Natural deep eutectic solvents and their application in natural product research and development

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Chapter 1

General Introduction

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Current organic solvents have several problems such as toxicity to humans and the environmental burden caused by their residues and their disposal. In food, pharmaceutical, cosmetic, and chemical industries, organic solvents are widely used for extracting, e.g. flavors, fragrances, medicines and dyes from plants. Plant metabolites differ in many properties such as polarity, stability, boiling point as well as quantity. An extraction of plants can have different goals: 1) complete extraction of single compounds for analysis or industrial production; 2) complete extraction of a group of compounds for analysis or industrial production; 3) extraction of all compounds present for metabolomics analysis. For each of these goals different characteristics of the solvent are required. A universal solvent does not exist. In addition, metabolites have interactions with other cellular components. So, different solvents mixtures are reported to adjust characteristics of solvents such as high cell penetration ability, broad or specific solubilizing capacity, and even cell wall break-down ability. For example, solvents of different polarities are needed in the extraction, separation, purification, and administration of various medicines. Solvents, such as alcohols, chloroform, ethyl acetate, etc. are generally applied for this purpose. However, organic solvents may result in organic impurities in extracts, requiring special assays in quality control of food and drugs. Moreover, organic solvents are often toxic, flammable, explosive or poorly biodegradable. To address these problems, new alternative solvents are required which are biodegradable, and have low toxicity, and at the same time have a broad solubilizing capacity in terms of selectivity and polarity.

With the aim of developing environmentally friendly solvents, ionic liquids (ILS) have attracted great attention because they have negligible vapor pressure at room temperature and features such as polarity and selectivity can be tailor-made for different applications. Compared with conventional organic solvents, ILs are low melting point organic salts ($<$100 ^{o}C), composed of a cation and an anion, of which at least one is an organic ion. ILs have many attractive physicochemical properties, such as chemical and thermal stability, non-flammability, high conductivity, and good solubility of various compounds. Because of their good physicochemical properties, ILs have been explored in many areas. In biology, they have been applied in the extraction and separation of a wide range of metabolites covering primary and secondary metabolites and macromolecules (e.g.

DNA, proteins and polysaccharides) and as alternative media to enzyme reactions. Particularly, some ILs can dissolve starch and cellulose which are not soluble in water without heating. All those applications are attributed to their special features, forming strong interactions with solutes, e.g. hydrogen or ionic bonds. So far most ILs have been obtained by chemical synthesis. Although ILs overcome some of the problems of organic solvents, their use is limited in food and pharmaceutical applications because of their high costs, the high toxicity of some of the ingredients, and their irritation properties. Another type of solvent with similar physical properties and phase behavior to ILs are deep eutectic solvents (DES). DES are obtained by mixing solid compounds forming a eutectic mixture with a melting point much lower than either of the individual components. For example quaternary ammonium salts, amides, organic acids, polyalcohols, etc. DES show advantages over ILs such as: simple preparation method, high purity, low cost, and low toxicity. These solvents have been used in organic reactions, enzyme reactions, and electrochemistry. However, the high melting point of most reported DES restricts their applications as a green solvent at room temperature.

In order to increase the number of candidates for ILs and DES and to overcome the problems of synthetic ILs and DES, our group explored natural products as a source for such solvents, i.e. primary metabolites, which include organic acids, amino acids, sugars, sugar alcohols, and amines. Natural products are indeed a plentiful and ideal source of ILs and DES due to their enormous chemical diversity, biodegradable properties, sustainability and pharmaceutically acceptable toxicity profile. Our group introduced the term Natural Deep Eutectic Solvents (NADES) for these liquids made from natural products. In addition to being the source of useful ILs and DES, with many potential applications, we hypothesize that the existence of NADES in organisms can explain many biological processes, which cannot be explained by water and lipids as the only liquids in living organisms. For example, the mystery of the biosynthesis, solubilization, storage, and transport of poorly water-soluble metabolites and macromolecules in the aqueous environment of cells may be explained by NADES (Choi, et al., 2011). Also, it may explain the survival of organisms in extreme drought and/or cold conditions. The many advantages of the natural ILs and DES suggests agreat potential for their application in food, cosmetics and pharmaceutical areas, resulting in economical as well as ecological advantages.

Aims of the thesis

Previously, we proposed the hypothesis about the role of NADES in nature. Abundant metabolites in plants might be the source of NADES and those NADES may have diverse biological roles as alternative liquid media to water and lipids in organisms, explaining the biosynthesis of non-water soluble metabolites in living cells and how organisms survive in extreme drought or cold conditions. In this thesis, the range of NADES is extended and these new solvents are explored for their physicochemical properties, their application in metabolite extraction and food chemistry, and their occurrence and possible function in plants (Fig. 1). In order to address the hypothesis the following objectives for this thesis are formulated.

1 develop a suitable production method for NADES, explore suitable combinations and ratio of ingredients in NADES with different types of primary metabolites;

2 determine the structure, physicochemical properties, and solubilizing capacity of NADES for metabolites in organisms and explore the effect of the water percentage in NADES on their structure and properties;

3 study the stability of compounds in NADES in combination with the effect of temperatures, light or long time storage and identify the major factors that affect the stability of compounds in NADES;

4 develop methods for analysis of extracts prepared with NADES, and explore the effect of NADES on the results;

5 develop extraction methods for phenolic compounds with NADES, compare the extraction ability of NADES with conventional solvents and explore the effect of water percentage in NADES on the extraction efficiency;

6 recover phenolic compounds from NADES;

7 explore the applications of NADES in pharmaceuticals；

8 explore the composition, position and function of NADES in plants and explain the relationship between the composition of NADES and their function in plants.

Outline of the Thesis

This thesis aims to expand the number of NADES, determine their physicochemical properties, explore their applications in metabolite extraction from plants and uncover their possible biological functions. In chapter 2, the synthesis, properties and application of synthetic ionic liquids and deep eutectic solvents in metabolite extraction, separation, and enzyme reactions are reviewed. The analytical methods and the combination of ionic liquids with other technologies are also reviewed.

In chapter 3 and 4, preparation of different combinations of NADES, their structures and physicochemical properties are described. Two preparation methods of NADES are compared. Solubility of poorly water-soluble metabolites and macromolecules is studied. In addition, the effect of the water percentage in NADES on the structure, properties, and solubilizing capacity of NADES is explored. In chapter 5, the stability of metabolites in some NADES are reported, with water and ethanol as references, under different conditions, including heat, light, and long-term storage. The effect of water content on the stability of compounds in NADES is also tested. The mechanism of stabilization ability of phenolic compounds by NADES is discussed.

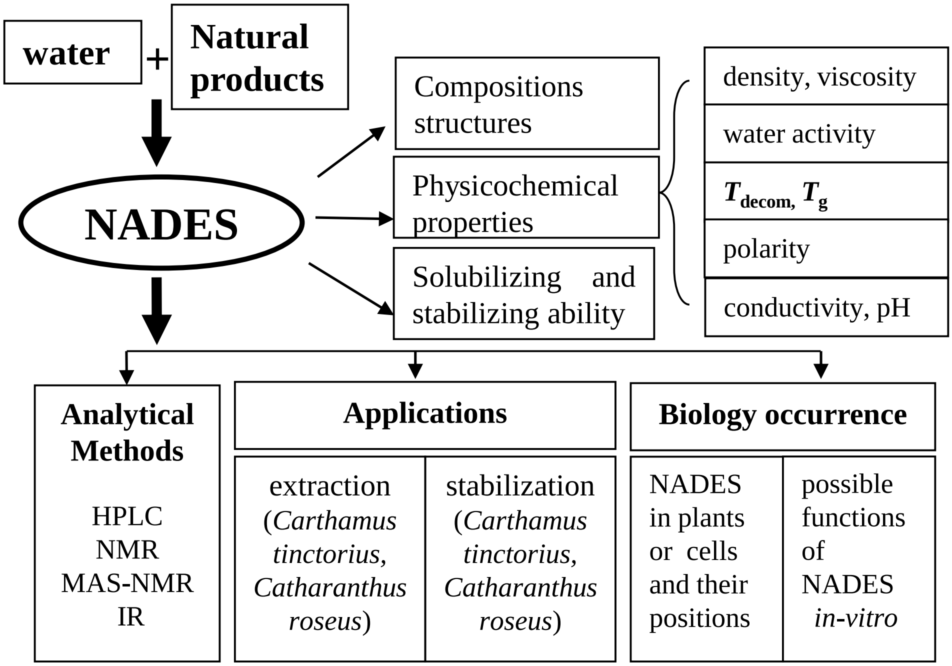

Fig. 1. Schematic diagam of this thesis

The studies in these chapters lay the basis for the application of NADES, which is explored in the following three chapters. In chapter 6 and 7, the application of NADES as solvents in the extraction of phenolic compounds is exemplified with flowers from *Catharanthus* roseus and *Carthamus tinctorius* L.. In chapter 6, the whole process from sample preparation to analysis is reported for anthocyanins from *Catharanthus* roseus, including extraction, analysis, and sample storage methods. Chapter 7 reports the difference in extraction ability of NADES and general solvents for phenolic compounds from *Carthamus tinctorius*, investigated by HPLC-UV based metabolomics. Furthermore, the extraction parameters of high extraction ability of NADES are optimized and the recovery method for phenolic compounds from NADES is studied.

The composition and function of NADES in plants are discussed in chapter 8. The NADES in different plants or plant secretions are analyzed. To explore the functions of NADES in plants, the diffusion between water and NADES, the hygroscopicity of NADES, and barley seeds are investigated. The behavior of liposomes in NADES is tested to explore the function of NADES in the cell membrane. The summary, final conclusion, and the future perspective of the results obtained in this thesis are discussed in chapter 9.