Synthesis and formulation of curcumin nanoparticles

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ABSTRACT: Abstract Nanoparticles comprised of carboxymethyl cellulose acetate butyrate (CMCAB) complexed with the poorly soluble drug Curcumin was produced by a rapid precipitation process and the formulation process and properties of nanoparticles were investigated. Two different particle synthesis methods were explored – a conventional precipitation method and a rapid precipitation in a multi-inlet vortex mixer (MIVM). The particles were processed by rotavapor followed by freeze drying or hot air oven drying. Particle diameters as measured by dynamic light scattering were dependent on the synthesis method used. The conventional precipitation method did not show desired particle size distribution. Whereas, particles prepared by the mixer showed well defined particle size ~ 130-300 nm before and after freeze drying respectively with lower polydispersity indices. Fourier-transform infrared spectroscopy showed chemical stability and intactness of entrapped drug in the nanoparticles. Differential Scanning Calorimetry showed that the drug was in amorphous state in the polymer matrix.

KEYWORDS: Curcumin; Polymer Drug Nanoparticles

INTRODUCTION [1-12]

There are many options for increasing the bioavailability of poorly soluble drugs like adding of ionized salts, solid dispersions, micronization technique, soft-gel technology etc.,. These methods have their own limitations in terms of drug loading capacity, toxicity, biodegradability, large dosages and environmental considerations. In the recent years, nanotechnology has emerged as a promising field in addressing these issues. One of the most active research areas of nanotechnology is nanomedicine, which applies nanotechnology to highly specific medical interventions for the prevention, diagnosis and treatment of diseases. Currently, nanomedicine is dominated by drug delivery systems, accounting for more than 75% of total sales. To improve its bioavailability, several strategies were developed like transported targeted delivery, where drug is attached to a ligand which is a substrate for specific nutrient transporter, developing analogues of prodrugs etc., Carboxymethyl Cellulose Acetate Butyrate (CMCAB) is a mixed cellulose ester. It is insoluble in water, water - swellable when partially ionized, but more soluble in common organic solvents than CA or CAB. It is a stable carrier for metallic pigments. CMCAB as nanocarriers is one of the thrust areas of research in nanotechnology. Amorphous matrix formulations of CMCAB with different drugs were prepared and dissolution tests were carried out. These studies showed that CMCAB enhanced the stability of the amorphous drug with respect to crystallization both in solid state and in solution. It has been observed that amorphous drugs in solid dispersions of CMCAB have greatly enhanced solubility as well as very fast release. Studies conducted to determine the performance of CMCAB in drug delivery with different drug formulations showed zero order release. The other advantage is that, CMCAB based drug nanoparticles can be prepared very easily through different methods like co-precipitation, spray drying and film casting because of its good solubility in organic solvents. Considerable work has been done to formulate polymer-anticancer drug nanoparticles with synthetic or semisynthetic polymers. More recently, studies have shown that polysaccharides are very promising for oral drug delivery due to their affinity for complexing with a variety of drugs which can suppress drug crystallization, their relatively high glass transition temperatures, and their biocompatibility.

Research Methodology

Materials Fresh Curcuma xanthorrhoea Roxb. known in Indonesia as temulawak was obtained from traditional market in Bantul, Yogyakarta, Indonesia. Citric acid (analytical grade, Sigma Aldrich) was used for raw material preparation, meanwhile technical grade of ethanol (76%, General Labora) was used for extraction process.

Procedures

Curcuma xanthorrhiza Roxb. rhizome was peeled, cleaned, and then wet-ground into small pieces using blender. An amount of 150 gram of the ground rhizome was adjusted to different pH values, i.e. 3, 5, and 7, by adding citric acid. After that, Curcuma xanthorrhiza Roxb. rhizome was macerated with 400 mL ethanol 76% for 1 hour and continued to the extraction process in 70 co. for additional 1 hour using the extraction tools set as described by Harjanti (2008). The extract was then separated by filtration proccess and concentrated using rotary vaccum evaporator until the volume was reduced to 100 mL.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to see the significant differences and Tukey's post hoc test was performed to compare the data. The significant difference was considered when the p value was less than 0.05.

Results and Discussion

Optimization of Extraction Process

Citric acid was added to Curcuma xanthorrhoea Roxb. before extraction process to prevent the browning reaction. Citric acid was used to control the acid condition because it is an acid which is safe for food and medical application as it is listed in Food and Drug Administration (FDA) (Shukla et al., 2017). From the appearances, the final extracts have different colour as shown in Figure 1. The change of colour is obtained from the reaction of nucleophilic groups in the curcumin which act as a proton acceptor to the positively charged hydrogen from dissociated citric acids. These finding highlights that altering the pH of curcumin extract into acid condition could decrease the possibility of curcumin degradation and prevent browning reaction. Curcumin stability decreases as the pH of the solution increases which further results in the formation of its degraded products such as ferulic acid(brownish-

yellow) and vanillin (Kuma vat et al., 2013). Figure 1 shows that increasing pH could change the colour of curcumin to be more brownish as a sign of curcumin degradation. When the colour remains yellow, curcumin stability in the presence of light is increased.

MATERIALS USED [13-16]

The Drug Used – Curcumin is present in turmeric also called as Curcuma longa. Curcumin is a natural poly- phenol.

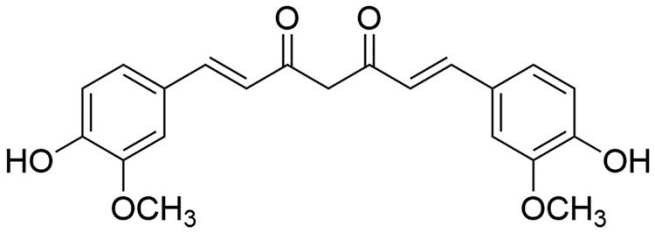

Fig: 1 Structure of curcumin

The structure of Curcumin Low absorption and poor bioavailability in animals and humans are the major challenges associated with using Curcumin. To combat the challenge, a number of nano-based approaches are being developed to improve curcumin's bioavailability and reduce perceived toxicity. These approaches include solid-lipid nanoparticles, nanosuspension, nano emulsion, cyclodextrin curcumin self-assembly, hydrogel nanoparticles, curcumin-phospholipid complex and curcumin incorporated within polymer nanoparticles. We have synthesized curcumin incorporated within polymer nanoparticles to combat the challenge.

Carboxy Methyl Cellulose Acetate Butyrate (CMCAB)

We have chosen CMCAB due to its unique properties like: CMCAB is insoluble in water, it is water soluble only when partially ionized. CMCAB is more soluble in organic solvent such as THF, ethanol, ethyl acetate or isopropanol. CMCAB also helps to keep constant drug release over 24 hours, and can be used to increase the solubility, and thus bioavailability of various drugs.

METHOD OF PREPARATION

We have utilised two main methods of synthesis:

- Nanoprecipitation Method or Conventional method.
- Flash Nanoprecipitation Method or MIVM method (Multiple Inlet Vortex Mixer).

Nanoprecipitation Method or Conventional Method In the conventional method,

first the drug and polymer were dissolved into the organic solvent, then this solution was added dropwise into the aqueous phase under the magnetic stirrer. Then keep it for overnight to remove the organic solvent completely under the magnetic stirrer and then the synthesized particles were processed by rotary evaporator followed by freeze drying or hot oven drying. The particle diameters as measured by Dynamic Light Scattering (DLS) were dependent on the drying method used. Drying is a major part of the synthesis. There are two methods of drying: Through Hot Air Oven and through Lyophilized or freeze drier. It was observed that by using conventional method of synthesis, undesired particle sizes were obtained.

After synthesizing these particles, we characterised them using the following:

- Particle Size by DLS.
- Drug Loading by UV –V is spectroscopy
- DSC to determine the amorphous nature of nanoparticle.
- FTIR for determining the interaction between the polymer and the drug.

Flash Nanoprecipitation Method (MIVM)

Flash Nano-Precipitation Provides:

- Micro-mixing that produces high super-saturation be accomplished in milliseconds.
- Timescale for block copolymer adsorption be tuned to the precipitation rate of the solute.
- If the polymer assembles too rapidly it forms micelles that deplete the concentration of stabilizing polymer and lead to poor particle stability.
- If the polymer assembly is too slow then the particles grow too large in size before adequate polymer is adsorbed and growth is quenched.
- Controlling time scales for micro-mixing, self-assembly, and nucleation and growth is the key to the success of the process. In the MIVM methods, the drug and polymer were carried out in four jet Multi Inlet Vortex Mixer that accommodates four streams. The THF solution of CMCAB was injected into the mixer along with the three other water streams. And the remaining process for drying was same as simple precipitation method.

Curcumin Nanoparticles for Brain Diseases

Curcumin chemical information [17]

Chemically, curcumin is a naturally polyphenol denominated (1E,6E)-1,7-bis(4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione) (Figure 1), which is isolated from the rhizomes of C. longa. From a structural point of view, there are three chemical entities in the molecule: two aromatic ring systems containing o-methoxy phenolic groups linked by a seven-carbon spacer consisting of an α, β-unsaturated β-diketone moiety.

Thermal analysis of curcumin [18]

Thermogravimetric analysis is a common complementary tool to describe this molecule. Therefore, we performed an evaluation of the thermal degradation of curcumin at a heating rate of 10 °C/min and under a nitrogen atmosphere. As can be observed in Figure 2a, the initial temperature of the mass loss is approximately 193 °C. This behaviour results in the decomposition of the turmeric powder; below this temperature, weight loss in the curcumin follows a gradual decrease related to the loss of moisture. In a complementary way, a differential scanning calorimetry Thermogram showed a melting temperature for curcumin of 174.05 ◦C, which is in agreement with data reported previously.

Ultraviolet-Visible Spectrophotometric Analysis of Curcumin [19,20]

The chemical reactivity and solubility of curcumin depends on the medium pH in which it is dissolved; that is, under acidic conditions, curcumin exhibits moderate solubility, and the solution maintains a yellow colour (Figure 3A), whereas at a neutral pH, curcumin is not fully soluble, as can be observed in Figure 3B. On the other hand, within the basic pH range, curcumin is more water-soluble than in the neutral form, and the colour of the solution changes to red (Figure 3C). The colour change under alkaline conditions could be an effect deriving from the deprotonation. It is known that the photophysical and photochemical properties of curcumin are related to the solvent's polarity because of the keto–enol structure of curcumin that involves intramolecular proton transfer. Kharat M. et al. mentioned the formation of condensation yellow products (such as feruloymethane) as a potential reason for this colour increment under an alkaline environment

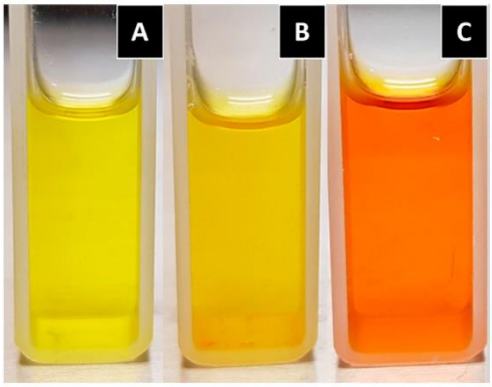

Fig: 2 Curcumin dissolved in different mediums.
(A) Curcumin in an acidic solution (pH 3.5); and (B) curcumin in a neutral solution (pH 7.4); both with the addition of 1% Tween 80 in order to increase solubility. (C) Curcumin in a basic solution (ph12)

Solubility [17]

Curcumin has poor solubility in water (an estimated of 3.21 mg/L at 25 ◦C); however, it is soluble in ethanol, dimethyl sulfoxide (DMSO), methanol, acetonitrile, chloroform, and ethyl acetate. The theoretical Hansen solubility parameters (HSP), which were calculated on the basis of the group contribution method, are δd = 17.46, δp = 3.66, δh = 13.84, and total = 22.46 for the enol form of curcumin [21]. The first pKa1 = 7.5–8.5 corresponds to the deprotonation of the enolic proton group, while pKa2 = 8.5–10.4 and pKa3 = 9.5–10.7 are for the phenolic protons, indistinctly. The log octanol–water partition coefficient (log Kow) is 3.29 (estimated), conferring hydrophobic characteristics on the molecule.

BIOLOGICAL ACTIVITY [21-25]

Curcumin has a broad spectrum of biological activities. For example, it has been reported as possessing antioxidant, anti-AD, anticarcinogenic, antimutagenic, and anti-inflammatory properties Curcumin also reduces low-density lipoprotein (LDL), and inhibits the oxidation of proteins and DNA. At the enzymatic level, curcumin inhibits lipoxygenase/cyclooxygenase and xanthine dehydrogenase/oxidase, which are two enzymes related to the generation of ROS, and upregulates superoxide dismutase and glutathione peroxidase, which are two first-line enzymes of defense against oxygen-free radicals. In AD, curcumin protects against Aβ-induced oxidative stress, prevents the formation and extension of Aβ fibrils, destabilizes Aβ fibrils, inhibits acetylcholinesterase, decreases neuroinflammation, and sequesters transition metals. A variety of structure–activity studies have proven that the three moieties in the chemical structure of curcumin play different roles in its interaction with the Aβ peptide: one of the hydroxyl substitutions in the aromatic end group is necessary for inhibition, while the other one of the hydroxyl substitutions is required for activity. Finally, the diketo chain contributes to the flexibility and correct length between aromatic rings. Curcumin has been extensively evaluated and possesses potential antioxidant and anti-inflammatory activity in AD. The most important mechanism of the anti-inflammatory action of curcumin is based on the inhibition of NF-kB, which leads to the decreased formation of cytochemokines and Aβ fibrils. Other molecular targets inhibited by curcumin are inducible nitric oxide synthase (iNOs), c-Jun N-terminal kinase (JNK) activation, and activating protein-1 (AP-1). In anticancer therapy, curcumin inhibits oxidative stress, reduces lipid peroxidation and DNA single-strand breakage, inhibits the COX-1 and COX-2 enzymes, suppresses NF-kB activation, and possesses antiproliferative effects. Moreover, it induces apoptosis by targeting mitochondria, and affects tumor protein p53 (p53)-related signalling. The specific molecular targets for curcumin that are therapeutically important in cancer-signalling pathways include cyclin-dependent kinases (CDKs), p53, Ras, phosphoinositide 3-kinase (PI3K), Protein kinase B (Akt), Wnt/β-catenin, and mammalian target of rapamycin (mTOR). During angiogenesis, curcumin can inhibit and/or downregulate the expression of various pro-angiogenic growth factors such as the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and the endothelial growth factor (EGF).

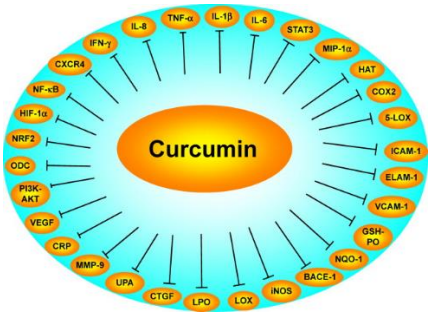

Fig: 3 Curcumin is a pleiotropic agent with multiple molecular targets. This molecule could modify the expression of genes, inflammatory cytokines, transcriptional and growth factors, enzymes, and receptors, among others.

Effect of Curcumin on Aggregation Protein [26-34]

Protein aggregation is the process by which misfolded proteins assume a conformation that cause their polymerization into aggregates and organized fibrils. The adequate aggregation of protein is a precise progression that requires extensive guidance from an excellent control network, which comprises approximately 800 proteins in humans. Many neurodegenerative diseases are associated with inappropriate protein aggregation. These neuronal diseases include disorders in which the aggregates may accumulate in the nucleus, such as for example in polyglutamine expansion diseases (such as spinocerebellar ataxias and Huntington's disease (HD)), which are pathologies that are characterized by inclusions in cytoplasm (for example, α-synuclein in PD), disorders in which the aggregates are found outside of the cell (prion diseases), or both intracellularly and extracellularly (such as Aβ in AD). The effect of curcumin on prion disease has been studied by several authors. Hafner-Bratkovi˘c et al. reported that the binding of curcumin to the α-intermediate could block conformational change into the β-structure, and that the binding of curcumin to prion fibrils could prevent further growth, thus, the formation of new seeds. Similarly, Caughey et al. concluded in their work that curcumin inhibits prion protein resistance (PrP-Res) accumulation in neuroblastoma cells infected with the scrapie agent. In addition, these authors reported the partial inhibition of the conversion of PrP into PrP-res. Additionally, Pandey et al. analyzed the curcumin effect both in vitro and in cell culture models of α-synuclein aggregation. The authors concluded that curcumin induces the inhibition of α-synuclein aggregation in a dose-dependent manner. Also, their results suggested that curcumin increased α-synuclein solubility in cells containing aggregates. The oligomerization of α-synuclein aggregates is structurally similar to the Aβ-protein aggregates of AD. Therefore, curcumin has been investigated as a potential AD treatment. Brahmkhatri et al. reported that curcumin-loaded gold nanoparticles inhibited Aβ aggregation, and that these were capable of dissolving aggregates. Likewise, Mithu et al. reported that curcumin disorganizes Aβ fibrils; the disruption of Aβ-fibrils was achieved by means of structural changes in the salt bridge region and near the C terminus. A more detailed report on the inhibition of Aβ aggregation revealed that, besides curcumin inhibiting fibril formation in vitro, it also inhibited the formation of Aβ oligomers and their toxicity in vivo. It has been reported that amyloid formation could be limited by mechanisms such as metal chelation and reducing the induction of the β-secretase enzyme (BACE1) by proinflammatory cytokines. It has been suggested that BACE1 has a main role in the initiation of the formation of Aβ; therefore, it is an attractive drug target for AD. The sequential proteolytic cleavage of the Aβ precursor protein (APP), which is a type I transmembrane protein, produced the formation of Aβ. Zhang et al. studied the interaction between curcumin and Aβ. These authors proposed the modulation of APP levels in the secretory pathway as the cellular mechanism by which curcumin reduces Aβ levels. In addition, they reported that the use of curcumin considerably increased the retention of immature APP in the endoplasmic reticulum. Furthermore, the authors suggested that APP endocytosis could be attenuated by treatment with curcumin. In order to identify the chemical features that are most important for preventing Aβ accumulation, Reinke et al. examined the effect of three features on the inhibition of amyloid aggregation: the presence of aromatic groups at both extremes of the molecule, the substitution pattern of these aromatics, and the distance and flexibility of the linker section. They demonstrated that the presence of just one single aromatic group did not decrease the protein aggregation; thus, the curcumin efficiency as an aggregation inhibitor could be related to its two phenyl groups. Also, their results suggested that the substitution of these aromatics groups is important for activity, since these are capable of taking part in hydrogen bonding. In addition, the authors reported the approximate distance between the docking sites, which are found between eight and 16 Å from each other; this is similar to the distance between the terminal aromatic regions of curcumin.

Conclusions

Curcumin is an example of an ancestral phytochemical whose health benefits have been confirmed and new applications have been discovered with a high impact for incurable diseases. The study of curcumin is one of the few cases of broad applications demonstrated under methodological principles and in a reproducible manner. Recently, the greatest impacts of this molecule are due to the novel technological proposals for its biological administration. In particular, the use of nanoparticles has made it possible to demonstrate benefits at the brain level, which can even revolutionize medicine. However, as with any chemical substance, it is convenient to emphasize toxicity issues in order to ensure the safety of all clinical trials. In addition to the clear results in brain models, the increase in benefits can be addressed with an improvement in the functioning of nanotechnological carriers.

Utilization of local Curcuma xanthorrhiza Roxb. (temulawak) to produce curcumin nanoparticle using solvent-antisolvent precipitation method was explored. Curcumin which has better stability and better nanoparticle size distribution can be produced by optimizing the extraction process and nanoparticle synthesize process. At pH value of 3, curcumin extract exhibited the expected yellow colour and the extract was also more stable to be compared to the extract obtained with other pH values. PSA result showed that all samples which were prepared at pH of 3, 5 and 7 contained nanoparticles in the range of 109.5 to 180.8 nm average size. Optimum stirring time for precipitation process was obtained at 45 minutes meanwhile the optimum stirring rate was obtained at 500 rpm.

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