



Faculty of Resource Science and Technology

**PREPARATION AND CHARACTERIZATION OF CURCUMIN-LOADED  
CELLULOSE NANOPARTICLES**

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**(Resource Chemistry)**

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## **Declaration**

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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## List of Abbreviations

<u>Symbols</u>	<u>Descriptions</u>
ASAM	Alkaline sulfite anthraquinone methanol
CNPs	Cellulose nanoparticles
CCNPs	Curcumin-loaded cellulose nanoparticles
EDA	Electron donor-electron acceptor
FTIR	Fourier Transform Infrared Spectroscopy
GMO	Glycerol monooleate
HSA	Human serum albumin
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ILs	Ionic Liquids
NMMO	N-methylmorpholine-N-oxide
NPs	Nanoparticles
NTU	NaOH/thiourea/urea
PCL	Poly- $\epsilon$ -caprolactone
PLA	Poly(lactic acid)
PLGA	Poly lactic-co-glycolic acid
SEM	Scanning Electron Microscope

TEM                      Transmission Electron Microscope

UV                        Ultra-Violet

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# **Preparation and Characterization of Curcumin-loaded Cellulose Nanoparticles**

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**Supervisor: Prof. Dr. Pang Suh Cem**

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# Preparation and Characterization of Curcumin-Loaded Cellulose Nanoparticles

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## ABSTRACT

Curcumin possesses diverse pharmacological activities such as anti-inflammatory, anti-carcinogenic and antioxidant but its uses are limited by its poor bioavailability and low water solubility. The objective of this study is to prepare curcumin loaded cellulose nanoparticles and to optimise the loading efficiency and loading capacity of curcumin in cellulose nanoparticles. Curcumin loaded cellulose nanoparticles with a mean size of 114 nm and size range of 73-263 nm in diameter were successfully regenerated by using *in situ* nanoprecipitation technique. Different parameters such as ratio of cellulose solution to ethanolic curcumin solution, concentration of ethanolic curcumin solution, concentration of surfactant (Tween 80), and loading durations were controlled to examine the loading efficiency and loading capacity of curcumin in cellulose nanoparticles. The UV-visible spectra showed a characteristic absorption peak at 470 nm. The functional groups of chemical structure, morphology, and mean size of curcumin-loaded nanoparticles were characterized by using FTIR, SEM and TEM. 2.38 mg/mg loading capacity and 30.57% loading efficiency of curcumin were obtained using 1:6 (v/v) phase ratio with 0.1 mg/mL ethanolic curcumin solution within 1 hour loading.

**Keywords:** Curcumin, curcumin loaded cellulose nanoparticles, *in situ* nanoprecipitation

## ABSTRAK

Kunyit mempunyai pelbagai aktiviti farmakologikal seperti anti-inflamasi, anti-karsinogenik, dan antioksidan tetapi kegunaannya terhad oleh kelemahannya dalam bioavailabiliti dan kelarutan. Objektif kajian ini adalah untuk menyediakan nanopartikel selulosa yang dimuat dengan kunyit dan mengoptimumkan muatan kapasiti dan muatan efisiensi kunyit dalam nanopartikel selulosa. Selulosa yang dimuat dengan kunyit yang mempunyai min saiz 114 nm dan julat saiz 73-263 nm dalam diameter telah dihasilkan dengan menggunakan teknik *in situ* nanopemendakan. Pelbagai pembolehubah seperti nisbah larutan selulosa ke larutan etanolik kunyit, kepekatan larutan etanolik kunyit, kepekatan surfactant (Tween 80), dan masa muatan telah dikawal bagi memeriksa muatan efisiensi dan muatan kapasiti kunyit dalam nanopartikel selulosa. UV-visible spektra menunjukkan ciri-ciri puncak penyerapan di 470 nm. Kumpulan berfungsi untuk struktur kimia, morfologi, dan min saiz bagi selulosa yang dimuat dengan kunyit telah dicirikan dengan menggunakan FTIR, SEM dan TEM. 2.38 mg/mg muatan kapasiti dan 30.57% muatan efisiensi bagi kunyit telah dihasilkan melalui 1:6 (v/v) fasa nisbah dengan 0.1 mg/mL larutan etanolik kunyit dalam 1 jam muatan.

**Kata Kunci:** Kunyit, nanopartikel selulosa dengan muatan kunyit, *in situ* nanopemendakan

## 1.0 Introduction

Curcumin is an extract of yellow polyphenol derived from the rhizome of turmeric, *Curcuma longa* (Hong *et al.*, 2011). Curcumin has attracted significant interest particularly for its pharmacological activities such as anti-inflammatory, anti-carcinogenic and antioxidant (Yallapu *et al.*, 2012). Previous studies have shown that curcumin are safe to be consumed even in large quantities (Rajasekaran, 2011). Regardless of these promising properties, the utilization of curcumin in clinical applications and development has not been completely achieved due to its weaknesses related to poor bioavailability and low water solubility. To overcome these aspects of curcumin, nanoparticle-based drug delivery systems have been developed to discover its potentiality due to its enhanced water solubility. Although curcumin is safe to be consumed by human beings, the efficacy of curcumin-loaded synthetic polymers has not been revealed (Pang *et al.*, 2014).

Lignocellulosic materials is defined as plant materials that are composed of hemicellulose, cellulose, and lignin (Sjostrom, 1993). Cellulose can be found in lignocelluloses of wastes streams, or in papers as partially purified cellulose, or in cotton as pure cellulose, or in citrus wastes as unpurified cellulose (Talebnia *et al.*, 2008). Environmental problems could be caused by the improper waste management of lignocellulosic materials. Hence, the conversion of lignocellulosic materials or waste papers into useful nano-scaled particles is a cost-saving approach for landfill wastes reduction.

Over the past decades, cellulose nanoparticles draw more and more research interest due to their low cost, low density, low toxicity, high water holding capacity, high crystallinity, high ratio of strength to weight, good processability and renewability (Yang *et al.* 2013; Zhang *et al.* 2011). Jantarat (2013) reported that various kind of materials have been used as nanoparticle carriers such as poly lactic-co-glycolic acid (PLGA), human serum albumin (HSA), chitosan, poly- $\epsilon$ -caprolactone (PCL), and glycerol monooleate (GMO). Due to some

of the promising characteristics such as solid state solubility, compatibility, biodegradability, and versatile degradation kinetics, PLGA is the most common materials being used for drugs encapsulation (Jantarat, 2013). However, usage of cellulose as nanoparticle carriers for drugs entrapment has remained uncertain.

According to Chidambaram and Krishnasamy (2013), polymeric nanoparticles can be produced by nanoprecipitation, dialysis, solvent evaporation, ionic gelation, salting out, nano spray drying, desolvation, and supercritical fluid process. Nevertheless, nanoprecipitation was a better choice because it is the easiest and cheapest approach to prepare polymeric nanoparticles.

In this study, the central goal is to prepare curcumin-loaded cellulose nanoparticles by loading curcumin into regenerated cellulose from paper waste or lignocellulosic wastes. Printed paper wastes were pretreated with alkaline pretreatment and dissolved in NaOH/thiourea/urea (NTU) aqueous solution. The curcumin loaded cellulose nanoparticles were regenerated using *in situ* nanoprecipitation method. Different parameters such as ratio of cellulose solution to ethanolic curcumin solution, concentration of ethanolic curcumin solution, concentration of surfactant (Tween 80) and loading durations were studied to examine the loading capacity and loading efficiency of curcumin in cellulose nanoparticles. The functional groups of chemical structure, morphology, and mean size of curcumin-loaded nanoparticles were characterized by using FTIR, SEM and TEM. The absorbance of absorption peak were determined at 470 nm using the UV-visible spectrophotometer.

## **1.1 Problem Statement**

Curcumin exhibits poor bioavailability and low water solubility. Environmental problems could be caused by the improper waste management of lignocellulosic wastes. Therefore, research on synthesizing cellulose nanoparticles through regenerating cellulose from paper waste or lignocellulosic wastes was carried out to encapsulate the curcumin in order to enhance its poor bioavailability and low water solubility.

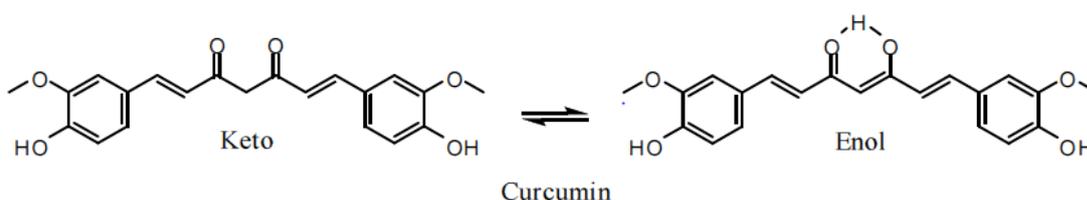
## **1.2 Objectives of Study**

1. To synthesize curcumin loaded cellulose nanoparticles using *in situ* nanoprecipitation method.
2. To optimize loading efficiency and loading capacity of curcumin in cellulose nanoparticles.
3. To characterize curcumin-loaded cellulose nanoparticles using UV-vis spectrophotometer, Scanning Electron Microscope, Transmission Electron Microscope and Fourier Transform Infrared Spectroscopy.

## 2.0 Literature Review

### 2.1 Curcumin

Shehzad *et al.* (2011) reported that curcumin was first extracted by *Vogel et al.* in 1815 and their diferuloylmethane structure was discovered by *Lampe et al.* in 1910. Curcumin is a natural diphenolic compound derived from the rhizome of the turmeric, *Curcuma longa* (Moorthil *et al.*, 2012). Due to its hydrophobic properties, curcumin has extremely low solubility in water (11 ng/mL) whereas it has high solubility in organic solvents like methanol, acetone, ethanol, and dimethylsulfoxide (Das *et al.*, 2010). Curcumin is chemically named as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) which exhibited keto-enol tautomerism in its bis- $\alpha,\beta$ -unsaturated  $\beta$ -diketone structure that formed a predominant keto form in acidic and neutral media and a stable enol form in alkaline as shown in Figure 1 (Jantarat, 2013).



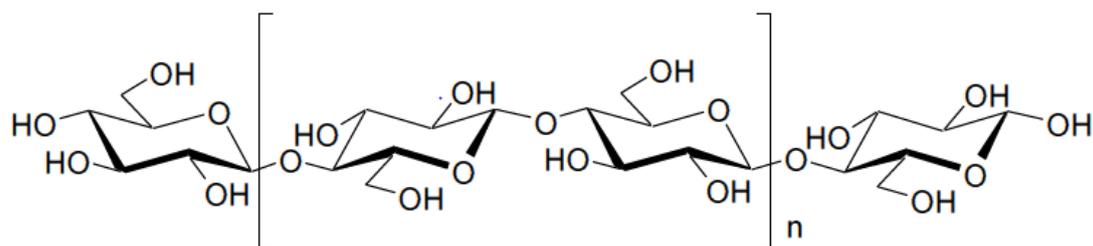
**Figure 1:** Keto-enol tautomerism of the chemical structure of curcumin (Jantarat, 2013).

Curcumin with molecular weight of 368.37 g/mol consists of a mixture of three curcuminoids which is mainly 77% curcumin, 18% demethoxycurcumin, and 5% bis-demethoxycurcumin (Yallapu *et al.*, 2013). Curcumin is more actively decomposed by first order degradation in acidic media than in neutral and alkaline media at 37°C (Wang *et al.*, 1997). On top of that, curcumin also undergoes photodegradation in the presence of oxygen and solvent system (Konatham *et al.*, 2011). Curcumin possesses wide variety of pharmacological effects, including anti-oxidant, anti-inflammatory, anti-cancer, antidepressant activities and wound healing properties (Sun *et al.*, 2013). In addition, studies

have demonstrated that curcumin possesses anti-cancer effects to cancer cells such as prostate, breast, pancreatic, colorectal, gastric and cervical carcinomas (Yallapu *et al.*, 2012). Curcumin is extremely safe even at very high doses because it has been found to be significantly higher in tumor cells than the uptake rate in normal cells (Dhule *et al.*, 2012). In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent. The major barriers to the clinical usefulness of curcumin in the treatment of cancer are poor bioavailability, poor aqueous solubility, highly degradable, and high metabolism rate (Yallapu *et al.*, 2011). To increase solubility and bioavailability of the curcumin, several efforts have been made through co-crystallisation, solid solution, co-solvency, cryogenic technique, eutectic mixture, floating granule, hydrotrophy, inclusion complex, micellar solubilization, microemulsion, micronization, pH adjustment, polymeric nanoparticle, self-emulsifying drug delivery system, solid dispersion, solid lipid nanoparticle, sonocrystallisation, super critical fluid process, microsphere, microcapsule and liposome (Chidambaram & Krishnasamy, 2013).

## **2.2 Cellulose**

According to Moran *et al.* (2008), cellulose has a long history in the pulp and paper industry, and nowadays, it draws more and more research interest in textile, material science and biomedical engineering due to its extensive characteristics such as broad chemical modifying capacity, chirality, hydrophilicity, biodegradability, and its capability of forming flexible semi-crystalline fiber morphologies. Cellulose constitutes the most abundant and renewable polymeric resource on the planet (Siqueira *et al.*, 2010). Siqueira *et al.* (2010) reported that cellulose fibers are generally formed by the cross-linked microfibrils structure. On top of that, cellulose is a linear polysaccharide polymer of glucose monomers that composed of  $\beta$ -D glucopyranose units bonded by  $\beta$ -1-4-linkage as shown in Figure 2 (Taherzadeh & Karimi, 2008).



**Figure 2:** Cellobiose repeat unit shown by the basic chemical structure of cellulose (Siqueira *et al.*, 2010).

Hydrogen bonds are formed by the hydroxyl groups found on the monomers that are used for determining the physical properties and crystalline packing of cellulose (John & Thomas, 2008).

According to Siqueira *et al.* (2010), there are two major polymorphs of cellulose which are cellulose I and cellulose II. Cellulose I is referred to the name of the native cellulose with crystalline structures. While, regenerated cellulose, namely cellulose II, is generally the precipitates formed in the alkali solutions (Siqueira *et al.*, 2010). Cellulose II is more thermodynamically stable than cellulose I because extra hydrogen bonding per glucose residue was formed within them (Saxena & Brown, 2005).

Basically, there are two forms of nanoparticles which are cellulose nanocrystals and microfibrillated cellulose (Siqueira *et al.*, 2010). Cellulose is insoluble in water due to its stabilized crystalline structures by the extensive intra and intermolecular hydrogen bonding (Thielemans *et al.*, 2009).

## 2.3 Cellulose Pretreatment

### 2.3.1 Pulping Process

Several basic approaches have been identified to isolate cellulose from raw materials into nano-scaled particles (Laskiewicz, 2000). Generally, presence of the lignin has restricted the separation of wood into its component fibers (Hubbe *et al.*, 2008). Thus, delignification methods such as pulping is necessary as first pretreatment for the preparation of cellulose nanoparticles. During the pulping process, slurry of paper mills and pulps which containing cellulose fiber was produced (Kumar & Velayutham, 2013). Pulps are prepared from wood by chemical and mechanical processes or semi-chemical pulping process. Mechanical pulping produced pulps mainly by refining of wood chips or by grinding of small diameter stem sections (Wegener, 1993). This can increase the size of pores and accessible surface area, and reduce the crystallinity and degrees of polymerization of the cellulose (Taherzadeh & Karimi, 2008). Semi-chemical pulping involved a two-stages pulping process, removing fibre-bonding substances or part of the lignin chemically and followed by separation of the fibres under mechanical refining (Manskinen *et al.*, 2011). There are three major types of chemical pulping process which are Kraft, sulfite and soda (Okwonna, 2013). However, Kraft pulping (sulfate pulping) and sulfite pulping are the more common types of chemical pulping being used recently. The main difference between the two types of chemical pulping is the types of solvent used in the delignification process (Brady *et al.*, 1998). Kraft process was introduced in Germany in 1879 and was initially applied to a Swedish mill in 1885 (Brady *et al.*, 1998). There are three main processes involved in Kraft pulping which are digestion, washing and chemical recovery process (Brady *et al.*, 1998). In digestion process, a hot solution of sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S) is used to solubilize the lignin component of the wood after progressively depolymerized and chemically substituted under pressure in a digester or pressurized vessel (Hubbe *et al.*, 2008). Washing

process is then carried out to separate the black liquor from the pulps. Eventually, sodium sulfide and sodium carbonate are recovered from the black liquor for reused. Brown pulps are generally produced by Kraft pulping. Thus, bleaching process is needed to lighten the pulps produced (Brady *et al.*, 1998). Sulfite pulping was developed in the United States in 1867 and started to be used in a mill by a Swedish chemist in 1874 (Brady *et al.*, 1998). A lighter pulp is produced by sulfite pulping compared to Kraft pulping. On top of that, most of the steps involved in sulfite pulping are similar to Kraft pulping. However, a mixture of sulfurous acid ( $\text{H}_2\text{SO}_3$ ) and bisulfite ion ( $\text{HSO}_3^-$ ) in the form of calcium, magnesium, sodium, or ammonium bisulfide instead of the mixture of NaOH and  $\text{Na}_2\text{S}$  is used to treat the wood chips in the digestion process of sulfite pulping (Brady *et al.*, 1998). This makes the main difference between sulfite pulping and Kraft pulping.

Sulfate pulping and sulfite pulping can produce pulps effectively. However, there are still some major disadvantages of sulfate pulping and sulfite pulping processes which are pollution, odour problems, and problematic bleaching (Wegener, 1993). Hence, alternative environmental friendly solutions of producing pulps were developed such as Organocell process, alkaline sulfite anthraquinone methanol (ASAM) pulping and Acetosolv process.

Organocell process is the process of utilizing methanol-water mixtures and the addition of sodium hydroxide with anthraquinone as catalyst to produce pulps which are free of sulfur contents (Wegener, 1993). In the bleaching process, oxygen/alkali, chlorine dioxide and peroxide instead of chlorine are being used as principal residual delignification agents. This implies that the use of chemical is reduced and eventually reducing the pollution problems. On top of that, the strength properties of pulps produced by Organocell process shows mostly between those of sulfite and sulfate pulps.

ASAM pulping was developed with some modification of sulfite pulping which involved alternative application of sodium carbonate and sodium hydroxide (Wegener, 1993). Pulps

produced by ASAM pulping has slightly better strength properties to Kraft pulps. The major advantages of the ASAM pulping compared to Kraft pulping is that it is easier and more effective bleachability in producing high brightness levels of pulps (Wegener,1993).

In 1992, a new concept of pulping process called Acetosolv was developed at Gschwend (Wegener,1993). Acetosolv process is a delignification process using diluted acetic acid followed by bleaching with oxidizing agents in acetic acid medium and eventually bleached with peroxyacetic acid or peroxide (Wegener, 1993).

Low yield of the cellulose is generally produced by the pretreatment of high pulping concentration. On top of that, concentration of the pulping medium also played an important role in affecting the density, thermal stability, porosity, moisture sorption capacity, degree of polymerization, loss on drying and fibers morphology of the obtained microcrystalline cellulose (Okwonna, 2013).

### **2.3.2 Alkaline Pretreatment**

In alkaline pretreatment, lignin and hemicellulose are significantly dissolved when the intermolecular ester bonds between xylan hemicelluloses and lignin or other hemicellulose are saponified (Zheng *et al.*, 2009). Besides that, Zheng *et al.* (2009) reported that the solubility of the cellulose and hemicellulose in alkaline pretreatment is improved by removing the acetyl and uronic acid substitutes on the hemicellulose. Due to the swelling of the lignocellulosic components, degree of polymerization and crystallinity are decreased, internal surface area is increased, structure of the lignin is destroyed, and lignin is separated from the carbohydrates in the alkaline treatment before undergoing mechanical process (Siro & Plackett, 2010).

On top of that, the deinking process is carried out more effectively by the increased electrostatic repulsion between the fibers and the ink particles due to the adsorption of

hydroxide ion while increasing the alkali concentration (Bajpai, 2014). Compared to high lignified softwoods, hardwoods that contain low lignin content is more affective in the alkaline pretreatment under lower temperatures and pressures (Zheng *et al.*, 2009). Siro and Plackett (2010) reported that the degradation of the cellulose is prevented by extracting the alkali carefully and followed by hydrolyzing only the surface of the fibers in order to extract the intact nanofibers. However, there is a drawback in alkaline pretreatment as salts produced cannot be recovered (Zheng *et al.*, 2009).

### **2.3.3 Acid Hydrolysis**

Taherzadeh and Karimi (2008) reported that there are two ways of acid pretreatment which are dilute-acid pretreatment and concentrated acid pretreatment. According to Taherzadeh and Karimi (2008), dilute-acid pretreatment is generally operated under a high temperature while concentrated-acid pretreatment is operated under low temperature. Although concentrated-acid pretreatment is more effective than dilute-acid pretreatment, it is very corrosive and dangerous (Taherzadeh & Karimi, 2008). Siqueira *et al.* (2010) reported that nanowhiskers are generally extracted from the crystalline regions of cellulose via acid hydrolysis process. Besides that, the amorphous regions of cellulose are disrupted by the acid attack in order to extract the cellulose nanocrystals (Siqueira *et al.*, 2010). Siqueira *et al.* (2010) stated that this is due to the hydrolytic cleavage of the glycosidic bonds through the penetration of hydronium ion into the amorphous regions. Shorter monocrystals with increased surface charge are produced by longer exposure time to the acid hydrolysis (Siqueira *et al.*, 2010).

According to Siqueira *et al.* (2010), sugar molecules are produced by the digestion of the cellulose under longer time of reaction. However, large aggregates of fibers are produced by shorter time of reaction. Besides that, more stable aqueous suspensions are generated by the

use of sulfuric acid rather than hydrochloric acid (Siqueira *et al.*, 2010). Siqueira *et al.* (2010) reported that negatively charged surface of cellulose nanocrystals are induced during the use of sulfuric acid. This is because charged sulfate group are formed by the esterification of the surface hydroxyl groups (Siqueira *et al.*, 2010).

## **2.4 Dissolution of Cellulose Fibers**

Cellulose is not soluble in common solvents and only can be melted by thermal degradation due to the strong inter- and intra-molecular hydrogen bonding (Jin *et al.*, 2007). Dissolving of cellulose requires the solvent molecules to intrude into the core of the cellulose crystal and break the inter-chain hydrogen bonding (Moran *et al.*, 2008). Traditionally, cuprammonium and viscose technology were used to produce cellulose fibers and films (Jin *et al.*, 2007). However, heavy metal residues from cuprammonium technology can lead to the environmental problems. Besides that, viscose technique involved the usage of harmful CS<sub>2</sub> and releasing of H<sub>2</sub>S to the environment (Jin *et al.*, 2007). Hence, Lyocell production which uses organic solvent N-methylmorpholine-N-oxide (NMMO) is developed for replacing viscose technology to produce Lyocell fibers (Moran *et al.*, 2008). However, this technology also produces undesired by-products and requires high cost to recover the solvent used.

### **2.4.1 NaOH/Urea and NaOH/Thiourea Aqueous Solutions**

More recently, NaOH/urea and NaOH/thiourea aqueous solutions are introduced to enhance the cellulose solubility quickly and directly. In NaOH/urea aqueous solution, 7-9 wt% NaOH aqueous solution is used for dissolving cellulose at low temperatures near 4°C due to the distortion of intermolecular hydrogen bonds (Zhou *et al.*, 2000). On top of that, Zhou *et al.* (2000) reported that intermolecular hydrogen bonding of polysaccharides were destroyed by the presence of urea. Zhang *et al.* discovered that compositions of 6% NaOH/4% Urea, 7%