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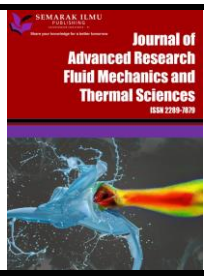

Laser Intensity of Thermo-Responsive Nanoparticles Size Measurement Using Dynamic Light Scattering

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ABSTRACT

Dynamic light scattering (DLS) or photon cross-correlation spectroscopy (PCCS) is a very potent means for analyzing the diffusion behaviour of supramolecules in their solution form. The diffusion coefficient, probed from the scattered light by supramolecules underexposure to incident light, allows the hydrodynamic radii to be calculated. However, the scattering values were recently misled by an unexpected interaction between the incident light and thermo-sensitive nanogels. Hence, in turn, it resulted in a miscalculation of the size of the nanogels by more than 100% of their expected values. The study fulfills a vital knowledge gap by investigating the effects of laser intensity on the size of thermo-sensitive Poly(N-Isopropyl Acrylamide-Vinyl Pyrrolidone-Polyethylene Glycol Diacrylate-(2-(Dimethyl Amino)Ethyl Methacrylate)), Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogels and subsequent count rates in DLS measurements. The Lower Critical Solution Temperature, LCST phase transition of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel can be observed using the DLS technique. The higher laser intensity was advantageous for measuring at high dilution more vigorously with varying laser intensities. Thus, a sufficient laser intensity should be chosen based on the light scattering characteristics of typical samples.

1. Introduction

Nanoparticle synthesis from green chemistry pathways has recently been preferred in nanotechnology research due to its natural biological reduction property, which reduces the utilization and exposure of toxic chemicals to the environment compared to physical and chemical methods [1]. Advance in nanotechnology has recently changed the biomedical drug delivery field, notably through nanogels. Nanogels are potential drug nanocarriers for site targeting and

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controllable anti-cancer drug release as they protect the cargo from degradation and elimination [2]. The nanogel, as its name implies, has small dimensions in the nanometre range. Due to this, the gels can reach the smallest capillary veins, allowing them to permeate tissues via transcellular pathways [3,4] and participate actively in the delivery process [5]. The bulk of stimulating nanogels under research is currently concentrated on temperature-sensitive or thermo-responsive nanogels [6-8] due to their effectiveness in overcoming the limitations of premature and insufficient drug release at target sites in drug delivery systems.

The thermo-responsive delivery system is a promising cancer-targeting strategy that inhibits reversible physical or chemical variations in response to temperature changes based on (LCST) [9, 10], subsequent to tumour site temperature. To date, the most studied temperature-sensitive polymers are NIPAAM and DMAEMA, as their LCST value is adjacent to the body temperature [11]. Thus, in a controlled drug delivery system for thermo-modulated drug encapsulation, a combination of NIPAAM and DMAEMA nanogels would provide good temperature responsiveness [12,13]. The LCST of nanogel can be studied using DLS, where a sudden drop in size can be observed [14].

The accurate determination of nanoparticle sizing is becoming increasingly important in nanoscience and nanotechnology. DLS is a standard method for measuring particle size distributions by the American Society for Testing and Materials [15], significant for measuring biopharmaceutical formulations and other colloidal dispersions. The application of DLS is well-established for evaluating the diffusion coefficient of dispersed particles in a liquid by measuring fluctuations in the intensity of scattered light using the correlation function provoked by Brownian motion [16,17].

As a result, information about the size distribution of particles could be acquired by processing the fluctuations in the intensity of scattered light. Furthermore, the concentration of nanogels in the solution might influence the intensity of photon scattering or countrates [18]. The factors that influence DLS results include sample preparation, sample concentration, coloured or fluorescent samples, the effect of agglomeration, shape of nanoparticles, rotational diffusion of nanoparticles, issues related to the cuvette, and maintenance of the instrument and laser diffraction intensity [19].

Laser diffraction is a well-known technique for determining particle size distribution among the factors that affect the measurement result [20, 21]. However, the influence of laser intensity has not been studied thoroughly. Laser intensity is a critical aspect concerning satisfactory colloidal analysis by DLS [22]. Herein, we present an important knowledge gap to study the effects of laser intensity on the size of thermo-sensitive nanogels and subsequent count rates in DLS measurements. The synthesis of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel via free radical co-polymerization by using gamma radiation-induced polymerization is employed in this research. PVP and PEGDA were copolymerized onto NIPAAM and DMAEMA to form nanogels with amphiphilic properties. Polymerization using this method does not involve the addition of harmful chemical initiators and crosslinkers [23] that are difficult to remove, which would result in a cleaner product.

2. Methodology

2.1 Materials

NIPAAM, 99%, PVP, 99%, PEGDA, and 98% DMAEMA were purchased from Sigma-Aldrich and used without prior purification. Ultrapure water was used throughout this work for all solution preparations.

2.2 Synthesis of Thermosensitive Nanogel

NIPAAM, PVP, PEGDA and DMAEMA were dissolved in water at specific molar ratios. The mixture was aliquoted into several vessels and had its oxygen content purged with nitrogen gas 30 min prior to gamma irradiation. Each vessel was irradiated at 20 kGy. Gamma radiation will be sourced from cobalt-60 (half-life 5.3 years, dose rate 0.6 Gy/s) in Sinagama Irradiation Facility in Malaysian Nuclear Agency. After irradiation, the Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogels were purged again with nitrogen gas for 30 min. Then, the Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogels were dialyzed against water using a 12,000 Da molecular cutoff membrane for 48 h. The dialysis procedure was monitored using Shimadzu UV-1700 at 280 nm wavelength before being lyophilized and resuspended in ultrapure water for subsequent characterization and testing. The samples were diluted into different concentrations.

2.3 Dynamic Light Scattering Study

Dynamic light scattering measurement was performed to obtain the size (hydrodynamic diameter) and photon scattering intensity of the resulting nanogel. The equipment used was Nanophox from Sympatec GmbH, a 10 mW HeNe laser beam at a wavelength of 632.8 nm and a 90° scattering angle at 25°C. The measurement condition was set according to previous reports. The lyophilized nanogel and the loaded nanogel were redispersed in ultrapure water and filtered with a 0.22 µM pore filter. Measurement was done using the 3D cross-correlation function on the scattering intensity and analyzed using cumulant analysis to obtain the particles' hydrodynamic diameter and dispersity information. The temperature used in the experiment was 25 and 40 °C.

3. Result and Discussion

The irradiated polymeric mixture of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel was expected to produce random copolymers as the thru effect on the polymers in the solution was negligible upon irradiation (Figure 1). The water phase is expected to absorb most energy imparted from gamma rays in a diluted solution [24]. So, its direct impact on the solution's polymers will be negligible. However, the ionizing energy will cause a chemical reaction in a diluted environment. The aqueous media absorbed the irradiation source's energy, forming radicals.

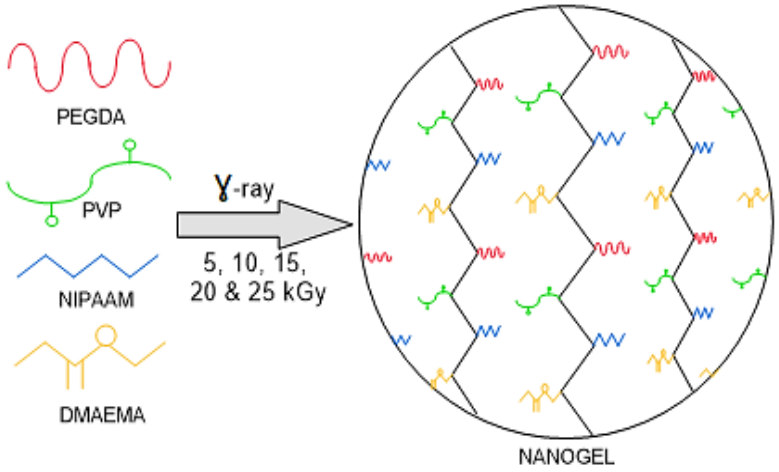

Fig. 1. Synthesis of Poly(NIPAAM-PVP-PEGDA-DMAEMA) Nanogel

Hydroxyl radicals react and concentrate faster than other species with macromolecules [25]. As hydroxyl radicals react with reactant monomers, macromolecules with active sites (macroradicals) are produced by polymers' hydrogen abstraction of the vinyl group. Molecular recombination is a reaction route for these unstable macroradicals [26]. During the recombination of the four different macroradicals, co-polymerization is expected to form grafted polymeric structures that will coil in water to form nanogels. Hydrogen atoms and solvated electrons react similarly to hydroxyl radicals [27]. The polymeric structures formed by the grafting were amphiphilic, with a hydrophobic core and a hydrophilic shell [28]. The grafted polymeric structures were estimated to be random with an amphiphilic characteristic of the hydrophobic core and hydrophilic shell. The proposed copolymer structure is shown in Figure 2.

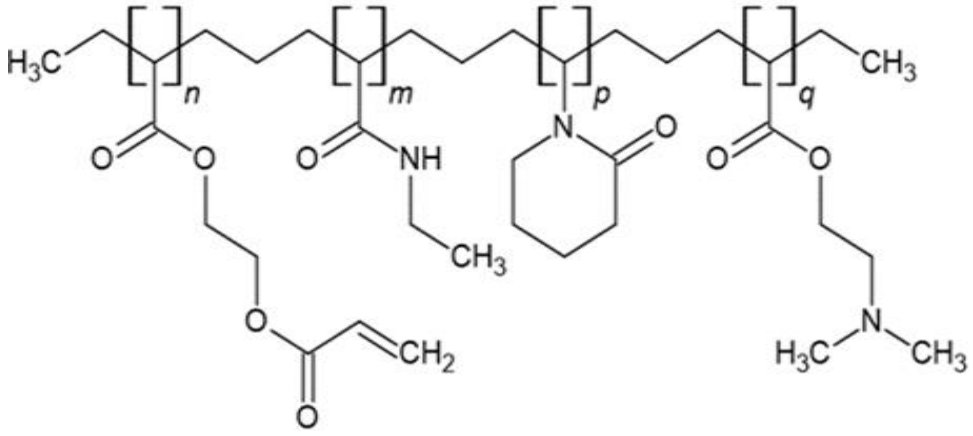

Fig. 2. Suggested Copolymer Structure of Poly(NIPAAM-PVP-PEGDA-DMAEMA) Nanogel from the Gamma-Induced Co-polymerization Reaction

The DLS technique tracks the LCST phase transition of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel, where a sudden decrease in size can be observed. However, the scattering values were misled by an unexpected interaction between the laser intensity and this thermo-sensitive Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogels. Figure 3 indicated a sudden increase in particle diameter and count rates from 95.87 nm to 1946.04 nm and 69.13 kcps to 530.26 kcps, respectively, as the laser intensity suddenly escalated from 10 % to 90 % in the DLS system. Thus, further study has been done to differentiate the experimental details that affect size measurement to enable a meaningful comparison of any instrumental factor.

Two parameters, size measurement and count rates with altered laser intensities, were tested, and the higher laser intensity was found to be beneficial to measure at high dilution more vigorously. The Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel was found to be ranging from 50.87 to 85.46 nm (Figure 4) in size as the laser intensity escalated, as expected theoretically [22]. These were similar to the accepted ideal size for nanogel in drug delivery applications, 70-200 nm [24, 29]. A rose in size measurement at a higher dose was observed, attributable to the aggregation of nanogels [30, 31].

Count rates of photon scattering intensity of irradiated monomer are directly related to the amount of nanogel in the solution [28]. A higher scattering rate clarifies a more significant number of nanoparticles formed in the solution. Hence, the highest count rate was better in this case. It was found that the control sample that was highest in temperature and concentration had the highest photon scattering rate, 206.42 kcps (Figure 5), whereas 12.99 kcps was obtained for 0.2 M at 25 °C samples.

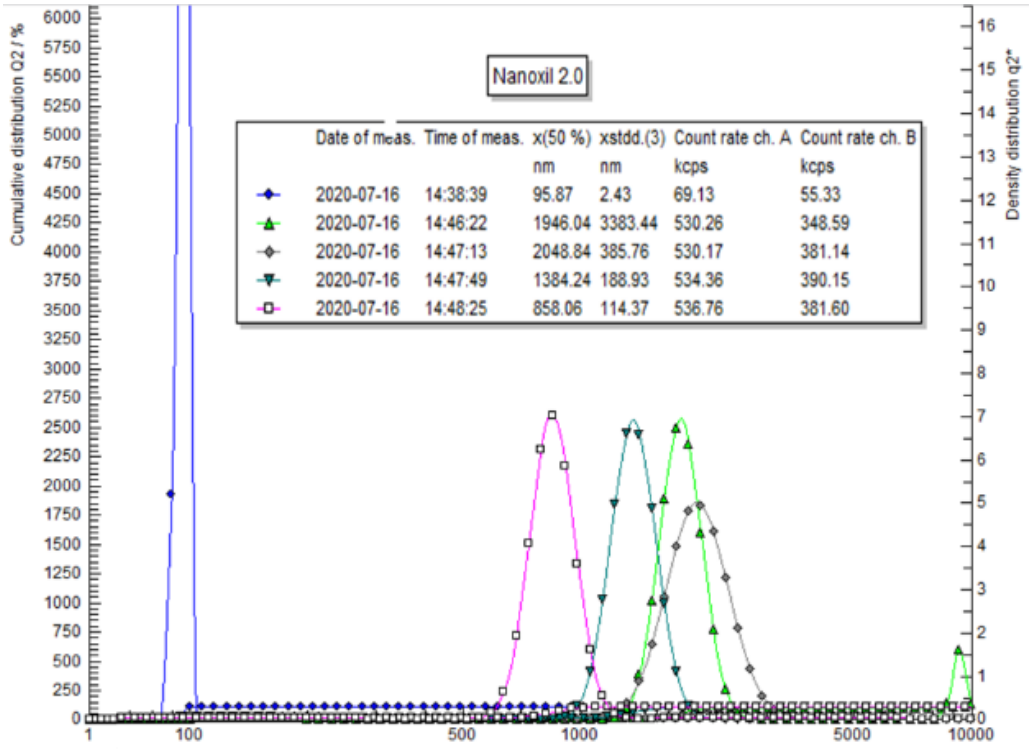

Fig. 3. Influence of Laser Intensity over Size and Count Rates of Nanogels

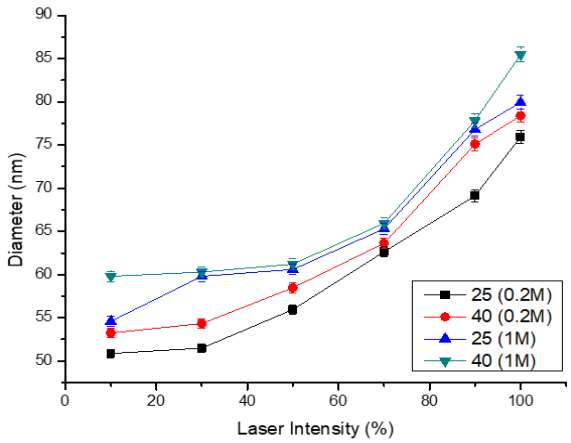

Fig. 4. Influence of Laser Intensity over Size of Nanogels

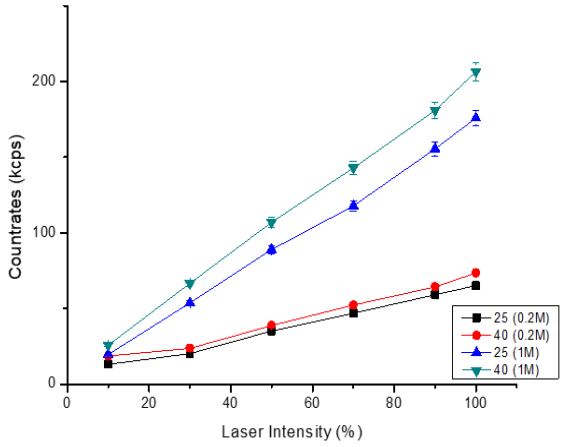

Fig. 5. Influence of Laser Intensity over Countrate of Nanogels

Mainly, the higher laser power enabled the measurement of such low concentrations of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogels with an experimental protocol that was still practical from an industrial viewpoint. The limits for the maximum applicable laser power rely on the absorption properties specific to the systems to be considered. High intensity may guide local heating and apparent smaller measured hydrodynamic radii if considerable absorption occurs [22].

4. Conclusions

Accurate nanoparticle sizing determination is becoming increasingly important in determining the LCST of thermo-responsive nanogels. The DLS technique is used to track the LCST phase transition of Poly(NIPAAM-PVP-PEGDA-DMAEMA) nanogel. Laser intensity is a critical aspect regarding adequate colloidal analysis by DLS. However, laser intensity and its implications on instrument sensitivity have not been studied well. Size measurement and count rates were tested with varying laser intensities. The higher laser intensity (Size: 85.46 nm, photon scattering rate: 206.42 kcps) was found to be beneficial for measuring at high dilution more vigorously. Given the light scattering characteristics of typical samples, a sufficient laser intensity should be selected. Elevated intensity may guide local heating and apparent smaller measured hydrodynamic radii if there is considerable absorption.

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References

[1] Hamrayev, Hemra, Kamyar Shameli, and Mostafa Yusefi. "Preparation of zinc oxide nanoparticles and its cancer treatment effects: A review paper." *Journal of Advanced Research in Micro and Nano Engineering* 2, no. 1 (2020): 1-11.

[2] Neamtu, Iordana, Alina Gabriela Rusu, Alina Diaconu, Loredana Elena Nita, and Aurica P. Chiriac. "Basic concepts and recent advances in nanogels as carriers for medical applications." *Drug Delivery* 24, no. 1 (2017): 539-557. https://doi.org/10.1080/10717544.2016.1276232

[3] Vashist, Arti, Ajeet Kaushik, Atul Vashist, Jyoti Bala, Roozbeh Nikkhah-Moshaie, Vidya Sagar, and Madhavan Nair. "Nanogels as potential drug nanocarriers for CNS drug delivery." *Drug discovery today* 23, no. 7 (2018): 1436-1443. https://doi.org/10.1016/j.drudis.2018.05.018

[4] Zaborniak, Izabela, Angelika Macior, and Paweł Chmielarz. "Smart, naturally-derived macromolecules for controlled drug release." *Molecules* 26, no. 7 (2021): 1918. https://doi.org/10.3390/molecules26071918

[5] Soni, Kruti S., Swapnil S. Desale, and Tatiana K. Bronich. "Nanogels: An overview of properties, biomedical applications and obstacles to clinical translation." *Journal of Controlled Release* 240 (2016): 109-126. https://doi.org/10.1016/j.jconrel.2015.11.009

[6] Aguilar, María Rosa, and Julio San Román. "Introduction to smart polymers and their applications." In *Smart polymers and their applications*, pp. 1-11. Woodhead Publishing, 2019. https://doi.org/10.1016/B978-0-08-102416-4.00001-6

[7] Niskanen, Jukka, and Heikki Tenhu. "How to manipulate the upper critical solution temperature (UCST)?." *Polymer Chemistry* 8, no. 1 (2017): 220-232. https://doi.org/10.1039/C6PY01612J

[8] Bordat, Alexandre, Tanguy Boissenot, Julien Nicolas, and Nicolas Tsapis. "Thermoresponsive polymer nanocarriers for biomedical applications." *Advanced drug delivery reviews* 138 (2019): 167-192. https://doi.org/10.1016/j.addr.2018.10.005

[9] Gray, Dominic M., Adam R. Town, Edyta Niezabitowska, Steve P. Rannard, and Tom O. McDonald. "Dual-responsive degradable core–shell nanogels with tuneable aggregation behaviour." *RSC advances* 12, no. 4 (2022): 2196-2206. https://doi.org/10.1039/D1RA07093B

[10] Yu, Yibin, Yi Cheng, Junye Tong, Lei Zhang, Yen Wei, and Mei Tian. "Recent advances in thermo-sensitive hydrogels for drug delivery." *Journal of Materials Chemistry B* 9, no. 13 (2021): 2979-2992. https://doi.org/10.1039/D0TB02877K

[11] Hajebi, Sakineh, Amin Abdollahi, Hossein Roghani-Mamaqani, and Mehdi Salami-Kalajahi. "Hybrid and hollow Poly (N, N-dimethylaminoethyl methacrylate) nanogels as stimuli-responsive carriers for controlled release of doxorubicin." *Polymer* 180 (2019): 121716. https://doi.org/10.1016/j.polymer.2019.121716
[12] Motaali, Soheila, Maryam Pashaeiasl, Abolfazl Akbarzadeh, and Soodabeh Davaran. "Synthesis and characterization of smart N-isopropylacrylamide-based magnetic nanocomposites containing doxorubicin anti-cancer drug." *Artificial cells, nanomedicine, and biotechnology* 45, no. 3 (2017): 560-567. https://doi.org/10.3109/21691401.2016.1161640
[13] Javed, Bilal, Xinyi Zhao, Daxiang Cui, James Curtin, and Furong Tian. "Enhanced Anticancer Response of Curcumin- and Piperine-Loaded Lignin-gp (NIPAM-co-DMAEMA) Gold Nanogels against U-251 MG Glioblastoma Multiforme." *Biomedicines* 9, no. 11 (2021): 1516. https://doi.org/10.3390/biomedicines9111516
[14] Zhang, Qilu, Christine Weber, Ulrich S. Schubert, and Richard Hoogenboom. "Thermoresponsive polymers with lower critical solution temperature: from fundamental aspects and measuring techniques to recommended turbidimetry conditions." *Materials Horizons* 4, no. 2 (2017): 109-116. https://doi.org/10.1039/C7MH00016B
[15] Maguire, Ciarán Manus, Matthias Rösslein, Peter Wick, and Adriele Prina-Mello. "Characterisation of particles in solution–a perspective on light scattering and comparative technologies." *Science and Technology of Advanced Materials* 19, no. 1 (2018): 732-745. https://doi.org/10.1080/14686996.2018.1517587
[16] Eskandari, Parvaneh, Zahra Abousalman-Rezvani, Hossein Roghani-Mamaqani, Mehdi Salami-Kalajahi, and Hanieh Mardani. "Polymer grafting on graphene layers by controlled radical polymerization." *Advances in Colloid and Interface Science* 273 (2019): 102021. https://doi.org/10.1016/j.cis.2019.102021
[17] Derkachov, G., D. Jakubczyk, K. Kolwas, K. Piekarski, Y. Shopa, and M. Woźniak. "Dynamic light scattering investigation of single levitated micrometre-sized droplets containing spherical nanoparticles." *Measurement* 158 (2020): 107681. https://doi.org/10.1016/j.measurement.2020.107681
[18] Ashfaq, Aiysha, Marie-Claude Clochard, Xavier Coqueret, Clelia Dispenza, Mark S. Driscoll, Piotr Ulański, and Mohamad Al-Sheikhly. "Polymerization reactions and modifications of polymers by ionizing radiation." *Polymers* 12, no. 12 (2020): 2877. https://doi.org/10.3390/polym12122877
[19] Bhattacharjee, Sourav. "DLS and zeta potential–what they are and what they are not?." *Journal of controlled release* 235 (2016): 337-351. https://doi.org/10.1016/j.jconrel.2016.06.017
[20] Pei, Yiwen, Beth A. Hinchliffe, and Caterina Minelli. "Measurement of the size distribution of multimodal colloidal systems by laser diffraction." *ACS omega* 6, no. 22 (2021): 14049-14058. https://doi.org/10.1021/acsomega.1c00411
[21] Giorgi, Francesco, Judith M. Curran, Douglas Gilliland, Rita La Spina, Maurice Whelan, and Eann A. Patterson. "Limitations of Nanoparticles Size Characterization by Asymmetric Flow Field-Fractionation Coupled with Online Dynamic Light Scattering." *Chromatographia* 84, no. 2 (2021): 199-206. https://doi.org/10.1007/s10337-020-03997-7
[22] Aleandri, Simone, Andrea Vaccaro, Ricardo Armenta, Andreas Charles Völker, and Martin Kuentz. "Dynamic Light Scattering of Biopharmaceutics—Can Analytical Performance Be Enhanced by Laser Power?." *Pharmaceutics* 10, no. 3 (2018): 94. https://doi.org/10.3390/pharmaceutics10030094
[23] Bhuyan, Md Murshed, Hirotaka Okabe, Yoshiki Hidaka, Nirmal Chandra Dafader, Nazia Rahman, and Kazuhiro Hara. "Synthesis of pectin-N, N-dimethyl acrylamide hydrogel by gamma radiation and application in drug delivery (in vitro)." *Journal of Macromolecular Science, Part A* 55, no. 4 (2018): 369-376. https://doi.org/10.1080/10601325.2018.1442177
[24] Hamzah, Yusof Bin, Shahrir Hashim, and Wan Aizan Wan Abd Rahman. "Synthesis of polymeric nano/microgels: a review." *Journal of Polymer Research* 24, no. 9 (2017): 1-19. https://doi.org/10.1007/s10965-017-1281-9
[25] Dispenza, Clelia, Maria Antonietta Sabatino, Natascia Grimaldi, Björn Dahlgren, Mohamad Al-Sheikhly, James F. Wishart, Zois Tsinas, Dianne L. Poster, and Mats Jonsson. "On the nature of macroradicals formed upon radiolysis of aqueous poly (N-vinylpyrrolidone) solutions." *Radiation Physics and Chemistry* 174 (2020): 108900. https://doi.org/10.1016/j.radphyschem.2020.108900
[26] Ashfaq, Aiysha, Jung-Chul An, Piotr Ulański, and Mohamad Al-Sheikhly. "On the Mechanism and Kinetics of Synthesizing Polymer Nanogels by Ionizing Radiation-Induced Intramolecular Crosslinking of Macromolecules." *Pharmaceutics* 13, no. 11 (2021): 1765. https://doi.org/10.3390/pharmaceutics13111765
[27] Mohamed, Abdul Aleem Seeni, Zi Ern Chong, Andrea Jia Xin Lai, Tomoya Tsuji, Wen Chun Siaw, Lian See Tan, Ragunath Bharath, and Taka-aki Hoshina. "Peng-Robinson Cubic Equation of State Based on Key Group Contribution and Calculation of Nitrogen Gas Solubility in MMA Dimer." *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 88, no. 1 (2021): 1-11. https://doi.org/10.37934/arfmts.88.1.111
[28] Hamzah, Mohd Yusof Bin, Shahrir Hashim, and Wan Aizan Wan Abd Rahman. "Gamma radiation-induced synthesis of nanocurcumin: Characterization and cell viability test." *International Journal of Polymeric Materials and Polymeric Biomaterials* 66, no. 18 (2017): 926-933. https://doi.org/10.1080/00914037.2017.1291512

[29] Asghar, Adnan, Teh Yuan Ying, and Khairy Zaimi. "Two-Dimensional Magnetized Mixed Convection Hybrid Nanofluid Over a Vertical Exponentially Shrinking Sheet by Thermal Radiation, Joule Heating, Velocity and Thermal Slip Conditions." *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 95, no. 2 (2022): 159-179.
[30] Rezaei, Seyed Jamal Tabatabaei, Mohammad Reza Nabid, Hassan Niknejad, and Ali Akbar Entezami. "Multifunctional and thermoresponsive unimolecular micelles for tumor-targeted delivery and site-specifically release of anticancer drugs." *Polymer* 53, no. 16 (2012): 3485-3497. https://doi.org/10.1016/j.polymer.2012.05.056
[31] Shafie, Nur Fathin Amirah, Mohd Yusof Hamzah, and Roshafima Rasit Ali. "Nanogels obtained by gamma radiation induced polymerization." *Polimery* 66, no. 9 (2021): 451-458. https://doi.org/10.14314/polimery.2021.9.1