PERFORMANCE EVALUATION OF A NEW MAGNETIC ADSORBENT FUNCTIONALIZED WITH DEEP EUTECTIC SOLVENTS FOR TETRACYCLINE ANTIBIOTICS REMOVAL FROM AQUEOUS SAMPLES

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by

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
BenzIm	1-benzylimidazole
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
ButIm	1-butylimidazole
ButIm-EG	1-butylimidazole-ethylene glycol
ChCl	Choline chloride
CTC	Chlortetracycline
DES	Deep eutectic solvent
DO	Doxycline
EG	Ethylene glycol
FE	Factor effect
FFA	Full factorial analysis
FTIR	Fourier Transform-Infrared
LBT	Larger the better
Fe_3O_4	Magnetic iron
Fe_3O_4@DES	Magnetic iron coated with deep eutectic solvent
Fe_3O_4@ButIm-EG	Magnetic iron coated with 1-butylimidazole and ethylene glycol
GFAAS	Graphite furnace-atomic absorption spectrophotometry
Gly	Glycerol
GO	Graphene oxide
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
IL	Ionic liquid
Im	Imidazole
KBr	Potassium bromide
LLE	Liquid-liquid extraction
LOD	Limit of detection
MetIm	1-Methylimidazole
MPBC	Magnetic modified poplar wood biochar
M_S	Magnetic saturation

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear magnetic resonance
OD	Optical density
OTC	Oxytetracycline
PFO	Pseudo-first order
PSO	Pseudo-second order
RSD	Relative standard deviation
SD	Standard deviation
SEM	Scanning electron microscope
S/N	Signal to noise ratio
SPE	Solid phase extraction
STB	Smaller the better
TBAC-PrOH	Tetrabutyl ammonium chloride and propionic acid
TC	Tetracycline
TEM	Transmission electron microscope
TGA	Thermogravimetric analysis
UV	Ultraviolet
UV-vis	Ultraviolet-visible
VSM	Vibrating sample magnetometer
XRD	X-ray diffraction
ZnO	Zinc oxide

LIST OF SYMBOLS

A	Absorbance
pK_a	Acid dissociation constant
q_{cal}	Adsorption capacity of calculation (mg g^{-1})
q_{exp}	Adsorption capacity of experiment (mg g^{-1})
m_i	Average of signal to noise ratio for each level
$\bar{x}$	Average of x
q_e	Binding/adsorption capacity at equilibrium (mg g^{-1})
cm	Centimeter
R^2	Coefficient of determination
ºC	Degree Celsius
Δ	Delta
R_L	Dimensional separation factor
emu	Electromagnetic unit
ΔH^o	Enthalpy
ΔS^o	Entropy
C_e	Equilibrium concentration of adsorbate (mg L^{-1})
R	Gas constant (J mol^{-1} K^{-1})
ΔG^o	Gibbs free energy
g	Gram
H^+	Hydrogen ion
–OH	Hydroxyl group
C_θ	Initial concentration of adsorbate (mg/L)
J	Joules
K	Kelvin
kJ	Kilojoules

L	Liter
$\overline{m}_i$	Means of m_i
μg	Microgram
μL	Microliter
μm	Micrometer
mg	Milligram
mm	Millimeter
mΩ	Milliohm
M	Molarity
mol	Mole
nm	Nanometer
Δq (%)	Normalized standard deviation
N_l	Number of L for each parameter
n	Number of replicates
π	Pi
k_1	Rate constant for PFO kinetic model (min^{-1})
x	Removal percentage
m^2	Square meter
θ	Theta
w/v	Weight per volume (100 mL)
λ	Wavelength, lambda

PENILAIAN PRESTASI PENJERAP MAGNETIK BAHARU DIFUNGSIKAN DENGAN PELARUT EUTEKTIK TERDALAM UNTUK PENYINGKIRAN ANTIBIOTIK TETRASIKLIN DARIPADA SAMPEL AKUEUS

ABSTRAK

Permintaan yang semakin meningkat untuk pemprosesan industri dan aplikasi analisis yang mampan menyokong perlunya alternatif yang lebih hijau dan mudah dilaksanakan berbanding kaedah konvensional dalam penyingkiran ubat-ubatan daripada badan air yang tercemar tanpa mencetuskan kesan yang lebih buruk kepada alam sekitar. Pelarut eutektik terdalam (DES) telah mendapat perhatian yang semakin meningkat kerana sifat hijaunya bersama dengan faedah tambahan seperti penggunaan yang mudah dan kos pengeluaran yang rendah. Dalam penyelidikan ini, DES yang baharu dan mesra alam sekitar yang terdiri daripada etilena glikol (EG) dan 1-butilimidazol (ButIm) telah dipilih sebagai pengubah dalam besi magnetik (II, III) oksida, Fe_3O_4. Sifat unik Fe_3O_4@DES diaplikasikan dalam penyingkiran yang pantas lagi cekap bagi empat jenis tetrasiklin; tetrasiklin (TC), klortetrasiklin (CTC), oksitetrasiklin (OTC), dan deoksisiklin (DO) daripada sampel akueus. Penjerap yang disintesis akan menyediakan pelbagai interaksi termasuk ikatan hidrogen, ikatan π-π, dan juga interaksi elektrostatik yang meningkatkan pertalian penjerap kepada analit tetrasiklin. Dengan tindak balas kepada magnet dari luar, magnetik DES tersebut boleh dikeluarkan dengan mudah, sekali gus menyumbang kepada prosedur penjimatan masa dan pendekatan yang lebih hijau dengan menggunakan sedikit penjerap tanpa menggunakan pelarut toksik yang berlebihan. Sifat kimia, fizikal dan struktur bahan penjerap yang disintesis dinilai melalui spektroskopi inframerah transformasi Fourier

(FTIR), spektroskopi resonans magnetik nuklear (NMR), mikroskop elektron pengimbas (SEM), mikroskop elektron transmisi (TEM), pembiasan sinar-X (XRD), magnetometer sampel bergetar (VSM), analisis termogravimetrik (TGA), dan penganalisis permukaan Brunauer-Emmett-Teller (BET). Enam parameter penting telah diambil kira untuk pengoptimuman – pH, masa interaksi, dos penjerap, isipadu sampel, kepekatan awal dan suhu. Selain itu, untuk mengatasi kaedah konvensional yang memakan masa, iaitu kaedah satu-pembolehubah-pada-satu-masa (OVAT), analisis statistik Taguchi digabungkan dengan spektrofotometri UV-vis sebagai kaedah pengesanan telah disepadukan dalam pengoptimuman pembolehubah. Peratusan penyingkiran maksimum diperoleh dengan kepekatan awal TC 5 mg L^{-1} dengan isipadu 5 mL, pH diselaraskan kepada 6, yang dijerap oleh 15 mg penjerap dan digoncang bersama selama 40 minit pada suhu 45°C. Secara ringkas, teori kinetik penjerapan berpadanan dengan baik dengan model pseudo tertib kedua manakala isoterma dan parameter termodinamik proses penjerapan ini bersesuaian dengan model Freundlich dan Halsey, serta penjerapan pada penjerap adalah eksotermik dan bersifat spontan. Penemuan ini akan membolehkan pemahaman lanjut tentang Fe_3O_4@DES terhadap interaksi tetrasiklin yang akan menyokong pembangunan penjerap baharu yang berpotensi dalam teknologi pengasingan. Teknik ini telah diaplikasikan dalam penyingkiran empat jenis tetrasiklin daripada pelbagai sampel air sisa farmaseutikal. Analisis sampel sebenar bagi penjerap yang dicadangkan mempamerkan penyingkiran tetrasiklin yang sangat baik daripada matriks sampel yang agak kompleks.

PERFORMANCE EVALUATION OF A NEW MAGNETIC ADSORBENT FUNCTIONALIZED WITH DEEP EUTECTIC SOLVENTS FOR TETRACYCLINE ANTIBIOTICS REMOVAL FROM AQUEOUS SAMPLES

ABSTRACT

The increasing demand for a sustainable industrial processing and analytical application advocates for greener and feasible alternatives to the conventional method of drug removal from contaminated water bodies without instigating further detrimental effect to the environment. Deep eutectic solvents (DESs) had garnered growing attention due to their green properties along with additional benefits such as simple and low cost production. In this research, a relatively new and environmentally benign DES comprised of ethylene glycol (EG) and 1-butylimidazole (ButIm) was selected as modifiers in magnetic iron (II, III) oxide, Fe_3O_4. The unique properties of Fe_3O_4@DES were employed to design a rapid, efficient removal of four tetracycline compounds; tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), and doxycycline (DO) from aqueous sample. The synthesized adsorbent will provide a wide range of interactions including hydrogen bonding, π-π bonding, and electrostatic interaction that increases the affinity of adsorbent to tetracycline analytes. In response to an external magnet, the magnetic DES can be drawn out with ease, thus contributing to a time-saving procedure and greener approach by using small amount of sorbent without the excessive use of toxic solvents. The chemical, physical and structural properties of the synthesized adsorbent were assessed via Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffractometer (XRD), vibrating sample magnetometer (VSM), thermogravimetric analysis (TGA), and Brunauer-Emmett-Teller (BET) surface analyzer. Six crucial parameters for the removal of TC were considered for optimization: pH, shaking time, adsorbent dosage, sample volume, initial concentration, and operating temperature. Moreover, to overcome the conventional time-consuming method of one-variable-at-a-time (OVAT) approach, Taguchi design statistical analysis combined with UV-vis spectrophotometry as a detection method was integrated in variable optimization. The maximum removal percentage was observed with 5 mg L^{-1} TC initial concentration in 5 mL of TC with pH adjusted to 6, subjected to 15 mg of adsorbent and shaken for 40 min at 45°C. Briefly, the kinetics theory complied well with pseudo-second-order model while the isotherm model and thermodynamic parameters revealed that the experimental data results exhibit Freundlich behavior and Halsey, and that the TC adsorption onto the adsorbent was exothermic and spontaneous in nature. These findings will allow further understanding on the Fe_3O_4@DES towards TC interactions that will support the development of new potential sorbent for separation technology. The technique was applied to remove four tetracycline compounds from various pharmaceutical wastewater samples. Real sample analysis of the proposed adsorbent exhibit an excellent removal of tetracyclines from relatively complex sample matrices.

CHAPTER 1

INTRODUCTION

1.1 General background

Tetracyclines are commonly used antibiotic in human and veterinary medicine. It is also used as a growth promoter in animals such as chicken, swine, and cattle (Osorio, Mezquita & Balcázar, 2023). Regulatory authorities have established maximum residual limits (MRLs) for tetracyclines in animal-derived food products. The MRL is the highest concentration of a pesticide or veterinary medicine residue that may be legally allowed in animal-derived food products receiving the treatment. These limits help to ensure that the concentration of tetracyclines does not exceed the level that are considered to be safe for human health, based on scientific data and risk assessments. The MRL for tetracycline differs depending on the country and region. Food and Agriculture Organization (FOA) of the United Nation established the MRLs allowed for tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) in kidney, liver and muscle were 1200 $\mu g\ kg^{-1}$, 600 $\mu g\ kg^{-1}$ and 200 $\mu g\ kg^{-1}$, respectively (for cattle, porcine, poultry, and sheep), while for egg is 400 $\mu g\ kg^{-1}$ (FAO, OMS & CODEX, 2015). For milk products, the MRL is set at 400 $\mu g\ L^{-1}$. In aquafarming, the MRL for fish and prawn is set only for OTC which is 200 $\mu g\ kg^{-1}$. On the contrary, the European Union implemented that the MRLs for tetracyclines (TC, CTC and OTC) are much lower: 600 $\mu g\ kg^{-1}$, 300 $\mu g\ kg^{-1}$, 200 $\mu g\ kg^{-1}$, and 100 $\mu g\ kg^{-1}$ for kidney, liver, eggs, and muscle (including fish), respectively, while for milk is 100 $\mu g\ L^{-1}$ (European Commission, 2009).

The regulatory limit for tetracycline antibiotics in water bodies such as rivers, lakes, and groundwater is not well established because unlike for food-producing animals, as it is not a common exercise to administer these antibiotics directly in water bodies. However, surface and groundwater may get contaminated by the excrements of the treated animals (Mutuku, Gazdag & Melegh, 2022). When tetracycline is ingested, it is not fully metabolized by the animal, and a significant amount is excreted in feces and urine (Yang, Huang & Huang, 2019). Therefore, it can enter the environment through agricultural runoff, wastewater treatment plants, and animal manure disposal (Gothwal & Shashidhar, 2015; Javid *et al.*, 2016). Excessive tetracycline in aquatic environments can have a severe impact on aquatic life. Tetracycline can inhibit the growth of algae and other photosynthetic organisms that form the basis of the aquatic food chain (Bashir & Cho, 2016). It can also reduce the diversity of aquatic invertebrates and inhibit the reproduction of fish, leading to long-term population declines (Li *et al.*, 2023).

Conversely, it is crucial to develop efficient methods to remove tetracycline from water bodies. Several methods have been developed to address the issue of tetracycline contamination in water sources. These methods include advanced oxidation processes, adsorption techniques, and membrane filtration. However, each of these methods has its limitations. One of the major challenges in removing tetracycline from water is the high cost associated with these treatment methods. Advanced oxidation processes, such as UV radiation or ozonation, require specialized equipment and consume significant amounts of energy, making them economically unfeasible (Han *et al.*, 2020). Furthermore, such treatment method can produce toxic transformation product of tetracyclines. Another limitation of current tetracycline removal methods is their low efficiency. Some of these techniques may not effectively remove tetracycline at low concentrations (Ahamad *et al.*, 2020). Adsorption techniques, such as activated carbon or other materials, can be costly and require frequent replacement, adding to the overall expenses (Oesterle *et al.*, 2020). They also consume chemicals during their activation (Thompson *et al.*, 2016).

Given the limitations of the existing approaches, there is a pressing need for an innovative and sustainable method to effectively and economically remove tetracycline from water sources. One promising approach is the use of advanced nanomaterials for tetracycline removal. Nanomaterials have unique properties that make them highly efficient in adsorbing and degrading contaminants. By designing nanomaterials with specific properties, such as high surface area, enhanced reactivity, and affinity towards tetracycline, it is possible to develop efficient and cost-effective systems for water treatment (Rana & Bassi, 2023).

Magnetic removal method involves the attraction of dissolved tetracycline molecules to the surface of the magnetic adsorbent, resulting in their adsorption and removal from the water. Magnetic adsorbents are materials that can be magnetized and used to capture dissolved contaminants from water. These materials have demonstrated excellent adsorption capacities for various types of contaminants, including tetracycline (R. Li *et al.*, 2020). However, magnetic adsorbents alone may not be efficient in removing tetracycline from water, especially when the concentration of the antibiotic is low. Hence, magnetic nanoparticle with deep eutectic solvents (DES) have emerged as promising candidates for the removal of various contaminants from water samples.

Deep eutectic solvents (DES) are environmentally benign analogue for ionic liquid that can dissolve organic compounds and enhance the adsorption capacity of magnetic adsorbents (Li, Wang & Row, 2017; Lim, Pang & Lau, 2023). DES are composed of two or more components that form a eutectic mixture, resulting in a lower melting point than any of the individual components. The coating of the magnetic adsorbent with DES will increase the affinity for tetracycline, resulting in enhancement in the adsorption capacity of magnetic adsorbents, by incorporating more diverse interactions into adsorption systems, including hydrogen bonding, π-π bonding, halogen bonding, and electrostatic interactions (Wei *et al.*, 2020). The advantages of using magnetic adsorbents coated with DES for tetracycline removal include high adsorption capacity, low cost, and simplicity, without the need to centrifuge or undergo filtration (Yao *et al.*, 2015; Zhou *et al.*, 2017; Wang *et al.*, 2019). The adsorbents can be easily removed from the water once tetracycline molecules are attached to their surface, and they can be reused by regeneration (Hassan *et al.*, 2020). However, their application in the removal of tetracycline from water has not been extensively explored.

The mechanisms involved in tetracycline removal using magnetic adsorbents coated with DES are still under investigation. However, based on the available literature, it appears that the DES may interact with the tetracycline molecules through hydrogen bonding and electrostatic interactions, resulting in enhanced adsorption capacity. Additionally, the magnetic adsorbent coated with DES is highly stable and can withstand repeated use without significant loss of adsorption capacity.

The conventional one-variable-at-a-time OVAT method poses several limitations as it tests only one variable at a time, which can be time-consuming and may not be able to account for interactions between variables that are critical to the outcome. Taguchi analysis is introduced to overcome this drawback by reducing the number of experiments required to obtain results.. Taguchi approach experimental design involves organizing the parameters affecting a process and their levels using orthogonal arrays. This method allows for the evaluation of the effects and possible interactions of several variables simultaneously. Traditional OVAT requires more experiments to be conducted, as each variable is tested separately. Taguchi analysis utilizes orthogonal arrays, which reduces the number of experiments needed to obtain the same information. Taguchi analysis allows for the identification of the optimal settings for each variable to achieve the desired outcome. The method includes an optimization step that takes into account the variation in the results due to each variable and identifies the settings that will result in the optimal outcome.

This thesis aims to investigate the potential of magnetic DES for the removal of tetracycline from water. The research will involve the synthesis and characterization of magnetic DES formulations optimized for tetracycline adsorption. The performance of the developed magnetic DES will be evaluated in terms of adsorption efficiency and reusability. Additionally, the impact of various parameters such as pH, shaking time, adsorbent dosage, initial tetracycline concentration, sample volume, and temperature on the adsorption process will be investigated. Moreover, there has been a limited amount of research reporting on the toxicology profile of magnetic DES. The potential toxicity of magnetic DES, including their impact on human health and the environment, remains largely unexplored. To address this knowledge gap, further scientific investigations and studies are done to thoroughly assess the cytotoxicological properties of the synthesized material. The outcomes of this research will contribute to the development of a sustainable and cost-effective method for the removal of tetracycline from water, thereby mitigating its potential harmful effects on human health and the environment. Furthermore, this research will provide insights into the potential application of magnetic DESs for the removal of other pharmaceutical contaminants from water, paving the way for future studies in this field.

1.2 Scope of study

This study aimed to analyze the removal efficiency of the synthesized adsorbent Fe_3O_4@ButIm-EG towards tetracycline antibiotics. The individual components of DES

(ButIm and EG) and the synthesized materials (ButIm-EG, Fe_3O_4, and Fe_3O_4@ButIm-EG) were characterized using Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffractometer (XRD), vibrating sample magnetometer (VSM), Brunauer-Emmett-Teller (BET) surface analyzer, and thermogravimetric analysis (TGA) to define their chemical and physical properties. A cell toxicology study was also generated. The synthesized Fe_3O_4@ButIm-EG adsorbent was used to remove TC from the aqueous samples. The optimization of the removal conditions using TC as the parent compound for tetracycline antibiotics via UV-vis spectrophotometry. The influence of temperature, shaking time, initial concentration, volume of solution, pH, and the amount of sorbent were optimized using Taguchi design with three replicates for each run. The adsorption kinetics, isotherm, and thermodynamics studies were also conducted and discussed. The method was validated and applied for the removal of four tetracycline compounds (TC, CTC, OTC, and DO) from possibly contaminated pharmaceutical environmental water samples.

1.3 Objective of the research

Main objective:

To elucidate the adsorption mechanism of tetracycline antibiotics in aqueous samples using a newly synthesized green magnetic adsorbent coated with DES (Fe_3O_4@ButIm-EG).

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Specific objectives:

1) To determine the optimum molar ratio of hydrogen bond acceptor to hydrogen bond donor in DES and iron oxide for the synthesis of Fe_3O_4@ButIm-EG.

2) To investigate the performance of Fe_3O_4 @ButIm-EG for the removal of tetracyclines from aqueous sample.
3) To analyze the capability of the prepared adsorbent under optimized conditions for the removal tetracyclines from environmental water samples.

1.4 Outline of the thesis

This thesis consists of five chapters. **Chapter 1** introduces the background, scope, and purpose of this research. **Chapter 2** contains the literature review, defining the major elements revolving around this study. **Chapter 3** outlines the methodology, including the origins of chemicals, reagents, and instruments used in this project, as well as the synthesis of adsorbents, storage procedures, sample preparation techniques, and execution of optimization process. This chapter describes the synthesis, characterization, and toxicity study of the material, followed by the adsorption study for the removal of tetracyclines antibiotics using the synthesized adsorbents and optimization of removal conditions. Findings, results, and outcomes obtained are presented in **Chapter 4**. This chapter reveals the characterization and cell toxicological study of synthesized materials and presents supporting evidences. Details of the outcomes of the optimization process, adsorption study, method validation, application on real environmental water sample, and reusability of the synthesized adsorbent are also portrayed. Finally, **Chapter 5** infers the overall findings of the project and proposes suggestions, improvements, and recommendations for future direction of the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Tetracycline

2.1.1 Background and usage

Tetracycline is a family of antibiotics capable of treating an array of bacterial infections. It is well known that tetracyclines have a broad spectrum of activity and are effective against infections caused by Gram-positive and Gram-negative bacteria, as well as certain anaerobes (Bougrini *et al.*, 2016). Tetracycline antibiotics operate by inhibiting the growth of bacteria, specifically by preventing their protein synthesis by binding to the 30S ribosomal subunit and block aminoacyl-tRNA molecules from attaching, which is vital for the creation of new peptide bonds during synthesis of protein, hence, preventing replication of the bacteria which ultimately results in bacterial death (Chopra & Roberts, 2001; Hong, Zeng & Xie, 2014).

Tetracycline name was derived from the four naphthalene ring structure as shown in **Figure 2.1(a)**. *Aureomycin*, which was later known as chlortetracycline (CTC), **Figure 2.1(b)**, was the earliest known member of the tetracycline family first recorded in 1948 by a team of scientists lead by Benjamin Duggar (Duggar, 1948). They were isolated from the bacterium *Streptomyces aureofaciens*, which occurs naturally in soil and were clinically commercialized in late 1940s and early 1950s (Darken *et al.*, 1960). Two years later after the first discovery of CTC, Finlay *et al.*, (1950) isolated *terramycin* or later known as oxytetracycline (OTC), **Figure 2.1(c)**, from the bacterium *Streptomyces rimosus*. Tetracycline (TC) itself, the nomenclature of the series, was synthesized by Lloyd H. Conover in 1953 through a series of chemical reactions involving reductive dechlorination of the naturally occurring compound CTC (Conover *et al.*, 1953). This discovery and development of tetracyclines revolutionized the development of other tetracycline antibiotics and led to a significant increase in their availability and obtain safer drugs with better therapeutic benefits (Nelson & Levy, 2011). The three tetracycline antibiotics, TC, OTC and CTC, were the basis for the development of new derivatives. For example, doxycycline (DO), **Figure 2.1(d)**, is a second-generation tetracycline that can be produced semi-synthetically from naturally occurring tetracyclines (Tan *et al.*, 2011). DO serves as a safe, cost-effective and readily available antibiotic with anti-inflammatory characteristics when added to standard of care treatments for COVID-19 patients and had been proven effective to decrease the requirement for ICU admission (Dhar *et al.*, 2023).

Tetracyclines are the world's second most prevalent antibiotic class, accounting for more than one-third of antibiotic manufacturing and use annually (Pulicharla *et al.*, 2017; Pattanayak *et al.*, 2022). They are considered safe drugs and have many favorable properties due to their broad-spectrum activity, low cost, ease of administration and relatively minor side effects (del Castillo, 2013). Tetracyclines are usually prescribed for infections ranging from acne to urinary tract infections, respiratory infections, and sexually transmitted infections while their anti-inflammatory properties makes it suitable to attenuate conditions such as rosacea and periodontitis (Borghi & Palma, 2014; Majewski, 2014; Tilakaratne & Soory, 2014). A recent study in Colombia revealed that 95.1% of prescribe tetracycline was DO, with more than half was prescribed for acne treatment (Valladales-Restrepo *et al.*, 2022).

Tetracycline is one of the four mainly used group of antibiotics in animal husbandry apart from β-lactams, fluoroquinolones and sulphonamides (Chen *et al.*, 2023). Among these, TC, CTC, OTC, and DO are often used in veterinary medicine to treat infections and prevent diseases in animals, and it is also a common practice to use them as food additive to act as growth promoter for livestock to cater for increasing demand for animal-derived food resources (Granados-Chinchilla & Rodríguez, 2017; Maghsodian *et al.*, 2022).

Granados-Chinchilla and Rodríguez (2017) added that before its permanent ban in the US in 2017, CTC and OTC had been approved as growth promoters for swine, cattle and calves while DO was mainly used in medical treatment for companion animals (such as cats and dogs) infected by *Rickettsia*, *Bartonella*, *Toxoplasma*, and *Anaplasma* as well as Lyme disease, salmon poisoning, leptospirosis, etc. In apiculture, beekeepers also may use tetracyclines especially OTC to treat bacterial infections in bees or to prevent the spread of diseases within the colony (Aljedani, 2022).

In the plantation sector, tetracycline had been used to treat bacterial infections in trees and plants. For instance, OTC has been demonstrated to be the most successful therapy for citrus greening disease, a crop disease that causes plants to produce green, deformed, and bitter citrus fruits unfit for sale caused by "*Candidatus* Liberibacter asiaticus", a bacterium spread by disease-infected insect called the Asian citrus psyllid (*Diaphorina citri* Kuwayama) (Grafton-Cardwell, Stelinski & Stansly, 2013; U.S. Department of Agriculture, 2018; Hijaz *et al.*, 2020).

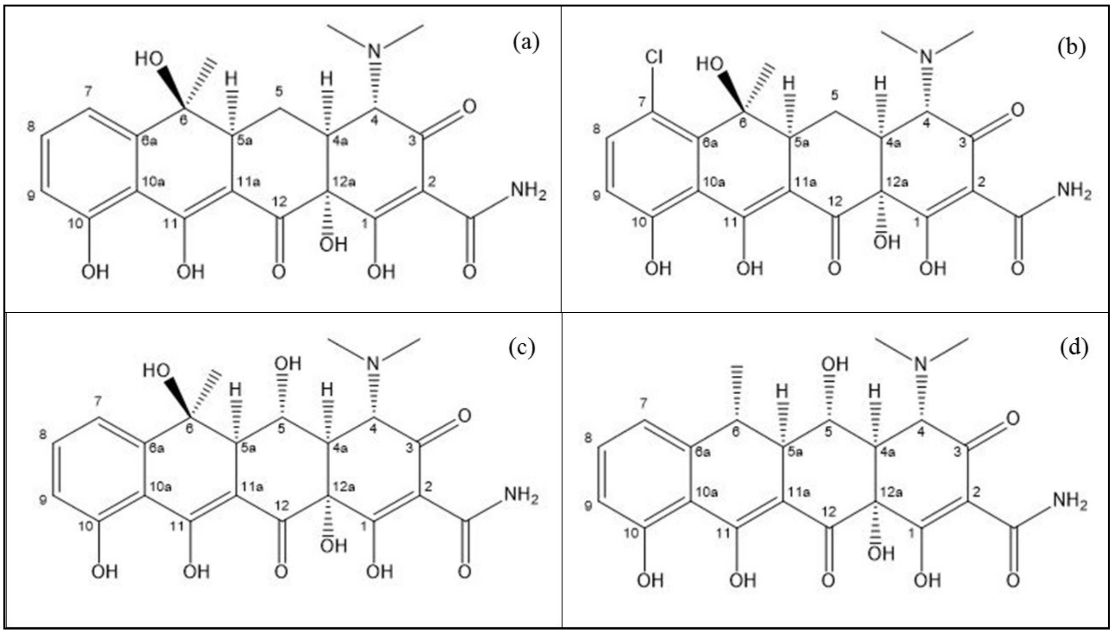

Figure 2.1: Structures of (a) tetracycline, (b) chlortetracycline, (c) oxytetracycline, and (d) doxycycline.

2.1.2 Tetracyclines contamination and implications to ecosystem

Tetracyclines may leach the environment in a number of significant ways. Similar to any medications, tetracycline antibiotics consumed by human can be excreted and may end up in the environment through means such as sewage and wastewater discharge from residences, medical facilities and other sources (Polianciuc *et al.*, 2020). This pathway also includes an improper disposal of the antibiotics, by flushing them down or by irresponsible disposal of unused antibiotics which subsequently causes landfill leachates (Ben *et al.*, 2019; Rogowska & Zimmermann, 2022). In addition, certain antibiotics may still be present in treated wastewater effluent since wastewater treatment facilities may not be able to completely eradicate these micropollutants (Hoff, Pizzolato & Diaz-Cruz, 2016; Zainab *et al.*, 2021).

For more than 70% of the time, tetracyclines are released in their active form via urine and feces from animals and humans, causing them to remain biologically active in the aquatic system (Daghrir & Drogui, 2013). Antibiotics consumed by livestock may not be completely metabolized and will be released into the environment through excrement. These manures were sometimes used as organic fertilizers in agronomies which leads to tetracyclines and their metabolites to be leeched into the soil, groundwater, and eventually surface waterways (Kang *et al.*, 2016). One of the key mechanisms that impacts the fate of tetracycline antibiotics in the environment is adsorption to soil particles since tetracyclines have a high tendency to bind with soils and sediment through electrostatic interaction between the positively charged –$NH(CH_3)$ group and negatively charged soil particles, surface complexation at the tautomeric C-11 and C-12 β-diketone system (under alkaline condition) with divalent metal ions that exist freely in soils (e.g., Ca^{2+}, Cu^{2+}, Fe^{2+}, etc.), van der Waals attraction, and hydrogen bonding (Wang & Wang, 2015; Chen *et al.*, 2017).

Antibiotic residues, including tetracyclines, from municipal and agricultural wastewater treatment plant effluents are frequently detected in surface waters, groundwater, soil and sediments (Daghrir & Drogui, 2013; Huang *et al.*, 2020). In 2019, Olaniran and colleagues compares TC concentration (administered to cows) in effluent discharge and surface water in which they found that higher concentration was detected in the later which demonstrates the accumulation and persistence of tetracyclines in water bodies (Olaniran, Sogbanmu & Saliu, 2019). Tetracycline leakage into the environment had been linked to the development of an increasing number of bacteria discovered to be resistant to tetracyclines (Jubeh, Breijyeh & Karaman, 2020). For example, Shahaza *et al*. (2017) found that *Escherichia coli* extracted from poultry and porcine showed a significant resistance to TC and DO. Honeybees are one of the perpetrators for harboring and spreading of tetracycline-resistance gene as revealed by Levy and Marshall (2013). Antibiotics used in beekeeping can then be transferred to the honey produced by the bees, as well as beeswax, propolis, larvae, and pollen (Aljedani, 2022).

Prolonged consumption of bee-products contaminated with traces of antibiotics not only caused drug-resistance, but also disturbance of gut flora, carcinogenicity, teratogenicity, bone marrow depression, and hypersensitivity, among other concerns (Sharma *et al.*, 2023).

There are three known type of resistance mechanisms exhibited by tetracycline; active efflux pump, ribosomal protection an enzyme inactivation (Thiang *et al.*, 2022). Antibacterial resistance occurs when bacteria are exposed to low doses of antibiotics, causing them to develop resistance via a process known as horizontal gene transfer (HGT). HGT is process where antibiotic-resistant bacteria spread their resistance genes (excluding gene transfer from parent to offspring) to other bacteria in the environment, causing bacterial mutation and evolution (Burmeister, 2015). Exposure to antibiotic, even at low concentration, will also causes some bacteria to survive, mutate and develop a population of bacteria with resistant strains which will become dominant in an environment over time. These bacteria will ultimately spread to animals and human, causing common antibiotics to be no longer effective and certain infections to be more difficult to cure. Inevitably, the use of a stronger, more potent type of antibiotics with higher dosage of will be obligatory.

Several antibiotics can persist for months to years in agricultural-related matrices, even though many of them have short half-lives ranging from days to weeks, especially at high concentrations (Massé, Saady & Gilbert, 2014). Antibiotics with an environmental half-life of more than 60 days to degrade are regarded as very persistent, whilst those with half-life of more than 14 days might pose environmental issues (Borghi & Palma, 2014). Environmental half-lives of tetracyclines were estimated between 2 days (TC, OTC ad DO) to 12 days (CTC) as demonstrated in a mesocosm experiment designed by Chabilan *et al.* (2023). Even though they were classified under non-persistent to moderately-persistent group in the experiment, findings by Massé and colleagues in 2014 implied that tetracyclines could persist for more than 60 days in manure storage.

Traces of the much more resilient tetracycline could exist for a long period of time in the ecosystem, enabling them to accumulate to a high concentration and eventually posing threat to the aquatic lives (Maghsodian *et al.*, 2022). The biodiversity of the microflora and bacterial populations in the environment may also be impacted by the presence of tetracycline antibiotics (Grenni, Ancona & Barra Caracciolo, 2018). Specific antibiotics, for instance, may selectively suppress the growth a certain group of bacteria while some others thrive. Besides, some antibiotics are known to be toxic to some aquatic organisms, including algae, crustaceans, and fish (Limbu *et al.*, 2018). They can accumulate in the tissues of these aquatic organisms especially in their life early stage and can compromise their reproductive and immune systems (Hao *et al.*, 2014).

Tetracyclines concentrations can differ widely, but they are typically measured in micrograms per liter $\mu g\ L^{-1}$. For instance, domestic wastewater contains low tetracyclines concentrations, around 1 $\mu g\ L^{-1}$, while hospital wastewater may have relatively higher levels, reaching up to 100 $\mu g\ L^{-1}$ (Fiaz, Zhu & Sun, 2021). The concentrations of common tetracycline antibiotics used in aquatic farm (e.g., TC, OTC, minocycline, DO, CTC, and demeclocycline) have been mostly found to be ranging from 1 ng L^{-1} to 100 $\mu g\ L^{-1}$ level (Liyanage & Manage, 2019; Yuan *et al.*, 2019). Tetracyclines can be adsorbed or ingested by aquatic organisms and enters the food chain through passive absorption by vegetables which later will be consumed by human (Gothwal & Shashidhar, 2015). This might result in unintentional antibiotic consumption which can lead to adverse health effects such as teeth discoloration in children from prolonged consumption of tetracycline-contaminated milk (Aalipour *et* *al.*, 2015). Other side effects include allergies, liver damage and gastrointestinal issues due to disruption of intestinal microbiota (Buzia, Ploscutanu & Elisei, 2019; Khataee *et al.*, 2020). Furthermore, antibiotics may undergo chemical and biological transformation such as hydrolysis, oxidation and photo degradation when exposed to the environment (Gothwal & Shashidhar, 2015). They can be metabolized by environmental microbes, which may result in by-products that are either more or less harmful than the original medication (Boxall, 2004; Capleton *et al.*, 2006; Gothwal & Shashidhar, 2015). Past studies had shown that the environmental degradation products formed as a result of UV light degradation, biodegradation and hydrolysis for CTC, OTC and tetracyclines (TC, CTC, OTC and DC), respectively, were found to be even more toxic to organisms in the environment than the parent compound (Guo & Chen, 2012; Li *et al.*, 2019; Zhong *et al.*, 2022).

Tetracycline and its derivatives are a useful class of antibiotics with wide variety of application in veterinary, agriculture and human therapy. Unfortunately, these antibiotics may accidentally be released into the environmental water body, either directly or indirectly via run off from farms, sewage, and wastewater treatment facilities. This may cause accumulation of tetracycline in the environment and result in a number of issues with the balance of the ecosystem and human health due to the emergence of bacteria resistant to antibiotics (Xue *et al.*, 2022). Therefore, studying the adsorption, separation, and removal of tetracycline antibiotics from the environmental water bodies is crucial to mitigate the detrimental impact of antibiotic resistance. The balance of the ecosystem depends on the continuing study and development of the many techniques for eliminating these antibiotics from contaminated water sources.

2.1.3 Occurrence of tetracyclines in Malaysia

Some of the ways in which tetracyclines can be introduced to water bodies and land environment in Malaysia include animal husbandry, agricultural runoff and aquaculture. More than 97 types of antibiotics had been approved as antimicrobial medicines for veterinary use in Malaysia, which include tetracyclines (Premarathne *et al.*, 2017; Department of Veterinary Services Malaysia, 2021). Tetracycline usage had been approved for treating and preventing bacterial infections in livestock (bovine and swine), poultry and household pets which includes feline, canine, small mammals, leporidae (rabbit), avian, reptiles, and equine (horse). Only TC, CTC, OTC, and DO were approved for the said purposes. Tetracyclines can be administered orally – by spiking the animals' drinking water or via injection.

The MRL established for the approved tetracyclines closely follows the values set by FOA, and the levels were specified in the first edition Malaysian Veterinary Antimicrobials Guidelines 2021 (Department of Veterinary Services Malaysia, 2021). In specific, the MRLs for TC and CTC administered orally in animal-derived food products for muscle, liver, kidney, and eggs are 200, 600, 1200, and 400 µg kg^{-1}, respectively, while for milk is 100 µg L^{-1}. The limits for OTC and DO are 50% lower which are 100, 300, 600, 10, and 200 µg kg^{-1} for muscle, liver, kidney, fat, and eggs, respectively, while for bovine milk is also 100 µg L^{-1}.

Premarathne *et al.* (2017) discovered more than 70% of *Campylobacter* spp. isolated from cattle fecal samples and beef meat obtained from various markets in Selangor, Malaysia were found to be ineffective against tetracycline. In the poultry aspect, two studies were conducted focusing on poultry farms scattered in three states in the East coast of Peninsular Malaysia regarding the prevalence of bacterial resistance (*Escherichia coli* and *Salmonella* spp.) towards certain classes antibiotic (Elmi *et al.*, 2021; Osman *et al.*, 2021). Even though low levels of tetracyclines were observed in surface waters, high levels of tetracyclines were still detected in poultry excrement and sediment samples of Kelantan resulting from agriculture run-off (Elmi *et al.*, 2021). Consequently, a high percentage of *E. coli* isolated from poultry fecal matter was found to be susceptible to TC (91.2%), OTC (89.1%) and DO (63%). Discovery of substantial number of *Salmonella* spp. susceptible to sulphonamides and tetracyclines by Osman et al. (2021) also implied that these two antibiotics as growth promoter is still a common practice in poultry business as well as agriculture in Malaysia. Ho *et al.* (2014) concurred the theory in which the practice of animal waste as fertilizer in agricultural contributes to the dissemination of tetracyclines into the environment, based on the samples collected in three states (Selangor, Negeri Sembilan and Melaka). Their study detected a significantly high DO residue, which is approximately 7.85×10^4 $\mu g\ kg^{-1}$ dry weight, in broiler manure. These residues were also found in the soils treated with the manure.

Aquafarming is one of the possible sources of tetracycline contamination in Malaysia. The use of this antibiotic has been associated with the development of antibiotic-resistant bacteria reported in west coast waters in Malaysia, which can be transmitted to humans through contaminated seafood (You, Bong & Lee, 2016; Faja *et al.*, 2019). Tetracycline had also been used in aquaculture industry mainly to treat Vibriosis outbreak, disease caused by *Vibrio* spp. and *Photobacterium* spp. that affect the mortality rate of shrimps in aquaculture industry (Sanches-Fernandes, Sá-Correia & Costa, 2022). In a recent research by Thiang *et al.* (2021), tetracycline is the most frequently detected class of antibiotics in 29 investigated marine aquaculture farms across Peninsular Malaysia. They specified that among these antibiotics, OTC, TC and minocycline are the three most detected tetracyclines, at ng L^{-1} level. In 2022, Thiang and colleagues investigated the presence of five tetracycline compounds (TC, CTC, OTC, DO, and minocycline) and the occurrences of bacteria carrying tetracycline-resistant genes in five aquaculture farms in west coast Malaysia (Thiang *et al.*, 2022). While their findings conclude that CTC and minocycline were not detected in all sampling locations, TC was detected at a higher level (14.4 ng L^{-1} to 25.6 ng L^{-1}) in two sampling sites. Compared to OTC and DC which were below limit of quantitation (LOQ), this shows that the use of TC is more dominant (at least in the studied regions) compared to the rest of the studied compounds.

Low *et al.* (2021) conducted a study at Larut and Sangga Besar River, Perak, Malaysia for the incidence of six classes of antibiotics (sulfonamide, macrolide, fluoroquinolone, phenicol, trimethoprim, and tetracycline) originating from different contamination sources (hospital, zoo and slaughterhouse wastewater effluent). While CTC was not detected in all samples, TC was detected at 66.94 – 1092.49 ng L^{-1} range in hospital wastewater at midstream Larut River. Meanwhile, the detection of OTC in zoo and slaughterhouse wastewater effluent were at 18.81 – 108.88 ng L^{-1} and 26.7 – 92.58 ng L^{-1} range, respectively.

2.2 Deep Eutectic Solvents (DES)

A new approach to remove pharmaceutical pollutants from environmental samples was proposed using low-melting point organic salts and ionic liquids. These were shown to have various characteristics, such as low vapor pressure, excellent thermal stability, and low flammability. However, the challenges associated with the process still remain. Recently, a new class of deep eutectic solvents (DESs) has emerged that have a comparable properties to ILs but with superior qualities such as lower toxicity, low cost, and easy preparation with no preparation required (Yao *et al.*, 2015; Wang *et al.*, 2019).

DESs are mixtures of Lewis acids or Brønsted bases and hydrogen bond acceptors. The term DES refers to liquids that are close to the eutectic composition of the mixtures, such as the molar ratio of the components that gives lower melting point than that of the starting components, generally under moderate heating (Abbott *et al.*, 2004, 2017). This keeps production costs comparatively low compared to conventional ILs and enables large-scale applications. Zhang *et al.* (2020) described the generation of eutectic mixtures as complexes formed between halide salts and a range of compounds such as alcohols, polyols, amides, amines, and carboxylic acids (Zhang, Zhang & Yu, 2020). DES contain large, asymmetric ions that have a low lattice energy and thus a low melting point. They are usually obtained by complexing a quaternary ammonium salt with a metal salt or a hydrogen bond donor (HBD). The charge shifts that results from the hydrogen bonds between, for example, a halide ion and the hydrogen donor moiety is responsible for the responsible for lowering the melting point of the mixture compared to the melting points of the individual components. The development of a task-specific solvent based on the combination of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) could lead to the efficient removal of pharmaceutical compounds from contaminated sources. While the individual components of DES are usually well characterized toxicologically, there is very little information on the toxicological properties of the eutectic solvents themselves, which needs further investigation by the scientific community.

2.2.1 Hydrophobic and hydrophilic DES

DES can be classified based on their hydrophilic or hydrophobic nature. The properties of DES can be adjusted by the correct selection of the individual components in terms of molecular structure, chemical nature, ratio and water content (Asfaram *et al.*, 2015).

Due to their hydrogen bonding ability, DES are generally hydrophilic and therefore dissolve relatively easily in an aqueous environment.

Hydrophobic DES are DESs that have low polarity and are not readily miscible in water, whereas hydrophilic DES are DESs that have high polarity and are water-soluble. The variation in polarity and solubility has major impact on their attribution and prospective usages. Hydrophobic DESs are a new class of water immiscible solvents that was first published in the year 2015 in two literatures (Ribeiro *et al.*, 2015; Van Osch *et al.*, 2015). Hydrophobic DES are primarily generated by combining an HBA with a non-polar HBD, such as a fatty acid or a long chain hydrocarbon that results in DES with low polarity (Chen *et al.*, 2021). Many applications have been made use of these solvents. DL-menthol:carboxylic acid, acid:thymol and terpenoids:carboxylic acid are a few examples of successfully synthesized hydrophobic DES in researches (Florindo *et al.*, 2017; Schaeffer *et al.*, 2018; Li *et al.*, 2022).

One advantage of hydrophobic DES is that the solvents are easily separable in water-containing matrices due to their difference in density without leaving residues, hence can be recycled and reused (Verma & Banerjee, 2018). Hydrophobic DES are superior solvents for treatment of extraction of non-polar compounds such as hydrophobic volatile organic compounds (e.g., toluene) and parabens which poses detrimental impacts on human health and environment (Chen *et al.*, 2021; Zelinski *et al.*, 2022). In pharmaceutical application, Fatty acid/alcohol-based hydrophobic DES have been employed as an alternative to traditional hydrophobic organic solvents (e.g., halogenated solvents) in liquid-liquid microextraction of levofloxacin and ciprofloxacin antibiotics (Tang, Dai & Row, 2018).

On the other hand, hydrophilic DESs are solvents that tend to be highly polar and have high affinity for water. Alcohols, acids, organic salts, and sugars are examples of hydrophilic compounds that make up hydrophilic DESs (Florindo, Branco & Marrucho, 2019). A HBA with polar functional groups, such hydroxyl (-OH) or carboxyl (-COOH) groups, is the main component of hydrophilic DES. Hydrophilic DES is water soluble because these functional groups have the ability to establish hydrogen bonds with water molecules. Choline chloride:EG, L-arginine:glycerol (Gly) and caprolactam: tetrabutylammonium bromide are a few examples of hydrophilic DESs produced in recent years (Lin *et al.*, 2014; Ren *et al.*, 2018; J. Zhang *et al.*, 2019). Most human medicines and drugs are polar in nature as they need to be soluble in water in order to be effectively transported throughout the bloodstream since water makes up about 60% of the human body (Mitchell *et al.*, 1945). Tetracyclines are polar in nature due to the presence of several polar functional groups in the molecule, namely the hydroxyl (–OH), carbonyl (–C=O), and amino (–NH_2) groups, which generate an overall uneven distribution of charge across the molecule (Shen *et al.*, 2019). Hence, tetracyclines are likely to be chemically compatible with polar DESs and less effective in non-polar ones. In one example, molecularly imprinted polymers (MIPs) were traditionally synthesized in organic solvents, causing them to have a considerably poor performance in aqueous environment (Wang, Qiao & Yan, 2021). Wang and colleagues developed an approach to overcome this predicament, by encompassing hydrophilic DES in the synthesis process of molecularly imprinted resin (MIR), abbreviated as DES-HMIR. This modification not only results in increased adsorbent specific surface area, but also overcome the water phase compatibility issue, which results in better selective molecular recognition and enhanced the overall performance of the adsorbent.

2.2.2 Imidazole-based DES

Imidazole (Im) is an organic compound composed of five-membered ring (heterocyclic aromatic molecule) with two nitrogen atoms in the ring. Im-based DESs are formed by combining the compound with hydrogen bond donors, typically choline chloride (ChCl), urea, Gly, amines, carboxylic acids and their homologs, and have been researched for many uses in chemistry including as solvents or catalyst for chemical reactions, as plasticizer, and in gas adsorption (Zdanowicz, Spychaj & Maka, 2016; Zhang *et al.*, 2017; Adamus *et al.*, 2018). Imidazoles have also been investigated for their potential in the other areas such as additives in membrane fabrication, separation chemistry and biomass conversion (Jiang *et al.*, 2018; K. Li *et al.*, 2021; Ruan *et al.*, 2021).

In comparison to conventional organic solvents, imidazole-based DES offers several advantages over traditional organic solvents, making them an attractive alternative for a wide range of applications. First off, DESs based on imidazole are renowned for being biodegradable and having minimal toxicity (Zdanowicz, Spychaj & Maka, 2016). Zdanowicz and colleagues studied the combination of ChCl as HBA with Im, Gly and carboxylic acids (citric or malic) as HBDs. They discovered the potential application of DES as plasticizer to replace conventional ILs and other organic solvents where they found that the combination of imidazole-based DES (ChCl/Im) is capable of lessening the polymer chain interaction and bind them together. In comparison to many conventional organic solvents, they are thus less hazardous to both human health and the environment. In the pharmaceutical sector, where the use of toxic solvents may be hazardous to both human health and the environment, this makes them more ecologically friendly and sustainable alternatives (Husin, Hashim, *et al.*, 2021; Husin, Muhamad, *et al.*, 2021).

Zhang *et al.* (2017) experimented on different combination of imidazole and its derivatives, Im, 2-methylimidazole, 2-ethylimidazole, and 2-propylimidazole as HBAs with Gly as HBD while Adamus *et al.* (2018) utilized ChCl as HBA with urea and Im as HBDs. The versatility of imidazole is evident in these examples. Most DESs based on imidazoles contain acidic and basic sites, which give them the ability to dissolve both polar as well as non-polar compounds (Luo *et al.*, 2021). This can be achieved through the modification the components of HBA and HBD (Jiang *et al.*, 2018). For instance, the lone pair of electrons on nitrogen in imidazole acts as HBA, while the hydrogen atom in N-H functions as HBD. It makes them a versatile solvent for a variety of processes, such as natural product extraction, separation procedures, and other chemical reactions (Jiao *et al.*, 2015; Balaraman & Rathnasamy, 2019; Qin *et al.*, 2020). This is due to the presence of HBA sites on the imidazole molecule which allows it to create strong connections with a broad variety of functional groups in different types of targeted analyte. In attempt to enhance the air and environmental quality, Ke Li *et al.* (2021) improved the existing method for capturing NH_3 by developing DES combining ammonium thiocyanate (NH_4SCN) and Im (K. Li *et al.*, 2021). The NH_4SCN-Im DES demonstrated an efficient NH_3 uptake capacity contributed by the dual active sites from the NH_4SCN and five-membered nitrogen-containing imidazole.

Finally, imidazole-based DES are not only non-flammable, but also have low vapor pressure and have a high thermal and chemical stability (Qin *et al.*, 2021). To overcome the risk of conventional solvents evaporating or causing a fire hazard in high-temperature reactions like catalysis and biomass conversion processes, they are the suitable choice of solvents for these type of processes. For example, Qin *et al.* (2019) devised a bifunctional acid-base tunable DES synthesized by combining imidazole and *p*-toluenesulfonic acid (PTSA) to replace toxic alkyl halides as a catalyst for generating long-chain ester in reactive extraction process. For the reasons stated above, the qualities of imidazole-based DESs are what makes them a viable option for industrial and environmental applications.

2.2.3 Polyol-based DES

Polyols are defined as multivalent alcohols, which means they are a class of organic compounds that contain several hydroxyl (–OH) groups (Ratna, 2022). In particular, polyols which contain two –OH groups are called diols (e.g., EG, 1,3-propanediol, 1,4-butanediol, etc.) while alcohols with three and four –OH groups are called triols (e.g., Gly, 1,2,5-pentanetriol, etc.) and tetrols (e.g., butane-1,2,3,4-tetrol), respectively. They may be employed as components in the creation of DES through coupling with different salts or organic acids such as ChCl (Wan Mahmood *et al.*, 2019). Polyol-based DES demonstrated a good biodegradability, low toxicity, emanate thermal stability, and establish strong solubility for a broad variety of chemicals for extraction (Huang *et al.*, 2017; Hossain *et al.*, 2021; Zdanowicz, 2021; Park *et al.*, 2022). They have been employed as DES in numerous applications, such as in biorefinery, removal of heavy metal contaminant, extraction of bioactive chemicals, enzymatic hydrolysis, etc. (Yao *et al.*, 2015; Mukhopadhyay *et al.*, 2016; Rodrigues *et al.*, 2021; Liang & Guo, 2022; Wang *et al.*, 2022). One of the most widely utilized polyols in deep eutectic solvents is EG, which has been demonstrated to generate stable DES with different salts or organic acids (Ibrahim *et al.*, 2019). Other polyols, such as Gly, pentaerythritol, propylene glycol, and 1,4-butanediol as well as natural polyols (e.g., biomass-derived polyols such as xylitol and sucrose) have also been utilized in the formation of DESs (Yao *et al.*, 2015; Alomar *et al.*, 2016; Kučan *et al.*, 2018; Azimzadeh-Sadeghi & Yavari, 2021; Hossain *et al.*, 2021).

2.3 Synthesis of magnetic nanoparticles, Fe_3O_4

Magnetic nanoparticles Fe_3O_4 can be synthesized by various methods which consist of, but not limited to co-precipitation (Anbarasu *et al.*, 2015), thermal decomposition (Patsula *et al.*, 2016), sol-gel (Akiba Fexy, 2018), microemulsion (Asab, Zereffa &