AN ULTRASONIC ASSISTED DISSOLUTION OF FEATHER KERATIN

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ULTRASONIC ASSISTED DISSOLUTION OF FEATHER KERATIN

ABSTRACT

Feather keratin is a biomass generated in excess from various livestock industries. Upon proper processing, it holds potential as a green source for degradable biopolymer that could possibly replace current fossil fuel-based materials. Several processing methods have been developed, but the report on the use of ultrasonication was not found. In this study, our work focuses on (i) optimising and comparing the dissolution process of turkey feather keratin through sonication and conventional processes, and (ii) generating a biodegradable polymeric material, as a value-added product, from the dissolved keratin that could be used in packaging and other applications. Sonication of feather keratin in pure ionic liquid (ILs) and mixtures containing ILs and different co-solvents were conducted under different applied acoustic power levels. It was found that ultrasonic irradiation significantly improved the rate of dissolution of feather keratin as compared to the conventional method, by approximately 90 % improvement in dissolution time. The amount of ILs required was also successfully reduced by introducing a suitable co-solvent. The keratin was then regenerated, and the characteristics was analysed using a combination of analytical techniques which are ATR-FTIR, PXRD, TGA, and DSC. From these techniques, it was observed that the protein backbone is still intact, and no major chemical changes of the polypeptide chains occur through ultrasonic method. This material holds the potential to be reused in various applications.

Keywords: Ultrasound, Regeneration, Keratin, Feather, Ionic liquids

PROSES PELARUTAN BULU PELEPAH BERKERATIN SECARA ULTRASONIKASI

ABSTRAK

Bulu pelepah berkeratin adalah sejenis biomas hasil lebihan daripada pelbagai jenis industri ternakan. Dengan cara pemprosesan yang bersesuaian, bulu pelepah berkeratin mempunyai potensi sebagai sumber alternatif untuk biopolimer boleh degradasi yang berupaya untuk menggantikan sumber bahan api fosil yang sedia ada. Beberapa cara pemprosesan telah direka tetapi penggunaan ultrasonikasi masih belum pernah lagi dikaji. Fokus utama dalam kajian ini adalah untuk (i) membandingkan dan mengoptimumkan proses pemelarutan bulu pelepah ayam belanda berkeratin melalui proses sonikasi dan konvensional, dan (ii) menjana bahan polimer biodegradasi daripada keratin yang telah larut untuk digunakan dalam pembungkusan dan pelbagai aplikasi lain. Proses sonikasi bulu pelepah berkeratin di dalam cecair ionik tulen dan campuran yang mengandungi cecair ionik dan pelarut bersama yang berlainan telah dijalankan di bawah tahap kuasa akustik yang berbeza. Hasil kajian mendapati bahawa penggunaan ultrasonik mempunyai kelebihan dalam kadar proses pemelarutan bulu pelepah berkeratin berbanding dengan proses konvensional, dengan penambahbaikan di dalam proses pelarutan sebanyak 90 %. Jumlah cecair ionik yang diperlukan juga dapat dikurangkan dengan adanya pelarut bersama. Bulu pelepah berkeratin akan dihasilkan semula, dianalisa dan dicirikan menggunakan gabungan beberapa teknik analitikal seperti ATR-FTIR, PXRD, TGA and DSC. Daripada teknik-teknik ini, dapat dibuktikan bahawa tulang belakang protein masih utuh and tiada perubahan kimia yang ketara pada rantaian polipeptida berlaku melalui proses ultrasonikasi. Bahan ini mempunyai potensi untuk digunakan semula dalam pelbagai jenis aplikasi.

Kata kunci: Ultrasonikasi, Penjanaan Semula, Keratin, Bulu Pelepah, Cecair Ionik.

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LIST OF SYMBOLS AND ABBREVIATIONS

[BMIM]Cl	:	1-butyl-3-methylimidazolium chloride
A	:	Ampere
Å	:	Angstrom
ATR-FTIR	:	Attenuated total reflectance Fourier-transform infrared spectroscopy
CH_3CN	:	Acetonitrile
cm	:	Centimetre
CO_2	:	Carbon dioxide
COOH	:	Carboxylic acid
CTAB	:	Cetyltrimethylammonium chloride
Da	:	Dalton
DMF	:	Dimethylformamide
DMSO	:	Dimethyl sulfoxide
DNA	:	Deoxyribonucleic acid
DSC	:	Differential scanning calorimetry
g	:	Gram
h	:	Hour
Hz	:	Hertz
IFs	:	Intermediate filaments
IUPAC	:	International Union of Pure and Applied Chemistry
ILs	:	Ionic liquids
J	:	Joule
k	:	Kilo
K	:	Kelvin
KI	:	Potassium iodide

M	:	Mega
m	:	Meter
MCLW	:	Metal-clad leaky waveguide
min	:	Minute
mL	:	Millilitre
mm	:	MillimetreMillimeter
ms	:	Millisecond
Na_2SO_3	:	Sodium sulphite
Na_2S	:	Sodium sulphide
NH_2	:	Amino
nm	:	Nanometre
NMR	:	Nuclear magnetic resonance
Pa	:	Pascal
P_B	:	Blake threshold
P_v	:	Function of the solution vapour pressure
P_0	:	System pressure
PXRD	:	Powder x-ray diffraction
R_0	:	Initial nanobubble radius
RNA	:	Ribonucleic acid
rpm	:	Revolutions per minute
S-S	:	Disulphide
SH	:	Thiol
SL	:	Sonoluminescence
SONAR	:	Sound navigation and ranging
T_b	:	Boiling temperature
TGA	:	Thermogravimetric analysis

USW	:	Ultrasound standing waves
UV	:	Ultraviolet
V	:	Volt
W	:	Watt
wt %	:	Weight percent
°C	:	Degree Celsius
α	:	Alpha
β	:	Beta
σ	:	Surface tension
μ	:	Micro
%	:	Percent
π	:	Pi
θ	:	Theta

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CHAPTER 1: INTRODUCTION

1.1 Background study

For the past few years, the ultrasonic wave has been gaining scientist's interest and has been extensively studied due to its broad application in many fields. In commercial applications, the ultrasonic wave has been applied in preparation of food products (Awad et al., 2012; Mason et al., 1996; McClements, 1995), pharmaceuticals (Chemat et al., 2017; Farooq, 2012), advanced treatment of waste water (Albero et al., 2015; Chemat et al., 2011; Hoffmann et al., 1996), oceanography and archaeology (Hill & Breckle, 1983; Hu et al., 2008) and domestic products (Leighton & Dumbrell, 2004; Peters, 1996; Price, 1990; Tiwari, 2015). The biomedical field (Cravotto & Cintas, 2006; Hill & Breckle, 1983) is also utilising the ultrasonic wave technology in diagnosis and therapy. The most common non-invasive diagnostic tool is the external foetal ultrasonic scanning in the medical field, with the frequency range of 3 to 10 MHz. The exploitation at higher frequencies (≥ 30 MHz) (Wear, 2005) of ultrasonic scanning discerned to be as non-destructive as X-rays which has been used to measure the sound speed and to investigate bone health and osteoporosis (Lee et al., 2003).

The phenomenon of acoustic cavitation is initiated when there is a mechanical interaction between sound waves and dissolved bubbles in liquids or solvents. During this process, the expansion and rarefaction cycles of ultrasound force the dissolved bubbles growth by rectified diffusion. This continues until it reaches its resonance size range and internally collapses, also known as implosion (Ashokkumar et al., 2007; Nor Saadah Mohd Yusof et al., 2016). Due to the increased pressure inside the bubbles, the collapse results in the generation or release of high pressure. This process causes strong physical impact as well as the generation of local high temperature. For this reason, these bubbles are also referred to as micro-reactors or hot-spots (Ashokkumar et al., 2007; Koda et al.,

2003). The generation of bubble clouds under the influence of an oscillating pressure field is due to the presence of dissolved gas nuclei or adventitious microbubbles inherently in the liquid which is caused by the ultrasound waves. Solvent and solute molecules exist within the bubbles are decomposed under these extreme conditions and produced highly reactive radicals (Ashokkumar et al., 2008). For example, when water is sonicated, cavitation activates the generation of H• and •OH radicals (Kirpalani & McQuinn, 2006). The radicals from the sonolysis of water vapour in the gas bubbles may scavenge and react with dissolved substances. Besides H• and •OH radicals formed from water, atoms of diatomic gases, such as oxygen and hydrogen, may be present in the bubbles which have probability to participate in the reactions with a solute (Hart & Henglein, 1985).

Since the discovery of petroleum-based materials, people has been using it in variety of applications. As the human population grows, the demand of this non-renewable materials keeps increasing; which creates urgency for renewable resources as alternatives. Biomaterials have been showing great potential alternative hence has gained interest amongst researcher especially in the polymer field. The characteristics possessed by the biopolymer-based protein fibre makes it a suitable candidate to replace current petroleum-based polyamides. Even though keratin can be found from abundant sources such as hair, nail, and animal hooves; cellulosic fibres has been gaining more attention compared to others in latest research (Azila Mohd Idris et al., 2014; Poole et al., 2008). Due to the greater availability of raw feather, studies of keratin dissolution mostly focused on feather keratin. The difference in the cysteine content is one of the significant differences between the types of keratin; feather and wool keratin types contain ~7 % and 11–17 % cysteine units in their amino acid sequences, respectively (Arai et al., 1983; Barone et al., 2005). Keratin is insoluble in polar solvents like water, weak acids and bases, as well as in apolar solvents (Horvath, 2009). However, due to the presence of cysteine and disulphide bond, keratin can be reduced, oxidised and hydrolysed. The higher cysteine

content makes these biomass to be more difficult to be dissolve (Azila Mohd Idris et al., 2013; Kakkar et al., 2014).

The stability of the protein structure in keratin from the internal interactions makes it difficult for the materials to be dissolved nor extracted. Thus, it is crucial for an efficient method to be develop on the dissolution process in order to regenerate the needed properties while maintaining the important core structure of the raw feather. Out of 90 % keratin in feathers, cysteine comprises 7 % of the total amount (Arai et al., 1983). The protein in feather is arranged in the α-helix and β-sheet manner. Due to its tight packing arrangement, it is impossible to dissolve feather keratin in normal organic solvent. This is because in order to break the crosslinking by the disulphide linkage, processes such as oxidation and reduction (Brovelli et al., 2010; Poole et al., 2008; Yamauchi et al., 1996), sulphitolysis (Cecil & McPhee, 1959) or oxidative sulphitolysis (Smith et al., 1991) is required. The ability to reduce the disulphide bonds is possessed by some common reagents. However, the reaction is plausible with only the right amount of reactivity and ability which are commonly found in a few types of reagent such as thiols and ammonium bisulphite. However, the major back draw from these reagents are the difficulty to remove or recycle, costly price and toxicity (MacLaren, 1962).

Salts which are liquid below 100 °C and comprised entirely of cations and anions is called an ionic liquids (ILs) (Forsyth et al., 2004; MacFarlane & Seddon, 2007). ILs is a unique salt as it is a type of salt which is possible to be design in endless amount of combinations as long as it is chemically possible. Hence, the properties of the ILs can be altered according to the end use functions of the salt. That is why ILs is favourable as the combinations of cation and anion may bring out the ideal properties required in a solvent such as low vapour pressure and high thermal stability (Domanska & Bogel-Lukasik, 2005). The dissolution of biopolymers such as cellulose, starch, wood, lignin, feather and wool has not been studied extensively as it is known to be insoluble in the common

organic solvents (Azila Mohd Idris et al., 2013). However, some studies show that certain type of ILs manage to dissolve cellulosic protein. Thus, more research has been done to study on whether it is possible to dissolve other types of biopolymer by using ILs (Azubuike et al., 2012; Biswas et al., 2006; Gao et al., 2012; Swatloski et al., 2002; Tan et al., 2009; Zakrzewska et al., 2010; Zavrel et al., 2009).

1.2 Objectives of research

The general aim of this study is to investigate the ability of ultrasound with incorporation of several types of solvent to dissolve feather keratin. Specific objectives of this research are as follows:

i. To investigate the effect(s) of ultrasonic method on the process of feather keratin dissolution.

ii. To compare between the ultrasonic assisted dissolution and the conventional (heating) method in the dissolution of feather keratin.

iii. To characterise and analyse the regenerated keratin materials from both methods.

The focus of this study was to investigate and compare the possibility of dissolving keratin by combining ultrasound to a known available method by the use of ILs. The optimisation of the dissolution process involves our attempts to reduce the usage of ILs by introducing suitable co-solvents and also to reduce the processing time required. A solvent with appropriate boiling point was chosen to ensure that the reaction is viable without degrading the structure of feather keratin. A combination of analytical techniques including Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), powder X-ray diffraction (PXRD), thermogravimetric analysis (TGA) and

differential scanning calorimetry (DSC) was used to investigate the physicochemical properties of the regenerated keratin materials and the degree of feather keratin solubility in these solvent systems and through the application of ultrasound.

1.3 Scope of research

The scopes of the current research are divided into two main sections; the dissolution and the regeneration of feather keratin by using ultrasonic and conventional method. The dissolution of feather keratin was carried out by choosing suitable solvents, finding optimum concentration of 1-butyl-3-methylimidazolium chloride, [BMIM]Cl in solvent, and the effects of ultrasonication power applied on the processing time. [BMIM]Cl was chosen as the main ILs because of its high chloride concentration which will aid in breaking the extensive hydrogen-bonding network present in feather keratin (Swatloski et al., 2003; Swatloski et al., 2002). Furthermore, it was shown by Xie *et.al* that the solubility of wool keratin in different anions of [BMIM] cation (Xie et al., 2005) and chloride anion shows the highest solubility in weight percentage. Hence, the aim of this experiment is to find the optimum condition in dissolving feather keratin. Two different methods; conventional (heating dissolution) and ultrasound were compared for dissolution of feather keratin.

For the second part, keratin was regenerated with the addition of water. Several types of characterisation were carried out to investigate the effect(s) on the chemical composition of regenerated keratin materials upon the application of ultrasound. ATR-FTIR, PXRD, TGA and DSC were done to compare the composition of raw feather keratin and regenerated keratin materials after dissolution and regeneration process. This process is crucial for this study as it will shows the ability of both processes to retain the protein backbone of feather keratin after the dissolution process. Such a study has never

been reported before. We believe that this could offer a new and improved dissolution method for biomass by using ultrasonic technology that can be utilised at a larger scale. The use of minimal amount of ILs will also lead to a positive environmental impact as well as reducing the financial and synergic cost of such process. Therefore, we believe that this study will be of great interest to chemists, engineers and environmental scientists.

1.4 Outline of dissertation

The dissertation is divided into five chapters. This chapter (Chapter 1) provides a brief overview on the topic of the research by the background study on several fields involved for the research. The objectives and scope of the study was also included and elaborated thoroughly in this specific chapter. Through this introduction, the significance on the use of ultrasound on the feather keratin dissolution process is explained.

Chapter 2 provides and discusses numbers of reviews on the previous studies that has been conducted on the feather keratin dissolution process. These reviews aided the design of the project, i.e the solvents to be used, especially ILs and several types of organic solvents as co-solvent for the solvent system. Furthermore, the analysis and determination of the optimum condition for the dissolution process was done through this particular chapter.

Chapter 3 outlines the methodology on how the experiments were conducted. This includes the ultrasonic physical and chemical effects' quantification (calibration), feather keratin dissolution, and regeneration methods. For a specific ultrasonic system, calibration is vital to determine the amount of power applied to the system and the amount of radical formed during the process based on acoustic power level meanwhile regeneration steps provide information on how the dissolved keratin is regenerated back. Hence, this chapter provided the background story for this research.

Chapter 4 provides the overall outcome of the study. First, result of the calibrations of the ultrasonic system reveals the trend in understanding on whether there are any necessary or unnecessary, as well as dominating impacts of ultrasound on the dissolution process. Furthermore, different parameters such as types of solvent systems and amount of delivered powers affecting the processing time were recorded and discussed. Finally, the results for the characterisation of regenerated products were further analysed and discussed in this chapter.

Chapter 5 concluded the research with results obtained throughout the study summarisation from the outcomes from the experiments and plausible future works as a continuation from this project.

CHAPTER 2: LITERATURE REVIEW

2.1 Keratin

Annually, poultry farming will produce an estimation of 5 million tonnes of feather as a by-product of from a reliable production pipeline (Poole et al., 2008), making it a dependable biopolymer feedstock. Instead of sending it to landfills, the development of alternative industrial uses of feather keratin will not only reduce environmental impacts and health hazards but will also increase the commercial value of the feathers (Saber et al., 2010). However, due to the limitation of the keratin fibre, feather keratin is often used for a low-grade feed stock or disposed as waste (Reddy & Yang, 2007). This due to the fact that at the temperatures above their crystalline melting point, concurrent crystalline melting and degradation will destroy the unique protein structure and fibrous nature (Senoz et al., 2012). As for now, the keratin as a natural monomer for use in eco-composites and bio-plastics is one of the appealing end-uses of this type of biopolymer (Pillai, 2010).

2.1.1 History of keratin biomaterials

The use of keratin in daily life has been documented as early as in the 16th century. Li Shi-Zhen, a Chinese herbalist has been using keratin in medicinal applications. In the span of 38-years period, Shi-Zhen managed to write more than 11,000 therapeutic prescriptions which is compiled in 800 books known as the Ben Cao Gang Mu. One of the prescriptions or widely known as Xue Yu Tan uses pyrolised human hair in a form of ground ash to accelerate wound healing and blood clotting (Li, 1978). To describe animal horns and hooves which is made up of hard tissues, “keratin” is used in history recording

comes from the Greek "kera" meaning horn. Back then, keratins is one of the "abnormal" proteins as normal methods such as burning and grinding were ineffective for solubilising keratin. From 1905 to 1935, oxidative and reductive chemistry has been utilised to develop the methodology design for the extraction of keratin (Breinl & Baudisch, 1907; Goddard & Michaelis, 1935; Lissizin, 1915, 1928; Neuberg, 1909; Stary, 1924). As the extracts shows good biological properties, keratin gained immense interest that led to the development of keratins for medical applications. Thus, many applications of keratin was found and among the first inventions were keratin powders for composites, cosmetics, and coatings for drugs (Beyer, 1907; Dale, 1932).

By 1970, more studies and publications has been extensively covered the physical forms of keratin which converted extracted keratins into powders, films, gels, coatings, fibres, and foams (Kawano & Okamoto, 1975; Okamoto, 1977). During the 1980s, medical applications observed the uprising use of collagen as biomolecule. Following after collagen, the use of other naturally derived molecules such as alginates from seaweed, chitosan from shrimp shells, and hyaluronic acid from animal tissues was also used (Rouse & Van Dyke, 2010). The first study describing the use of a keratin coating on vascular grafts as a way to eliminate blood clotting (Noishiki et al., 1982), as well as experiments on the biocompatibility of keratin (Ito et al., 1982) was published in 1982 by a Japanese scientist.

2.1.2 Structure of keratin

Feather keratin is made up of ordered α-helix (Figure 2.1) or β-sheet structures (Figure 2.2) with other disordered structures. The percentage of α-helix is higher as compared to β-sheet was observed for feather keratin (Schmidt & Jayasundera, 2004). The presence of about 7 % cysteine causes the formation of disulphide bonds (Arai et al., 1983). The

presence of the disulphide bonds contributed to the strength and stiffness of keratin in its solid state (Barone et al., 2006). However, keratin is also bound by strong internal interactions (non-covalent interactions such as electrostatic forces, hydrogen bonds, hydrophobic interaction) that stabilises the protein structure, therefore making it harder to be dissolved or extracted (Onifade et al., 1998; Xie et al., 2005). Hence, it is vital to develop a technique to dissolve keratin and to produce solution that is industrially scalable, able of re-crosslinking, and have minimal to none destruction of the primary protein chains.

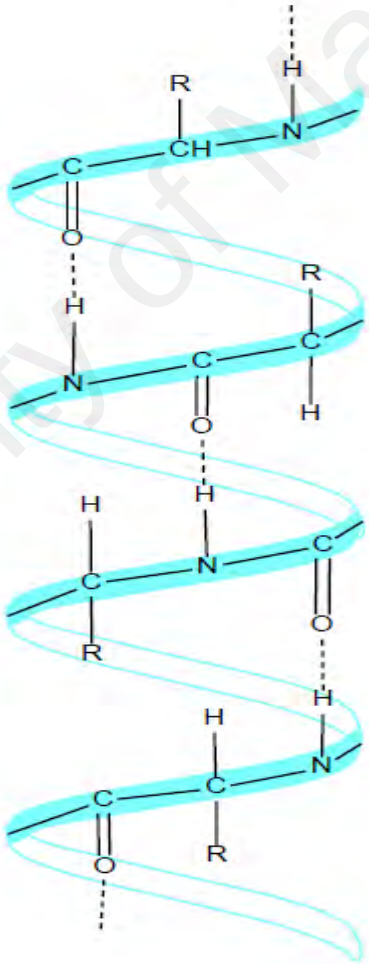


Figure 2.1: Alpha helix structure of keratin

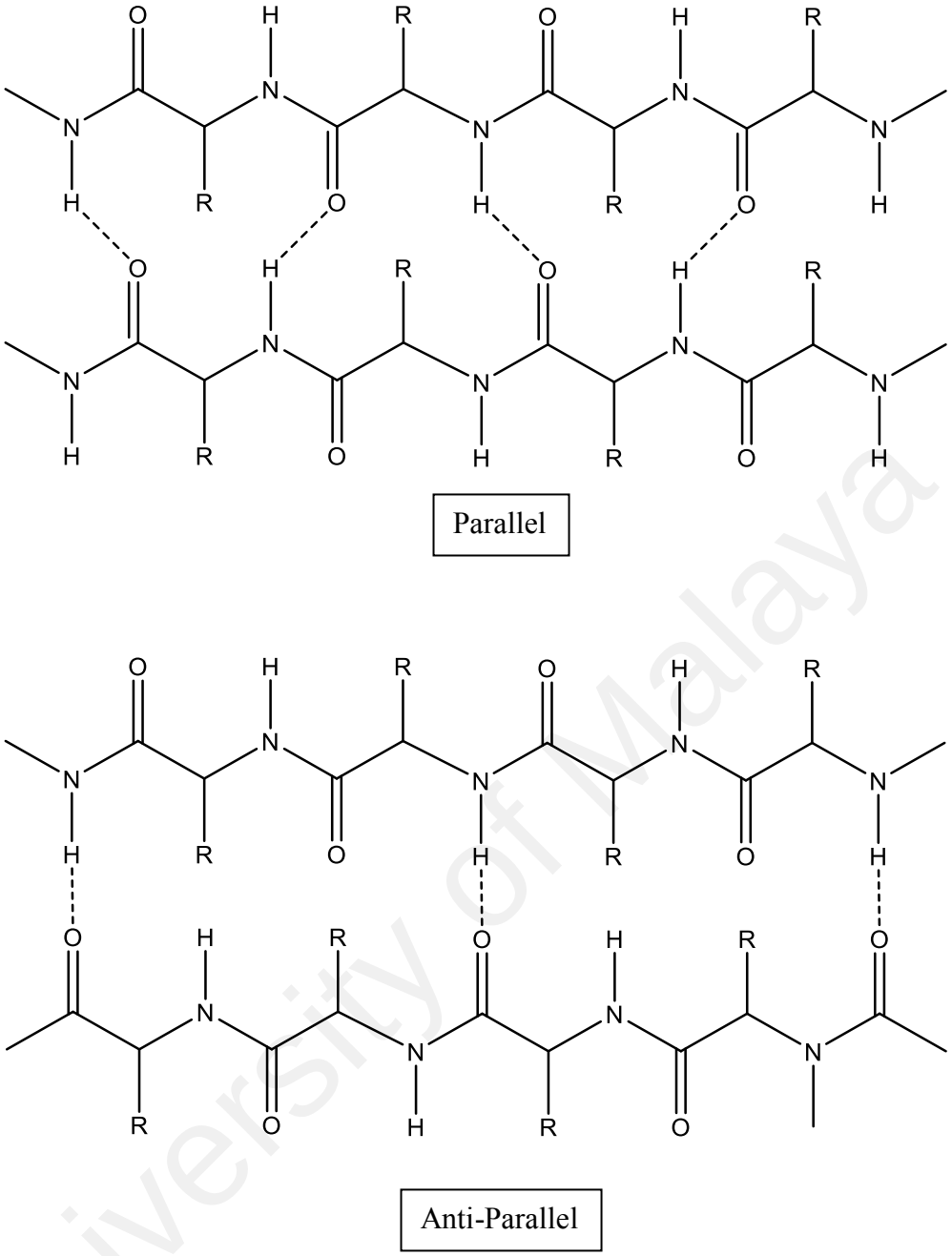


Figure 2.2: Beta sheet structure of keratin

The presence of keratin protein several functional groups such as disulphide (-S-S), amino ($-NH_2$) and carboxylic acid (–COOH) makes it chemically reactive. Apparently, keratin have no solubility in water thus very low chemical reactivity. However, with the aid of some reducing agent (i.e. Na_2SO_3 or Na_2S) at acidic pH and high temperature, its solubility in water can be enhanced. The unique properties of biodegradability and non-toxic nature makes keratin protein versatile which enable modification and development

of keratin into various forms, for instance, gels, films, beads and nano or micro-particles. Numerous applications was found in green chemistry, food sciences, pharmaceutical, and cosmetic industries post-modification for keratin (Khosa & Ullah, 2013).

2.1.3 Extraction of keratin

For the past decades, varieties of sources have been used for the extraction of keratin such as hair, hooves, shells, fingernails, and feathers. By using Shindai method, most keratin was extracted by many researchers from poultry feathers under reduced conditions (Arai et al., 1983; Goddard & Michaelis, 1934; Moore et al., 2006; Muhammad Arshad Khosa et al., 2013; Schrooyen et al., 2001; Yamauchi et al., 1996). For extraction of keratin, there are three main steps which is important for the process; ethanol pre-treatment, hydrochloric acid pre-treatment and 2-mercaptoethanol deoxidisation (Yin et al., 2013). Furthermore, the process of cleaving the disulphide bonds with the aid of 2-mercaptoethanol as a source of thiols is also known as a process of dissolution of keratin without damaging its primary protein chain (Anfinsen & Haber, 1961).

The disulphide bonds can also be chemically reduced or oxidised using thioglycolic acid (Kuzuhara & Hori, 2013), or hydrogen peroxide (H_2O_2) (Hogg et al., 1994), respectively. Wool treated with thioglycolic acid was found to dissolve five times faster in ILs compared to wool without any treatment (Kuzuhara & Hori, 2013). With hydrogen peroxide, the wool surface is oxidised. Hence, the cuticle fully swelled in shorter time than for the raw fibre when the wool surface is oxidised. Interestingly, with the aid of hydrogen peroxide, simultaneous dissolution occur for both the cuticle and the cortex (Chen et al., 2014).

However, these methods will consume a large quantity of reagents such as acids or reductants and inability of these reagents to be recycled is among a few of the serious shortcomings for extraction of keratin. Thus, finding simple and eco-friendly processing methods to dissolve feather keratin has been a primary focus for researchers. Some recent works have been reported regarding the use of ILs in dissolution and regeneration of keratin fibre (Hameed & Guo, 2010; Xie et al., 2005). Regardless of the higher cost of ILs than that of inorganic reagents, they can be reused and recycled thus improving the productivity of whole process, leading to lowering the overall cost of the process (Wang & Cao, 2012).

Keratin after extraction, was potentially used to prepare powders, films, gels, coatings, fibres and foams by many researchers such as Krystayna *et al.* (Wrześniewska-Tosik et al., 2011) studied on fibrous composites; Anker (Anker, 1972) prepared films and coating material from keratin; and Kawano (Kawano & Okamoto, 1975) synthesised keratin-based film and gelatin.

2.1.4 Keratin-based biomaterials

The usage of keratin in medical field as a biomaterial has been widely explored (Mogoşanu et al., 2014; Noishiki et al., 1982; Rouse & Van Dyke, 2010). The development of keratin-based biomaterials is dependent upon several key properties of keratin such as physical, chemical and biological behaviour of these biomaterials. Table 2.1 summarised the sources and how keratin experimental feature can be applied for selective keratin-based biopolymers.

Table 2.1: Summary of sources, experimental features and applications of selective keratin-based biopolymers.

Keratin Source	Experimental feature	Application
Animal horns[1]	Using lime	Keratin-based gel
Horns, hooves, wool, human hair[2]	Reductive and oxidative agents	Powders, films, gels, coatings, fibres, foams
Wool and hair[3]	Self-assembly of keratin solution	Biological function in tissue engineering
Wool[4]	Determination of cell compatibility	Cultivation of mouse fibroblasts on the surface of film
Hair fibres[5]	Improved disulphide crosslinking	Glue for increased toughness to hair fibres
Human hair[6]	Ground ash from human hair	Wound healing and blood clotting

[1] (Hofmeier, 1905)

[2] (Anker, 1972; Kawano & Okamoto, 1975)

[3] (Izawa & Inagaki, 2006; Magin et al., 2007)

[4] (Yamauchi et al., 1998)

[5] (Schweizer et al., 2006)

[6] (Li, 1978)

Keratin Source	Experimental feature	Application
Glycerol containing keratin films[7]	Addition of chitosan for making chitosan-keratin films	Antibacterial and good substrate for cell culture
Silk fibroin-keratin composite[8]	Transition from random coil to β-sheets in secondary structure of composite	Biomaterial with intrinsic biocompatibility and biodegradability
Keratin/PEO film[9]	Improved structural properties	Scaffolds, wound dressing & drug delivery membranes
Keratin-Polyamide 6 composite[10]	Preparation of keratin-based material	Biomedical devices, water filtration, textile fibres
CF & bio-modified cellulose[11]	Fabrication of keratin-based material with high sorption capacity	Hygienic fabrics, green chemistry

Table 2.1, continued

[7] (Tanabe et al., 2002)

[8] (Lee & Ha, 1999)

[9] (Tonin et al., 2007)

[10] (Zoccola et al., 2008)

[11] (Wrześniewska-Tosik et al., 2011)

Keratin Source	Experimental feature	Application
CF[12]	Extraction of CF keratin & their films	Controlling drug release in medical
CF[13]	Modification & characterization of CF	Removal of As (III) in green chemistry

Table 2.1, continued

2.2 Ionic liquids (ILs)

Salt with a melting temperature below the boiling point of water and is liquid at room temperature is a suitable definition from historical or even practical point of views of ILs. However, the only general part emitted from the definition of ILs is that most ILs have organic cations and inorganic anions. There are several synonyms used for ILs. The common term and broadly applied for ionic compounds are 'molten salts'. Yet, the term 'ionic liquids' has been used in literatures which also represented low melting salts (Inman & Lovering, 1981). Even though differences between ILs and molten salts is not that significant, but the practical differences are sufficient to put a definite barrier and justify a separately identified niche for room temperature liquid salts.

In practice, the ILs usually may be treated like ordinary solvents. There are also some fundamental features that are not often seen in higher temperature molten salts that is part of ILs properties, such as strong ion–ion interactions. Furthermore, new types of solvents,

[12] (Yin et al., 2013)

[13] (Muhammad Arshad Khosa et al., 2013)

or older materials that are introducing new applications as solvents in recent years is also given a term as 'neoteric solvent' which also applicable for ILs. Process solvents such as supercritical fluids and ILs are good examples of neoteric solvents that have been known for a long time with recent new applications. The solvent properties of ILs are the bridge that connected ILs and green chemistry.

2.2.1 History of ionic liquids

Ethyl ammonium nitrate; a protic ILs is the first room-temperature ILs which was discovered by Paul Walden (Walden, 1914) and first published by Welton (Welton, 1999). Due to the larger organic cation compared to an inorganic salt, Paul concluded that the low melting point (13–14 °C) was a result of the decrease in the degree of ion association. Even though $AlCl_3$ based ILs on can be considered as the first generation of ILs, the main obstacle on the development especially for open-air applications is due to their hygroscopic nature. Consequently, the synthesis of water and air-stable dialkylimidazolium-based ILs were discovered by Hussey and Wilkes as they investigated the effect of changing the cation (Green & Long, 2009; Wilkes & Zaworotko, 1992). The group combined the 1-ethyl-3-methylimidazolium cation, $[C_2MIM]^+$ with $[BF_4]^-$, $[NO_3]^-$, $[NO_2]^-$, and acetate anions and ($[C_2MIM]^+$ is still one of the most broadly used cations today. They revealed that through the use of more hydrolytically stable anions such as stated previously, the water sensitivity was significantly reduced thus the preparation and storage of ILs could be conducted outside of an inert atmosphere.

Initially, the common example of a fluorinated anion used is $[NTf_2]^-$ anion (Bonhote et al., 1996; Koch et al., 1995), and it is attractive for ILs electrolytes for the reasons stated above as the battery community has been incorporating it in for many years (originally just as the lithium salt, $Li[NTf_2]$). Although phosphonium cations can impart

better stability and becoming increasingly common, predominantly nitrogen-based cations are still used to make ILs (Fraser & MacFarlane, 2009). 'Tunability' is another significant feature to be studied for ILs. By understanding that incorporation of large charge-diffuse ions will be able to decrease the melting point of high-temperature molten salts, ILs can be synthesised from unusually huge number of ions. Therefore, we will be able to produce ILs which will fit the need of our applications by 'tuning' the ILs based on properties of the different ions (MacFarlane et al., 2017).

In 1934, ammonium salts ability to dissolve cellulose was first remarkably recognised (Graenacher, 1934; Plechkova & Seddon, 2008). Following after that, the ability of 1-butyl-3-methyl imidazole chloride ([BMIM]Cl) to dissolve cellulose produced a new class of cellulose solvent systems. By studying the interaction of ILs with solute species, Welton focused on trying to find more benign alternatives to replace environmentally damaging solvents (Welton, 1999). Other than reaction medium, ILs can also act as reagent or catalyst in some reactions or processes with the addition of functional groups as reported by Jessop *et al.* or also known as "task-specific ILs." A safer in situ solvent is plausible with the addition of pressurised carbon dioxide into an organic mixture which transforms it into an ILs (Jessop et al., 2005).

2.2.2 Properties of ionic liquids

ILs consist of positive (cation) and negative (anion) charges, similar to salts that is bonded together by electrostatic interaction. However, in contrary to solid salts that packed neatly together to form a crystalline structure, ILs possess freedom in movement

due to their loosely packed ions, thus allowing the salt to take on the property of flow (Marr & Marr, 2016).

One of the advantages of ILs is that it has negligible vapour pressures due to its high thermal stability hence it can withstand high temperature reactions. In contrast to traditional volatile organic compounds, they are called 'green' solvents because of their negligible vapour pressure and recyclable properties (Seddon, 1997; Zhu et al., 2006). Since major method of molecular solvent discharge into the environment is through evaporative loss, the use of selected ILs may offer environmental advantages in industrial processes to which they are suitable. Next, a range of substrates can be dissolved by ILs, and a growing number of studies reported that ILs can provide improvements in product yields, selectivity or ease of product recovery when used in place of molecular solvents (Davis & Fox, 2003).

The ability to choose the anion or the cation makes large number of combination of salts up to 10^{18} ILs are theoretically possible (van Rantwijk & Sheldon, 2007). These ions can be combined and functionality can be introduced, thus allowing specifically designed functional ILs (Marr & Marr, 2016). Table 2.2 and 2.3 show examples of cation and anion by (MacFarlane et al., 2017) that is commonly used for combination of ions to produce ILs.

Table 2.2: List of common cations for combination ILs.

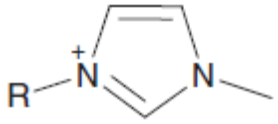
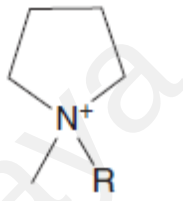
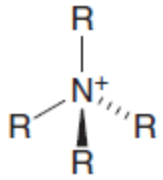
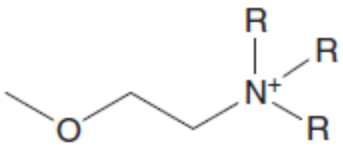
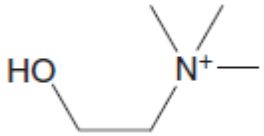
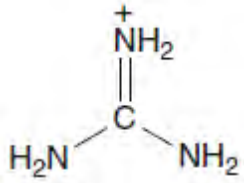
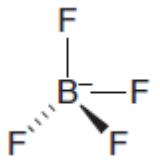
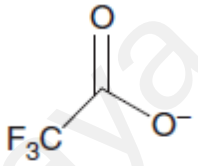
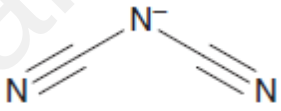
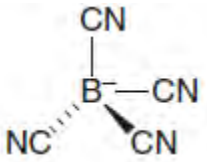
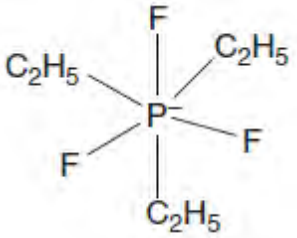
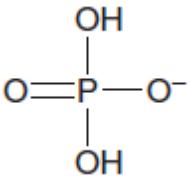
Quarternary cation	Abbreviation	Structure
Alkylmethylimidazolium	$[C_n\text{mim}]^+$	
Alkylmethylpyrrolidinium	$[C_n\text{mpyr}]^+$	
Ammonium	$[N_{n,n,n,n}]^+$	
Ether-functionalized	$[N_{R,R,R,2O1}]^+$, etc.	
Cholinium	$[\text{Ch}]^+$	
Guanidium	$[\text{Gdm}]^+$	

Table 2.3: List of common anions for combination ILs.

Quarternary anion	Abbreviation	Structure
Tetrafluoroborate	$[BF_4]^-$	
Trifluoroacetate	$[tfa]^-$	
Dicyanamide	$[N(CN)_2]^-$ or $[dca]^-$	
Tetracyanoborate	$[B(CN)_4]^-$	
Fluoroalkylphosphates	$[fap]^-$, $[efap]^-$, etc.	
Dihydrogenphosphate	$[H_2PO_4]^-$ or $[dhp]^-$	

Compared to conventional solvents such as ethanol and hydrochloric acid, ILs have a higher magnitude of viscosity. The high viscosity of ILs is due to the bonding presence, such as van der Waals forces, hydrogen bonding, Coulombic forces, and electrostatic forces. The formation of viscous ILs is contributed largely to the ability of fluorinated anions such as BF_4^- and PF_6^- to form of hydrogen bonds (Kosmulski et al., 2004). In acidic mixture, the presence of $AlCl_4^-$ and $Al_2Cl_7^-$ will form weaker hydrogen bond hence reduced viscosity. In fact, due to stronger van der Waals forces, more energy is required for molecular motion. The viscosity of ILs can be reduced effectively by mixing it with molecular solvents. However, it will decrease the advantages of properties of ILs such as electrochemical stability, an increase in vapour pressure, flammability, and much more (Mallakpour & Dinari, 2012). Lastly, viscosity is highly affected by temperature by decreasing relatively rapidly and this should be taken into account for the smoothness of handling either in lab scale or larger scale commercial reactor design (MacFarlane et al., 2017).

As ILs vapour pressure remained negligible near ambient temperatures, they are indeed considered as non-volatile for practical purposes (Kubisa, 2004). It has been reported that under significantly reduced pressure and at very low distillation rate, that ILs can be distilled at a range of 200–300 °C. Generally, with ILs negligible volatility, the release of excess toxic gaseous and causing air pollution will not be the main concern with the use of ILs. Consequently, at ambient and higher temperatures, ILs are considered as non-volatile and non-flammable. However, at elevated temperatures, the probability of release of ILs vapours (or decomposition products) must still be considered when ILs are used (Mallakpour & Dinari, 2012).

To evaluate an ILs, its melting point is the key criterion to be observed. Although ILs have been classified as salt which the melting points below 100 °C, data must be treated with caution. Commonly, the melting point of ILs is measured by using DSC. As they undergo considerable supercooling, the melting point will be uncertain for many ILs. Depending on the condition of analysis (heated or cooled) and the potential presence of impurities, the temperature of the phase change can differ substantially (Olivier-Bourbigou et al., 2010). ILs melting point is highly dependent on the structure and chemical composition of an ILs. Through manipulation of combination on cation and anion, this physical property can be altered, and both cations and anions make a significant contribution to the low meting points of ILs. The decrease in melting point is commonly due to the increase in anion size. Furthermore, the melting point can be increased by the addition of branching on the alkyl chain (Mallakpour & Dinari, 2012).

For ILs applications, there is a specific range for thermal properties which is crucial. The ILs is analysed through heating at a controlled rate and the decrease in mass of the sample by using TGA. Generally, TGA is used to determine the high thermal stability for many ILs, commonly more than 350 °C. ILs such as [EMIM]BF_4, [BMIM]BF_4, and 1,2-dimethyl-3-propylimidazolium bis(trifluorosulfonyl)imide are stable up to temperatures of 445 °C, 423 °C, and 457 °C, respectively (Olivier-Bourbigou et al., 2010). Thermal decomposition for the different cations appears similar for most ILs but will somehow started to decrease as the anion hydrophilicity increases (Chiappe & Pieraccini, 2005; Mallakpour & Dinari, 2012).

Primarily, the electrochemical properties are the reason why ILs is used extensively in batteries, supercapacitors, fuel cells, solar cells, electrowinning, and much more. The fact that ILs is consisted of solely ions makes it predictable to have high conductivity. As the size of ions affect the ion mobility, the conductivity of ILs will reduce with larger size

ions. Furthermore, reduced conductivity is also contributed by ion pair formation and/or ion aggregation. In general, the wide electrochemical windows of ILs enable electrodeposition on metals and semiconductors which have opened the door for such applications which previously depends only from high temperature molten salts (Chiappe & Pieraccini, 2005; Endres & El Abedin, 2006; Mallakpour & Dinari, 2012; Ohno, 2005).

For classification and characterisation purposes, polarity is one of the most important properties as it is crucial to know how the chemical reactions will be affected by the solvent. Variety of methodologies has been addressing the subject of ILs polarity. One of the approach is to measure the solvent polarity is by the measurement of keto-enol equilibria in which is reliant on the polarity of the medium (Mallakpour & Dinari, 2012; van Rantwijk et al., 2003). As ILs is known for their non-volatile properties, this become the main reason for researchers to believe that ILs is non-toxic and has a potential to be a green substitute for conventional volatile organic solvents.

Air and moisture stability are one of the characteristics of most ILs. Commonly, imidazolium and ammonium salts are hydrophilic which makes hydration certainly take place if they are used in open vessels. Increasing the length of the alkyl chain will increase the hydrophobicity of an ILs. Furthermore, Wasserscheid *et al.* (Wasserscheid et al., 2002) pointed out poor stability in water and production of toxic and corrosive species such as hydrogen fluoride or hydrochloric acid was observed generally for ILs containing halogen anions (Kitazume et al., 2000; Mallakpour & Dinari, 2012).

By “low-cost” starting materials or from renewable feedstock, new families of ILs have been studied and major concerns have been on the cost and biodegradability of these ILs. Biomaterials is the main composition of these new “Bio-ILs” (Fukaya et al., 2007). In combination with suitable anion, alternative cation such as choline has been selected to generate this Bio-ILs. As reported in (Yu et al., 2008), the study on the biodegradable

properties of these ILs has been conducted. By introducing an ester group into long alkyl chains, the main aim is to reduce toxicity and improve ecotoxicity of ILs which is deemed to be advantageous in the synthesis of ILs. The inclusion of an ester group with the imidazolium shows improved biodegradability as the cation side chain. Several ammonium ILs based on choline has been discovered and reported which these type of ILs can be readily prepared and is biodegradable (Coleman & Gathergood, 2010; Mallakpour & Dinari, 2012).

2.2.3 Applications of ionic liquids

A range of ILs and their applications could be find in the literature as it can be tuned to exhibit varieties of behaviour, i.e., to have functional pharmaceutical therapeutic ability (Carter et al., 2004; Davis et al., 1998), providing support for cells and enzymes (Sheldon et al., 2002; van Rantwijk & Sheldon, 2007) , to be used in drug delivery (Williams et al., 2014), or to kill microbes (Gilmore et al., 2013), designed to be biodegradable (Garcia et al., 2005; Gathergood et al., 2004; Gathergood et al., 2006) and synthesised from amino acids which is a renewable resources (Ohno & Fukumoto, 2007) or biomass (Ferlin et al., 2013), or last longer by making it more durable and also to facilitate the clean-up and removal of environmental pollutants such as carbon dioxide (CO_2) (Bates et al., 2002; Ramdin et al., 2012; Smiglak et al., 2014) and for metal extraction (Abbott et al., 2011; Nockemann et al., 2006; Visser et al., 2002). However, misuse in any of these applications can lead to the potential environmental damage (Pham et al., 2010). ILs can also be hydrophobic, hydrophilic, acidic or basic (Marr & Marr, 2016).

Electrochemical stability is obviously one of the most important characteristics of some ILs proven by their extensive applications in electrochemical devices such as electrowinning, water splitting and many more (Abbott et al., 2008; MacFarlane et al., 2016; MacFarlane et al., 2014). ILs is a great advantage to the field of energetic materials due to the huge structural variability of ILs (Zhang & Shreeve, 2014) that gains properties such as low vapour pressure, wide liquid range, and good thermal stability. As they commonly used as explosives and propellants, energetic materials with a large amount of stereochemical energy that can be released is advantageous to be use for shock, heating, or applying friction. 'Hypergolic ILs' which is one of classes of ILs which in presence of a suitable oxidiser is designed to ignite (MacFarlane et al., 2017). Another potential field for the use of ILs in the commercial application is its usage in sensor. The utilisation is referred to electrochemical sensors, where a considerable advantage of ILs is their wide electrochemical window and non-volatility (Abdul Rehman & Zeng, 2012; MacFarlane et al., 2017).

Finally, the use of ILs in dissolution of biomass has been extensively studied. One of the earliest biomass that has been dissolved with the aid of ILs is cellulose. The dissolution process has been done by using cellulose-dissolving pulps (from cellulose acetate, lyocell, and rayon production lines), fibrous cellulose (Aldrich), and Whatman cellulose filter papers. A range of anions were selected to experiment with the dissolution rate and [BMIM]Cl shows the highest solubility for cellulose (Swatloski et al., 2002). Furthermore, several studies on cellulose dissolution by using ILs has been reported (Farouk Ibrahim et al., 2015; Parviainen et al., 2014). The dissolution of other biomass such as collagen fibre (Meng et al., 2012), nut shell (Parviainen et al., 2014), and wool (Azila Mohd Idris et al., 2014) have also been explored. For keratin, several studies have been done that shows the dissolution process by using ILs. These studies (Azila Mohd

Idris et al., 2013; Ji et al., 2014; Wang & Cao, 2012) show the usage of different types of ILs with different experiment setup and varieties of outcome.

2.3 Ultrasound sonochemistry and its history

2.3.1 Sonochemistry and its history

Discovery of the modern time generation of ultrasound is pioneered by Jacques and Pierre Curie in 1880 through its origin in the piezoelectricity (Curie & Curie, 1880; Mould, 2007). These researchers showed that under mechanical stress, some crystals especially quartz can generate electrical polarisation. During World War I in 1917, an ultrasonic submarine detector developed by Paul Langev was the first practical application of ultrasound; who had been one of Pierre Curie's students. The device was used to detect the returning echo which consisted of two steel plates with thin quartz crystals glued in between, along with a hydrophone. The distance to the object can be determined by measuring the time taken to hear the echo of the sound waves from a submarine object when the transducer emits a pulse of ultrasound. SONAR (Sound Navigation and Ranging) is the result of subsequent developments of ultrasound in underwater range finding (Dadras et al., 2011).

Formation of vapour bubbles may happen when propagation of wave pressure with sufficient intensity passes through a liquid. The first occurrence was claimed (Young, 1999) to be observed by cavitation in low-pressure regions in a thin layer of water between two rolling pieces of glass by Isaac Newton. The discovery happened when he was examining rings formation between a plane glass surface and a convex lens. However, it is widely known that in 1895, Sir John Thornycroft and Sidney Barnaby initiated stepping stone in cavitational studies that comes from observations of the poor performance of a newly built destroyer, HMS Daring (Thornycroft, 1895). Due to the

rapid movement of the propeller blade in water, it was found that the formation of microbubbles at trailing edge is due to sufficient negative pressure. The extreme effects on the metal surfaces exposed to the cavitating liquid is because of the sudden growth and collapse of those vapour cavities. Through modification of the propeller surface, Thorneycroft and Barnaby envisaged a solution to increase its performance by decreasing its angular velocity and therefore decreasing bubble formation (Bremner, 1990; Dadras et al., 2011).

In sonochemistry, there are three sites for a cavitation bubble as shown in Figure 2.3 (Mason, 1999) and will be further explained in the next sub chapter. There is the interior of a bubble, the interface region at around the bubble surface and the liquid region outside the interface region. The liquid region which is usually at ambient temperature is where chemical species with a relatively long lifetime such as H_2O_2, chemically react with solutes by diffusing out of the interface region. Radicals with a relatively short lifetime such as •OH and •O react with solutes or radicals themselves at the interface region; as the temperature dramatically rises at this region due to the thermal conduction from the heated interior of a bubble. Several authors have estimated the actual temperature in the interface region but it still remained unknown (Storey & Szeri, 2000; Suslick et al., 1986; Yasui, 1996). Heat and radical attack can dissociate surfactants that is absorbed at the bubble surface at the interface region (Sostaric, 1999). In the interior of a bubble, high temperature causes volatile solutes to evaporate into the region (Yasui, 2002).

Three following phenomena originated the effects of acoustic cavitation. First, collapsing bubbles produced liquid jets (microjetting) impacting on the solid surface through asymmetrical collapse (Leighton, 1994). Next, shock waves released from collapsing bubbles (Holzfuss et al., 1998; Pecha & Gompf, 2000; Weninger et al., 2000).

Lastly, microstreaming is induced by pulsating bubbles during cavitation and implosion near the solid surface (Elder, 1959; Leighton, 1994).

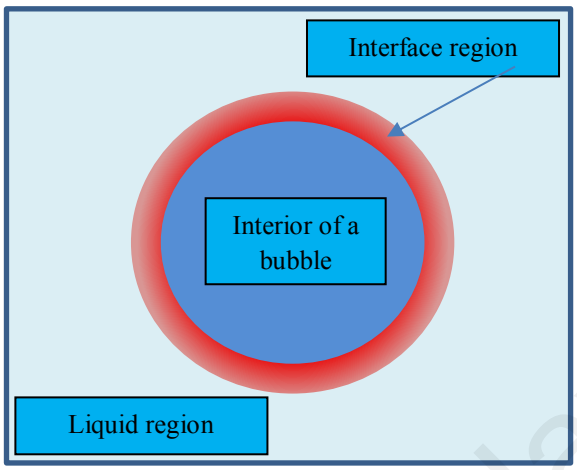


Figure 2.3: Three sites for chemical reactions for a cavitation bubble.

A spherical shock wave is emitted from a bubble into the surrounding liquid when symmetrical collapse of bubbles happened. Furthermore, microstreaming occurs when a pulsating bubble induces a liquid flow around the bubble. Hence, these effects improve a mass transfer toward or from the solid surface. Increasing the limiting current density in electrolysis is one of the examples of enhancement in mass transfer by acoustic cavitation (Walton & Phull, 1996).

2.3.2 Types of ultrasound

Ultrasound has been widely used in various industrial and life applications, especially in diagnostic and therapeutic medicine (Cavalieri et al., 2011; Zhou et al., 2016). Sound waves above the frequency of human hearing (≥ 20 kHz) is referred to as ultrasound. Depending on the wave frequency, ultrasound is divided into three categories, namely

power ultrasound (20–100 kHz), high frequency ultrasound (100 kHz–1 MHz), and diagnostic ultrasound (1–500 MHz) (Wu et al., 2013).

To destroy cellular structures or to enhance or inhibit activities within foods, high intensity (low frequency, high power) ultrasound is commonly used (Mason et al., 1996). These intense acoustic waves can produce high pressures inside liquid foods, causes streams of fast-moving microbubbles and causing bubbles in liquids to implode violently (Mann & Krull, 2004). Hence, (Povey & Mason, 1998) described high-power ultrasound as "material-altering", in parallel to the low-intensity ultrasound as descriptor of "non-destructive". In the food processing arena, numerous applications are found for the material-altering applications of high-intensity ultrasound (Mason, 2003).

2.3.3 Acoustic cavitation process

Strong sonic vibration occurs when ultrasonic waves propagate through a medium. Mild to severe structural damage can occur if the sonication medium contains solid particles, for example, plant materials such as seeds. This is also the basis of its utilisation in cleaning and extraction applications. Ultrasound generates acoustic streaming within liquids on top of the mechanical vibration effect (Manasseh et al., 2010), which enhances effective mass transfer leading to improved productivities in some industrial processes. The propagation of ultrasonic waves in liquid medium results in a phenomenon known as acoustic cavitation (Ashokkumar & Mason, 2007).

The acoustic cavitation is a process of creating cavity in the system where it involves microbubbles growth in the solution with the resonating acoustic waves and collapses once it reaches its maximum resonance size. The pressure built during the microbubble growth is released through the collapse of cavitation bubbles, resulting in the generation

of very high local temperature which in turns aid in the formation of highly reactive radicals through the pyrolysis. For an example, due to high temperature conditions, hydrolysis of water molecules within bubbles can generates H• and HO• radicals (generally referred to as primary radicals). Secondary reducing radicals can be produced in the presence of suitable solutes through lysis by the primary radicals (Nor Saadah Mohd Yusof & Ashokkumar, 2015).

Acoustic cavitation can be classified into two types; transient cavitation and stable cavitation (Leighton, 1994; Yasui et al., 2010). The bubbles shape stability is what defined transient and stable cavitation bubbles. In transient cavitation, temporal fluctuation in the number of bubbles occurs when bubbles sporadically fragment into daughter bubbles, coalesce with each other, and are nucleated. It is studied that stable cavitation bubbles are relatively large bubbles of about 10 mm or more in radius or tiny bubbles of a few mm in ambient radius (Yasui, 2002). They are classified according to former definition which is into stable cavitation bubbles or also called "high energy stable cavitation" bubbles. On the other hand, they can also be called "repetitive transient cavitation" bubbles which classified into transient cavitation bubbles. Whenever the terms transient and stable cavitation are used, it is important to specify which definition is used; activity or shape stability.

Furthermore, when acoustic amplitude is lower and ultrasonic frequency is higher, stable cavitation bubbles are more regularly observed (Ashokkumar et al., 2009). Moreover, transient cavitation bubbles are more commonly seen when ultrasonic frequency is lower and acoustic amplitude is higher. The addition of a surfactant to the liquid makes it possible to manage the population of stable cavitation bubbles relative to that of transient ones because coalescence of bubbles is strongly retarded by a surfactant

and for stable cavitation bubbles, the ambient radius of bubbles becomes adequately small (Lee, J. et al., 2005; Lee et al., 2007; Yasui et al., 2010).

Nucleation or formation of a bubble in acoustic cavitation can be based on these three mechanisms (Leighton, 1994). First mechanism is the nucleation at the solid surface such as a liquid container, motes or particles in liquid. The surface of a gas pocket is concave in a crevice, hence the pressure inside a pocket is reduced by the surface tension of a gas pocket. This explained the stability of a gas pocket, responsible in preventing its dissolution into the liquid because comparing to a gas pocket, the partial pressure of dissolved gas in the liquid is possibly higher. When the liquid is irradiated by ultrasound, the pressure inside a pocket further decreases in a gas pocket in a crevice during the rarefaction phase which causes gas to diffuse into the pocket from the surrounding liquid. During the compression phase of ultrasound, the gas pressure increases in a pocket and a gas pocket shrinks which resulted in the diffusion of gas out of a pocket into the liquid. Furthermore, as the volume of gas diffusing into the pocket during the rarefaction is more than that diffusing out of the pocket during the compression, it causes growth of gas pocket. Finally, when the gas pocket sufficiently grows, a gas bubble is formed from a crevice (Borkent et al., 2008; Madanshetty & Apfel, 1991; Tuziuti et al., 2005).

The second mechanism for nucleation is by impurities or the initially present bubble nuclei which its surface is covered with surfactants present in the liquid and hence stabilised against dissolution. Exceptional for liquid with supersaturated gas, within a few seconds, bubbles with radius smaller than 1 mm should dissolve in the absence of ultrasound without the presence of surfactants (Yount et al., 1984). This happened because due to surface tension of a bubble, the gas pressure inside a bubble is larger than the partial pressure of the dissolved gas in the liquid. Therefore, the gas inside a bubble gradually dissolves into the bulk liquid.

The third mechanism is through fragmentation of active cavitating bubbles (Young, 1999). New nuclei for cavitation bubbles are formed and unstable bubble is fragmented into several daughter bubbles. Asymmetric acoustic environment induced instability of a bubble through the presence of a neighbouring bubble, liquid surface, solid object, or an asymmetric liquid container or a traveling ultrasound (Bremond et al., 2006; Calvisi et al., 2007; Wang et al., 2003). Under some condition, new nuclei can also be formed when a bubble jets many tiny bubbles (Lee et al., 2007; Young, 1999).

Growth of a bubble in acoustic cavitation can be explained through two mechanisms (Leighton, 1994). First is coalescence of bubbles. The other is the gas diffusion into a bubble or also known as rectified diffusion. Furthermore, there are two mechanisms for the coalescence of bubbles. One is secondary Bjerknes force or the attractive radiation force between bubbles. The other radiation force which also called the primary Bjerknes force drives active bubbles to the pressure antinode of a standing wave field. Acoustic amplitude and frequency are factors that strongly affect the bubble growth rate due to rectified diffusion (Crum, 1980; Lee, Judy et al., 2005). Coalescence of bubbles may be the key mechanism of the bubble growth after acoustic cavitation is fully started (Iida et al., 2010; Yasui, 2002). Conversely, rectified diffusion may be the main mechanism at the initial development of acoustic cavitation, as the rate of coalescence is proportional to the square of the number density of bubbles which should be minor at the initial stage of acoustic cavitation (Yasui, 2011).

2.3.4 Effects of ultrasound

The occurrence of different types of reactions is due to the three different regions that have been postulated to exist during cavitation process. One is a gaseous phase where in the final stage of bubble collapse, high temperatures (several thousand degrees Kelvin)

and high pressures are produced and thus typical combustion actions occur. In this region, H• and •OH radicals are formed by the thermal decomposition of water (hydrolysis) hence any volatile solute in this region will participate in these combustion reactions. The second region is where large gradients of temperature and pressures as well as large radical concentrations exist. This region is the interface between the hot gas bubble and the bulk of the solution. The third region is the bulk solution, where the observed products are similar to those observed in aqueous radiation chemistry and where radical scavenging reactions follow the kinetics observed in radiolysis (Alegria et al., 1989).

Microbubbles of various sizes present in a liquid could be forced to oscillate in response to alternating pressure waves of ultrasound. Such oscillation generates shear forces and enhances fluid flow and mass transfer that could be used in various chemical processes. In addition, specific applications such as cleaning of surfaces and various materials is possible as ultrasound itself generates mechanical agitation that could be used for such processes. When the oscillating bubbles cavitate under specific experimental conditions, much stronger forces are generated with the addition of the direct mechanical forces generated by ultrasound or oscillating bubbles (Nor Saadah Mohd Yusof et al., 2016).

During acoustic cavitation, mass transfer effects is generated. This causes a variety of possible chemical reactions to be induced by the primary and secondary radicals and the rate of chemical reactions can be enhanced. Several industrial processes use the mechanical properties of ultrasound as well as the physical effects generated during acoustic cavitation for specific applications. Examples include underwater communication, diagnostic medicine, extraction and emulsification (Zhou et al., 2013).

The acoustic cavitation is also accompanied by the emission of light, referred to as sonoluminescence (SL). Other mechanical and physical effects, such as shock wave

formation and turbulent motion of the liquid are also generated during cavitation alongside the heat and light produced during bubbles collapse. Regions of high and low pressure are created as this pressure wave passes through the medium. The amplitude of the pressure wave or the acoustic pressure which denote the size of these pressure variations, is directly proportional to the amount of energy applied to the system. The wave will dissipate this energy in the form of viscous flow as this wave passes through a viscous medium. This is also known as "steady streaming" (Riley, 2001).

Under the influence of the acoustic field, bubbles formed through cavitation will begin to expand and collapse. Otherwise, inertial cavitation can occur for certain bubble sizes and acoustic pressures, the bubble expansion phase is prolonged and is followed by a violent collapse back to a smaller bubble size. When the cycle persists for many hundreds of acoustic cycles this mode of bubble oscillation is referred to as stable, or repetitive transient cavitation.

Alternatively, the bubbles grow and collapse spectacularly within a very few acoustic cycles with higher acoustic amplitude and the collapsed bubble then fragments into a mass of daughter bubbles (Leighton, 1994; Yasui, 2002). These daughter bubbles collapse rapidly as they are often small and so to complete obliteration of the original bubble (Crum & Nordling, 1972; Strasberg, 1959). This phenomenon generally observed at low frequencies (20–100 kHz) and is referred to as unstable or transient cavitation. The resonance size is defined as the size range at which transient cavitation occurs. Transient cavitation is particularly relevant to many food applications as the occurrence of the cavitation within the proximity of a solid surface as the bubble collapses asymmetrically. This incidence is referred as the emission or microjet of fluid or bubbles (Lee et al., 2007). The solid surface is the direction of this microjet and this can lead to pitting and erosion. Dislodging of particles attached to the surface and segregation of large to small particles

is part of the surface action of microjetting (Hagenson & Doraiswamy, 1998; Suslick, 1988).

Conversely, the water vapour pressure inside the cavitating bubbles increased with increasing external temperature. The bubble collapse event is subdued because of this water vapour acting as a "cushion" for the collapse. Hence, at temperatures significantly above ambient levels, ultrasound is found to be less effective. Finally, the energy is ultimately converted to heat regardless of the mechanism for dissipation of acoustic energy, may it be steady streaming, transient cavitation, microstreaming, or microjetting. This means that unless cooling is simultaneously applied, all applications of ultrasound will result in surge in temperature. In most circumstances, the temperature effect is relatively mild, only with an increase of a few degree celsius, but it is still important to take system design into consideration (Luther et al., 2001).

Generation of enormous temperatures at a localised level (>5,000 K) is theoretically possible when the violent collapse events occurs during transient and repetitive transient cavitation (Ashokkumar & Mason, 2007). Inside a collapsing bubble, the high temperatures resulted in the formation of primary radicals. When the temperature inside the collapsing bubble is at a maximum in a single bubble, the number of radicals generated is high. By increasing the sonication power, decreasing the external (solution) temperature, or increasing the external pressure, local temperature can be altered. The size of the cavitation bubble also affected the amount of heat generated. In a multi bubble field, the number of active bubbles generated is also another factor for the total number of primary radicals generated; not only by the bubble temperature. In fact, to control the radical yield, it has been shown that the number of bubbles generated is the dominant factor (Ashokkumar et al., 2007).

2.3.5 Applications of ultrasound

In industrial application such as pharmaceutical or fine chemical industry, shorter reaction time and higher yields is advantageous factors for chemical reaction by using ultrasound. Sonochemistry is beneficial in the synthesis of expensive products or for fast reactions. Hence, establishment of a commercialised process is the most important factor for development of ultrasound application in industrial scale. In pharmaceutical formulations, an ultrasonic extraction method which is rapid, sensitive, and accurate has been established for nicotine. Compared with the conventional cold extraction technique, the ultrasonic extraction can shortened the extraction time greatly from 24 hours to less than 20 minutes (Zuo et al., 2004). The amount of solvent consumed is six times lower than similar conventional extraction methods which is contradicted with ultrasonic extraction (Kanbe et al., 1993). The effect of ultrasound on the synthesis of drugs also have been studied by many and it is well documented (Emery et al., 2005; Mason et al., 1997).

There are several interesting potentials in the development of ultrasound-induced chemical processes by the possibility of cavitation at high pressures, such as, precipitation polymerisation in CO_2, phase-transfer catalysis in liquid, and bulk ethylene polymerisation by using ultrasound. Radical polymerisation is possible to be carried out without the addition of initiator or catalyst. However, the increase in viscosity causes the yield obtained for a bulk polymerisation to be rather low. Cavitation is hindered by a high viscosity and subsequently the production of radicals. Precipitation polymerisation can be a solution to overcome this conversion limitation (Ando et al., 1984; Price, 1996). In the high-pressure setup, there are two types of precipitation systems are used; one of it is the systems in which the monomer can acts as reacting species and as anti-solvent for the polymer, e.g., carbon dioxide is applied as anti-solvent in ethylene systems (Adeoya-Osiguwa et al., 2003; Farooq, 2012; Zhu et al., 1993).

Application of ultrasound has improved extrusion if terms of dispersing of nanocomposites in polymer melts. To lower the resistance of the shaping channels or reduce the viscosity of polymer melts with the application of ultrasound field has been approved to be a very efficient way to melt polymer in the shaping zone. Guo *et al.* demonstrated significant changes in the properties of the polymeric materials by the application of ultrasound to the polymer melt during extrusion process. Ultrasonic oscillations in the direction parallel to the flow of polymer melt have been applied by Guo *et al.* (Guo et al., 2003). On top of dispersing nanocomposites; increase of crystallinity, enhancement of mechanical properties, and reduction of structural defects have been observed with the application of ultrasound to polymer processing (Paradkar & Dhumal, 2012).

Considerable interest has been shown in recent years on the application of ultrasound for hazardous chemical destruction, including the degradation of chlorinated hydrocarbons, pesticides, aromatic compounds, explosives, surfactants, and dye (Emery et al., 2005). Ultrasound waves are irradiated into a liquid medium to destroy the contaminants or this process is also known as sonication. By means of ultrasonic irradiation at 20 kHz, the degradation of a toxic compound typically found in effluents from the pharmaceutical industry, triphenylphosphine oxide (TPPO) in water is plausible (Zhu et al., 1993).

In advanced water and wastewater treatment processes, membrane filtration plays a vital role due to high removal capacity, ability to meet stringent treatment goals and small footprint of the technology. Membrane fouling is one of the main barriers to its greater applications despite the major advantage of the technology. Generally, membrane fouling is a limitation of salts on the membrane surface and/or within the membrane pores caused by the accumulation of water impurities (i.e., membrane foulants), such as colloidal

particles, organic matters, and microorganisms. Over filtration time, the membrane will consequently get clogged. Both the quantity (permeate flux) and the quality (solute concentration) of the product water are affected by membrane fouling (Zhu & Elimelech, 1997).

Compared to conventional cleaning methods, ultrasonic technique has significant advantages for membrane fouling control. On top of requiring high energy cost; increasing crossflow rate, bubble scouring, and back washing are not very effective. The strongly adhered foulants on the membranes such as organic matters are also very tedious to be removed. In addition, the membrane may be damage by chemical cleaning and may induce secondary pollution (Li et al., 2002). Effectiveness, chemical free, and simultaneous membrane cleaning and fouling prevention during the filtration process are among the advantages of ultrasound. No downtime of filtration is necessary by using this method. Results indicated that throughout the duration of filtration with the assistance of ultrasound, the clean water permeate flux of the membrane can be maintained (Chen et al., 2006).

Another interesting industrial application field of power ultrasound is specific heating at the junction between the pieces of material or also known as welding of plastics or metals (Mason et al., 2003) and it is extremely important to develop new transducers design for welding devices. This occurrence resulted a shorter bonding time and increasing the speed of the wire bonders with the application of higher frequency of the transducers (Parrini, 2001). Chemical compatible is necessary if any two plastic materials to be welded together. There can be no chemical bonds if they are not compatible, even when they melt at the same temperature. A large number of factors such as the amplitude of the vibrations of the working and of the waveguide, welding time, frequency of mechanical vibrations, and static pressure caused by the clamping force of the waveguide

on the welded parts are dependent for the high quality of welding outcome (Gutnik et al., 2002).

2.3.6 Ultrasound and ionic liquids

For the last decade, incredible development of the field of ILs has been observed and well established in green chemistry of modern world. They are generally composed of a bulky organic cation and an organic or inorganic anion. Organic chemistry, electrochemistry, and inorganic chemistry are among fields that ILs have been used in a wide range of applications (Estager, 2012), and are now being applied in different industrial processes (Plechkova & Seddon, 2008). In term of sonochemistry, the very low vapour pressure of ILs can be very helpful to reduce the tendency of the solvent to undergo cavitation or to avoid nebulisation phenomena (Flannigan et al., 2005). Different areas of chemistry have been utilising the coupling of ILs and ultrasound.

First and foremost, the synthesis of ILs using ultrasound have been reported in different publications (Lévêque et al., 2007). In the recent years, various sono-assisted organic reactions have been incorporating ILs in the reactions. For example, the synthesis of quinoline in butylimidazolium tetrafluoroborate have been described by Heravi (Mohammad Reza Poor Heravi, 2009). This tandem addition or annulation reaction of o-amino-aryl ketones and α-methyleneketones used ILs to play the role of acidic catalyst and the medium managed to be recycled twice without any loss of acidity. The same catalyst has also been used for the Biginelli reaction on the synthesis of 3,4-dihydropyrimidin-2-(1H)-ones (Gholap et al., 2004) or the sono-assisted synthesis of 1,8-dioxo-octahydroxanthene derivatives (Venkatesan et al., 2008). For instance, the acetalisation of alcohol by using ILs without these acidic properties have been studied (Gholap et al., 2003); an easy work-up is enabled with ILs, or for which the imidazolium

cation acted as a platform for the reaction of the benzoin condensation via its C2 position (Estager et al., 2007).

The synthesis of organised soft material consisting of imidazolium ILs and single-wall carbon nanotubes have also been utilising coupling of ultrasound and ILs, making use of the interaction of π electron on the surface of the nanotubes and the imidazolium ring that leads to gel with fascinating properties (Fukushima & Aida, 2007). Finally, ILs and ultrasound have some very interesting synergies which is crucial to be noted. For example, extraction and/or solubilisation of materials makes ILs very useful as it can be designed to tune their solubility of different compounds. In both cases, reduction of particles size for solubilisation and enhanced mass transfer and microemulsion formation for extraction can play a major role in these processes as the physical effects of ultrasound. As an example, the solubilisation of cellulose in 1-allyl-3-methylimidazolium chloride or in $[C_4mim]Cl$ has been utilising ultrasound and ILs in the studies (Dadras et al., 2011; Estager, 2012; Mikkola et al., 2007). Interestingly, even though there has been a study on dissolution of cellulose with the aid of ultrasound (Mikkola, Kirilin et al., 2007), there has not been any study or research that focuses the dissolution of keratin with the aid of sonication.

CHAPTER 3: MATERIALS AND METHOD

3.1 Materials

3.1.1 Preparation of feather keratin

Cleaned turkey feathers were purchased from Spotlight, Selangor, Malaysia. At this stage, only the barb from rachis part was used. The content of cysteine bond for different part of feather is known to be different according to their structure and specific uses (McKittrick et al., 2012; Reddy & Yang, 2007; Wang et al., 2016). Thus, the barb part was used for constant purposes throughout the study. A fixed amount of feather (500 mg) was used for each dissolution throughout the study.

3.1.2 Preparation of [BMIM]Cl:DMSO solvent

Through literature reviews and preliminary studies, the ILs chosen for the context of this study is 1-butyl-3-methylimidazolium chloride ([BMIM]Cl, 98% purity), purchased from Sigma Aldrich and dimethyl sulfoxide (DMSO) was purchased from Merck. Throughout the study, the feather dissolution experiments were carried out in several concentrations ratio of [BMIM]Cl:DMSO. An optimum concentration of 35:65 mixture of [BMIM]Cl:DMSO solvent was obtained.

3.2 Dissolution of feather keratin in [BMIM]Cl and [BMIM]Cl:DMSO solvent

3.2.1 Keratin dissolution via conventional heating method

The solubility experiments were conducted in vial as shows in Figure 3.1. The vial was heated in an aluminum heating block until 130 °C and remained constant on a hot plate.

A total amount of 500 mg feather keratin to be dissolved was kept constant throughout the study for each experiment. The dissolution process was quantified by gradual addition of 50 mg of feather upon dissolution and mechanically stirred until they were completely dissolved in the solvent. The complete dissolution of the feather was confirmed visually and presence of small particles approaching complete dissolution is detected via light scattering by using laser beam. The dissolution time was recorded.

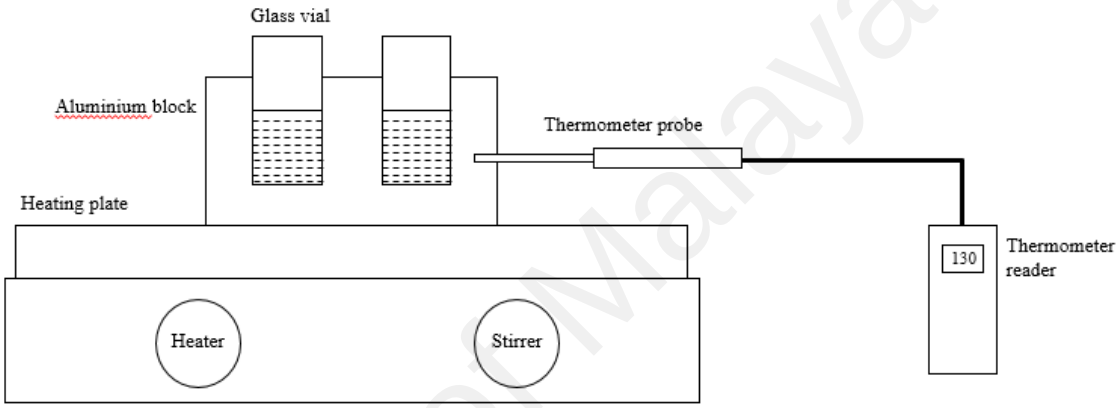


Figure 3.1: Experimental setup for conventional (heating) method

3.2.2 Keratin dissolution via ultrasonication

The solubility experiments were conducted in 50 mL beaker as shown in Figure 3.2. The ultrasound waves were transmitted in the solvent system by a probe transducer with a constant depth of 2 cm in the solvent system at a frequency of 20 kHz with applied powers of 120 W, 200 W and 280 W by using Branson Sonifier 450. A fixed amount of 500 mg feather keratin was determined for dissolution as a constant throughout the experiment. Small incremental amounts of 50 mg of feather were gradually added to the solvent system to quantify the solubility and mechanically stirred until they were completely dissolved in the solvent. The complete dissolution of the feather was

confirmed as the technique mentioned before. The dissolution time for each applied power was recorded.

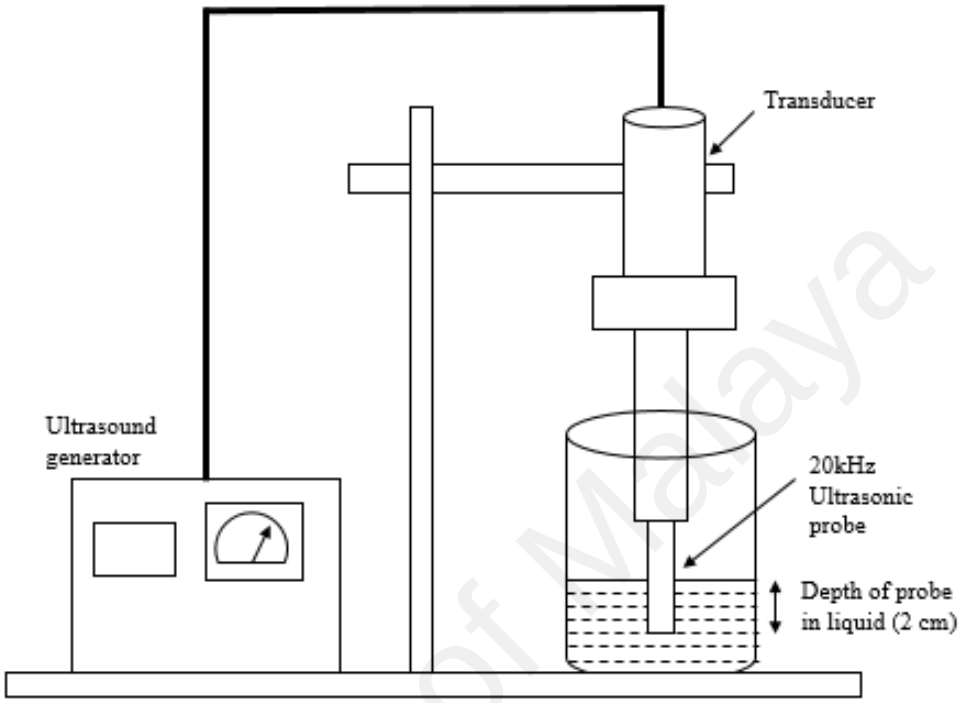


Figure 3.2: Experimental setup for ultrasonication method

3.2.3 Regeneration of dissolved keratin material

Figure 3.3 shows the processes involved from raw turkey feather to regenerated keratin after both conventional and ultrasonic assisted dissolution process. The keratin regeneration was carried out by addition of water at room temperature, and the regenerated keratin precipitated from the solution immediately through the action of stirring. The regenerated keratin was separated by centrifugation for 20 minutes at 4000 rpm and vacuum dried in oven at 60 °C for 3 days. A brown-coloured solid is obtained as the regenerated keratin materials (Figure 3.3).

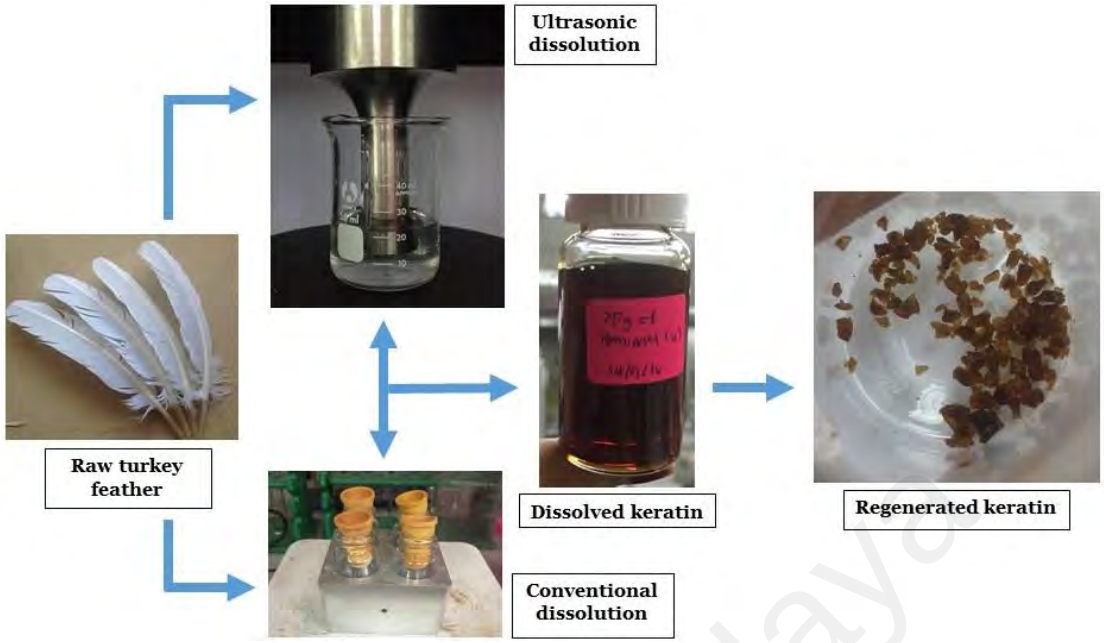


Figure 3.3: Summary of dissolution and regeneration processes of raw turkey feather through ultrasonic-assisted and conventional methods.

3.2.4 Solubility of feather keratin in organic solvents

The conventional heating method was studied with the dissolution of feather by using [BMIM]Cl as the main solvent. The solubility experiments of keratin in organic solvents was carried out using conventional method before proceeding with the sonication method. Another important aspect of this study is to reduce the use of ILs. Therefore, several dissolution attempts were done by mixing [BMIM]Cl with other organic solvents at various ratio. Several organic solvents were chosen as a co-solvent for the solvent system and the results of the dissolution process is as tabulated in Table 3.1 below.

From Table 3.1, it can be observed that dissolution of feather is not possible with most of the organic solvents trialed except DMSO. Even though DMF and acetonitrile shows partial dissolution, when they were used for sonication method, full dissolution is not plausible. Hence, only DMSO was chosen to act as co-solvent with ILs for the dissolution process.

Table 3.1: The summary of solvent systems and the result of dissolution process.

Medium of reaction	Result
2.0 M of [BMIM]Cl in Ethanol	Unsuccessful
2.0 M of [BMIM]Cl in H_2O	Unsuccessful
2.0 M of [BMIM]Cl in DMF	Partial dissolution
2.0 M of [BMIM]Cl in Acetonitrile	Partial dissolution
2.0 M [BMIM]Cl in DMSO	Successful
5.0 M [BMIM]Cl in DMSO	Successful

3.3 Ultrasound calibration

3.3.1 Physical effect of ultrasonication

The power applied to the system were varied from 120 W to 280 W. However, the power delivered to the system depends on different factors such as the power lost through heat or the light emitted by microbubbles collapse (sonochemiluminescence). Therefore, the power delivered by the reactor has to be calibrated. This is done by measuring the difference in temperature for an interval set of time. The ultrasonic power dissipated into the reaction liquid was measured by using the following equation:

Equation 3.1: Power (W) = dT/dtCpm,

where Cp is the heat capacity of water (4.2 J g-1) and m is the mass of water (g). (dT/dt) is the temperature rise per second. The sample volume was 20 cm^3 and the initial temperature rise was measured at room temperature by using a digital thermometer, which was immersed in the sample solution and held at the half height of the solution.

3.3.2 Chemical effect of ultrasonication

By indirectly measuring the amount of radicals produced in a system within a specific application of power, the intensity of chemical effect by the application of ultrasound can be determined. The measurement can be done through radical quantification. For the experimentation part, three types of solutions were prepared; solution A (0.4 M potassium iodide + 0.05 M sodium hydroxide + 1.6×10^{-4} M ammonium heptamolybdate tetrahydrate), solution B (0.1 M potassium hydrogen phthalate) and 20 mL sonicated deionised water. The deionised water was sonicated for a set interval of time and afterward, 1 mL of sonicated deionised water was mixed with each 1 mL of solution A and B respectively. The freshly mixed solution was then irradiated with UV to get an absorbance value at 355 nm.

When an aqueous KI solution is mixed with deionised water that is irradiated with ultrasound, I^-ions are oxidised to give I_2. I_2 reacts with the excess I^-ion to form I_3^-ion when excess I^- ions are present in solutions (Koda et al., 2003). Concentration of KI was 0.1 mol dm^{-3}. The absorbance of I_3^-at 355 nm was measured (ε = 26,303 $dm^3\ mol^{-1}cm^{-1}$).

3.4 Characterisation methods

The characterisations of the regenerated keratin were done by using analytical instrumentations such as ATR-FTIR, PXRD, DSC and TGA. This part of the study is crucial to make sure that the protein backbone of raw feather keratin is maintained even after the dissolution and regeneration process.

3.4.1 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

Fourier transform infrared spectra were obtained using a Perkin-Elmer 400 RX1 FT-IR spectrophotometer. The FTIR was performed in the wavenumber range from 400 to 4000 cm^{-1}. The spectra were recorded with a resolution of 4 cm^{-1} with 30 scans. Spectra were baseline corrected.

3.4.2 Powder X-ray diffraction (PXRD)

At 22 ± 2 °C using a Panalytical Empyrean powder diffractometer, the powder X-ray diffraction (PXRD) patterns were obtained. Approximately, 1 – 2 g of the finely ground sample was placed randomly on a locally designed flat brass sample holder for each analysis. CuKα1 radiation (λ = 1.540 Å) was produced at 40 kV and 25 mA. By using a 2θ-range of 5 to 50.0 ° (2θ) range, the data were collected in the Highscore Plus (θ/2θ) horizontal geometry using with a step size of 0.02 ° 2θ and an accompanying scan rate of 0.5° min^{-1}.

3.4.3 Thermogravimetric analysis (TGA)

For TGA analysis, samples were placed in a flowing nitrogen atmosphere in a Thermogravimetric Analyser, TGA 6 (Perkin Elmer, USA) between 30 and 700 °C at a heating scan rate of 10 °C min^{-1}. This analysis was carried out to investigate the thermal stability of the raw turkey feather and regenerated materials. The samples were first dried under vacuum in an oven at a temperature of 60 °C. Before running each experiment, these samples were then loaded in ceramic pans, equilibrated and the heating is started at the starting temperature of 30 °C.

3.4.4 Differential scanning calorimetry (DSC)

DSC analysis was conducted on a Diamond DSC (Perkin Elmer, USA) with 5 – 10 mg of sample in closed aluminium pans, at a ramp rate of 10 °C per minute. All samples were analysed from room temperature to 250 °C, then cooled to 50 °C before heated for the second time to 250 °C. The first heating was done to diminish any thermal history and the second heating was done for the analysis of the materials. Transition temperatures are reported using the peak maximum of the thermal transition.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Dissolution of feather keratin in [BMIM]Cl and [BMIM]Cl:DMSO solvent

For this subchapter, both conventional and sonication dissolution process will be explained and discussed in depth. The summary of the organic solvents used in order to determine the suitable co-solvent to be mixed with ILs is also elaborated here. Further justifications are included on the reasons of choosing DMSO as co-solvent. Lastly, the effect(s) of power of ultrasound on the dissolution process was explained extensively.

4.1.1 Dissolution of feather keratin in [BMIM]Cl and [BMIM]Cl:DMSO solvent

The initial aim of the project was to compare the efficiency of feather keratin dissolution, particularly the dissolution time required, in the absence and presence of ultrasonic irradiation. The experiment was conducted by immersing the probe transducer into a reaction beaker (containing 50 mg of feather in 20 mL of solvent) at a fixed depth of 2 cm and 120 W, 200 W and 280 W power supplied by the ultrasound generator. For each power, the experiments were repeated at least three times and the mean time taken for the dissolution was recorded. Initially, it took a few minutes for the dissolution of the first portion of feather keratin. The process was continued with the sequential addition of 50 mg feather keratin until all 500 mg of the feather keratin was dissolved completely in the solvent yielding in a viscous solution. Upon dissolution, no precipitation was observed even after the solution mixture was cooled to room temperature.

Then the dissolution rate started to become constant as shown in Figure 4.1 as each batch of feather keratin was added to the system. The visible viscosity of the ILs remains unchanged based on visual observation but the colour of the solution becomes darker as

time passed. It is deduced that ILs can dissolve the feather keratin by mainly breaking the hydrogen bonds in keratin fibre. The dissolution is due to the role of chloride ions and $[BMIM]^+$ ions in ILs that disrupted the hydrogen bonding in the keratin material. The chloride ions associated with the hydrogen of protein backbone and $[BMIM]^+$ ion complexes with the protein oxygen as shown in Figure 4.1. This interaction disrupts hydrogen bonding in feather keratin, leading to the dissolution of keratin. The keratin fibre can then move freely and finally dissolves in ILs.

The mechanism of keratin dissolution in pure ILs has been reported in the literature by Chen and coworkers (Chen et al., 2014). It has been suggested that ILs are suitable for the dissolution of keratin at high temperatures due to the presence of chloride ions in cellulose and other biopolymers (Azubuike et al., 2012; Meng et al., 2012; Swatloski et al., 2002; Zakrzewska et al., 2010). In addition, at elevated temperatures, the keratin structure is unfolded (Horvath, 2009) allowing a strong interaction between keratin and the ILs as reported by Zhang et al. (Xie et al., 2005).

A clear comparable result of ultrasonic assisted feather keratin dissolution and the conventional method is shown in Figure 4.2. It is clear that the use of sonication in the dissolution process has significantly shortened the dissolution time of feather keratin by 90 %. This will be discussed in subsection 4.1.4. The conventional heating method was carried out at 130 °C, as referring to the literature (Azila Mohd Idris et al., 2013). It has also been reported that the dissolution of feather keratin was found to be less efficient at temperature lower than 130 °C (Xie et al., 2005). The dissolution of feather keratin by this method is at its optimal at 130 °C due to the result reported by Xie *et al.* that showed optimum solubility of wool keratin over the span of 10 hours was 11 wt % at 130 °C as compared to 4 wt % at 100 °C (Xie et al., 2005). On the other hand, conventional method with heating at temperature higher than 130 °C was found to generate more water soluble

peptide and amino acids, thus resulting is lower yield of regenerated material (Ghosh et al., 2014). However, for sonication method in this study, no temperature control was used.

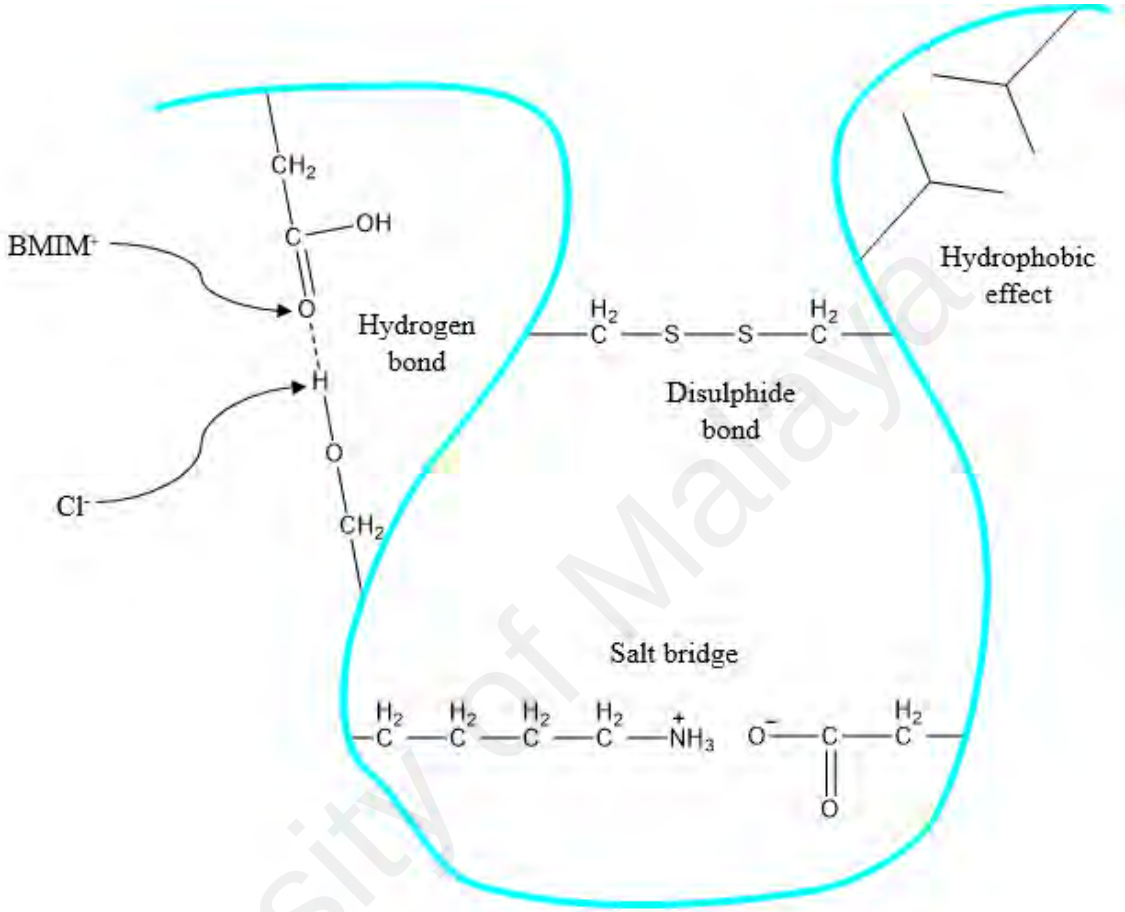


Figure 4.1: Mechanism on role of ILs in dissolution of feather keratin

As can be seen in Figure 4.2, the time taken for the dissolution of the first 50 mg of ultrasound (5 minutes) is way faster than the conventional method (15 minutes). The total dissolution through conventional heating method requires almost 2 hours (114 minutes) for 500 mg feather keratin to be completely dissolved in 5 mg of [BMIM]Cl as compared to the ultrasonic method (11 minutes). This proves its structural strength. Feather keratin consists of two distinct regions, the cuticle (sulphur-rich outer layer) and the cortex (inner component). The cuticle is composed of three layers, the epicuticle, exocuticle and endocuticle with exocuticle being the highest sulphur content. The lengthy dissolution

time is due to the strong cuticle structure which protects the cortex, hence retaining the original structure of the feather. However, when a similar experiment was carried out in the presence of sonication, the processing time was reduced by about 90 % from almost 2 hours to 11 minutes at much lower temperatures. It can then be suggested that the observed enhancement in the dissolution efficiency is may be due the physical forces and the mass transfer effects generated during acoustic cavitation. The dissolution of cortex happens within the first few minutes of heating, however the complete dissolution of the cuticle takes much longer time as reported by Chen and coworkers (Chen et al., 2014).

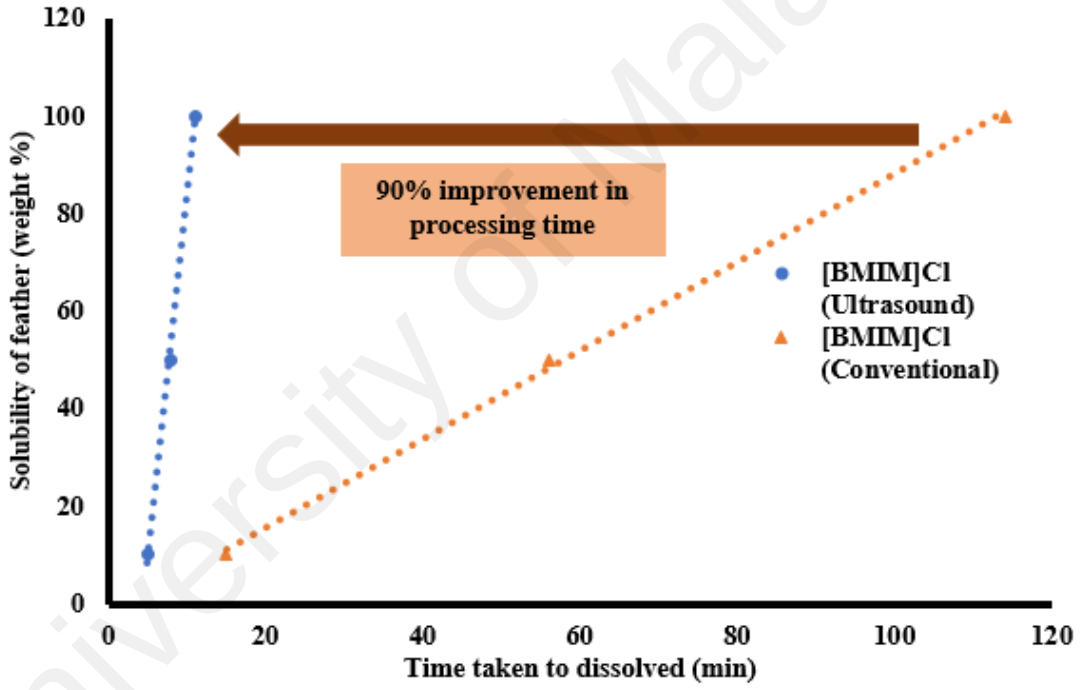


Figure 4.2: Rate of dissolution of feather keratin in [BMIM]Cl (ultrasound, 200 W of power) and [BMIM]Cl (conventional heating, in the absence of sonication).

4.1.2 Summary of solubility of feather keratin in organic solvents

In the case of sonication, both physical (shear) and chemical (radicals) effects are generated by acoustic cavitation. In order to see which of these effects contribute to the effective dissolution of feather keratin, further experiments were carried out. In parallel, efforts were taken to reduce the amount of ILs in order to lower the overall cost of the

process. For this purpose, attempts to dissolve feather keratin were carried out in different organic solvents, namely, pure [BMIM]Cl and a mixture of [BMIM]Cl and water, ethanol, propanol, dimethylformamide (DMF), acetonitrile (CH_3CN) or dimethylsulfoxide (DMSO). [BMIM]Cl was also mixed with an aqueous solution of cetyltrimethylammonium chloride (surfactant) or simple salts such as sodium salicylate. The [BMIM]Cl:solvent systems were used to investigate the plausibility of the solvent system for dissolution of feather keratin by using conventional method. Only systems with viable solubility undergo ultrasonic method and a small amount of feather keratin dissolution was observed in some of the systems.

However, a complete dissolution was only achieved with either pure [BMIM]Cl or [BMIM]Cl:DMSO. From Table 4.1, the first experiment was carried out with the use of only pure water as the solvent. The dissolution process was deemed unsuccessful after two trials. The following system were followed by the use of low concentration surfactant and simple salt but with no positive result. Further increase of concentration of the surfactant does not show any improvement in terms of dissolution. This concludes that the surfactant and common organic solvents are not powerful enough to dissolve the feather keratin. Furthermore, mixed solvent systems of [BMIM]Cl:DMSO were used in low concentrations (0.2 M and 0.5 M) but only partial dissolution was observed. Further increase in concentration (2.0 M) resulted in the full dissolution of the feather keratin. However, higher concentration (5.0 M) doesn't show any signification improvement in the rate of dissolution. Hence, it can be concluded that the 2.0 M [BMIM]Cl:DMSO solution is the most optimum solvent to be trialed for the study of mixed solvent system. The dissolution process is further optimised in an open system dissolution as reported in literature (Zhou et al., 2013) to obtain maximum efficiency of ultrasonication. Further details are provided in the Table 4.1 below.

Table 4.1: List of reaction medium for ultrasonic reaction.

Medium of reaction	Result
Water – 50 ml (2 trials), Power: 200 W	Unsuccessful[14]
0.015 M CTAB + 0.015 M sodium salicylate (1:1 ratio, 50 ml), Power: 200 W	Unsuccessful
0.1 M of CTAB (50 ml), Power: 200 W	Unsuccessful
0.0 5M of CTAB + 0.05 M of sodium salicylate + 0.1 M of propanol (50 ml), Power: 200 W	Unsuccessful
0.015 M of CTAB + 0.015 M of sodium salicylate + 1 M of propanol (50 ml), Power: 200 W	Unsuccessful
0.2 M of [BMIM]Cl in DMSO (50 ml), Power: 200 W	Unsuccessful
0.5 M of [BMIM]Cl in DMSO (50 ml), Power: 120 W	Unsuccessful
0.5 M of [BMIM]Cl in DMSO (50 ml), Power: 200 W	Unsuccessful
2.0 M of [BMIM]Cl in DMF (50 ml), Power: 200 W	Unsuccessful
2.0M of [AMIM]Cl in DMF (50 ml), Power: 200 W	Unsuccessful

[14] "Unsuccessful": the feather was undissolved

2.0 M [BMIM]Cl in DMSO (20 ml), Power: 200 W	Successful[15]
5.0 M [BMIM]Cl in DMSO (20 ml), Power: 200 W	Successful
2.0 M [BMIM]Cl in 2-propanol (20 ml), Power: 200 W	Successful
20 g [BMIM]Cl (20 ml), Power: 200 W	Successful

Table 4.1, continued

4.1.3 DMSO as co-solvent

The choice of DMSO as a co-solvent is another aspect that has not been discussed yet. Amongst different co-solvents trialed, only DMSO was found as an effective co-solvent for the dissolution process. In order to explain this observation, we considered the physical properties of various solvents used in this study. Among various properties, the vapour pressure of DMSO was found to be much lower than other co-solvents trialed (the vapour pressure of DMSO is 55.6 Pa meanwhile DMF = 516 Pa, Propanol = 1333.2 Pa, Ethanol = 5950 Pa, H2O = 2338.8, Acetonitrile = 5332.9 Pa). It is well known that the collapse intensity of bubbles during acoustic cavitation strongly depends upon the vapour pressure of the solvent (Adewuyi, 2001). With lower vapour pressure, the cavitation intensity is increased thus reducing the amount of vapour diffusing into the bubbles to cushion the cavitational collapse (Adewuyi, 2001; Destaillats et al., 2003).

[15] "Successful": the feather was dissolved

Solvents with low viscosity, high vapour pressure, and low surface tension formed cavities more readily (Adewuyi, 2001). However, due to more vapour entering the bubbles, less violent bubbles collapse will happen with higher vapour pressure (Peters, 1996). Hence, cavitation in a low vapour pressure solvent would generate stronger physical forces and mass transfer effects. Due to these effects, the disruption of hydrogen bonds happened in shorter period of time; hence causes the dissolution of the cuticle of feather keratin to be enhanced. Further mechanism will be explained in subchapter 4.2. An attempt was also made by carrying out the experiment in only pure DMSO. However, no dissolution of feather keratin was observed with the use of only pure DMSO. The dissolution efficiency was monitored by mixing DMSO with pure ILs at various proportions. At 2.0 M of [BMIM]Cl:DMSO, full dissolution of feather keratin was achieved. This proves that the contribution of ILs is still crucial due to the presence of chloride ion and [BMIM]+ ion which plays a major role in disrupting the hydrogen bond in keratin thus making dissolution of feather keratin with addition of organic solvent plausible. Further studies on acoustic power effect were carried out using [BMIM]Cl:DMSO system.

4.1.4 Effects of power on ultrasound application

The data shown in Figure 4.3 compares the dissolution efficiency of pure [BMIM]Cl and 35:65 mixture of [BMIM]Cl:DMSO solvents in the presence of ultrasound at 120 W, 200 W and 280 W. It could be seen that the rate of dissolution significantly decreases when pure ILs is replaced with the mixed solvent system by 33.5 minutes. However, this could be considered as a compromise between the rate of dissolution and cost effectiveness. This is because even though rate of dissolution is lower with mixed solvent systems, the amount of ILs used was way less compared to the pure ILs system which is

beneficial in term of cost. In addition, a reduction in the amount of ILs would also result in lowering environmental impact. Hence, from an industrial application point of view, the amount of ILs could be reduced by 65 % with mixed solvent systems and a 55 % increase in rate of dissolution as compared to the conventional method.

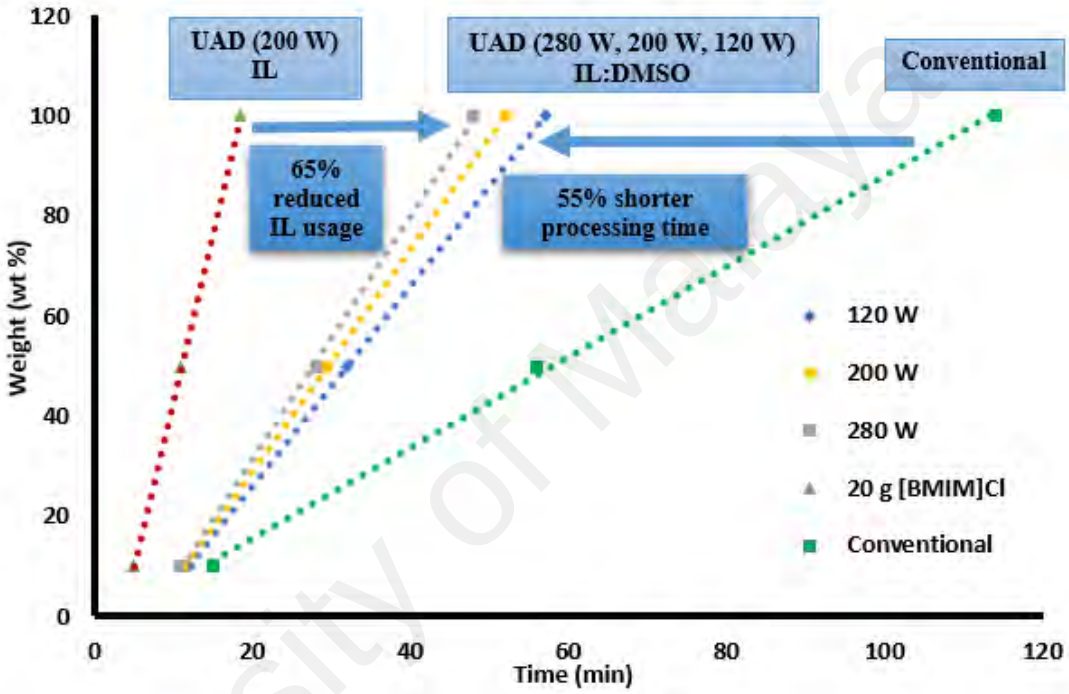


Figure 4.3: Rate of dissolution of feather keratin in 2.0 M of [BMIM]Cl:DMSO at applied sonication power of 120 W, P=200 W, P=280 W and pure [BMIM]Cl at P=200 W.

Figure 4.3 data also considers the effect of ultrasonic power on the dissolution rate of keratin in pure [BMIM]Cl and 35:65 mixture of [BMIM]Cl: DMSO solvents. This series of experiments were carried out in order to find an optimal ultrasonic power level, again, keeping possible industry applications in mind. In order to provide a clear picture of the power effect, the data presented in Figure 4.3 is summarised in Table 4.2.

Table 4.2: Time taken for dissolution of 500 mg of feather in 35:65 mixture of [BMIM]Cl:DMSO solvent at different power levels.

Power	Duration[16]
P = 0 (no US)	297.0
P=120 W	57.0
P=200 W	52.0
P=280 W	48.0

The observed power effect is of interesting and important. It could be seen from Figure 4.3 and Table 4.2, the time taken for the complete dissolution at different applied powers are not changing much, with approximately 15 % processing time difference (within experimental errors) when the power was varied from 120 W to 280 W. Even at the lowest power level used, the maximum dissolution rate could be achieved. This indicates the possibility that the optimum ultrasonic power applied could be achieved at even lower power. The power effect is also important to understand the possible role of ultrasound in enhancing the dissolution rate.

Despite the ultrasonic power level applied to the system, the power delivered is usually much less than the power received by the system. Due to that, it can be observed from calorimetric power data (see Appendix A) that the amount of energy delivered to the system increases with an increase in power. This can be concluded from the calorimetry power data when the amount of energy received for 120 W is 1.2406 $\frac{\mathrm{W}}{\mathrm{mL}}$ and increased to 1.3777 $\frac{\mathrm{W}}{\mathrm{mL}}$ for 280 W. With increasing power intensity delivered, the time taken for

[16] Time taken for complete 500 mg of feather keratin dissolution (min)

dissolution decreased. This is due to the higher physical damages induced by the sonication.

Next, calibration for H_2O_2 yield data is measured to determine the amount of radicals produced within a system. From H_2O_2 yield data (Appendix B), it is clear that the amount of radicals produced increases with an increase in acoustic power. The amount of radicals produced increased from 6×10^{-9} for 120 W to 8×10^{-9} for 280 W. Combining these observation with that of Table 4.2, it could be concluded that radicals (chemical effects) do not play significant role in improving the rate of dissolution (Hart & Henglein, 1985). It can then be suggested that the physical forces and the mass transfer effects generated during acoustic cavitation are responsible for the observed enhancement in the dissolution efficiency. Also, the mass transfer effects generated at the lowest power (120 W) is more than sufficient to achieve maximum efficiency. Further details on the mechanism are provided below.

4.2 Dissolution mechanism through conventional and sonication method

Despite the significant improvement of ultrasonic assisted dissolution process as compared to the conventional heating method, a concrete mechanism requires an extensive experimentation. First, let us look at the properties of feather keratin. Feather keratin consists of two distinct regions, the sulphur-rich outer layer known as the cuticle and the inner component called the cortex. The cuticle contains numerous disulphide bonds which connect the peptide chains and is composed of three layers, the epicuticle, exocuticle and endocuticle. Of the three layers, the exocuticle has the highest sulphur content which makes it a tougher structure and harder to dissolve. The mechanism of this dissolution process starts with the swelling of the cuticle followed by swelling of the cortex. Then the cortex starts to dissolve in a few minutes, however confined in the swollen cuticle. The time taken for the dissolution of the cortex is significantly shorter

(less than 5 minutes) than that to completely dissolve the cuticle which can take up to hours (Chen et al., 2014). High content of disulphide bonds within the cuticle is the main reason of the extended time required to dissolve the cuticle. The swelling of the keratin during dissolution suggests that the cortex may remain inside the fibre even in the dissolved state. The single fibre *in situ* observation technique is applied as reported by Chen *et al.* (2014) to prove that indeed the cortex remains inside the casing of the cuticle even in the dissolved state. Finally, the cuticle was dissolved, and both cuticle and cortex were dispersed in the solvent system. The dissolution process is illustrated in Figure 4.4. The disulphide bonds can also be chemically reduced or oxidised using thioglycolic acid (Kuzuhara & Hori, 2013), or hydrogen peroxide (H_2O_2) (Hogg et al., 1994), respectively.

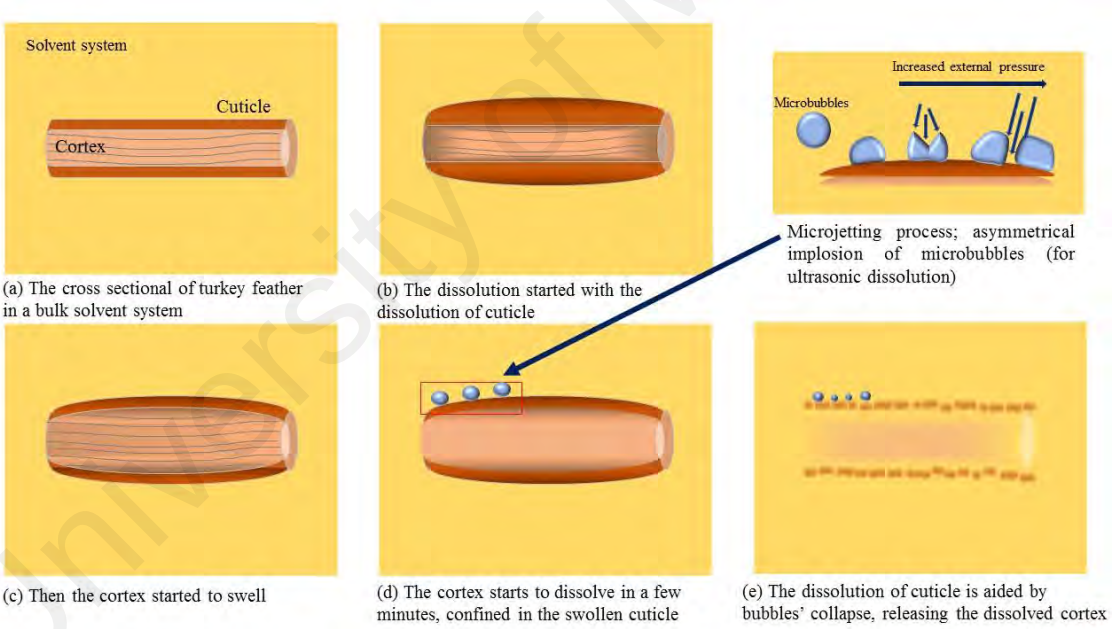


Figure 4.4: The illustration of the dissolution mechanism of feather keratin by using ILs.

Based on the characterisation data collected, and the fact that the presence of ILs is still crucial in all the dissolution attempts, it is best for now to speculate that the mechanism of ultrasonic assisted dissolution works similar to the conventional heating. However, the shorter processing time required is due to physical forces generated. During bubble

oscillation and bubble collapse, microstreaming of the solution is generated, resulting in an increase of mass transfer. At the same time, when the bubble attaches to the cuticle, asymmetric collapse may take place, resulting in a phenomenon known as microjetting that may help weakening the cuticle structure. Microjetting is a process where the collapse of bubbles is asymmetric near to the interfaces that hits the solid surface (Mason, 1998). Pitting and erosion can happen when the microjet is directed toward the solid surface. Particles attached to the surface can be dislodge by the surface action and large aggregates can be broken down into smaller particles (Hagenson & Doraiswamy, 1998; Suslick, 1988). Specifically, when the microjetting occurs with asymmetric bubble collapse, a few millimeters of the surface is where the acoustic streaming patterns around solid objects are strongest (Feng et al., 2011). Once the cuticle is broken at different places, the feather structure could dissolve instantaneously shortening the required time for dissolution.

Despite the ability of ultrasound in speeding up the keratin dissolution process, we are also concerned on preserving the protein backbone of the keratin. To confirm that the protein backbone is preserved, the dissolved keratin in the absence and presence of ultrasound were regenerated, analysed and compared by different techniques as will be discussed in the next section.

4.3 Regenerated keratin materials from ILs

The regeneration process of keratin is done mainly to study whether the regenerated keratin material obtained has the similar physical and chemical characteristic as the raw feather. Therefore, it is crucial to regenerate and characterise the keratin structure to investigate whether its protein backbone is retained or disrupted. If the protein backbone is disrupted, the chemical properties of the keratin will differ greatly than the raw feather or degradation may has already occur during the dissolution process. After the dissolution of feather keratin from both procedures, the regeneration of keratin was carried out

following a method published in the literature (Chen et al., 2014). This regenerated keratin material holds the potential for various application as in biomedical and textile industry (Balaji et al., 2012; Evazynajad et al., 2002; Li et al., 2009; Xu, 2014; Xu et al., 2014; Yu et al., 2015). These materials were characterised further.

4.4 Characterisation of regenerated keratin

Multiple characterisation methods were carried out to determine that the regenerated keratin materials obtained from ultrasonic method have the similar physical and chemical properties as the raw feather keratin. This is important to ensure that the dissolution through ultrasound process did not affect the properties of keratin.

4.4.1 ATR-FTIR studies

ATR-FTIR measurement can be used to assess the structural changes in proteins. Hence, the regenerated (water insoluble) keratin materials from the ILs can be further understood through this analysis. Through the infrared absorption spectra, the absorption bands of peptide bonds (-CONH-) are usually the main characteristic band detected for the regenerated keratin materials. The vibrations in the peptide bonds originate from bands known as Amide A, Amide I, II, and III (Aluigi et al., 2007). From these spectra, the orientation of the constituents and the conformation of the polypeptide chains are the information that will be obtained through this analysis. For raw keratin, due to the N–H stretching or also known as the Amide A vibrations, a medium absorption bands of peptide bond was observed at 3286 cm^{-1} (Wojciechowska et al., 1999). The regenerated keratin materials show identical stretching frequencies (Figure 4.5 (b) to (e)) at 3292, 3289, 3385 and 3317 cm^{-1} respectively. Amide I, the most intense absorption band in proteins, is useful for the analysis of the protein secondary structure and contributed mainly from C=O stretching. Strong absorption bands were observed at between 1600

and 1700 cm^{-1} in both raw feather and regenerated keratin materials. The Amide I peak predominantly consists of peptide carbonyl stretching vibrations of the α-helix (one strong band at 1633-1634 cm^{-1}) and β-sheet (one strong band at 1615-1638 cm^{-1}) (Barth, 2007). The absorption band in Amide I of regenerated keratin materials shifted towards a lower frequency were indicative of increased amounts of ordered *β*- sheet structures (Khurana & Fink, 2000). Amide II shows a strong absorption peak at 1532 cm^{-1} for raw feather and it was attributed to C–N stretching and N–H bending vibrations. The regenerated keratin materials bands were observed at 1538, 1533, 1536 and 1541 cm^{-1} respectively, similar to the raw feather. The C–N and C–C stretching, N–H plane bending and C=O bending vibration resulted in a weak absorption band that was observed at 1220–1300 cm^{-1} which was due to Amide III for both raw feather and regenerated keratin materials. It can be observed from the spectra that the raw feather and regenerated keratin materials are similar and there are no signatures of new functional groups appearing in the regenerated keratin materials (Aluigi et al., 2007; Azila Mohd Idris et al., 2014; Azila Mohd Idris et al., 2013; Barth, 2007; Ghosh et al., 2014; Jackson & Mantsch, 1995; Schor & Krimm, 1961; Sun et al., 2009; Surewicz et al., 1993; Ullah et al., 2011; Wang & Cao, 2012; Wojciechowska et al., 1999; Zoccola et al., 2009).

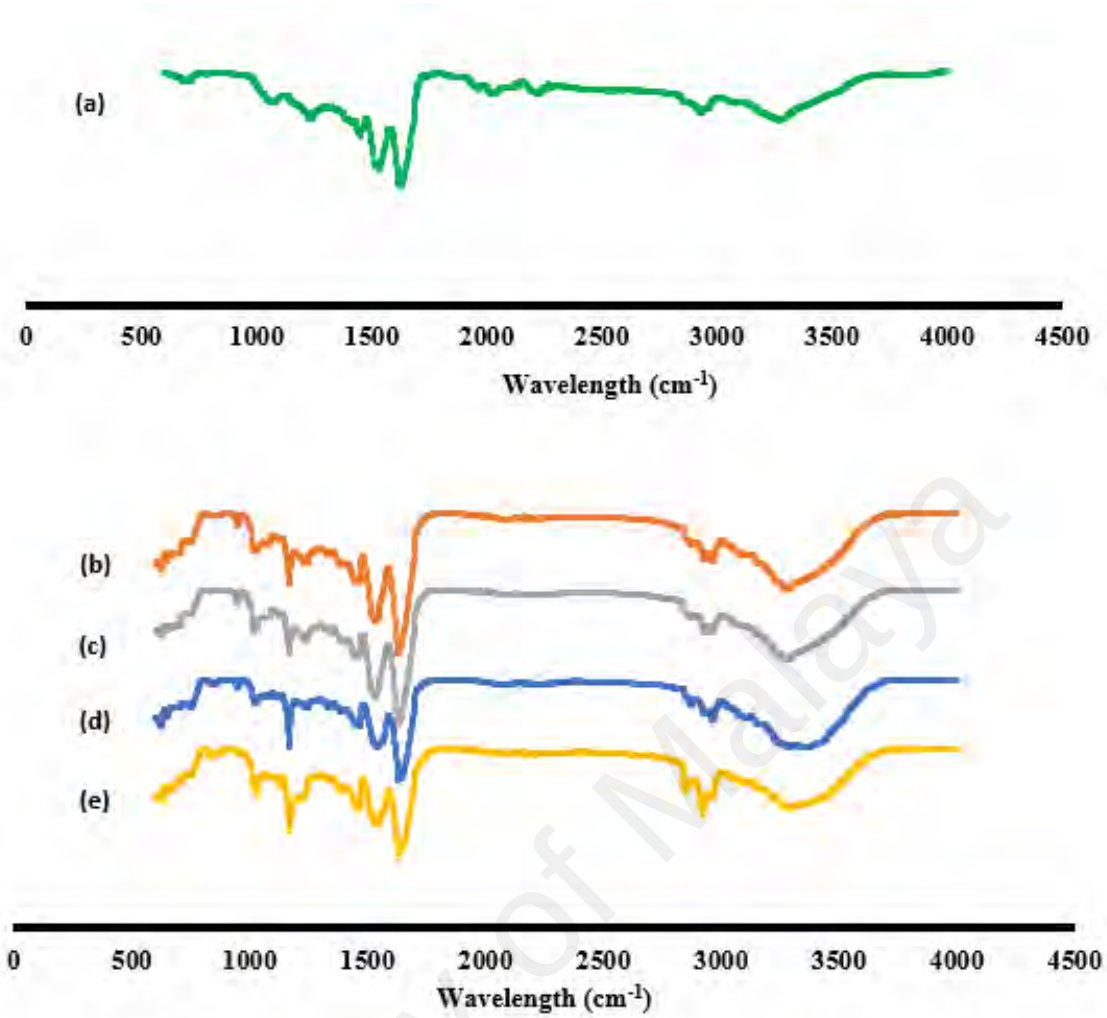


Figure 4.5: ATR-FTIR spectra of (a) raw feather and regenerated keratin materials from 2.0 M of [BMIM]Cl in DMSO (b) P=120 W, (c) P=200 W, (d) P=280 W and (e) 20 g of [BMIM]Cl.

4.4.2 XRD studies

XRD analysis was carried out for raw feather and regenerated keratin materials. Comparison of the XRD patterns are as shown in Figure 4.6. There are three noteworthy peaks that should be observed for keratin which expressed the crystallinity of the material. A high intensity 2θ peak at about 9 ° (0.98 nm) and 17.8 ° (0.51 nm) as shown in these figures has been assigned to α-helix meanwhile typical of the β-sheet structure peak is also at 9 ° (0.98 nm) and at about 19 ° (0.47 nm) (O'Shaughnessy, 1956). Hence, these peaks should be taken into consideration during the analysis of the regenerated keratin

materials. However, in the regenerated keratin materials (b), (c) and (d), the peak at 9 ° disappeared which indicates that during the dissolving process, the crystallinity was reduced and is not obtained back during the regeneration process (Azila Mohd Idris et al., 2014; Xie et al., 2005). Only regenerated material (e) has the 9 ° peak but with lower intensity.

Based on the spectra, the substantial 2θ peak at about 9 ° (0.98 nm), 17.8 ° (0.51 nm) and 19 ° (0.47 nm) represent the crystallinity in the raw feather. However, due to the overlapping signals at about 17.8 ° and 19 ° from the α-helix and β-sheet structures, only single peak is obtained as in the spectra due to both the α-helix and β-sheet peak cannot be assigned accurately. From Figure 4.6, similar diffraction patterns were obtained for the regenerated keratin materials exhibit, at around 20.3 °, 20.2 °, 23.2 ° and 21.7 ° for (b) to (e) respectively proving that the crystallinity pattern of the regenerated keratin materials is similar as the raw feather keratin. Moreover, broader peaks are observed in all regenerated keratin materials, which may be due to the overlapping of the β-sheet and β-turn peaks. However, the peaks at about 20 ° are significantly less in intensity for the regenerated material (b), (c) and (d), suggesting a lower content of the β-sheet structure hence reduced crystallinity. This observation contradicted with material (e) which produced higher intensity peak which resulted higher crystallinity (Azila Mohd Idris et al., 2013; Azila Mohd Idris et al., 2014; O'Shaughnessy, 1956; Rao & Gupta, 1992; Sun et al., 2009).

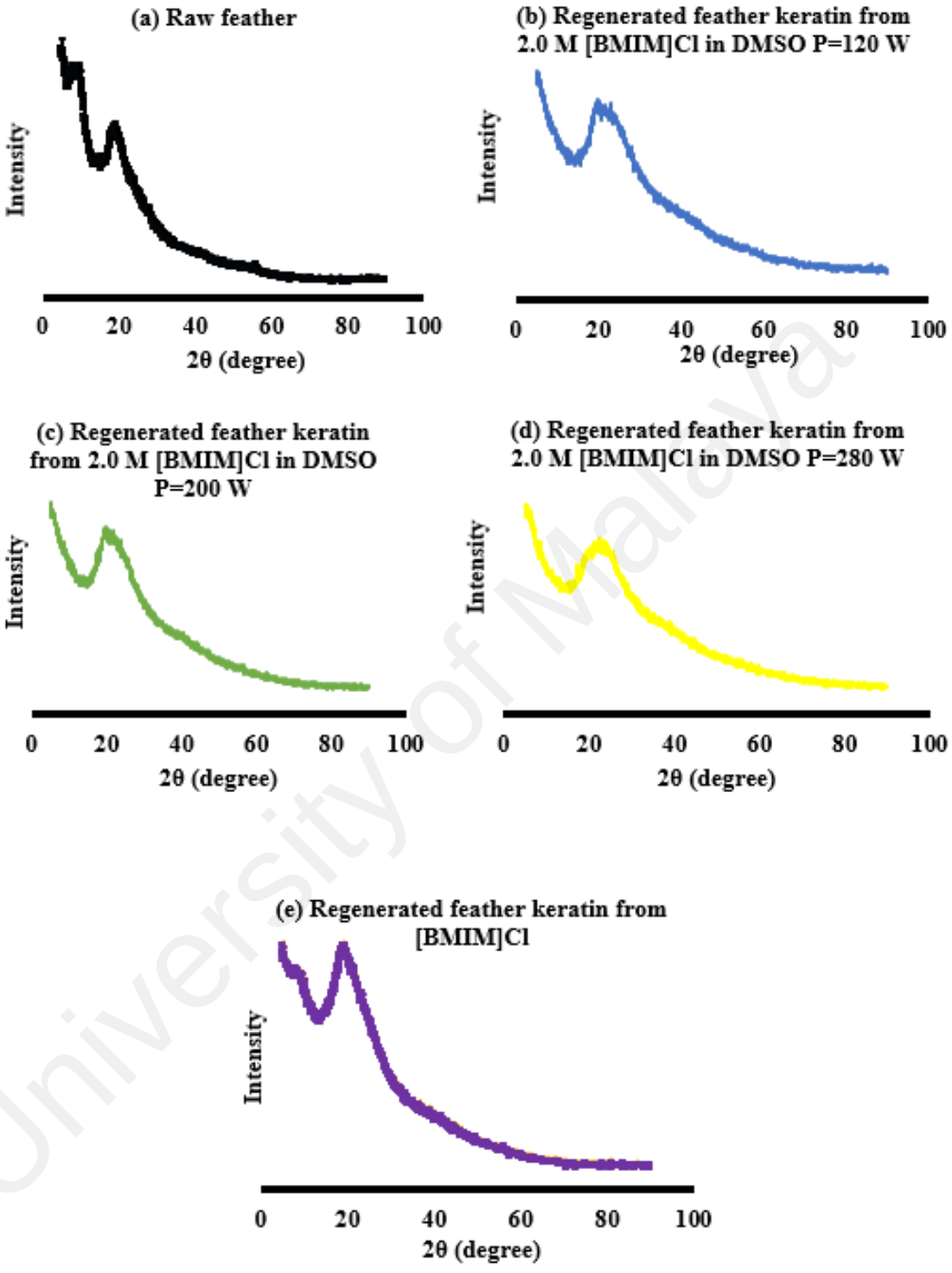


Figure 4.6: Results from XRD analysis of (a) raw feather and regenerated keratin materials from 2.0 M of [BMIM]Cl in DMSO (b) P=120 W, (c) P=200 W, (d) P=280 W and (e) [BMIM]Cl.

4.4.3 Thermal stability and phase behaviour

TGA was conducted to analyse the thermal decomposition of the raw feather and regenerated keratin materials (Figure 4.7). The raw feather and regenerated keratin materials show thermal stability up to more than 200 °C. In all samples, there are two stages of decomposition transitions that can be observed. The first stage occurred at about 100 °C which shows a small weight loss that could be due to the evaporation of residual moisture or solvent that was bound in the regenerated keratin materials. For the second stage, the degradation of the keratin materials occurred between 250 °C and 400 °C. It is understood that the rupture of the helical conformation and disulphide bond breakage is associated with the keratin degradation (Azila Mohd Idris et al., 2013; Azila Mohd Idris et al., 2014; Davies et al., 2000; Ullah et al., 2011).

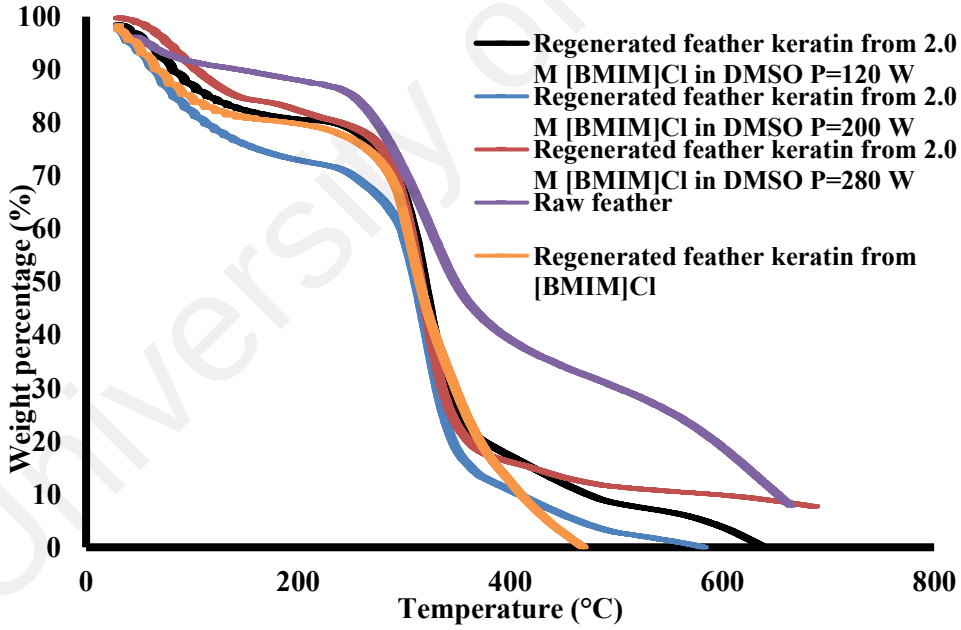


Figure 4.7: Single heating scan TGA traces of raw feather and regenerated keratin materials after various dissolution conditions.

The phase behaviour of the raw feather and regenerated keratin materials were studied by using DSC. Three consecutive heating and cooling cycles were carried out to ensure

that no moisture was present in the samples and to remove any thermal history for the sample. The typical heat flow curves of the 3rd heating cycle of the DSC traces are as shown in Figure 4.8. An exothermic peak around ~230 °C is observed for the raw feather as reported by Azila Mohd Idris *et al*. and Wu *et al*. (Azila Mohd Idris et al., 2013; Wu et al., 2014) which is usually assigned to crystalline melting (Ullah et al., 2011) and α-helix denaturation and disordering (Spei & Holzem, 1987). However, the DSC traces of regenerated keratin materials show no peaks at the reported area as in Figure 4.7.

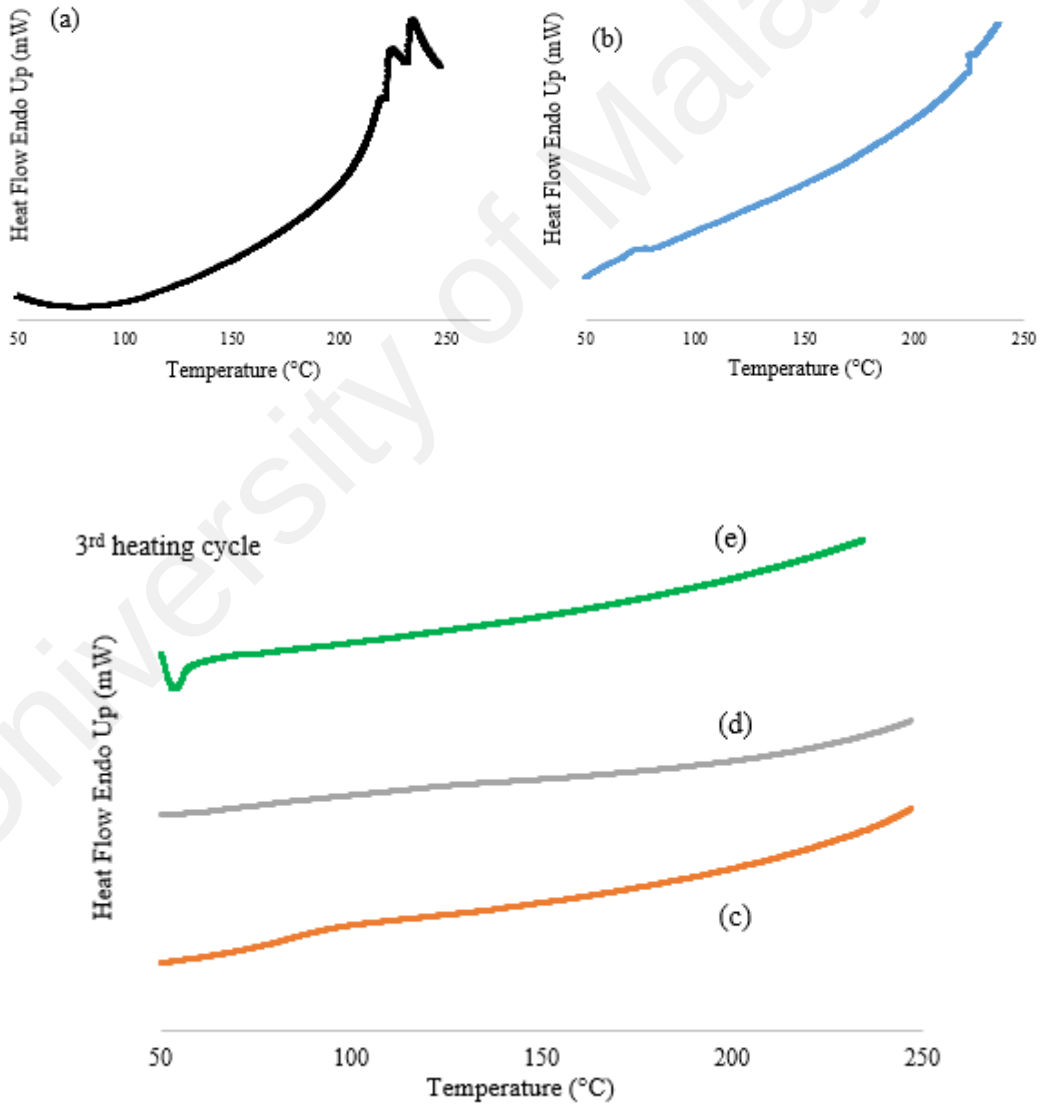


Figure 4.8: DSC results of (a) raw feather and regenerated keratin materials from (b) [BMIM]Cl and 2.0 M of [BMIM]Cl in DMSO with (c) P=120 W, (d) P=200 W and (e) P=280 W.

These results are consistent with the XRD results which shows reduced crystallinity for regenerated keratin materials. No obvious glass transition (T_g) was observed in the raw feather and regenerated keratin materials.

CHAPTER 5: CONCLUSION AND FUTURE WORKS

5.1 Conclusion

In summary, low frequency high power sonication was found to be effective in dissolving feather keratin by disrupting the hydrogen bonding in the material in pure ILs and ILs:DMSO solution. Furthermore, the advantages of ultrasonication on feather keratin dissolution as compared to the conventional heating method are;

(a) Significantly shorter processing time from 2 hours to 11 minutes.

(b) The dissolution system does need to be heated up, unlike conventional method that need heating to 130 °C.

(c) The amount of ILs required can be reduced by 65 %

(d) Reduced cost as less ILs used and easier process as no heating is needed.

Dissolution process is aided mainly by physical (shear) effect upon sonication; radicals generated by sonication (chemical effect) is minimal and does not affect the protein backbone structure. Through ATR-FTIR, XRD, TGA and DSC analyses, it was observed that the protein backbone is still intact and no major chemical changes of the polypeptide chains occur through ultrasonic method. The thermal stability of regenerated keratin materials is stable up to 200 °C.

5.2 Recommendation for future studies

By ultrasonic application, the dissolution of feather keratin was successfully achieved. This new dissolution methodology can open-up new route to convert the excess biomass into a value-added biopolymer. As the method is still new and many modifications is needed to be done due to some experimental restriction, it still can be an interest to engineers and chemist in the related field. Moreover, this method has proven to be more

efficient in term of processing time, amount of ILs used and ease of process which will reduce the overall cost of the process. Currently, regenerated keratin has been gaining interest in biomedical and textile field (Balaji et al., 2012; Evazynajad et al., 2002; Li et al., 2009; Xu, 2014; Xu et al., 2014; Yu et al., 2015). Hopefully through this method, this material will attain more applications especially on the biopolymer field.

Hence, following are some suggestions for future works:

i) Study the physical properties of the regenerated keratin materials by the formation of film which can be moulded into various shapes to test the flexural, impact or tensile strength for possible biopolymer applications.

ii) Study the dissolution process by using deep eutectic solvents which is lower in cost compared to the current solvent to reduce the overall cost.

iii) Study the ultrasound application by using high frequency to compare the efficiency in term of dissolution of feather keratin.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Nur Afiqah Azmi, Azila Mohd Idris, & Nor Saadah Mohd Yusof. (2018). Ultrasonic technology for value added products from feather keratin. *Ultrasonics Sonochemistry, 47*, 99-107.