of composition change in multicomponent batch distillation. *Chem. Eng. Sci.* **1990**, 45, 1207.

<sup>24</sup> Dussel, R.; Sticklmair, J. Separation of azeotropic mixtures by batch distillation using an entrainer, *Comput. Chem. Eng.* **1995**, 19, 113.

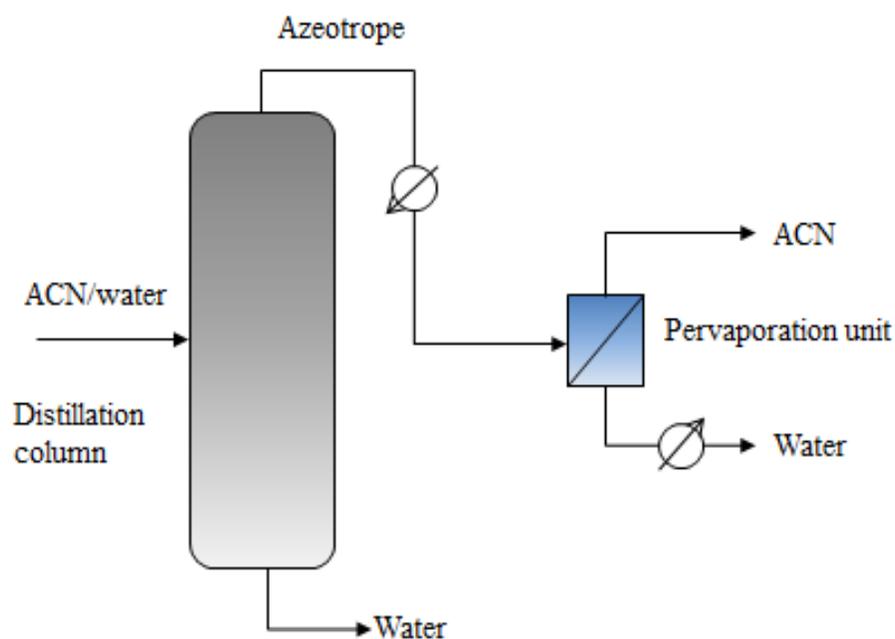
<sup>25</sup> Rodriguez-Donis, I.; Gerbaud, V.; Joulia, X. *Ind. Eng. Chem. Res* **2001**, 40, 2729.

<sup>26</sup> Rodriguez-Donis, I.; Esquivarosa, J. A.; Gerbaud, V.; Joulia, X. *AIChE Journal* **2003**, 49, 3074.

<sup>27</sup> Rodriguez-Donis, I.; Pardillo-Fontdevila, E.; Gerbaud, V.; Joulia, X. *Comput. Chem. Eng.* **2001**, 25, 799.

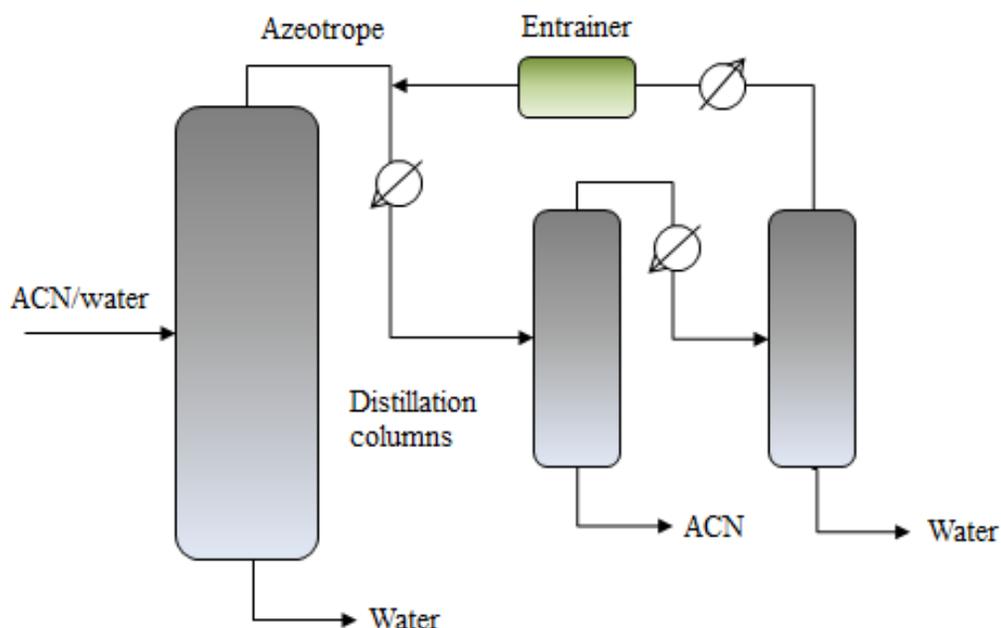
complete overview of entrainer selection for homogeneous azeotropic batch distillation and screened several alternative entrainers by simulation via the Universal Functional Activity Coefficient (UNIFAC) method, which is a semi-empirical system for the prediction of non-electrolyte activity in non-ideal mixtures. UNIFAC uses the functional groups present on the molecules that make up the liquid mixture to calculate activity coefficients.<sup>28</sup> They studied the separation of ACN–water systems using heterogeneous batch distillation, identifying two potential entrainers, hexylamine and butyl acetate, from various amines, alcohols, esters, ketones and aromatics.

**Figure 11: Hybrid distillation/pervaporation**



<sup>28</sup> Fredenslund, A.; Jones, R.L.; Prausnitz, J.M.; Group-Contribution Estimation of Activity Coefficients in Nonideal Liquid Mixtures”, *AIChE Journal* **1975**, 21, 1086.

**Figure 12: Azeotropic distillation**



### **1.7 Low temperature induced phase separation:**

A method of the purification of acetonitrile by low temperature phase separation<sup>29</sup> can be used as a process for separation and recovery of substantially pure acetonitrile from crude waste organic streams containing acetonitrile, water and one or more additional organic components.

Temperature studies revealed that by lowering the temperature for 50-50 % (v/v) mixtures of ACN-water, acetonitrile may be concentrated to greater than 80 % by weight in the organic phase at -7 °C to -9 °C. At -14 °C the water begins to form ice in the organic phase but the exact freezing point depends on the composition of the ACN-water mixture. Normally, mixtures of water and acetonitrile are miscible at room temperatures. Robert Lee Hartnett in his patent<sup>29</sup> mentioned that using the Clausian-Clapeyron equation one would predict that a 50-50 mixture of acetonitrile and water would freeze at -53 °C without any prior phase separation but the equation cannot predict

<sup>29</sup> Hartnett, R L (Monsanto Co., USA). Method for the purification of acetonitrile by low temperature phase separation. European patent EP0113331, DEC 28, 1983.

occurrence of phase separation in ACN-water mixture. This method of separation was further applied for purification of proteins<sup>30</sup> and other biomolecules.<sup>31</sup> In most cases, azeotropic mixtures require special methods to facilitate their separation.

## **1.8 Mass separating agents:**

The introduction of a mass separating agent (MSA) into a homogenous solution to create a second immiscible phase is a promising alternative for solvent extraction, purification and recovery of solvents such as acetonitrile.

### **1.8.1 Salts as mass separating agents:**

Use of salts as MSAs is a widely studied and employed technique. It is most commonly referred to as ‘*salting-out*’ or ‘*salting-out assisted liquid-liquid extraction*’ (*SALLE*). It has long been known that the addition of an inorganic salt into mixture of water and a water miscible organic solvent causes a separation of the solvent from mixture and the formation of two phase system.<sup>32</sup> Frankforter and Fray first investigated ternary systems of water-miscible organic liquids, salts, and water. Their findings were that potassium carbonate ( $K_2CO_3$ ) was the only salt that would cause *salting-out* of methanol-water, while several salts would cause the *salting-out* of ethanol-water. The dehydration of acetone was very similar to that of alcohol and ether. In most cases the dehydration of organic liquids is accompanied by the formation of two definite layers, one organic and the other

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<sup>30</sup> Gu, T.; Gu, Y.; Zheng, Y.; Wiehl, P. E.; Kochick, J. J. Phase separation of acetonitrile-water mixture in protein purification. *Sep. Technol.* **1994**, *4*, 258–260.

<sup>31</sup> Gu, T.; Zheng, Y.; Gu, Y.; Haldankar, R.; Bhalerao, N.; Ridgway, D.; Wiegl, P. E.; Chen, W. Y.; Kopchick, J. J. Purification of a pyrogen-free human growth hormone antagonist. *Biotechnol. Bioeng.* **1995**, *48*, 520–528.

<sup>32</sup> (a) Frankforter, G.B.; Cohen, L. *J. Am. Chem. Soc.* **1914**, *36*, 1103-1134.; (b) Frankforter G B.; Fray F C. *J. Phys. Chem.* **1913**, *17*: 402-473.; (c) Frankforter G B.; Temple S. *J. Am. Chem. Soc.* **1915**, *37*: 2697-2716.

aqueous. Tabata *et al.*<sup>33</sup> investigated fourteen water-miscible polar solvents for the separation from their aqueous solutions by *salting-out* using sodium chloride.

Due to relevance to the project in this thesis, only ACN-water system and its salting out techniques will be discussed. Early in 1965, J. A. Renard<sup>34</sup> published an investigation concerned with the *salting-out* characteristics system of acetonitrile-water-salt. This investigation included the development of an infrared analysis technique by which liquid-liquid equilibrium in the system of ACN-water-salt could be obtained. Renard *et al.* were the first to comprehensively study the effect of potassium salts on the ACN-water system. Their results showed that  $K_2CO_3$ , KF and KBr were effective in causing *salting-out*, whereas KI was ineffective while KCl and  $K_2SO_4$  precipitated from aqueous solutions of water after addition of ACN. In the case of a one-phase binary mixture of water and organic solvent, the addition of a salt soluble only in the water will lower the vapor pressure of water but raise the vapor pressure of the organic compound. This will cause the binary liquid to separate into two liquid phases which are in equilibrium. The vapor pressure of each component will be the same in the two phases, and the extent of *salting-out* will be determined by the resulting vapor pressures. The reason for effective phase separation by the salts  $K_2CO_3$ , KF and KBr is that they are insoluble in acetonitrile, but, they are effective in lowering the vapor pressure of water. Salts KCl and  $K_2SO_4$  are ineffective in phase separation because they are both insoluble in acetonitrile and they do not lower the vapor pressure of water. The reason that KI does not cause phase separation is due to an appreciable amount of solubility in acetonitrile and also it does not lower the vapor pressure of water.

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<sup>33</sup> Tabata, M; Kumamoto, M; Nishimoto, J. Chemical Properties of Water-Miscible Solvents Separated Salting-out and Their Application to Solvent Extraction. *ANALYTICAL SCIENCES* JUNE 1994, VOL. 10.

<sup>34</sup> Renard, J.A.; Oberg, A.G. Ternary systems: Water-Acetonitrile-Salts *Journal of Chemical and Engineering Data* 1965, 10, 152-155.

Research on sodium and ammonium salts<sup>35</sup> showed that salts such as  $(\text{NH}_4)_2\text{SO}_4$  has the greatest *salting-out* power followed successively by  $\text{Na}_2\text{S}_2\text{O}_3$  (sodium thiosulfate),  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  (trisodium citrate),  $\text{Na}_2\text{CO}_3$  and  $\text{Na}_2\text{SO}_4$ . Other salts such as  $\text{NaCl}$  and  $\text{NH}_4\text{Cl}$  precipitated from aqueous solutions of water after addition of ACN.

Further Renard *et al.* investigated *salting out* characteristics for the fluoride, chloride, iodide, bromide, acetate, carbonate, sulfate and nitrate salts of lithium.<sup>36</sup> Their results showed that the salts  $\text{LiCl}$ ,  $\text{LiC}_2\text{H}_3\text{O}_2$  (lithium acetate) and  $\text{Li}_2\text{SO}_4$  were effective in causing *salting-out*. The salts  $\text{LiBr}$ ,  $\text{LiI}$  and  $\text{LiNO}_3$  were ineffective while  $\text{LiF}$  and  $\text{Li}_2\text{CO}_3$  precipitated from aqueous solutions of water after addition of ACN and thus were ineffective.

The application of salt-induced phase separation of ACN-water mixtures was further extensively used in extraction of various chemicals and biochemicals where homogeneous liquid-liquid extraction of water soluble compounds such as highly charged metalloporphyrin, cannot be extracted into conventional solvents such as chloroform and carbon tetrachloride<sup>37</sup>

The mechanism of  $\text{NaCl}$  induced phase separation of ACN-water mixture at the molecular level was investigated in detail by Takamuku *et al.*<sup>38</sup> using large-angle X-ray scattering (LAXS) and small-angle neutron scattering (SANS) methods. Their study revealed that in ACN-water mixtures, water molecules can preferentially solvate both  $\text{Na}^+$  and  $\text{Cl}^-$ , but, acetonitrile molecules do not because both electron donor and acceptor properties (i.e., the hydrogen bonding properties) of water are stronger than those of acetonitrile. The hydration shells of  $\text{Na}^+$  and  $\text{Cl}^-$  increase in acetonitrile-

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<sup>35</sup> Renard, J.A. Ternary systems: Water- Acetonitrile-Salts. *Journal of Chemical and Engineering Data* **1966**, 11, 169-171.

<sup>36</sup> Renard J.A.; Heichelheim, H.R. Ternary systems: Water-Acetonitrile-Salts. *Journal of Chemical and Engineering Data* **1967**, 12, 33-36.

<sup>37</sup> Tabata, M.; Kumamoto, M.; Nishimoto, J. Ion-pair extraction of metalloporphyrins into acetonitrile of determination of copper (II). *Analytical Chemistry* **1996**, 68, 758–762.

<sup>38</sup> Takamuku, T.; Yamaguchi, A.; Matsuo D.; Tabata, M.; Kumamoto, M.; Nishimoto, J *et al.* Large-angle X-ray scattering and small angle neutron scattering study on phase separation of acetonitrile–water mixtures by addition of  $\text{NaCl}$ . *J Phys Chem B*. **2001**, 105, 6236–6245.

water-NaCl mixtures when the NaCl concentration increases. In addition, the number of linear hydrogen bonds among water molecules increases with increasing salt concentration, indicating that the hydrogen-bonded network of water is likely to be evolved around both hydrated ions. Hence, for the NaCl-induced phase separation of acetonitrile-water mixtures, the hydrogen-bonded network of water is evolved by hydration of  $\text{Na}^+$  and  $\text{Cl}^-$  at ambient temperatures which acts as a main driving force for phase separation. Moreover, in the phase separation process, water and acetonitrile aggregates are not completely separated to form neat acetonitrile and water phases since the dipole-dipole interaction among acetonitrile and water molecules partly remains even in a strong electrostatic field of the added ions. With a further increase in salt concentration the acetonitrile-water interactions are ruptured, and the phase separation gradually progresses toward an increase in volume and content of acetonitrile for the acetonitrile-rich phase. Later in 2006, Takamuku *et al.*<sup>39</sup> extended their study to other alkali chlorides induced phase separation of ACN-water mixture by LiCl and KCl along with NaCl using small-angle neutron scattering (SANS) experiments. Their results indicated that the hydration shell of  $\text{Na}^+$  is most suitable to form water clusters among the alkali ions due to sufficient electrostatic interaction between  $\text{Na}^+$  and water molecules and the size of the hydration shell. Thus, the formation of clusters in the acetonitrile–water–alkali chloride salt mixtures is governed by two opposite effects. The electrostatic force of the alkali metal ions to attract water molecules is stronger in the order of  $\text{Li}^+ > \text{Na}^+ > \text{K}^+$ , while the formation of hydrogen bonds among water molecules around the hydration shell of the ions is easier in the order of  $\text{K}^+ > \text{Na}^+ > \text{Li}^+$ . Consequently, the overall effects result in the phase separation of acetonitrile–water mixtures are more easily induced by NaCl among the alkali chlorides. More recently, Haramaki *et*

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<sup>39</sup> Takamuku, T.; Noguchi, Y.; Yoshikawa, E.; Kawaguchi, T.; Matsugami, M.; Otomo, T. Alkali chlorides-induced phase separation of acetonitrile-water mixtures studied by small-angle neutron scattering. *J. Mol. Liq.* **2007**, 131/132, 131–138.

*al.*<sup>40</sup> studied phase separation of alkali halide–acetonitrile–water mixtures by cooling using a combination of SANS, infrared and NMR techniques. Ligette *et al.*<sup>41</sup> illustrated that ACN–water phase separation can be induced at room temperature with addition of NaCl and can be applied for extraction of nitroaromatics, nitroamines, and nitrate esters such as trinitrotoluene (TNT) and cyclotrimethylenetrinitramine, commonly called as RDX and compared efficiencies of ACN and dichloromethane both with and without added salts. Le *et al.*<sup>42</sup> studied extraction of erythromycin from fermentation broth using salt-induced phase separation using the ACN-water system. The results demonstrated that the mixture was separated into two phases by adding an inorganic salt, such as NaCl or Na<sub>2</sub>SO<sub>4</sub>. Erythromycin was extracted from broth to the organic phase and finally recovered from solvents by vacuum distillation and crystallization. Their study showed that compared with traditional liquid–liquid extraction, the new extraction processes have advantages of rapid and higher extraction efficiency of more than 98.5% by a one-stage extraction. Gu *et al.*<sup>43</sup> after successfully applying low temperature phase separation in ACN-water system for protein extraction further demonstrated that various salts could induce the phase separation of ACN-water system more effectively at the near-zero temperature with a relatively small amount of salt added. Furthermore, the phase separation can be enhanced significantly by coupling salt addition and cooling procedures. Among the tested salts, they found that K<sub>2</sub>HPO<sub>4</sub> (dipotassium phosphate/potassium phosphate dibasic) was the suitable salt for phase separation, followed by KH<sub>2</sub>PO<sub>4</sub> (potassium phosphate/potassium phosphate monobasic), NaCl or NH<sub>4</sub>HCO<sub>3</sub> (ammonium bicarbonate), and KCl, respectively. But they observed that K<sub>2</sub>HPO<sub>4</sub> alone may lead to a dramatic increase of pH so they

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<sup>40</sup> Haramaki, H.; Shimomura, T.; Umecky, T.; Takamuku, T. SANS, infrared, and <sup>7</sup>Li and <sup>23</sup>Na NMR studies on phase separation of alkali halide-acetonitrile-water mixtures by cooling, *J. Phys. Chem. B.* **2013**, 117, 2438–2448.

<sup>41</sup> Leggett, D. C.; Jenkins, T. F.; Miyares, P. H. Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water, *Anal Chem.* **1990**, 62, 1355

<sup>42</sup> Le QH, Shong LG, Shi YH. Extraction of erythromycin from fermentation broth using salt-induced phase-separation processes. *Sep Purif Technol* **2001**;24:85-91.

<sup>43</sup> Gu, Y.; Shih, P.-H. Salt-induced phase separation can effectively remove the acetonitrile from the protein sample after the preparative RP-HPLC. *Enzyme Microb. Technol.* **2004**, 35, 592.

replaced it with a potassium phosphate buffer (pH 7.0), which not only maintained the phase separation efficiency but also neutralized the pH of the water-rich phase. Their study also concluded that NaCl could initialize the phase separation of a 50% (v/v) of ACN–water solution at 25 °C while the molar concentration was about 331 mM. However, with the same ACN concentration, much less NaCl was required to prompt the phase separation at 4 °C with a molar concentration of 109 mM. Also, K<sub>2</sub>HPO<sub>4</sub> exhibited much better induction for phase separation than NaCl and other salts at 4 °C, in which a lesser amount of K<sub>2</sub>HPO<sub>4</sub> was required for the same phase-separation efficiency in comparison with NaCl. The results also showed that KH<sub>2</sub>PO<sub>4</sub> was better than NaCl at a low salt concentration, but its advantage disappeared at the high salt concentration. They estimated that about 64% of ACN could be successfully removed from a 50% (v/v) of ACN solution at 4 °C by K<sub>2</sub>HPO<sub>4</sub> at molar concentration of 150 mM, whereas the same mole fraction of NaCl could remove only 33% of ACN. On the other hand, they noticed that the salt accumulated fully in the water-rich phase, whereas the conductivity was barely responsive in the ACN-rich phase, suggesting that the preferential hydration of salt ions might be implicated as the major driving force to draw the water molecules from the weak dipole–dipole interactions with acetonitrile molecules, and acetonitrile molecules were expelled from the water aggregates, resulting in the phase separation. Thus, the modified phase-separation technique by coupling cooling and salt addition offered the more economic, effective, and reliable method in removing ACN.

Zhang *et al.*<sup>44</sup> investigated extraction of platinum, palladium and rhodium in a single step using salting-out induced three-liquid-phase system of diisopentyl sulfide acetonitrile-NaCl-water. Experimental results indicated that Pd(II), Pt(IV) and Rh(III) were selectively concentrated into the diisopentyl sulfide organic top phase, acetonitrile-rich middle phase and NaCl aqueous bottom

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<sup>44</sup> Zhang, C.; Huang, K.; Yu, P.; Liu, H, Salting-out induced three-liquid-phase separation of Pt(IV), Pd(II) and Rh(III) in system of S201-acetonitrile-NaCl-water, *Separation and Purification Technology* **2011**, 80, 81–89.

phase, respectively. The *salting-out* induced two-phase splitting of acetonitrile–water mixtures was the main driving force for three-liquid-phase partition of platinum metal ions.

Thus, various researchers highlighted the adept ability of salts to induce phase separation in ACN-water system. However, one inconvenience when using this technique is the need to use a pH buffering agent to protect sensitive components such as enzymes. The use of salts may also be problematic in that they, under certain conditions, may precipitate out of solution, leading to fouling problems in process equipment. Furthermore, the prospect of unwanted reactions with impurities and complications due to corrosion may negate the use of salts as mass separating agents. Furthermore, if analysis of these extraction processes involves mass spectrometric techniques, the analyte may react with the inorganic salts, and depending on the type of salt added, these may impact mass spectrometric ionization.

### 1.8.2 Sugars as Mass separating agents:

Sugars as mass separating agent have gained considerable attention over last few years. Dr. Hao Feng's lab in University of Illinois at Urbana-Champaign first reported use of sugars as mass separating agents.<sup>45</sup> This application related to use of sugars as mass separating agent was mostly extended to extraction of important analytes from ACN-water system.

In 2008 B. Wang *et al.*<sup>45</sup> made a novel observation that by adding a monomeric sugar (glucose, xylose, arabinose or fructose) or a disaccharide (sucrose or maltose) into an ACN-water mixture, ACN separated out from water to form a new upper phase. They termed this phenomenon as '*sugaring out*' and as a new phase separation method used to obtain high purity ACN from an ACN-water mixture. The results showed that ACN concentration in the upper phase increases when

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<sup>45</sup>(a)Wang B., Ezejias T., Feng H., Blaschek H. Sugaring-out: A novel phase separation and extraction system. *Chemical Engineering Science* **2008**, 63, 2595-2600.; (b)Wang, B.; Feng, H.; Ezeji, T.; Blaschek, H. Sugaring-out separation of acetonitrile from its aqueous solution. *Chem. Eng. Technol.* **2008**, 31, 1869-1874.

more sugar is added into the ACN-water mixture and also as temperature was reduced. Results also showed that addition of polysaccharides (starch and dextran) did not result in phase separation. The important results were expressed in terms of phase ratio (Table 1) and percent purity of ACN obtained in upper phase after addition of different sugars (Table 2). Glucose and xylose were the most promising sugars in terms of phase ratio and percent of ACN obtained in upper layer. The results showed that when 50 g/L glucose or xylose was added into a 1:1 (v/v) ACN–water solution at 1 °C, the phase separation was triggered immediately after mixing. After 24 h, results from GC analysis showed that the upper phase in glucose- and xylose-induced two-phase systems contained 95% and 91% (wt/wt) ACN, respectively, with most water remaining in the lower phase. The ACN concentration in lower phase of the glucose and xylose induced two-phase systems was 16% and 26% (wt/wt), respectively. They postulated that an increase in the sugar concentration increased the volume of the upper phase and that the sugar molecules may have replaced the ACN molecules in the water by forming hydrogen bonds with water molecules. As a result, more ACN molecules were “*pushed out*” from the water into the upper phase when more sugar molecules were introduced into the system. Moreover sugars behave as non-ionic kosmotropes, stabilizing the effect of hydrogen bonds between the hydroxyl groups or the carbonyl oxygen of the sugars and the polar water molecules. They concluded such an ACN–water two-phase system will find applications in the extraction of compounds that have different solubility in water and in ACN or those that cannot be effectively extracted by conventional liquid-liquid extraction method.

**Table 1: Effect of sugar concentration on ACN-water phase separation<sup>45</sup>**

Concentration (g/L)	5	10	15	20	25	30	35	40	45	50
Sugar	Phase ratio ( $R = V_{upper}/V_{lower}$ )									
Glucose	-	-	-	-	0.36 ± 0.02	0.39 ± 0.04	0.40 ± 0.02	0.46 ± 0.03	0.47 ± 0.02	0.51 ± 0.03
Xylose	-	-	0.08 ± 0.02	0.20 ± 0.04	0.22 ± 0.00	0.27 ± 0.02	0.28 ± 0.01	0.30 ± 0.04	0.36 ± 0.01	0.42 ± 0.00
Arabinose	-	-	-	-	0.21 ± 0.04	0.22 ± 0.01	0.28 ± 0.00	0.33 ± 0.02	0.36 ± 0.01	0.40 ± 0.03
Fructose	-	-	-	-	0.19 ± 0.03	0.23 ± 0.01	0.30 ± 0.01	0.36 ± 0.02	0.38 ± 0.00	0.40 ± 0.01
Sucrose	-	-	-	-	-	0.26 ± 0.03	0.27 ± 0.01	0.35 ± 0.00	0.38 ± 0.05	0.43 ± 0.03
Glucose + xylose mixture (1:1 wt/wt)	-	-	0.16 ± 0.02	0.21 ± 0.01	0.22 ± 0.02	0.28 ± 0.00	0.38 ± 0.01	0.45 ± 0.06	0.48 ± 0.01	0.48 ± 0.01

**Table 2: ACN concentration (% wt/wt) in the sugared-out upper phase<sup>45</sup>**

Concentration (g/L)	5	10	15	20	25	30	35	40	45	50
Sugar	ACN concentration (% wt/wt)									
Glucose	-	-	-	-	87.6 ± 1.3	89.0 ± 1.9	90.6 ± 4.2	91.7 ± 2.5	92.6 ± 6.1	95.4 ± 2.1
Xylose	-	-	74.5 ± 0.6	81.4 ± 1.8	84.6 ± 2.5	84.5 ± 3.8	87.7 ± 4.5	92.6 ± 4.3	90.1 ± 2.2	90.9 ± 3.2
Arabinose	-	-	-	-	91.1 ± 5.7	90.1 ± 4.3	94.2 ± 1.3	94.9 ± 5.0	92.4 ± 6.3	89.8 ± 5.4
Fructose	-	-	-	-	81.4 ± 3.7	80.7 ± 5.0	82.2 ± 3.3	85.2 ± 6.0	87.0 ± 4.1	89.1 ± 5.1
Sucrose	-	-	-	-	-	81.7 ± 3.8	84.9 ± 8.0	85.4 ± 7.8	87.0 ± 6.5	90.4 ± 6.2
Glucose+xylose mixture (1:1 wt/wt)	-	-	81.2 ± 4.0	84.7 ± 5.5	83.4 ± 2.1	81.3 ± 0.7	83.1 ± 3.6	84.5 ± 7.0	88.3 ± 7.6	94.9 ± 3.1

B. Wang *et al.* after studying the formation of ACN–water two-phase systems at different sugar concentrations, they investigated the distribution of value added chemicals such as furfural, 5-hydroxymethyl furfural (HMF), ferulic acid, syringic acid, and *para*-coumaric acid in the two phases formed by *sugaring-out*. Once a two-phase system was formed, the syringic acid or furfural was drawn into the upper ACN phase. As the concentration of sugar increased, more syringic acid or furfural was extracted into the upper phase. They further studied the separation of *para*-coumaric acid, ferulic acid, and HMF from an ACN–water two-phase system formed by addition of glucose, xylose, or glucose + xylose. The ACN–water two-phase system formed using glucose as an MSA was the most effective in the extraction of the three organic compounds from sugar solutions. It was thus determined that glucose was a better mass separating agent relative to other monosaccharides and disaccharides.

Dhamole *et al.*<sup>46</sup> studied phase separation conditions which were favorable for the potential applications of this method for the extraction of high value products such as biomolecules (proteins and antibiotics). They showed that *sugaring-out* uses sugars which do not react with the components of the system, do not change the environment conditions (e.g., pH) and can occur at higher temperature (> 277 K). Thus, the application of sugar and relatively high temperature (> 277 K) for phase separation makes this method better than existing methods such as salting out techniques. They further extended their study for potential application for separation of acetonitrile from the proteins and antibiotics samples after a preparative RP-HPLC was explored in this study.<sup>47</sup> Reverse phase-high pressure liquid chromatography (RP-HPLC) with an acetonitrile–water mixture as the eluent is widely used for purification of proteins. The separation of acetonitrile (ACN) in RP-HPLC

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<sup>46</sup> Dhamole, P. B., Mahajan, P., Feng, H. Phase separation conditions for sugaring out in acetonitrile-water systems. *J.Chem .Eng .Data.* **2010**, 55, 3803-3806.

<sup>47</sup> Dhamole, P. B., Mahajan, P., Feng, H. Sugaring out: A new method for removal of acetonitrile from preparative RP-HPLC eluent for protein purification *Process Biochemistry* **2010**, 45,1672-1676.

eluent is important for protein recovery. The effect of glucose amount, temperature, and initial concentration of ACN on the phase separation behavior, as can be seen from the phase ratio, was investigated. Addition of glucose into an ACN-water mixture resulted into phase separation at relatively low temperature (6-18 °C). Their conclusions were as follows:

1. Phase ratio (*volume of upper phase / volume of lower phase*) and ACN extraction increased with an increase in glucose concentration (105-165 g/L) and with a decrease in temperature (from 18 °C to 6 °C).
2. More than 95 % (wt/wt) proteins were retained in the aqueous phase after sugaring-out separation of ACN.
3. Hydrophobic erythromycin (65 % wt/wt) was extracted in the ACN-rich phase and 80-90 % (wt/wt) streptomycin and 91-94 % (wt/wt) nalidixic acid were retained in the aqueous phase.

Later in 2010, Tsai *et al.*<sup>48</sup> applied *sugaring-out* assisted liquid-liquid extraction method combined with high performance liquid chromatography with fluorescence detection (HPLC-FL) for the extraction and determination of sulfonamides in honey. Their results concluded that under the sugar/water/ACN system, ACN could be used as an acceptor phase to extract both the parent sulfonamides and the fluorescamine-derivatized sulfonamides. Using sulfonamides as a model, the sugaring-out assisted liquid-liquid extraction procedure could be applied to the determination of sulfonamides at trace levels in honey by HPLC-FL. The proposed method is simple, inexpensive and highly sensitive with a low limit of detection.

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<sup>48</sup> Tsai, W.; Chuang, H.; Chen, H.; Wu, Y.; Cheng, S.; Huang, T; Application of sugaring-out extraction for the determination of sulfonamides in honey by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A.* **2010**, 1217, 7812–7815.

As a following paper by Zhang, C *et al* in 2011 on use of salt such as NaCl in extraction of PGM (platinum group metals),<sup>46</sup> Zhang, C *et al.*<sup>49</sup> proposed a novel *sugaring-out* assisted three-liquid-phase system (TLPS) of diisopentyl sulfide-acetonitrile-glucose-water to extract and separate platinum, palladium and rhodium from their hydrochloric acid solution. Experimental results indicated Pd(II), Pt(IV) and Rh (III) respectively, could be concentrated and separated into the diisopentyl sulfide organic top phase, sugaring-out acetonitrile- rich middle phase and aqueous bottom phase of the TLPS. Compared with the traditional salting-out phase separation process, sugaring-out assisted three-liquid-phase system had many advantages, especially the sugar concentration required for phase separation was much less than that for salt. Therefore, the use of sugar avoided the problems of equipment corrosion and fouling caused by salt.

In 2013, Zhang, J *et al.*<sup>50</sup> studied *sugaring-out* assisted liquid/liquid extraction with water miscible acetonitrile and successfully demonstrated in the determination of eight compounds in human plasma. The method was used to validate two antiviral drugs for lopinavir and ritonavir in human then compared with a previously validated method using *salting-out* assisted liquid/liquid extraction with acetonitrile.

### 1.8.3 Ionic liquids as mass separating agents:

Ionic liquids have attracted significant attention recently as potential azeotrope breakers for a wide range of systems. They are essentially salts which are liquid at or near room temperature and are typically made up of organic cations and inorganic anions, thereby combining some of the properties of organic entrainers with those of inorganic salts. The advantages of ionic liquids include

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<sup>49</sup> Zhang, C.; Huang, K.; Yu, P.; Liu, H. Sugaring-out three-liquid-phase extraction and one-step separation of Pt(IV), Pd(II) and Rh(III) *Separation and Purification Technology* **2012**, 87, 127-134.

<sup>50</sup> Zhang, J; Myasein, F; Wu, H; El-Shourbagy, T A, Sugaring-out assisted liquid/liquid extraction with acetonitrile for bioanalysis using liquid chromatography–mass spectrometry, *Microchemical Journal* **2013**, 108, 198-202.

their negligible vapor pressures,<sup>51</sup> making their separation and recycling relatively straightforward, based on the fact that their physical and thermodynamic properties can be tuned by varying the cation-anion combination and hydrocarbon chain lengths. It has been estimated that there are over 106 possible ionic liquids, each with different physicochemical properties.<sup>52</sup> Therefore, with the emergence of appropriate computational screening technologies, the potential exists to optimally design ionic liquids as azeotrope breakers for a given separations problem. The lack of extensive toxicity data for novel ionic liquids is a major barrier to their direct use in pharmaceutical processing in the short to medium term but they have potential to be used as azeotrope breakers where the ACN is to be recycled externally to non-cGMP processes. A detailed review of the application of ionic liquids as azeotrope breakers has been recently published by Pereiro *et al.*<sup>53</sup> Here the authors cite references for the use of ionic liquids as azeotrope breakers in a range of water + alcohol, water + THF, alcohol + ester, alcohol + ketone, alcohol + hydrocarbon, alcohol + halogen and aromatic + aliphatic systems, whilst also offering a comparison with published data regarding more conventional entrainers. Some major disadvantages of ionic liquids include high cost and environmental toxicity<sup>54</sup> associated with them.

Based on the use of ionic liquid induced separation of ACN-water system, Okana *et al.*<sup>55</sup> reported that phase separation of water/ACN mixture to a biphasic system can be induced by the presence of an acidic ionic liquid such as 1-methyl-3-(butyl-4-chlorosulfonyl) imidazolium chlorosulfate ([MBCIm]SO<sub>3</sub>Cl), which allows to produce *pseudo* ternary phase of ACN-water-[MBCIm]SO<sub>3</sub>Cl system at room temperature. This system was established for catalytic dehydration

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<sup>51</sup> Fredlake, C. P.; Crosthwaite, J. M.; Hert, D. G.; Aki, S. N. V. K.; Brennecke, J. F. *J. Chem. Eng. Data.* **2004**, 49, 954.

<sup>52</sup> Luis, P.; Ortiz, I.; Aldaco, R.; Irabien, A. *Ecotoxicol. Environ. Saf.* **2007**, 67, 423.

<sup>53</sup> Periero, A. B.; Araujo, J. M. M.; Esperanca, I. M.; Marrucho, I. M.; Rebelo, L. P. N. Ionic liquids in separations of azeotropic systems - A review *J. Chem. Thermodynamics* **2012**, 46, 2–28.

<sup>54</sup> Crombie, Janet. Ionic liquids toxic to fish. “*Chemical Science.*” October **2005**.

<sup>55</sup> Okano, T.; Qiao, K.; Bao, Q.; Tomida, D.; Hagiwara, H.; Yokoyama, C; Dehydration of fructose to 5-hydroxymethylfurfural (HMF) in an aqueous acetonitrile biphasic system in the presence of acidic ionic liquids, *Applied Catalysis A: General* **2013**, 451, 1-5.

of fructose to 5-hydroxymethylfurfural (HMF) in the presence of [MBCIm]SO<sub>3</sub>Cl at low temperature in a shorter period of time.

#### 1.8.4 Carbon dioxide (CO<sub>2</sub>) as a mass separating agent:

In 1950s it was first discovered that a homogeneous aqueous solutions of alcohols or other polar solvents can be split into two liquid phases by pressurized gases<sup>56,57</sup> where CO<sub>2</sub> was most popular and widely used<sup>58</sup> as a mass separating agent. Adrian *et al.* have extensively studied this technique for various applications like extraction of biomolecules<sup>59</sup> and various natural product recovery.<sup>60</sup> Separation of ACN –water mixture induced by CO<sub>2</sub> have recently found an application as Organic-Aqueous Tunable Solvents (OATS) process. OATS consist of miscible mixtures of an aprotic organic solvent (some examples are 1,4-dioxane, acetonitrile, or tetrahydrofuran) and a polar protic solvent (water) tunable solvent mixtures<sup>61</sup> used as a homogeneous reaction media. The mixture is chosen such that upon the addition of an antisolvent gas, e.g., CO<sub>2</sub>, a phase split occurs yielding a biphasic liquid-liquid system which takes place because CO<sub>2</sub> is completely soluble in most organics but only slightly soluble in aqueous media. Ye *et al.*<sup>62</sup> developed a novel process to separate azeotropic mixtures by tuning the phase behavior using pressurized CO<sub>2</sub> as a tunable

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<sup>56</sup> Baker, L. C. W.; Anderson, T. F. Some phase relationships in the 3-component liquid system CO<sub>2</sub>-H<sub>2</sub>O-C<sub>2</sub>H<sub>5</sub>OH at high pressures. *J. Am. Chem. Soc.* **1957**, 79, 2071–2074.

<sup>57</sup> Elgin, J. C.; Weinstock, J. J. Phase equilibrium at elevated pressures in ternary systems of ethylene and water with organic liquids. Salting out with a supercritical gas. *J. Chem. Eng. Data.* **1959**, 4, 3-12.

<sup>58</sup> Adrian, T.; Wendland, M.; Hasse, H.; Maurer, G. High-pressure multiphase behaviour of ternary systems carbon dioxide water polar solvent: Review and modeling with the Peng-Robinson equation of state. *J. Supercrit. Fluids* **1998**, 12, 185–221.

<sup>59</sup> Adrian, T.; Freitag, J.; Maurer, G. High pressure multiphase equilibria in aqueous systems of carbon dioxide, a hydrophilic organic solvent and biomolecules. *Fluid Phase Equilib.* **1999**, 160, 685–693.

<sup>60</sup> Adrian, T.; Freitag, J.; Maurer, G. A new high-pressure liquid-liquid extraction process for natural products recovery. *Chem. Eng. Technol.* **2000**, 23, 857–860.

<sup>61</sup> Blasucci, V.M.; Husain, Z.A.; Fadhel, A.Z.; Donaldson, M.E.; Vyhmeister, E.; Pollet, P.; Liotta, C.L.; Eckert, C.A. Combining Homogeneous Catalysis with Heterogeneous Separation Using Tunable Solvent Systems. *J. Phys. Chem. A.* **2010**, 114, 3932–3938.

<sup>62</sup> Ye, K.; Freund, H.; Sundmacher, K. A New Process for Azeotropic Mixture Separation by Phase Behavior Tuning Using Pressurized Carbon Dioxide. *Ind. Eng. Chem. Res.* **2013**, 52, 15154–15164

solvent and then validated the fundamental idea to separate azeotropic mixtures of ACN-water system.

### 1.8.5 Urea as a mass separating agent:

The ternary system of ACN-water-urea was reported by Francis *et al.*<sup>63</sup> as a part of comprehensive study on a ternary system with acetonitrile. However the first phase separation behavior was reported by Loeser *et al.*<sup>64</sup> in 2011. This phase separation behavior was only observed in higher ACN range of 77 to 86 % v/v of ACN-water mixture. Their study reported that the mixtures divided into two liquid phases. The more dense layer contained a roughly 2:2:1 mole ratio of water/ACN/urea, while the less dense layer was primarily ACN with small amounts of water and urea. Within the concentration range of 77 to 86 % (v/v) of ACN-water, the volume of upper and lower layers showed slight change but the composition remained essentially constant. Thus they hypothesized that the water-rich phase is saturated in both urea and ACN and the driving force for the phase separation is explained as the tendency for urea to promote self-association of the individual water and ACN components.

## 1.9 Basic thermodynamic concept:

To interpret and correlate the nature of intermolecular forces it is necessary to have some understanding of the thermodynamic properties of solutions. Similarly, thermodynamic properties of a mixture depend on intermolecular forces that operate between the molecules of the mixture. The case of a mixture, however, is necessarily more complicated because consideration must be given

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<sup>63</sup> Francis, A. W. Ternary Systems of Acetonitrile. *J. Chem. Eng. Data* **1965**, 10, 145–151.

<sup>64</sup> Loeser, E.; Delacruz, M.; Madappalli, V. Solubility of urea in acetonitrile-water mixtures and liquid liquid phase separation of urea-saturated acetonitrile-water mixtures. *J. Chem. Eng. Data*. **2011**, 56, 2909–2913

not only to interaction between molecules belonging to the same component, but also to interaction between dissimilar molecules.

### *Equilibrium and Free Energy*

Underlying all molecular interactions, and in fact all aspects of organic and inorganic behavior, are the principles of thermodynamics.

Consider the association of two molecules, **A** and **B**, which combine to form a non-covalent complex **A•B**. The complex will exist in equilibrium with the individual molecules:



Their affinity for one another can be measured by the equilibrium constant for the association,  $K_a$ , also called the **association constant**,

$$K_a = \frac{[A \cdot B]}{[A][B]}$$

The size of that equilibrium constant is a measure of the affinity of two molecules for one another. At a given temperature, the size of the association constant is solely dependent upon the difference in free energy ( $\Delta G$ ) between the complex and the separated molecules

$$K_a = e^{-\Delta G^\circ/RT}$$

Where  $R$  is the ideal gas constant  $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ,  $T$  is the temperature in units of Kelvins and  $\Delta G^\circ$  is defined as  $\Delta G \text{ products} - \Delta G \text{ reactants}$  where all compounds are at standard state (1 M for solutes). Thus, for two molecules to combine with a high association constant, the free energy of the product must be low in comparison to the free energy of the reactants.

The Gibb's free energy equation shows that the change in free energy can be related to the difference in two more fundamental thermodynamic parameters

$$\Delta G = \Delta H - T\Delta S$$

Where  $\Delta H$  is the change in the enthalpy associated with a process and  $\Delta S$  is the change in entropy.

*The van't Hoff Equation:*

The van't Hoff equation provides information about the temperature dependence of the equilibrium constant. The van't Hoff equation may be derived from the Gibbs-Helmholtz equation, which gives the temperature dependence of the Gibbs free energy. For the standard Gibbs free energy, the Gibbs-Helmholtz equation is

$$\left[ \frac{\delta(\Delta G^\circ / T)}{\delta(1/T)} \right]_p = \Delta H^\circ$$

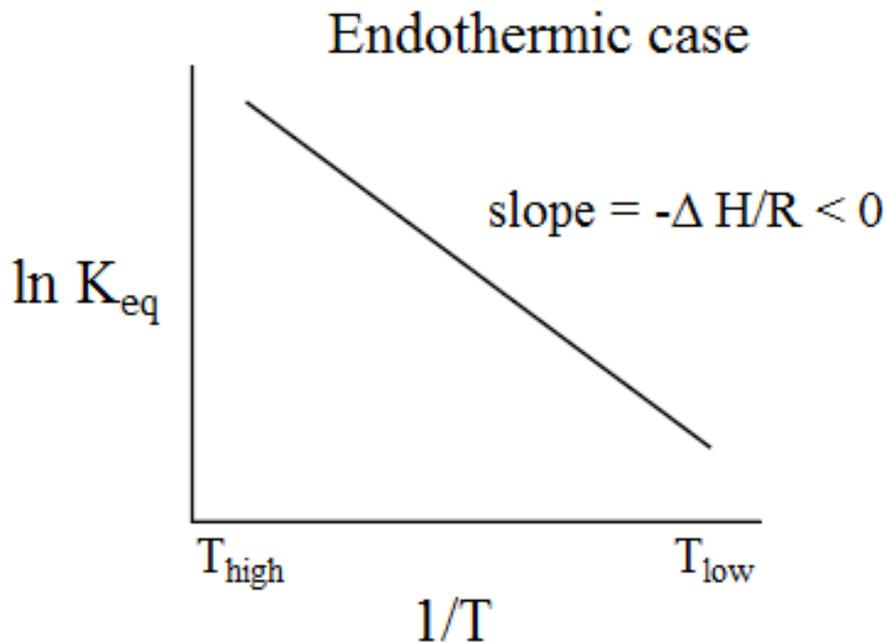
Substituting the expression  $\Delta G^\circ = -RT \ln K_{eq}$  and dividing both sides by  $-R$ , the first form of the van't Hoff equation is obtained,

$$\left[ \frac{\delta \ln K_{eq}}{\delta(1/T)} \right]_p = - \frac{\Delta H^\circ}{R}$$

From this form of the van't Hoff equation, we see that at constant pressure, a plot with  $\ln K_{eq}$  on the y-axis and  $1/T$  on the x-axis has a slope given by  $-\Delta H/R$ . This is the origin of Le Chatelier's Principle for the heat absorbed or evolved during the course of a chemical reaction.

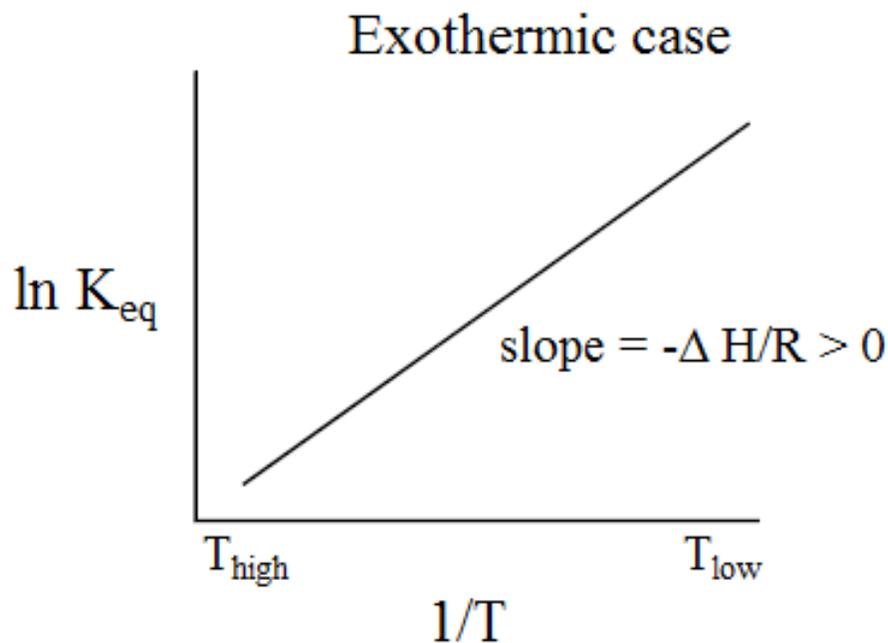
For an endothermic reaction, the slope is negative and so as the temperature increases, the equilibrium constant increases, as illustrated in Figure 11

**Figure 13: Typical van't Hoff plot for an endothermic reaction.**



For an exothermic reaction, the slope is positive and so as temperature increases, the equilibrium constant decreases, as illustrated in Figure 12

**Figure 14: Typical van't Hoff plot for an exothermic reaction.**



Finally, an additional linear form of the van't Hoff equation may be obtained starting from the relation

$$\Delta G^{\circ} = - RT \ln K_{\text{eq}}$$

Solving for the natural log,

$$\ln K_{\text{eq}} = - \frac{\Delta G^{\circ}}{RT}$$

At any given temperature, the change in Gibbs free energy may be written from the definition of the Gibbs free energy as

$$\Delta G^{\circ} = \Delta H - T\Delta S^{\circ}$$

Finally the linear form of the van't Hoff becomes,

$$\ln K_{\text{eq}} = - \frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R}$$

This equation indicates that a plot with  $\ln K_{\text{eq}}$  on the y-axis and  $1/T$  on the x-axis has a slope given by  $-\Delta H/R$  and an intercept given by  $\Delta S/R$ , Thus using van't Hoff's equation one can extract  $\Delta H$ ,  $\Delta S$  and  $\Delta G$  values.

**Table 3: List of patents on ACN purification/ recovery/ dehydration**

<b><u>Patent No</u></b>	<b><u>Filing date</u></b>	<b><u>Publication date</u></b>	<b><u>Applicant</u></b>	<b><u>Title</u></b>
US2417862	Dec 01, 1943	Mar 25, 1947	US Vanadium Corp	Dehydration of acetonitrile by decantation and distillation
US2560931	Feb 26, 1946	Jul 17, 1951	Socony Vacuum Oil Co Inc	Dehydration of acetonitrile
US3203974	Sep 05, 1962	Aug 31, 1965	Universal Oil Prod Co	Purification of acetonitrile and propionitrile
US3328458	Sep 10, 1964	Jun 27, 1967	Universal Oil Prod Co	Purification of nitriles
US3451899	Dec 21, 1965	Jun 24, 1969	Badger Co	Purification of acetonitrile by an azeotropic distillation method
US4105688	Apr 26, 1971	Aug 08, 1978	Lonza, Ltd.	Process for the production of malonic acid dinitrile and purification thereof
US3870746	Jan 14, 1972	Mar 11, 1975	Degussa	Process for recovery of pure acetonitrile
US4119497	Oct 12, 1976	Oct 10, 1978	Instituto Mexicano Del Petroleo	Process for the purification of acetonitrile
US4269667	Aug 02, 1979	May 26, 1981	Standard Oil Company	Acrylonitrile purification by extractive distillation
US4308108	Mar 19, 1980	Dec 29, 1981	Asahi Kasei Kogyo Kabushiki Kaisha	Process for purification of crude acetonitrile

<u>Patent No</u>	<u>Filing date</u>	<u>Publication date</u>	<u>Applicant</u>	<u>Title</u>
US4287134	Jun 26, 1980	Sep 1, 1981	E. I. Du Pont De Nemours And Company	Purification of acetonitrile by caustic extraction
US4362603	Jan 02, 1981	Dec 7, 1982	The Standard Oil Company	Continuous acetonitrile recovery process
EP0113331A 1	Dec 28, 1983	Jul 11, 1984	Monsanto Company	Method for the purification of acetonitrile by low temperature phase separation
US5292919	May 21, 1993	Mar 8, 1994	Cryodyne Technologies, Inc.	Method for purification of acetonitrile
US5426208	Nov 19, 1993	Jun 20, 1995	Cryodyne Technologies, Inc.	Method of purification of acetonitrile
US5440068	Dec 30, 1993	Aug 8, 1995	The Standard Oil Company	Acetonitrile purification via an adsorption-based process
US5629443	Jan 11, 1995	May 13, 1997	Asahi Kasei Kogyo Kabushiki Kaisha	Highly purified acetonitrile and process for purifying crude acetonitrile
EP0890572A 1	Jul 08, 1997	13 Jan 1999	The Standard Oil Company	Purification of acetonitrile by a distillative recovery/ion exchange resin treatment process
EP0937707A 2	Feb 02, 1999	25 Aug 1999	The Standard Oil Company	Improved purification and recovery of acetonitrile

<b><u>Patent No</u></b>	<b><u>Filing date</u></b>	<b><u>Publication date</u></b>	<b><u>Applicant</u></b>	<b><u>Title</u></b>
US6843890	Jul 23, 1999	Jan 18, 2005	The Standard Oil Company	Purification and recovery of acetonitrile from waste solvent acetonitrile
US6843890	Jul 23, 1999	Jan 18, 2005	The Standard Oil Company	Purification and recovery of acetonitrile from waste solvent acetonitrile
US6395142	Oct 14, 1999	May 28, 2002	Chemcycles, Inc.	Method and apparatus for purifying low grade acetonitrile and other constituents from hazardous waste
US6326508	Sep 05, 2000	Dec 4, 2001	The Standard Oil Company	Purification and recovery of acetonitrile
US6780289	Jul 16, 2001	Aug 24, 2004	The Standard Oil Company	Process for the purification and recovery of acetonitrile
US6508917	May 24, 2002	Jan 21, 2003	Chemcycles, Inc.	Method and apparatus for purifying low grade acetonitrile and other constituents from hazardous waste
US20080073 201	Oct 28, 2004	Mar 27, 2008	Ucb, S.A.	Acetonitrile Recycling Process

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<b><u>Patent No</u></b>	<b><u>Filing date</u></b>	<b><u>Publication date</u></b>	<b><u>Applicant</u></b>	<b><u>Title</u></b>
US20090090894 A1	Oct 05, 2007	Apr 9, 2009	Bin Wang, Hao Feng, Thaddeus Ezeji, Hans Blaschek	Separation and extraction system
US8609879	Jul 16, 2010	Dec 17, 2013	Basf Se, The Dow Chemical Company	Method for the separation of acetonitrile from water

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## CHAPTER 2:

# POLYOL-INDUCED EXTRACTION (PIE) OF WATER FROM ORGANIC LIQUIDS

*Preface:* Below, I present a chapter incorporating Patent which was filed based on research work done under guidance of Dr Sowa and Dr Murphy.<sup>65</sup> The chapter covers method of separating water from organic solvent with special emphasis on acetonitrile. It also includes list of possible polyols and solvents involved and various experiments thereof to exemplify the research work.

*I highly appreciate Seton Hall University's contribution to fund patent related expenses.*

## **2.1 Background of invention:**

There is an extensive body of literature on the purification of organic compounds especially in the practice of the removal of water from organic liquids.<sup>66</sup> Conventional processes include distillation, use of dehydrating agents, and recrystallization, which are used alone or in combination. However, distillation is ineffective for organic liquids that form lower boiling azeotropes. Azeotropic distillation results in the formation of byproducts that are used to break the azeotrope. For example, benzene and toluene are used for the azeotropic distillation of ethanol resulting in hazardous waste mixtures of benzene/water and toluene/water requiring further treatment. Dehydrating agents such as alumina, magnesium sulfate, molecular sieves and sodium sulfate are also established methods for removal of water from organic liquids and from solutions of solutes in organic solvents.

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<sup>65</sup> Sowa, J. R., Jr.; Murphy, W.R.; **Deshpande, M.** *Polyol induced extraction of water from organic solvent.* U.S. Patent Application 14/209,228, filed March 2013.

<sup>66</sup> (a) Williams *et. al.*, *J. Org. Chem.* **2010**, 75,:8351-8354.; (b) Maslan *et. al.*, *J. Phys. Chem.* 1956, 60(8):1146-1147

For example, acetonitrile is a polar solvent with a high dielectric constant that has a strong affinity for polar compounds, such as organometallic complexes, metal chelates, ion pairs and organic solutes. Acetonitrile is miscible with water in all proportions. Acetonitrile is an excellent organic solvent and has become widely used in organic synthesis and manufacturing, as well as for purity and quality control analysis of organic compounds used in pharmaceuticals, cosmetics, personal care products and many other applications in research and development. In the latter aspects, the miscibility of acetonitrile in water renders it one of the most useful solvents for reverse phase high pressure liquid chromatography (RP-HPLC). Several events have resulted in a shortage in acetonitrile causing prices of this solvent to rise dramatically.<sup>67</sup> Thus, there is unprecedented interest in the recovery of acetonitrile from research and manufacturing and RP-HPLC waste streams.<sup>68</sup> However, like many other organic solvents, acetonitrile forms an azeotrope with water, which as persons skilled in the art appreciate, is a liquid mixture the individual components of which cannot be separated from each other by distillation. According to the literature, since the azeotrope boils at 76 °C and is composed of 86% acetonitrile and 14% water, there is no possibility of obtaining acetonitrile of purity above 86% by distillation and more costly processes are required to purify acetonitrile to a level higher than the azeotrope.

U.S. Patent Application Publication 2009/0090894, to Wang *et al.*, teaches a method for separating acetonitrile from water that utilizes a saccharide (monosaccharide or disaccharide), and that the acetonitrile in the so-called "sugared-out" upper phase can reach a purity of 95.4%.

Notwithstanding a greater need for recycling, concern persists that unimpeded use of our natural resources is wasting essential resources and contributing toward global warming which if unchecked, will dramatically impact daily life. Emergent initiatives known as "sustainable

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<sup>67</sup> (a)Majors, *et al.*, *LC-GC North America* **2009**, 27, 458-471.; (b) McConvey *et al.*, *Org. Proc. Res. Dev* **2012**, 16, 612-624.; (c)Venkatasami, Sowa, *Anal. Chim. Acta.***2010**, 665,:227-230

<sup>68</sup> Yesong *et al.*, *Enzyme and Microbial Technology*, 35:592-597 (2004).

manufacturing", "sustainability", "green manufacturing", "green technology" and "green chemistry" are designed to encourage better and more economical use of natural resources and to minimize any negative impact on the environment. They also aim towards making manufacturing process more economical, reducing costs of disposal through recycling and providing "green" products. In the area of chemical manufacturing there is a great need to improve research and manufacturing processes by developing new sustainable, green chemical processes.<sup>69</sup>

## **2.2 Brief summary of invention:**

The present invention is directed to a method of separating water from a composition containing an organic liquid and water, which entails adding to the composition a polyol that causes separation of the composition into at least two phases, wherein the first phase is aqueous and the second phase is organic (*e.g.*, greater than about 80 % pure organic liquid).

The methods of the present invention are advantageous in several respects. The extraction agents, *i.e.*, the polyols, are (with rare exception) non-toxic, freely available, inexpensive, easy to use and reusable. More specifically, the "green" properties of polyols are exploited. With the arguable exception of ethylene glycol, they are non-toxic as indicated by their LD<sub>50</sub> values (ethylene glycol (8.5 g/kg), propylene glycol (25 g/kg), glycerol (greater than 20 g/kg), xylitol (22 g/kg), sorbitol (16 g/kg)). In addition, they are biodegradable and recyclable. Further, the high boiling points of polyols (*e.g.*, ethylene glycol is 196-198 °C; propylene glycol is 187 °C and glycerol is 180 °C at 20 mmHg) facilitate their recovery by distillation of the more volatile components that include residual organic liquid and water. Polyols which are solid at room temperature may be also recovered by distillation of the more volatile components and recrystallization.

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<sup>69</sup> Anastas, Warner "Green Chemistry Theory and Practice", Oxford, 2000

As shown in the working examples, the present invention is successful in extracting water from various organic liquids such as acetonitrile to achieve purity levels that reach about 98% or greater and the polyol is recovered in greater than about 95% yield. Broadly, the present invention may be used to separate any organic liquid from water, provided that upon contact with the polyol, a phase separation occurs. Without intending to be bound by any particular theory of operation, the present inventors believe that phase separation occurs via a competition between the organic liquid and the polyol for water. When the strength (as indicated by enthalpy or free energy) of the interaction between the polyol and water is greater than that of the organic liquid and water, phase separation occurs resulting in an aqueous phase and an organic phase. The aqueous phase contains water and polyol and has lesser amounts of the organic liquid than the amount originally present in the composition. The organic phase typically contains substantially the organic liquid which for purposes of the present invention means that the organic phase typically contains relatively smaller amounts of water, *e.g.*, less than about 20%, 15%, 10%, 5% or less

Water is frequently difficult to remove from organic liquids. The present method provides a practical solution in that the water content in the original composition is substantially reduced on addition of the polyol to obtain an organic liquid of suitable purity to enable it to be recycled. The polyol can be also recovered for re-use. Moreover, the invention may also be viewed as a practical way to purify water when it is present in admixture with an organic liquid such that the water can be subjected to further treatment for re-use or for environmentally responsible disposal.

Formally the phase separation process is an equilibrium process where the Gibbs free energy equation applies (*e.g.*, 1):

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} \quad (1)$$

Where,  $\Delta$  is the observed change from the initial state to the state at equilibrium,  $G^{\circ}$  is defined as the free energy at standard temperature and pressure (STP),  $H^{\circ}$  is the enthalpy at STP,  $T$  is temperature given in Kelvin, and  $S^{\circ}$  is entropy at STP.

The phase separation reaction is given by the following reaction equation (eq 2):



Where, Org is the organic liquid, P is the polyol, (l) indicates a pure liquid phase or, if appropriate (s) in the case of a pure solid phase, (aq) indicates aqueous solution, respectively, of the organic liquid or polyol.

Thus, the equilibrium constant for phase separation ( $K_{PS}$ ) is given by eq 3:

$$K_{PS} = \frac{[\text{Org}_{(l)}] [\text{P}_{(aq)}]}{[\text{Org}_{(aq)}] [\text{P}_{(l)}]} \quad (3)$$

Where, for example, the brackets  $[\text{Org}_{(aq)}]$  indicate concentration of the organic liquid; however, concentration terms are often expressed as activity,<sup>70</sup> *i.e.*,  ${}^a\text{Org}_{(aq)}$ . By definition, a pure liquid or solid has an activity of 1. Thus, eq 3 reduces to eq 4.

$$K_{PS} = \frac{[\text{P}_{(aq)}]}{[\text{Org}_{(aq)}]} \quad (4)$$

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<sup>70</sup> Moore, "Physical Chemistry, 3rd ed.," Prentice Hall, 1963, p. 349

As indicated by the Gibbs equation (eq 5), an increase in  $K_{PS}$  leads to a decrease in value of  $\Delta G^{\circ}$  which is interpreted as a decrease in free energy. Thus, phase separation is driven by a decrease in free energy of the reaction.

$$\Delta G^{\circ} = -RT \ln K_{PS} \quad (5)$$

where,  $R$  is the gas constant ( $8.314 \text{ J/T}^* \text{ mole}$ );  $J$  is Joules and  $T$  is temperature in Kelvin ( $K$ )

Persons skilled in the art may determine whether a given polyol will cause phase separation in a composition containing water and a given organic liquid by employing Le Chatelier's Principle<sup>71</sup> to drive the equilibrium in eq 2 toward the products ("to the right"). According to Le Chatelier's Principle, factors that control this equilibrium process are: a) free energy of the interaction of water with the organic liquid and water with the polyol (which can be roughly estimated by one skilled in the art by solubility), concentration and temperature. If the solubility of the organic liquid in water is low, the reaction (eq 2) will be driven to the right. If the solubility of the polyol in water is high, the reaction will be driven to the right. Furthermore, if the solubility of the polyol in the organic liquid is low, the reaction will also be driven to the right. An increase in concentration of the organic liquid and polyol relative to water will drive the reaction to the right. A decrease in temperature will decrease the free energy of interaction between water and the organic liquid and, thus, increase phase separation.

Broadly, the organic liquids from which water can be extracted are those in which water is at least partially soluble and which form a single homogeneous phase when mixed with water. Suitable organic liquids include industrial solvents which, because of their expense and environmental toxicity, are candidates for recycling. The organic liquids also include solvents, reagents and intended or unintended byproducts, used or generated in research and development. The organic

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<sup>71</sup> Umland, Bellama, "General Chemistry, 3rd ed.," Brooks/Cole, 1999

liquids further include industrial products such as oil, petroleum, petroleum distillates, and fuels such as gasoline, kerosene, diesel and biodiesel.

Organic liquids suitable for use in the present invention can be categorized into three basic classes: non-polar ("np"), polar aprotic ("pap," which as known in the art, do not have a labile proton) and polar protic ("pp," which as known in the art, have a labile proton that can be donated to a reagent).<sup>72</sup> The miscibility of the organic liquid in water is not critical provided that a phase separation occurs, the present method can separate organic liquids that are only slightly miscible with water to those that are completely miscible with water.

Representative examples of non-polar organic liquids that may be suitable for use in the present invention include hexanes, cyclohexane, carbon tetrachloride, emulsified crude oil, crude oil, gasoline, kerosene, petroleum, petroleum distillates, biodiesel, and diesel fuel, all of which have slight miscibility with water. For example, the organic liquid, hexanes, is a mixture of isomers having the formula  $C_6H_{14}$ . Hexanes are miscible with water in very small proportions (1.1 g/100 mL).<sup>73</sup> It is frequently necessary for purposes of recycling and for practical use as an anhydrous solvent and for chemical reactions including combustion to remove even traces of water that are present in these liquids.

Representative examples of polar aprotic organic liquids that may be suitable for use in the present invention include dibutylphthalate, dimethylphthalate, t-butylmethylether, mesitylene, toluene, benzene, p-xylene, diethyl ether, chloroform, ethyl acetate, propylene carbonate, 1,2-dimethoxyethane, methyl benzoate, tetrahydrofuran, 2-methyltetrahydrofuran, halogenated alkanes such as dichloromethane, cyclopentanone, 2-butanone (methylethylketone), nitromethane, acetone and cyanated alkanes such as acetonitrile and propionitrile. Acetonitrile is an excellent

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<sup>72</sup> Reichardt, "Solvents and Solvent Effects in Organic Chemistry, 2nd ed.," VCH, 1990

<sup>73</sup> Riddick, Bunger, "Organic Solvents, Physical Properties and Methods of Purification, 3<sup>rd</sup> ed., Vol. II," Wiley, 1970, p. 78-79

example of a polar aprotic organic liquid that is completely miscible with water. Acetone, dioxane, and tetrahydrofuran are also completely miscible with water. Examples of polar aprotic organic liquids that are only partially miscible with water include dichloromethane and chloroform (in which only traces of water dissolve). Water dissolves to a greater extent (*e.g.*, greater than 1 to 10 % wt/v) in organic liquids such as diethyl ether, ethyl acetate, benzene and toluene.

Representative examples of polar protic organic liquids that may be useful in the practice of the present invention include alkanols, *e.g.*, 1-octanol, cyclohexanol, isopentanol (3-methyl-1-butanol), 2-pentanol, 1-butanol, t-butanol, 1-propanol and isopropanol (2-propanol). Alkanols are a broad class of organic liquids that are pp and range from completely miscible with water to partially miscible. Those that are completely miscible include methanol, ethanol, 1-propanol, isopropanol (2-propanol), t-butanol. Those that are partially miscible include 1-butanol, 2-butanol, pentanol, cyclopentanol, cyclohexanol, hexanol, heptanol, and 1-octanol.

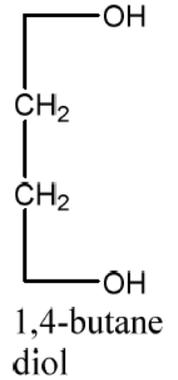
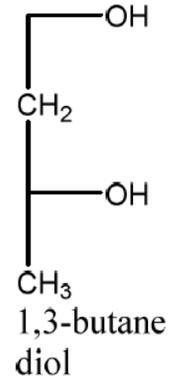
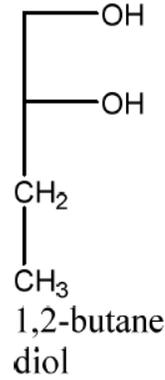
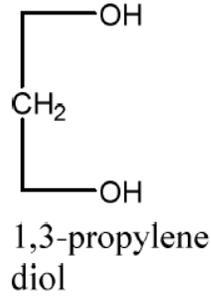
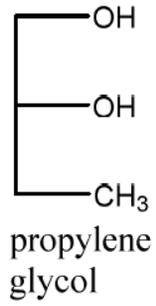
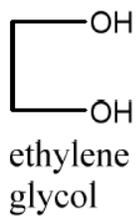
Polyols useful as extraction agents in the present invention may be described in terms of the general formula:



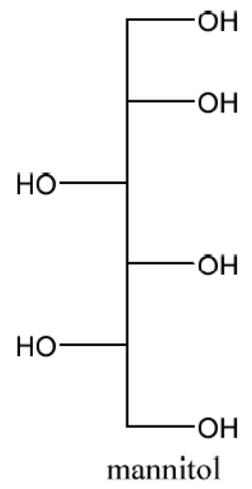
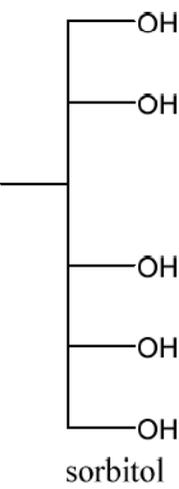
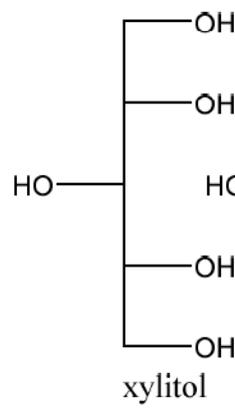
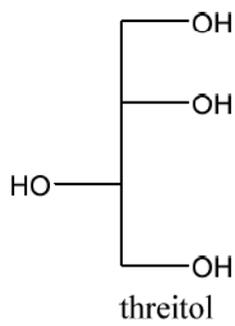
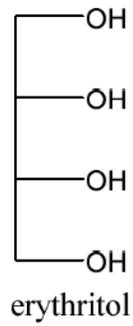
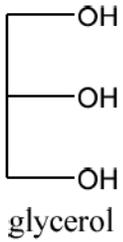
wherein  $R^1$  and  $R^2$  each independently represent hydrogen, a linear or branched  $C_1$ - $C_8$  alkyl (*e.g.*, methyl, ethyl) group or an aryl group (*e.g.*, phenyl and naphthyl), or  $R^1$  and  $R^2$  taken together represent a bond such that the polyol is cyclic; wherein x, y and z are each independently integers including 0 to 4, and wherein the sum of  $x+y+z$  is less than or equal to 4 (*i.e.*, 0, 1, 2, 3, or 4), provided that when  $R^1$  and  $R^2$  taken together represent a bond, the sum of x, y, and z is 4; and  $R^3$  represents hydrogen when y is 1, and when y is 2, 3 or 4, each  $R^3$  is hydrogen or one of the  $R^3$  groups is pyranosyl or polypyranosyl and the other one, two, or three  $R^3$  groups is hydrogen (*i.e.*, in some embodiments is only hydrogen and in some embodiments  $R^3$  is one pyranosyl or one

polypyranosyl group and the remaining R<sup>3</sup> groups are hydrogen). As persons skilled in the art appreciate, hydrogenation of disaccharides such as maltose and lactose produces polyols that are linked to one pyranosyl group via an internal or terminal hydroxyl group namely pyranosyl-substituted polyols. In addition, hydrogenated polysaccharides produce polyglycitols such as Maltotriitol, Maltotetraitol and hydrogenated starch hydroxylate (polyglucitol) namely polypyranosyl-substituted polyols. Moreover, persons skilled in the art appreciate that when R<sup>1</sup> and R<sup>2</sup> together represent a bond, the polyol is cyclic *e.g.*, an inositol. (*e.g.*, *myo*-inositol which is the predominantstereo-isomer) The pyranose-substituted polyols, polypyranosyl-substituted polyols, and inositols are considered polyols within scope of this invention. For purposes of the present invention, "polypyranosyl" refers to 2-4 pyranosyl groups linked together.

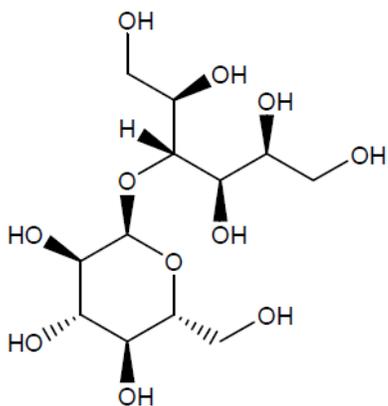
**Figure 15: C<sub>2</sub>-C<sub>4</sub> diols**



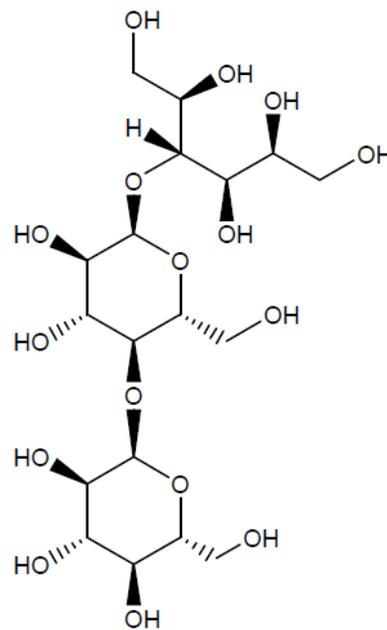
**Figure 16: C<sub>3</sub> triols to C<sub>6</sub> hexaols**



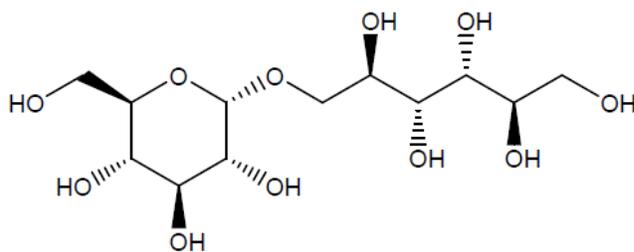
**Figure 17: Polyols having cyclic sugar (pyranosyl) groups**



Maltitol, 4-O-1-D-glucopyranosyl-D-glucitol  
or 4-O- $\alpha$ -glucopyranosyl-D-sorbitol

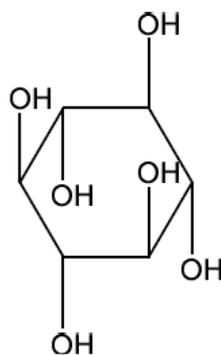


maltotriitol, O-1-D-glucopyranosyl-(1,4)-O-1-D-glucopyranosyl-(1,4)-D-glucitol



Isomalt, 1-O- $\alpha$ -D-glucopyranosyl-D-mannitol

**Figure 18: Cyclic polyol, inositol.**



myo-inositol

As shown in Figures 13-16, the polyol moieties in this formula are indicated as "CHOR<sup>3</sup>" groups when R<sup>3</sup> represents OH. These groups may be linked to each other in a continuous fashion from the initial carbon atom to the last carbon atom in the chain. The simplest polyol applicable to

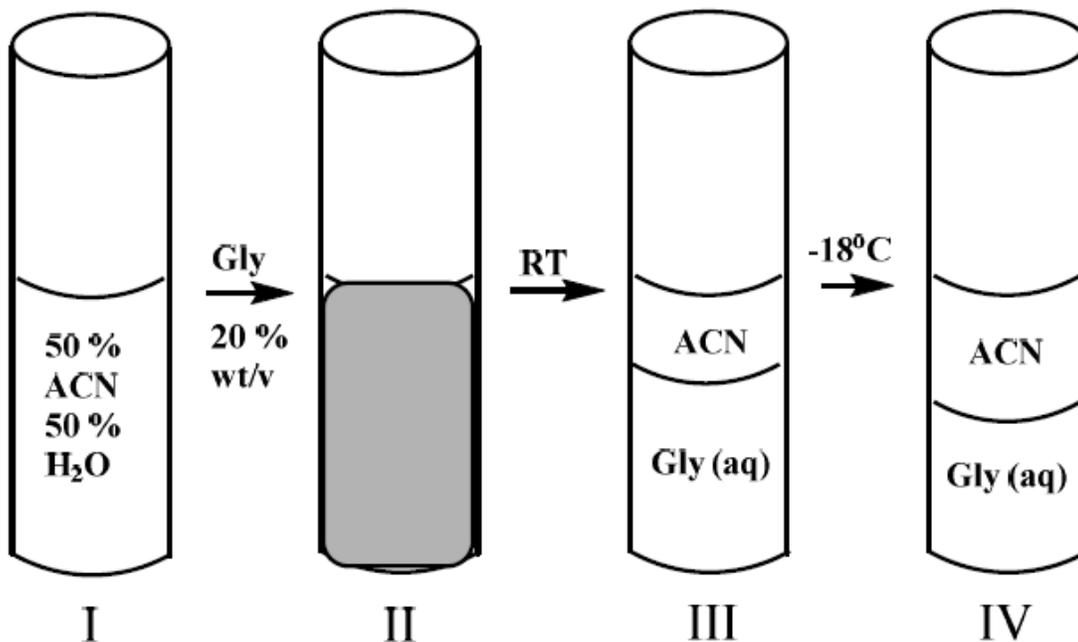
the general formula is ethylene glycol as shown in Figure 13 where  $R^1$  and  $R^2$  are H,  $R^3$  is OH, and subscripts x, y and z are 0. In addition, there may be an alkyl group ( $R^1$ ) at the beginning of the chain where, for example,  $R^1$  is methyl as in the case of propylene glycol (Figure 13). In the case of 2,3-butanediol (not shown),  $R^1$  is methyl,  $R^3$  is OH, x, y, and z are each zero, and  $R^2$  is methyl. Methylene  $(CH_2)_y$  groups may also be present between the "CHOH" moieties where subscript x indicates the number of methylene groups. In the case of 1,3-propanediol, x is 1 (Figure 13). In the case, of 1,4-butanediol, x is 2 (Figure 13).

Glycerol (Figure 14) contains three contiguous "CHOH" moieties. In the case of sorbitol (Figure 14), there are six contiguous "CHOH" moieties where  $R^1$  and  $R^2$  are H; x and z are 0, y is 4 and  $R^3$  is OH. Additional polyols schematically illustrated in Figure 14 include erythritol, threitol, xylitol and mannitol. Polyols included in this invention may contain a pyranosyl such as glucopyranosyl or a polypyranosyl moiety connected to one of the hydroxyl groups of the linear polyol chain. As shown in Figure 15, the pyranosyl polyols, Maltitol and Isomalt, and polypyranosyl-substituted polyol, Maltotriitol, may also be useful in the practice of the present invention. Examples of other polypyranosyl-substituted polyols that may be useful include Maltotetraitol and polyglucitol. Cyclic polyols, inositol (also known as myo-inositol) that may be useful is shown in Figure 16.

Ethylene glycol, propylene glycol and glycerol as well as other polyols useful in the present invention are liquids at room temperature, which is advantageous in that they are easily poured or pumped into a reaction vessel. Moreover, these liquids more readily dissolve and/or are dispersed in organic liquids. Even though other polyols such as erythritol, xylitol, sorbitol, mannitol and Isomalt are solid at room temperature, they are also applicable in this invention. Although solid polyols

require time and heat to dissolve in an organic liquid, in some cases, they are more effective than the liquid polyols.

**Figure 19: Illustrates a schematic flow diagram of an exemplary embodiment of the present invention.**



As illustrated in Figure 17, phase separation may be easily monitored by visual inspection as the refractivity of the organic layer is different from the aqueous layer. Initially, on mixing the polyol into the composition of the organic liquid (I), the organic liquid is rendered insoluble and the mixture becomes cloudy (II). The amount of the polyol sufficient to cause the composition to become cloudy is called the "cloud point" as that term is understood in the art.<sup>74</sup> In a few minutes, the cloudy mixture begins to separate forming an aqueous and an organic layer (III). In some embodiments, cooling may cause the phase separation to increase (IV). Phase separation may be more easily visualized by using a dye such as Sudan IV which stains the organic phase red and does

<sup>74</sup> Imani *et al.*, *J. Chem. Thermodynamics* **2010**, 41,893-896

not stain the aqueous phase. Alternatively, fluorescein can be used to stain the aqueous phase fluorescent green.

The amount of polyol used in the methods of the present invention depends on variables such as the nature of the organic liquid, the percentage of water in the composition containing the organic liquid, the specific polyol used as the extraction agent, and the total volume of the composition from which the organic liquid is to be separated. Typically, the compositions containing water and the organic liquid that are generated from industrial or research processes (*e.g.*, water/acetonitrile mixtures generated from high pressure liquid chromatographic procedures) contains about 30 to about 90% organic liquid. The methods of the present invention may be effective in extracting about 80 to about 99% of the water, thus generating organic liquids having about 80 to about 99 % purity.

Evaluation of solubility of water in an organic liquid may be readily assessed by one skilled in the art by performing a solubility test. Also, extensive data on water solubility are available in the literature.<sup>75</sup> In general, in embodiments where the water is very slightly miscible with the organic liquid, the amount of the polyol typically ranges from about 0.01 to about 1% wt/v of the polyol relative to the amount of water in the composition. In these cases, where water concentration is very low, it may be advantageous to use an excess of the polyol relative to the amount of water present where a convenient amount is 5% wt/v. In embodiments where water is partially or infinitely miscible with the organic liquid, the amount of polyol typically ranges from about 5 to about 10 % wt/v, from about 10 to about 25 % wt/v and from 25-50 % wt/v relative to the total volume of the composition, *e.g.*, where the preferred range is about 10 % to about 25 % wt/v.

Water concentration in the final product (*e.g.*, either the aqueous or organic phase) can be easily determined by persons skilled in the art using standard techniques such as Karl-Fischer

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<sup>75</sup> a) The Merck Index, 12th Ed.; b) Reichardt, "Solvents and Solvent Effects in Organic Chemistry, 2nd ed.," VCH, 1990.

titration, nuclear magnetic resonance (NMR) spectroscopy (*e.g.*, quantitative NMR), density measurements, and infrared spectroscopy preferably with an attenuated total reflectance probe (ATR-IR).<sup>76</sup> By way of illustration, the purity of the resultant acetonitrile layer may be determined over the spectral range of 4000 to 600  $\text{cm}^{-1}$ . Within this range, key absorbances are 1640  $\text{cm}^{-1}$  ( $\text{H}_2\text{O}$ , bending vibrational mode), 3500  $\text{cm}^{-1}$  (OH stretch) and 2250  $\text{cm}^{-1}$  (C-triple bond-N stretch of acetonitrile).

As disclosed above and illustrated in Figure 29, in some embodiments, lowering the temperature increases the phase separation and thus the amount of water extracted. In general, the extraction may be conducted at a temperature between about 40 °C and about -78 °C, and in some embodiments from about 25 °C to about 2 °C, and in other embodiments, from about 25 °C to about -25 °C. In the upper end of the range, phase separation is observed with solid polyols in which the composition is heated to dissolve the polyol. In the low end of the range, the temperature may be lowered such that the aqueous layer freezes; however, the temperature must be above the freezing point of the organic liquid such that the organic liquid remains as a liquid. In some embodiments, once a phase separation is obtained, the composition of the phase remains stable even when warmed to room temperature. Examples include acetonitrile/glycerol, acetonitrile/xylitol, acetonitrile/sorbitol, and isopropyl alcohol/sorbitol. This phenomenon is advantageous as the phase separation may be optimized at low temperature and then stored at room temperature until the layers are mechanically separated. In some embodiments, the phases are not temperature stable. Thus, once phase separation is obtained at low temperature, mechanical separation must be performed at low temperature. Examples include acetonitrile/ethylene glycol, and acetonitrile/propylene glycol, acetonitrile/mannitol, and acetone/sorbitol.

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<sup>76</sup> Kassis, Bhawtankar, and Sowa, *J. Pharm. Biomed. Anal.* **2010**, 53,269–273

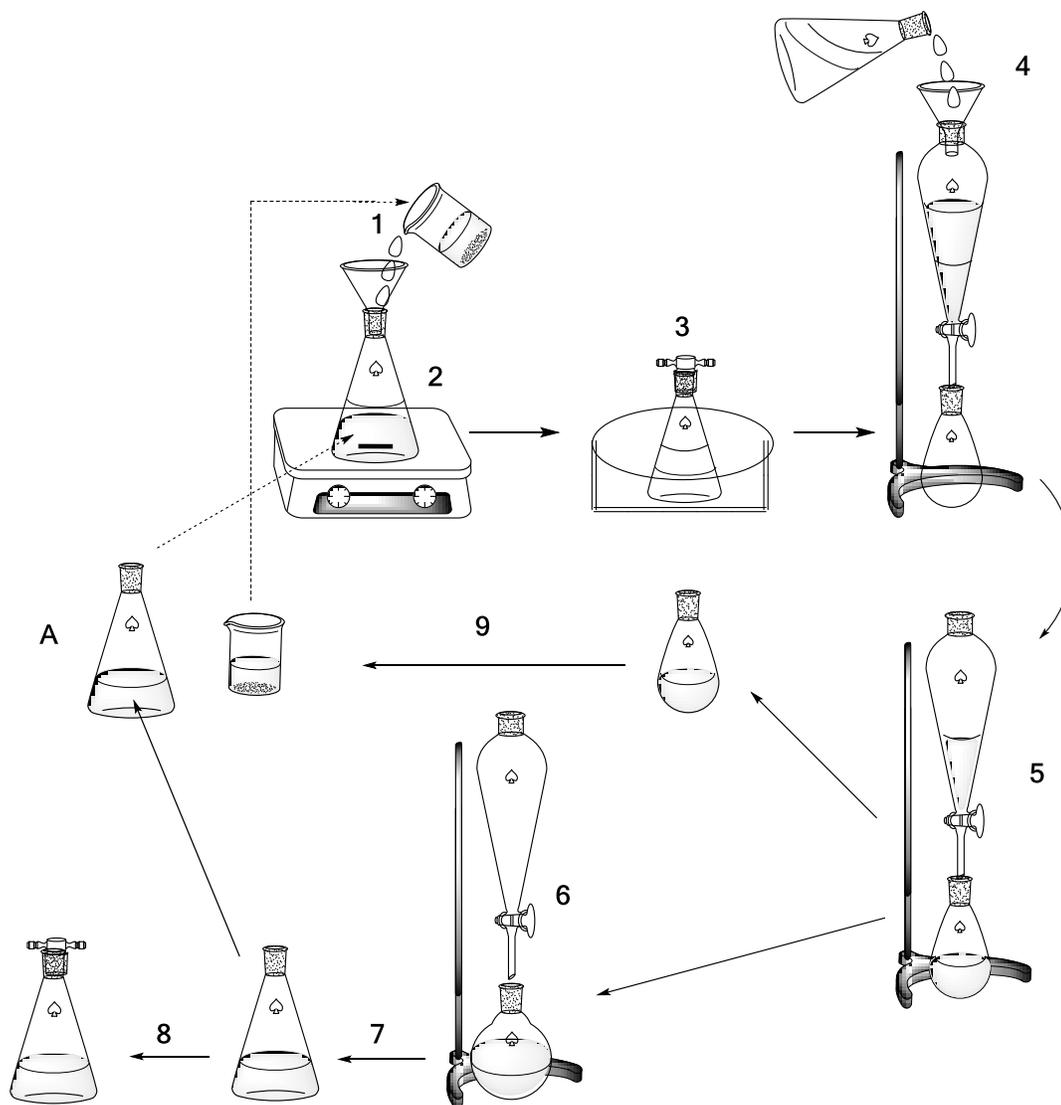
The volume of the original composition may affect the amount of polyol used. For instance, in some embodiments, relatively small volumes of the composition may require slightly more polyol to maximize phase separation relative to large volumes. As described in the working examples, a 10 mL volume of acetonitrile/water (50% v/v) at room temperature 21% wt/v glycerol gave maximum phase separation, whereas for a 1 L volume of the same, 17% wt/v of glycerol gave maximum phase separation. Without intending to be bound by any particular theory of operation, the present inventors believe that the relationship between volume and maximum phase separation is related to the surface of the reaction vessel which may compete with binding of the polyol. In this regard, addition of glass beads to a composition requires that more of the polyol be added to the composition to induce phase separation. In addition, slightly more polyol (1-5% wt/v) is required when a glass vessel is used versus a polypropylene vessel.

In some embodiments, pressurization of the composition with carbon dioxide gas may enhance the phase separation and the purity of the organic-rich upper phase. Thus, in some embodiments, when a composition of acetonitrile and water (50:50, v/v) is initially separated with glycerol (20%) at 0 °C an acetonitrile-rich upper layer is obtained which is approximately 30% of the total volume. Upon pressurization with carbon dioxide to 20 psi while stirring with a stirring bar, the volume of the acetonitrile-rich upper layer is increased to 40% of the total volume and the purity of the acetonitrile is 98%. Without intending to be bound by any particular theory of operation, the present inventors believe that the CO<sub>2</sub> enhancement is due to the formation of carbonic acid (CO<sub>2(aq)</sub>) which as shown in eq 6 drives the equilibrium to the right. The CO<sub>2(g)</sub> enhancement is greater than acetonitrile/water/CO<sub>2</sub> or acetonitrile/water/glycerol alone.



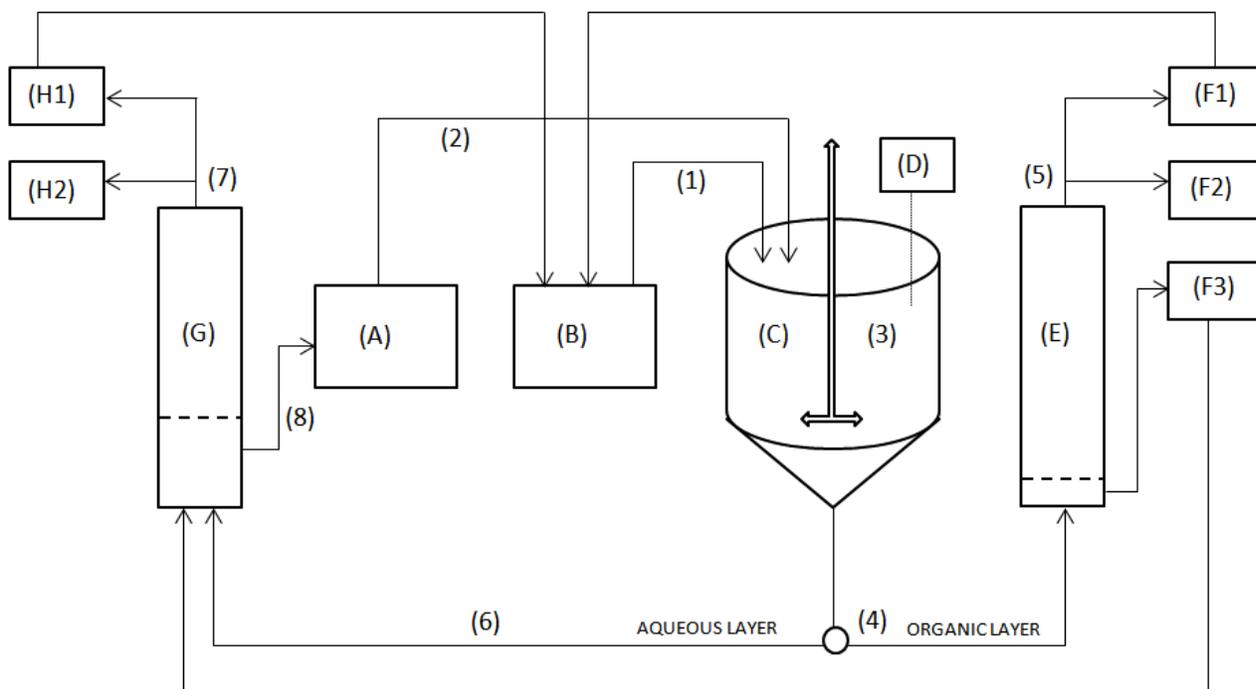
Depending on these other variables, the amount of time required for optimal extraction may also vary, generally between about 2 min and about 2 hours for separations conducted at room temperature and from about 1 hour to about 12 hours for separations that require cooling. In some embodiments, separations with high viscosity liquids (greater than about 1000 poise) such as diesel fuel, petroleum distillates and cyclohexanol, about 12 hours to about 48 hours may be required for separation or use of centrifugation may be necessary.

**Figure 20: Schematic diagram illustrating an embodiment of a polyol induced extraction (PIE), in accordance with the present invention, which can be conducted manually.**



An embodiment of the present invention is now illustrated by reference to Figure 18, which features a separation performed manually in 9 steps, for example, in a research laboratory setting. A composition of organic liquid containing water (Erlenmeyer flask) and a liquid or solid polyol (beaker) is obtained (A). In Step 1, the polyol is added to the composition, and, in step 2, the polyol is mixed into the composition such that the polyol dissolves or is dispersed in the composition. If the polyol is a solid, the mixture is heated to reduce the time it takes to dissolve the polyol. At this point, separation may spontaneously occur. In Step 3, the composition containing the polyol is cooled below room temperature to increase separation or to induce separation if it has not already occurred. In Step 4, the phase separated mixture is poured into a separatory funnel. In Step 5, one skilled in the art may easily determine the whether the upper or lower phase contains the organic liquid. Assuming that the upper phase is the desired organic phase (due to its lower density), the lower phase is drained into a round-bottomed flask and, in step 6, the upper phase is drained into a different round-bottomed flask. In Step 7, the organic phase is distilled by simple, fractional or vacuum distillation (*e.g.*, using a rotary evaporator) to obtain a pure organic liquid (typically, greater than about 95% purity, less than about 5% water). In Step 8, if the organic phase is of insufficient purity, *e.g.*, typically containing greater than about 5 to 25% water, it is subjected to additional treatments with the polyol. In Step 9, the polyol is recovered from the aqueous solution by distillation or recrystallization and may be used again for PIE.

**Figure 21: Schematic diagram illustrating an embodiment of the PIE process, in accordance with the present invention, which may be conducted on an industrial scale. The diagram is specifically illustrated for glycerol induced extraction of water from acetonitrile**



An embodiment of the invention is now illustrated in by reference to Figure 19, which is described in the context of the glycerol-induced extraction of water from a composition containing water and acetonitrile (ACN) on an industrial scale. In step 1, the acetonitrile/water mixture is added to Reaction Vessel C. In step 2, glycerol is added to Reaction Vessel C. In step 3, the acetonitrile/water and glycerol components are mixed. In step 4, one skilled in the art may easily substitute glycerol for another liquid polyol or for a solid polyol. However, if the polyol is a solid, the mixture is heated as needed for dissolution of the polyol. The mixture is cooled to induce separation or to increase separation as needed. Reaction Vessel C also enables contents to settle into distinct aqueous and organic phases. The phases are either detected by visual inspection or through the use of a sensor such as an ATR-IR probe. The sensor will also allow for determination of the purity of each phase or aliquots can be withdrawn to determine the composition. In step 5, the

aqueous layer and organic layer are directed to the respective distillation apparatus I and II. In cases where the aqueous layer is denser than the organic layer, such as acetonitrile, the aqueous layer will be the bottom layer. One skilled in the art will be able to determine cases where the aqueous layer is less dense than the organic layer, such as alkyl halides including dichloromethane, methyl iodide and carbon tetrachloride. In step 6, the organic layer containing acetonitrile is subjected to distillation. Fractions are collected such that impure lower boiling fractions containing acetonitrile/water are returned to B for further recovery; pure acetonitrile is collected in vessel E; residue in D containing glycerol is directed to distillation apparatus II for recovery of glycerol. In step 7, the aqueous layer containing mostly water, glycerol and smaller amounts of acetonitrile is subjected to distillation for recovery of glycerol. In step 8, the more volatile component of the distillate is collected and the distillate containing water is collected in vessel G. In step 9, glycerol is directed to vessel A for further use. If necessary, glycerol is removed for purification by distillation, recrystallization, charcoal treatment, *etc.*, before it is directed to vessel A.

For purposes of the present invention, the term "about" means  $\pm$  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% of the numerical value with which it is used.

The present invention will now be described in terms of the following, non-limiting examples.

### 2.3.1 Example 1. Evaluation of suitability of organic liquids for PIE:

To a 35 mL culture tube was added 10 mL of the organic liquid. To aid in observation of the phase separation a trace (less than 1 mg) of Sudan IV was added which stained the organic liquid red. Water was added by dropwise addition until the organic liquid was saturated with water. If the organic liquid was completely miscible with water then only 2.5 to 10 mL of water was added to make 20–50% aqueous solutions. To these solutions, was added 20% wt/v of the polyol. The culture

tube was capped and the contents were mixed to dissolve the polyol. For solid polyols, the culture tube was heated with warm tap water (40-50 °C) until the solid dissolved. If the organic liquid was partially miscible with water such that saturation was obtained with 5–20% water, 20% wt/v of the polyol was also added. If the organic liquid was slightly miscible with water such that saturation was obtained with one drop of water (0.05 to 0.1 mL), then only 5% wt/v of the polyol was added. The mixtures were allowed to settle at room temperature for 5 to 10 min and phase separation was observed by the formation of a distinct aqueous and organic phase wherein the organic phase was stained red and the aqueous phase was clear and colorless. If phase separation did not occur at room temperature (RT), then the culture tube was placed in the refrigerator (2 °C) or the freezer (-18°C) for 12 h (overnight) and evaluated for phase separation. The results are shown in Table 4.

**Table 4: Organic Liquids Tested for Polyol induced Extraction (PIE).**

Organic Liquid	Type <sup>a</sup>	Gly Solu- bility <sup>b</sup>	Composition (% H <sub>2</sub> O) <sup>c</sup>	Polyol <sup>d</sup>	wt/v <sup>e</sup> Temp <sup>f</sup>
dimethylsulfoxide	pap	s	50%	nr	
poly(ethylene glycol)butyl ether	pap	s	50%	nr	
Acetonitrile	pap	i	20%	EG	20,-18°C
			20%	PG	20,-18°C
			50%	Gly	20%, RT
			50%	Ery	20%, RT
			50%	Xyt	20%, RT
			50%	So	20%, RT
			50%	Man	1%, <sup>g</sup> -10°C
			50%	Isom	20%, RT
			50%	Malt	20%, RT
			50%	Ino	Nr
dimethylformamide	pap	s		nr	
N-methylpyrrolidinone	pap	s		nr	
dimethylacetamide	pap	s		nr	

tri(ethylene glycol)	pp	s		nr	
2-butanone	pap	i	50%	Gly	20, RT
acetone	pap	s	40%	So	20%,-18°C
Cyclopentanone	pap	i	50%	Gly	20%, RT
2-ethoxyethanol	pap	s		nr	
t-butanol	pp	s	25%	Gly	30,-18°C
1-butanol	pp	i	25%	Gly So	20%, RT 20%, RT
isopentyl alcohol (3-methyl-1-butanol)	pp	s	50%	Gly	20%, RT
cyclohexanol	pp	s	~10%	Gly	20%, RT
1-octanol	pp	i	Sat	Gly	5%, RT
Pyridine	pap	s		nr	
dichloromethane	pap	i	Sat	Gly	5%, RT
tetrahydrofuran	pap	s	20%	Gly	20%,-18°C
tri(ethylene glycol) dimethyl ether	pp	i		nr	
1,2-dimethoxyethane	pap	i	~ 10%	Gly	20%, RT
methyl benzoate	pap	i	Sat	Gly	5%, RT
propylene carbonate	pap	i	Sat	Gly	5%, RT
ethyl acetate	pap	i	Sat	Gly So	5%, RT 5%, RT
Chloroform	pap	i	Sat	Gly	5%, RT
diethyl ether (ethyl ether)	pap	ss	Sat	Gly So <sup>h</sup>	5%, RT 5%, RT
Mesitylene	pap	i	Sat	Gly	5%, RT
p-xylene	pap	i	Sat	Gly	5%, RT
Benzene	pap	i	Sat	Gly	5%, RT
toluene	pap	i	Sat	Gly So	5%, RT 5%, RT
carbon tetrachloride	np	i	Sat	Gly So	5%, RT 5%, RT

t-butylmethyl ether	pap	ss	Sat	Gly	5%, RT
dimethylphthalate	pap	i	Sat	Gly	5%, RT
dibutylphthalate	pap	i	Sat	Gly	5%, RT
crude oil(petroleum)		i	Sat	Gly	5%, RT
emulsified crude oil				Gly	5%, RT
Gasoline	np	i	Sat	Gly So	20%, RT 20%, RT
Kerosene	np	i	Sat	Gly So	20%, RT 20%, RT
Diesel	np	i	Sat	Gly So	20%, RT 20%, RT
petroleum distillates	np	i	Sat	Gly So	20%, RT 20%, RT
Cyclohexane	np	i	Sat	Gly So	5%, RT 5%, RT
hexanes	np	i	Sat	EG PG Gly So <sup>g</sup> Man Ino	5%, RT 5%, RT 5%, RT 5%, RT 1%, RT 1%, RT

<sup>a</sup>Type of organic liquid; pap is polar aprotic; pp is polar protic; np is nonpolar.

<sup>b</sup>Solubility of glycerol in the pure organic liquid at RT.

<sup>c</sup>% of water in the organic liquid/water composition. If sat (saturated) is stated, water is < 1%.

<sup>d</sup>nr is no separation observed at RT on addition of greater than 20% Gly and on cooling to -18°C.

Polyols are ethylene glycol (EG), propylene glycol (PG), glycerol (Gly), erythritol (Ery), xylitol (Xyl), sorbitol (So), mannitol (Man), Isomalt (Isom), Maltitol (Malt), inositol (Ino).

<sup>e</sup>Amount of polyol added (wt) relative to the volume (v) of the composition to initiate phase separation.

<sup>f</sup>Initial addition of polyol was at RT (room temperature), cooling to -18°C was done if separation was not initially observed at RT.

<sup>g</sup>Amount of mannitol is limited due to low solubility.

<sup>h</sup>On addition of sorbitol, a viscous aqueous solution was formed.

### 2.3.2 Example 2. Determination of amount of glycerol required to obtain maximum phase separation of different compositions of acetonitrile/water mixture by titration

Six glass vials were prepared each containing a 10 mL (v/v) mixture of 95%, 90%, 75%, 50%, 25%, and 10% of acetonitrile in water, respectively. Glycerol was added in incremental aliquots at room temperature. The mixtures were shaken and observed for minimum amount of glycerol required for maximal phase separation. The mixtures were then cooled in freezer at -18°C overnight for further separation and analyzed for percent purity of acetonitrile by ATR-IR spectroscopy in the upper layer of the separated phases.

**Figure 22: Graph showing an infrared spectral analysis series of the acetonitrile (ACN) layer of glycerol induced phase separation (PS), wherein water concentration was monitored by measuring the peak area at 1640 cm<sup>-1</sup>**

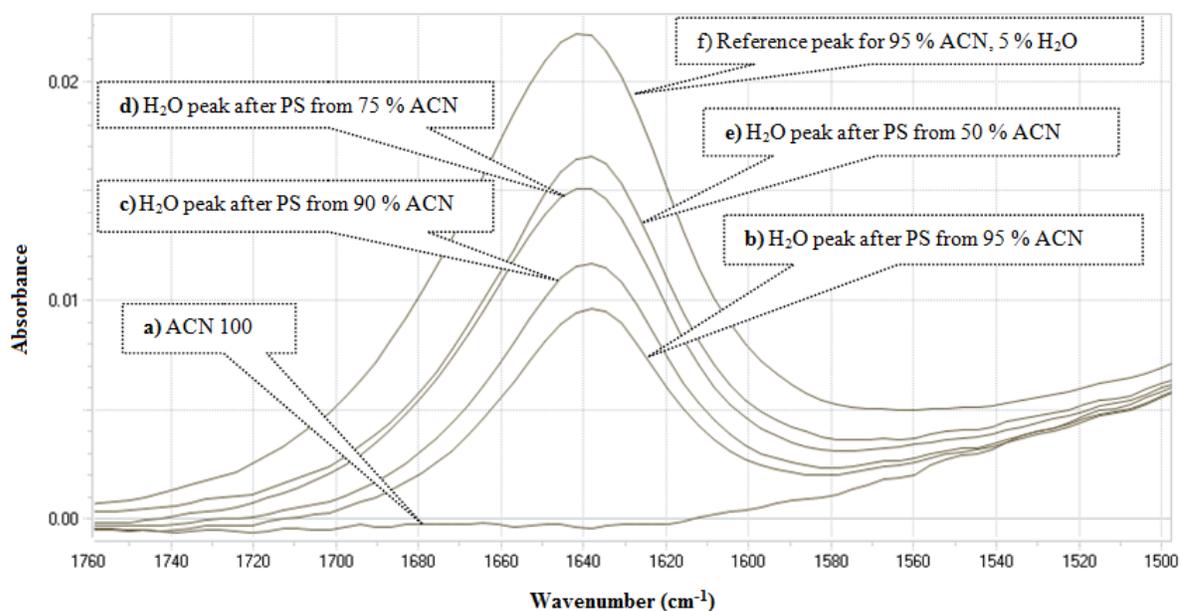


Figure 20 is a graph showing an infrared spectral analysis series of the acetonitrile (ACN) layer of glycerol-induced phase separation (PS), wherein water concentration was monitored by measuring the peak area at 1640 cm<sup>-1</sup>.

**Table 5: Minimum amount of glycerol required for maximum phase separation of acetonitrile and water.**

Entry	Percent composition of ACN <sup>a</sup> (v/v)	Minimum amount(g) of Gly <sup>a</sup> for maximum PS <sup>a</sup>	% Gly at maximum PS (wt/v%)	% purity <sup>b</sup> of ACN, after cooling to -18°C
1	95	0.8	8	98%
2	90	1.0	10	98%
3	75	1.4	14	97%
4	50	1.8	18	97%
5	25	More than 10 g	More than 100%	NA
6	10	More than 10 g	More than 100%	NA

<sup>a</sup>ACN is acetonitrile; Gly is glycerol; PS is phase separation.

<sup>b</sup>Purity determined by ATR-IR (see Figure 20).

The results are listed in Table 5. A spectral analysis is presented in Figure 20, which shows a cut out of the  $1640\text{ cm}^{-1}$  absorbance wherein the amount of water in the resulting acetonitrile layer is less than 5% for four treatments of acetonitrile/water mixture with glycerol.

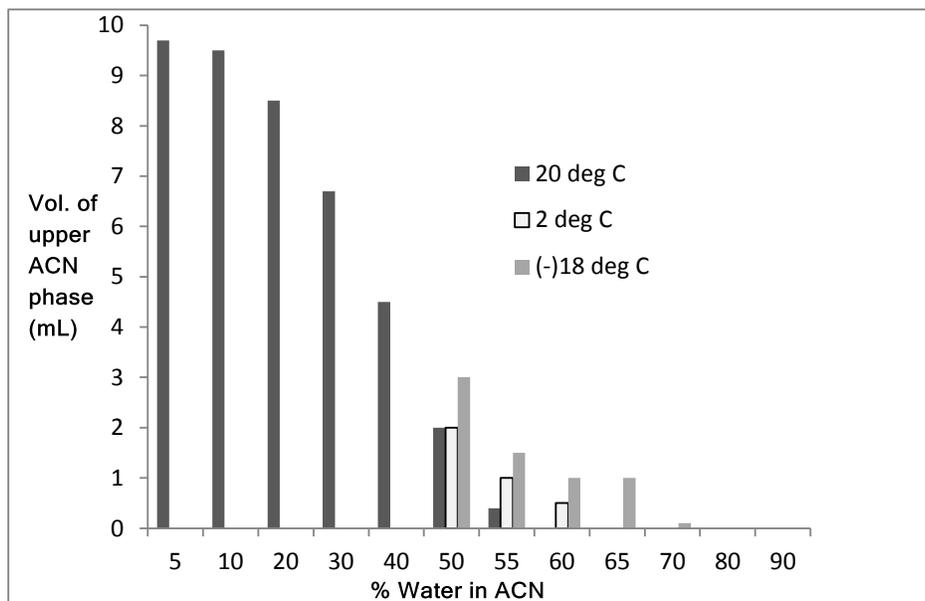
These results clearly indicate the amount of polyol for optimal separation depends on the % water in the original composition as illustrated by the successful separation of water from acetonitrile by glycerol from compositions ranging from 5-50% v/v water. Above 75% v/v water, separation was not observed at room temperature.

### 2.3.3 Example 3. Determination of the effect of % water and temperature on glycerol-induced extraction of water from acetonitrile

Twelve plastic (polypropylene) graduated centrifuge tubes were prepared each containing 10 mL of 95%, 90%, 80%, 70%, 60%, 50%, 45%, 40%, 35%, 30%, 20%, and 10% acetonitrile/water (v/v), respectively. Control samples of 100% acetonitrile and 100% water were also prepared. To each tube was added 2.0 g glycerol (20% wt/v) and the mixtures were shaken. The mixtures were allowed to stand at room temperature ( $20^{\circ}\text{C}$ ) for 15 min to allow phase separation to reach equilibrium. The volume of the upper acetonitrile phase was measured. The tubes were then cooled overnight to  $2^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$  and the volume of the upper phases was measured at each temperature.

The results are illustrated by spectral analysis in Figure 21. At room temperature, phase separation was observed at 55% water. However, decreasing temperature to  $2^{\circ}\text{C}$  resulted in phase separation for 60% water and at  $-18^{\circ}\text{C}$ , separation was observed at 70% water. At 55% water, there was an excellent indication of the effect of temperature. Surprisingly, at room temperature 0.4 mL of ACN was obtained. Lowering the temperature to  $2^{\circ}\text{C}$  resulted in 1.0 mL and by lowering to  $-18^{\circ}\text{C}$ , 1.5 mL was obtained.

**Figure 23: Bar graph demonstrating the effect of the amount % water and temperature on phase separation.**



#### 2.3.4 Example 4: Glycerol-induced extraction of water from acetonitrile

To 1.0 L of a 1:1 mixture of acetonitrile and water was added 200 g of glycerol (20% w/v) and the mixture swirled until the glycerol dissolved. Initially, two layers formed consisting of acetonitrile as the less dense top layer and an aqueous solution of glycerol as the bottom layer. The mixture was placed in a freezer at  $-18^{\circ}\text{C}$  overnight to increase the phase separation. The cold mixture was poured into a separatory funnel and the bottom aqueous layer removed. The top layer resulted in 378 mL (75.6% yield) and contained 97% acetonitrile with 3% water. The top layer was treated again with glycerol 76 g (20% w/v), swirled and placed in freezer at  $-18^{\circ}\text{C}$  overnight for further separation of water. The cold mixture was again separated using a separatory funnel. The yield of the top layer was 355 mL (71%) and contained 98% acetonitrile, 1-2% water and 0.53% glycerol (1.9 g). This layer was distilled at atmospheric pressure and 99% pure acetonitrile was collected at  $81^{\circ}\text{C}$  (352 mL, 70.4% yield). The glycerol was recovered by fractional distillation.

Residual acetonitrile in the distillate was collected between 76 – 99°C and water was collected at 100°C leaving anhydrous glycerol in the distillation pot (190 g, 95% recovery).

### 2.3.5 Example 5: Sorbitol-induced extraction of water from acetonitrile

To 1.0 L of a 1:1 mixture of acetonitrile and water was added 200 g of sorbitol (20% w/v) and the mixture was swirled until the sorbitol dissolved. Initially, two layers formed — acetonitrile as the less dense top layer and an aqueous solution of sorbitol as the bottom layer. The mixture was placed in a freezer at -18°C overnight. The cold mixture was poured into a separatory funnel and the bottom aqueous layer was removed. The yield of the top layer was 380 mL (76%) and contained 92% acetonitrile with 8% water. The top layer was treated again with sorbitol 76 g (20% w/v), mixed and placed in freezer at -18°C overnight for further separation of water. Sorbitol acted mostly as a dehydrating agent, absorbing most of the water and forming solid lumps. The remaining organic layer was again separated to analyze for its purity. The yield of the top layer was 345 mL (69%) and contained 97% acetonitrile, 2-3% water and 0.26% sorbitol (0.90 g). This layer was distilled at atmospheric pressure and the pure acetonitrile was collected at 81°C (342 mL, 68.4% yield and contained 98% pure acetonitrile). The sorbitol was recovered by vacuum distillation using a rotary evaporator (40 °C, 230 mbar) leaving anhydrous sorbitol in the distillation pot (190 g, 95% recovery).

### 2.3.6 Example 6: Sorbitol-induced extraction of water from acetone

To a 1 L glass bottle was added acetone (515 mL), water (300 mL) (36.8% v/v) and the contents were mixed to form a clear colorless solution. A trace amount (ca. 1 mg) of Sudan IV was added to give a pink-red clear solution. Sorbitol was then added in 5–25 g increments until a total of 170 g was obtained (21 % wt/v). After addition of each increment, the mixture was agitated until the

sorbitol completely dissolved. As the mixture became saturated with sorbitol, the last increment (ca. 10 g) did not completely dissolve. After agitation, the mixture was allowed to settle at room temperature (30 min). Initially, the saturated mixture appeared cloudy and slowly as the solids settled, the mixture formed a less dense top layer of acetone which was red in color due to Sudan IV and a denser, colorless bottom aqueous layer. The mixture was placed in a freezer at -18°C overnight resulting in a well-separated top layer (500 mL) and bottom layer (400 mL) wherein the extra volume was caused by the sorbitol. Upon additional storage at -18°C, the sorbitol crystallized out of the aqueous layer. The cold fluid was poured into a separatory funnel and the layers were separated. The yield of the top layer was 450 mL and contained 80% acetone with 20% water. This layer was distilled at reduced pressure (40°C, 550 mbar) using a rotary evaporator (230 mL, 45% yield) and contained 95% pure acetone by IR spectral analysis.

### 2.3.7 Example 7: Glycerol-induced extraction of water from hexanes

To 500 mL of hexanes was added 50 mL of water and a trace of indicator Sudan IV. The contents were mixed to yield a slightly pink colored top layer which was spotted with droplets of water and a bottom layer which was clear and colorless. The bottom layer was removed using a separatory funnel. The top layer was treated with 10 g of glycerol and mixed to give a top layer that contained droplets of aqueous glycerol and a bottom layer of aqueous glycerol. The bottom layer was removed. Then the top layer was drained into a clean, dry Erlenmeyer flask leaving behind the droplets of aqueous glycerol on the walls of the separatory funnel. The fluid in the Erlenmeyer flask was decanted into a clean, dry separatory funnel to give a clear pink-red solution with no spotting on the walls. A residual amount (1 mL) of aqueous glycerol was removed. The top layer was drained and distilled under reduced pressure (40 °C, 330 mbar) using a rotatory evaporator. The first 30 mL of distillate was discarded and 430 mL (86% yield) of greater than 99% water-free hexanes (as

indicated by IR spectral analysis which showed the absence of an absorption at  $1640\text{ cm}^{-1}$ ) was obtained.

### 2.3.8 Example 8: Sorbitol-induced extraction of water from hexanes

To 500 mL of hexanes was added 50 mL of water and a trace of indicator Sudan IV. The contents were mixed to yield a slightly pink colored top layer which was spotted with droplets of water and a bottom layer which was clear and colorless. The bottom layer was removed using a separatory funnel. The top layer was treated with 1.0 g of sorbitol and mixed to give a liquid bottom layer of aqueous sorbitol and droplets of aqueous sorbitol on the walls of the flask. The top layer was decanted into an Erlenmeyer flask and then decanted a second time leaving droplets of aqueous sorbitol in the original Erlenmeyer flask. The decantant was distilled at atmospheric pressure at  $65\text{--}68^\circ\text{C}$  to obtain 450 mL of greater than 99% water-free hexanes (as indicated by IR spectral analysis which showed the absence of absorption at  $1640\text{ cm}^{-1}$ ).

### 2.3.9 Example 9: Comparison to traditional method: Azeotropic distillation of acetonitrile/water

The following example was conducted to demonstrate ability of distillation as a method of purification of ACN from mixture of ACN-water. The results were indicated in terms of purity level of ACN achieved as oppose to yield in terms of quantity.

1.0 L of a 1:1 mixture of acetonitrile and water was subjected to traditional azeotropic distillation. For demonstration purpose, two separate distillates were collected where first distillate was collected between  $76\text{--}81^\circ\text{C}$  (550 mL) and was analyzed to be 86% pure acetonitrile. The volume of second distillate was collected between  $81\text{--}99^\circ\text{C}$  (250 mL) and was analyzed to be only 75% pure

acetonitrile. The experiment clearly demonstrated that maximum purity level achieved by distillation was 86% pure acetonitrile

### 2.3.10 Example 10: Glycerol-induced extraction of water from a commercial formulation of a non-acetone nail polish remover

To a clear, 12 oz., plastic (PETE) bottle was added 200 mL of non-acetone nail polish remover. The principal ingredients of the formulation were indicated as ethyl acetate, alcohol, water and propylene carbonate. Then 20 mL of glycerol was added and the mixture was shaken which resulted in a cloudy suspension that separated into two layers in 15 - 30 min at room temperature. The layers were separated by piercing a small hole ( $< 0.1 \text{ mm} \times 5 \text{ mm}$ ) in the base of the bottle with a knife and inverting the bottle such that the liquid laid at the base of the bottle. The cap was loosened and the lower aqueous layer was allowed to drip out (50 mL) through the hole. Once the separation of the aqueous layer was completed, the remaining upper organic phase was poured into a separate bottle for storage (150 mL).

### 2.3.11 Example 11: Determination of cloud point and effect of container on phase separation

To an 8 dram glass vial, 125 mL glass Erlenmeyer flask and 1 L glass bottle was added 10, 100, and 1000 mL of acetonitrile/water (50:50 % v/v), respectively. To a 15 mL polypropylene centrifuge tube, 150 mL and 1 L plastic bottle was added 10, 100, and 1000 mL of acetonitrile/water (50:50 % v/v), respectively. To an 8 dram glass vial and a 15 mL polypropylene centrifuge tube was added 1.0 g of glass beads (2 mm diameter) and 10 mL of acetonitrile/water (50:50 % v/v). To each sample, glycerol was added in aliquots and the solution was mixed to dissolve the glycerol. The

amount of glycerol corresponding to a cloudy appearance of the solution was determined to be the cloud point. The results are shown in Table 6.

**Table 6: Cloud point and effect of container on phase separation**

Entry	Container	Volume (mL, 50:50, v/v ACN <sup>a</sup> /water)	Amount(g) of Gly <sup>a</sup> at cloud Point	Relative % change in % Gly at cloud point
1	Glass/8 dram	10	2.08	
2 <sup>b</sup>	Glass/ 8 dram	10	2.13	1.13% <sup>c</sup>
3	Plastic/ 15 mL	10	2.00	-4.00%
4 <sup>b</sup>	Plastic/ 15 mL	10	2.05	-1.44%
5	Glass/125 mL	100	19.5	-6.25%
6	Plastic/ 150 mL	100	19.0	-9.47%
7	Glass/1 L	1000	170	-18.3%
8	Plastic/ 1 L	1000	165	-20.7%

<sup>a</sup>ACN is acetonitrile, Gly is glycerol.

<sup>b</sup>Glass beads (1.0 g, 2 mm dia) added to container.

<sup>c</sup>Calculation performed relative to entry 1 (%Gly 20.8):  $(\%Gly \text{ entry } x - 20.8)/20.8$ . A positive value indicates an increase in %Gly is required to reach cloud point and a negative value indicates a decrease in %Gly is required.

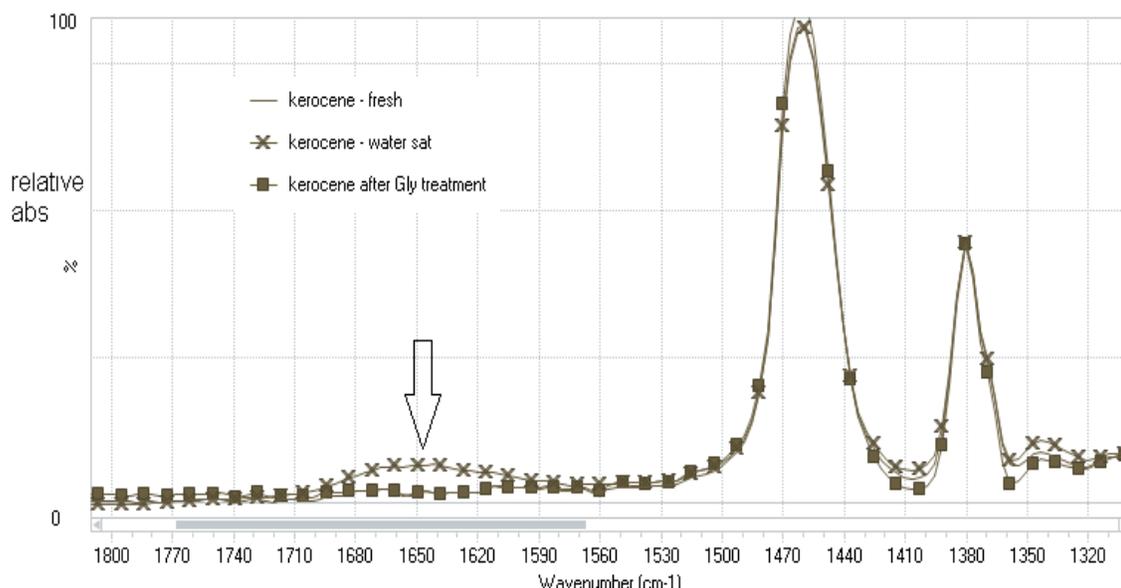
### 2.3.12 Example 12: Glycerol-induced extraction of water from diesel fuel and extraction of diesel fuel from water

To 1.0 L of diesel fuel (obtained from a local gas station) was added 100 mL of water. The contents were mixed and allowed to settle for 1 h to yield a top organic layer and an initial bottom aqueous layer (100 mL, AQ 1). The bottom layer was removed using a separatory funnel. The top

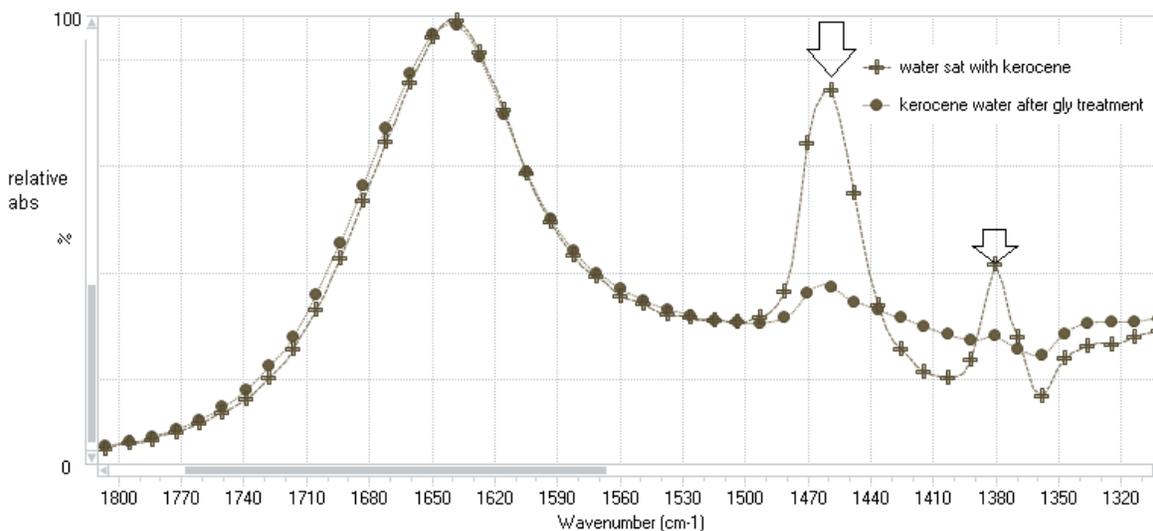
layer was treated with 100 g of glycerol and mixed and allowed to settle for 1 h. After the glycerol containing bottom layer (AQ 2) was removed, the upper layer of diesel fuel (950 mL, 95 % yield) was analyzed by IR spectroscopy and showed the absence of absorption at  $1640\text{ cm}^{-1}$  which indicated the water was completely removed from the diesel fuel. The initial aqueous layer (AQ 1) was examined by IR spectroscopy which showed absorbances at  $1445$  and  $1380\text{ cm}^{-1}$  characteristic of alkane hydrocarbons present in diesel fuel. Glycerol (20 g, 20%) was added to AQ 1 and upon dissolution of the glycerol, droplets of diesel fuel appeared indicating that the glycerol was extracting the diesel fuel from the water. Analysis of the AQ 1 by IR spectroscopy treated with glycerol indicated a significant reduction (75 %) in the hydrocarbon absorbances.

### 2.3.13 Example 13: Glycerol-induced extraction of water from kerosene and extraction of kerosene from water

**Figure 24: Spectral analysis of the glycerol-induced extraction of water from kerosene as described in example The spectrum of fresh kerosene overlays with the spectrum of the glycerol treated kerosene.**



**Figure 25: Spectral analysis of the glycerol induced extraction of kerosene from water by addition of glycerol as described in example**



To 1.0 L of kerosene (obtained from a local hardware store) was added 100 mL of water. The contents were mixed and allowed to settle for 1 h to yield a top organic layer and a bottom aqueous layer (AQ 3). The bottom layer was removed using a separatory funnel. The top layer was treated with 100 g of glycerol and mixed and allowed to settle for 1 h. After the glycerol containing bottom layer (AQ 4) was removed, the upper layer of kerosene (980 mL, 98% yield) was analyzed by IR spectroscopy and showed the absence of an absorption at 1640 cm<sup>-1</sup> (Figure 22) which indicated the water was completely removed from the kerosene. The initial aqueous layer (AQ 3) was examined by IR spectroscopy which showed absorbances at 1445 and 1380 cm<sup>-1</sup> (Figure 23) characteristic of alkane hydrocarbons present in kerosene. Glycerol (20 g, 20%) was added to AQ 3 and upon dissolution of the glycerol, droplets of kerosene appeared indicating that the glycerol was extracting the kerosene from the water. Analysis of the AQ 3 by IR spectroscopy treated with glycerol indicated a significant reduction (80 %) in the hydrocarbon absorbances.

### 2.3.14 Example 14. Quick and simple PIE test

To a 50 mL polyethylene centrifuge tube was added 12 mL of a mixture of acetonitrile and water (50/50% v/v) and one "sugar-free" breathmint tablet (1.8 g) containing sorbitol as the major ingredient (*i.e.*, listed first on the ingredient label). The tube was capped and the contents mixed until the tablet dissolved. The contents were allowed to settle for 5 min and separation of the layers was observed as the contents settled to obtain 7.5 mL of a lower aqueous layer and 4.5 mL of an upper organic layer.

## **2.4 CLAIMS**

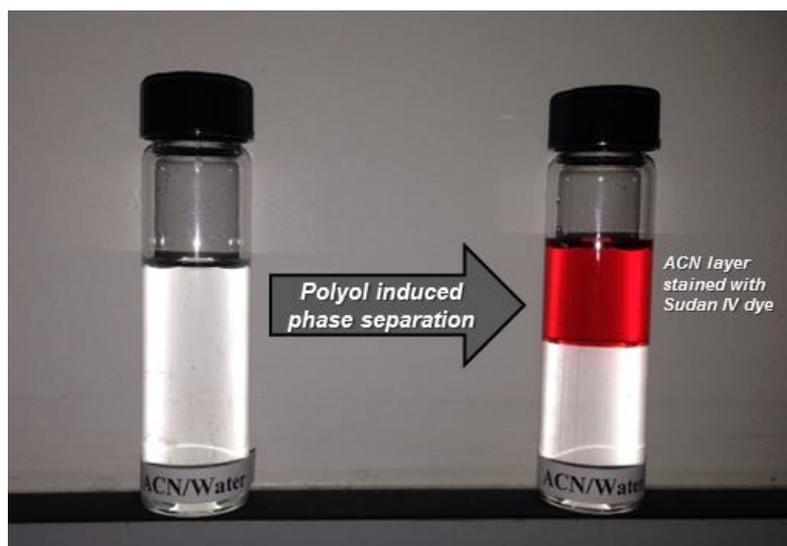
1. A method of separating water from an organic liquid from a composition containing water and the organic liquid, comprising adding to the composition a polyol that causes separation of the composition into at least two phases, wherein a first phase is aqueous and the second phase is organic, and wherein the polyol is  $R_1(CHOR_3)(CH_2)_x(CHOR_3)_y(CH_2)_z(CHOR_3)R_2$  wherein  $R_1$  and  $R_2$  each independently represents hydrogen (H), a linear or branched C1-C8 alkyl group or an aryl group, or wherein  $R_1$  and  $R_2$  taken together represent a bond such that the polyol is cyclic; wherein  $x$ ,  $y$  and  $z$  are each independently integers including 0 to 4, and wherein the sum of  $x+y+z$  is less than or equal to 4, nprovided that when  $R_1$  and  $R_2$  taken together represent a bond, the sum of  $x$ ,  $y$ , and  $z$  is 4; and  $R_3$  represents hydrogen when  $y$  is 1, and when  $y$  is 2, 3 or 4, each  $R_3$  is hydrogen or one of the  $R_3$  groups is pyranosyl or polypyranosyl and the other  $R_3$  groups are hydrogen.
2. The method of claim 1, wherein the polyol is glycerol.
3. The method of claim 2, wherein the organic liquid comprises a polar aprotic liquid which is acetonitrile, toluene, ethyl acetate, 2-methyltetrahydrofuran or tetrahydrofuran.
4. The method of claim 2, wherein the organic liquid comprises a polar protic liquid which is 1-butanol, t-butanol, 2-pentanol, isopentyl alcohol, cyclohexanol or 1-octanol.
5. The method of claim 2, wherein the organic liquid comprises a non-polar liquid which is hexane, cyclohexane, kerosene, diesel fuel, biodiesel fuel, a petroleum distillate or gasoline.
6. The method of claim 1, wherein the polyol is sorbitol.
7. The method of claim 6, wherein the organic liquid comprises a polar aprotic liquid which is acetonitrile, acetone, ethyl acetate, toluene or diethyl ether.

8. The method of claim 7, wherein the organic liquid comprises a polar protic liquid which is isopropanol or 1-butanol.
9. The method of claim 6, wherein the organic liquid comprises a non-polar liquid which is carbon tetrachloride, diesel fuel, biodiesel fuel, gasoline, kerosene or a petroleum distillate.
10. The method of claim 1, wherein the polyol is erythritol, threitol or xylitol.
11. The method of claim 10, wherein the organic liquid comprises a polar aprotic liquid which is acetonitrile or acetone.
12. The method of claim 10, wherein the organic liquid comprises a nonpolar liquid which is carbon tetrachloride, diesel fuel, biodiesel fuel, gasoline, kerosene or a petroleum distillate.
13. The method of claim 10, wherein the organic liquid comprises a polar protic liquid which is isopropanol or 1-butanol.
14. The method of claim 1, wherein the polyol is ethylene glycol.
15. The method of claim 14, wherein the organic liquid comprises a polar aprotic liquid which is acetonitrile or acetone.
16. The method of claim 14, wherein the organic liquid comprises a nonpolar liquid which is hexane, cyclohexane, kerosene, diesel, biodiesel, petroleum distillate or gasoline.
17. The method of claim 1, wherein the polyol is propylene glycol or inositol.
18. The method of claim 1, wherein the polyol is Maltitol, Isomalt, or Inositol.
19. The method of claim 1, wherein the polyol is a polyglycitol.
20. The method of claim 19, wherein the polyglycitol is Maltotriitol, Maltotetraitol, or polyglucitol.

## CHAPTER 3:

### Thermodynamic investigation of phase separation in acetonitrile-water (ACN-H<sub>2</sub>O) system using 'glycerol' as mass separating agent (MSA)

**Figure 26: Glycerol induced phase separation in ACN-water mixture (1:1 ratio % v/v) at 0 °C.**

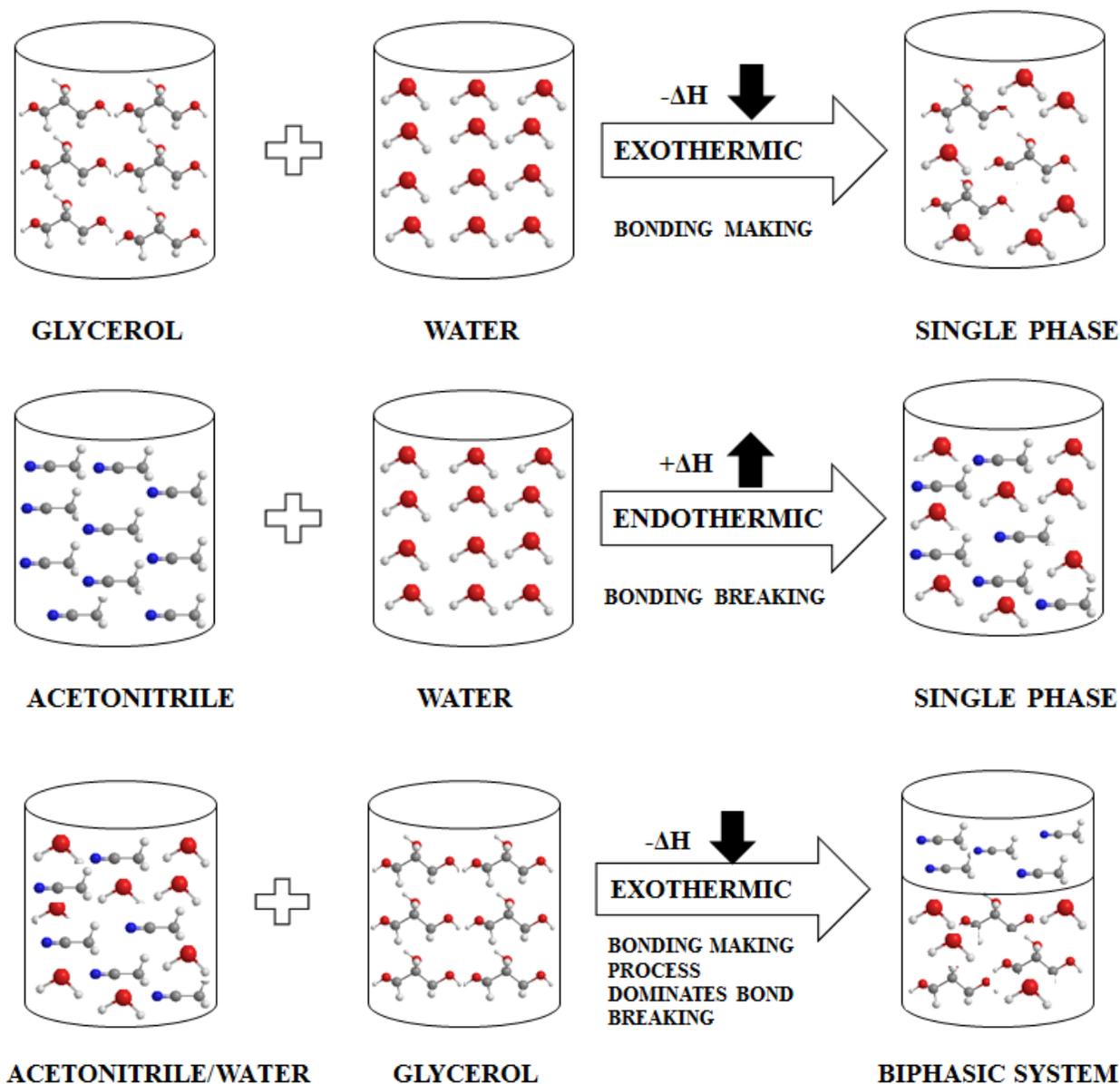


### 3.1 Introduction:

The present research article is directed to a method of separating water from a mixture of acetonitrile and water, where adding a polyol such as glycerol and sorbitol causes separation of the composition into at least two phases, wherein the lower phase is substantially aqueous (and contains, on a percentage basis (v/v), less acetonitrile than what was originally present prior to the addition of the polyol) and the upper phase is substantially organic. Since a successful separation will depend on the judicious selection of mass separating agent, therefore it is necessary to study thermodynamic properties associated with the system as a phase separation process is essentially a thermodynamic

process. Any chemical reactions proceed with the evolution or absorption of heat. This heat flow represents differences in chemical energy associated with the rearrangement of atoms in molecules, the making and breaking of bonds to form new substances. When this measured at constant pressure is the *enthalpy change* ( $\Delta H$ ) for the reaction.

**Figure 27: Thermodynamics of dissolution of different components in acetonitrile-water-glycerol system**



To interpret and correlate the nature of intermolecular forces it is necessary to have some understanding of thermodynamic properties of solutions. The case of a mixture, however, is necessarily more complicated because consideration must be given not only to interaction between molecules belonging to the same component, but also to interaction between dissimilar molecules. Figure: 27 shows different thermodynamic and molecular interactions associated with acetonitrile-water-glycerol system.

## **3.2 Materials and methods:**

### **3.2.1. Chemicals:**

BAKER ANALYZED HPLC Ultra gradient ACN ( $\text{CH}_3\text{CN}$ ) was purchased from J.T Baker (Phillipsburg, NJ), water ( $\text{H}_2\text{O}$ ) HPLC grade was from EMD chemicals (Gibbstown, NJ ), glycerol( $\text{C}_3\text{H}_8\text{O}_3$ ) was purchased from Sigma Aldrich Chemicals (St Louis, MO), glucose( $\text{C}_6\text{H}_{12}\text{O}_6$ ) was purchased from Aldrich Chemical (Milwaukee, WI) and sodium chloride ( $\text{NaCl}$ ) Crystal, Reagent A.C.S grade were purchased from Spectrum Chemical MFG CORP (New Brunswick, NJ).

### **3.2.2 Glycerol induced phase separation:**

Glycerol at concentration of 5 – 250 g/L with 5 g/L increments was dissolved in 1:1 (v/v) ratio of ACN-water mixture (5 mL of ACN and 5 mL of distilled water) in a capped 15 mL graduated VWR polypropylene plastic tube. The mixture was mixed with gentle vortexing and observed for phase separation at 20 °C and 4 °C after incubating overnight for each of the temperatures. The temperature was controlled using a water bath.

The results were expressed in terms of phase ratio (PR) and percent purity of ACN separated

$$PR = \frac{\text{volume of upper rich ACN phase}}{\text{volume of lower rich water phase}}$$

### 3.2.3 Comparison of different MSA:

After determining 20% of glycerol as optimum amount of MSA to cause phase separation of ACN-water mixture. Following example was performed to compare efficiencies of different MSAs such as glycerol, sorbitol, glucose, and sodium chloride. To five different graduated VWR polypropylene plastic tubes. (15 mL) containing each 10 mL of a 1:1 mixture of ACN and water was added 2g of glycerol (20% wt/v) which is the MSA. The mixtures were mixed with gentle vortexing until the MSAs dissolved and subjected to temperature controlled environment at 20 °C, 10 °C, 0 °C, -10 °C and -20 °C for 120 minutes.

Similar procedure was repeated for above mentioned MSAs For most of MSAs two layers were immediately formed of upper layer consisting mostly of ACN and more dense water and MSA as the lower layer. The results were based on percent purity of ACN, phase ratio and percent yield obtained:

$$PR = \frac{\text{volume of upper rich ACN phase}}{\text{volume of lower rich water phase}}$$

$$\text{Percent yield} = \frac{\text{volume of upper phase}}{\text{initial volume of ACN added}} \times 100 \times \text{percent purity of ACN}$$

### 3.2.4 Thermodynamic evaluation of glycerol induced phase separation:

Thermodynamic investigation is one of the most important criteria to understand the scientific principal governing the project.

We have derived the expression for equilibrium constant which is given by following equation

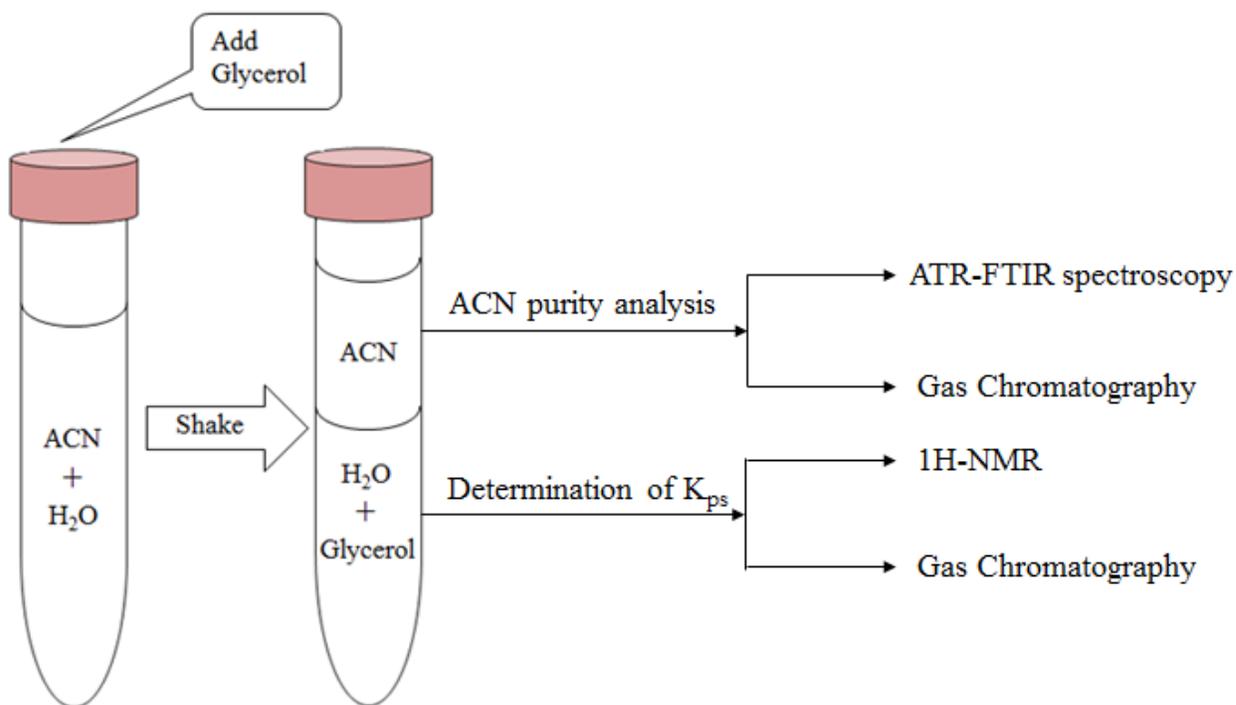
$$K_{ps} = \frac{[P_{(aq)}]}{[Org_{(aq)}]}$$

Based on above mentioned expression, equilibrium constant ( $K_{ps}$ ) was determined by measuring concentration of ACN and glycerol in lower layer of phase separation at different temperatures. The van't Hoff equation was plotted to determine  $\Delta H$ ,  $\Delta G$  and  $\Delta S$  values for the phase separation process

Enthalpy ( $\Delta H$ ) was further determined separately using direct calorimetric calculation using EasyMax reaction calorimetry.

### 3.2.5 Analysis:

**Figure 28: Schematic for analysis of glycerol induced phase separation.**



For percent ACN analysis:

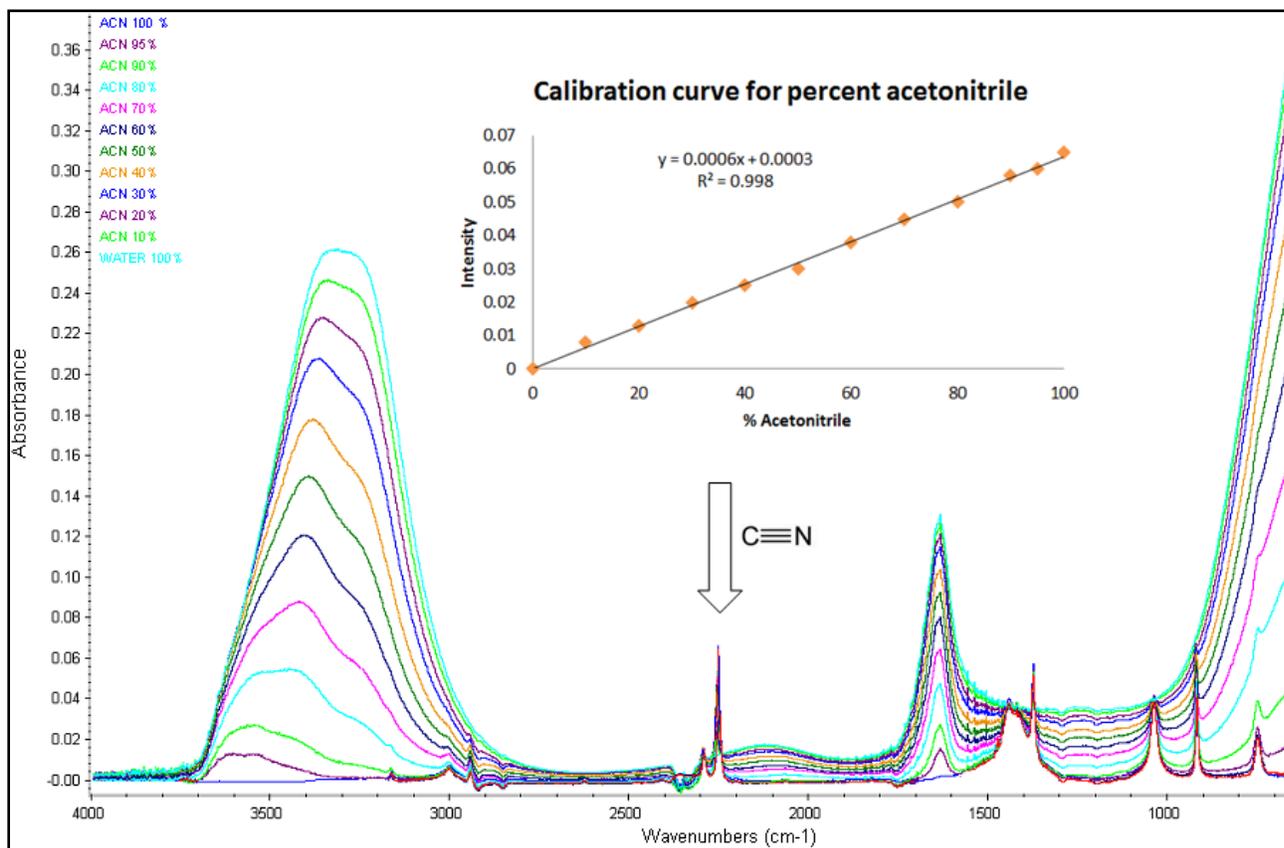
The percent purity of ACN in upper phase was determined using an infrared spectrometer with an attenuated total reflectance (ATR) accessory in absorbance mode. The results were compared to acetonitrile concentration determined by a gas chromatograph unit (GC) equipped with a thermal conductivity detector (TCD).

For determination of Equilibrium constant:

Amount of glycerol and acetonitrile in lower layer of the phase separated mixture was analyzed using  $^1\text{H-NMR}$  and results were compared with analysis from GC-TCD.

ATR-FTIR analysis:

**Figure 29: ATR-FTIR analysis for ACN purity using calibration curve**



For ATR-FTIR analysis, a thin film of liquid sample was carefully overlain, in direct contact, on the ZnSe crystal mounted on a Nicolet 4700 FTIR spectrometer (Thermo Electron Corporation, USA). The resolution was set to 4 wavenumbers. The optical range used by the system was: 4000–650  $\text{cm}^{-1}$ . The gain adjustment was set to normal (1 $\times$ ) and the apodization method was set to Happ-Genzel. The spectrophotometer was linked to a personal computer equipped with Nicolet Omnic software version 7.1 allowing the continuous automated collection and subsequent manipulation of spectra. ACN content determinations were based on absorption band intensities (areas of spectral bands) between 2300  $\text{cm}^{-1}$  and 2200  $\text{cm}^{-1}$  where absorption band at 2253  $\text{cm}^{-1}$  was specific for ACN corresponding to its nitrile group ( $\text{C}\equiv\text{N}$ ). The calibration curves were constructed using the ratio of absorbance intensities in the investigated regions. Using appropriate baseline correction the linearity of the calibration curves was maintained over the entire investigated ACN content regions and used to calculate unknown concentration of ACN.

#### GC analysis:

Acetonitrile concentration was determined using a HP 6890N Gas Chromatograph (GC) equipped with a HP7683B Series Autoinjector and TCD detector. The column used was Restex Rtx®-Wax ,30 m, 0.25 mm ID, 0.50  $\mu\text{m}$ . The oven temperature was programmed at 50  $^{\circ}\text{C}$  (hold 7 min) to 250  $^{\circ}\text{C}$  at 10 $^{\circ}\text{C}/\text{min}$  (hold 15 min). The injector and detector temperature was set to 250  $^{\circ}\text{C}$  and TCD @ 250  $^{\circ}\text{C}$  respectively. The carrier gas was He at 1.0  $\text{ml min}^{-1}$  flowrate and ethyl caproate was used as an internal standard. The data was interpreted using Agilent Technologies GC Chemstation software (Agilent Technologies, Germany).

***Analysis of upper phase:***

The percent purity of acetonitrile in upper phase was determined using the direct integration results from Agilent's Chemstation software.

***Analysis of lower phase:***

The lower phase was analyzed to determine the moles of acetonitrile and glycerol which were used to calculate  $K_{SP}$  at different temperatures.

Preparation of the internal standard: To approximately 1 g of water, acetonitrile, and glycerol was added 2 mL of ethyl caproate (internal standard) in a 10 mL volumetric flask. The mixture was further diluted to the 10 mL mark with methanol..

Preparation of samples from lower phase: To 1 mL of the lower phase was added 2mL of internal standard in 10 mL volumetric flask. The mixture was further diluted up to 10 mL mark using methanol

The response factor (RF) of each specific compound of interest (SC) in relation to the internal standard (IS) was determined by following equation from the GC results obtained:

$$\text{Internal Response Factor} = \frac{\text{area}_{\text{IS}} \times \text{amount}_{\text{SC}}}{\text{amount}_{\text{IS}} \times \text{area}_{\text{SC}}}$$

IS= Internal standard

SC= Specific compound of interest

Once the response factors were determined, the amounts of acetonitrile, water, and glycerol in the lower phase were quantitated through the use of the following equation:

$$\text{Amount of Specific Compound} = \frac{\text{amount}_{\text{IS}} \times \text{area}_{\text{SC}} \times \text{IRF}_{\text{SC}}}{\text{area}_{\text{IS}}}$$

IS= Internal standard

SC= Specific compound of interest

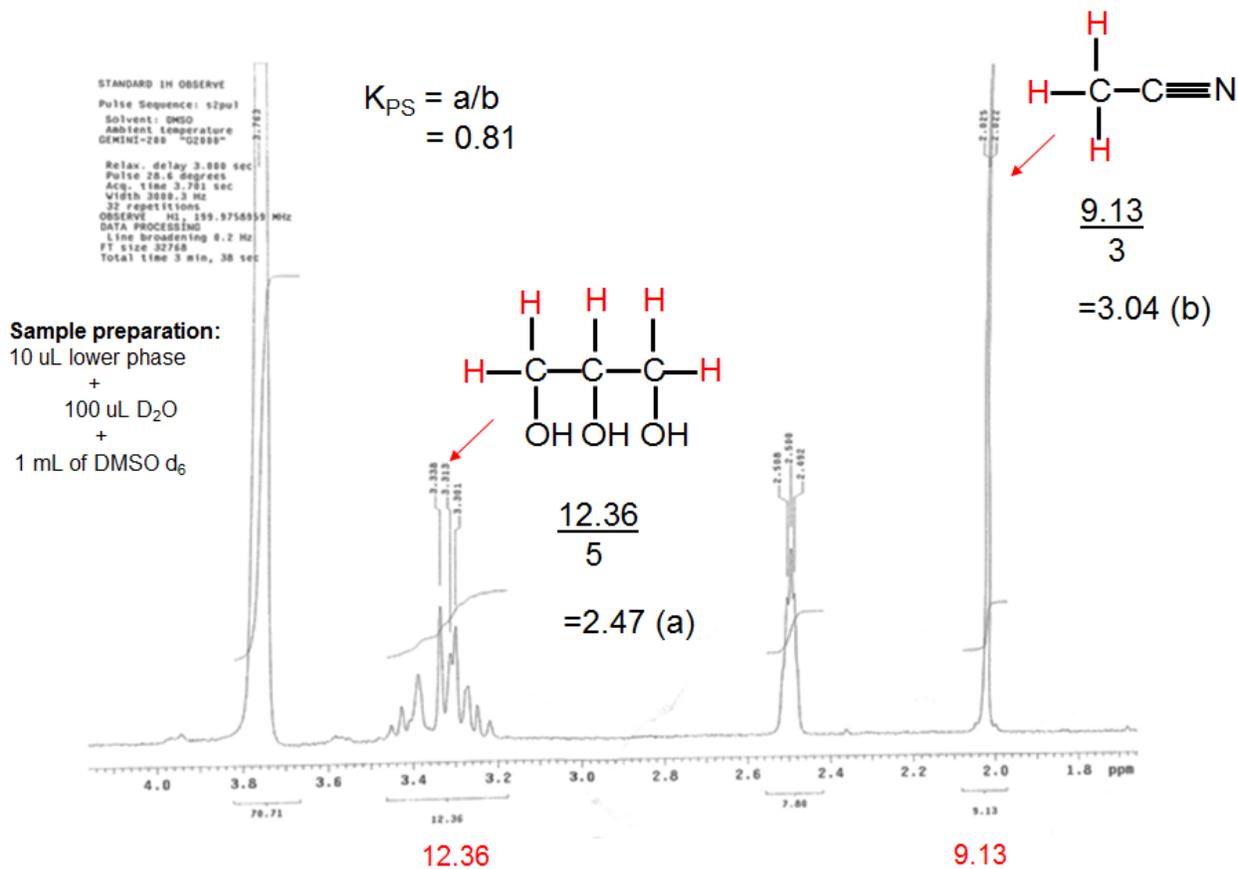
IRF= Internal response factor

*<sup>1</sup>H-NMR analysis:*

To determine the amount of ACN and glycerol in lower phase, analysis was performed using a Varian Gemini 2000 NMR, <sup>1</sup>H Frequency = 200 MHz. For quantitative determination, the number of moles of relative proton in each molecule was determined based on integration of each peak obtained after <sup>1</sup>H-NMR analysis.

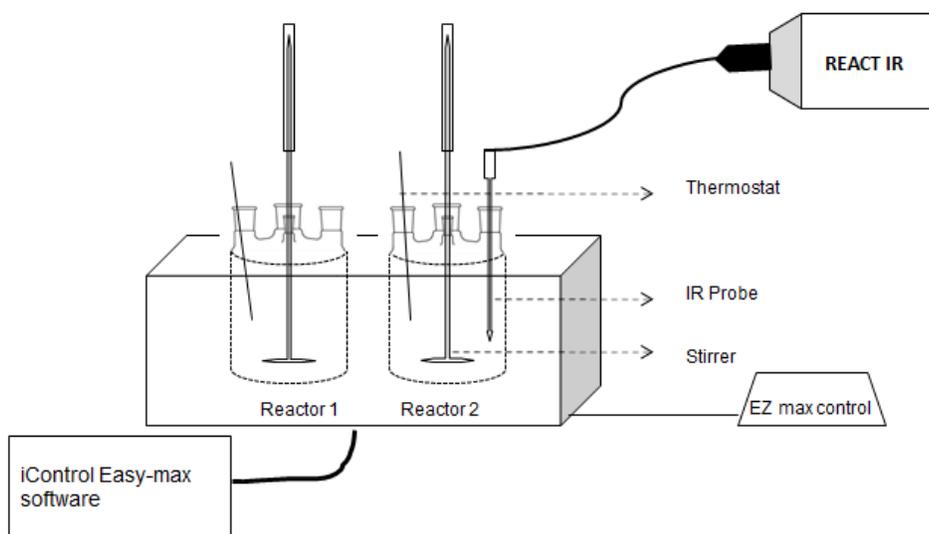
Figure 30 shows an example of how the <sup>1</sup>H-NMR technique was used to determine K<sub>PS</sub>. In the following example (glycerol induced phase separation of ACN-water at -20 °C) the sample was initially prepared by adding 100 μL deuterated water (D<sub>2</sub>O) and 1 mL of deuterated dimethyl sulfoxide ((DMSO-d<sub>6</sub>)) to 10 μL of lower phase. The sample was subjected to <sup>1</sup>H-NMR analysis to obtain peaks relevant to composition of sample content. The peak near 2.02 ppm corresponded to ACN whereas peaks near 3.30 ppm were relevant to carbonyl proton on glycerol. Both the peaks were integrated to obtain values which corresponded to relative number of protons of ACN and glycerol present in the composition, which further gave number of moles of ACN ('b'- Figure 30) and glycerol ('a'- Figure 30). The equilibrium constant (K<sub>PS</sub>) was then calculated by dividing number of moles of glycerol with moles of ACN as shown in Figure 30

**Figure 30: Example of determination of  $K_{PS}$  using  $^1\text{H-NMR}$  analysis**



Enthalpy determination using Mettler toledo's EasyMax automated reaction monitoring system:

**Figure 31: EasyMax reaction calorimeter**



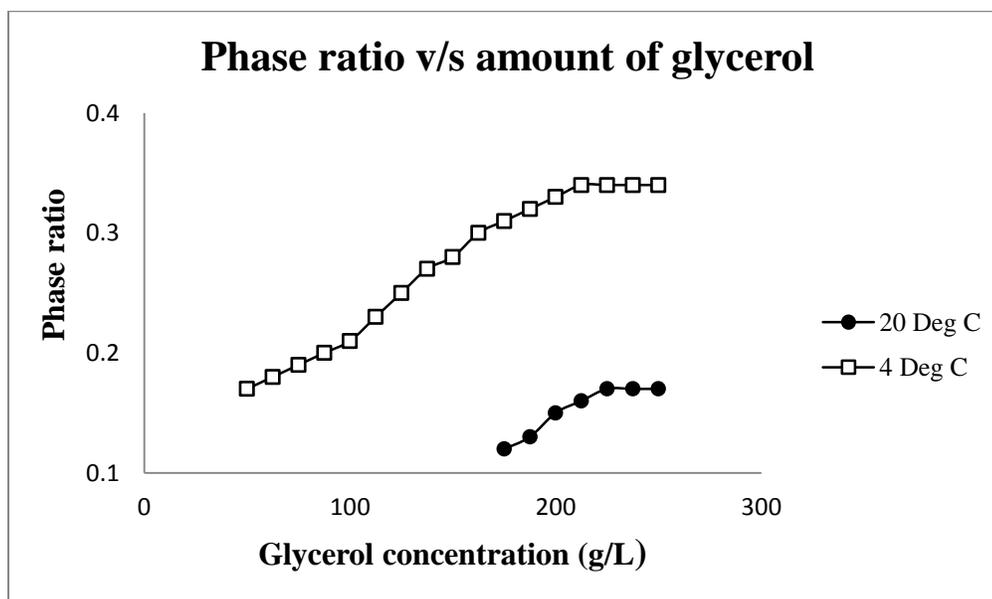
EasyMax was used to determine enthalpy of phase separation at different temperatures where 20 % of MSA was added to 100 mL mixture of ACN-water, 1:1 (v/v) ratio. Following parameters were employed for the experiment:

Stirring rate	300 rpm
Working Volume	100 mL
Pressure	ambient pressure
Temperature	-20° C to 20° C
Heating/Cooling	Solid state thermostat
Control	iControl PC software and touchscreen
Software	iControl 4.1

### **3.3 Results and discussion**

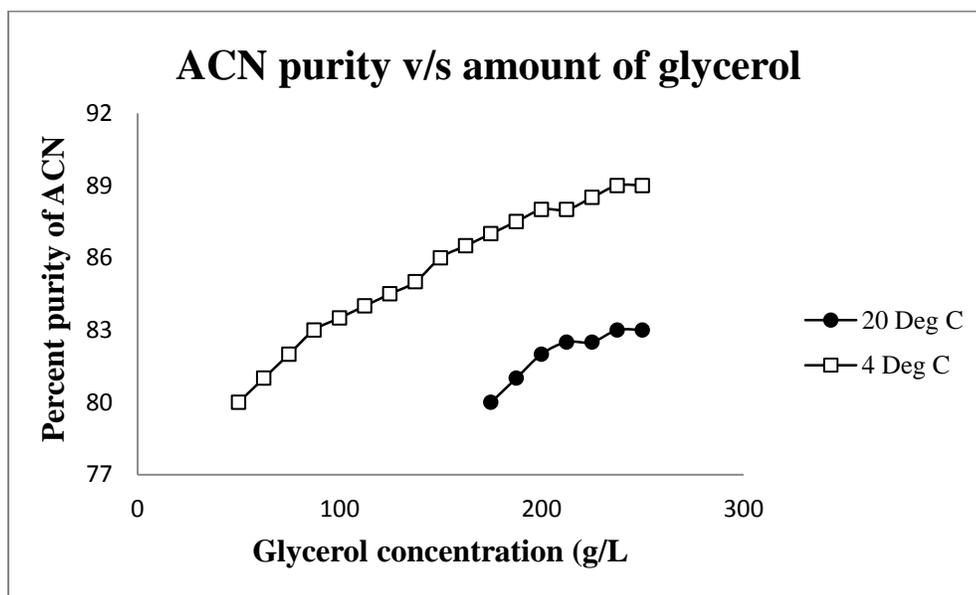
#### **3.3.1 Effect of temperature and glycerol concentration on phase separation and acetonitrile recovery:**

**Figure 32: Phase ratio of glycerol induced phase separation of an ACN-water mixture 1:1 (v/v) at 20 °C and 4 °C**



It was observed that when glycerol was added to an ACN-water mixture, phase separation occurred with upper rich acetonitrile and lower aqueous layer. In this study we found that as concentration of glycerol increased the phase ratio improved over the entire range. It was further observed that the phase separation could be further enhanced by combining glycerol addition and cooling procedures. Figure.32, clearly shows that glycerol could initiate phase separation of 1:1 (v/v) ratio of ACN-water mixture at 20 °C when its concentration was 175 g/L of ACN-water mixture and the phase ratio reached a plateau after a certain limit. However at 4 °C only 50 g/L of glycerol was able to initialize phase separation which showed higher phase ratio throughout the range as compared to that at higher temperature until it reached a plateau.

**Figure 33: The percentage purity of ACN obtained after glycerol induced phase separation of an ACN-water mixture 1:1 (v/v) at 20 °C and 4 °C**



When upper rich acetonitrile layer was analyzed for ACN concentration, it revealed that purity had significantly improved to 90 % with most of the ACN recovered in upper phase. Figure.33 shows that glycerol induced phase separation enriches ACN to above 82 % with most being at 96 % at much lower temperature condition (see table 7)

The formation of phase separation by glycerol can be explained based on interaction between ACN, water and MSA which was also reported earlier by other authors in salting out and sugaring out systems,<sup>77</sup> where ACN molecules form three dimensional clusters. These clusters were surrounded by water molecules through hydrogen bonding and dipole-dipole interaction between adjacent ACN molecules. The intermolecular interaction between ACN-water competes with water-glycerol where existing hydrogen bond between ACN-water molecules were replaced by water-glycerol due to stronger hydrogen bonding ability leaving free ACN molecules to form separate phase.

### 3.3.2. Effect of temperature on phase separation.

It has been previously reported that a 1:1 (v/v) ratio of ACN-water mixture undergoes phase separation without addition of MSA only at temperatures below  $-1.32\text{ }^{\circ}\text{C}$ <sup>78</sup> and. Our experiments indicated that addition of glycerol not only enhances the phase separation at subzero temperatures but also show phase separation above subzero temperatures. Under low temperature conditions the experiment clearly shows that lower temperature favors the phase separation of ACN-water mixture.<sup>79</sup> The phase separation improved significantly when temperature was reduced from  $20\text{ }^{\circ}\text{C}$  to  $4\text{ }^{\circ}\text{C}$  with higher phase ratio and initiation of phase separation at lower glycerol concentration. The analysis of upper phase ACN concentration also revealed that purity was enhanced to 90 % at  $4\text{ }^{\circ}\text{C}$  and up to 96 % pure ACN at much lower temperatures (see table 7)

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<sup>77</sup>(a) Cringus, D.; Yeremenko, S *et al.* Hydrogen and Vibration energy relaxation in water-acetonitrile mixture *J.Phys.Chem* **2004**,108, 10376-10387.; (b) Takamuku, T.;Yamaguchi, A.; Matsuo, D.; Tabata, M.; Kumamoto, M.; Nishimoto, J *et al.* Liquid structure of acetonitrile-water mixture by X-ray diffraction and Infrared Spectroscopy.*J Phys Chem B* **1998**; 102: 8880-8888.;(c) Pence, D.N.; Gu, T.Y. Liquid-Liquid equilibrium of the acetonitrile-water system for protein purification. *Sep Technol.* **1996**; 6, 261-264

<sup>78</sup> Hartnett, R L (Monsanto Co., USA). Method for the purification of acetonitrile by low temperature phase separation. European patent EP0113331, DEC 28, **1983**.

<sup>79</sup> Dhamole, P. B., Mahajan, P., Feng, H. Phase separation conditions for sugaring out in acetonitrile-water systems. *J.Chem .Eng .Data.* **2010**, 55, 3803-3806.

It can be thus hypothesized that the degree of hydrogen bonding depends on temperature. At lower temperatures, the distance between adjacent ACN and water molecules increases. Thus MSA molecules will find more chance to replace ACN molecules to associate with water molecules and essentially forming separate phase for ACN.

### 3.3.3 Temperature studies of glycerol-induced extraction of water from acetonitrile (ACN) and determination of $K_{PS}$

**Table 7: Effect of temperature on glycerol (20% wt/v) induced phase separation on 1:1 (v/v) mixture of ACN-water**

<b>Temp</b>	<b>20 °C</b>	<b>10 °C</b>	<b>0 °C</b>	<b>-10 °C</b>	<b>-20 °C</b>
<sup>a</sup> Volume of Upper phase in mL	*1.5±0.05	2.5±0.08	3.0±0.18	3.5±0.18	4.0±0.32
<sup>b</sup> Volume of Lower phase in mL	10.0±0.50	9.0±0.27	8.5±0.50	8.0±0.40	7.5±0.60
Percent purity of ACN in upper phase (%)	82±0.58	85±1.53	89±1.00	93±1.53	96±1.00
<sup>a/b</sup> Phase ratio	0.15±0.01	0.28±0.01	0.35±0.02	0.44±0.02	0.57±0.05
$K_{ps} = [P_{(aq)}/Org_{(aq)}]$	0.38±0.02	0.48±0.02	0.60±0.03	0.69±0.02	0.81±0.06

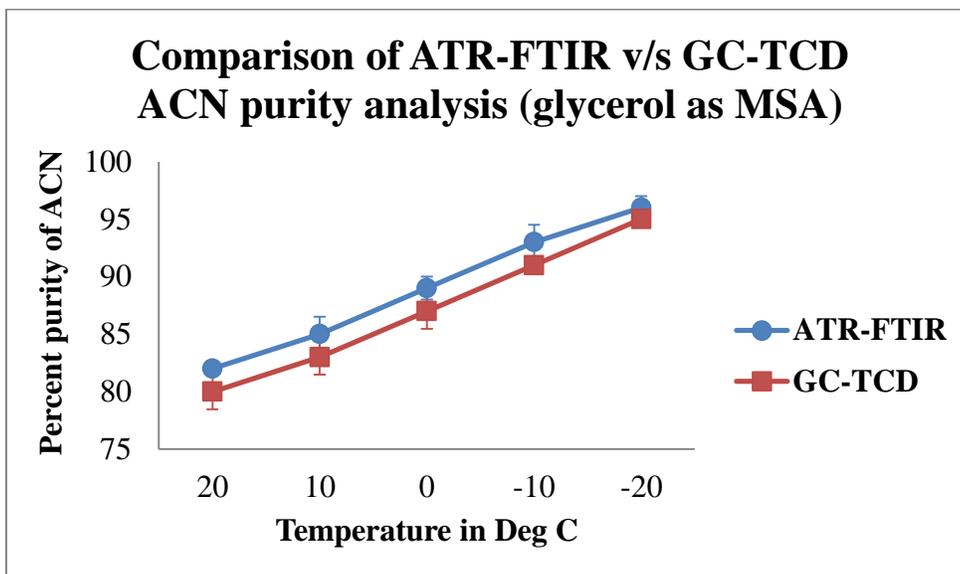
\*± = Standard deviation, n=3

Table 7 shows effect of glycerol (20% wt/v) induced phase separation on a 10 mL ACN-water mixture, 1:1 (v/v) ratio at five different temperatures (20 °C, 10 °C, 0 °C, -10 °C and -20 °C). The results obtained clearly showed that as temperature was lowered from 20 °C to -20 °C, there was enhancement in the phase ratio and percent purity of ACN in upper phase. The maximum phase ratio of 0.57 and 96 % pure ACN was obtained at -20 °C which indicate that lower temperature favored better phase separation. The equilibrium constant ( $K_{PS}$ ) values obtained by analyzing amount of

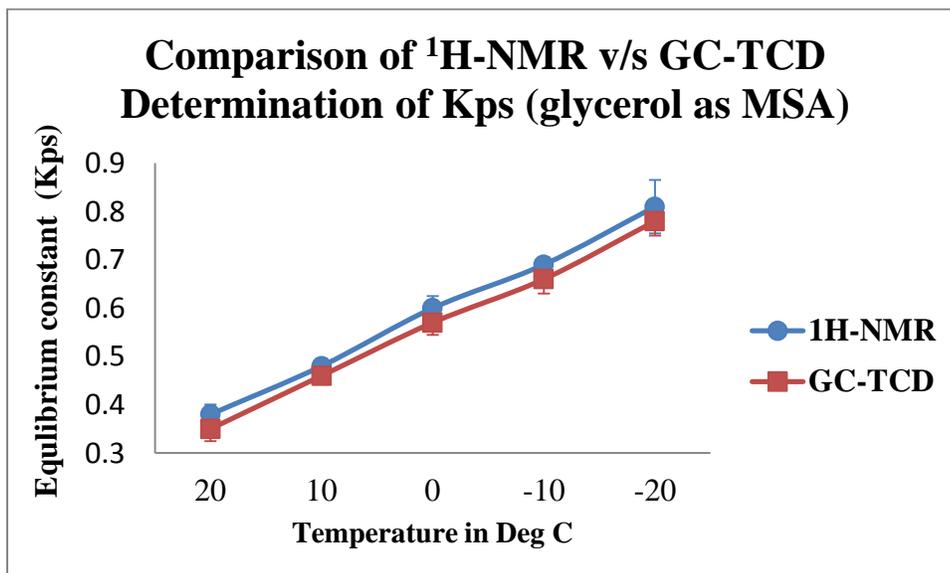
glycerol and ACN in lower phase showed linear increment approaching  $K_{PS}=1$  as temperature was lowered from 20 °C to -20 °C.

Analysis of ACN purity (Figure 34) and determination of  $K_{PS}$  (Figure 35) by the GC-TCD technique showed comparable results to ATR-FTIR and  $^1\text{H-NMR}$  methods.

**Figure 34: ACN purity analysis using ATR-FTIR and GC-TCD.**



**Figure 35: Determination of  $K_{PS}$  using  $^1\text{H-NMR}$  and GC-TCD.**



### 3.3.4 Temperature studies of sorbitol-induced extraction of water from acetonitrile (ACN) and determination of $K_{PS}$

**Table 8: Effect of temperature on sorbitol (20% wt/v) induced phase separation on 1:1 (v/v) mixture of ACN-water**

Temp	20 °C	10 °C	0 °C	-10 °C	-20 °C
<sup>a</sup> Volume of Upper phase in mL	*3.5±0.06	3.5±0.00	4±0.06	4±0.06	4±0.00
<sup>b</sup> Volume of Lower phase in mL	7.5±0.00	7.5±0.06	7±0.00	7±0.06	7±0.06
Percent purity of ACN in upper phase (%)	83±1.00	85±0.57	88±1.15	91±1.00	96±1.00
<sup>a/b</sup> Phase ratio	0.46±0.01	0.46±0.01	0.57±0.00	0.57±0.01	0.57±0.00
$K_{ps} = [P_{(aq)}/Org_{(aq)}]$	0.38±0.01	0.43±0.01	0.50±0.01	0.57±0.01	0.66±0.01

\*± = Standard deviation, n=3

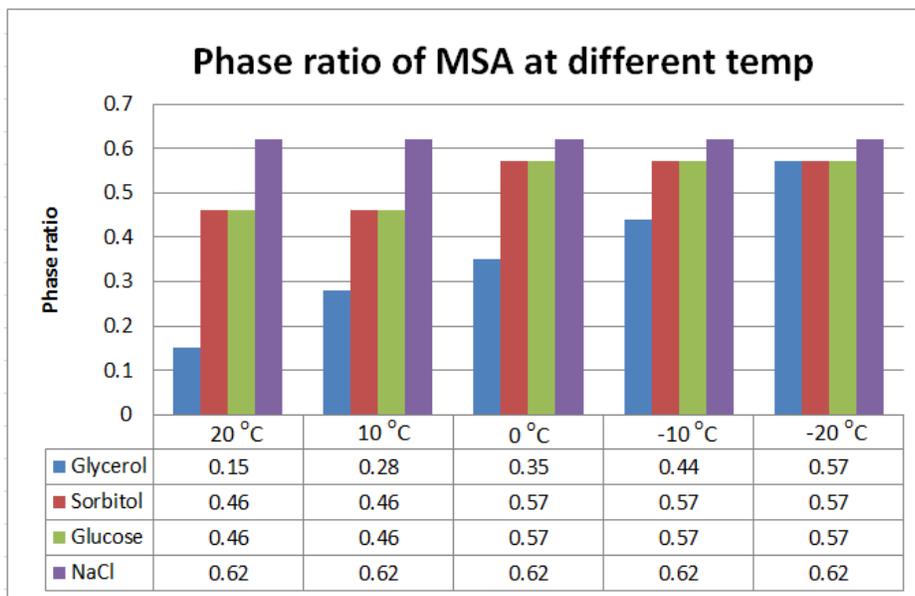
Table 8 shows the effect of sorbitol (20% wt/v) induced phase separation on 10 mL ACN-water mixture, 1:1 (v/v) ratio at five different temperatures (20 °C, 10 °C, 0 °C, -10 °C and -20 °C). Similar trend of results for sorbitol was seen as that of glycerol induced phase separation where there was enhancement in phase ratio and percent purity of ACN in upper phase as temperature was decreased.

The phase ratio for sorbitol induced phase separation was better than glycerol at higher temperature but was less so at lower temperature. The maximum phase ratio of 0.57 and 96 % pure ACN was obtained at -20 °C which indicated that lower temperature favored better phase separation. The equilibrium constant ( $K_{PS}$ ) values obtained also showed a linear increase as temperature was lowered from 20 °C to -20 °C.

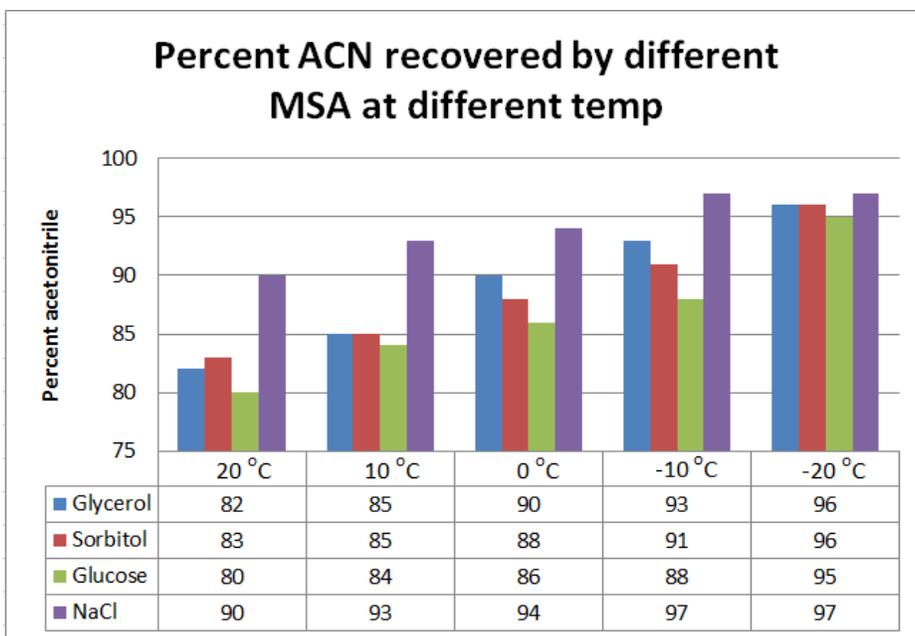
### 3.3.5 Comparison of different MSAs:

Figure 36, 37 and 38 show comparisons of different MSAs based on parameters such as phase ratio, ACN purity analysis and percent yield of ACN obtained.

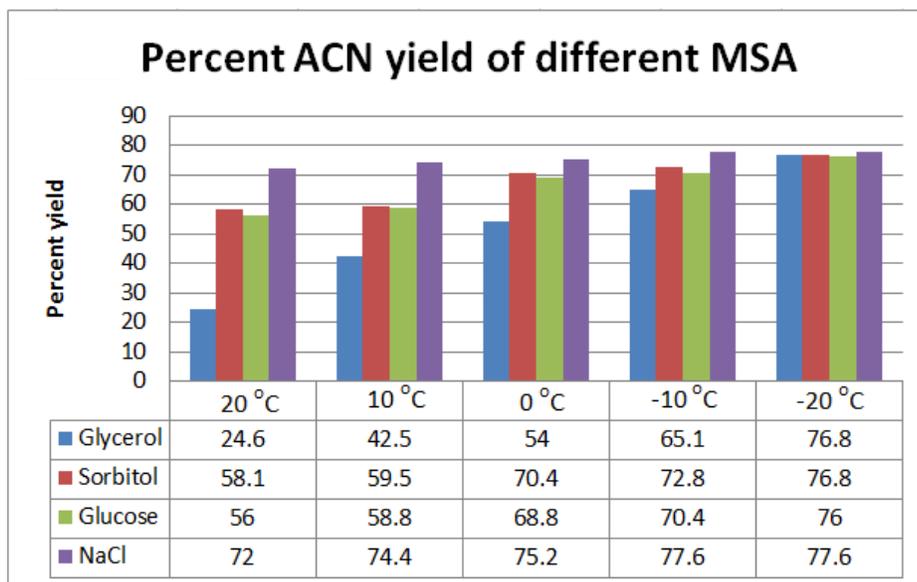
**Figure 36: Phase ratio comparison of different MSAs at different temperatures.**



**Figure 37: ACN purity analysis of different MSAs at different temperatures.**

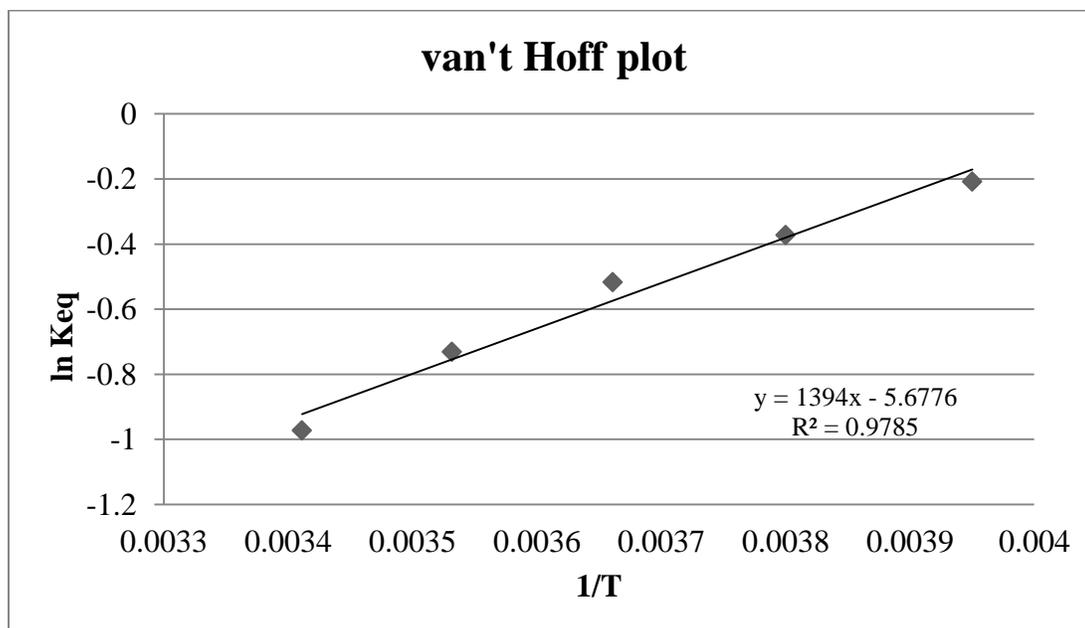


**Figure 38: Percent yield of ACN recovered with different MSA at different temperatures.**



### 3.3.6 Thermodynamic investigation of glycerol induced phase separation

**Figure 39: van't Hoff plot for glycerol induced phase separation.**



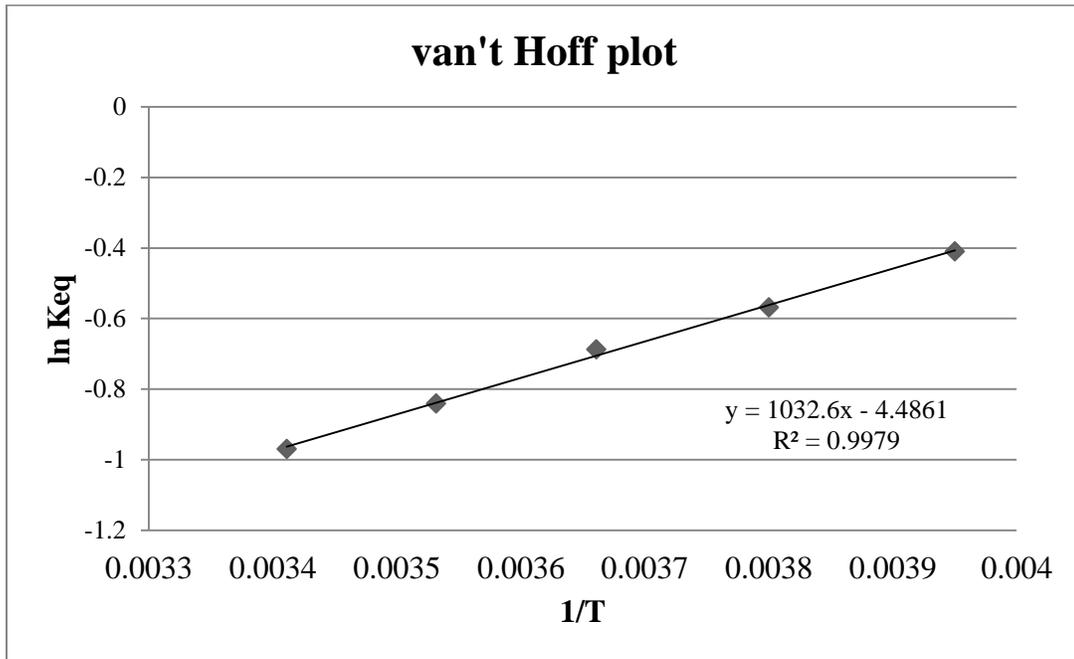
**Table 9: Thermodynamic parameters for glycerol induced phase separation**

Temp in °C	$K_{PS}$	$\Delta H(\text{kJ/mol})$ (van't Hoff)	$\Delta H(\text{kJ/mol})$ (EZMAX)	$\Delta S(\text{J/mol})$ (van't Hoff)	$\Delta G(\text{J})$ (van't Hoff)	$\Delta G(\text{J})$ - $RT\ln K_{eq}$
20	0.38±0.02	-11.59	-2.06±0.08	-47.20	2246.97	2371.49
10	0.48±0.02		-3.46±0.11		1774.97	1720.96
0	0.60±0.03		-5.04±0.07		1302.97	1174.90
-10	0.69±0.02		-7.07±0.06		830.97	815.28
-20	0.81±0.06		-74.99±1.09		358.97	437.61

\* ± = Standard deviation, n=3

**3.3.7 Thermodynamic investigation of sorbitol induced phase separation**

**Figure 40: van't Hoff plot for sorbitol induced phase separation.**



**Table 10: Thermodynamic parameters for sorbitol induced phase separation**

Temp in °C	K <sub>PS</sub>	ΔH(kJ/mol) (van't Hoff)	ΔS(J/mol) (van't Hoff)	ΔG(J) (van't Hoff)	ΔG(J) -RTlnK <sub>eq</sub>
20	0.38±0.01	-8.59	-37.29	2346.53	2363.69
10	0.43±0.01			1973.63	1980.07
0	0.50±0.01			1600.73	1562.24
-10	0.57±0.01			1227.83	1243.26
-20	0.66±0.01			854.93	863.39

\* ± = Standard deviation, n=3

When two or more substances are mixed, ΔG is defined as the difference between the Gibbs free energy of the solution and the pure compounds. If ΔG ≤ 0, forms a stable single phase solution, but if ΔG ≥ 0, the homogeneous solution is unstable and the system is forced to split into two or more phases in order to minimize the Gibbs free energy.

**As indicated by the Gibbs equation, an increase in K<sub>PS</sub> leads to a more decreasing value of ΔG which is interpreted as a decrease in free energy. Thus, phase separation is driven by a decrease in free energy of the reaction.**

$$\Delta G = -RT \ln K_{PS}$$

Where R is the gas constant (8.314 J/T mole), J is Joules and T is temperature in Kelvin (K)

The values determined by <sup>1</sup>H-NMR, van't Hoff plot and calorimetric calculations with EasyMax clearly support the proposed theory behind phase separation process.

The effects of temperature on glycerol induced phase separation on acetonitrile-water system showed that the miscibility region was reduced as temperature decreased, indicating an exothermic phase separation process. These features clearly suggest that phase separation is more influenced by enthalpic than entropic contributions.

### **3.4 Conclusion:**

Phase separation induced by addition of MSAs such as glycerol is a useful method for the removal of water from ACN-water mixture. With appropriate concentration and temperature conditions, we were able to demonstrate that glycerol can improve purity of ACN up to 96 % which was comparable with those reported by other MSAs in sugaring out and salting out techniques. Our thermodynamic investigation based on determination of change in free energy ( $\Delta G$ ) and change in enthalpy ( $\Delta H$ ) revealed that glycerol and sorbitol induced phase separation is driven by decrease in free energy of process and that phase separation is primarily an exothermic process influenced by enthalpic contribution.

## CHAPTER 4:

### APPLICATIONS AND FUTURE WORK

#### 4.1 Extraction and separation system: Polyol induced extraction (PIE)

Liquid-liquid extraction (LLE) also known as solvent extraction, is one of the most well established and studied method of separating compounds having different solubility in two immiscible liquids. This method has been widely employed in hydrometallurgical, nuclear, pharmaceutical, and food industries to separate target components from liquid mixtures.<sup>80</sup> The two liquids are typically water and a nonpolar organic solvent such as hexane, diethyl ether, methyl tertiary butyl ether (MTBE), dichloromethane and ethyl acetate. However, the conventional LLE procedures are time consuming, generally labor intensive and require large quantities of expensive, toxic and environmentally unfriendly organic solvents. Another drawback of the use of nonpolar, water-immiscible organic solvents is that due to their low dielectric constants, they are relatively poor at the extraction of very polar or highly charged solutes, particularly for highly water-soluble pharmaceuticals that may require extractions at very low or very high pH values.<sup>81</sup> Acetonitrile is an organic solvent less harmful than the conventional LLE solvents used, this makes it more favorable within a green chemistry context. Furthermore its polarity is favorable to the extraction of a wide range of compounds and it is compatible with gas and liquid chromatography but since acetonitrile is miscible with water in all the proportions it cannot be used for conventional LLE.<sup>82</sup>

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<sup>80</sup> Thornton, J. D. Science and practice of liquid-liquid extraction (II): process chemistry and extraction operations in the hydrometallurgical, nuclear, pharmaceutical, and food industries. *Oxford: Clarendon Press* **1992**.

<sup>81</sup> Majors R.E , *LCGC* 26(12), **2008**,p. 1158–1166

<sup>82</sup> Majors R.E , *LCGC*, **2009**,p. 526-533

Valente et al.<sup>83</sup> have reviewed salting-out assisted liquid-liquid extraction in ACN-water system. Their review aims to discuss in more detail the salting-out phenomenon applied to the homogeneous liquid-liquid mixtures, with particular emphasis to water-acetonitrile, for further application in the extraction of relevant compounds such as  $\alpha$ -dicarbonyl compounds in beverages and analysis by high performance liquid chromatography with spectrophotometric detection (HPLC-UV).

Our investigations have shown that polyols possess similar activity as salts in salting-out and sugars in sugaring-out processes. Thus ‘*polyol induced extraction*’ (*PIE*) can be adapted as a method of extraction of various analytes from organic solvents such as acetonitrile.

#### 4.1.1 Extraction of metals complexes

The scarcity in natural resources of platinum group metals (PGMs), especially platinum (Pt), palladium (Pd) and rhodium (Rh), attracts a worldwide interest to develop various new techniques for recovering and refining Pt, Pd and Rh.<sup>84</sup> These metals have been extensively used in automobile catalytic converters due to their outstanding catalytic activity. Three liquid phase system (TLPS) and one-step separation of Pt(IV), Pd(II) and Rh(III), have been successfully investigated by Zhang *et al.* using salting-out,<sup>85</sup> sugaring-out<sup>86</sup> and ionic liquids<sup>87</sup> in acetonitrile-water. Their experimental results have demonstrated that Pd(II), Pt(IV) and Rh(III) can be separated in a one-step extraction process, respectively, into a diisopentyl sulfide organic top phase, mass separating agent induced acetonitrile-

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<sup>83</sup> Valente I. M., Gonclaves L.M., Rodrigues J.A., Another glimpse over the salting-out assisted liquid-liquid extraction in acetonitrile/water mixtures *J. Chromatogr. A*, **2013** 1308, p. 58-62

<sup>84</sup> Bernardis, F.L.; Grant, R.A.; Sherrington, D.C.; A review of methods of separation of the platinum-group metals through their chloro-complexes, *React. Funct. Polym* **2005**, 65 205–217.

<sup>85</sup> Zhang, C.; Huang, K.; Yu, P.; Liu, H, Salting-out induced three-liquid-phase separation of Pt(IV), Pd(II) and Rh(III) in system of S201-acetonitrile-NaCl-water, *Separation and Purification Technology* **2011**, 80, 81–89.

<sup>86</sup> Zhang, C.; Huang, K.; Yu, P.; Liu, H; Sugaring-out three-liquid-phase extraction and one-step separation of Pt(IV), Pd(II) and Rh(III) *Separation and Purification Technology* **2012**, 87, 127-134.

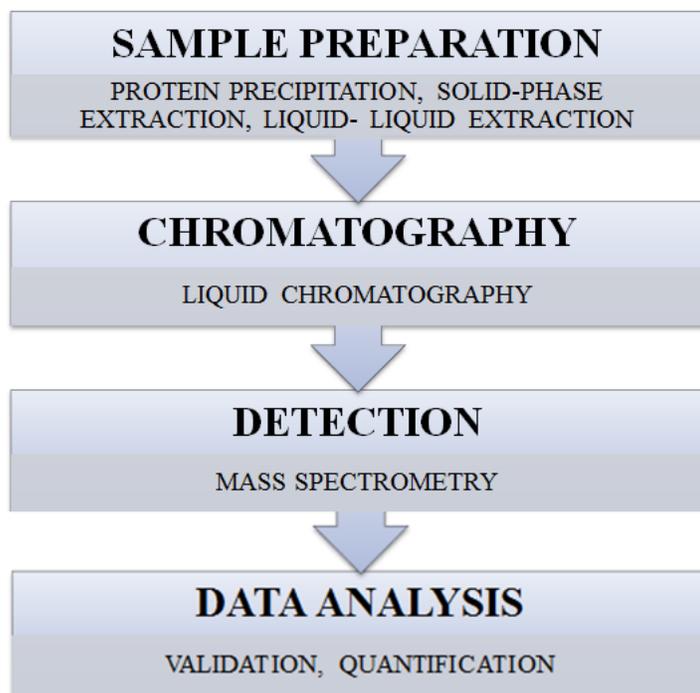
<sup>87</sup> Zhang, C.; Huang, K.; Yu, P.; Liu, H; Ionic liquid based three-liquid-phase partitioning and one-step separation of Pt(IV), Pd(II) and Rh(III) *Separation and Purification Technology* **2013**, 108, 166-173.

rich middle phase and aqueous bottom phase of the TLPS. Based on their experimental results and similarity in activity possessed by various mass separating agents, PIE can be effectively used as a novel method in extraction of PGMs using similar TLPS.

#### 4.1.2 Improved sample extraction technique in bio analysis:

Bioanalysis is the quantitative determination of drugs and their metabolites in biological systems. In the pharmaceutical industry it is used to provide a quantitative measure of the active drug and/or its metabolite(s) for the purpose of pharmacokinetics, toxicokinetics, bioequivalence and pharmacodynamics studies. Bioanalysis widely uses liquid chromatography–mass spectrometry (LC–MS–MS) as the main separation and quantification technique due to its sensitivity, selectivity, specificity, and robustness and typically batch sizes can consist of up to several hundred samples, standard, and QC injections. Although bioanalysis has been performed using these advanced analytical instruments, most of these instruments cannot handle the sample matrices directly because of the multitude of substances present in the biological samples, such as proteins, salts, acids, bases, and numerous organic components with chemical properties similar to those of the analytes, these could potentially interfere with the analysis. Thus, the introduction of more sensitive methods like mass spectrometers has further increased the burden on sample preparation and generation of cleanest extract as possible. The first analytical problem in the development of bioanalytical methods is the removal of macromolecular interferences to prevent blockage in the analytical column. Thus, sample preparation is usually necessary before analysis to clean up a sample and/or to concentrate a sample to improve its detection, greatly influencing the reliability and accuracy of results. LLE is a common sample preparation choice in regulated bioanalysis. LLE can generate a high analyte recovery and clean extraction. Depending on the compound being analyzed, the method still needs to be optimized.

**Figure 41: Basic steps involved in bio analytical sample assay**



It has long been known that the addition of an inorganic salt into a mixture of water and a water-miscible organic solvent causes a separation of the solvent from the mixture and the formation of a two-phase system<sup>88</sup> but a potential concern of the method for the subsequent LC–MS/MS analysis of biological samples is that a portion of the added salt (typically of high concentration) might be extracted and affect the chromatographic separation and ionization in a mass spectrometer.<sup>89,90</sup> Therefore, there is need of a mass separating agent (MSA) that not only has the potential for minimal interference in analysis but can be easily regenerated and recycled for

<sup>88</sup> Chang M.S., Ji Q.C., Zhang J., El-Shourbagy T.A., Historical review of sample preparation for chromatographic bioanalysis: pros and cons *Drug Dev. Res.* **2007**, 68,,107–133.

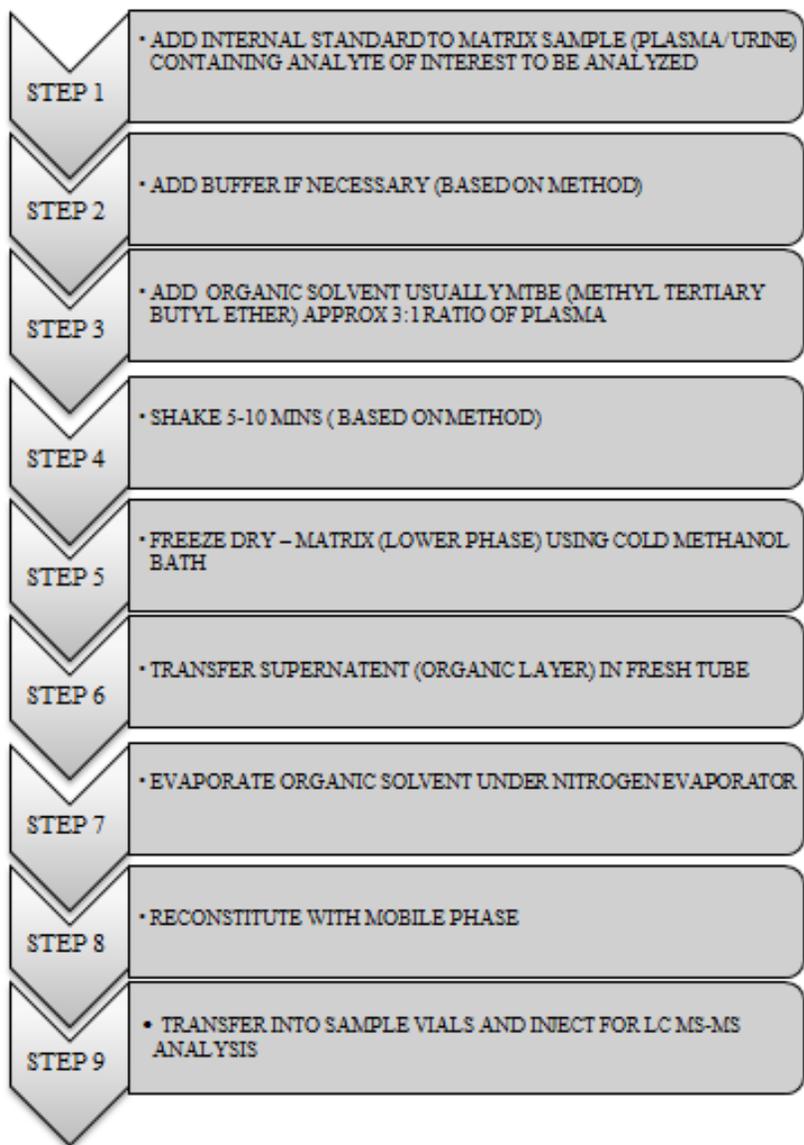
<sup>89</sup> Zhang J., Wu H., Kim E.J., El-Shourbagy T.A., Salting-out assisted liquid/liquid extraction with acetonitrile: a new high throughput sample preparation technique for good laboratory practice bioanalysis using liquid chromatography-mass spectrometry. *J. Biomed. Chromatogr.* **2009** . 23, 419–425

<sup>90</sup> Wu H., Zhang J., Norem K., El-Shourbagy T.A., Simultaneous determination of a hydrophobic drug candidate and its metabolite in human plasma with salting-out assisted liquid/liquid extraction using a mass spectrometry friendly salt...*J. Pharm. Biomed. Anal.* **2008**, 48, 1243–1248

environmental and economic reasons. Figure 1 and 2 shows a general protocol for sample analysis and the proposed novel sample preparation technique.

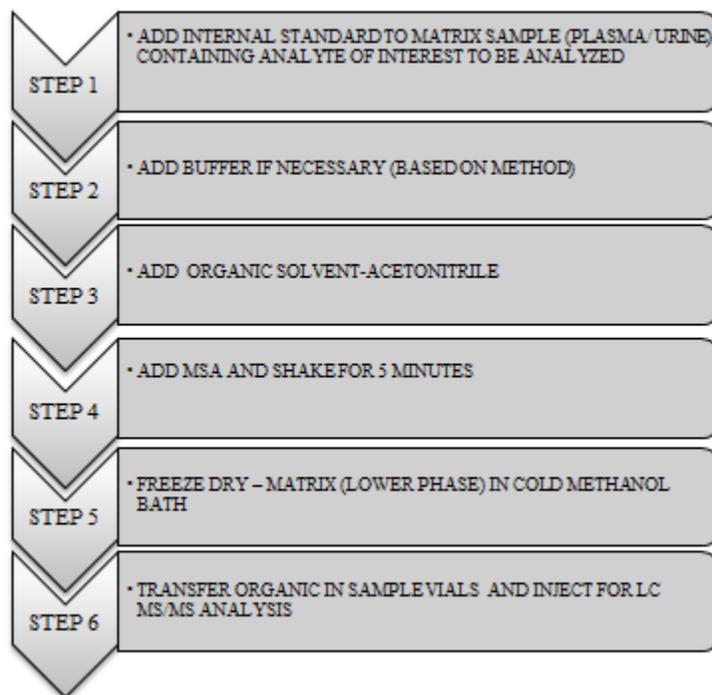
**Figure 42: Traditional LLE sample preparation technique in bioanalytical sample assay**

**TRADITIONAL PROTOCOL FOR SAMPLE PREPARATION  
USING LIQUID-LIQUID EXTRACTION**



**Figure 43: Proposed novel PIE sample preparation technique in bioanalytical sample assay.**

#### NOVEL METHOD FOR SAMPLE PREPARATION



## **4.2 MSA-induced extraction of water from ionic liquids**

Room-temperature ionic liquids (RTILs) are organic salts that are liquid at room temperature. They have unique chemical and physical properties including extremely low vapor pressure, tunable structure, high thermal and chemical stability, and excellent solvent power for organic and inorganic compounds. Because of these properties, they can serve as a “green” recyclable alternative to the volatile organic compounds that are traditionally used as industrial solvents.<sup>91,92</sup> These RTILs have been successfully used as solvents in many applications including organic and inorganic

<sup>91</sup> Welton, T.; Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. Chem Rev. 1999 Aug 11;99(8):2071-2084.

<sup>92</sup> Spear, S. K.; Visser, A. E.; Willauer, H. W.; Swatoski, R. P.; Griffin, S. T.; Huddleston, J. G.; Rogers, R. D. "Green Separations Science & Technology: Replacement of Volatile Organic Compounds in Industrial Scale Liquid-Liquid or Chromatographic Separations," In *Green Engineering*; Anastas, P. T.; Heine, L. G.; Williamson, T. C., Eds.; ACS Symposium Series 766, American Chemical Society: Washington, DC, 2001; pp 206-221.

syntheses,<sup>93,94</sup> to chemical separations;<sup>95,96</sup> however, due to the use of large amounts of solvents in synthesis processes of RTILs, solvent impurities cannot be avoided. Those solvent impurities mainly are water and moreover both hydrophilic RTILs and hydrophobic RTILs have the property to absorb water due to their hygroscopic nature. The remains of water and organic solvents not only reduce the purity of RTILs but also influence the physicochemical properties of RTILs. For example, the presence of water can change the conductivity, the viscosity, and the density of RTILs. Moreover, the presence of water can make the catalysts in ILs inactive and interfere with the intended chemical reaction.<sup>97</sup>

*Proof of concept:*

The following example was performed to determine efficiency of different MSAs such as glycerol, sorbitol, glucose, ethylene glycol and polyethylene glycol. To each 10 mL of a 1:1 mixture of ionic liquid (IL), 1-butyl-3-methylimidazolium tetrafluoroborate, and water was added 1 g of the above mentioned MSAs (10% w/v) and the mixture was swirled until the MSA dissolved. The mixture was then kept in temperature controlled environment at 0 °C for 10 min. For most of the MSAs, two layers were immediately formed consisting of an upper layer consisting mostly of water and MSA and a denser IL as the bottom layer. The results are shown below in Table 11.

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<sup>93</sup> Wu, H-H.; Yang, F.; Cui, P.; Tang, J.; He, M-Y.; An efficient procedure for protection of carbonyls in Bronsted acidic ionic liquid [Hmim]BF<sub>4</sub> *Tetrahedron Lett.*, **2004**, *45*, 4963-4965.

<sup>94</sup> Song, C. E.; Shim, W. H.; Roh, E. J.; Lee, S.; Choi, J. H. liquids as powerful media in scandium triflate catalysed Diels-Alder reactions: significant rate acceleration, selectivity improvement and easy recycling of catalyst *Chem. Commun.*, **2001**, 1122-1123.

<sup>95</sup> Rogers, R. D.; Visser, A. E.; Swatloski, R. P.; Hartman, D. H. "Metal Ion Separations in Room Temperature Ionic Liquids: Potential Replacements for Volatile Organic Diluents," In *Metal Separation Technologies Beyond 2000: Integrating Novel Chemistry with Processing*; Liddell, K. C.; Chaiko, D. J., Eds.; The Minerals, Metals & Materials Society: Warrendale, PA, 1999; pp 139-147.

<sup>96</sup> Huddleston, J. G.; Willauer, H. W.; Swatloski, R. P.; Visser, A. E.; Rogers, R. D. "Room Temperature Ionic Liquids as Novel Media for 'Clean' Liquid-Liquid Extraction," *Chem. Commun.* **1998**, 1765-1766.

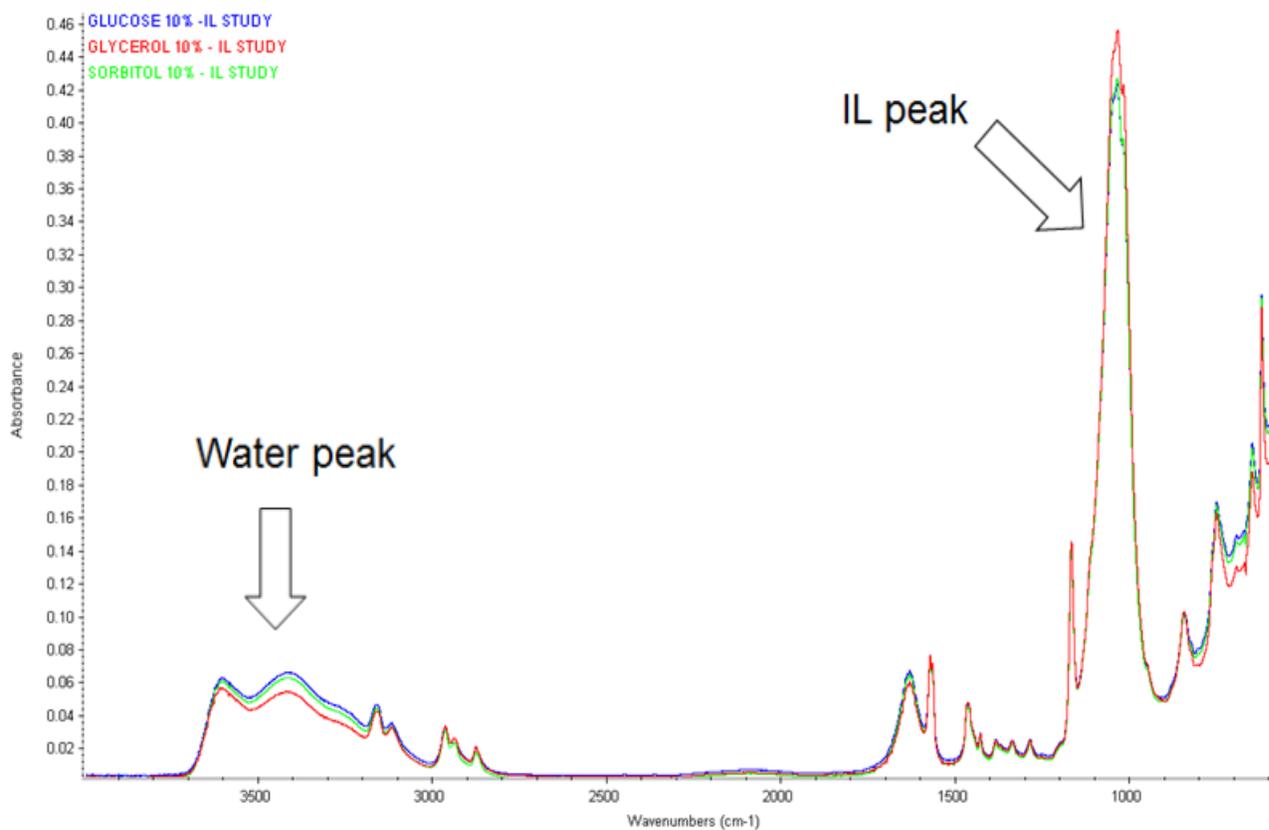
<sup>97</sup> Ren, S.; Hou, Y.; Wu, W.; Liu, W. Purification of Ionic Liquids: Sweeping Solvents by Nitrogen. *Journal of Chemical & Engineering Data* **2010** *55* (11), 5074-5077

**Table 11: Polyol induced phase separation of water-ionic liquid**

MSA	Glycerol	Sorbitol	Glucose	Ethylene glycol	Polyethylene glycol
<sup>a</sup> Volume of Upper Phase(mL)	5.5	5	5	NPS	NPS
<sup>b</sup> Volume of Upper Phase(mL)	5.5	6	6	NPS	NPS
Percent purity of IL obtained	86%	84%	84%	NPS	NPS
<sup>a/b</sup> Phase ratio	1	0.83	0.83	NPS	NPS

*\*NPS: no phase separation*

**Figure 44: IR spectra for extraction of water from ionic liquid by different MSA.**



The application was further extended to other ILs such as

1. 1-ethyl-3 methyl imidazolium methane sulfonate
2. 1-ethyl-3 methyl imidazolium ethyl sulfonate
3. 1-butyl-3 methyl-imidazolium tetrafluoroborate.

Only 1 butyl-3 methyl-imidazolium tetrafluoroborate showed phase separation with glycerol.

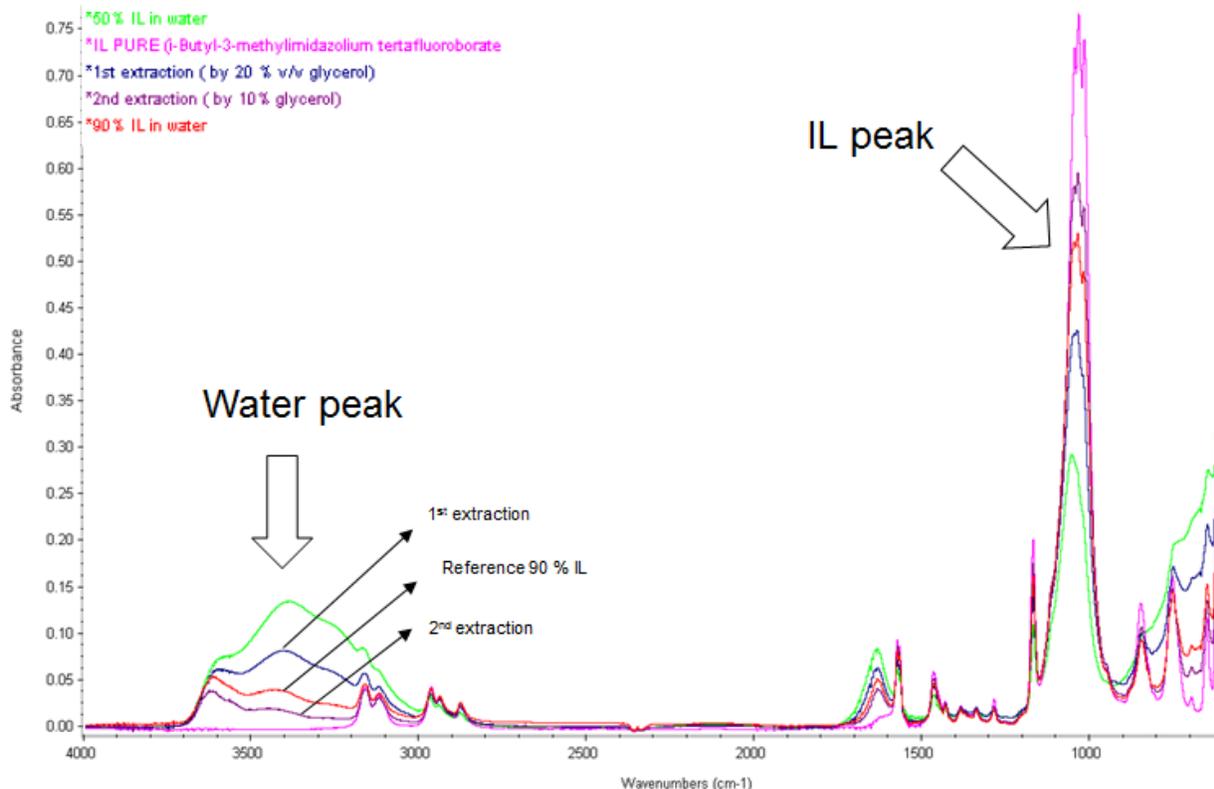
For different IL/water ratios of 10% / 90% , 25 %/ 75%, 50% /50% , 75%/25% and 90%/10% v/v phase separation was seen only at 25 %/ 75%, 50% /50% & 75%/25% v/v ratios of IL/water at room temperature.

The study was further extended to phase separation at lower temperature (-18 °C). The results indicate that phase separation between 1 butyl-3 methyl-imidazolium tetrafluoroborate and water with glycerol not only occurred at different ratios but even the percent purity of IL was significantly increased thus indicating lower temperature favors phase separation.

#### 4.2.1 Glycerol-induced extraction of water from Ionic liquid

To 40 mL of a 1:1 mixture of ionic liquid (IL), 1-butyl-3-methylimidazolium tetrafluoroborate, and water was added 8 g of glycerol (20% w/v) and the mixture was swirled until the glycerol dissolved. Two layers were immediately formed consisting of an upper layer consisting mostly of water and glycerol and a more dense IL as the bottom layer. The bottom layer resulted in 19 mL and contained 85% IL. The bottom layer was treated again with glycerol 4 g (10% w/v), swirled and observed for further separation of water. The yield of the bottom layer was 17 mL and contained 93% IL.

**Figure 45: IR spectra for extraction of water from ionic liquid(1-Butyl-3-methylimidazolium tetrafluoroborate) by glycerol**



### **4.3 Dehydration of organic solvents**

In chemical processes, synthesizing and isolating an organic compound often results in an organic compound or solution contaminated with traces of water. For instance, in extractions comprising of immiscible or partially immiscible solvents such as hexane and dichloromethane some water will be transferred into the organic phase because of the partial miscibility of the organic phase and water. Commonly used techniques in organic chemistry usually include addition of saturated aqueous sodium chloride and solid drying agents.

#### **Saturated Aqueous Sodium Chloride:**

In this method the bulk of the water can often be removed by shaking or "washing" the organic layer with saturated aqueous sodium chloride (also known as brine). The salt water works to

pull the water from the organic layer to the water layer. This is because of stronger attraction of salts for water than organic solvents and the equilibrium favors distribution of water into the concentrated salt solution.

### **Solid Drying Agents:**

Several inorganic salts such as calcium chloride,  $\text{CaCl}_2$ , calcium sulfate,  $\text{CaSO}_4$  (Drierite), magnesium sulfate,  $\text{MgSO}_4$ , potassium carbonate,  $\text{K}_2\text{CO}_3$  and sodium sulfate,  $\text{Na}_2\text{SO}_4$ , have been traditionally used to remove final traces of water from organic solution. These salts are commonly termed as drying agents which readily take up water to become hydrated.

Polyols can be an alternative in extraction of residual water from organic solvents. Because of the stronger hydrogen bonding ability with water, polyols present themselves as a potential candidates as liquid or solid dehydrating agents.

## **4.4 Extraction of essential oils.**

Essential oils are complex mixtures of lipophilic substances containing volatile aromatic compounds derived from plants. In general, essential oils differ from fixed or vegetable oils because of their higher volatility, and the term “essential” refers to the volatile aromatic substances with an intense, pleasant odor. Essential oils are often used for their therapeutic effect or as a fragrance for use in perfume, cosmetics, food, medicine and house cleaning products. Essential oils, like all organic compounds, are made up of hydrocarbon molecules and can further be classified as terpenes, alcohols, esters, and aldehydes.

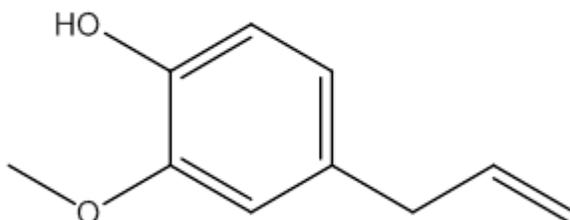
Liquid–liquid extraction is one of the various extraction methods used in the manufacture and extraction of essential oils which often employ dangerous chemical solvents, such as methylene chloride, chloroform and ethers. Use of a mass separating agent induced phase separation in water miscible solvent system is an alternative approach to avoid use of such dangerous chemicals in

liquid-liquid extraction. Thus, polyol induced phase separation can be implemented in the extraction of essential oils.

*Proof of concept:*

Cloves contain the phenolic compound 4-allyl-2-methoxyphenol ( $C_{10}H_{12}O_2$ ) commonly called 'Eugenol'.

**Figure 46: Structure of eugenol**



The following example was performed to determine *PIE* of eugenol from cloves:

To a mixture of 10 mL ACN-water 1:1 v/v were added 5-6 clove buds and soaked overnight for extraction of volatile components. On the following day, 20% wt/v of glycerol was added as an MSA to obtain a phase separation of upper ACN rich layer and lower aqueous layer. GC analysis of upper layer determined that most of the eugenol was extracted in upper phase where  $K_D$  (distribution coefficient) of eugenol was greater than 1.

#### **4.5 Conclusion:**

Thus, the above proposed studies and some preliminary investigations have exemplified use of polyol as a potential novel mass separating agent in extraction and separation system. This promises to be an effective future application of the PIE process.