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CHOLINIUM SALICYLATE AS A HYDROTROPIC MEDIUM FOR IMPROVED RESVERATROL SOLUBILIZATION IN WATER AND PRESERVATION OF ANTIOXIDANT ACTIVITY

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ABSTRACT

Resveratrol (RSV) is a naturally occurring polyphenolic compound that has attracted considerable attention in recent decades due to its pronounced antioxidant activity and therapeutic potential, including anti-inflammatory, cardioprotective, and anticancer effects. However, its broader application is significantly limited by poor aqueous solubility. In this study, aqueous solutions of cholinium salicylate ([Ch][Sal]) were investigated as a potential medium for improving RSV solubilization. The influence of ionic liquid concentration on RSV solubility was evaluated to gain insight into the interactions governing the dissolution process. Additionally, the antioxidant activity of RSV in [Ch][Sal] aqueous media was assessed to determine whether the ionic liquid environment affects its functional properties.

INTRODUCTION

Resveratrol is widely recognized for its diverse biological activities, which have contributed to its growing interest in pharmaceutical, nutraceutical, and cosmetic applications [1]. This compound is biologically active in its trans configuration; however, under exposure to light, elevated temperature (above ~37 °C), oxygen, and basic media, it readily undergoes isomerization to the cis form, which exhibits significantly reduced biological activity [2]. Additionally, the practical application of RSV remains limited by its poor aqueous solubility, which leads to low bioavailability and challenges in formulation within water-based systems.

Various strategies have been explored over the years to overcome these limitations for RSV and other poorly water-soluble molecules. More recently, ionic liquids (ILs) have emerged as promising alternative media for enhancing the solubility of such compounds [3]. Owing to their highly tunable physicochemical properties, amphiphilic character, and ability to establish specific intermolecular interactions, ILs can act as efficient hydrotropic systems

capable of significantly improving the dissolution behavior of hydrophobic molecules.

Among different IL families, cholinium-based ionic liquids have attracted particular interest due to their favorable environmental profile and relatively low toxicity [4]. Their ability to participate in hydrogen bonding and stabilize dissolved molecules in aqueous media makes them attractive systems for solubilization of bioactive compounds.

Therefore, in this study, the influence of aqueous solutions of cholinium salicylate ([Ch][Sal]) on RSV solubilization behavior was investigated. Initially, the concentration of IL was varied from ~2 to ~25 wt% to evaluate its influence on RSV solubilization enhancement. In order to ensure that the functional properties of RSV were preserved under these conditions, its antioxidant activity was additionally evaluated in IL media at both lower (5 wt%) and higher (20 wt%) concentrations. Overall, the proposed method represents a potential solution to the problem of poor solubility and the practical utilization of bioactive compounds in aqueous formulations.

EXPERIMENTAL

Cholinium salicylate was synthesized via a neutralization reaction using cholinium hydroxide (46 wt% in water) solution (Sigma-Aldrich - USA) and salicylic acid (purity ≥ 99%, Sigma-Aldrich - USA), in an equimolar ratio. The obtained solution was stirred for an additional period to ensure reaction completion, followed by water removal under reduced pressure using a rotary evaporator (R-210 Rotavapor System, Büchi Labortechnik AG, Switzerland).

Resveratrol concentration was determined using a high-performance liquid chromatography (HPLC) system (Azura HPLC, Germany) equipped with a C18 column and diode-array detector (DAD). Separation was performed using a methanol/water gradient elution at a flow rate of 1 mL/min, injection volume of 20 μL, and column temperature of 30 °C. Quantification was performed at 306 nm, and all solutions were prepared in 0.1% formic acid in water (pH = 2.70).

The antioxidant activity of RSV in IL media was evaluated using the DPPH radical scavenging assay. A DPPH stock solution (0.2 mM) was prepared in methanol. An aliquot of 10 μL of RSV solution at its maximum solubility in aqueous ionic liquid media (containing 5 and 20 wt% IL) was mixed with 990 μL DPPH solution and incubated in the dark at room temperature for 30 min. Antioxidant activity was simultaneously evaluated in ethanol at the same RSV concentrations to assess the influence of the IL medium. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer (LLG-uniSPEC2, Germany).

RESULTS AND DISCUSSION

The presence of IL in aqueous media significantly enhanced RSV solubility, achieving up to a 50-fold increase compared to water (Figure 1). This confirms their strong solubilizing (hydrotropic) effect through RSV-IL and IL-water interactions that disrupt water structure and favor solute stabilization. Solubility enhancement can be potentially attributed to π-π interactions between the aromatic salicylate anion and RSV and hydrogen bonding, further stabilizing the solute in solution.

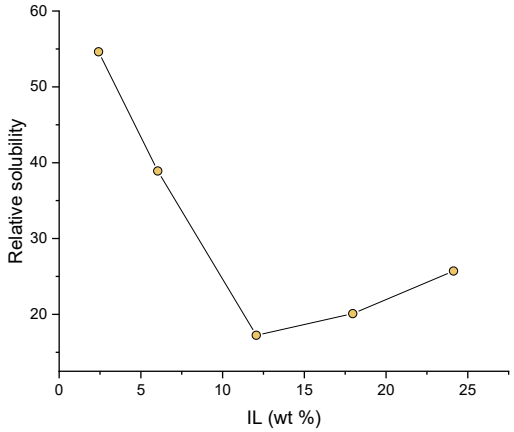


Figure 1. Dependence of the relative solubility of RSV on the IL mass percentage in aqueous media.

Additionally, IL concentration had a clear impact: the solubility increase was more pronounced at lower concentrations, where RSV-IL interactions dominate. At higher concentrations, enhanced IL-IL self-association likely reduces the availability of free IL species for direct interaction with RSV, leading to a less efficient incremental increase.

The system operates in a hydrotropic regime within the studied range (≤1 M), as no distinct minimum hydrotrope concentration was observed and only a weak sigmoidal trend was detected. This suggests gradual solubilization without formation of well-defined IL-rich aggregates.

After establishing that the ionic liquid significantly enhances the solubility of RSV, its antioxidant activity was subsequently evaluated.

The inhibition percentage of RSV in 5 wt% IL media at its maximum solubility resulted in 40.12% inhibition, while in 20 wt% IL media the inhibition was 33.06%. For comparison, at the same RSV concentrations in ethanol, inhibition values of 39.45% and 33.56% were obtained, respectively. These results indicate that the IL medium does not adversely affect the antioxidant activity of RSV, confirming that its radical scavenging capability is preserved in IL-based systems.

CONCLUSION

Ionic liquids demonstrated a significant enhancement of RSV aqueous solubility, achieving up to a 50-fold increase compared to water, confirming their strong hydrotropic capability and tunable solvation behavior. The influence of IL concentration was found to be crucial, with more pronounced solubilization effects observed at lower concentrations. Importantly, despite the strong solubilization effect, the antioxidant activity of RSV remained preserved in IL media. This indicates that the IL environment does not negatively affect the intrinsic radical scavenging properties of RSV. Overall, the results highlight the functionality of ILs as efficient solubilizing media that maintain the bioactivity of RSV, supporting their potential application in the formulation and processing of poorly water-soluble bioactive compounds.

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