Vortex Fluidic Mediated Fabrication of Natural Fluorescence Hydrogels Based on Quercetin and Gelatine with Tuneable Properties

Master Thesis

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DECLARATION

I certify that this work does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Danielle Wong

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EXECUTIVE SUMMARY

Aluminium ions (Al^{3+}) are a neurotoxin that is nowadays primarily found in foods such as baking products and consumer products. According to the Food and Drug Administration (FDA) and World Health Organisation (WHO), the minimum concentration of Al^{3+} allowed in a consumer product is between the range of 0.05-0.2 mg/L in drinking water. The limit of a person daily intake is approximately 3-10 mg depending on geographic and demographics. Some of the long term effects on human health include the development of Alzheimer disease and neurodegenerative diseases when humans ingest an excessive amount of Al^{3+}. ((ATSDR), 2008, (WHO), 2010)

Widespread efforts are made to improve food safety and Al^{3+} detection such as developing food preservatives, antimicrobial packaging, and stimuli-responsive materials developed for *in situ* detection of food quality. Some traditional ways for sensing for Al^{3+} include fluorescent probes, test papers, chemo-sensors, and atomic absorption spectroscopy. However, these methods have some disadvantages, such as being time-consuming, expensive, and tedious synthetic processes. They are not always conventional, sensitive, accurate and robust in Al^{3+} detection.

The investigation of using a novel quercetin-gelatine hydrogel with natural fluorescence properties for the sensing/detecting of Al^{3+} will be done for this research. Where quercetin, a natural polymer with sensing properties will be used to detect Al^{3+}. Gelatine is a natural polymer known to have heat-reversible ability; therefore, the effect of temperature can influence its physical properties.

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Aggregation Caused Quenching	ACQ
Aggregation-Induced Emission Hyperbranched Polymer	AIE-HBP
Aggregation-Induced Emission	AIE
Aluminium Ion	Al^{3+}
Excited-State Intramolecular Proton Transfer	ESIPT
Extracellular Matrix	ECM
Gelatine Hydrogel (NO VFD)	G (NO VFD)
Photoluminescence	PL
Poly Vinyl Alcohol	PVA
Quercetin Gelatine Hydrogel (1000RPM)	QG 1000 RPM
Quercetin Gelatine Hydrogel (2000RPM)	QG 2000 RPM
Quercetin Gelatine Hydrogel (3000RPM)	QG 3000 RPM
Quercetin Gelatine Hydrogel (4000RPM)	QG 4000 RPM
Quercetin Gelatine Hydrogel	QG (NO VFD)
Quercetin Solution (1000RPM)	Q 1000 RPM
Quercetin Solution (2000RPM)	Q 2000 RPM
Quercetin Solution (3000RPM)	Q 3000 RPM
Quercetin Solution (4000RPM)	Q 4000 RPM
Quercetin Solution	Q (NO VFD)
Rotations Per Minutes	RPM
Tetrahydrofuran	THF
Vortex Fluidic Device	VFD

INTRODUCTION

Aluminium is a very reactive element and is never found as a free metal in nature. In this way, it is frequently discovered combined with different components, for example, oxygen, silicon, and fluorine. Numerous aluminium compounds are utilized in the industry, medications and food added substances. Most aluminium compounds are solids showing high melting point. Nonetheless, the dissolvability of aluminium salts is dictated by pH, since aluminium ions (Al^{3+}) has a solid partiality for the hydroxide ion, which advances precipitation. (Krewski et al., 2007, Ganrot, 1986) Al^{3+} is classified as toxic and is harmful to biological life if an excessive amount is found in the body. Primarily, Al^{3+} is nowadays found in food such as baking product, food additives and food prepared with the use of aluminium potassium sulphate. ((WHO), 2010, Krewski et al., 2007, Huang et al., 2020, Ganrot, 1986, He et al., 2018b)

With growing concerns in limiting human exposure to Al^{3+}, efforts are being made to improve Al^{3+} detecting materials and techniques. Such as atomic absorption spectroscopy, chemo-sensors solution, fluorescence probes, test papers are traditional ways to detect Al^{3+}. However, these methods are not always conventional and do not always provide accurate and robust *in-situ* results. Another major drawback is how time-consuming these techniques are to obtain the metal ions detection result and the use of toxic or acidic pH chemicals for detecting Al^{3+}.

To approach this problem, a novel quercetin-gelatine hydrogel fabricated by a vortex fluidic device (VFD) will be demonstrated experimentally. The hydrogel's properties will be tuned via VFD at different rotational speeds, to determine whether quercetin's sensing properties will be enhanced for the detecting of Al^{3+}. The hydrogel's swelling properties and mechanical properties will be tuned via VFD at different rotational speeds to determine which specimens have the best swelling rate and capacity to hold the Al^{3+} solution in its polymer matrix, along with which specimens have the best mechanical properties for product handling.

Literature Overview

The literature review overview includes an outline of the background information on aluminium ions (Al^{3+}) and traditional ways/techniques for detecting them. Background information of hydrogels, their mechanism of network formation and classification of hydrogels based on types of cross-linking will be covered in the literature review. An overview of traditional ways of preparation of hydrogels, ideal characteristic and hydrogel application will be included in the literature review.

Background information on gelatine will be included in the literature review. Along with the literature overview of the development of gelatine hydrogel and problems with gelatine hydrogel by traditional techniques will be covered.

A literature overview of quercetin and how it detects Al^{3+} due to forming a chemical bonding called 'complex compound' will be covered. Along with how the development of quercetin films has helped to improve *in-situ* Al^{3+} detection.

The hydrogel fabrication via VFD, how it tunes the properties and characteristic of the hydrogels will be covered in the literature review.

Scope of the study

The importance of this research is to fabricate a low-cost *in-situ* hydrogel for Al^{3+} sensing using natural/ green chemistry polymers. These polymers are gelatine, quercetin, ethanol, and water which are commonly found in nature, and they do not require to be synthetically fabricated.

The investigation begins with the preparation of a quercetin-gelatine hydrogel, where gelatine is the aggregation/mixing agent and quercetin is the sensing agent being employed in a VFD. A hydrogel product will be yielded through the employment of the VFD. After the synthesising of the hydrogel have been fabricated, various tests are to be performed to investigate the characteristic of the novel hydrogel. These tests include physical, mechanical, fluorescence properties and sensing properties to Al^{3+} of the hydrogel. After obtaining the result data of those tests, the information is to be analysed and studied.

The traditional techniques for sensing Al^{3+} ions are atomic optical spectroscopy and ion-selective electrodes ion. The current conventional methods for metal ions detections are often time-consuming, require labour training and high-cost equipment. These conventional techniques sometimes use toxic chemicals and do not always provide accurate *in-situ* results when employed. Therefore, extensive efforts are made for developing low-cost *in-situ* materials that provide accurate metal ion sensing.

The importance of this proposed research can bring about a new low-cost natural/green chemistry polymer *in-situ* Al^{3+} detecting hydrogel which provides high sensitivity and fluorescence emission along with enhancing swelling and mechanical properties when employed in a VFD.

Problem Statements

Aluminium ion (Al^{3+}) is classified as a neurotoxin and is primary found in food nowadays. When an excessive amount of Al^{3+} is ingested, it is toxic to human and can lead to the development of Parkinson's disease, Alzheimer's disease, neurodegenerative diseases, bone disease and anaemia. ((ATSDR), 2008, Krewski et al., 2007, Zhi-Yan et al., 2019) Food and food addictive often have aluminium potassium sulphate, which can contain residual Al^{3+}. Traditional ways of sensing Al^{3+} include the use of fluorescent probes, chemo-sensors, and test paper. However, these traditional techniques are not conventional and do not always provide *in-situ* detection. Traditional fluorescent probe often suffers from aggregation-caused quenching (ACG) effect, and traditional fluorescent dye are known to be toxic to organism, therefore limiting their application in food quality and safety. Extensive efforts are being made to improve food safety and Al^{3+} detection such as developing food preservatives, antimicrobial packaging and stimuli-responsive materials developed for in situ detection of food quality. (He et al., 2018b, Huang et al., 2020)

Gelatine and Quercetin are food graded products; therefore, they are safe, edible, biodegradable and biocompatible. Gelatine is also known to not harm the human tissue, due to not showing any antigenicity, macrophage activities making it suitable for biomedical application. Quercetin has anti-inflammatory, antioxidant, and anti-bacterial properties, making it ideal for many biomedical applications as well. (He et al., 2018b, Kelly, 2011, Rubini et al., 2020, Nathiya et al., 2014) However, the major drawback for gelatine is its solubility in aqueous media and mechanical properties. (Diniz et al., 2020, Van Nieuwenhove et al., 2016) Quercetin's major drawback is its solubility in water and their instability in physiological medium, therefore limiting the use of them. (Nathiya et al., 2014, Rubini et al., 2020, Vedakumari et al., 2017)

Objective

The objectives are to fabricate a natural fluorescent hydrogel based on quercetin and gelatine with tuneable properties via VFD for aluminium ion (Al^{3+}) sensoring. The strategies include using a VFD, where gelatine mixtures will be infused with quercetin in a rapidly rotating tube of the VFD platform bringing about the creation of a hydrogel product. The examinations of the physical and mechanical properties, alongside fluorescence emission properties will be performed on the novel hydrogel end-product. Gelatine, quercetin, and ethanol are all green chemistry polymers. Thus, the objective is to fabricate a conventional *in-situ* green chemistry hydrogel that can be used for the sensing of Al^{3+} by tuning the hydrogel's properties via VFD.

Hypotheses

Based on several research articles on VFD fabricated hydrogels, it is hypothesized that the nanoparticles of the hydrogel mechanical properties will be enhanced significantly when employed in the VFD. The physical and morphological properties will also be enhanced due to high shear stress exhibited by the VFD when rotating. It is also hypothesized that the fluorescence properties and the sensitivity for Al^{3+} detection of the novel hydrogel will improve due to high shear stress exhibited by the VFD when rotating. At different VFD rotational speeds, it is hypothesized that the different hydrogel properties can be enhanced, tuned, and controlled.

Methodology outline

The methodology involves micro-mixing the quercetin and gelatine mixtures in the VFD platform, which will yield a hydrogel end-product.

The beginning of the experiment includes the photoluminescence (PL), absorption spectra, transmission of the Quercetin solution employed at different RPMs will be done to determine whether its fluorescence emission can be tuned, as well as whether the employment of a VFD, hinders the absorption wavelength of the specimens at different RPM.

To determine whether the hydrogel specimens at different RPM can absorb and hold the Al^{3+} solution in its polymer matrix, a swelling test is done in this research. For the swelling test, the dry weight is recorded, and the swelling weight was recorded every 30 minutes till 300 minutes (i.e. 5 hours) is reached. The methodology mentioned were repeated for the hydrogel at different rotation speeds up to 4000 RPM of the VFD and the hydrogels via without VFD.

Data will be collected for the photoluminescence (PL) with different concentration of Al^{3+} solution of the Quercetin-Gelatine Hydrogel via Cary Eclipse Fluorescence Spectrometer and Cary 50 & Agilent 60 UV-Vis Spectrophotometer. This is to determine whether the specimens have sensing properties to detect Al^{3+}. The Absorption spectra without Al^{3+} of the hydrogel specimens will be done to determine whether the employment of a VFD, hinders the absorption wavelength of the samples.

For the mechanical test, the specimens were cut to approximately 25 mm x 35 mm. An elongation rate of 5 mm/min was used for the various specimens for soft hydrogels. Pre-measurement will be made when the specimens are loaded onto the grippers before mechanical

testing. Post measurement will be done after the specimens completed mechanical testing. The methodology mentioned were repeated for the hydrogel at different rotation speeds up to 4000 RPM of the VFD and the hydrogels via without VFD. This test was done to determine which hydrogel specimen will have the ideal mechanical properties for product handling.

An optical microscopy images of different hydrogel samples were obtained by the employment of a microscope. This test was done to determine whether the surface morphology and porosity of the hydrogel specimens can be tunable via VFD at different RPMs.

Study Outline

The structure of the thesis includes investigating quercetin solution for Al^{3+} sensing and Quercetin-Gelatine Hydrogel for Al^{3+} sensing.

The research aims to fabricate a natural fluorescence properties hydrogel based on quercetin and gelatine with tuneable properties by the employment of a VFD.

The quercetin for Al^{3+} sensing includes the investigation of whether quercetin fluorescence, transmission and absorption spectra properties can be tuned via VFD at different RPMs when in a solution form, as well as investigating the fluorescence properties of the quercetin solution when different Al^{3+} concentrations are dropped onto the specimens.

The quercetin-gelatine hydrogel for Al^{3+} sensing includes the investigation of a swelling test to identify whether the hydrogel can hold the Al^{3+} solution in its polymer matrix and how fast it can absorb the solution via VFD at different rotational speeds, as well as investigating the fluorescence and sensitivity properties of the hydrogel when different Al^{3+} concentrations is dropped onto the specimens.

A comparison of the photoluminescence intensity comparison of the end-products at different Al^{3+} concentrations will be investigated.

The investigation of a mechanical test will be done to identify whether the hydrogels specimens' mechanical properties will be enhanced with the employment of a VFD at different RPMs. As well as to determined which specimens will have the best mechanical properties for product handling.

An optical microscopy of the hydrogel specimens will be done to identify whether the surface morphology can be tuned and controlled via VFD.

LITERATURE REVIEW

The fabrication of hydrogel by VFD is a recent innovative concept, and very few experiments have been verified. Gelatine has been used in biomedical, pharmaceutical, and wound dressing application, however, due to the difficulties of modifying its physical and mechanical properties via traditional methodologies. The use of gelatine is limited in specific applications and fields. Recently, quercetin has been used for biosensing application, and extensive efforts are made in research to improve its natural fluorescence properties.

With the increasing demand for improving food safety and products by detecting Al^{3+}, ongoing efforts are being made to improve conventional *in-situ* material which can provide can replace unconventional non-*in-situ* Al^{3+} detection techniques.

To gain an in-depth understanding of these chemical components, methodologies and research field, a literature review of existing methods and past researches was done.

Aluminium Ion (Al^{3+})

Aluminium ion (Al^{3+}) is classified as a neurotoxin and is primary found in food nowadays. When an excessive amount of Al^{3+} is ingested, it can disturb the human immune system and the nervous system, which can lead to the development of Parkinson's disease, Alzheimer's diseases, bone disease and anaemia. Food and food addictive often have aluminium potassium sulphate, which can contain residual Al^{3+}. Traditional ways of sensing Al^{3+} includes the use of atomic optical spectroscopy, fluorescent probe, chemo-sensors, and test paper. However, these conventional techniques for sensing Al^{3+} does not always provide an *in-situ* result, and often acidic or toxic chemicals are used to detect Al^{3+}. Extensive efforts are being made to improve food safety and Al^{3+} detection such as developing food preservatives, antimicrobial packaging and stimuli-responsive materials developed for in situ detection of food quality.

(He et al., 2018b, Ganrot, 1986, (ATSDR), 2008, (WHO), 2010, Krewski et al., 2007)

According to the Food and Drug Administration (FDA), the minimum excessive of Al^{3+} in drinking water should be approximately 0.05 – 0.2 mg/L. World Health Organization (WHO) suggested that the daily intake of Al^{3+} should be approximately 3-10 mg per person. The minimum consumption guideline of Al^{3+} for a person per year should not exceed 30-50 mg/L. ((ATSDR), 2008, (WHO), 2010, Krewski et al., 2007)

Traditional Method for Detecting Aluminium Ion (Al^{3+})

Some traditional method for detecting metal ions include ion-selective electrodes and atomic optical spectroscopy. However, these methods have some disadvantages such as being time-consuming, expensive and tedious synthetic processes. (Bings et al., 2010)

There are two types of atomic optical spectroscopy, which are atomic emission spectroscopy and atomic absorption spectroscopy. Both techniques methodology achieves micro-analysis, where different chemical elements will have its unique spectra. (Bings et al., 2010)

Atomic optical spectroscopy is used to identify and quantify different species and quantity of the elements in a sample. The sample requires preparation and employment to an atomizer, which is a high-cost component of the device that provides energy, dry vaporize and atomize the prepared sample. The sample's element component can be determined through a spectra detector after being processed by the atomizer. The use of spectroscopy is not hard, and the result can be immediately obtained once procedures are done. However, the disadvantage of atomic optical spectroscopy is the cost of the sample preparation, technical training and operation required. (Bings et al., 2010)

Development of Modern Sensors for Al^{3+} Detection – Fluorescence Probes

Fluorescence probe is a novel method in the fields of chemical and biomedical sciences, which have qualitative and quantitative detection. Numerous fluorescent sensors and probes are used for detecting metal ions, nanoparticles, biological structures, and molecules. They are influential and valuable indicators for studies and research. (Huang et al., 2020, Hong et al., 2009, He et al., 2018b, Zhi-Yan et al., 2019, Fu et al., 2019)

Aggregation-Caused Quenching (ACQ)

A certified fluorescent probe ought to have high detecting and imaging affectability which are controlled by the illumination of the fluorescent probes as well as the difference of its fluorescence before and after analyte binding.

In any case, plenty of fluorogens, with the pi-pi stacking structure, have the corresponding issue called aggregated-caused quenching (ACQ). They are normally quenched at high concentration or in an aggregated state (See Figure 1).

Even though these fluorescent probes have high emission in the solution state, they perform weak emission or even non-emission in the aggregated state. Therefore, this impact has a contrary effect on the analysis because of an extraordinary restriction for the labelling degrees of fluorophores. (Huang et al., 2020, Hong et al., 2009, He et al., 2018b, Zhi-Yan et al., 2019, Fu et al., 2019)

Research are pushed to use fluorescent probes in diluted solution, which causes incredible difficulties to analyse desired chemical components. Likewise, it causes ACQ effects in the diluted solution since the fluorescent probes have the propensity of gathering in the surface, prompting the high focus in the specific zone. This is the explanation to why most fluorescent probes have low effectiveness and work in a 'turn-off' mode, therefore limiting the opportunity for rational applications such as food safety and quality control (See Figure 1). (Huang et al., 2020, Hong et al., 2009, He et al., 2018b, Zhi-Yan et al., 2019, Fu et al., 2019)

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Figure 1 - Fluorescence photographs of solutions/suspensions of (a) DDPD and (b) HPS in THF-water mixtures with different water content..(Huang et al., 2020)

Aggregation-Induced Emission (AIE)

Aggregation-induced emission (AIE) is an abnormal phenomenon observed with certain organic luminophores (i.e. fluorescent dyes). Aggregation-induced emission fluorogens (AIEgens) performs at a higher emission in the aggregated state. The typical AIEgens structure is a propeller-shaped like rotor, as shown in Figure 2. The connection of benzene is a carbon-carbon single bond between benzene, as shown in Figure 2. As the molecules are isolated, this structure empowers AIEgens undergoing a low-frequency torsional moment and allows the structure to perform as the molecules are isolated. Though, when the AIEgen aggregates, the unique structure confines the intermolecular rotation, which shows high emission and has strong fluorescence in the aggregated state. (Hong et al., 2009, Huang et al., 2020)

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Figure 2 - Structure of ACQ and AIE (Hong et al., 2009)

AIEgens have been widely used in several biomedical engineering and biological applications such as differentiation of protein monomers, monitoring of cell apoptosis and metal ion detection. (Hong et al., 2009, He et al., 2018a, He et al., 2018b, Huang et al., 2020, Zhi-Yan et al., 2019)

Hydrogel

Mechanism of Network Formation

The linking of macro-molecular chains together which at first prompts progressively larger branched yet soluble polymers relying upon the structure and adaptation of the beginning material is allude to as gelation. Sol is depicted as the blend of such poly-disperse soluble branched polymer. Continuation of the linking process brings about expanding the size of the branched polymer with diminishing solubility. This 'infinite polymer' is known as the 'gel' or 'network' and is saturated with finite branched polymers. The progress from a framework with a finite branched polymer to boundless molecules is called 'sol-gel transition' or 'gelation'. Gelation can occur either by physical linking (physical gelation) or by chemical linking (chemical gelation), see Figure 3. Physical gels can be sub arranged as strong physical gels and weak gels. The strong physical gel has strong physical bonds between polymer chains and is adequately lasting at a given arrangement of experimental conditions. Consequently, strong physical gels are undifferentiated from chemical gels. Weak physical gels have reversible links formed from the transitory relationship between chains. Conversely, chemical gelation comprises the formation of covalent bonds and continuously results in a strong gel. Condensation, vulcanisation, and addition polymerisation are the three main chemical gelation processes. (Ahmed, 2013, Gulrez et al., 2011)

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Figure 3- Classification of Gelation Mechanism and Relevant Examples, (Ahmed, 2013)

Classification of Hydrogels Based on Types of Cross-Linking

The two categories that hydrogels are classified into are Permanent/ Chemical gel and Reversible/ Physical gel. ‘Permanent' or ‘chemical’ gels are covalently cross-linked, where hydrogen bonds are replaced by stronger and stable covalent bond networks. The equilibrium swelling state depends on the polymer-water interaction parameter and the crosslink density. 'Reversible' or 'physical' gels are when the networks are held together by molecular entanglements, and/or secondary forces including ionic, hydrogen bonding or hydrophobic interactions. In physical cross-linked gels, disintegration is forestalled by physical interactions, which exist between various polymer chains. These interactions are reversible and can be disturbed by changes in states of conditions or utilization of stress. (Ahmed, 2013, Gulrez et al., 2011)

Preparation of Hydrogels via Traditional Methods and The Problems.

The preparation methods used for the fabrication of hydrogels include polymerization grafting, chemical or physical cross-linking (See Figure 4) and radical cross-linking. (Ahmed, 2013, Gulrez et al., 2011, Inamuddin and Mishra, 2018)

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Figure 4 - (a) Chemical Cross-Linked Polymers And (b) Physically Cross-Linked Polymers (Inamuddin and Mishra, 2018)

Traditional techniques still used today are freeze-thawing, solvent casting, grafting and electrospinning. Freeze-thawing involves the formation of microcrystals in the structure to achieve physical cross-linking of the hydrogel polymer. (Ahmed, 2013, Gulrez et al., 2011) However, these techniques lead to a random fibrous or porous structure and are primarily employed to control the bulk morphology. (Tavakoli et al., 2020b) Solvent casting includes the procedure for forming a polymer test by plunging a mould into a solution of the polymer and

drawing off the solvent to leave a polymer film adhering to the mould. (Awais and Sintayehu Mekuria, 2019) Nevertheless, it not only involves at least two steps but lacks control over surface morphology, mechanical and physicochemical properties. To the best of today's knowledge, many traditional techniques prompt a random fibrous or permeable structure and lack control over the surface morphology, mechanical and physicochemical properties of the hydrogel. Consequently, there is very little methodology that can control these properties of hydrogel films precisely. (Tavakoli et al., 2020b) The most significant challenge for hydrogel fabrication is the critical control of particle size distribution controlled by synthesis processes such as the regulation of monomer/polymer/cross-linker ratio and the perfect adjustment of experimental conditions (i.e. pH, ionic strength, temperature). (Inamuddin and Mishra, 2018) Subsequently, a new methodology of using a VFD has been introduced for the fabrication of hydrogel. However, this concept has very limited in-depth hydrogel experimental demonstrations. (Tavakoli et al., 2020b)

Ideal Characteristic

The ideal hydrogel material characteristics are having high absorption capacity (maximum equilibrium swelling) in liquid, the desired rate of absorption (preferred particle size and porosity) depending on the application requirement, their highest absorbency under load, low soluble content and residual monomer, low in prices, high durability and stability in the swelling environment and during the storage. Other ideal characteristic includes having high biodegradability without the formation of toxic species following the degradation, have pH-neutrality after swelling in water, photostability, and have re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it, depending on the application requirement. Colourlessness, odourlessness and non-toxic characteristics are also ideal for a hydrogel. (Ahmed, 2013, Gulrez et al., 2011)

Hydrogel Application

Hydrogels have many applications in agriculture, drug delivery system, sealing, coal dewatering, pharmaceuticals, biomedical applications, tissue engineering, regenerative medicines, biosensor, and wound dressing. Hydrogel ideal characteristic may vary in each application fields. (Ahmed, 2013, Gulrez et al., 2011)

Gelatine

Background

- Chemical structure, physical properties, and mechanism of gelatine

One of the most abundant structural protein found in mammals is a type of collagen called gelatine. (Neves and Reis, 2016, Van Nieuwenhove et al., 2016, Osorio et al., 2007) The amino acid structure of gelatine is similar to its parent collagen. In any case, the source and type of collagen along with the manufacturing procedure, essentially influence the material qualities of the inferred gelatine. (Buschow, 2001, Neves and Reis, 2016) The significant components of gelatine are hydroxyproline, glycine, and proline. (Neves and Reis, 2016)

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Figure 5 - Basic Chemical Structure of Gelatine (Kommareddy et al., 2007)

- Physical Properties of Gelatine in Water and Heat

Gelatine behaves like a synthetic polymer, and its physical properties are a strong function of the molecular weight distribution. One of the critical properties is solution viscosity, which is significant for fluid handling and coating requirements. Solution viscosity is influenced not just by the molecular weight distribution of the gelatine, but also by concentration, temperature, pH, ionic quality, and the existence of added substances. Another important property of gelatine is the ability to form heat-reversible gels. When a gelatine solution is cooled to below 35–40 °C, the viscosity of the solution increases, gradually from the start, then rapidly as the gel point is reached. This gelation procedure is thought to proceed through three phases. The main stage includes the steric rearrangement of gelatine particles to produce regions of ordered, helical arrangement. The second stage consists of the association of helical locales in discrete particles to frame a three-dimensional network. The third stage, or maturing of the gel, provides stabilization of the network through inter-chain hydrogen bonding. The rigidity or resistance of the gel to mechanical deformation is a significant physical property and is measured by subjective yet standardized conditions. This property is customarily called gel strength and is regularly utilized as a general proportion of physical quality. However, gelatine is entirely soluble in water that is above 35-40 °C, pH and the presence of some organic solvents, making this its major drawback factor. (Buschow, 2001, Osorio et al., 2007)

Development of Gelatine Scaffolds/Hydrogels

The development of gelatine scaffolds/hydrogel began with the aim to replace synthetic polymer hydrogel with natural polymers. As the difficulties with synthetic polymers are to eliminate unpolymerized monomer residue, which is highly toxic for biomedical and pharmaceutical applications. (Wang et al., 2019) The development of non-synthetic scaffolds/hydrogel resembling natural Extracellular Matrix (ECM) is favoured, in order to replicate as closely as possible, the natural aqueous condition that cells experience in the human body. Therefore, gelatine is often applied as natural building blocks in hydrogels, because they closely represent both the protein and polysaccharide constituent of the natural ECM. (Van Nieuwenhove et al., 2016) Natural polymers are mainly made from natural polymer-based materials such as protein and polysaccharides. This is because natural polymers are lower in cost, non-toxic, less-abrasive, non-cytotoxic, biocompatible, biodegradable and are environmentally friendly. (Diniz et al., 2020, Reno et al., 2013, Van Nieuwenhove et al., 2016, Tavakoli, 2017, Osorio et al., 2007)

The fabrication of alginate/gelatine blended hydrogel fibres cross-linked by Calcium ions (Ca2+) and oxidized starch through wet spinning. It was found that excessive additive amount of gelatine diminished the mechanical properties of the hydrogel matrix. From Figure 6. the results indicated that an appropriate amount of gelatine would lead to better compatibility between alginate and gelatine. Whereas, the additive amount of gelatine arrived in higher content, the worse compatibility between alginate and gelatine formed. This indicated that the ratio of gelatine used in hydrogel fabrication might reduce the mechanical properties of it. It was found that optimal additive amount of gelatine to improve mechanical properties, swelling properties, and water absorption and retention properties of alginate/gelatine blended hydrogel fibres. From Figure 6, that the optimal mass ratio of Sodium Alginate and Gelatine at 10:2, gave the highest tensile strength index. (Wang et al., 2019)

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Figure 6 - Mechanical Properties of Alginate Hydrogel Fibres And Alginate/Gelatine Blended Hydrogel Fibres With Different Mass Ratio For Sodium Alginate To Gelatine (Wang et al., 2019)

The development of gelatine- and starch-based hydrogels that were prepared through covalent crosslinking found that both the mechanical properties and the swelling extent of the hydrogel can be controlled by varying the chemical composition, and the degree of substitution of the methacrylamide-modified gelatine (gel-MA) applied. From Figure 7, hydrogel films comprising of gel-MA 95% are described by a higher G'- values as these systems are more crosslinked, and the G' shifts to higher qualities for the interpenetrating polymer networks (IPNs) with a starch-substance of 10%. The mechanical properties are in this manner enhanced upon introducing an additional starch phase in the gelatine network. However, the film casting of gelatine-and-starch-based hydrogels outlined that when a critical amount of starch was added, the gel fraction and mechanical properties decreases due to the starch domains leaching out during incubation, these domains can be considered as starch-only hydrogels as shown on Figure 7.(Van Nieuwenhove et al., 2016)

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Figure 7 - 3D Plot Representing The Storage Modulus G' Of The Various Hydrogels (Z-Axis) As A Function Of The Starch Content (%) (X-Axis) And The Degree Of Substitution Of Methacrylamide-Modified Gelatine (Gel-MA) (Y-Axis) (Van Nieuwenhove et al., 2016)

Problems with Gelatine Hydrogels via Traditional/Outdated Methodologies

Gelatine is not used alone because it does not form stable hydrogel due to their poor mechanical properties. Therefore, they are often mixed with other synthetic polymers to overcome this problem. (Bemiller, 2009, Hassan et al., 2018) Hydrogel engineered through electrospinning, freeze-drying and solvent casting has shown that the methods lack control over surface morphology and physicochemical properties of the hydrogel and lead to a random fibrous or porous structure. These traditional methods also lack control in enhancing the mechanical properties of hydrogels. (Tavakoli et al., 2020b)

These researches indicated that the outdated/traditional methodologies lack control in enhancing the mechanical and physicochemical properties of hydrogels. The control of gelatine-based hydrogel mechanical properties solely depends on the ratio of the chemical components (i.e. starch and gelatine) and degree of substitution. (Van Nieuwenhove et al., 2016, Wang et al., 2019)

Quercetin

Background

Quercetin is classified as a flavanol, a family of plant compounds that share a similar flavone backbone, as shown in Figure 8. It consists of a three-ringed molecule with hydroxyl [OH] group attached. Flavonoids occur as either glycosides (with attached sugars [glycosyl groups]) or as aglycones (without attached sugars). (Kelly, 2011, Vedakumari et al., 2017, He et al., 2018a, He et al., 2018b, Rubini et al., 2020)

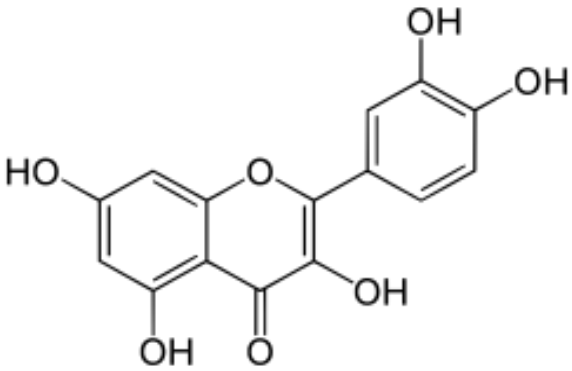

Figure 8 - Quercetin Chemical Structure , (Kelly, 2011)

Quercetin has pharmaceutical effects such as antioxidant, anti-inflammatory, anti-cancer, anti-bacterial anti-toxic and immunomodulatory effects. Researchers have shown that quercetin has good biocompatibility properties, therapeutical values and are often used to treat wounds due to the aid of increasing fibroblast proliferation while decreasing fibrosis and scar formation. (Althans et al., 2014, He et al., 2018a, He et al., 2018b, Kelly, 2011, Nathiya et al., 2014, Vedakumari et al., 2017, Rubini et al., 2020) Research has also discovered quercetin to have sensing characteristic with for Al^{3+} due to its ability to form a complex compound with Al^{3+}. (Cornard and Merlin, 2002)

Problems with Quercetin.

Some problems with quercetin are their poor solubility in water, low bioavailability and the hydrophobic nature and poor permeability. Along with their instability in physiological medium, therefore limiting the use of them. (Nathiya et al., 2014) Quercetin is soluble in some organic solvent and alcohol solution, such as methanol and ethanol. (Althans et al., 2014, Vedakumari et al., 2017, Rubini et al., 2020) The stability of quercetin is pH and temperature-dependent. It is proposed that quercetin is very unstable in some organic solution (e.g. acetonitrile and methanol) at a pH greater than 7. Other factors such as metal ions could affect the chemical stability (e.g. oxidation and degradation) of quercetin. (Wang et al., 2016)

How Quercetin Detects Al^{3+}.

In a study on the spectroscopic and structural analysis of complexes of quercetin with Al^{3+}. The experiment was done by adding quercetin into aluminium trichloride ($AlCL_3$) solution, where $AlCL_3$ contains residual of Al^{3+} when dissolved in water. Researchers have proposed that the mechanism for quercetin detecting Al^{3+} is because of how quercetin can bond to the Al^{3+} complexes forming a complex compound, as shown in Figure 9. A complex compound is when a chemical structure in which a central metal atom is surrounded by non-metal atoms/groups of atoms, called ligands, joined to it by chemical bonding. (Cornard and Merlin, 2002)

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Figure 9 – Proposed Mechanism For Quercetin Detecting Al3+ Complexes. (Cornard and Merlin, 2002)

Development of Quercetin Hydrogels/Film

The possibility of toxic food additives found in food, along with food safety and food storage, have become major global concerns, especially in developing countries. An example would be the preparation of traditional Chinese snacks such as deep-fried dough sticks and steamed buns that are usually prepared using aluminium potassium sulphate, which means that these foods always contain residual Al^{3+}. (He et al., 2018b, Bai et al., 2019)

Recent research aims to detect food containing residual Al^{3+} by using a multifunctional film incorporating the natural fluorescence quercetin via physically mixing quercetin and poly (vinyl alcohol) (PVA). From this research, the quercetin's fluorescence emission was observed at approximately 500-700 nm (See Figure 10). The results obtained from the study shows that the film was transparent and showed an excellent fluorescence enhancement when exposed to foods containing Al^{3+} residues, as shown in Figure 10. When Al^{3+} concentration is increased, the fluorescence properties of the quercetin increased, as shown in Figure 10. The film was also used for the detecting of Putrescine (i.e. a foul-smelling organic chemical) and shown to have good fluorescence enhancement when exposed to the film, as shown in Figure 10. (He et al., 2018b)

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Figure 10 - (a) Fluorescence spectra of quercetin (10 μg/mL) upon the addition of different concentrations of Al3+ in THF/H2O (20:80) (excitation wavelength = 360nm). (b) Fluorescence spectra of quercetin (10 μg/mL) upon the addition of different concentrations of putrescine in THF/H2O (20:80) (excitation wavelength = 360nm). (c) Images of QACF upon the addition of different concentrations of Al3+ (0.6-4.8 mg/mL) and different concentrations of putrescine (5-700 ppm). (He et al., 2018b)

Not only was the natural fluorescence properties of quercetin composite able to detect Al^{3+} residual and putrescine in food but the film showed good antibacterial and antioxidant activities, which enabled it to be successfully used as a coating to extend food storage times (Figure 11). (He et al., 2018b)

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Figure 11 - Images of bananas over 5 days without coating (top), coated with PVA (centre), and coated with QACF (bottom). (b) Images of apples at 0 and 2 h without coating (left), coated with PVA (centre), and coated with QACF (right). (He et al., 2018b)

However, the major drawback for the research was the use of PVA, which is a synthetic polymer and therefore, cannot be sourced naturally in the environment. Thus, PVA can be expensive to manufacture and requires safe disposal protocols. PVA can release toxic vapours if overheated. (He et al., 2018b)

Quercetin Loaded Gelatine Film

A recent study investigated quercetin loaded gelatine film with the aim of developing materials with tailored antioxidant, mechanical and stability properties. Quercetin-Gelatine films were prepared by adsorption in different amounts of quercetin (0.5, 1, 1.5 and 2 g/L.) and were labelled EtOH 05, EtOH 1, EtOH 15, EtOH 2, whereas reference sample with no quercetin was labelled EtOH 0. (Rubini et al., 2020)

It is observed in this research that the film's fluorescence emission was at 510-542 nm. It was found during the fluorescence microscopy of the films that the EtOH seems not completely uniformed in the distribution of the quercetin content, therefore research concluded that the quercetin was not homogenously distributed as there were regions where the film was more densely packed. (Rubini et al., 2020)

A swelling test was done by this research and was found that an increase in quercetin content concentration provokes a reduction of swelling degree. (Figure 12)

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Figure 12 - Swelling curves of (a) EtOH films. (Rubini et al., 2020)

The research has shown that the values of stress at break and deformation increase significantly on increasing quercetin concentration up to 1.5 g/L. However, at a quercetin concentration of 2g/L, there was a significant worsening of the mechanical, mostly due to the non-homogeneous distribution of flavonoid inside the films (Figure 13). (Rubini et al., 2020)

Image removed due to copyright restriction.

Figure 13 - Typical stress-strain curves of EtOH gelatine films at different quercetin content.(Rubini et al., 2020)

This research, however, did not demonstrate any investigation on whether their hydrogels were able to detect Al^{3+} and whether its intensity will be hindered by the solvents (i.e. ethanol) used.

Hydrogel Fabrication via VFD

VFD background

The VFD consists of inlet feeding tubes, collection trough, outlet tube, reaction tube and electric motor (see Figure 14). Solutions will be fed through the inlet feed tubes before entering the reaction tube that is rotating due to the generated electric motor. As the reaction tube rotates, the solutions will move upwards (i.e. laminar flow) into the collection trough, and the product can be obtained through the outlet tube. VFD works under continuous flow by conveying reagents to the base of a rapidly rotating tube that is titled comparative with the level position. The viscous drag from the Stewartson and Ekman layers provide shear, where this mechanoenergy can be harnessed for different applications. In addition, the intense micro-mixing and the resulting thin film (down to an estimate of 250 microns) delivers high heat and mass transfer bringing about all molecules being treated similarly. (Technologies, 2015) The innovation works by accurately controlling various parameters that influence fluid dynamics and the shear forces experienced by these fluids. These parameters include types of solvents, rotation, the direction of spin, temperature, rate of injection, tilt angle and modes of operation. (Tavakoli et al., 2020b, Technologies, 2015)

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Figure 14 - Structural Schematic Diagram and Components of the VFD (D'Alonzo et al., 2017)

The utilization of VFD covers a developing number of processing capabilities, from little small molecule synthesis through to processing advanced materials and controlling single-cell living beings. (Technologies, 2015) However, one of the fields that are least explored is the fabrication of hydrogel via VFD. (Tavakoli et al., 2020b)

Hydrogel Fabrication via VFD

Broad efforts have been centred around the introduction of hydrogels with enhanced physical, mechanical and biomedical properties for a broad scope of applications. The concept of fabricating hydrogel via VFD began with the intentions to control the surface morphology and to reduce post-treatment process that conventional methodologies required. It is also practically impossible to control the surface morphology of hydrogels through traditional methods. Only a few research has been done on the fabrication of hydrogel have shown that the characteristic of the hydrogel was able to be tuned and controlled via VFD. (Tavakoli et al., 2020b)

Hydrogel Characteristic via VFD

The phenomena of how the critical control of particle size distribution and control of surface morphology was explored through the employment of the VFD. The fabricated PVA hydrogel has shown to display different surface morphologies at different rotational speeds. These observations identify that the formation of different surface morphologies in PVA hydrogel strongly depends on the magnitude of the applied centrifugal force generated by the rotation of the VFD tube. A 60% improvement in fracture stress was observed when VFD was employed for the preparation of PVA-based self-healing hydrogel, compared to the traditional method of hydrogel preparation. It was observed that the formation of the hydrogel particles was a more homogenous structure when the VFD was utilized. The exact mixing of PVA and borax solution, which was promptly reachable by utilizing the VFD, prompted better spatial movement of borax particles inside the PVA chains. This, thusly, brought about the development of a progressively uniform structure that was not indistinguishable from that of PVA hydrogel arranged by the traditional approaches. The surface morphology, alongside their self-recuperating property, could be fundamentally adjusted, with higher speeds essentially expanding the extensibility and self-fracture stress of the hydrogels, while changing both the rotational speed of the VFD and crosslinking density during the fabrication of hydrogels. Therefore, this novel methodology achieved the critical control of particle size distribution and control of surface morphology, as shown in Figure 15. Consequently, the direct control of surface morphology, as an advantage of utilization of VFD, opens new opportunities for biological and material studies. (Tavakoli et al., 2020b)

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Figure 15 - Similar surface morphologies were observed for PVA hydrogels at the same rotation speeds (1000 – 4000 rpm) using different batches. (Tavakoli et al., 2020b)

Another recent research was done by investigating whether the fluorescence properties of an aggregation-induced emission hyperbranched polymer (AIE-HBP) was able to be tuned by the employment of a VFD. The fabrication of the AIE-HBP was employed at different RPMs, and research has shown that the fluorescence properties of the AIE-HBP were able to be tuned. It is shown in Figure 16 that the fluorescence properties significantly improved when higher RPM was employed. Researchers have proposed that the rapid rotational speed that conveys high shear stress achieved the critical control of particle size distribution of the AIE, and therefore affected the fluorescence properties of the AIE-HBP. (Tavakoli et al., 2020a)

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Figure 16 - The effect of rotation speed on the relative intensity of AIE–HBP (1 mM). (Tavakoli et al., 2020a)

Due to the few research done on the utilization of VFD, a few examples of areas that are not explored includes whether the swelling and mechanical properties can be tuned by VFD. (Tavakoli et al., 2020a, Tavakoli et al., 2020b)

VFD fabricated Hydrogel Research Gaps

The first few experimental demonstrations for VFD fabricated hydrogel, a number of research gaps and future studies that can be undertaken. Such examples include having little discussion or results for the mechanical properties, physicochemical, swelling properties, as these first-time experiments solely investigated the tuning and controlling of surface morphologies and fluorescence properties.

The evaluation of mechanical behaviours of the hydrogels was excluded from the study; accordingly, there is little conclusion of whether the mechanical and swelling properties can be tuned by the employment of the VFD. (Tavakoli et al., 2020b, Tavakoli et al., 2020a)

Research Gap Summary

The following research gaps were identified and are summarized into dot-points:

- Gelatine – physical properties and solubility in aqueous media, highly hydrophilic and poor mechanical behaviours. (Bemiller, 2009, Buschow, 2001, Van Nieuwenhove et al., 2016)
- Gelatine are not used alone because it does not form stable hydrogel due to their poor mechanical properties. They are often mixed with other polymers to overcome this problem. (Hassan et al., 2018, Bemiller, 2009)
- Quercetin have poor solubility in water, low bioavailability and the hydrophobic nature and poor permeability.(Nathiya et al., 2014)
- Quercetin are soluble in alcohol solution, however it is purposed that the stability can be hinder by organic solvent (i.e. methanol) (Althans et al., 2014, Vedakumari et al., 2017, Rubini et al., 2020, Wang et al., 2016)
- Traditional methods have the lack control over surface morphology and physiochemical properties of the hydrogel and leads to a random fibrous or porous structure. These traditional methods also lack control in enhancing the mechanical properties of hydrogels.(Tavakoli et al., 2020b)
- Traditional methods for Al^{3+} detection are time-consuming, require equipment trainings and does not always provide accurate *in-situ* results.(Bings et al., 2010)
- A few examples of areas that are not explored includes whether the swelling and mechanical properties can be tuned by VFD. (Tavakoli et al., 2020a, Tavakoli et al., 2020b)
- Research has been done on the fabricating a gelatine-based hydrogel via traditional method, but not through the employment of a VFD.

- VFD research has shown that it can tune surface morphology and fluorescence properties of hydrogel but very few researches done on the tuning of swelling and mechanical properties. (Tavakoli et al., 2020a, Tavakoli et al., 2020b)
- Investigating whether Quercetin-Gelatine Hydrogel's fluorescence emission, swelling properties and mechanical properties can be tuned via VFD at different RPMs.(He et al., 2018b, Kelly, 2011, Nathiya et al., 2014) (Tavakoli et al., 2020b)
- Investigating whether Quercetin-Gelatine Hydrogel can perform *in-situ* Al^{3+} detection.

Literature Review Summary

To summarize the literature review, the background information of Al^{3+}, health risks and regulations was covered. The traditional method, along with modern techniques for detecting Al^{3+}, was mentioned in the literature review.

The hydrogel mechanism of network formation, classification of hydrogel based on types of cross-linking, preparation of hydrogel via traditional methods, ideal characteristic, hydrogel properties and applications were outlined. The background information of gelatine was also discussed, along with a detailed overview of past research done for the development of gelatine scaffolds/hydrogel. The overview of quercetin and its proposed sensing mechanism was covered in the literature review. The VFD background, VFD fabricated hydrogels, and purposed future research direction was also mentioned.

From the literature review, the research gap that can be adapted into this research is the fabrication of quercetin-gelatine hydrogel use for the sensing application of Al^{3+} by tunning the fluorescence properties of it by adjusting the rotational speed of the VFD.

By undertaking these adaptations, there could be an improvement with the quercetin-gelatine hydrogel properties and characteristics such as enhanced swelling, mechanical and fluorescence properties via VFD. As mentioned in the literature review, one of the main drawbacks is that the mechanical properties of quercetin-gelatine hydrogel and the weak fluorescence properties of quercetin, along with quercetin's poor solubility in water. Past research has identified that the formation of different surface morphologies and fluorescence properties in hydrogels strongly depends on the magnitude of the applied centrifugal force generated by the rotation of the VFD tube. By applying the principle of the magnitude of the applied centrifugal force

generated by the rotation of the VFD onto the Quercetin-gelatine hydrogel, various properties, such as the swelling, mechanical, and fluorescence properties could be controlled and tuned.

The contribution that this research can make is the advancement of *in-situ* Al^{3+} detection hydrogel uses as biomedical and biosensing engineering application, as well as bringing awareness for the fabrication of hydrogels through a recent VFD methodology. The outcome of this research can be useful for biomedical engineering, mainly on the focus of improving food safety and *in-situ* detecting material for Al^{3+} sensing.

PROJECT METHODOLOGY / EXPERIMENTAL SECTION

MATERIALS

The material required is Gelatine Powder (i.e. McKenzie's gelatine powder from a local food store. Quercetin (≥95% (HPLC), solid) and Aluminium Potassium Sulphate were purchased from Sigma-Aldrich. Ethanol 100% undenatured was purchased from Chem-supply. The VFD and VFD heating jack are required for this experiment. Milli-Q water was used to prepare all aqueous solutions. Cary Eclipse Fluorescence Spectrometer and Cary 50 & Agilent 60 UV-Vis Spectrophotometer are used for the photoluminescence (PL), absorption spectra, and transmission of the Quercetin-Gelatine hydrogel. As well as use for the PL, absorption spectra and transmission of Quercetin solution.

Mechanical testing was done using a universal testing machine called Instron, and 'Bluehill 3' was used to record the force-elongation data, and the stress-strain diagrams were produced.

Microscopy images of the hydrogel specimens were obtained by an Aunet NMM - 800TRF microscope.

METHODOLOGY AND TESTING

Material Preparation

Quercetin Solution

Non- VFD Preparation

Quercetin (weight = 0.05g (i.e. 0.5g/L)) is added to the solution and is dissolved in 100 mL of a mixture of water/ethanol (50/50) at 45-degree Celsius. The solution was mixed for 30 minutes via magnetic stirrer.

VFD Preparation

Quercetin (weight = 0.05g (i.e. 0.5g/L)) is added to the solution and is dissolved in 100 mL of a mixture of water/ethanol (50/50) at 45-degree Celsius. The solution was mixed for 5 minutes via magnetic stirrer. 4 ml of the solution is added into the quart tube and mix for 30 mins at 1000 RPM, 45-degree Celsius, rotating clockwise and at 45-degree angle via VFD. Repeat the steps, however increasing the VFD RPM by 1000 RPM increments, till 4000 RPM is reached.

Hydrogel Fabrication

Non-VFD Hydrogel Preparation

Gelatine Hydrogel

5g of gelatine powder is dissolved in 100 mL of a mixture of water/ethanol (50/ 50) at 45-degree Celsius. The solution was mixed for 30 minutes via magnetic stirrer. Add 4 ml of the solution into petri dishes. Allow the solution to set at room temperature under the fume hood.

Quercetin-Gelatine Hydrogel

5g of gelatine powder is dissolved in 100 mL of a mixture of water/ethanol (50/ 50) at 45-degree Celsius. Quercetin (weight = 0.05g (i.e. 0.5g/L)) is added to the solution and mix for 30 mins via magnetic stirrer. Add 4 ml of the solution into petri dishes. Allow the solution to set at room temperature under the fume hood.

VFD Quercetin-Gelatine Hydrogel Preparation

Quercetin- Gelatine Hydrogel

To begin with, 5g of gelatine powder is dissolved in 100 mL of a mixture of water/ethanol (50/ 50) at 45-degree Celsius. Quercetin (weight = 0.05g (i.e. 0.5g/L)) is added to the solution and mix for 5 mins via magnetic stirrer. 4 ml of the solution is added into the quart tube and mix for 30 mins at 1000 RPM, 45-degree Celsius, rotating clockwise and at 45-degree angle via VFD. Allow the solution to set in petri dishes at room temperature under the fume hood. Repeat the steps, however increasing the VFD RPM by 1000 RPM increments, till 4000 RPM is reached.

Methodology Tests

Photoluminescence of Quercetin Solution and Quercetin-Gelatine Hydrogel

The Photoluminescence (PL) intensity of the quercetin solutions and Quercetin-gelatine hydrogels was measured using the Cary Eclipse Fluorescence Spectrometer, where the wavelength range is 200-800nm, and the excitation wavelength is 380nm.

The PL intensity value of Quercetin solution without VFD and with VFD (i.e. 1000 – 4000 RPM) was measured. The fluorescence emission was observed at 500-550 nm.

All measurement was made in triplicate and averaged.

Transmission and Absorption Spectra of Quercetin Solution and Quercetin-Gelatine Hydrogel

The transmission and absorption spectra of the specimens were measured using the Cary 50 & Agilent 60 UV-Vis Spectrophotometer, where the wavelength range is 200-800nm.

The result of hydrogel specimens, along with quercetin solution specimens, were also measured.

All measurement was made in triplicate and averaged.

Sensing of Quercetin Solutions and Quercetin-Gelatine Hydrogels to Al^{3+}

The PL intensity, absorption, and transmission of the specimens at different Al^{3+} concentrations were determined using Cary Eclipse Fluorescence Spectrometer and Cary 50 & Agilent 60 UV-Vis Spectrophotometer. The wavelength range is 200-800 nm, and the excitation wavelength is 380nm for the spectrometer settings.

The fluorescence emission was observed at 500-550 nm.

All measurement was made in five determinations and averaged.

Al^{3+} solution of 0.6, 1.2, 2.4, 3.6, 4.8 mg/mL was prepared by mixing aluminium potassium sulphate with de-ionised water.

A total of 25 microliters of Al^{3+} solution (0.6, 1.2, 2.4, 3.6, 4.8 mg/mL) was dropped into the 2.5ml of quercetin solution without VFD employment for sensing.

A total of 25 microliters of Al^{3+} solution (0.6, 1.2, 2.4, 3.6, 4.8 mg/mL) was dropped on the 10 mm X 10 mm hydrogel specimens for sensing.

Swelling Testing Methodology

The hydrogel specimens were dried for 12 hours at room temperature under the fume hood before the swelling test.

The initial weight of the hydrogels in the dry state was recorded before immersing it into 100mL deionised water at room temperature. The swelling weight was recorded every 30 minutes till 300 minutes (i.e. 5 hours) was reached. The wet samples were wiped with filter papers to remove excess liquid and weighted. The steps were repeated for the hydrogel at different rotation speeds up to 4000 RPM of the VFD and the hydrogels via without VFD. All measurement was made in triplicate and averaged.

The swelling ratio %, is calculated using Equation 1.

$$Swelling\ Ratio\ \% = \frac{W_s - W_d}{W_d} \times 100\ , \qquad Equation\ 1$$

Where, W_s is the weight of the hydrogel in swollen state and W_d is the weight of the hydrogel in dry state.

Mechanical Testing Methodology

Gelatine hydrogel (i.e. without VFD), Quercetin-Gelatine hydrogel (i.e. without VFD) and Quercetin-Gelatine hydrogel (i.e. with VFD) were cut into approximately 25 mm x 35 mm (i.e. $L \times W$).

For delicate hydrogels, an elongation rate of 5 mm/min was used for the various specimens.

The thickness, length and width of the specimens were measured, after properly load the hydrogel into the Instron. Once the hydrogel is loaded and measurement are done, apply Tensile loading onto the specimens. Record the data of the Tensile force, Ultimate Tensile Strength and Young Modulus into the computer software or excel. The steps were repeated for the hydrogel products fabrication at different rotation speeds up to 4000 RPM of the VFD and hydrogels via without VFD.

The stress and strain can be calculated by:

Strain:

$$\varepsilon = \frac{L_c - L_o}{L_0}, \qquad \textit{Equation 2}$$

Where, ε is the strain, L_c is the change in length of the specimens and L_o is the original length of the specimens.

Stress:

$$\sigma = \frac{F}{A}, \qquad \textit{Equation 3}$$

Where, σ is the stress, F is the applied force and A is the cross-section area of the specimen.

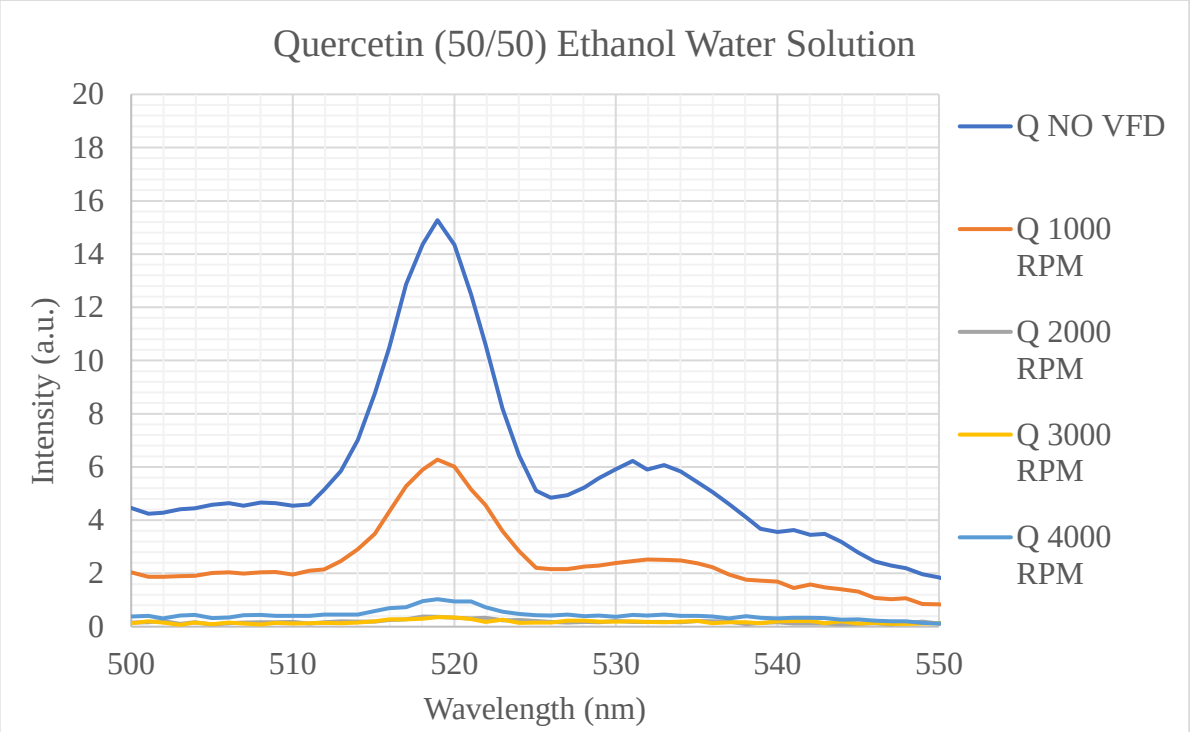
All measurement was made in triplicate and averaged.

Optical Microscopy Methodology

Hydrogel specimens (i.e. No VFD and with VFD at 1000 – 4000 RPM) images were obtained by an Aunet NMM - 800TRF microscope with 5x objective. All measurement was made in triplicate.

RESULTS AND DISCUSSION

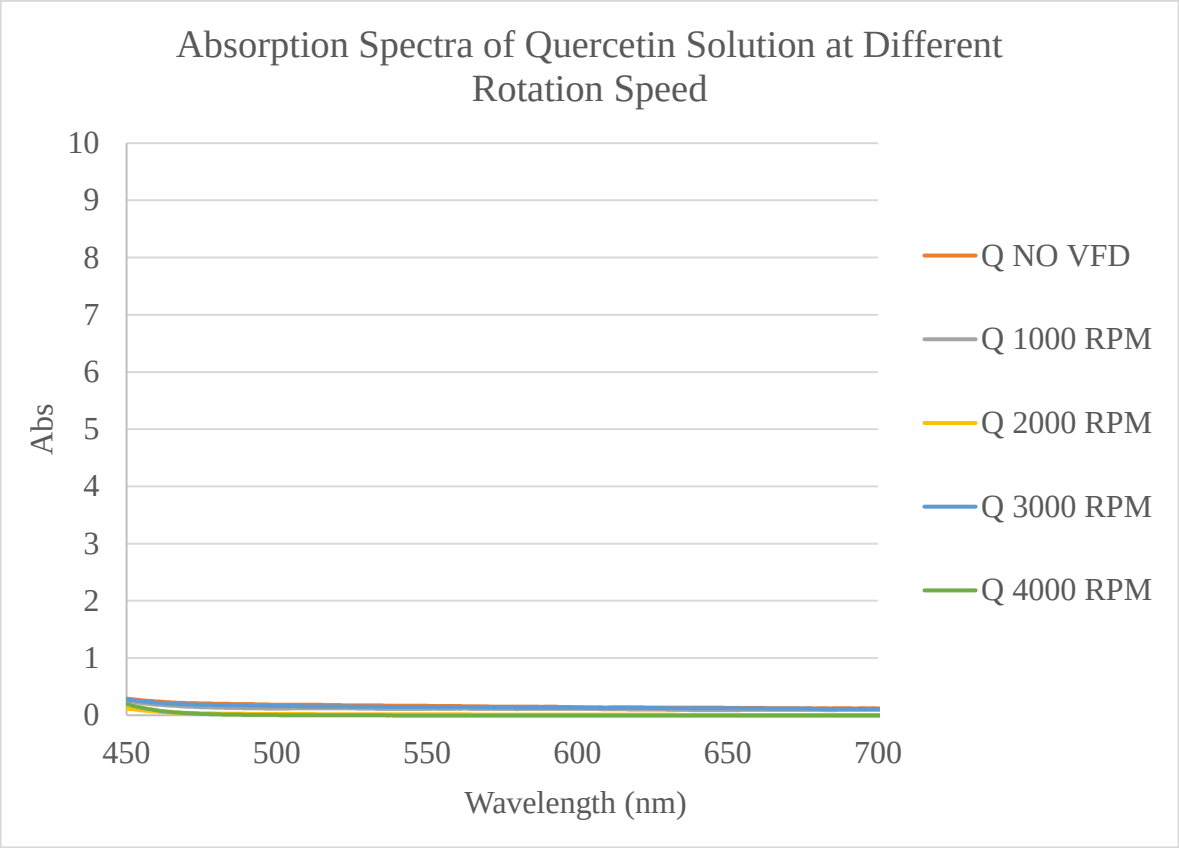
Photoluminescence Properties of Quercetin Solution at Different RPM



Graph 1 - Quercetin Solution Fluorescence Emission

Graph 1 shows that as the RPM of the increases, the intensity of the quercetin solution decreases. The result indicates that quercetin solution without the employment of the VFD had the highest intensity peak and Q 1000 RPM intensity was decreased by approximately 60%. Whereas the intensities of Q 2000 RPM, Q 3000 RPM and Q 4000 RPM were decreased by approximately 90%. From Graph 1, in terms of sensing application, the Q solution without the employment of the VFD and Q 1000 RPM would be the ideal to further investigate for sensing of Al^{3+} in this research. Therefore, the investigation of VFD employed at 2000 RPM, 3000 RPM and 4000 RPM was excluded because it exhibits little to no fluorescence emission for the photoluminescence of Al^{3+} sensing. Overall, the results indicate that the high shear stress and micro-mixing from the employment of VFD at different rotational speed does hinder the intensity of the quercetin solution.

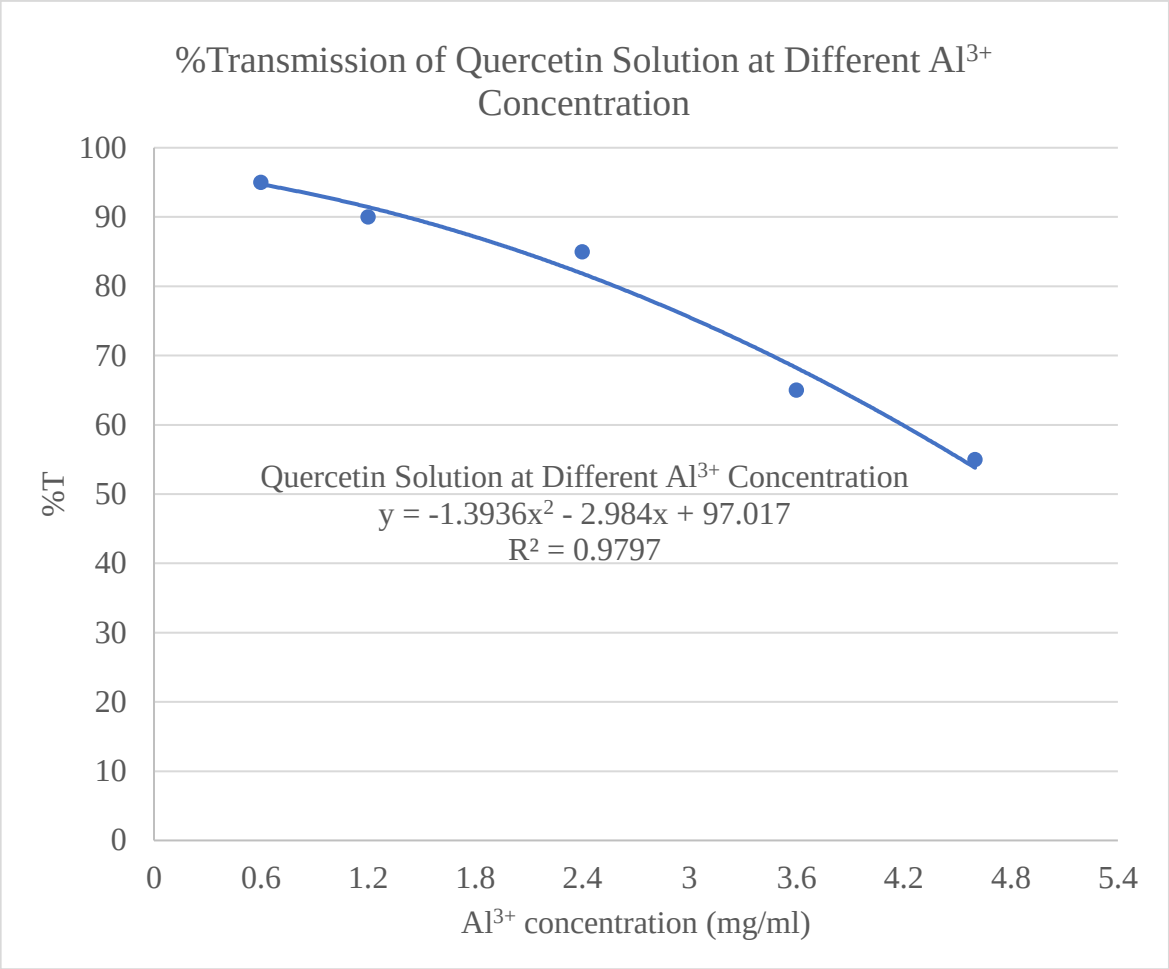
Absorption Spectra Properties of Quercetin Solution Without Al^{3+} Concentration



Graph 2 - Absorption Spectra of Quercetin Solution at Different Rotation Speed

The purpose of absorption spectroscopy is to quantify the absorption of radiation as a function of frequency or wavelength due to its interaction with specimens. The specimens absorb photons (i.e. energy) from the radiating field. The visible light spectrum range that the human eye can detect is approximately between the wavelength of 400 – 700 nm. From Graph 2, the quercetin solution at different RPMs, along with quercetin solution did not have any peaks in the visible light spectrum. This indicates that the specimens did not absorb any photons (i.e. energy) from the radiating fields and that the quercetin solution specimens are transparent in colour. Overall, the data shows that even with the employment of the VFD at different RPMs, it did not alter the absorption spectra of the specimens.

%Transmission Properties of Quercetin Solution with Al^{3+} Concentration



Graph 3 - % Transmission of Quercetin Solution at Different Al^{3+} Concentration

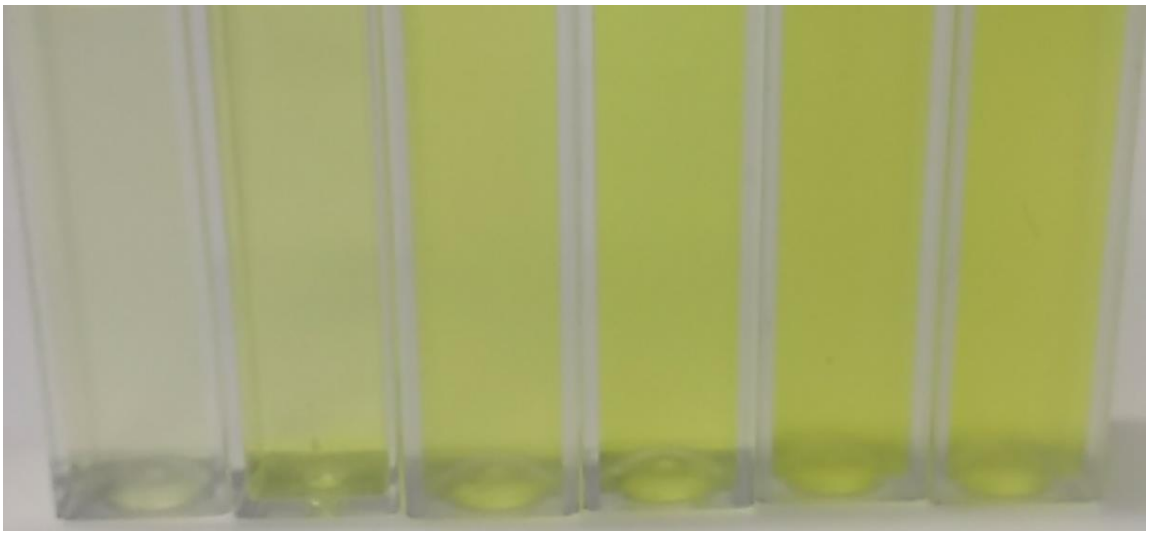


Figure 17 - Quercetin Solution at Different Al^{3+} Concentration

(Left to Right) - Quercetin solution without Al^{3+}, Quercetin solution with Al^{3+} 0.6 mg/ml, Al^{3+} 1.2 mg/ml, Al^{3+} 2.4 mg/ml, Al^{3+} 3.6 mg/ml, and Al^{3+} 4.8 mg/ml.

From Graph 3, when a concentration of 0.6 mg/ml of Al^{3+} was added to the quercetin solution, the %transmission was approximately 95. As the concentration of the Al^{3+} increases, the %T of the quercetin solution decreases. The result indicates that at a lower concentration of Al^{3+}, the solution is quite transparent or light in colour, and higher transmission of light can pass through the quercetin solution. When at higher concentration, the solution became darker in colour, and therefore, a lower transmission of light can pass through the quercetin solution.

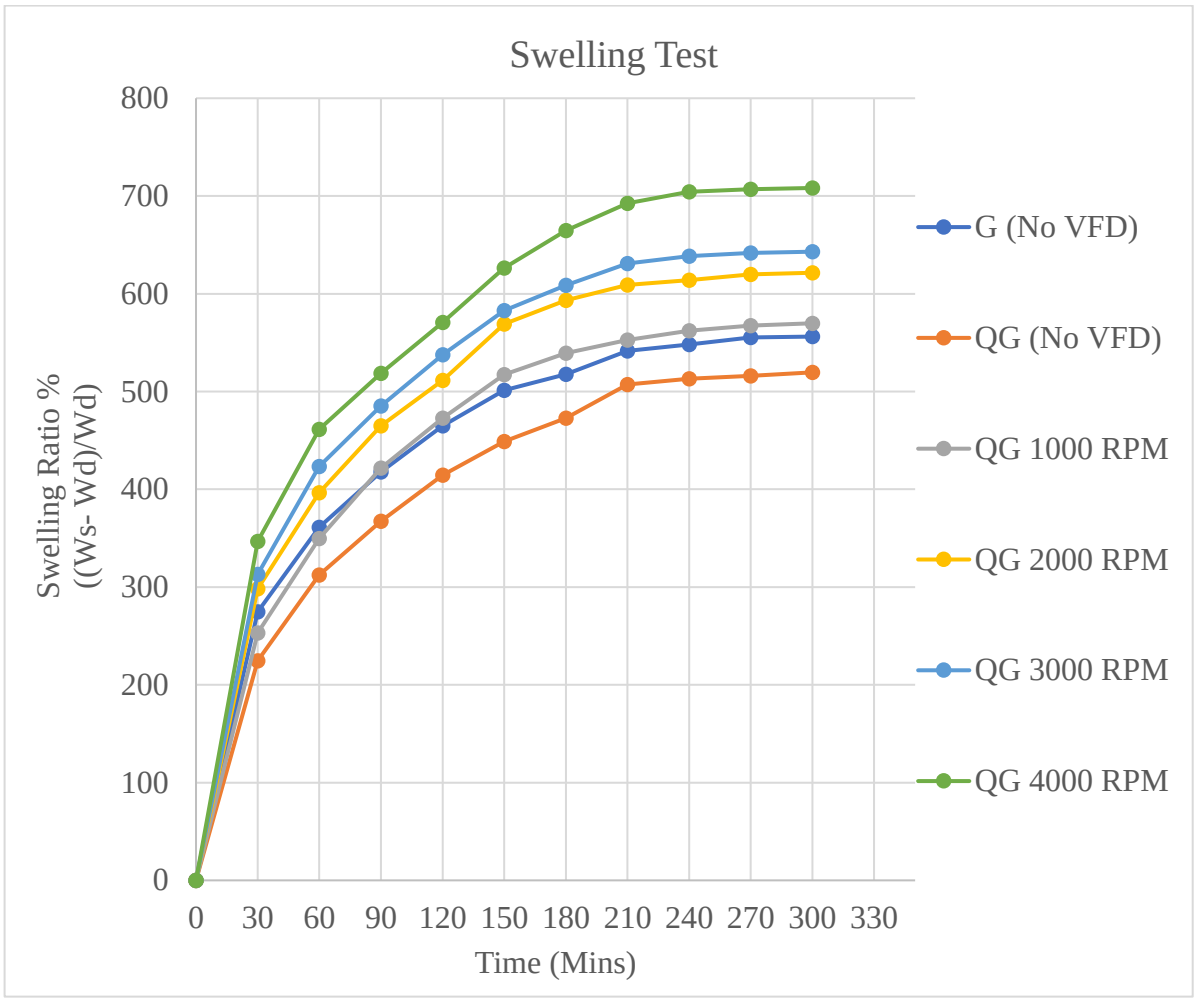
The significance of this result indicates that quercetin solution will be darker when higher Al^{3+} concentration is added, and therefore, it can be visibility detected by the human eyes. (see Figure 17) This results also indicates that quercetin sensing properties functions as a 'Turn On' mechanism which is like AIE properties.

Swelling Test

The swelling ratio %, is calculated using Equation 1.

$$Swelling\ Ratio\ \% = \frac{W_s - W_d}{W_d} \times 100\ , \qquad Equation\ 1$$

Where, W_s is the weight of the hydrogel in swollen state and W_d is the weight of the hydrogel in dry state.



Graph 4 – The Swelling Ratio Result for The Different Rotational Speed Hydrogel Films and The Control Hydrogel Films

The purpose of the swelling test was to identify whether the hydrogel can hold the Al^{3+} solution in its polymer matrix. As well as to identify how fast the hydrogels can absorb the solution when different concentrations of Al^{3+} is dropped into it. From Graph 4, the QG 4000 RPM had

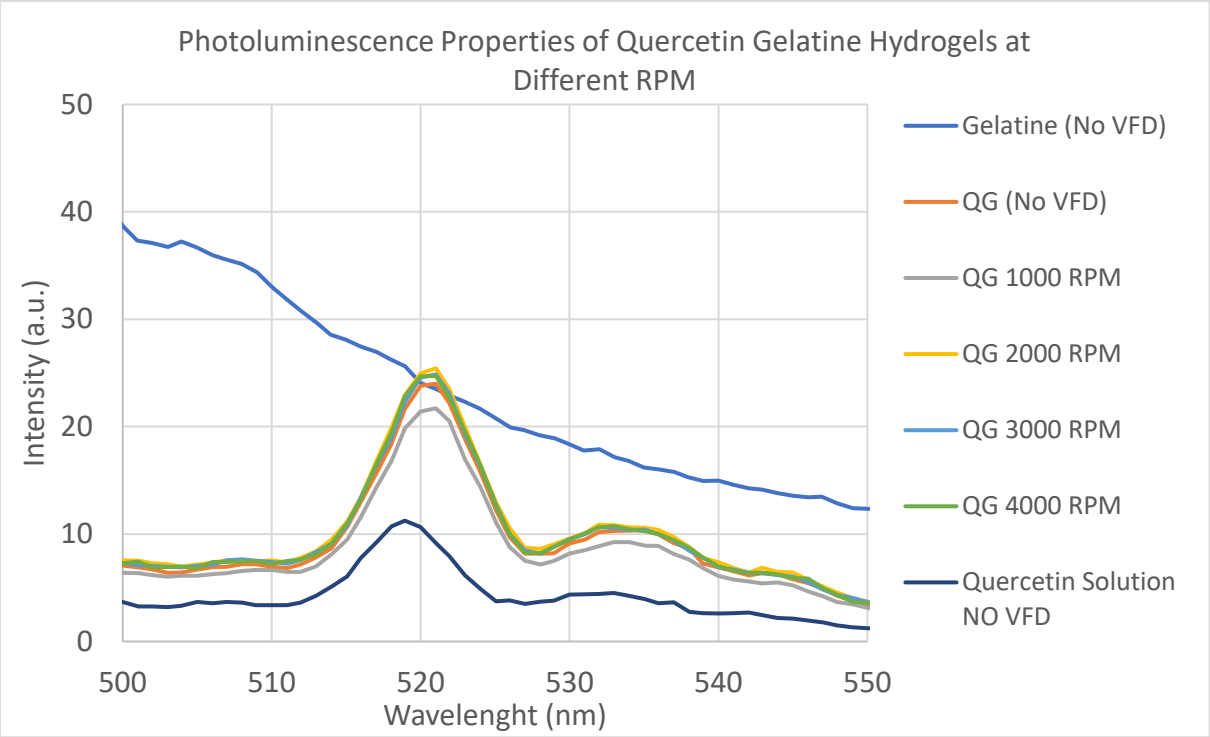
the stiffest swelling ratio, QG 3000 RPM second stiffest swelling ratio and QG 2000 RPM third stiffest swelling ratio. QG 1000 RPM swelling ratio has improved when comparing it with QG (NO VFD), which has the lowest swelling ratio.

QG 4000 RPM displayed a swelling ratio of about 700%, which was the highest among the specimens after 240 minutes. Whereas QG (NO VFD) displayed a swelling ratio of about 510%, which was the lowest of all specimens after 240 minutes.

From Graph 4, Gelatine (NO VFD) swelling ratio is higher than QG (NO VFD). The QG (No VFD) shows with the addition of quercetin content in gelatine hydrogel films, has influenced the swelling ratio. Therefore, it can be said that the addition of quercetin in films decreases the swelling properties of the films in deionized water.

The key findings from this swelling test indicated that the high shear stress and micro-mixing from the employment of VFD at different rotational speed could tune and control the swelling properties of the film. The absorption rate and capability of the hydrogels are improved via increasing the RPM of the VFD.

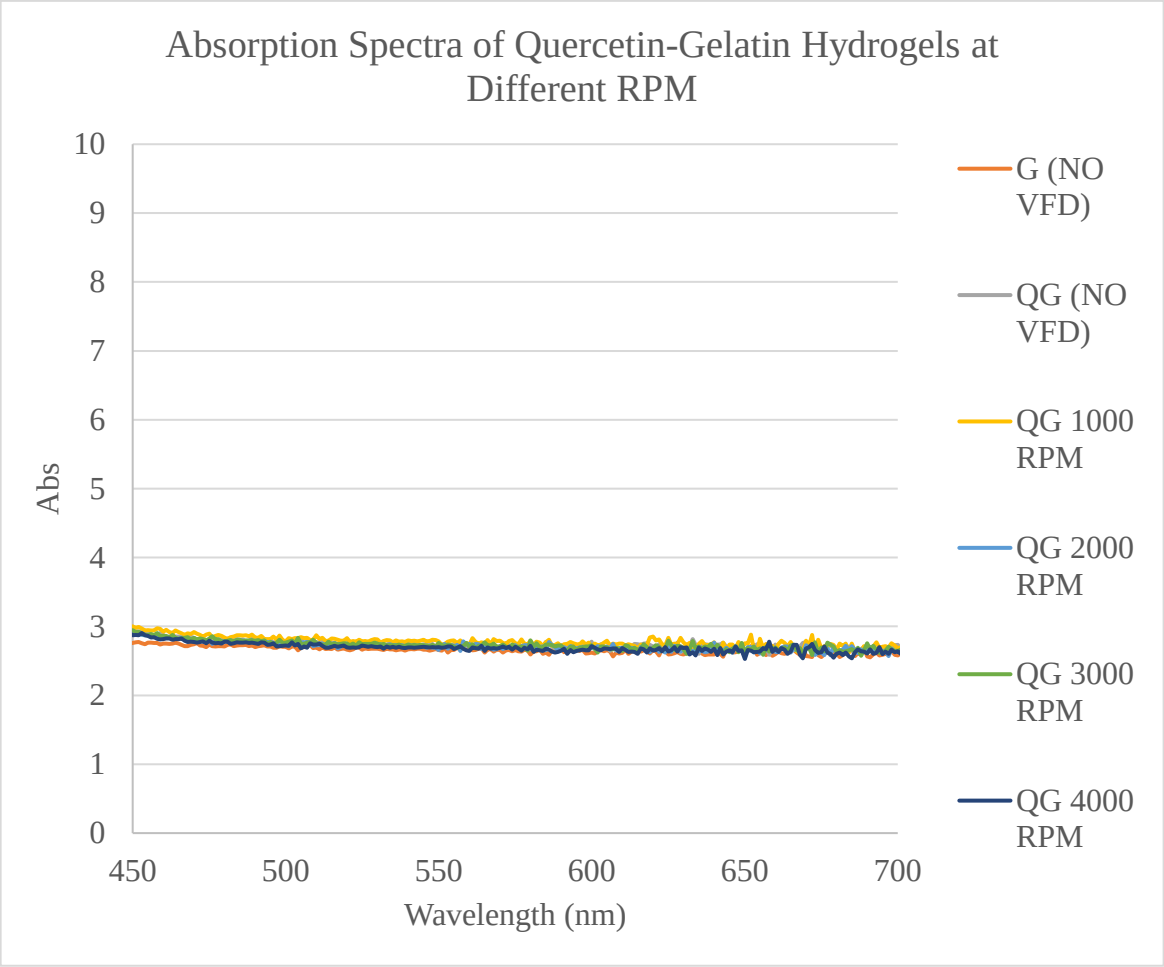
Photoluminescence Properties of Quercetin Gelatine Hydrogels at Different RPM



Graph 5 - PL Properties of Quercetin Gelatine Hydrogel at Different RPM

Graph 5 shows the PL intensity comparison of the hydrogel specimens at different RPM with quercetin solution as well. Quercetin when in solution form exhibits a low intensity compared to when it is in hydrogel state. The hydrogels employed at different RPM, as shown in Graph 5 did not show any visible tuning effect when not used for Al^{3+} sensing. From Graph 5, there is an improvement of approximately 40% for the quercetin-gelatine hydrogels when compared to the quercetin solution fluorescence emission. Overall, the key finding is that the fluorescence properties of the quercetin exhibit a better intensity when in hydrogel form compared to when it is in solution. This could be due to quercetin being isolated, therefore this structure empowers AIEgens going through a low-frequency torsional moment and allows the structure to perform. Though, when the AIEgen aggregates, the unique structure confines the intermolecular rotation, which shows high emission and has strong fluorescence in the aggregated state. (Hong et al., 2009, Huang et al., 2020)

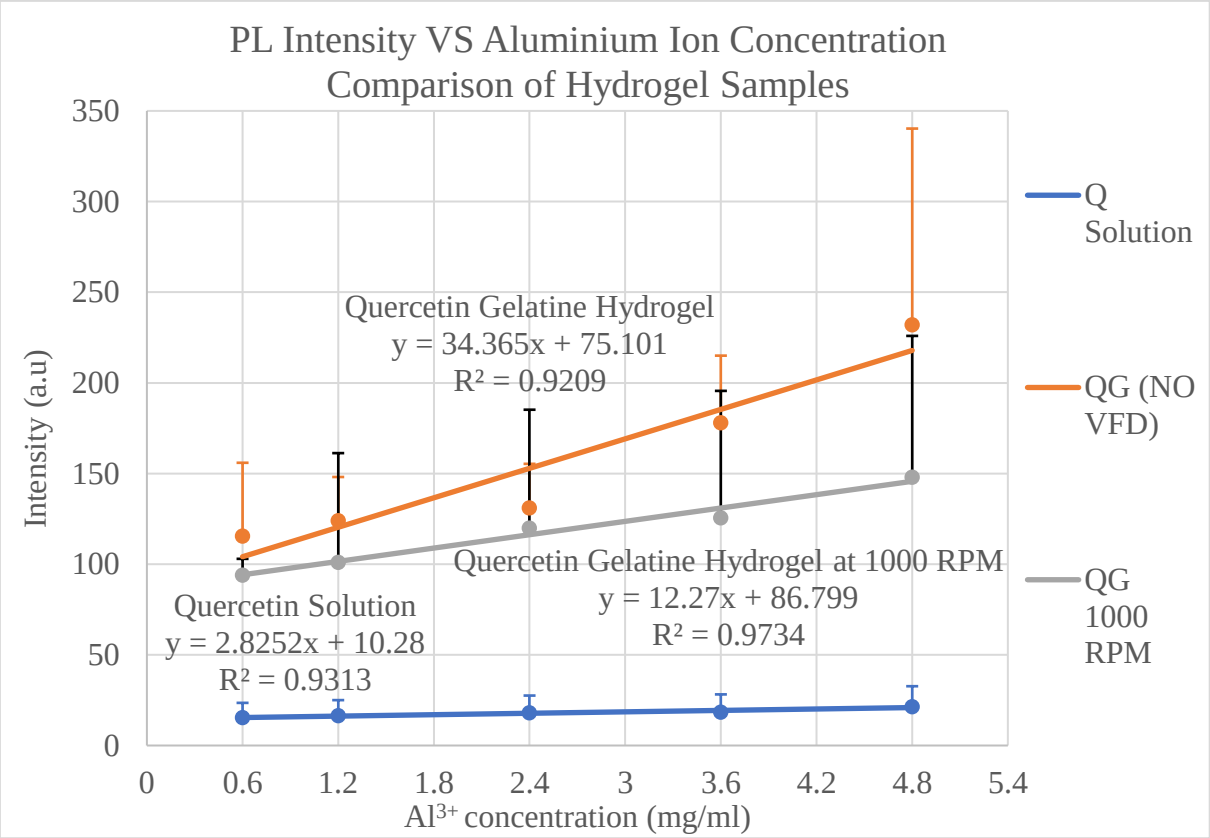
Absorption Spectra of Quercetin-Gelatine Hydrogels at Different RPM



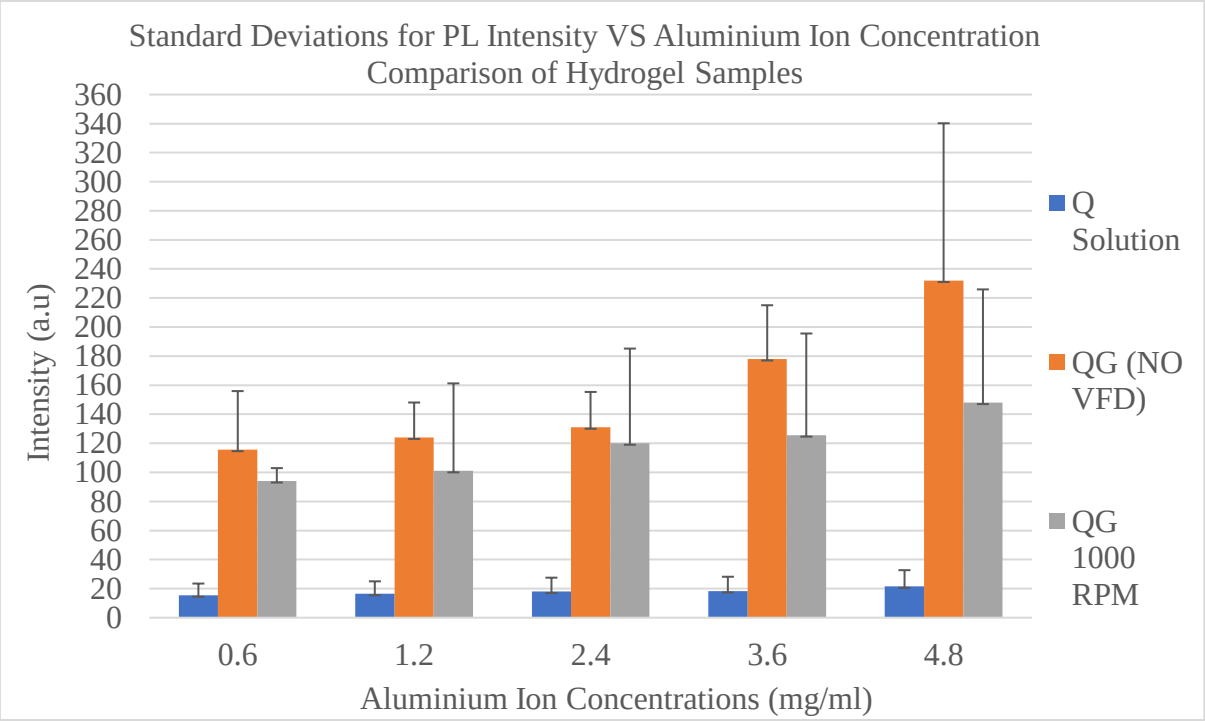
Graph 6 - Absorption Spectra of Quercetin- Gelatine Hydrogels at Different RPM

As mentioned, the visible light range that the human eye can recognize is between the frequency of 400 – 700 nm. From Graph 6, the quercetin-gelatine hydrogels at different RPM, along with quercetin hydrogel did not have any peaks in the visible light spectrum. This indicates that the specimens did not absorb any photons (i.e. energy) from the radiating fields. Overall, the result shows that even with the employment of the VFD at different RPMs, it did not alter the absorption spectra of the specimens radiating fields. Overall, from the absorption spectra shown in Graph 6, the quercetin hydrogel specimens employed at different RPM are transparent in colour or does not have a distinct colour.

Sensing of Quercetin-Gelatine hydrogels to Al^{3+} Comparison



Graph 7 - Comparison PL Intensity of QG Hydrogels at Different Al^{3+} Concentrations



Graph 8 - Standard Deviation for PL Intensity VS Aluminium Ion Concentrations Comparison of Hydrogel Samples

Table 1- Standard Deviations for PL Intensity Vs Aluminium Ion Concentrations Comparison of Hydrogel Samples

Aluminium Ion Concentrations (mg/ml)	Q Solution PL Intensity Average	Q Solution, Standard Deviation	QG (NO VFD) PL Intensity Average	QG (NO VFD), Standard Deviation	QG 1000 RPM PL Intensity Average	QG 1000 RPM, Standard Deviation
0.6	15.4	8.11	115.6	40.33	94	8.94
1.2	16.4	8.62	124	24.08	101	60.25
2.4	17.98	9.56	131	24.34	120	65.19
3.6	18.36	9.83	178	37.01	125.6	69.98
4.8	21.5	11.19	232	108.25	148	77.91

As mentioned, due to quercetin solution exhibiting little to no fluorescence emission, when employed in a rotation speed of 2000, 3000 and 4000 RPM, the hydrogels at that RPM were excluded in the Al^{3+} sensing comparison (see Graph 1). Little to no fluorescence emission for the sensing of Al^{3+} is not ideal for the aim of this research.

Graph 7 shows a comparison of the PL intensity of quercetin solution, QG (NO VFD) and QG 1000 RPM. The result indicates that when a low concentration of Al^{3+} was added to specimens, the intensity of the specimens is at its lowest. The intensity of the specimens increased when a higher concentration of Al^{3+} was dropped to the solution. This result also indicates that the quercetin's sensing mechanism works as a 'turn on' switch when a higher concentration of Al^{3+} is dropped. This results also suggest that quercetin sensing properties functions as an AIE mechanism for the quercetin solution and quercetin-gelatine hydrogel specimens.

The sensitivity of the specimens can be determined from the slope of the specific specimen's equation stated in Graph 7. It can also be seen in Graph 7 that the QG (NO VFD) has better sensitivity in sensing Al^{3+} compared to the QG 1000 RPM. The quercetin solution had the

lowest sensitivity in sensing Al^{3+}. For the QG 1000 RPM, it can be noted in Graph 7 that the employment of the VFD affected the sensitivity and intensity of the hydrogel. The sensitivity of the QG 1000 RPM was reduced to approximately 50%, which is similar to the quercetin solution behaviour when employed in a VFD shown in Graph 1. This results also show that quercetin in hydrogel form have better fluorescence emission than when it is in solution form.

Graph 7, Graph 8 and Table 1 shows the standard deviation for the PL intensity versus aluminium ion concentration comparison of hydrogel samples. From Graph 8 and Table 1, Quercetin solution has the lower sensitivity to Al^{3+} concentration and the lowest standard deviation. QG (NO VFD) have the best sensitivity to Al^{3+} concentration and lower standard deviation compared to QG 1000 RPM. QG 1000 RPM has the highest standard deviations in comparison to quercetin solution and QG (NO VFD). From Graph 8 and Table 1, when comparing the QG (NO VFD) and QG 1000 RPM standard deviations, the QG (NO VFD) standard deviations were 40.33 for Al^{3+} concentration of 0.6 mg/ml and 108.25 for Al^{3+} concentration of 0.6 mg/ml. Whereas the QG 1000 RPM standard deviations were 8.94 for Al^{3+} concentration of 0.6 mg/ml and 77.91 for Al^{3+} concentration of 0.6 mg/ml. Graph 7, Graph 8 and Table 1 indicates that it is better to employ QG without the employment of a VFD for Al^{3+} sensing due to having best sensitivity to Al^{3+} concentration and lower standard deviation.

It can be proposed that high shear stress and micro-mixing decrease the quercetin's fluorescence and sensitivity properties. The result indicates that when quercetin is in a hydrogel state, the sensitivity is significantly improved and therefore, the employment of a quercetin-gelatine hydrogel is better for the sensing of Al^{3+}. From the result, it is better to employ QG without the employment of a VFD for Al^{3+} sensing.

Mechanical Test

The stress and strain can be calculated by:

Strain:

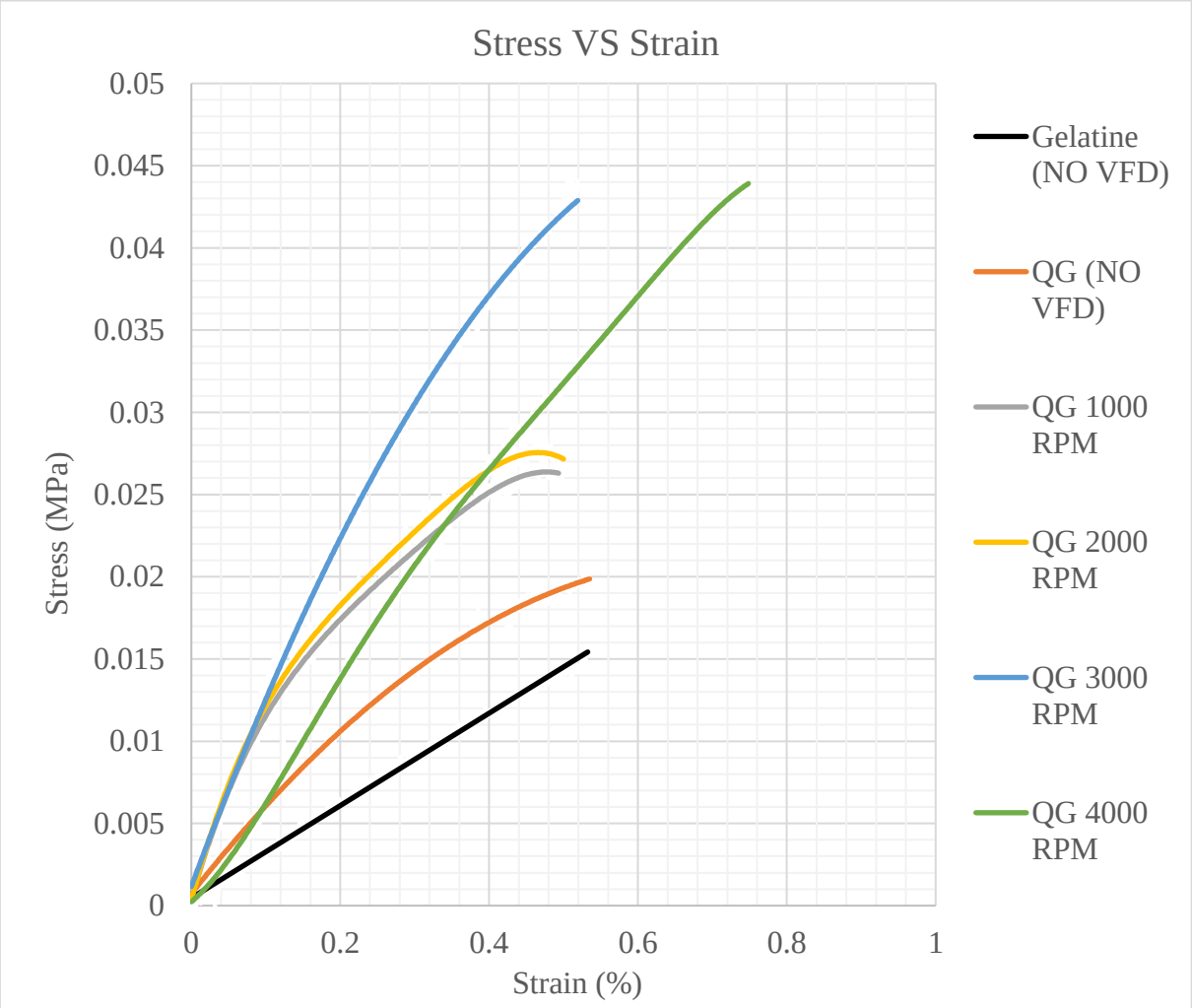
$$\varepsilon = \frac{L_c - L_o}{L_0}, \qquad \textit{Equation 3}$$

Where, ε is the strain, L_c is the change in length of the specimens and L_o is the original length of the specimens.

Stress:

$$\sigma = \frac{F}{A}, \qquad \textit{Equation 4}$$

Where, σ is the stress, F is the applied force and A is the cross-section area of the specimen.



Graph 9 - Comparison of Quercetin-Gelatine Hydrogels at Different RPM

The mechanical test was employed to determine which hydrogel specimens would exhibit an excellent mechanical property that could be employ as the Al^{3+} sensing product when handling. The mechanical properties of the quercetin-gelatine hydrogels at different RPMs were compared against the gelatine and quercetin-gelatine without VFD employment.

As shown in Graph 9, gelatine hydrogel had the lowest Young's Modulus (i.e. slope), fracture point and ultimate strength, thus indicating it has poor mechanical properties. The quercetin-gelatine hydrogel shows that with the addition of quercetin, the mechanical properties of the hydrogel improved as there was an improvement on the Young's Modulus, fracture point and ultimate strength (see Graph 9).

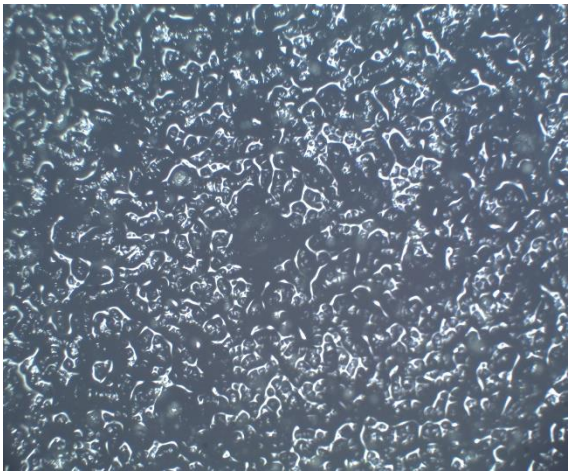
From Graph 9, the Young's Modulus (i.e. slope), the ultimate strength and fracture point were improved with the employment of the VFD. As the RPM increases for the hydrogel specimen, Young's modulus increases indicating that the stiffness and strength of the specimens have improved. QG 1000 RPM to QG 2000 RPM shows there was no significant improvement to the strain properties when compared to the quercetin-gelatine and gelatine hydrogel that was not employed via VFD. However, for QG 4000 RPM, the strain properties have improved by approximately 20%, and the ultimate fracture point is similar to QG 3000 RPM. When comparing QG 3000 RPM to the quercetin-gelatine and gelatine hydrogel that was not employed via VFD, there was no significant improvement on the strain properties, but Young's modulus and fracture point had significant improvement. This indicates that QG 3000 RPM and QG 4000 RPM are ideal for product handling, but delicate handling must be considered as the fracture point is approximately 0.042 MPa.

The hydrogels specimens demonstrated that it exhibits elastic deformation and does not display significant plastic deformation properties is shown in Graph 9. Therefore, indicating that the specimens have brittle mechanical behaviour and do not have ductile mechanical properties.

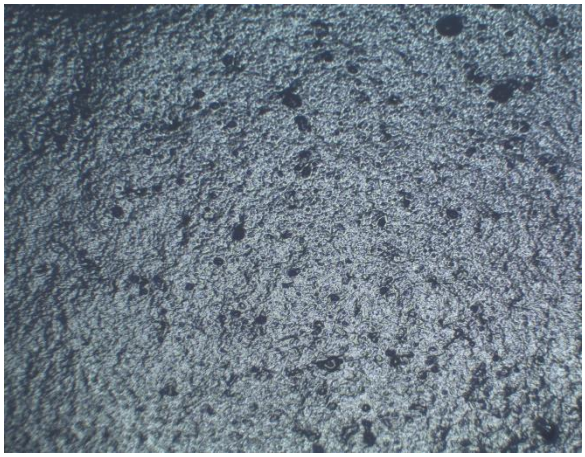
As shown in Graph 9, both the QG 3000 RPM and QG 4000 RPM mechanical properties would be ideal to have for the Al^{3+} sensing product when handling. The key finding from the mechanical testing is that the employment of the VFD can tune the mechanical structure/ properties of the hydrogel specimens.

Optical Microscopy

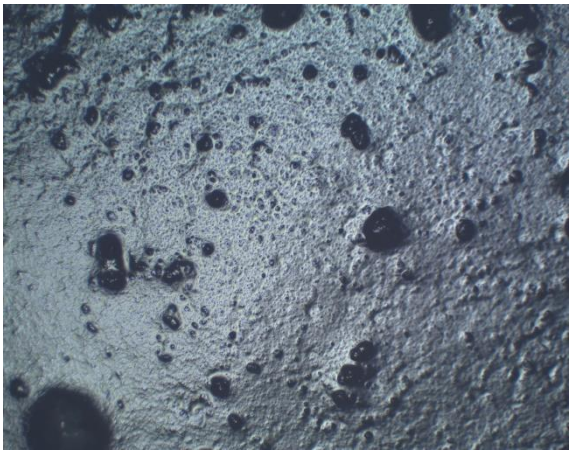
Gelatine Hydrogel (NO VFD)



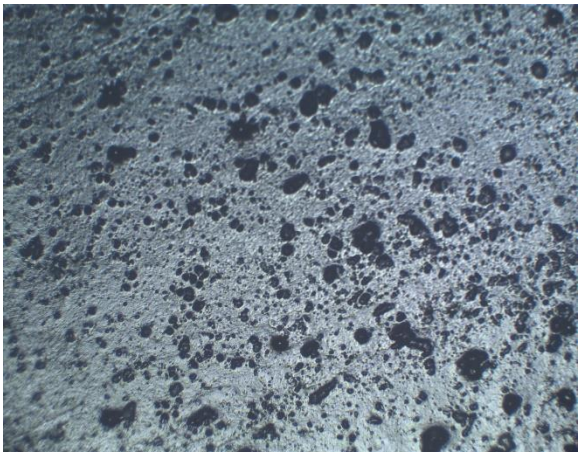
Quercetin-Gelatine Hydrogel (NO VFD)



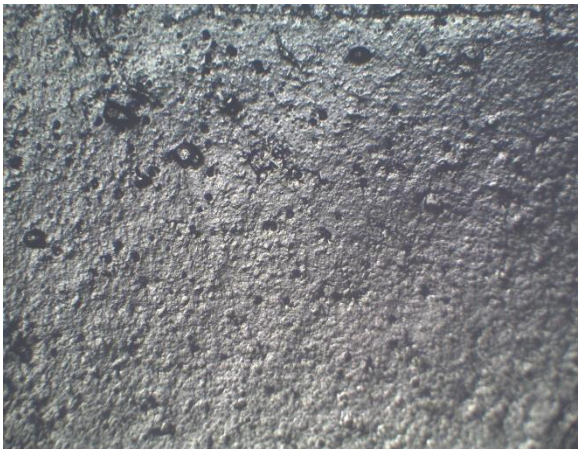
Quercetin-Gelatine Hydrogel (1000 RPM)



Quercetin-Gelatine Hydrogel (2000 RPM)



Quercetin-Gelatine Hydrogel (3000 RPM)



Quercetin-Gelatine Hydrogel (4000 RPM)

Figure 18 – Optical Microscopy Images of (Top, left to right) Gelatine hydrogel, QG (NO VFD) , (Middle, left to right) QG 1000 RPM, QG 2000 RPM, (Bottom, left to right) QG 3000 RPM and QG 4000 RPM.

From Figure 18, the gelatine hydrogel has a smooth surface and even molecule size distribution compared with the quercetin-gelatine hydrogel samples. The QG (NO VFD) indicated that with the addition of quercetin, the surface of the hydrogel has a rougher surface morphology and an uneven molecule size distribution is observed. The QG (NO VFD) surface also looked grainy when comparing to the quercetin-gelatine hydrogels with the employment of VFD at different RPMs and the gelatine hydrogel. From Figure 18, it can be indicated that when the RPM of the VFD increases, the surface morphology of the quercetin-gelatine hydrogel specimens become smoother and the crosslinking density improves. As appeared in Figure 18, with increasing RPM, the molecule size distribution of the specimens becomes smaller as well.

The optical microscopy result also supports the swelling and mechanical test results as shown in Graph 4 and Graph 9. With increasing RPM, the molecule size distribution and crosslinking density of the hydrogel specimens improves, which alters the porosity and mechanical properties of the hydrogel specimens. This is due to the high shear stress and micro-mixing employed by the VFD tuning these hydrogel properties. Therefore, these phenomena explain the improvement of the mechanical properties/structure (i.e. Young's Modulus) and the swelling capacity and rate for the hydrogel specimens Figure 18, Graph 4 and Graph 9.

Accordingly, as appeared in Figure 18, this VFD approach accomplished the basic control of molecule size distribution and control of surface morphology of the hydrogel specimens due to the high shear stress and micro-mixing employed by the VFD.

CONCLUSION

Al^{3+} is primarily found in food nowadays, and the exposure of these harmful metal ions can lead to the development of neurodegenerative diseases in human. Traditional methods for sensing Al^{3+} are not always convenient, requires extensive equipment training and does not always provide *in-situ* results. As mentioned, gelatine exhibits poor mechanical properties and often traditional methods have difficulties in controlling its physical and mechanical properties. Quercetin has natural fluorescence properties; however, they are insoluble in water and instability in physiological medium, therefore limiting the use of them. Therefore, the aim of the research is to fabricate a natural fluorescence properties hydrogel based on quercetin and gelatine with tuneable properties by the employment of a VFD for the sensing of Al^{3+}.

From the PL properties of Quercetin solution at different RPMs, the main findings were that went the RPM was increased, the PL intensity of the quercetin solution decreases. This indicates that the high shear stress and micro-mixing employed by the VFD hinders the PL intensity of quercetin, therefore decreasing quercetin's fluorescence properties.

The employment of the VFD did not tune the absorption spectra properties of the quercetin solution and quercetin-gelatine hydrogels without Al^{3+} concentration. Therefore, the main findings were at different RPMs, the colour of the specimens were all transparent or 'clear'. The main findings for the transmission properties of quercetin solution show that when a higher concentration of Al^{3+} was increased, the colour of the solution increases to a darker colour.

A swelling test was done to identify which hydrogel specimens had the best swelling rate and capacity to hold the Al^{3+} solution when dropped. The main findings were that when RPM increases, the hydrogels' swelling capacitance and rate properties enhanced.

From the PL properties of Quercetin-gelatine hydrogels and quercetin solution when different Al^{3+} concentration solutions were dropped, the main findings were that when the concentration was increased, the PL intensity of the specimens increases. The results show that QG 1000 RPM fluorescence and sensitivity was decreased when compared to QG without VFD employment. This demonstrated that the employment of the VFD could tune the fluorescence intensity and sensitivity to Al^{3+}.

From the mechanical test, the main findings show that when the RPM increases, the Young's Modulus of the specimens increases, with the exclusion of QG 4000 RPM. This indicates that the VFD can tune the stiffness of the specimens.

Accordingly, the optical microscopy result indicated that the basic control of molecule size distribution and control of surface morphology of the hydrogel specimens can be tuned at different RPMs. The optical microscopy result also supports the swelling and mechanical test results. This is due to the high shear stress and micro-mixing employed by the VFD hindering the molecule size distribution and surface morphology of the hydrogel specimens.

Overall, the results show that the employment of the VFD can tune the properties of the specimens. From the results, quercetin-gelatine hydrogel without the employment of a VFD shows to have better fluorescence emission and sensitivity to Al^{3+} at different concentrations compared to the QG 1000 RPM. Therefore quercetin-gelatine hydrogel without VFD should be employed for Al^{3+}. However, in terms of swelling properties, it is recommended to employ the QG 4000 RPM, as it will have a better absorption capacitance and rate in holding the Al^{3+} solution in its polymer matrix when dropped. In terms of mechanical properties, it is ideal to employ QG 3000 RPM and QG 4000 RPM for product handling.

From this conclusion, it would be ideal to employ the gelatine-hydrogel solution in the VFD at 4000 RPM and later mixing the quercetin solution into the gelatine-hydrogel solution after thorough mixing to obtain a Quercetin-Gelatine Hydrogel with ideal fluorescence, swelling and mechanical properties for Al^{3+} sensing.

FUTURE RESEARCH DIRECTION

From the literature review, studies have shown that a specific ratio of gelatine and starch can optimize the hydrogel to have an ideal mechanical property. Therefore, a future direction includes adding starch (i.e. 10 % content) and gelatine (i.e. 10:2 content) to the quercetin-gelatine hydrogel via VFD to improve the mechanical properties and potentially the swelling properties as well. (Tavakoli, 2017, Van Nieuwenhove et al., 2016, Wang et al., 2019)

It is proposed that quercetin is very unstable in some organic solution (e.g. acetonitrile and methanol) in the literature review. Therefore, another recommendation is to use Tetrahydrofuran (THF) as a solvent and repeat this experiment to identify whether the fluorescence properties of quercetin enhances. (He et al., 2018b)

Quercetin has been proven by researchers that it has good antioxidant, anti-inflammatory, anti-bacterial, biocompatibility properties and therapeutical values that are often used to treat wounds, due to the aid of increasing fibroblast proliferation while decreasing fibrosis and scar formation. (Althans et al., 2014, He et al., 2018a, He et al., 2018b, Kelly, 2011, Nathiya et al., 2014, Vedakumari et al., 2017, Rubini et al., 2020) Another possible future direction is to further investigate on tuning its therapeutical properties and employ this research product as multipurpose Al^{3+} detecting, drug delivery and wound dressing applications.

As mentioned in the conclusion, it would be ideal to employ the gelatine-hydrogel solution in the VFD at 4000 RPM and later mixing the quercetin solution into the gelatine-hydrogel solution after thorough mixing to obtain a Quercetin-Gelatine Hydrogel with ideal fluorescence, swelling and mechanical properties.

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