Interdisciplinary Social Studies
P-ISSN: 2808-0467
E-ISSN: 2808-5051

The nanoparticle formula optimization from Temulawak extract (*Curcuma xanthorrhiza*) as adjuvant therapy

Greesty Finotory Swandiny*, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, Indonesia
Safira Nafisa, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, Indonesia
Rahmatul Qodriah, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, Indonesia
Gumilar Adhi Nugroho, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, Indonesia
Muhammad Taher Bakhtiar, Kuliyyah of Pharmacy, International Islamic University Malaysia, Kuantan Pahang, Malaysia

*Email for Correspondence: greestyfinotory@univpancasila.ac.id

Keywords:

nanoparticle formula
Temulawak
adjuvant therapy

ABSTRACT

Temulawak extract was synthesized using temulawak's sodium alginate as a base material. It was characterized by scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. The size of Alg-Curcuma microbeads measured at SEM was 296 m. FTIR spectra showed that the size of the resulting beads was microparticle in size. The particle size distribution of the sample was also analyzed. The results show that the nanoparticle formula was obtained from temuwak extract material with parameters and characterization that have been analyzed and tested.

This is an open access article under the CC BY-SA license.

INTRODUCTION

Currently, a nanomedicine method has been developed that can target drugs to tumor cells specifically, namely nanoparticle therapy (Aghebati‐Maleki et al., 2020; Liu et al., 2007; Oroojalian et al., 2021; Tang et al., 2021; Thakkar et al., 2020). Nanoparticles are colloidal particles that can be amorphous or crystalline in shape that have a size smaller than 1 micron (Croissant et al., 2020; Dong et al., 2020; Ijaz et al., 2020; Rastogi & Asa-Awuku, 2022; Shrestha et al., 2020). The use of nanoparticles in cancer management aims to increase bioavailability and drug distribution (Chivere et al., 2020; Gavas et al., 2021; Hafeez et al., 2021; Raj et al., 2021; Wang et al., 2020), as well as improve drug targeting and release to tumor cells, so that it is expected to increase efficacy and reduce side effects (Ali et al., 2021; de la Torre et al., 2020; Gomes et al., 2021; Kashkooli et al., 2020; Zein et al., 2020). Therefore, it is necessary to develop a nanoparticle formula of temulawak extract which is expected to increase the effectiveness of temulawak extract as an anticancer.

The main ingredient used is standard temulawak powder. Other ingredients used were sodium alginate powder with a molecular weight of 216.12 g/mol purchased from Sigma-Aldrich® (Darmstadt, Germany), calcium chloride anhydrous powder (CaCl2.2H2O) purchased from Merck (Darmstadt, Germany) and distilled water. All materials have an analytical level.

The research would like to analyze the nanoparticle formula optimization from Temulawak extract (*Curcuma xanthorrhiza*) as adjuvant therapy. It is expected to give more insight into the formula optimization process of herbs in Indonesia, especially Temulawak since it is rarely explored.

METHOD

The equipment used in manufacturing microbeads is a syringe pump from KD Scientific® (Massachusetts, USA), an AC automatic voltage regulator from OET® (Australia), a high voltage gauge (Ormond Beach, Florida), a voltage regulating cable, an analytical scale, a magnetic stirrer, a hotplate (Gyeonggi-do, Republic of Korea), a homogenizer (Staufen, Germany), aluminum foil, a petri dish, a 23 needle, a 10cc/mL Terumo Syringe (Leuven, Belgium), parchment paper and other glassware. For grain analysis, the equipment used is Fourier Transform Infrared Spectroscopy (FTIR), UV-Vis Spectrophotometer, Zetasizer Light Scattering Instrument, SEM (Oregon, USA) and Oven.

Encapsulation Process

Preparing Microbeads Curcuma-Alginat

1) The sample prepared was 250 mL.
2) Temulawak powder as much as 15% weighed and dissolved in 100 mL aquades then stirred using a glass rod.
3) 3.5% alginate powder was weighed and dissolved in the remaining 150 mL of distilled water using a homogeniser at a speed of 4000 rpm.
4) After the homogenized sodium alginate solution is produced, the temulawak solution is mixed. The mixture is then stirred with a homogeniser at a speed of 4000 rpm until thoroughly mixed for approximately 5 minutes.
5) Next, the mixture is stirred continuously on a magnetic stirrer with a low temperature (the first temperature on the hotplate) for approximately 30 minutes.
6) The prepared mixture is then set for the dripping process using a syringe pump. The syringe pump is equipped with a 10 cc/mL syringe, size 23 needle and collecting wire (black wire), and the distance between the tip and the collecting solution is set at a constant distance of 10 cm.
7) A 2% w/v solution of CaCl2 is prepared by dissolving it in 250 mL of distilled water. It is used as a crosslinking solution for the formation of microbeads.
8) Prepare a petri dish with aluminum foil for conductivity from the soil (green wire) and pour the poured CaCl2 into it along with a small magnetic stirrer.
9) The mixture is sprayed into the CaCl2 solution with a certain flow speed and drop tension.
10) The droplet flow rate is set first, then the voltage is set until the desired cone-jet is generated. Table 1 shows the droplet flow rate values and voltages used.
11) The resulting microbeads are then stored in CaCl2 solution in falcon tubes.

Table 1. The Values of Dripping Flow Rate and Voltages Used

Sample	Dripping Flow Rate	Adjusted Voltage	Duration
First batches of synthesis			
Sample 1	10μL/min	24 kV	1 hour
Sample 2	20μL/min	18.2 kV	1 hour
Sample 3	30μL/min	16.66 kV	1 hour
Sample 4	40μL/min	16.52 kV	45 minutes
Sample 5	50μL/min	16.48 kV	45 minutes
Second batches of synthesis			
Sample 1	0.1 mL/hr	19.45 kV	20 minutes
Sample 2	0.2 mL/hr	16.5 kV	20 minutes
Sample 3	0.3 mL/hr	19.0 kV	20 minutes
Sample 4	0.4 mL/hr	23.7 kV	20 minutes

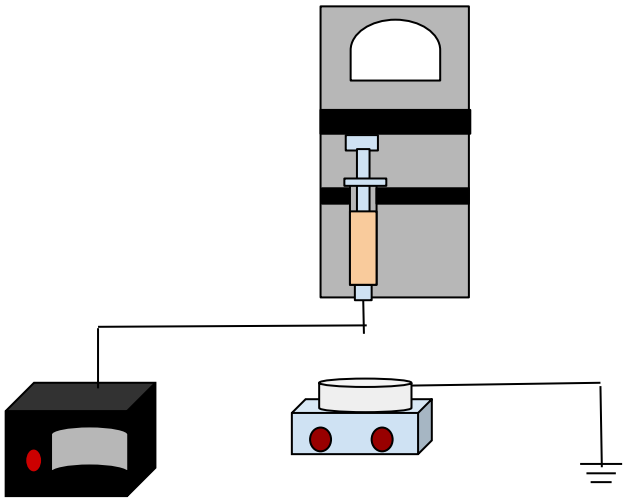


Figure 1. Schematic Diagram of Electrospray Experimental Setup



Figure 2. Real Setup of Electrospray Experiment

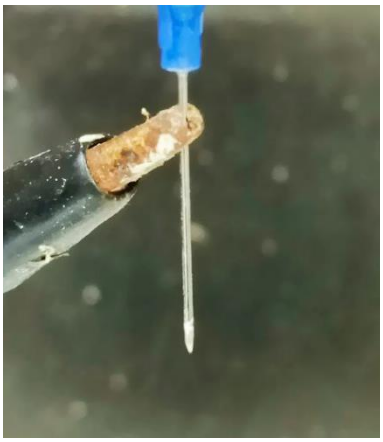


Figure 3. The Desired Cone-Jet

Alginat-Temulawak Analysis

SEM Analysis

1) Some dry microbeads are taken for SEM analysis and taken to KOM for coating and analysis.

2) Morphological analysis of the surface of microbeads at a specific magnification.

UV-Vis spectrophotometry analysis

1) UV-Vis spectrophotometry is first set with the desired wavelength of 350 nm to 800 nm.
2) CaCl2 solution is prepared and used as a blank.
3) 1 mL of CaCl2 solution and 1 mL of wet alginate-curcumin microbeads in CaCl2 solution for each sample were then analyzed.

FTIR greining

1) Alginate-curcumin microbeads are filtered using filter paper and rinsed using aquades.
2) The filtered microbeads are transferred to parchment paper and put in the oven to dry at 37°C for approximately 24 hours.
3) After drying is complete, several dry samples are taken for FTIR analysis.
4) Samples of sodium alginate powder and temulawak powder were also prepared.
5) This sample is carried out for FTIR analysis using FTIR spectroscopy.

Particle size analysis

1) 1 ml of alginate-curcumin solution is introduced into the cuvette.
2) The particle size distribution of the sample is analyzed.

Zeta potential analysis

1) Using a syringe, 1 ml of alginate-curcumin solution is introduced into the zeta cells of the Malvern Panalytical DTS1070 fold capillaries.
2) The zeta potential of the sample is analyzed.

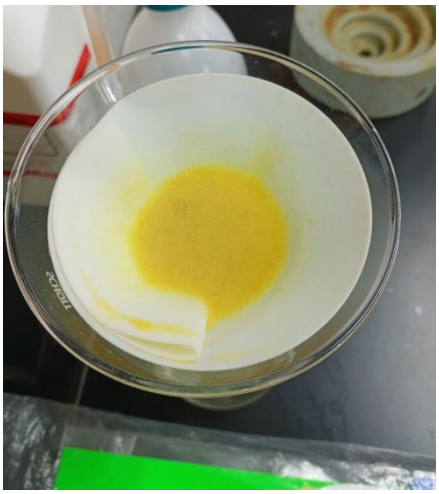


Figure 4. Filtered Alginate Curcuma



Figure 5. Dried Alginate Curcuma

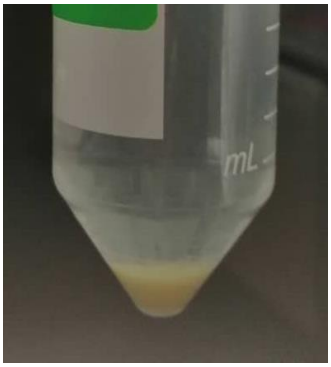
1) Constraints: It is necessary to optimize the carakerized nanoparticle formula.
2) Completion or Completion Plan: The supervision of MF members who are experts in nanoparticle formulation is very helpful in terms of the process of completing activities.

RESULTS AND DISCUSSION

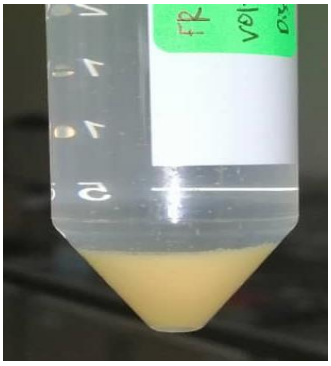
A nanoparticle formula was obtained from Temulawak extract material with parameters and characterization that have been analyzed and tested.

Visual observation

Alg-Curcuma was first synthesized at flow rates of 10, 20, 30, 40, and µL/min (first batch synthesis). However, the particle size varies greatly and can be observed with the naked eye.



(a) 10 µL/min sample



(b) 20 µL/min sample



(c) 30 µL/min sample



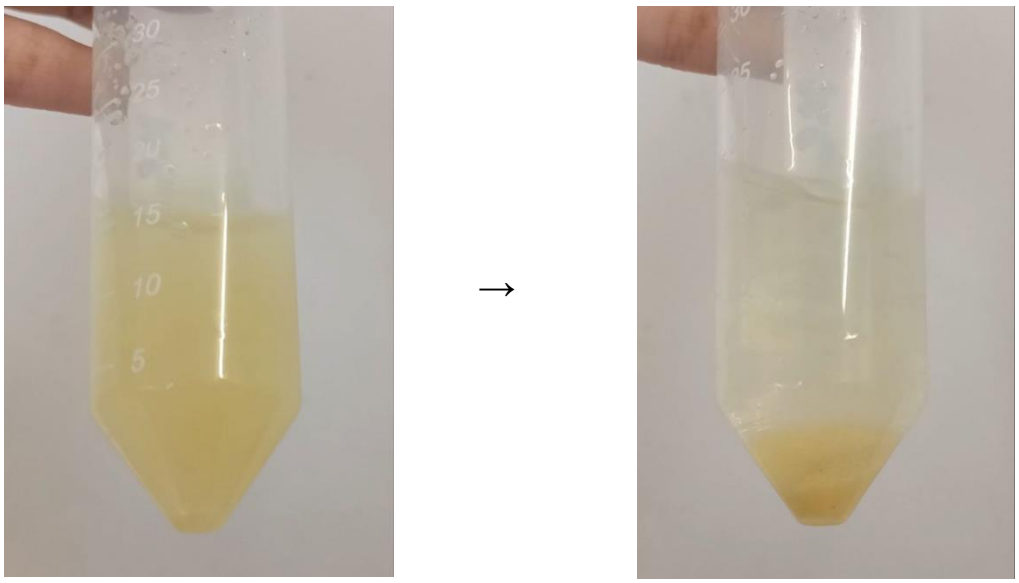
(d) 40 µL/min sample



(e) 50 µL/min sample

Figure 6. Synthesised Alg-Curcuma at different flow rate. (a) 10 µL/min. (b) 20 µL/min. (c) 30 µL/min. (d) 40 µL/min sample. (e) 50 µL/min.

The flow rate and voltage are then adjusted according to Alallam et al (2020). The study reported that the recommended parameter is 12.5 kV with a flow rate of 0.1 mL/h. Therefore, Alg-Curcuma is synthesized with flow rates of 0.1, 0.2, 0.3, and 0.4 mL/h (second wave synthesis).



(a) *(b)*

Figure 7. (a) Alg-Curcuma with flow rate 0.1 ml/hr. (b) Alg-Curcuma (0.1 ml/hr) sedimented rapidly within 8 seconds

SEM analysis

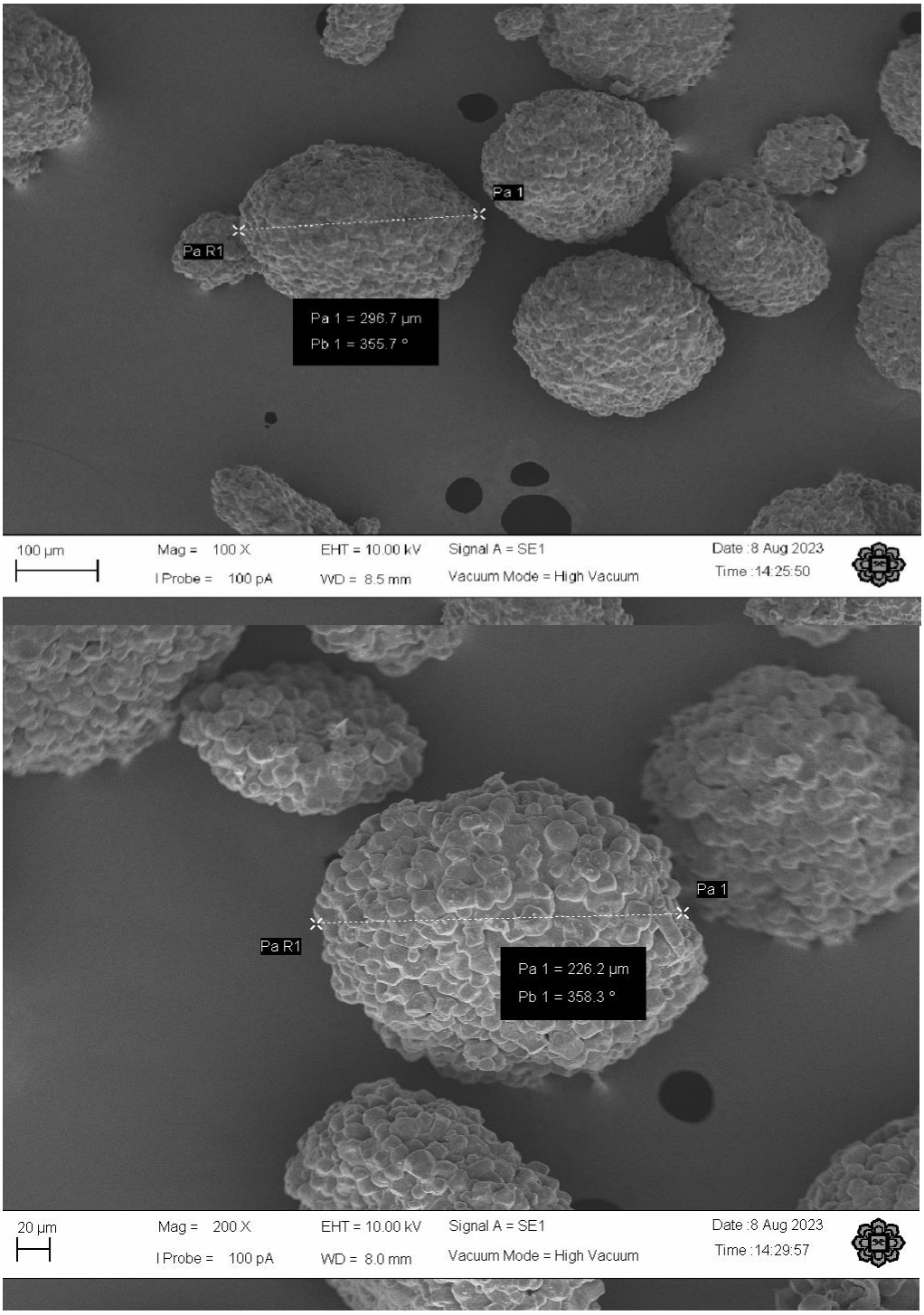


Figure 8. Image of Alg-Curcuma under SEM

The sizes of Alg-Curcuma microbeads measured at SEM were 296 µm and 226 µm.

UV-Vis spectrophotometry analysis

UV-Vis spectral readings at 10, 20, 30, 40 and 50 µL/min.

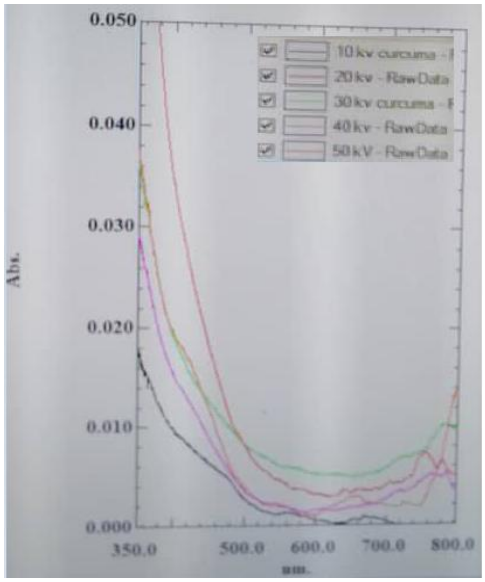


Figure 8. UV-vis spectra of 10, 20, 30, 40 and 50 µL/min**.** No peak observed for all of the samples.

UV-Vis spectral readings of 0.1, 0.2, 0.3, and 0.4 mL/h

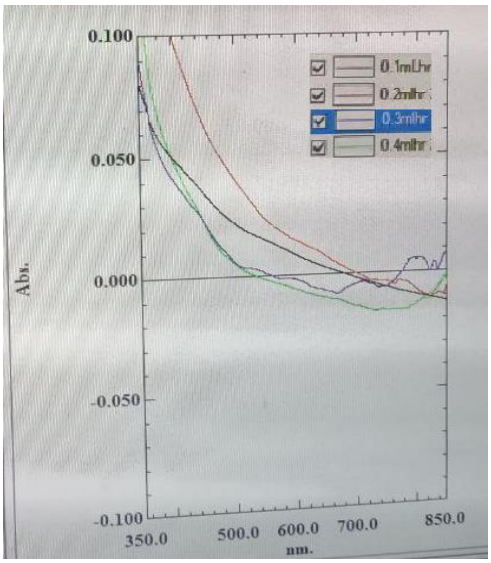


Figure 9. UV-Vis spectra of 0.1, 0.2, 0.3, and 0.4 mL/hr

Table 1. Peak and absorbance observed for each flow rate.

Flow rate	Peak at wavelength (nm)	Abs
0.1 mL/hr	363.2	0.071
0.2 mL/hr	No peak	-
0.3 mL/hr	839.8 828.2 824.8 815.4	0.003 0.004 0.002 0.004

0.4 mL/hr	360.6	0.097

FTIR greining

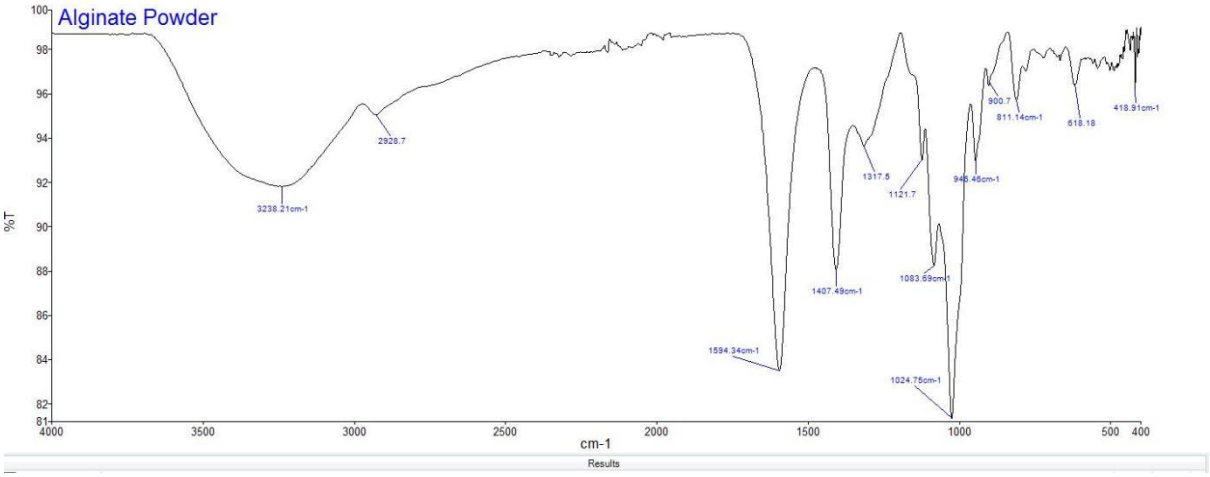


Figure 10. FTIR Spectrum of Alginate Powder

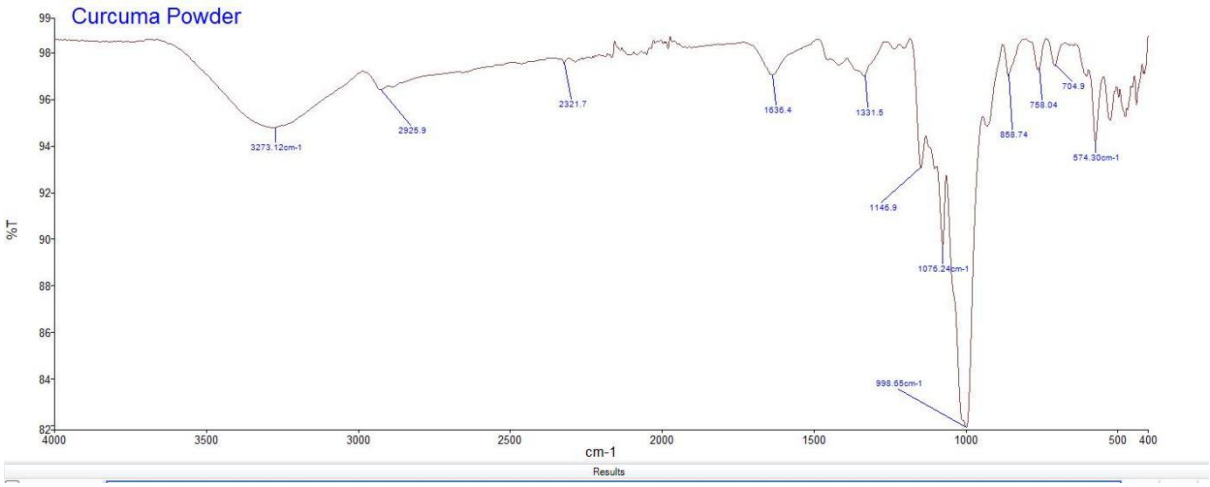


Figure 11. FTIR Spectrum of Curcuma Powder

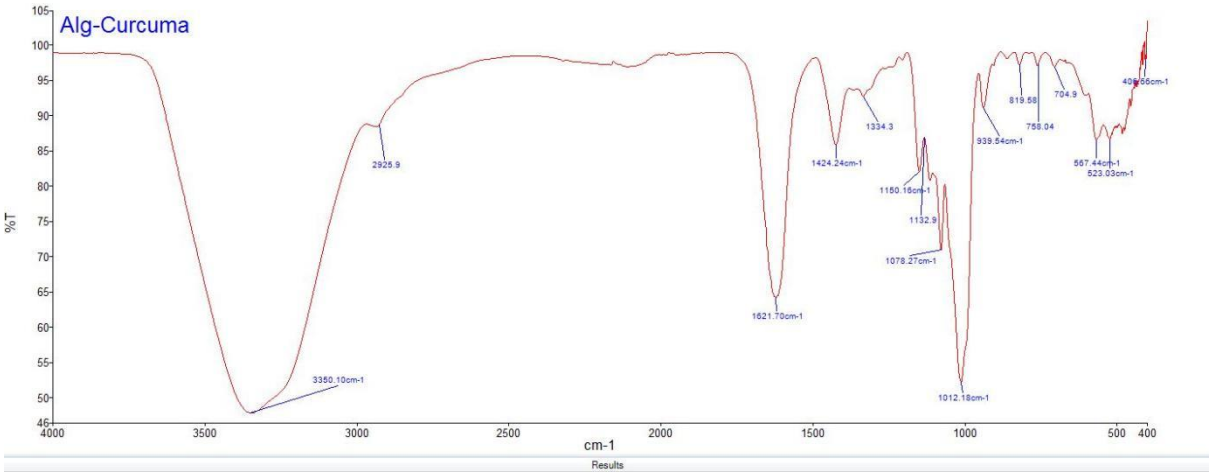


Figure 12. FTIR Spectrum of Alg-Curcuma

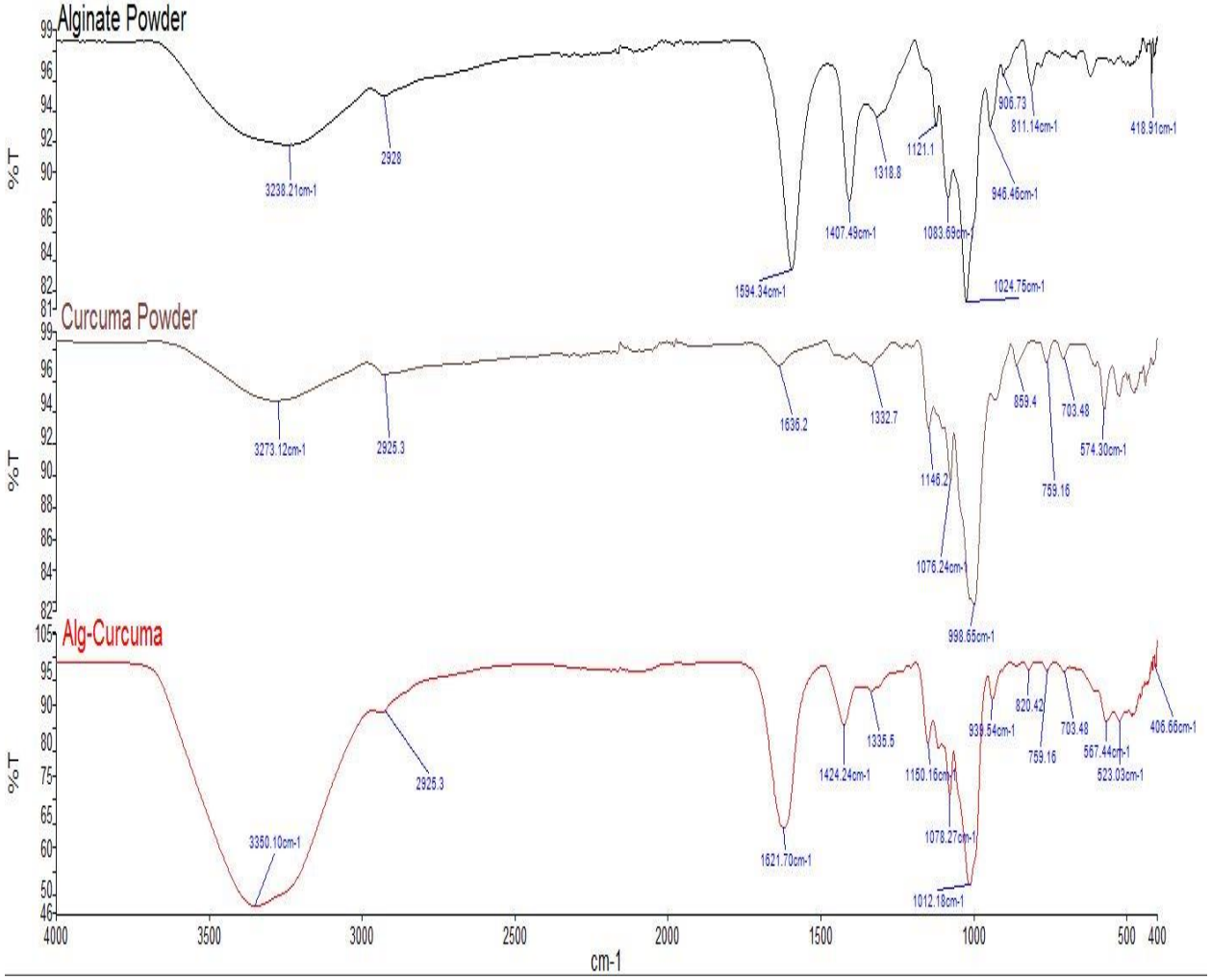
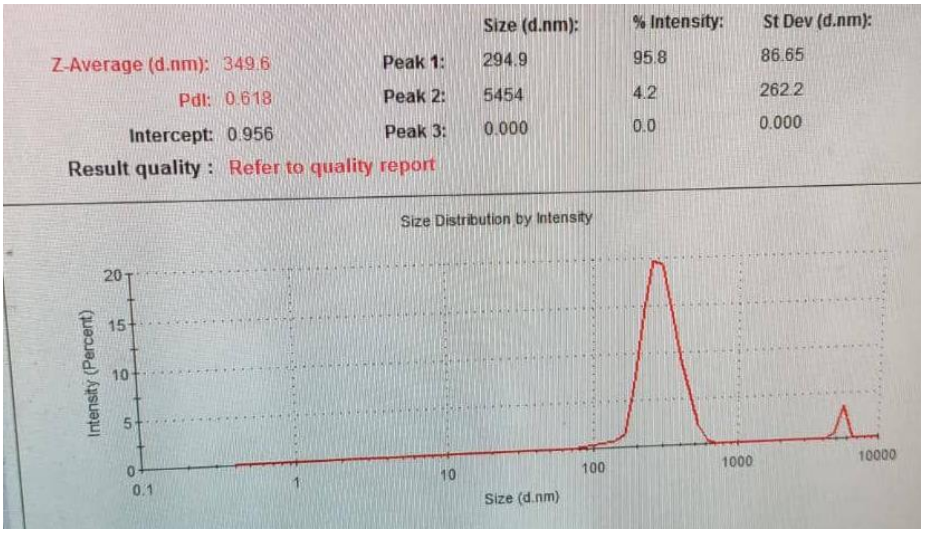


Figure 13. FTIR Spectra of Alginate Powder, Curcuma Powder and Alg-Curcuma

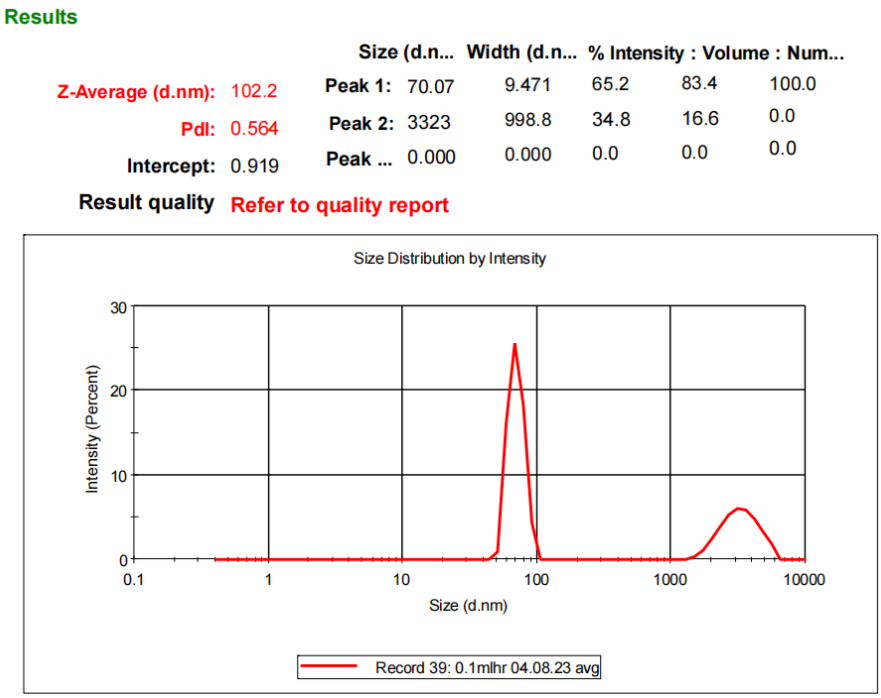
Particle Size Analysis

1) Flow rate: 10 µL/min



The size for 20, 30, 40 and 50 µL/min was not analysed.

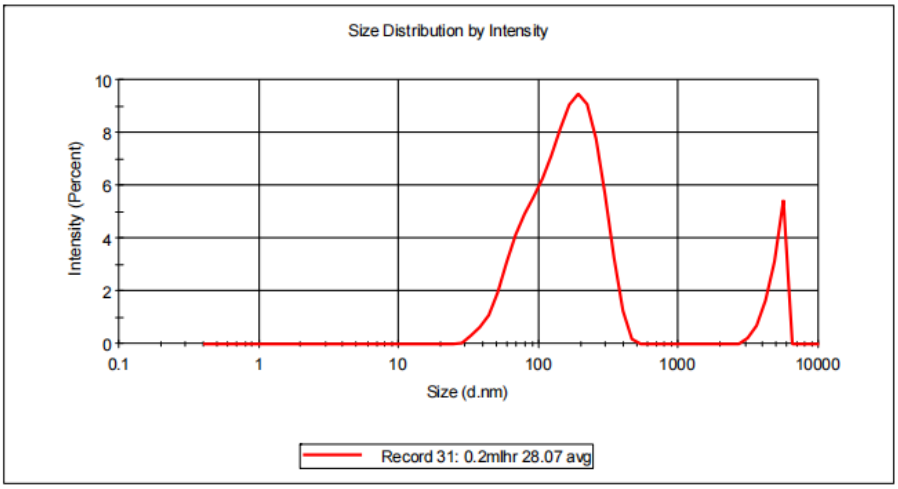
2) Flow rate: 0.1 ml/hr



3) Flow rate: 0.2 ml/hr

Results

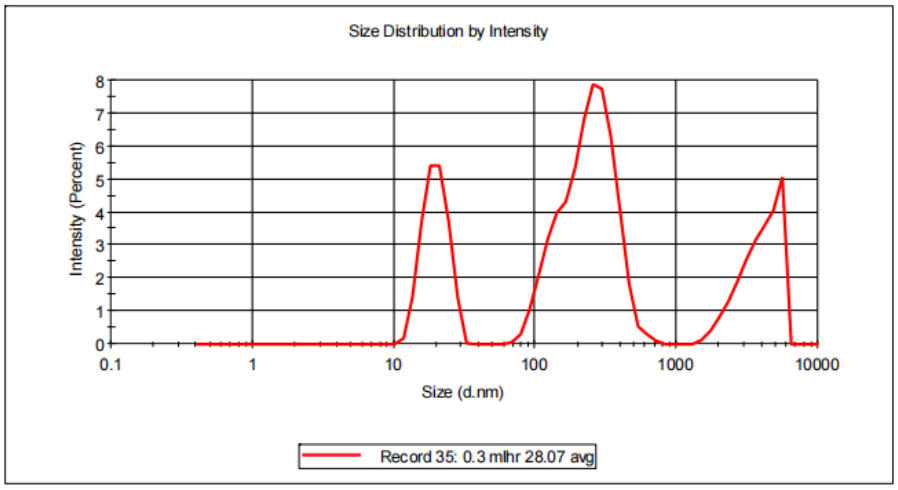
			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	187.6	Peak 1:	167.1	88.9	83.21
PdI:	0.596	Peak 2:	4965	11.1	684.4
Intercept:	0.920	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				



4) Flow rate: 0.3 ml/hr

Results

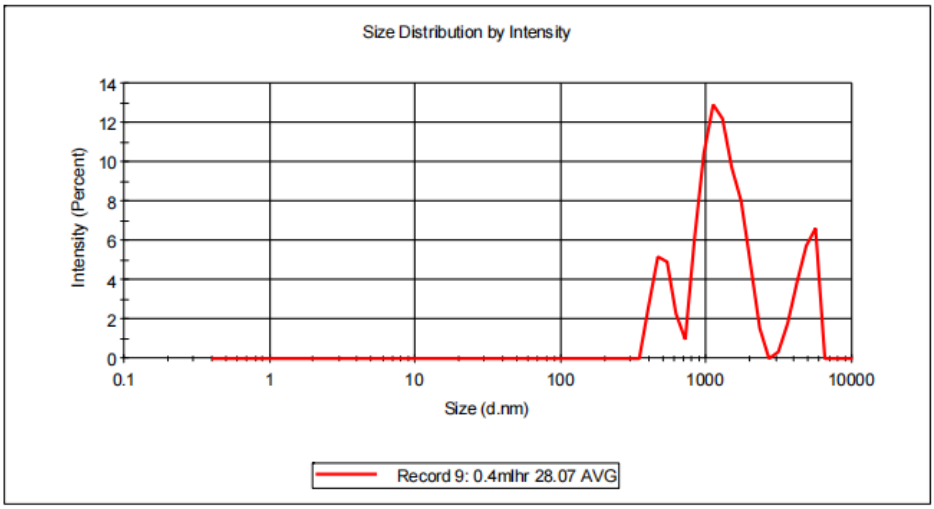
			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	144.1	Peak 1:	249.5	55.8	101.5
PdI:	0.883	Peak 2:	4020	22.9	1160
Intercept:	0.972	Peak 3:	19.85	21.3	3.968
Result quality	Refer to quality report				



5) Flow rate: 0.4 ml/hr

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1226	Peak 1:	1304	66.0	368.1
PdI:	0.579	Peak 2:	4786	18.1	705.0
Intercept:	0.912	Peak 3:	508.2	15.8	84.71
Result quality	Refer to quality report				



All the results for particle size analysis did not meet quality criteria due to the presence of large sedimenting particles.

Zeta potential analysis

Flow rate: 0.1 ml/hr

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	4.41	Peak 1:	0.00	0.0	0.00
Zeta Deviation (mV):	0.00	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	31.8	Peak 3:	0.00	0.0	0.00
Result quality	See result quality report				

*Graphs cannot be generated by the computer.

The zeta potential for other flow rates (0.2, 0.3 and 0.4 ml/hr) was not performed because the zeta potential data was unreliable.

Limitation

1) During the synthesis process, the voltage exceeds 25 kV, giving rise to an electric spark. Sometimes, even at 20 kV it can produce sparks. Therefore, there is a limit to obtaining the desired voltage at a given droplet flow rate. Therefore, optimized parameters may be difficult to obtain.
2) The synthesized beads are microparticle in size. CaCl2 solutions containing microbeads are not colloidal and tend to settle quickly.
3) During voltage adjustment, the size of the resulting microbeads varies.
4) Dry samples do not spread well after drying. They become lumpy and need to be separated manually. This can affect the surface morphology of microbeads.

CONCLUSION

Temulawak's sodium alginate synthesis results in microparticle size, not nanoparticle size. Microparticle sizes also vary thus contributing to high PdI which indicates a very wide particle size distribution. In addition, the zeta potential of the particle is 4.41 mV which means the suspension is unstable and the particles tend to aggregate. Based on the results obtained, it can be seen that temulawak sodium alginate microbeads are quickly precipitated and not well dispersed. Therefore, there are several recommendations that can be done to optimize the synthesis of temulawak sodium alginate. First, although ginger powder is dissolved in distilled water, it is not so stable to be homogenized well over a long period of time in alginate. This may be the main reason why synthesized alginate microgranules can precipitate quickly. Therefore, the use of ethanol as a solvent needs to be considered to dissolve Temulawak powder because it is also known as the best solvent. Secondly, since the nanoparticle size of temulawak alginate granules was not obtained, it is recommended to add chitosan to the solution because it can help reduce and control the resulting sample size (Phan et al., 2021).

REFERENCES

Aghebati-Maleki, A., Dolati, S., Ahmadi, M., Baghbanzhadeh, A., Asadi, M., Fotouhi, A., Yousefi, M., & Aghebati-Maleki, L. (2020). Nanoparticles and cancer therapy: Perspectives for application of nanoparticles in the treatment of cancers. *Journal of Cellular Physiology*, *235*(3), 1962–1972.

Ali, E. S., Sharker, S. M., Islam, M. T., Khan, I. N., Shaw, S., Rahman, M. A., Uddin, S. J., Shill, M. C., Rehman, S., Das, N., Ahmad, S., Shilpi, J. A., Tripathi, S., Mishra, S. K., & Mubarak, M. S. (2021). Targeting cancer cells with nanotherapeutics and nanodiagnostics: Current status and future perspectives. *Seminars in Cancer Biology*, *69*. https://doi.org/10.1016/j.semcancer.2020.01.011

Chivere, V. T., Kondiah, P. P. D., Choonara, Y. E., & Pillay, V. (2020). Nanotechnology-based biopolymeric oral delivery platforms for advanced cancer treatment. *Cancers*, *12*(2). https://doi.org/10.3390/cancers12020522

Croissant, J. G., Butler, K. S., Zink, J. I., & Brinker, C. J. (2020). Synthetic amorphous silica nanoparticles: toxicity, biomedical and environmental implications. *Nature Reviews Materials*, *5*(12). https://doi.org/10.1038/s41578-020-0230-0

de la Torre, P., Pérez-Lorenzo, M. J., Alcázar-Garrido, Á., & Flores, A. I. (2020). Cell-based nanoparticles delivery systems for targeted cancer therapy: Lessons from anti-angiogenesis treatments. In *Molecules* (Vol. 25, Issue 3). https://doi.org/10.3390/molecules25030715

Dong, X., Wu, Z., Li, X., Xiao, L., Yang, M., Li, Y., Duan, J., & Sun, Z. (2020). The Size-dependent Cytotoxicity of Amorphous Silica Nanoparticles: A Systematic Review of in vitro Studies. *International Journal of Nanomedicine*, *15*. https://doi.org/10.2147/IJN.S276105

Gavas, S., Quazi, S., & Karpiński, T. M. (2021). Nanoparticles for Cancer Therapy: Current Progress and Challenges. *Nanoscale Research Letters*, *16*(1). https://doi.org/10.1186/s11671-021-03628-6

Gomes, H. I. O., Martins, C. S. M., & Prior, J. A. V. (2021). Silver nanoparticles as carriers of anticancer drugs for efficient target treatment of cancer cells. *Nanomaterials*, *11*(4). https://doi.org/10.3390/nano11040964

Hafeez, M. N., Celia, C., & Petrikaite, V. (2021). Challenges towards targeted drug delivery in cancer nanomedicines. *Processes*, *9*(9). https://doi.org/10.3390/pr9091527

Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chemistry Letters and Reviews*, *13*(3). https://doi.org/10.1080/17518253.2020.1802517

Kashkooli, F. M., Soltani, M., & Souri, M. (2020). Controlled anti-cancer drug release through advanced nano-drug delivery systems: Static and dynamic targeting strategies. *Journal of Controlled Release*, *327*. https://doi.org/10.1016/j.jconrel.2020.08.012

Liu, Y., Miyoshi, H., & Nakamura, M. (2007). Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *International Journal of Cancer*, *120*(12), 2527–2537.

Oroojalian, F., Beygi, M., Baradaran, B., Mokhtarzadeh, A., & Shahbazi, M. A. (2021). Immune Cell Membrane-Coated Biomimetic Nanoparticles for Targeted Cancer Therapy. *Small*, *17*(12). https://doi.org/10.1002/smll.202006484

Raj, S., Khurana, S., Choudhari, R., Kesari, K. K., Kamal, M. A., Garg, N., Ruokolainen, J., Das, B. C., & Kumar, D. (2021). Specific targeting cancer cells with nanoparticles and drug delivery in cancer therapy. *Seminars in Cancer Biology*, *69*. https://doi.org/10.1016/j.semcancer.2019.11.002

Rastogi, D., & Asa-Awuku, A. (2022). Size, Shape, and Phase of Nanoscale Uric Acid Particles. *ACS Omega*, *7*(28). https://doi.org/10.1021/acsomega.2c01213

Shrestha, S., Wang, B., & Dutta, P. (2020). Nanoparticle processing: Understanding and controlling aggregation. *Advances in Colloid and Interface Science*, *279*. https://doi.org/10.1016/j.cis.2020.102162

Tang, L., Mei, Y., Shen, Y., He, S., Xiao, Q., Yin, Y., Xu, Y., Shao, J., Wang, W., & Cai, Z. (2021). Nanoparticle-mediated targeted drug delivery to remodel tumor microenvironment for cancer therapy. *International Journal of Nanomedicine*, *16*. https://doi.org/10.2147/IJN.S321416

Thakkar, S., Sharma, D., Kalia, K., & Tekade, R. K. (2020). Tumor microenvironment targeted nanotherapeutics for cancer therapy and diagnosis: A review. *Acta Biomaterialia*, *101*. https://doi.org/10.1016/j.actbio.2019.09.009

Wang, Y., Pi, C., Feng, X., Hou, Y., Zhao, L., & Wei, Y. (2020). The influence of nanoparticle properties on oral bioavailability of drugs. *International Journal of Nanomedicine*, *15*. https://doi.org/10.2147/IJN.S257269

Zein, R., Sharrouf, W., & Selting, K. (2020). Physical Properties of Nanoparticles That Result in Improved Cancer Targeting. *Journal of Oncology*, *2020*. https://doi.org/10.1155/2020/5194780