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Preparation, characterization, and bioactivity evaluation of curcumin-loaded poly (lactic-co-glycolic acid) nanoparticles

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Abstract

Background: One of the main challenges with curcumin is its hydrophobic nature, which limits its solubility and bioavailability. This issue can be addressed by using poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs). The small size and large surface area of these NPs significantly enhance drug delivery systems by improving the solubility and bioavailability of the drug. **Objective**: This project focuses on the preparation, characterization, and bioactivity evaluation of curcumin loaded in PLGA NPs, intended for the delivery of curcumin extracted from *Curcuma xanthorrhiza*, commonly known as 'temulawak' or 'Java turmeric'. **Methodology**: Curcumin was extracted and stored at 4°C for testing. PLGA-curcumin NPs were synthesized using the single emulsion method. Nanoparticle morphology was analyzed using SEM, while particle size and zeta potential were measured with a Zetasizer. Entrapment efficiency and drug loading capacity were calculated. *In vitro* release studies in phosphate buffer were conducted using UV-visible spectrophotometry. The cytotoxicity of the curcumin-loaded NPs was tested on MCF-7 breast cancer cells using the MTT assay. Statistical analyses were performed using Minitab 14, and Microsoft Excel was used for graphical representations, with significance set at $p < 0.05$. **Results**: The mean particle size of the curcumin NPs was 498.9 nm ± 597.4 nm. The entrapment efficiency and drug loading capacity were 50% and 5%, respectively. The average zeta potential was recorded as -28.7 mV ± 6.19 mV. The *in vitro* release study did not produce significant results as low concentrations of curcumin were detected. However, the bioactivity of the curcumin-loaded PLGA NPs demonstrated lower cell viability compared to the curcumin extract, suggesting that the PLGA formulation is more effective at inducing cancer cell death. This indicates its potential as a more efficient therapeutic option in cancer treatment. **Conclusion**: The single emulsion method managed to produce nano-sized particles with good zeta potential and bioactivity on MCF-7 cells. However, further study needs to be done to produce better formulation which can increase entrapment efficiency, drug loading capacity and also *in vitro* release profile.

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Introduction

Nowadays, NPs have become one of the popular terms used in drug delivery systems. NPs are particulate materials with at least one dimension less than 100 nm (Khan et al., 2019). The small size and large surface area of NPs have exhibited great advantages in drug delivery systems such as increasing solubility of the drug which will increase the bioavailability of the drug. Many drugs show problems in their solubility which now can be counteracted with the technology of NPs. By using NPs, more specific drug-targeted activity can be achieved which will increase the therapeutic value of a drug for the consumers. Nano-curcumin has been found to be an ideal choice in drug delivery systems compared to free curcumin. Organs that curcumin can hardly reach can be accessed by nano curcumin (Karthikeyan et al., 2020). It was discovered that nano curcumin may have a greater capability for intracellular absorption than regular curcumin. Targeting intracellular microorganisms for infectious diseases requires this property as well. When compared to free curcumin, it has been found that nano curcumin has a higher level of systemic bioavailability in the plasma and tissues. When compared to the therapy of native curcumin in an investigation with rat models, nano curcumin delivers a 60-fold increase in the biological half-life, increasing the in vivo bioavailability and distribution of the tissues (Karthikeyan et al., 2020).

One commonly used copolymer in drug delivery systems is poly (lactic-co-glycolic acid) (PLGA). It possesses excellent biodegradability and biocompatibility, which has enabled it to gain approval from the Food and Drug Administration. Based on its name, PLGA is made from the copolymer of polyglycolic acid (PGA) and polylactic acid (PLA). In the field of production of bone substitute structures, the co-polymer PLGA is typically favored over its component homopolymers PLA and PGA because it provides better control over degrading qualities by adjusting the ratio between its monomers. The degree of crystallinity is decreased when the PLA is copolymerized with the crystalline PGA, which results in faster rates of hydration and hydrolysis (Lanao et al., 2013). The PLGA has also been used in the treatment of cancer, the healing of wounds, and for antibacterial, antioxidant, and anti-inflammatory purposes (Guo et al., 2023). The ratio between the two monomers will produce different types of PLGA. The most common type used is the ratio of 50:50. Since it is more hydrophobic than glycolic acid, lactic acid is often the monomer that is most numerous in PLGA. Lactic acid-rich PLGA polymers have slower rates of degradation and drug release. The copolymer with a 50:50 ratio is an outlier since it has the fastest breakdown rates and the shortest half-life (Lanao et al., 2013). The manufacture of PLGA particles involves several methods, such as single and double emulsion solvent evaporation (ESE), nanoprecipitation, spray-drying, microfluidics, and hydrogel templating (Garms et al., 2021). Bottom-up and top-down methods can be used to produce the NPs. When using top-down techniques, including emulsion solvent evaporation (single- or double-phase emulsions), pre-synthesized polymer chains, like PLGA, are used to physically produce the NPs (Operti et al., 2021). Meanwhile, PLGA NPs can be chemically synthesized using lactide and glycolide monomers through bottom-up techniques.

One of the favorable methods that can be used is the top-down method of single and double-emulsion solvent evaporation due to its simplicity and efficiency. Simply put, a polymer dissolves the medication or emulsifies it with a polymer in an organic phase before the aqueous phase is added. Particles are cleaned and collected using centrifugation after the solvent has evaporated in preparation for lyophilization and long-term storage (McCall & Sirianni, 2013). The benefit of this method is the flexibility to regulate the release kinetics of the substance that is encapsulated as well as particle size and polydispersity (Garms et al., 2021). The NPs are obtained by ultracentrifugation after the suspension is kept under stirring to allow the organic solvent to evaporate. This method provides for easy scaling up and enables particle size management.

Curcuma xanthorrhiza also known as *temulawak*, Java ginger, Javanese ginger, or Javanese turmeric

has been used traditionally in jamu (traditional medicine from Indonesia). According to data from 2019, *C. xanthorrhiza* was grown in Indonesia on a grand scale, with yields of 29,637,119 kg produced on a demolished harvested area of more than 13,042,873 m^2 (Rahmat et al., 2021). Javanese turmeric is known to possess various pharmacological activities such as antibacterial, antifungal, antioxidant, antihypertensive, antihepatotoxic, and antidiuretic properties (Salleh et al., 2016). This has become one of the factors which contribute to the usage of Javanese turmeric in jamu to treat certain diseases such as liver disease, anticancer, arthritis, and hypertension. The mRNA synthesis of pro-inflammatory mediators, including cytokines and related enzymes like cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), is reduced by curcumin (Hassanzadeh et al., 2020). Terpenoids and curcuminoids were shown to be the most prevalent essential phytochemicals in the *C. xanthorrhiza* rhizome, according to scientific investigations (Rahmat et al., 2021). Curcuminoid is a polyphenol substance which produces the yellow color of turmeric. The activity of curcumin also emerges from curcuminoid. From the rhizome of *C. xanthorrhiza*, several curcuminoids, including octahydrocurcumin, 1- hydroxy-1,7-bis(4-hydroxy-3-methoxypheny1)-6-heptene-3,5-dione, dihydrocurcumin, hexa-hydrocurcumin, curcumin, monodemethoxy curcumin, bis-demethoxy curcumin, 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4- hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,4-dione, 3-demethoxycyclocurcumin, and 1,7-bis(4-hydroxy-3- methoxyphenyl)-heptane-3,5-diol has been successfully extracted and classified (Rahmat et al., 2021). Curcuminoids make up about 2%–6% (w/w) of turmeric. In the latter, there is 2% bisdemethoxycurcumin, 18% demethoxycurcumin, and 80% curcumin (Gupta et al., 2013). The active substance of curcumin which can be found in Javanese turmeric possesses one problem which is hydrophobicity. One of the main problems with turmeric or curcumin is it possesses a hydrophobic characteristic which makes it possess low solubility which will reduce the availability of curcumin in drug delivery systems (Dei Cas & Ghidoni, 2019; Karthikeyan et al., 2020). Curcumin has a log P of 3.2, thus it is nearly insoluble in water. Curcumin possesses 30nM water solubility (Hegde et al., 2023). The aim of this experiment is to develop and evaluate curcumin-loaded PLGA NPs to enhance the solubility, bioavailability, and therapeutic efficacy of curcumin extracted from *Curcuma xanthorrhiza*.

Materials and methods

Curcuma xanthorrhiza (obtained from Mega Mendung, West Java, Indonesia) in September 2023, PLGA Lactide: Glycolide (75:25, MW: 66000-107,000) (Sigma-Aldrich), Dichloromethane, PVA (1% w/v) (Sigma-Aldrich), deionized water, sonicator (Qsonica), centrifuge (Supra 22K), analytical balance, spatula, filter paper, stainless steel grinder (XINGANBANGLE 3000W), rotary evaporator (BÜCHI Rotavapor R-205), Zetasizer, UV-vis spectrophotometer, MTT [3-(4, 5-dimethythiazol-2-yl)- 2,5- diphenyl tetrazolium bromide] assay, phosphate buffer saline, Dulbecco's Modification of Eagle's Medium, TrypLE Express (Gibco), 75 cm^2 tissue culture flask (Avantor), 25 cm^2 tissue culture flask (SDL), Tamoxifen (Sigma-Aldrich).

Extraction of Curcuminoids

The extraction of curcuminoids was done using the methodology of maceration with the aid of stirring. 58.6 g of turmeric rhizome powder sample was used and put into the beaker and 150 ml of ethanol 95% was added and the curcumin within the turmeric rhizome powder was extracted into the ethanol. This process was left for 1 day. The next day, the mixture was filtered using filter paper, and the dark brown extract was collected. The remaining powder was extracted two more times using the same solvent with the aid of a magnetic stirrer and left to stir for 2 hours. The collected extraction was cooled, condensed separately under reduced pressure using a rotary evaporator, and kept at 4°C prior to further testing.

Preparation of Curcumin Loaded PLGA

The PLGA-curcumin NPs were prepared using the single emulsion method retrieved from Arzani et al. (2018) with slight modification.100 mg of PLGA lactide: glycolide (75:25, MW: 66000-107,000) was dissolved within 10 ml of dichloromethane. 10 mg of C. xanthorriza extract was added to the PLGA-DCM mixture and allowed to dissolve completely. 100 ml of PVA (1% w/v) in water was introduced with a PLGA-curcumin mixture drop by drop while intermittent vortexing at a high setting. The mixture was sonicated at 40 amplitude for three minutes to form a fine emulsion after the PLGA-curcumin had been fully incorporated. The resulting dispersion of NPs was rotated on a magnetic stirrer at 800 rpm for 4 hours. The NPs were gathered by centrifugation for 25 minutes at 15000 rpm and washed 3 times with deionized water. The NPs were freeze-dried. The produced NPs were kept in storage until further use.

Evaluation and characterization of curcumin-loaded PLGA and bioactivity.

Morphology study using scanning electron microscope

The morphology of PLGA-curcumin NPs was observed using an SEM (Carl Zeiss AG - EVO®50) for particle visualization. Greater beam intensities may cause the sample to heat up locally, changing the particle's surface shape. At a magnification of 200X, microparticles may be viewed, and at 3,000X, they can be distinguished. The sample was coated with gold using an ion sputter after it had dried to the point of coating. This method was retrieved from McCall & Sirianni, (2013).

Nanoparticle size and zeta potential measurement

The size and polydispersity index of the nano-formulated curcumin particles were determined using a Zetasizer. The prepared NPs (1 mg/50 ml) at 40 W and 4°C for 10 minutes. Every measurement was made three times, and the average of the measurement was taken.

Determination of Entrapment Efficiency and Drug Loading Capacity

10 mg of curcumin NPs were diluted in 10 milliliters of distilled water and put into centrifugation tubes, which were then filled with 30 milliliters of water and centrifuged for thirty minutes at a speed of 10,000 rpm. This method is retrieved from Arozal et al., (2022). The EE and DLC were computed after obtaining the sample weights. EE calculation:

$$\text{Entrapment efficiency (EE)} = \frac{(C0-C1)}{C0} \boldsymbol{x}\ \mathbf{100}\% \qquad (1)$$

C0 = the weight of the active compound at first (mg)

C1 = free active compound weight (mg)

Calculation of DLC:

$$\text{Drug Loading Capacity: } \frac{(C0-C1)}{Ctotal} \boldsymbol{x}\ \mathbf{100}\% \qquad (2)$$

C0 = weight of active compound at first (mg)

C1 = free active compound weight (mg)

Ctotal = total weight of NPs (mg)

The yield of curcumin NPs was also calculated using the formula:

$$\text{Yield (\%)} = \frac{(m\ drug + m\ polymer)}{(m\ nanoparticles)} \boldsymbol{x}\ \mathbf{100}\% \qquad (3)$$

In vitro release of curcumin

A 500 mL container is filled with a quantity of the dissolution medium, which is 0.1 M phosphate buffer at 25℃ and pH 6.8. The dialysis membrane which contained 5 ml of curcumin NPs was inserted into the dissolution tester with a rotation of 50 rpm. The stopwatch was set in motion simultaneously, and the 3 ml of samples were taken at intervals of 15, 30, 45, 60, 90, 120, 150, 180, and 360 minutes. 3 mL of samples were taken from the area in the middle of the container, at least 1 cm from the wall's surface, and replaced by the same amount of fresh PBS. Every sample taken from the above time points was measured using a 1900-UV UV-visible spectrophotometer (Shimadzu, Japan) set to 423 nm.

The derived linear regression equation was then used to compute the concentration. Curcumin concentrations were also determined based on the amount of curcumin that was liberated from the matrix during testing. This method is retrieved from Arozal et al., 2020.

Cytotoxicity assay

The cytotoxicity study was carried out on breast cancer cells (MCF-7). The viability of the cells (3 × 105/well) was examined using the MTT [3-(4, 5-dimethythiazol-2-yl)- 2,5- diphenyl tetrazolium bromide] assay. This method is retrieved from Gnanamangai et al., (2019). The cells were sown in 96-well plates and cultured in a CO_2 incubator overnight. Following the application of curcumin extract and curcumin-loaded PLGA at a variety of concentrations, the cells are rinsed with phosphate buffer saline (PBS). Untreated cells are regarded as negative control and the cells treated with Tamoxifen (Sigma Aldrich) are considered as positive control. Then, each well received 20µl of MTT (5 mg/mL), and the wells were incubated at 37 °C for 3 to 4 hours. 100 µl of dimethyl sulfoxide (DMSO) was used to dissolve the purple and blue MTT formazan precipitate. The absorbance was measured using the ELISA reader at 570 nm wavelength. The following formula was used to determine the triplicate values:

$$\% \text{ of cell viability} = (\text{OD of treated cells}/\text{ OD of control cells}) \times 100 \quad (4)$$

Statistical Analysis

The mean ± SD was used to present the data. P-values below 0.05 were regarded as significant. Statistical analyses were performed using Minitab 14. Microsoft Excel was used to create graphical representations.

Results and discussion

Evaluation and characterization of curcumin-loaded PLGA and bioactivity.

Morphology study using scanning electron microscope.

The SEM examination results are shown in Figure 1 which is 1a: the NPs at 1000X, 1b: the NPs at 2000X magnification, 1c: the NPs at 3000X magnification and 1d: the NPs at 3000X magnification. The SEM image of these curcumin-loaded PLGA shows a round and spherical shape.

Because emulsion-based production processes are easier to process and optimize, they are preferred for the formation of polylactic acid NPs (PLGA NPs) for drug delivery applications (Garms et al., 2021). With the usage of a single emulsion method, the particles within the range of nanometers managed to be obtained. The submicron-sized particles function as carriers that mediate the drug model so that the drug goes straight to the destination in drug delivery models with controlled-release targets (Arozal et al., 2020). However, some particles are micrometers in size. This can be due to the formulation process. The molecular weight of PLGA used in this experiment is big which is 66000-107,000 Da. This produced polydispersity in the size of the particles produced. Another factor that can affect the production of microparticles is the possibility of an error in the single emulsion method, leading to non-uniform particle sizes. In addition, a short sonication time may increase the polydispersity of the particles. According to Halayqa & Domańska (2014), increasing the concentration of PLGA leads to larger NPs for both drugs, likely due to the higher viscosity of the organic phase. This increased viscosity reduces shear stress, resulting in larger droplets during emulsification. Moreover, higher viscosity reduces the dispersion of the organic phase into the aqueous phase, further contributing to larger nanoparticle size. Increasing the drug amount in the organic phase results in larger NPs, and higher sonication power significantly affects droplet size, leading to larger NPs and increased polydispersity (Halayqa & Domańska, 2014). By increasing the sonication power, temperature increases promoting droplet coalescence and subsequent nanoparticle size increase. From the research, it can be hypothesized that by reducing the concentration of PLGA, reducing amounts of drug in organic phase and low sonication power can reduce the size of the NPs.

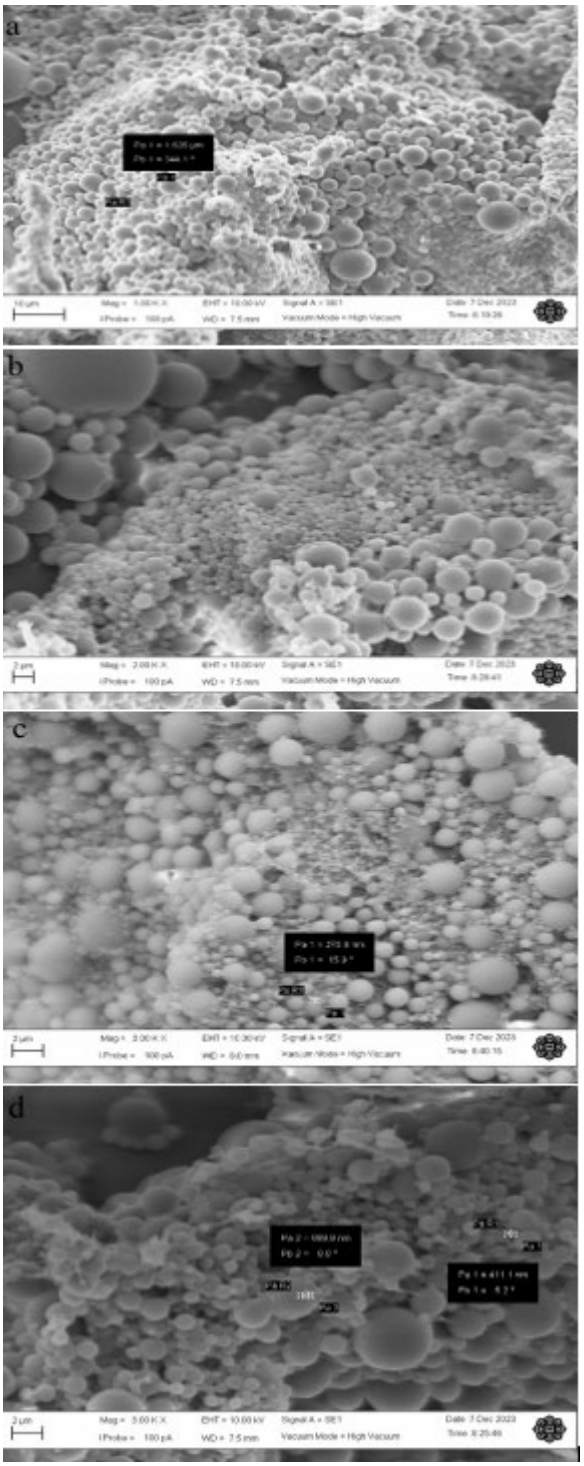

Fig. 1: Scanning electron microscopic images of curcumin-loaded PLGA NPs. 1(a) shows the NPs at 1000X. At this magnification, microparticle-sized PLGA can be seen with a size of 1.626 µm. At 2000X magnification (1b), smaller size molecules can be seen alongside big molecules (micro molecules). At 3000X magnification (1c and 1d), nanosized particles become more apparent with sizes ranging from 270.9 nm to 669.9 nm, respectively.

It is also stated in research done by Garms et al., (2021) that when comparing samples generated at a given concentration, SEM showed greater NP aggregation than TEM did. As a result, particles smaller than 50 nm were not visible in the SEM images, although 30 nm-sized particles were visible in the TEM images. Many formulation variables can be varied using the single emulsion approach, and these variations can change the characteristics of the NPs. For instance, bigger NPs with a wider size distribution will often be produced when dichloromethane (DCM) is used as the solvent instead. Because ethyl acetate (EtAc) is miscible with water, the polymer droplet in the main emulsion has less surface tension, which results in smaller NPs (McCall & Sirianni, 2013).

Curcumin has very poor solubility in water. In this experiment, PLGA and curcumin were diluted using dichloromethane. After the curcumin was dissolved into PLGA, the mixture was then dropped one drop at a time in distilled water where PVA had been dissolved.

The SEM examination shows that the NPs are round and sphere-shaped. The spherical shape indicates well-formed curcumin NPs that are more likely to pass across membranes. Round NPs are the most appropriate shape for drug delivery applications out of all of them (Arozal et al., 2020). From the usage of ImageJ, an image (Figure 1a) has been chosen to analyze how many NPs and microparticles are present. Based on the histogram in Figure 2a, it stated 315 particles of 356 particles are less than 1000 nanometers (1 micrometer) in diameter. This represents 88% of the particles that are nanometers in size. The mean of the 356 particles is 498.9 nm ± 597.4 nm. The size is bigger compared to a study done by Arzani et al. (2018) in which the mean size of the particle was 123 nm. The difference between the methods is the usage of chloroform compared to dichloromethane as the dissolution solvent of PLGA. Arzani et al. (2018) also stirred the solution of CUR-PLGA-PVA for a longer duration of 8–10 hours.

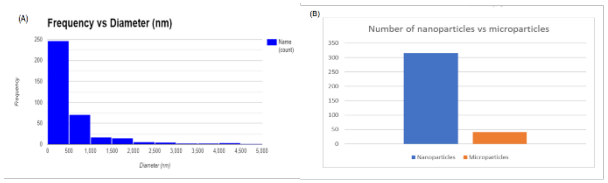

Fig. 2: Frequency of microparticles and nanoparticle molecules. (A) Frequency vs Diameter (in nm); (B) number of NPs vs microparticles.

Nanoparticle size and zeta potential measurement

The result of the size distribution by the intensity and zeta potential curcumin loaded PLGA are given in Figure 3 (a) and 3 (b) respectively. The Z-average of the curcumin-loaded PLGA is 444.7 nm with PDI 0.372. The zeta potential is -28.7 mV. ± 6.19 mV.

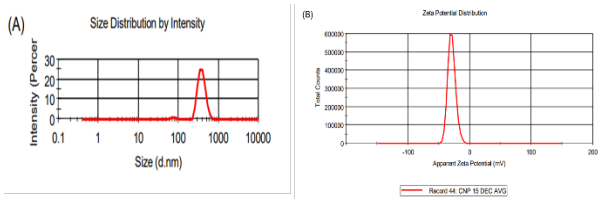

Fig. 3: Size distribution by intensity and zeta potential distribution for curcumin-loaded PLGA. (A) Size distribution by intensity; (B) Zeta potential distribution.

Arzani et al. (2018) obtained a polydispersity indice (PDI) of less than 0.27 and a zeta potential of about -40 mV due to the carboxyl group. Feczkó et al. (2011) found that the PDI from their distribution data ranged from 0.05 to 0.15 across different trials, typically centering around a PDI of 0.10. Mainardes and Evangelista (2005) achieved a PDI of 0.19 with a 5-minute sonication time. Tahara et al. (2017) obtained zeta potential of -40 mV and their particle size was 200 nm. The PDI was measured in this experiment as a parameter to show the homogeneity of nanoparticle droplet size and uniformity of particle size distribution. A PDI value of 0–1 indicates a uniform distribution; the closer to 0, the better (Arozal et al., 2020). The PDI value of the NPs in this experiment shows a result of 0.372. Meanwhile, the zeta potential of the NPs is -28.7 mV ± 6.19 mV. These readings are further from 0. One important metric that sheds light on the stability of colloidal dispersions, especially those containing NPs, is zeta potential. Because of the electrostatic

repulsion between the particles, NPs with larger absolute values of zeta potential—whether positive or negative—are generally more stable. It appears that the majority of the NPs' surface is negatively charged based on the negative zeta potential. An excellent degree of electrostatic repulsion between particles is indicated by a strong negative zeta potential, such as -28.7 mV ± 6.19 mV, which lowers the chance of aggregation.

Determination of Entrapment Efficiency and Drug Loading Capacity of Curcumin-loaded PLGA NPs.

The yield of the NPs is 99.01%. The initial drug used, which is curcumin, was 10 mg and after centrifugation, there were 5 mg of unentrapped drug.

$$\text{Entrapment Efficiency (\%)} = (10\text{mg}/5\text{mg}) \times 100\% = 50\%.$$

Meanwhile, drug loading capacity which is the percentage of the drug weight that is encapsulated about the total weight of the NPs is:

$$\text{Drug Loading Capacity (\%)} = (5\text{mg}/100\text{mg}) \times 100\% = 5\%$$

The entrapment efficiency of NPs shows a result of 50%. This indicates that only half of the curcumin was encapsulated in the PLGA particles while the remaining half might have been lost during loading or not incorporated properly. Drug-carrier interaction, solvent selection, preparation technique, and other formulation parameters could all have an impact on the entrapment efficiency. Meanwhile, the drug loading capacity of the NPs shows a result of 5%. This indicates that curcumin makes up 5% of the weight of all PLGA particles. A loading capacity that is deemed acceptable may vary depending on several factors, including the drug's solubility in the carrier and the formulation's stability.

Based on the study by Arzani et al. (2018), stated that increasing the amount of curcumin in the organic phase led to enhanced encapsulation efficiency (EE) and drug loading capacity (LC) of curcumin-loaded PLGA, with the increase being insignificant ($P > 0.05$). Specifically, raising the curcumin amount from 3 to 10 mg resulted in an increase in EE from 77.81% to 89.87% and LC from 7.86% to 10.53%. Utilizing low concentrations of curcumin is more suitable for achieving smaller particle sizes while maintaining an optimal level of drug entrapment (Arzani et al., 2018).

In vitro release of curcumin

To ascertain the release mechanism and quantity of curcumin released from the curcumin nanoparticle matrix system, the release of curcumin NPs was conducted using a dialysis membrane. The release test was conducted for 3 hours and repeated four times. From this, a graph was plotted (Figure 4a), and the mean of the reading has been made. From the absorbance data, the concentration of the released curcumin was determined which is shown in Figure 4b. From the figure it can be seen that the reading is not consistent, however it shows that the curcumin release had reached the plateau at 45 minutes and above.

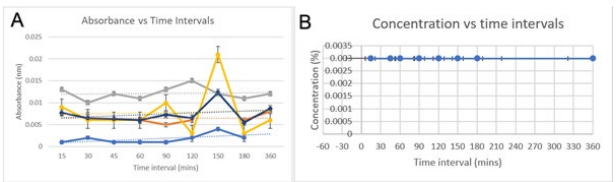

Fig. 4: In vitro release of curcumin NPs based on absorbance and concentration. (A) Absorbance vs time intervals; (B) Concentrations vs time intervals.

Based on the result of *in vitro* release using a dialysis membrane, it can be seen that only a small amount of curcumin was released from the dialysis membrane, which shows low reading. In this experiment, a dialysis tubing cellulose membrane (Sigma-Aldrich) with an average diameter of 16 mm and an average flat width of 25 mm has been used. The molecular weight cut-off of this dialysis membrane is 14000 Da which means the membrane will allow molecules smaller than 12,000–14,000 Da to pass through. The release of curcumin into the buffer may be affected by the size of curcumin, which could be too large for the pores of the dialysis membrane. One of the other reasons which affect the result is the low amount of curcumin being released into the buffer which makes the reading unreadable

by the UV-Vis spectrophotometry. To replicate the physiological circumstances of the small intestine, a pH of 6.8 has been selected. By selecting a buffer strength of 0.1 M, the ionic strength is guaranteed to be high enough to preserve the stability of the buffer solution. The interactions between molecules and the buffer's overall ability to maintain pH can both be impacted by the ionic strength. In an experiment done by (Halayqa & Domańska, 2014), they mention that larger NPs with higher encapsulation efficiency exhibit slower drug release for both drugs. The size of the NPs affects their dissolution rate, with smaller NPs showing faster dissolution due to increased surface area availability. The result of this experiment is not the same as Arzani et al. (2018) which managed to obtain the result of approximately 50% curcumin being released from the polymeric NPs in a rapid burst phase within the first 12 hours. Compared to Mogollon (2016), at room temperature (22°C), the release of curcumin occurs at a much slower rate, extending over approximately 96 hours. This slow release is primarily due to the reduced frequency of nanoparticle collisions at the organic-water interface. The percentage of accumulative release in the first 10 hours is around 25%.

Bioactivity of curcumin-loaded NPs.

The result from the effect of curcumin-loaded PLGA at different concentrations and the maximum cell viability of MCF-7 cells were studied at 48 hours. The relationship between the different concentrations of curcumin extract, curcumin NPs, negative control, and positive is given in Figure 5. There is a fluctuation that happened for every sample type except negative control. However, it can be seen that curcumin-loaded PLGA NPs showed a lower cell viability percentage compared to curcumin extract. There were no statistically significant differences between curcumin NPs and curcumin extract ($p > 0.05$). However, significant differences were observed between NPs and untreated cells ($p < 0.05$), as well as between NPs and cells treated with tamoxifen ($p < 0.05$). This aligns with findings from Arzani et al. (2018), indicating that curcumin-loaded PLGA is more effective than free curcumin. Similarly, a study by Tabatabaei Mirakabad et al. (2016) reported improved cell cytotoxicity with curcumin-loaded PLGA.

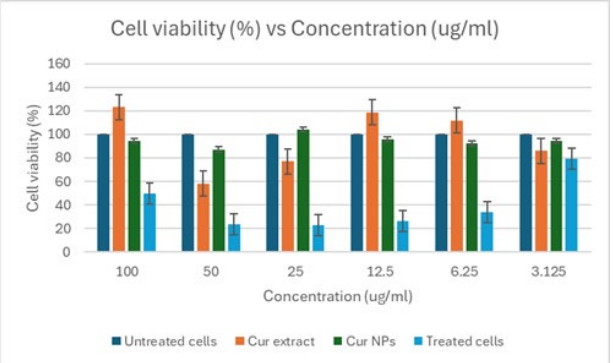

Fig. 5: Viability of cells vs series dilution against MCF-7

From the cytotoxicity study, it can be seen that the cell viability when treated with an extract of curcumin shows quite a variable reading compared to curcumin NPs. The cell viability of cytotoxic cells is lower compared to the viability of cells when treated with curcumin extract. This could be due to the hydrophobic characteristics of curcumin extract compared to curcumin-loaded PLGA NPs. Despite this cytotoxicity assay result, further testing needs to be done to show the cytotoxic effect of *C. xanthorrhiza*. There is currently little scientific information available regarding *C. xanthorrhiza's* mutagenic potential, genotoxicity, carcinogenicity, and even reproductive toxicity (Rahmat et al., 2021). Therefore, future research on the safety and effectiveness of *C. xanthorrhiza* extract and its active ingredients in specific therapeutic areas must be done.

Conclusion

The investigation aimed to formulate curcumin-loaded PLGA analyze the characteristics of curcumin-loaded PLGA and focus on their bioactivity. In this experiment, a single emulsion method was used to prepare curcumin-loaded PLGA. This process managed to produce NPs with an average size of 498 nm with a Z-average of 444.7 nm with PI 0.372 and a zeta potential of -28.7 mV ± 6.19 mV. However, the EE is quite low with 50% and DLC of 5%. The released study in vitro did not produce significant results as low concentrations of

release curcumin were detected. The bioactivity of curcumin-loaded PLGA shows lower viability of cells compared to curcumin extract which suggests that the PLGA formulation is more effective at promoting cell death. Further research needs to be done to further solidify the data on the release profile of curcumin from curcumin NPs and also an improved formulation needs to be done which increases the entrapment efficiency and drug loading capacity of curcumin inside the NPs.

Authors contributions

M.H.E: Investigation, Data Acquisition, Writing – Original Draft, Visualization. **M. T., D.S.:** Conceptualization, Methodology, Validation, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **S.**: Research materials, Resources. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

I have not used any AI tools or technologies to prepare this assessment.

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