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Original research paper

EXTRACTION OF THIACLOPRID FROM HONEY USING AQUEOUS BIPHASIC SYSTEMS BASED ON POTASSIUM PHOSPHATE AND IONIC LIQUIDS

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Abstract: Thiacloprid, a widely used neonicotinoid pesticide, poses a significant health risk when present in honey. Conventional extraction techniques are often complex and time-consuming, highlighting the need for more efficient methods. This study investigated the application of aqueous biphasic systems (ABS) based on ionic liquids (ILs) in combination with the kosmotropic salt potassium phosphate (K_3PO_4) for extracting thiacloprid from honey, intending to enhance extraction efficiency and simplify the process. Two commercially available ionic liquids, 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]) and tetrabutylammonium chloride ([N_{4444}][Cl]), were assessed for their phase-forming properties and extraction efficiencies. Both systems achieved extraction efficiencies exceeding 90%, with the [C_4mim][Cl] system demonstrating superior performance, attaining an extraction efficiency (*EE%*) of 98.11 ± 1.26%. Additionally, spectrophotometric detection was applied, providing a faster, simpler, and more cost-effective alternative to chromatographic methods. The results underscore the potential of IL-based ABS systems with K_3PO_4 as a sustainable and effective alternative to traditional extraction methods, demonstrating a selective, rapid, and environmentally friendly approach for extracting thiacloprid from a complex matrix such as honey.

Key words: *honey, thiacloprid, ionic liquids, aqueous biphasic systems*

INTRODUCTION

Honey is a natural substance produced by bees from the nectar of flowers, and it plays a significant role in both human diets and agriculture. Its composition and quality can be influenced by various environmental factors, including pesticide exposure (Cianciosi et al., 2018; El-Nahhal, 2020; Olas, 2020; Ward et al., 2022).

Thiacloprid is a neonicotinoid insecticide used to control a range of pests on crops. The use of thiacloprid has raised concerns due to its potential effects on non-target species,

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including pollinators like bees, which are crucial for eco-system health. Honey, produced by bees, is especially vulnerable to pesticide contamination, as residues from pesticides like thiacloprid can accumulate in honey, potentially impacting both its quality and safety (Siede et al., 2017).

Conventional extraction techniques consume significant amounts of time and require relatively large quantities of samples, toxic reagents, and organic solvents. Recent research aims to replace these techniques with more environmentally friendly approaches that streamline sample preparation and reduce solvent volumes, toxic reagent quantities, and analysis time (Masiá, Suarez-Varela, Llopis-Gonzalez & Picó, 2016). Solid-phase extraction (SPE) is one such technique that has emerged as an alternative to conventional methods, offering notable advantages due to reduced consumption of organic solvents. However, SPE often necessitates expensive equipment and precise optimization of parameters, such as sorbent composition and solvent selection, to achieve desired results. Although SPE has improved compared to conventional extraction, its limitations in selectivity, reproducibility, and robustness highlight the need for ongoing research to enhance its performance and address these issues (Bunno & Yabuki, 2020; Kerkich, Bouargane, el Laghdach, Souhail & Kadmi, 2023).

The analytical methods for the detection and quantification of thiacloprid in honey are well-developed, primarily utilizing high-performance liquid chromatography (HPLC) coupled with diode-array detection (DAD) or tandem mass spectrometry (MS/MS) (Kerkich et al., 2023; Ligor, Bukowska, Ratiu, Gadzała-Kopciuch & Buszewski, 2020; Tu & Chen, 2021). However, there remains potential for advancing eco-friendly chemistry approaches in sample preparation. One promising avenue of research involves the use of ionic liquids.

Ionic liquids (ILs) are organic salts characterized by an asymmetric organic cation and an organic or inorganic anion, with melting points generally below 100 °C and thermal stability extending up to around 400 °C (Bahrani, Raeissi & Sarshar, 2015). Various organic cations, such as imidazolium, sulphonium, ammonium, pyridinium, phosphonium, triazolium, pyrazolium, guanidinium and others, with different substituent groups, can be combined with anions of interest in the process of designing ionic liquids (Kaur, Kumar & Singla, 2022). Unlike conventional salts, like sodium chloride, ionic liquids have an unsymmetrical structure of their constituent ions, which results in lower melting points due to weaker ionic interactions compared to the tightly packed symmetric ions of traditional salts (Welton, 2004). The growing interest in ionic liquids and their applications can be attributed to their ability to form a wide range of inter-molecular interactions (strong and weak ionic, hydrogen and van der Waals bonds, dispersive and π-π interactions), which contributes to better solvation properties compared to conventional organic solvents (Hejazifar, Lanaridi & Bica-Schröder, 2020). Ionic liquids also exhibit several advantageous properties, such as low vapour pressure, nonflammability, nonvolatility, high thermal stability, and notable electrical conductivity. These characteristics, along with their tunable nature enabled by modifying the cation and anion components, substituent groups, and alkyl chain length have earned them the designation of "designer solvents" and "solvents of the future" (Egorova, Gordeev & Ananikov, 2017; Ghorbanizamani & Timur, 2018). Ionic liquids have been employed to create biphasic aqueous systems with amino acids, carbohydrates, inorganic/organic salts, organic buffers, and polymers (Freire et al., 2012; Richu, Sharmhal, Kumar & Kumar, 2022). Among these, ionic liquid-salt systems are particularly well-studied due to their ability to induce phase separation, resulting in the formation of two distinct aqueous phases. The salting-out effect arises from the formation of water-ion complexes that lead to the dehydration of the dissolved ionic liquid, thereby increasing the surface tension within the aqueous medium (Freire et al., 2009). When high charge density salts are introduced into aqueous ionic liquid solutions, they preferentially hydrate over the ionic liquid ions, causing phase separation and resulting in an ionic liquid-rich phase. Ionic liquids, due to their delocalized ions and low charge symmetry, are less hydrated in water compared to conventional inorganic salts (Marić et al., 2023; Zafarani-Moattar & Hamzehzadeh, 2010).

The objective of this study was to investigate the effect of various commercially available ionic liquids on the formation of aqueous biphasic systems (ABS) and their application for the extraction of thiacloprid from honey. The study examined the impact of the anions of these ionic liquids on the formation of biphasic systems using potassium phosphate (K_3PO_4) as the salting-out agent. This agent was chosen due to its strong salting-out capacity, as indicated by its position in the Hofmeister series, as well as considerations of biocompatibility and environmental impact. A key feature of this study is the use of spectrophotometric measurements, which offer a streamlined and accessible alternative to more complex methods commonly found in the literature. This approach provides a rapid and efficient means of analysing the extraction process. The novelty of this research resides in the method's flexibility and practicality, facilitating rapid implementation without the need for specialized equipment or extensive resources, thereby enhancing its applicability across various research contexts.

MATERIALS AND METHODS

Material

The honey solution was prepared by dissolving 10 g of honey in 50 mL of distilled water with a magnetic stirrer (Witeg, Germany) set at 500 rpm for 10 minutes. The mixture was then filtered through a 0.45 μm nylon microfilter (Amtast, Lakeland, FL, USA). All chemicals used are of at least analytical reagent grade.

The analytical standard of thiacloprid was obtained from Sigma-Aldrich (Germany). The solution of thiacloprid was prepared at 0.01 g/L by dissolving in 20% v/v honey solution. The 1-butyl-3-methylimidazolium chloride [C_4mim][Cl] and tetrabutylammonium chloride [N_{4444}][Cl] were provided by Merck (Germany). Potassium phosphate was supplied from Sigma Aldrich (St. Louis, MO, USA). The structures of the investigated ionic liquids are shown in Fig. 1.

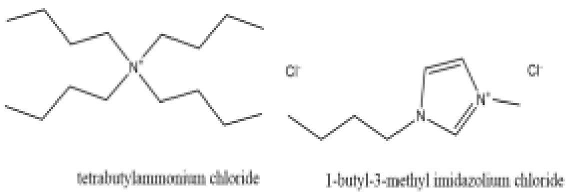

Figure 1. Structures of applied ionic liquids

Determination of phase diagrams

The binodal curves' construction was based on the cloud point titration method at temperature (T = 298 ± 1 K) and atmospheric pressure. A solution of potassium phosphate dissolved in a 20% v/v solution of honey (ω_{salt} = 40%) was added dropwise to a solution of ionic liquid prepared in a 20% v/v honey solution (ω_{IL} = 80%) until a turbid mixture was obtained, and the amount of added salt was measured by an analytical balance (AND HA-180M, Tokyo, Japan) with an uncertainty of ± 10^{-4} g. Furthermore, an aqueous solution was added to the mixture until the mixture became apparent, and the amount of added honey solution was measured using an analytical balance. After each addition, the mixture was stirred using a vortex mixer (Boeco V1 plus, Hamburg, Germany). This procedure was repeated until sufficient points were obtained to construct binodal curves. Ternary phase diagrams were fitted using the Merchuk equation (Merchuk, Andrews & Asenjo, 1998):

$$Y = A\exp(BX^{0.5} - CX^3) \quad (1)$$

where Y and X are the mass fractions of ionic liquids and salts, while the coefficients of the polynomial equations A, B, and C were determined by regression analysis. These values along with the correlation coefficients (R^2) are given in Table 1.

Table 1.
Correlation parameters of Merchuk equation with standard deviation for the IL + K_3PO_4 + 20% (v/v) honey solution

Merchuck parameters					
IL	**Salt**	**A ± σ**	**B ± σ**	**C ± σ (10^{-5})**	**R^2**
[N_{4444}]Cl	K_3PO_4	73.4 ± 1.0	-0.37 ± 0.006	8.81 ± 0.29	0.973
[C_4mim]Cl		72.9 ± 4.8	-0.30 ± 0.020	4.13 ± 0.41	0.995

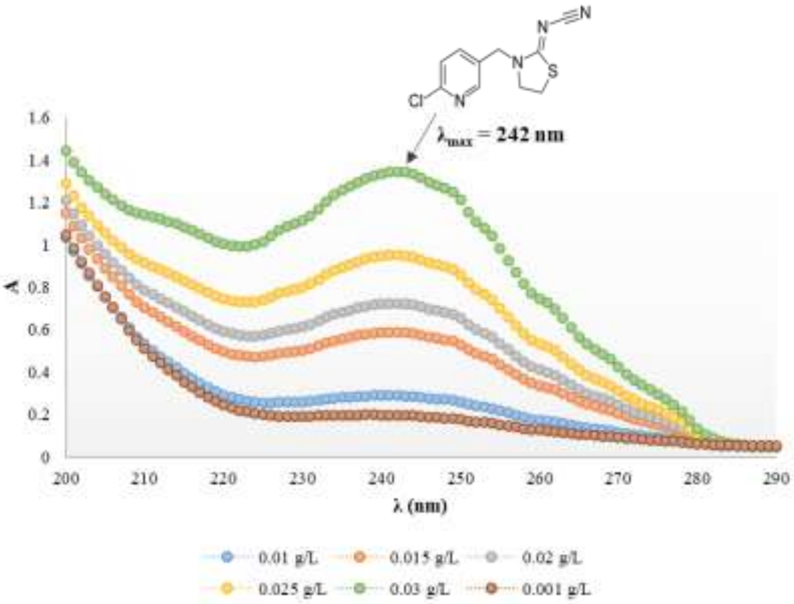

Figure 2. UV-Vis absorption spectrum of thiacloprid, indicating maximum absorption at 242 nm

Extraction of thiacloprid using aqueous biphasic systems

A ternary mixture within the biphasic region was prepared containing approximately 14% of IL, 26% of K_3PO_4 and 60% of the 20% (v/v) honey solution with thiacloprid and shaken for 3 min using a vortex mixer at 2500 rpm and left to equilibrate for 12 h. The final concentration of thiacloprid in the system was 0.01 g/L. The partitioning solute quantification, in both phases, was accomplished by UV spectroscopy (Shimadzu UV-1700 spectrophotometer, Kyoto, Japan), at the wavelength of 242 nm, using a calibration curve previously established (y = 43.221 x, R^2 = 0.9997).

The wavelength used for the thiacloprid quantification corresponds to the maximum absorption peak of solute (Fig. 2). Interferences of both the inorganic salt and the IL with the analytical method were taken into account and found to not be significant at the magnitude of the dilutions performed. Three samples of each aqueous phase were precisely quantified and the respective standard deviations determined. Moreover, both phases were additionally weighted. The partition coefficient (K) of thiacloprid was determined as the ratio of the concentration of thiacloprid in the IL and in the salt aqueous phases, according to Equation 2:

$$K = \frac{[Thiacloprid]_{IL}}{[Thiacloprid]_{Salt}} \quad (2)$$

where $[\text{Thiacloprid}]_{IL}$ and $[\text{Thiacloprid}]_{Salt}$ are the concentrations of thiacloprid in the IL and in the K_3PO_4 aqueous phases, respectively. The extraction efficiency (% *EE*) of thiacloprid was determined according to Equation 3:

$$\%EE = \frac{[Thiacloprid]_{IL} \times w_{IL}}{[Thiacloprid]_{IL} \times w_{IL} + [Thiacloprid]_{Salt} \times w_{Salt}} \times 100 \quad (3)$$

where w_{IL} and w_{Salt} are the weight values of the IL-rich phase and of the potassium phosphate-rich phase, respectively, and $[\text{Thiacloprid}]_{IL}$ and $[\text{Thiacloprid}]_{Salt}$ are the concentrations of thiacloprid in the IL-rich phase and in the K_3PO_4 phase, respectively.

RESULTS AND DISCUSSION

Phase diagrams of aqueous biphasic systems

The solubility curves of ionic liquids with chloride anions and distinct cationic cores, as shown in Fig. 3, characterize the phase behaviour of these systems. Curves positioned closer to the origin indicate that lower concentrations of IL and/or salt are sufficient to induce phase separation, resulting in an expanded biphasic region. This behaviour underscores the enhanced salting-out efficiency and phase-splitting capacity of the ILs, which are pivotal parameters in the thermodynamic optimization of aqueous biphasic systems for targeted separation and extraction processes (Dimitrijević et al., 2019).

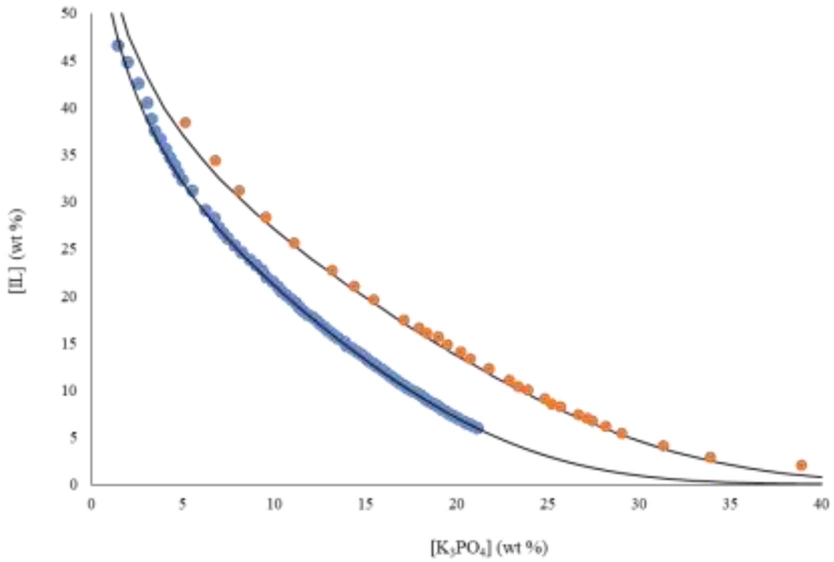

Figure 3. Ternary phase diagrams of the studied systems {IL + K_3PO_4 + 20 % v/v honey solution} at T = 296.15 K and atmospheric pressure (p = 0.1 MPa). Legend: ● [N_{4444}][Cl]; ● [C_4mim][Cl]

From the comparison of the binodal curves shown in the Fig. 3, an order for the ABS forming ability of ILs can be established: [N_{4444}][Cl] > [C_4mim][Cl]. This result is in accordance with the results of Bridges, Gutowski & Rogers (2007) and Marques et al. (2013) who investigated the phase behaviour of quaternary ammonium and imidazolium ionic liquids with kosmotropic salts. There are several reports in the literature describing ABS comprising imidazolium-based ILs (Najdanovic-Visak, Lopes, Visak, Trindade & Rebelo, 2007; Pei, Wang, Liu, Wu & Zhao 2007). Tetrabutylammonium chloride [N_{4444}][Cl], quaternary onium salt, has highly shielded charges, located mostly on the heteroatom surrounded by four butyl chains and hence possess a low affinity for water (Nie, Zheng, Lu, Yao & Guo, 2022).

The smaller the affinity for water and/or the more extensive hydrophobic nature of the ILs, the more prone it is to be salted-out. The imidazolium salts have charge diffuse cations, which lead to the depressed melting points. The charge is dispersed between the two nitrogen atoms and the C_2 carbon, thus exposing the charge to the solvent and allowing for more interactions between the cation and water (Izgorodina, Seeger, Scarborough & Tan, 2017). The interactions lead to a relative increase in the structuring of the water which is characteristic of kosmotropic salts (Berton et al., 2019; Louros et al., 2010).

Extraction of thiacloprid from honey

The partition coefficients of thiacloprid in aqueous biphasic systems containing various ionic liquids, K_3PO_4, and a 20% v/v honey solution are illustrated in Fig. 3. All experiments were performed in triplicate, and mean concentrations were used for further analysis. The data indicate that the highest partition coefficient for thiacloprid was observed in the ABS containing [C_4mim][Cl], while a lower partition coefficient was noted for the ABS with [N_{4444}][Cl] (Fig. 3). These findings suggest that thiacloprid extraction can be effectively achieved in a single-step procedure by optimizing the choice of ionic liquid in the ABS formulation. Given that thiacloprid has an octanol-water partition coefficient ($LogP_{o/w}$) value of 1.26 (ChemSpider, accessed 17 August 2024), classifying it as a medium-polar pesticide ($1 < LogP_{o/w} < 2$) (Dimitrijević et al., 2017), the results reflect the impact of the ionic liquid's properties on the partitioning behavior of thiacloprid. The enhanced partition coefficient observed with [C_4mim][Cl] can be attributed to several factors. The [C_4mim]$^+$ cation, featuring a butyl group, imparts significant hydrophobic characteristics to the ionic liquid (Jha, Kumar & Vankatesu, 2015), which promotes the preferential distribution of thiacloprid into this phase. This hydrophobic environment aligns well with thiacloprid's medium-polar nature, facilitating its extraction. Conversely, the $[N_{4444}]^+$ cation, with its bulkier structure comprising four butyl groups (Carreira et al., 2021), does not interact as favourably with thiacloprid, leading to a lower partition coefficient. The less compact nature of $[N_{4444}]^+$ results in reduced interaction efficiency with thiacloprid compared to the more compact $[C_4mim]^+$.

Additionally, the imidazolium-based ionic liquid $[C_4mim][Cl]$ is known for its ability to engage in strong π-π interactions and hydrogen bonding with thiacloprid, which further enhances its partitioning into the IL phase. Moreover, $[C_4mim][Cl]$ tends to form a more distinct IL-rich phase compared to $[N_{4444}][Cl]$, which aids in the effective separation and extraction of thiacloprid.

This underscores the importance of selecting and tailoring the ionic liquid to significantly enhance the efficiency of thiacloprid extraction in a single-step process.

Despite the observed differences in partition coefficients, both $[C_4mim][Cl]$ and $[N_{4444}][Cl]$ demonstrate high extraction efficiencies for thiacloprid, with values of 98.11 ± 1.26 % and 92.08 ± 0.79 %, respectively (Fig. 3). This indicates that while the partition coefficient reflects the relative distribution of thiacloprid

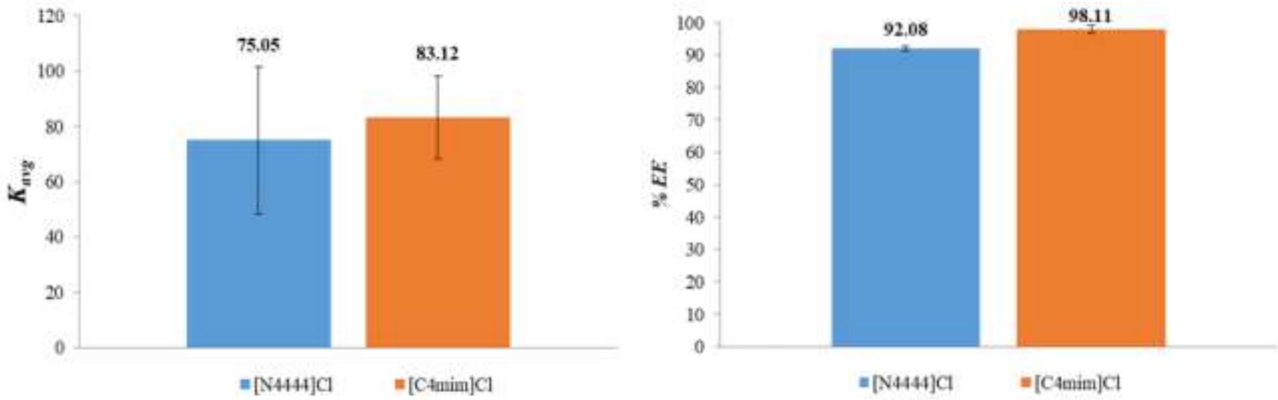

Figure 4. Partition coefficients (K_{avg}) and extraction efficiencies (% *EE*) with standard deviations of thiacloprid extraction from honey solution using ABS

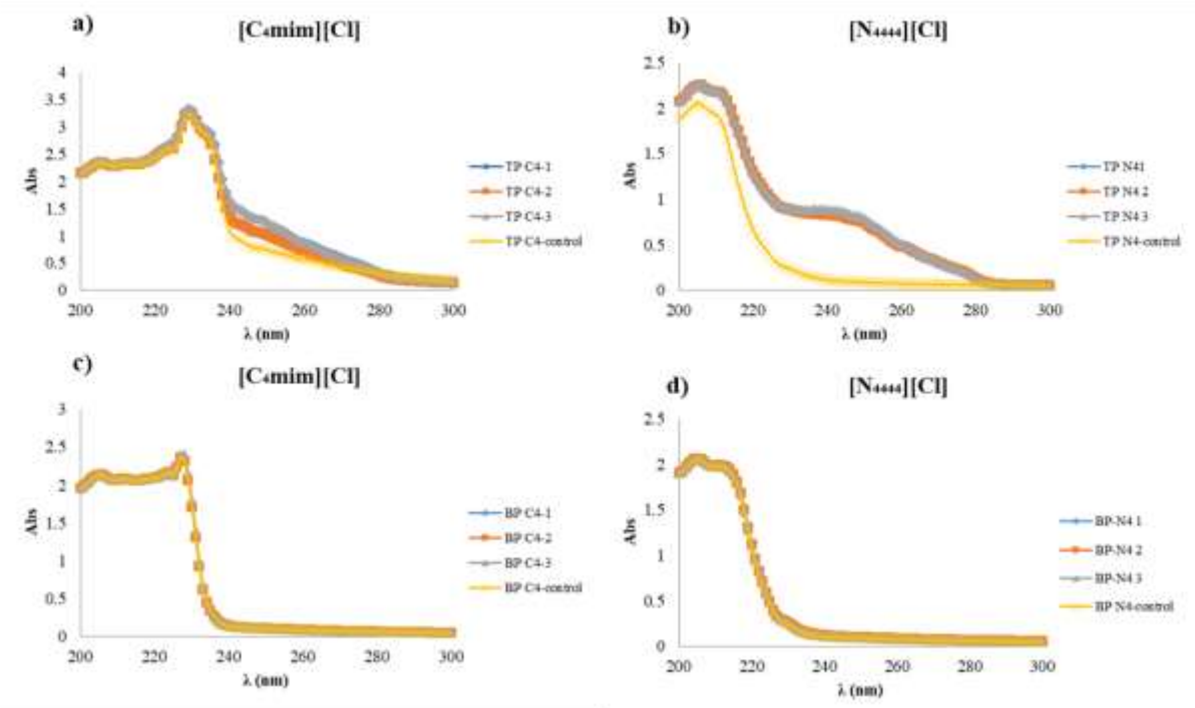

Figure 5. Spectrophotometric detection of thiacloprid: absorption spectra in ionic liquid: a), b) and salt: c), d) phases compared to control sample

between the phases, the overall extraction efficiency highlights the effectiveness of both ionic liquids in capturing thiacloprid from honey solution. In this study, spectrophotometric analysis was utilized to assess the presence of thiacloprid in the aqueous biphasic systems containing [C_4mim][Cl] and [N_{4444}][Cl].

As shown in Fig. 5, the absorption spectra of thiacloprid in the ionic liquid phase exhibited significant deviations from the control sample. This indicates that thiacloprid has successfully migrated to the IL phase, suggesting that the IL phase facilitates efficient extraction of thiacloprid. In contrast, the absorption spectra in the salt phase closely matched those of the control sample. This similarity suggests that thiacloprid behavior in the salt phase remains unchanged, confirming that thiacloprid is predominantly transferred to the IL phase. These observations validate the use of spectrophotometric analysis for evaluating the extraction of thiacloprid. The differences observed in the IL phase and the consistency in the salt phase highlight the successful extraction of thiacloprid into the IL phase. Future research could further elucidate the molecular interactions responsible for this extraction and provide a deeper understanding of thiacloprid behavior in different phases.

Various studies have explored the application of ionic liquids for pesticide extraction from different samples. Ravelo-Pérez, Hernández-Borges, Herrera-Herrera & Rodríguez-Delgado (2009) utilized 1-hexyl-3-methylimidazolium hexafluorophosphate ([C_6mim][PF_6]) for dispersive liquid-liquid microextraction (DLLME) of pesticide residues in grapes and plums. Zhang, Chen, Liu, Chen & Pan (2012) applied vortex-assisted DLLME with 1-octyl-3-methylimidazolium hexafluorophos-phate ([C_8mim][PF_6]) for organophosphorus pesticides in apple and pear samples. Additionally, ionic liquids such as specifically silica modified with propyltrioctylammonium chloride ([Si][N_{3888}]Cl) were used for the extraction of thiacloprid from aqueous media (Francisco, Almeida, Sousa, Neves & Freire, 2022). In the context of honey analysis, Yang, Ran, Xu, Ren & Yi (2019) developed an advanced in situ ionic liquid dispersive liquid-liquid microextraction method, utilizing the hydrophobic ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]) for the extraction of neonicotinoid insecticides, including thiacloprid, from honey samples. This method demonstrated high extraction efficiency with recoveries ranging from 81.0% to 103%, which is in agreement with the results of our study.

CONCLUSIONS

Using aqueous biphasic systems with ionic liquids to extract thiacloprid from honey represents an advanced method that offers several advantages over conventional extraction techniques. The experimental results demonstrate that both [C_4mim][Cl] and [N_{4444}][Cl] ionic liquids effectively facilitate the extraction of thiacloprid, with [C_4mim][Cl] showing a higher partition coefficient and slightly better extraction efficiency of 98.11 ± 1.26% compared to 92.08 ± 0.79% for [N_{4444}][Cl]. These findings confirm that the choice of ionic liquid significantly impacts the extraction efficiency, with the [C_4mim][Cl] proving more effective due to its hydrophobic properties and strong π-π interactions with thiacloprid.

This method is particularly effective due to the unique properties of ionic liquids, such as their ability to form distinct biphasic regions when combined with kosmotropic salts like potassium phosphate. The method facilitates efficient extraction, reducing both time and complexity compared to conventional multi-step methods. The ability to tailor ionic liquids for specific analytes, combined with environmental and operational benefits, makes this approach especially suitable for extracting compounds like thiacloprid from complex matrices such as honey.

Furthermore, the practical applications of this method extend beyond thiacloprid extraction and could be utilized for honey quality control and pesticide residue monitoring. Although this study focused on a single analyte, its principles could be applied to the extraction of other similar pesticides or contaminants, making it a versatile tool for broader analytical purposes. The results of this study underscore the importance of selecting the appropriate ionic liquid to maximize extraction efficiency and ensure a more sustainable and effective analytical method. Looking ahead, future studies will focus on the use of biodegradable ionic liquids of the latest generation, which are environmentally friendly and designed to be less toxic while decomposing in nature. This makes them a more sustainable alternative to traditional organic solvents for the extraction of a wide range of pesticides.

AUTHOR CONTRIBUTIONS

Conceptualization, A.Z.M. and S.B.G.; Methodology, A.Z.M. and P.T.J.; Investigation, formal analysis, validation, A.Z.M., B.B.Đ, and B.Đ.R.; Writing-original draft preparation, A.Z.M., B.B.Đ., and B.Đ.R.; Writing-review and editing, M.B.S. and A.Z.M.; Supervision, S.B.G, M.B.S, and P.T.J.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Bahrani, S., Raeissi, S., & Sarshar, M. (2015). Experimental investigation of ionic liquid pretreatment of sugarcane bagasse with 1,3-dimethylimadazolium dimethyl phosphate. *Bioresource Technology*, *185*, 411–415. https://doi.org/10.1016/j.biortech.2015.02.085

Banno A, & Yabuki Y. (2020). Simultaneous analysis of seven neonicotinoid pesticides in agricultural products involving solid-phase extraction and surrogate compensation using liquid chromatography-tandem mass spectrometry. *Journal of Pesticide Science*, *45*, 29–38. https://doi.org/10.1584/jpestics.D19-055

Berton, P., Kelley, S. P., Bridges, N. J., Klingshirn, M. A., Huddleston, J. G., Willauer, H. D., Baldwin, J. W., Moody, M. L., & Rogers, R. D. (2019). Water in solutions of chaotropic and kosmotropic salts: A differential scanning calorimetry investigation. *Journal of Chemical & Engineering Data*, *64*, 4781–4792. https://doi.org/10.1021/acs.jced.9b00222

Bridges, N. J, Gutowski, K. E., & Rogers, R. D. (2007). Investigation of aqueous biphasic systems formed from solutions of chaotropic salts with kosmotropic salts (salt-salt ABS), *Green Chemistry*, *9*, 177–183. https://doi.org/10.1039/B611628K

Carreira, A. R. F., Rocha, S. N., e Silva, F. A., Sintra, T. E., Passos, H., Ventura, S. P. M., & Coutinho, J. A. P. (2021). Amino-acid-based chiral ionic liquids characterization and application in aqueous biphasic systems. *Fluid Phase Equilibria*, 542–543, 113091. https://doi.org/10.1016/j.fluid.2021.113091

ChemSpider-Search and Share Chemists, https://www.chemspider.com, 2024, accessed 17 August 2024.

Cianciosi, D., Forbes-Hernández, T. Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P. P., Zhang, J., Lamas, L. B., Florez, S. M., Toyos, P. A., Quiles, J. L., Giampieri, F. & Battino, M. (2018). Phenolic compounds in honey and their associated health benefits: A review. *Molecules, 23,* 2322. https://doi.org/10.3390/molecules23092322

Dimitrijević, A., Ignjatović, L., Tot, A., Vraneš, M., Zec, N., Gadžurić, S., & Trtić-Petrović, T. (2017). Simultaneous extraction of pesticides of different polarity applying aqueous biphasic systems based on ionic liquids. *Journal of Molecular Liquids*, *243*, 646–653. https://doi.org/10.1016/j.molliq.2017.08.077

Dimitrijević, A., Jocić, A., Zec, N., Tot, A., Papović, S., Gadžurić, S., Vraneš, M., & Trtić-Petrović, T. (2019). Improved single-step extraction performance of aqueous biphasic systems using novel symmetric ionic liquids for the decolorisation of toxic dye effluents. *Journal of Industrial and Engineering Chemistry*, *76*, 500–507. https://doi.org/10.1016/j.jiec.2019.04.017

Egorova, K. S., Gordeev, E. G., & Ananikov, V. P. (2017). Biological activity of ionic liquids and their application in pharmaceutics and medicine. *Chemical Reviews*, *117*, 7132–7189. https://doi.org/10.1021/acs.chemrev.6b00562

El-Nahhal, Y. (2020). Pesticide residues in honey and their potential reproductive toxicity. *Science of the Total Environment, 741*, 139953. https://doi.org/10.1016/j.scitotenv.2020.139953

Francisco, R., Almeida, C., Sousa, A. C. A., Neves, M. C., & Freire, M. G. (2022). High performance of ionic-liquid-based materials to remove insecticides. *International Journal of Molecular Sciences*, *23*, 2989. https://doi.org/10.3390/ijms23062989

Freire, M. G., Carvalho, P. J., Silva, A. M. S., Santos, L. M. N. B. F., Rebelo, L. P. N., Marrucho, I. M., & Coutinho, J. A. P. (2009). Ion specific effects on the mutual solubilities of water and hydrophobic ionic liquids. *The Journal of Physical Chemistry B, 113,* 202–211. https://doi.org/10.1021/jp8080035

Freire, M. G., Cláudio, A. F. M., Araújo, J. M. M., Coutinho, J. A. P., Marrucho, I. M., Lopes, J. N. C., & Rebelo, L. P. N. (2012). Aqueous biphasic systems: a boost brought about by using ionic liquids. *Chemical Society Reviews*, *41*, 4966. https://doi.org/10.1039/c2cs35151j

Ghorbanizamani, F., & Timur, S. (2018). Ionic liquids from biocompatibility and electrochemical aspects toward applying in biosensing devices. *Analytical Chemistry*, *90*, 640–648. https://doi.org/10.1021/acs.analchem.7b03596

Hejazifar, M., Lanaridi, O., & Bica-Schröder, K. (2020). Ionic liquid based microemulsions: A review. *Journal of Molecular Liquids*, *303*, 112264. https://doi.org/10.1016/j.molliq.2019.112264

Izgorodina, E. I., Seeger, Z. L., Scarborough, D. L. A., & Tan, S. Y. S. (2017). Quantum chemical methods for the prediction of energetic, physical, and spectroscopic properties of ionic liquids. *Chemical Reviews*, *117*, 6696–6754. https://doi.org/10.1021/acs.chemrev.6b00528

Jha, I., Kumar, A., & Venkatesu, P. (2015). The overriding roles of concentration and hydrophobic effect on structure and stability of heme protein induced by imidazolium-based ionic liquids. *The Journal of Physical Chemistry B, 119,* 8357–8368. https://doi.org/10.1021/acs.jpcb.5b04660

Kaur, G., Kumar, H., & Singla, M. (2022). Diverse applications of ionic liquids: A comprehensive review. *Journal of Molecular Liquids*, *351*, 118556. https://doi.org/10.1016/j.molliq.2022.118556

Kerkich, K., Bouargane, B., el Laghdach, A., Souhail, B., & Kadmi, Y. (2024). Recent advances in the extraction, purification and analysis of emerging pesticides in honey products: A review. *Journal of Food Composition and Analysis*, *127*, 105947. https://doi.org/10.1016/j.jfca.2023.105947

Ligor, M., Bukowska, M., Ratiu, I. A., Gadzała-Kopciuch, R., & Buszewski, B. (2020). Determination of neonicotinoids in honey samples originated from Poland and other world countries. *Molecules*, *25*, 5817. https://doi.org/10.3390/molecules25245817

Louros, C. L. S., Cláudio, A. F. M., Neves, C. M. S. S., Freire, M. G., Marrucho, I. M., Pauly, J., & Coutinho, J. A. P. (2010). Extraction of biomolecules using phosphonium-based ionic liquids + K_3PO_4 aqueous biphasic systems. *International Journal of Molecular Sciences*, *11*, 1777–1791. https://doi.org/10.3390/ijms11041777

Marić, A., Jovanov, P., Gadžurić, S., Trtić-Petrović, T., Sakač, M., Tot, A., Bertić, M., & Vraneš, M. (2023). Application of biodegradable cholinium ionic liquids for the extraction of 5-hydroxymethylfurfural (HMF) from honey. *RSC Advances*, *13*, 32714–32721. https://doi.org/10.1039/D3RA06077B

Marques, C. F. C, Mourão, T., Neves, C. M. S. S., Lima, A. S., Boal-Palheiros, I., Coutinho, J. A. P., & Freire, M. G. (2013). Aqueous biphasic systems composed of ionic liquids and sodium carbonate as enhanced routes for the extraction of tetracycline. *Biotechnology Progress*, *29*, 645–654. https://doi.org/10.1002/btpr.1708

Masiá, A., Suarez-Varela, M. M., Llopis-Gonzalez, A., Picó, Y. (2016). Determination of pesticides and vete-rinary drug residues in food by liquid chromatography-mass spectrometry: A review. *Analytica Chimica Acta*, *936*, 40–61. https://doi.org/10.1016/j.aca.2016.07.023

Merchuk, J. C., Andrews, B. A., & Asenjo, J. A. (1998). Aqueous two-phase systems for protein separation. *Journal of Chromatography B: Biomedical Sciences and Applications*, *711*, 285–293. https://doi.org/10.1016/S0378-4347(97)00594-X

Najdanovic-Visak, V., Lopes, J., Visak, Z., Trindade, J., & Rebelo, L. (2007). Salting-out in aqueous solutions of ionic liquids and K_3PO_4: Aqueous biphasic systems and salt precipitation. *International Journal of Molecular Sciences, 8*, 736–748. https://doi.org/10.3390/i8080736

Nie, L., Zheng, Z., Lu, M., Yao, S., & Guo, D. (2022). Phase behavior of ionic liquid-based aqueous two-phase systems. *International Journal of Molecular Sciences*, *23*, 12706. https://doi.org/10.3390/ijms232012706

Olas, B. (2020). Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? *Nutrients*, *12*, 283. https://doi.org/10.3390/nu12020283

Pei, Y., Wang, J., Liu, L., Wu, K., & Zhao, Y. (2007). Liquid−liquid equilibria of aqueous biphasic systems containing selected imidazolium ionic liquids and salts. *Journal of Chemical & Engineering Data*, *52*, 2026–2031. https://doi.org/10.1021/je700315u

Ravelo-Pérez, L. M., Hernández-Borges, J., Herrera-Herrera A. V., & Rodríguez-Delgado, M. Á. (2009). Pesticide extraction from table grapes and plums using ionic liquid based dispersive liquid-liquid microextraction. *Analytical and Bioanalytical Chemistry*, *395*, 2387–2395. https://doi.org/10.1007/s00216-009-3133-x

Richu, Sharmhal, A., Kumar, A., & Kumar, A. (2022). Insights into the applications and prospects of ionic liquids towards the chemistry of biomolecules. *Journal of Molecular Liquids*, *368*, 120580. https://doi.org/10.1016/j.molliq.2022.120580

Siede, R., Faust, L., Meixner, M. D., Maus, C., Grünewald, B., & Büchler, R. (2017). Performance of honey bee colonies under a long-lasting dietary exposure to sublethal concentrations of the neonicotinoid insecticide thiacloprid. *Pest Management Science*, *73*, 1334–1344. https://doi.org/10.1002/ps.4547

Tu, X., & Chen, W. (2021). Overview of analytical methods for the determination of neonicotinoid pesticides in honeybee products and honeybee. *Critical Reviews in Analytical Chemistry*, *51*, 329–338. https://doi.org/10.1080/10408347.2020.1728516

Ward, L. T., Hladik, M. L., Guzman, A., Winsemius, S., Bautista, A., Kremen, C., & Mills, N. J. (2022). Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. *Science of the Total Environment*, *831*, 154697. https://doi.org/10.1016/j.scitotenv.2022.154697

Welton, T. (2004). Ionic liquids in catalysis. *Coordination Chemistry Reviews*, *248*, 2459–2477. https://doi.org/10.1016/j.ccr.2004.04.015

Yang, C., Ran, L., Xu, M., Ren, D., & Yi, L. (2019). In situ ionic liquid dispersive liquid–liquid microextraction combined with ultra-high performance liquid chromatography for determination of neonicotinoid insecticides in honey samples. *Journal of Separation Science*. *42*, 1930–1937. https://doi.org/10.1002/jssc.201801263

Zafarani-Moattar, M. T., & Hamzehzadeh, S. (2010). Salting-out effect, preferential exclusion, and phase separation in aqueous solutions of chaotropic water-miscible ionic liquids and kosmotropic salts: effects of temperature, anions, and cations. *Journal of Chemical & Engineering Data*, *55*, 1598–1610. https://doi.org/10.1021/je900681b

Zhang, L., Chen, F., Liu, S., Chen, B., & Pan, C. (2012). Ionic liquid-based vortex-assisted dispersive liquid-liquid microextraction of organophosphorus pesticides in apple and pear. *Journal of Separation Science*, *35*, 2514–2519. https://doi.org/10.1002/jssc.201101060

EKSTRAKCIJA TIAKLOPRIDA IZ MEDA PRIMENOM VODENIH BIFAZNIH SISTEMA NA BAZI KALIJUM-FOSFATA I JONSKIH TEČNOSTI

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Sažetak: Tiakloprid, široko korišćen neonikotinoidni pesticid, predstavlja značajan zdravstveni rizik kada je prisutan u medu. Konvencionalne tehnike ekstrakcije su često složene i vremenski zahtevne, što ukazuje na potrebu korišćenja efikasnijih metoda. U ovoj studiji istražena je primena vodenih bifaznih sistema (VBS) zasnovanih na jonskim tečnostima (JT) u kombinaciji sa kosmotropskom soli kalijum-fosfatom (K_3PO_4) za ekstrakciju tiakloprida iz meda, s ciljem unapređenja efikasnosti ekstrakcije i pojednostavljenja procesa. Ispitivane su dve komercijalno dostupne jonske tečnosti, 1-butil-3-metilimidazolijum-hlorid ([C_4mim][Cl]) i tetrabutilamonijum-hlorid ([N_{4444}][Cl]), u kontekstu njihove sposobnosti formiranja VBS i efikasnosti ekstrakcije formiranih sistema. Obe JT su postigle efikasnost ekstrakcije veću od 90%, pri čemu je sistem [C_4mim][Cl] pokazao superiorne performanse, ostvarujući efikasnost ekstrakcije (*EE%*) od 98,11 ± 1,26%. Spektrofotometrijska detekcija primenjena u ovom radu predstavlja bržu, jednostavniju i isplativiju alternativu u odnosu na hromatografske metode. Rezultati ukazuju na potencijal DBS zasnovanih na JT u kombinaciji sa K_3PO_4, kao održivih i efikasnih alternativa tradicionalnim metodama ekstrakcije, nudeći selektivan, brz i ekološki prihvatljiv pristup za ekstrakciju tiakloprida iz kompleksnih matriksa, kao što je med.

Ključne reči: *med, tiakloprid, jonske tečnosti, vodeni bifazni sistemi*

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