Characterization of curcumin-loaded nano thermo-sensitive hydrogel

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Abstract. Breast cancer is one of the most common cancers affecting women. Breast cancer therapy is one of the targets of the Sustainable Development Goals (SDGs), namely reducing death rates and ensuring that people receive safe and quality medicines. This research aims to characterize thermosensitive nano hydrogel preparations with curcumin as a model drug based on the characteristics of the preparation. Thermosensitive nano hydrogels were formulated with various concentrations of curcumin, namely formulations F1 (10 mg/L), F2 (20 mg/L%), and F3 (40 mg/L). The results showed that the particle size in F I was 472 ± 2.50 nm; F II 423 ± 3.02 nm and F III 455 ± 4.81 nm statistically show a p value <0.05. Data from the zeta potential test results show FI -7.95 ± 1.00 mV, F II -12.47 ± 0.91 mV, and F III -13.33 ± 0.64 mV, with p-value < 0.05 and pH testing shows that F I is 4.73 ± 0.01; FII 4.76 ± 0.01; F III 4.83 ± 0.02, with p-value <0.05. Meanwhile, the stability test of the flask-sensitive hydrogel preparation showed a curcumin F I content of 97.6 ± 0.02%; F II 98.3 ± 0.015%; F III 98.9 ± 0.01%, with p-value <0.05. For the test data, the sol-gel transition time F I was 114 ± 2.08 seconds; F II 112 ± 4.58 seconds; III 154 ± 5.51 seconds with p-value <0.05. F I viscosity data 3,335.98 ± 374.11 cps; F II 2,734.62 ± 428.33 cps; F III 1,923.14 ± 149.86 cps with p value > 0.05. This study concluded that the concentration of poloxamer-407 could reduce particle size, polydispersibility index, zeta potential, and viscosity.

1 Introduction

Breast cancer attacks women with an incidence rate of 15% of all cancers [1]. The causes of breast cancer are influenced by genetic, hormonal, environmental, and reproductive factors [2]. Breast cancer therapy is carried out to prevent the development of cancer cells. This is in line with the special Sustainable Development Goals (SDGs) target number 3 relating to reducing the death rate by one-third due to cancer by 2030 achieving quality health services and providing easy access to quality medicines [3]. It is hoped that breast cancer treatment will be accessible to the public using various methods, such as surgery, radiotherapy, and chemotherapy. Chemotherapy is currently the main choice in breast cancer therapy [4]. Chemotherapy drugs such as 5-fluorouracil and cyclophosphamide can cause intensive and

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systemic side effects, such as anemia, liver toxicity, and hemorrhagic cystitis [5], [6]. For this reason, it is necessary to develop chemotherapy drugs that provide good therapeutic effects and minimize toxicity effects. One natural ingredient proven to have anticancer activity in the breast is curcumin.

Curcumin is an active substance found in turmeric plants (Curcuma longa. In addition to inhibiting the proliferation of breast cancer cells, curcumin induces programmed cell death (apoptosis) [7], [8]. It has been shown in vitro that curcumin induces the death of breast cancer cells via a p53-dependent pathway in MCF-7 (Michigan Cancer Foundation-7) cells [9]. Curcumin at 2 uM and 5 uM concentrations significantly induced MCF-7 cell death, according to research conducted by Wang [10]. A nano thermos-gel formulation of curcumin is used to increase its effectiveness in treating breast cancer.

Nano thermo-gels are a sol-gel transformation system that has nanoparticle size and is influenced by temperature to control drug release [11]. Nano-thermogels are formulated from curcumin and poloxamer-407 as anticancer agents. Due to chitosan's biodegradable nature, the body can eliminate it easily. Nanoparticles are created by crosslinking and stabilizing chitosan amine groups with sodium tripolyphosphate, which acts as a stabilizer and crosslinker. In addition, poloxamer-407 is expected to exhibit activity at body temperature, so if injected, localization will occur to reduce systemic distribution. Therefore, the development of curcumin nano thermo-gels preparations based on modified curcumin and poloxamer-407 aims to determine the formulation and physical properties of the preparation which are good for increasing the effectiveness of therapy on cancer cells thereby minimizing the occurrence of systemic side effects.

2 Material and Methods

2.1 Physical Properties Test of Curcumin Nano-Thermosensitive Hydrogels

2.1.1 Determination of Particle Size and Zeta Potential

The determination of particle size and zeta potential aims to ascertain nanoparticle size and particle charge. Particle size determination is carried out at a temperature of 25°C, with a refractive index of 1.42; and absorption of 0.001; using water as the dispersing medium with a refractive index of 1.330; and viscosity of 0.8872 cP. Disposable cuvets are used for particle size determination, while a zeta dip cell is employed for zeta potential testing [12].

2.1.2 pH Testing

pH testing is conducted on formulas I, II, and III at room temperature using a calibrated pH meter. Rinse the probe with distilled water and the sample to be tested. Take samples of formulas I, II, and III, then insert the probe into the sample, ensuring the electrode is submerged approximately 2 cm. Record the pH value after reaching a constant reading on the monitor [13].

2.1.3 Determination of Curcumin Concentration using UV-VIS Spectrophotometry

Determination of maximum wavelength: Prepare accurately diluted standard solutions, starting from concentrations of 1 to 5 ppm, then measure their absorbance at 425.6 nm using a Shimadzu UV-1900 Spectrophotometer. Ethanol is used as the blank [14].

Measure the absorbance of curcumin nano-sensitive hydrogels formulas I, II, and III at 425.6 nm using a Shimadzu UV-1900 Spectrophotometer. Ethanol is used as the blank. Perform triplicate measurements for each concentration, then use the equation y = bx + a to determine the actual concentration of the sample [14].

2.1.4 Gelation Time of Curcumin Nano-Thermosensitive Hydrogels

Testing For measuring gelation time, place formulas I, II, and III in a water bath maintained at 37°C ± 0.5°C. Record the gelation time when there is no flow observed upon turning the test tube upside down [15].

2.1.5 Viscosity Testing

Viscosity testing is performed using the Rheosys Merlin viscometer. A certain amount of curcumin nano-sensitive hydrogels to approximately 1 mm distance from the formulation. The temperature is set at 37°C, with a delay time of 30 seconds, integration time of 10 seconds, 5 steps, and spindle rotation speed ranging from 2 to 80 rpm. The obtained data is then used to determine the viscosity using the kv and fk values obtained from testing standard solutions, expressed in centipoise (cps) [16].

2.1.6 Data Analysis

Data analysis is conducted using descriptive analysis to determine the sample's standard deviation. ANOVA testing is performed using SPSS 25.0 for Windows after checking for homogeneity and normality.

3 Result and Discussion

3.1 Particle Size, Size Distribution, and Polydispersity Index Testing

Drug delivery in nanoparticle sizes must remain within the bloodstream for a sufficient duration. The size and surface characteristics of nanoparticles will govern the fate of the injected nanoparticles. The size of cancer cell blood vessels can vary from 100 to 600 nm [17]. Therefore, the ideal size of nanoparticles designed for cancer cell drug delivery should fall within the range of 100 to 600 nm. The results of particle size determination can be seen in Table 1, where the acquired data includes particle size diameter, particle size distribution, and polydispersity index.

Table 1. Particle Diameter; Size Distribution (d.nm); Polydispersity Index and Zeta Potential

Sample	Particle Diameter (d.nm) ($\overline{X}$ ± SD)	Size Distribution (n.m) $\overline{X}$ ± SD	Polydispersity Index $\overline{X}$ ± SD)	Zeta Potential (mV) ($\overline{X}$ ± SD)
Formula I	472 ± 2.50	218 ± 100.9	0.465±0.038	-7.95 ± 1.00
Formula II	423 ± 34.05	131 ± 48.46	0.589±0.093	-12.47 ± 0.91
Formula III	455 ± 3.02	550 ± 236.6	0.304±0.024	-13.33 ± 0.64

Based on Table 1, the results of particle size testing using PSA show that the polydispersity index (PDI) values fall within the range of 0.2-0.6. This range is considered good as it is below 0.7. PDI values are used to determine the uniformity and homogeneity of particles. A lower PDI indicates more uniform particle sizes. A high PDI value, if greater than 0.7, indicates highly polydisperse particles with a broad size distribution due to sedimentation and particle aggregation [18].

The results of particle size determination using PSA, as shown in Table 2, reveal that formula I have an average size of 472 ± 2.50 d.nm with a PDI of 0.465, formula II has a size of 423.0 ± 34.05 d.nm with a PDI of 0.589, and formula III has a size of 455.9± 3.02 d.nm with a PDI of 0.304. Based on Figure 8, the particle size testing results for formula I indicate the presence of floating particles and some lack of homogeneity, thus the particle size is represented by a distribution of particle diameter sizes: 218 ± 100.9; 131 ± 48.46; 550 ± 236.6 nm. From the particle size testing results, the average size of all formulations remains below 600 nm, making them ideal for cancer cell delivery.

In Table 1, it is observed that the zeta potential of curcumin nano thermos-sensitive hydrogels is negatively charged, with values around -7.95, -12.47, and -13.33 mV for formulas I, II, and III respectively. These values depict stable nanoparticles with fewer particle aggregations. Generally, particle charge is a determinant of stability, and zeta potential within the range of > +30 mV and < -30 mV is considered ideal for physical stability [19]. In this testing, chitosan with a concentration of 0.02% w/v was used, dissolved in 1% w/v acetic acid. A low concentration of chitosan requires a higher amount of NaOH to achieve a pH of 4.00 ± 2.5. Consequently, the addition of a base to the chitosan solution leads to the particles acquiring a negative charge. Additionally, the negative charge on the particle surface is due to curcumin, which is in a basic condition when in solution [20]. A negative zeta potential supports the specific accumulation of the formulation, enhancing the Enhanced Permeability and Retention (EPR) effect by avoiding recognition by the phagocyte system, such as macrophages and the immune system, due to low protein binding on the particle surface [21].

3.2 Physical Property Testing

The results of the physical property testing of the formulation, including pH, concentration, gelation time, and viscosity. Data on the pH of the curcumin nano thermos-sensitive hydrogel formulations are shown in Figure 1.

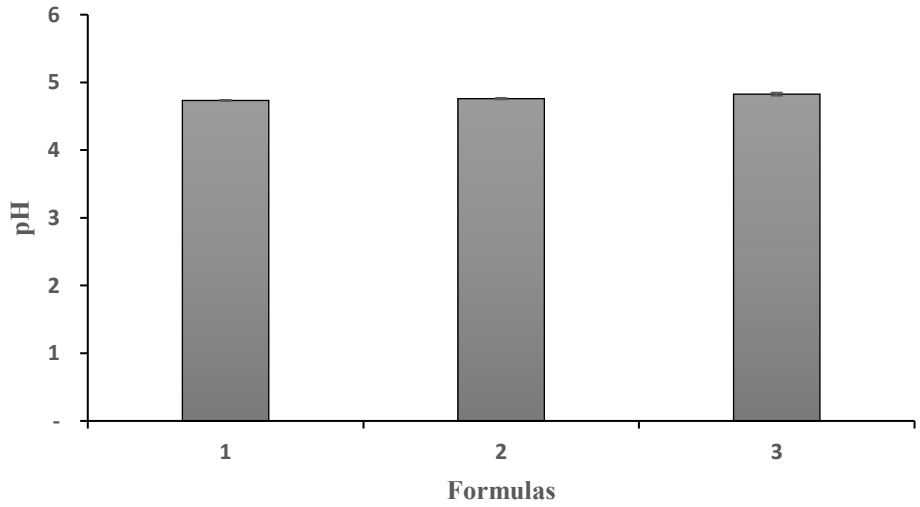

Fig. 1. pH of curcumin loaded thermosensitive hydrogels base.

Based on Figure 1, it can be observed that the pH in formulas 1, 2, and 3 is 4.73, 4.76, and 4.83, respectively, with the pH increasing progressively from formula 2 to formula 3. This will have an impact on drug penetration into cancer cells, as the pH in the cancer cell environment is slightly lower than in normal tissues due to the overexpression of matrix metalloproteinase (MMP) [22]. The acidic nature of the cancer cell microenvironment, where amino groups from the nanogel get protonated rapidly to induce electrostatic repulsion of the nano-gels and nucleus swelling for drug release [23]. Nanoparticles consist of amphiphilic polymers containing tertiary amine groups that can ionize for pH responsiveness [24]. Once the particles accumulate in cancer cells, the weakly acidic cancer cell environment triggers a sharp size transition. When the particles reach their target, the weakly acidic environment will drive PAMAM dendrimers, thus improving drug penetration efficiency and achieving localized drug delivery to cancer cells [25]. Therefore, it can enhance extravasation and particle/drug accumulation through the EPR effect, indicating efficient diffusion within cancer cells. The pH testing results of the nano-thermo-sensitive curcumin show an average pH of around 4, which is an acidic pH, making it suitable for drug delivery formulations to cancer cells.

Meanwhile, the statistical test results show that the Mann-Whitney test between formula 1 and formula 2 yielded a significance value of $0.046 \leq 0.05$, between formula 1 and formula 3 $0.046 \leq 0.05$, and between formula 2 and formula 3 $0.050 \leq 0.05$. The significant differences between these formulas are due to the increase in curcumin concentration in the nano-thermo-sensitive curcumin formulation, which leads to an increase in the pH value due to the deprotonation of curcumin, causing the loss of H+ ions and thus an increase in pH.

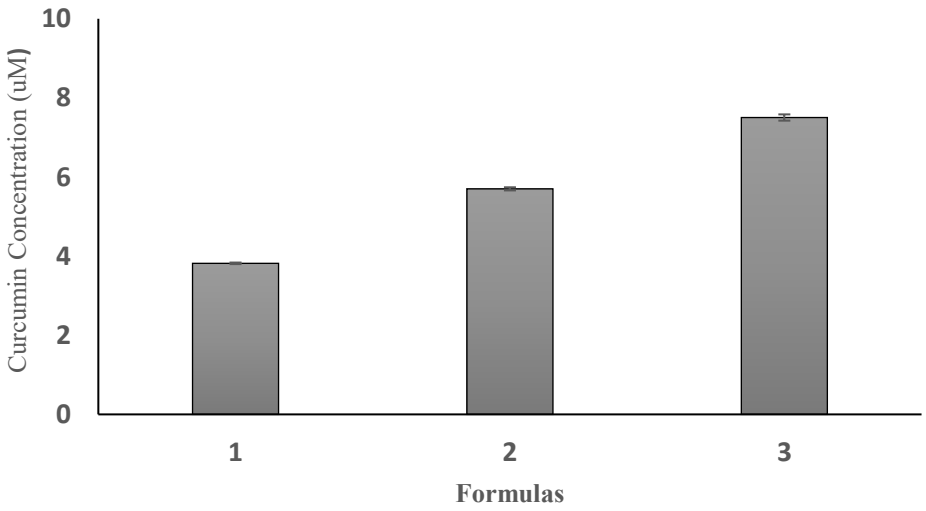

Fig. 2. Concentration of curcumin loaded thermosensitive hydrogels base.

The data Figure 2 showed a decrease in the curcumin content in the formulation after production. This is attributed to the degradation of curcumin due to light and heat exposure during the production process. The extent of curcumin degradation is as follows: in formula 1, it is 97.6%, in formula 2, it is 98.325%, and in formula 3, it is 98.9%. In the IC_{50} testing of curcumin nanoparticles, the concentrations of curcumin as an anti-breast cancer agent were found to be 3.80 μM and 5 μM for MCF-7 cells, 7.60 μM and 6.63 μM for MDA-MB-231 cells, and 6.81 μM and 10.58 μM for MCF10A cells [10], [26]. Statistical analysis showed that the pair sample t-test correlations yielded a significance value of $0.000 < 0.05$, indicating

a correlation between the curcumin content before and after production. Further analysis with the pair sample test revealed a significance value of 0.000 < 0.05, signifying a difference in curcumin content before and after the production process

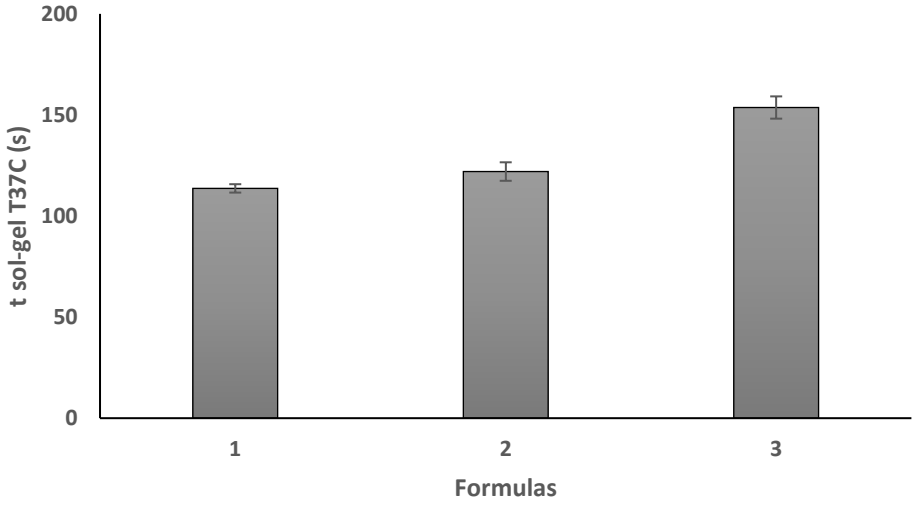

Fig 3. Tsol-gel time of curcumin loaded thermosensitive hydrogels base.

The data from the sol-gel testing in Figure 3. reveals that the nano-thermo-sensitive curcumin formulations in formulas 1, 2, and 3 form a gel at 37°C in 114, 122, and 154 seconds, respectively. Based on Figure 11, it's clear that the sol-gel time follows the order formula 1 < formula 2 < formula 3, indicating an increasing trend in the testing results. This characteristic ensures that the formulation forms a gel at the target cells quickly upon administration.

As shown in Figure 4, the formulation undergoes a transition from sol at 25°C to a gel at 37°C. The results of the gel time test at 25°C and 37°C can be seen in Figure 1. The blending of chitosan and poloxamer 407 aims to enhance reversible cross-linking in forming a hydrogel. Nanoparticles in the poloxamer hydrogel act to sustain the release of hydrophobic drugs, and the poloxamer hydrogel can act as a barrier to extend the drug release period [27].

Fig 4. Sol-Gel Transition of Nano-Thermogel Curcumin Formulation, (a) Sol at 25°C; (b) Gel at 37°C

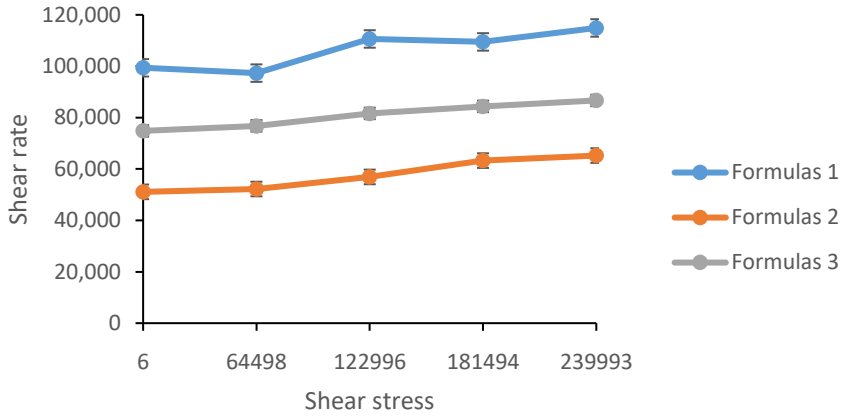

Fig 5. Viscosity of curcumin loaded thermosensitive hydrogels base.

Based on Figure 5, it demonstrates the flow behavior of the three formulations as being plastic. This is further confirmed by the calculation of the correlation coefficient (r) between log shearing stress vs.log shearing rate, which is smaller than the correlation coefficient (r) between shearing stress vs. shearing rate. Additionally, the rheogram does not pass through the point (0,0), known as the yield value, indicating a plastic flow type. The yield value is the minimum shear stress required to initiate flow. It occurs due to the contact between closely packed particles driven by van der Waals forces.

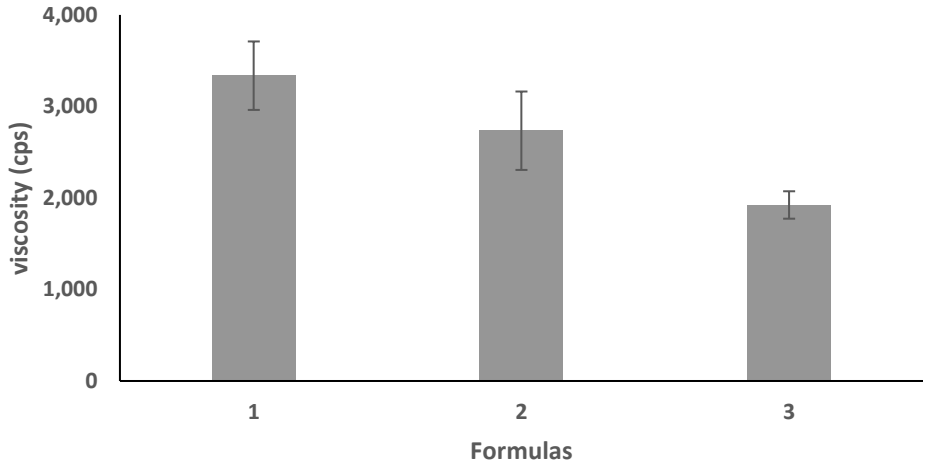

Fig 6. Shear rate vs. Shear stress viscosity test of curcumin nano thermosensitive hydrogels

Poloxamer 407 exhibits gel formation with increasing temperature and subsequently aggregates into micelles to minimize the free energy of the solution. At lower temperatures, Poloxamer 407 exists as monomers in the solution. The sol-gel transition temperature of Poloxamer 407 is defined as the point where the elastic modulus, G', is halfway between the values of the solution and the gel.

The concentration of polymer in the solution also affects viscosity. Higher viscosity in the formulation can increase the contact time between the formulation and the mucosal

membrane, thereby enhancing drug permeation (Ahmad et al., 2020). According to Table XIV, the viscosity of nano-thermo-sensitive hydrogels curcumin in formulas 1, 2, and 3 is 3,335.984; 2,734.623; 1,923.135 cps, respectively. The addition of more gel base, Poloxamer 407, increases the viscosity of the formulation. Therefore, the viscosity values of nano-thermo-sensitive hydrogel curcumin are in the order of formula 1 > formula 2 > formula 3. Poloxamer 407, being the thermosensitive gel base, thickens at higher temperatures.

These results indicate that Poloxamer 407 in water forms micelles due to hydrophobic interactions between poly(propylene oxide) molecules. Furthermore, as the temperature rises, the poly(propylene oxide) cores dehydrate. Conversely, the poly(ethylene oxide) shells, which are hydrophilic, hydrate, and swell, lead to the formation of a clear and robust hydrogel. Therefore, the higher the addition of Poloxamer 407, the higher the viscosity [22]. The viscosity testing results of nano-thermo-sensitive hydrogel curcumin show high viscosity, which is expected to increase the contact time of the formulation and drug permeation.

4. Conclusion

The physical characteristics of the thermosensitive hydrogel curcumin preparation formula are influenced by the comparative concentration of poloxamer 407 and chitosan, where the characteristics of formula II are better than formulas I and III.

5. Acknowledgments

The author would like to thank the financial support from the Grant of the Directorate of Research, Technology and Community Service (DRTPM) of the Directorate General of Higher Education, Research and Technology (Ditjen Diktiristek) of the Ministry of Education, Culture, Research and Technology (Kemendikbudristek).

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