The Parameter Pharmacokinetics of Caffeine Nanoparticle Preparations from Robusta Coffee Extracted Using PLGA and PVA

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Abstract: **Coffee is one of the beverages favoured by the public. Coffee contains caffeine which is able to increase concentration and this ability takes place depending on the dose received by the body. The purpose of this study is to isolate caffeine from coffee tree and characterize nanoparticles caffeine in polymerized PLGA-PVA and Its pharmacokinetics (V_d, k_e, $t_{1/2}$ dan *Cl*)**

Preparing of caffeine nanoparticles using ingredients form Robusta coffee beans that have been dried and roasted and extracted using digestive method, to obtain caffeine. Then the caffeine is made into a formulation of polylytic nanoparticles PLGA and PVA with emulsion solvent evaporation method. The formulation of caffeine nanoparticles is characterized particle size PSA, zeta potential and morphology is performed by using TEM. In vivo test, using 24 rats in four groups, gave 0,9 mg /200mgBW sample test versus control. Amount 0,5 ml of blood sample test is obtain at time series: 15, 30, 45, 60, 120, 180, 240, 360 and 480 via *vena orbitalis* to have (k_a;C_{max};T_{max}:AUC;V_d, k_e, $t_{1/2}$ and *Cl*) parameters. As the result of characterization is obtained an average diameter of 173.8 nm, zeta potential -16.2 mV and PDI of 0.148. We had V_d is 1954,159 ml. k_e is 0,00119 min^{-1}. $t_{1/2}$ is 580,979 min^{-1} ; *Cl* is 2,331 mg/min in T_{max} 30 minutes. Its morphology has a spheric surface shape. So that nanoparticle caffeine formulated based Robusta coffee beans can be produced using PLGA and PVA polymers using emulsion solvent evaporation method for topical pharmaceutic and cosmetics proposed.

Keywords: **Pharmacokinetics, Nanoparticle, caffeine, PLGA , PVA.**

I. INTRODUCTION

Coffee beans are the second most important ingredient in the world after oil. Robusta coffee has a higher caffeine content of Arabica coffee. Caffeine content in Robusta coffee beans is varies depending on the type of coffee beans, how to roast and how to study.[1,2] Robusta coffee (*Coffea canephora var*. Robusta) from Pagar Alam City of South Sumatra Province, is one of the best Robusta coffee in Indonesia.[3] Coffee is the second most popular drink after water and is consumed worldwide, with a daily consumption of about 1.6 billion cups. This is because caffeine is believed to be able to increase concentration, memory, and mind. [4]

In Indonesia as many as 31.5% has consume caffeinated beverages at least once a day.[5] Caffeine intake per person should not exceed 150 mg per day. As per SNI 01-7152-2006, the caffeine limit is 150 mg per day and 50 mg per serving. However, if you consume 100mg of caffeine per day, this can lead to caffeine addiction to keep the concentration of caffeine constant in the plasma, it is necessary to be made in nanoparticles using polymers so that caffeine is released slowly so that it can last a long time and have a long effect as expected.[6,7]

Besides, treatment of wounds using coffee grounds has been known to humans as one traditional medicine in coffee plantations all over the world. Resident on coffee plantations in Indonesia have for decade last year. The news of treating wound with coffee has been conveyed by doctors who served by doctors

Currently there have been studies of nanoparticles using polymer systems that are able to increase the bioavailability of drugs, for example in the research of amoxicillin-based polymer nanoparticles that show the results that the use of this polymer is able to control drug release, increase the solubility of bioactive drugs, make them more stable in the gastrointestinal (GI) tract, and suitable for oral administration. This process uses a nanotechnology system which will involve the synthesis of particles from nanoparticles ranging in size from 20-200 nanometers and this is also now widely used in the food, pharmaceutical and cosmetic industries.[8,9]

Nanoparticles vary in size, but typically range from 100 to 500 nanometers. With these nano particles capable of delivering drugs to specific tissues, allowing controlled treatment, the drug will precisely hit its target thereby reducing the toxicity caused by the drug.These nanoparticles can also increase patient adherence to taking the drug because with these nanoparticles are able to make the drug awake levels for a long time so that the patient does not need to repeat the dose at any time.[10]

There are many reasons why the use of nanoparticles is important for the advancement of therapeutic and diagnostic drug delivery. On the one hand, oral and injectable traditional medicines today are not necessarily designed with the correct prescription for each of its products. Polymer-based nanoparticles can increase the bioavailability of targeted drug

delivery and controlled drug delivery. By modifying the system, internal enzymes can prevent drug damage.[11] The purpose of the study was to sintesized and characterize caffeine nanoparticles from Robusta coffee bean extracted.

II. MATERIAL AND METHOD

Material

Robusta coffee beans, Ethyl acetate, Sigma, Distilled water, Na_2CO_3 (Sigma), PLGA-, Poly(D,L-lactide-*co*-glycolide) Resomer® RG 752 H Sigma Aldrich, PVA Poly(vinyl alcohol) Cat-341584 Sigma Aldrich, aqua for injection.Elisa Kit for Vd, Cl

Erlenmayer, rotary evaporator, paper filter, spatula, glass beaker, vial , *magnetic stirrer*, Capiller tube, Plate TLC , PSA (*Particle Size Analyzer)* HORIBA SZ-100, mikroscope elektron transmision (TEM Jeol 1010), analytic balance , *hot plate*, *sonicator bath*, micro pipet, ELISA kit Biovision

Sample Collection

The sample is Robusta coffee beans obtained directly from farmers in Pagar Alam City, South Sumatra Province, Indonesia. The sample has been identified at the Plantation Office of Pagar Alam City. The sample is dried in low temperature, then roasted and mashed. The fine powder of Robusta coffee is ready for extraction

Extraction

Robusta coffee powder is extracted by a digestion process., with 50 gram the coffee bean grounded and mixed with distilled water 250 mL, heated. Then filtered using filter paper and added with Na_2CO_3 into the filtrate then heated. Filtered again using a paper bag. To the filtrate add Pb Acetate 10%, to have precipitated. Separate solid phase, and next to used the filtrate. Add salfuric acid 10%. Then filtered, fractionated the filtrate using Chloroform for three times repetitions. The chloroform obtained was evaporated using an evaporator to produce a thick extract and dried to produce caffeine powder , Re-crystal with acetone.

Sample Preparation

. PVA solution, PVA powder is weighed as much as 250 mg and then dissolved in 10 mL with aqua pro injection at a temperature of 60°C. The mixture was then stirred for 24 hours, speed 100 rpm to produce a clear and homogeneous. PLGA solution, 100 mg of PLGA powder was dissolved in 2.5 mL of ethyl acetate and homogenized using a stirrer for 4 hours, speed 175 rpm at room temperature. Caffeine powder was weighed 50 mg and dissolved with 2 mL of ethyl acetate then homogenized and stir with 175 rpm for 4 hours.

Nanoparticle Caffeine Plga-Pva Formulated

The manufacture of nanoparticles was carried out using PLGA polymer and PVA emulsion solvent evaporation method. A total 2 ml of the caffeine solution was then added with 2.5 ml of PLGA solution mixed using a magnetic stirrer at an oil phase speed of 750 rpm The solution was pipetted and dripped by drop into the aqueous phase, 1.6 mL of PVA solution and stirred for 1 hour at a speed of 750 rpm The process was continued by homogenizing the suspension using sonication for 5 minutes with a wave power of 42 kHz. In the final stage, 25 ml of distilled water was added, then followed by the evaporation process by stirring for 24 hours to produce a suspension.

Characteristic Nanoparticle

The resulting nanoparticles were characterized using PSA (Particle Size Analyzer) to determine of diameter, PDI and Zeta Potential. This test uses a PSA brand Horiba SZ-100 with the dynamic light scattering (DLS) method. The process is by taking 50µL of nanoparticle suspension and diluting it using distilled water in a ratio of 1:100 then inserting it into a 50µL PSA cuvette and then the PSA tool will shoot monochromatic light which will be captured by the detector so as to produce the PDI value, diameter and zeta potential of the nanoparticle

Morphology Determine Particle

The morphology was observed at 30.000x using a Transmission Electron Microscope (TEM) TEM JEOL 1010 with a voltage of 80.0 kV

IN VIVO TEST PROCEDURE

1. Twenty four rat divided in four groups, in house animal. using one cage for one sample..
2. Preparing caffeine sample test nanoparticle-formulated versus non nanoparticle formulated
3. One group control negative to have 1 mL aquadest only
4. Sample test caffeine nanoparticle formulated gave (0,9 mg/200gBB)/ mL
5. One Group as Control positif to have cafein standar 0,9 mg /1 ml aqua pro injection.
6. One Group test (3) gave non-nanoparticle caffeine 0,9 mg / 1 ml aqua pro injection .
7. One Group test (4) gave nanoparticle caffeine : 0,9 mg /1 ml aqua pro injection.
8. Euthanasia with chloroform to have 0,5mL of blood sample test via orbitalis at time series.
9. Blood test with drawl in serial time (minutes) : 15, 30, 45, 60, 120, 180, 240, 360 and 480 via *vena orbitalis* using *pasteur* and capiler pipette collect into *microtube*
10. Centrifuge in 3000 rpm for 20 minute to have serum.
11. Serum 200 µL is used to test V*d*;k*e*; Cl.and T1/2 .
12. In preparation of Vd, ke Cl.and T1/2 is used *competitive* ELISA method ELISA kit Biovision

III. RESULT

The size of nanoparticles resulting with an average diameter is 173.8 nm. The zeta potential measurement have an surface charge of -16.2 mV. The size distribution observed with the poly density index (PDI) value obtained was 0.148. PSA, PDI and Zeta Value data can be seen in Table 1.

Table 1. Characteristic of Nanoparticle Caffeine PLGA-PVA Formulated

Characteristic	Value
Particle Size (nm)	173,8 nm
PDI	0,148
Zeta Potensial (mV)	-16,2

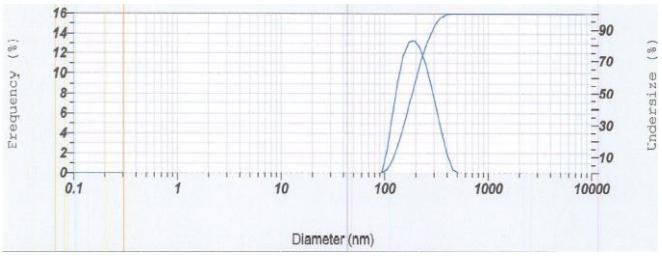

Figure 1. Nanoparticle size of Caffeine PLGA-PVA Formulated

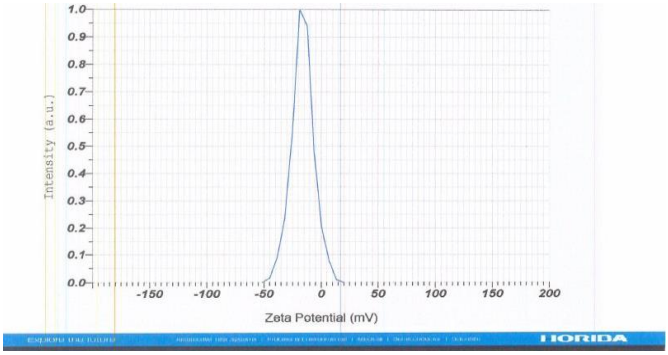

Figure 2 Zeta Potensial nanoparticle of caffeine formulated PLGA-PVA

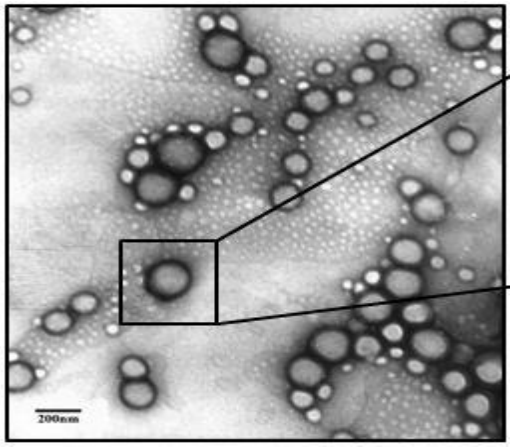

Figure 3. TEM (Morphology Nanosize of Caffeine formulated PLGA_PVA) has published

Characterization using Transmission Electron Microscope (TEM) aims to see the surface morphology of nanoparticles. The surface characterization of the nanoparticles at 30,000x magnification showed that the surface of the caffeine nanoparticles was spheric (Figure 3).

From this studied is obtained parameter of pharmacokinentic caffeine nanoparticle formulated and non nanoparticle formulated after using the procedural test Kit.

Table 2. The Average Body Weight of Animal Test

Group	Body Weigh (g)	*p-value*
1	218,17	0,360
2	213,83	0,283
3	218,17	0,167
4	227,83	0,120

Saphiro-Wilk test, p > 0,05

TABEL 3.PARAMETER of PHARMACOKINETICS CAFFEINE (V_d, k_e, $t_{1/2}$)

No	Variable	Caffeine Standard	Non NP formulated	NP formulated
1	V_d (ml)	2790,736	2660,150	1954,159
2	k_e minute^{-1}	0,00076	0,00108	0,00119
3	$t_{1/2}$ (min)	909,147	637,047	580,979
4	*Cl* (mg/min)	2,127	2,894	2,331

NP = nanoparticle, NNP =non nanoparticle

Table 4. PARAMETER PHARMACOKINETICS CAFFEINE (k_a;C_{max};T_{max}:AUC)

No	Variable	Unit	Caffeine Standard	NNP	NP
			average	average	Average
1	k_a	menit^{-1}	11,242	13,091	0,0823
2	C_{maks}	µg/ml	0,677 ± 0,199	0,582 ± 0,249	1,69 ± 1,18
3	T_{maks}	menit	30	30	30
4	AUC_{0-480}	µg.menit.ml^{-1}	13,68,816	12,70,626	18,72,484

NP = nanoparticle, NNP =non nanoparticle

IV. DISSCUSSION

To complete data of this research, after a part of the characteristic nanoparticle data that has been known and published previously by the authors, we are going now to continue perform data pharmacokinetics ((k_a;C_{max};T_{max}:AUC) of caffeine nanoparticle formulated. Particle size measurement is important in the manufacture of nanoparticle formulations where the smaller the particle size, the better the drug release power. Particle size is one of the factors that affect the efficiency of the compound or drug. Small size (nano size) can help the drug distribution process reach its target. Nano size also has high solubility and absorption efficiency. From the results of the particle size distribution using the PSA tool, the particle size is 173.8 nm (Figure 1),

which means that it is in the nanoparticle size range because it is known that nanoparticles are said to be in the range of 100 to 200 nm.[16]

From the results of the particle size distribution using the PSA tool, a particle size is obtained, of 173.8 nm which means that caffeine PLGA –PVA formulated perform in nanoparticle size. Nanoparticle size ranges from 100 to 500 nm. From the results of the zeta potential test, a value of -16.2 mV was obtained and this indicated that the suspension was stable because nanoparticles with a zeta potential value of less than -30 mV and greater than +30 mV had higher stability (Figure 2).[17]

PDI assessment is perform to see whether or not the size of a particle is uniform and from the test results obtained a PDI value of 0.148, this means that the distribution is homogeneous because it is close to a value of 0 where if the value is 1 it indicates a very wide size distribution and contains large particles and can undergo sedimentation (Table 1). In terms of morphology,[18] the surface shape is spherical (Figure 3), this indicates that the drug is well coated and the release power will be slow and it will make it easier for the drug to be given via a certain route because in drug delivery systems that use particles as conductors such as microparticles, the ability to coat drugs is high with small particle sizes, and a uniform spherical shape is preferable.[19] This will facilitate the administration of drugs through certain routes such as intravenous, intranasal and other.[20,21]

The particle size test was performed for the sample test. As based on the transmittance percentage test,. The result of PSA revealed that the particle size is 173.8 nm. Considering this, it fits the characteristic of an acceptable where the nanoparticle size ranges from 100 to 200 nm is better depend on the drug design using.

The PDI value of sample is measured at 0 .148. This value refers to the distribution of the particle size. If the PDI value is close to 0, the dispersity of the particle size is homogenous. If the PDI value is greater than 0.5, the heterogeneity is considered high. Samples with a PDI value greater than 0.7 have a very wide size distribution. This signifies that the size of the sample has a homogenous particle size dispersion. PDI measures the homogeneous of nanoparticles, the smaller the pdi the more homogeneous nanoparticles. Nanoparticles with pdi smaller than 0.4 is considered acceptable for drug deliver.[19,22] This is because differences among particle sizes are impactful on particle characterization.[23]

According to V*d* situation seems V*d*, is going to decrease in nanoparticle size formulated compare to non nanoparticle formulated Showed Profile pharmacokinetics of this study in Table 3. Since K*e* is leading to increase in nanoparticle formulated. These related to theory said, more time to eliminate more less drug in Volume distribution. T*max* showed in 30 minutes in nanoparticle preparation compare to non nanoparticle formulated, It mean that product is easy to absorbs and more rapidly enter to the blood circulation but there is a difference in volume distribution between nanoparticle versus non nanoparticle formulated and more rapidly to eliminate from the body (Table 4). Related to T_{max} and K_a (absorbtion) caffeine of nanoparticle formulation had inferior to non nanoparticle, we assumed that can be ease goes to the side of in event the body when its needed .The elimination pharmacokinetic profile of a drug can change due to the performance of presence size and formulated of the drugs. By this reason, particle size and also drug formulated can caused to change the paramater pharmacokinetic of drugs, likes absorption, distribution, metabolism and excretion of a drug[24,]

It can be seen from that data that there is a decrease in the Vd of the nanoparticle extract when compared to the non-nanoparticle extract. This is consistent with the study of researcher which showed a decrease in the volume of distribution of curcumin-PLGA nanoparticles. The volume of distribution describes the theoretical amount of fluid required in the body to contain the drug at the plasma drug concentration.[26,27]. The increasing in Vd indicated that there was an increasing drug in the plasma concentration of the caffeine-PLGA nanoparticle preparations related to the data of $T^{1/2}$ and k elimination of the test . From This study can be consider to produced in pharmaceutical and cosmetics preparation regarding to the objective of used preparation

V. CONCLUSION

Its has been successfully to have caffeine nanoparticles formulated from Robusta coffee bean extract. Formulated by using PLGA and PVA polymers through the emulsion solvent evaporation method. We obtained diameter value of 173.8 nm, zeta potential of -16.2 mV, PDI of 0.148. And the surface morphology looks spheric. Based on the research, the pharmacokinetics paramater ((k_a;C_{max};T_{max}:AUC) of nanoparticle caffeine formulated performance is better then non nanoparticle formulated. Suggested to produce caffeine nanoparticle formulated in pharmaceutics and cosmetics preparation for topical used.

CONFLICT of INTEREST

There is no Conflict of Interest Refers to this article

REFERENCES

[1]. Aka F, Angelo, Augustin A, et al. *The Pharma Innovation Journal 2016; 5(12): 01-05 Acute Toxicity of the Aqueous Extract of Roasted and Ground Beans of Coffea Canephora Robusta in the Wistar Rat.* ; 2016.

[2]. Cappelletti S, Daria P, Sani G, Aromatario M. Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug? *Current Neuropharmacology*. 2015;13(1):71-88. doi:10.2174/1570159x13666141210215655

[3]. Syafaruddin, Pabendon M, Penelitian B, Industri T, Penyegar D. Keragaman Genetik Antar Klon Kopi Robusta Lokal Pagar Alam Berdasarkan Analisis Marka Ssr Genetic Variability Among Indigenous Robusta Coffee Clones From Pagar Alam Based On Ssr Markers Analysis.

[4]. Vignoli JA, Viegas MC, Bassoli DG, Benassi M de T. Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Research International*. 2014;61:279-285. doi:10.1016/j.foodres.2013.06.006

[5]. Kemenkes RI. 2013. Riset Kesehatan Dasar. Jakarta : Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia

[6]. Zarwinda I, Sartika D. Pengaruh Suhu Dan Waktu Ekstraksi Terhadap Kafein Dalam Kopi. *Lantanida Journal*. 2019;6(2):180. doi:10.22373/lj.v6i2.3811

[7]. Hodali, H. A. Rawajfeh, R. S. Allababdeh, N. A., Caffeine loading into micro- and nanoparticles of mesoporous silicate materials: in vitro release kinetics. Journal of Dispersion Science and Technology. Doi 10.1080/01932691.2016.1239540

[8]. Enes .Development and characterization of polymeric-based nanoparticles for sustained release of amoxicillin – an antimicrobial drug. Artificial Cells, Nanomedicine, and Biotechnology. Published 2014. Doi 10.1080/21691401.2018.1476371

[9]. Nitthikan N, Leelapornpisid P, Natakankitkul S, et al. Improvement of Stability and Transdermal Delivery of Bioactive Compounds in Green Robusta Coffee Beans Extract Loaded Nanostructured Lipid Carriers. *Journal of Nanotechnology*. 2018;2018:1-12. doi:10.1155/2018/7865024

[10]. Biswas, A. K., M.R. Islam, Z.S. Choudhury, A. Mostafa, and M.F. Kadir (2014). Nanotechnology based approaches in cancer therapeutics. Advances in Natural Sciences: Nanoscience and Nanotechnology, 5(4). Doi 10.1088/2043- 6262/5/4/043001

[11]. Junwei Zhang, Saltzman M. Engineering biodegradable nanoparticles for drug and gene delivery. ResearchGate. Published March 2013.

[12]. Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The potential of arabica coffee grounds nanoparticles as an active compound of pharmaceutical preparations. *IOP Conference Series: Earth and Environmental Science*. 2020;425:012034. doi:10.1088/1755-1315/425/1/012034

[13]. Murdock, R.C., Braydich-Stole, L., Schrand, A.M., Schlager, J.J. & Hussain, S.M. 2008, Characterization of Nanoparticle Dispersion in Solution Prior to In Vitro Exposure using Dynamic Light Scattering Tehnique. Toxicol, Sci, 101: 239-253.

[14]. Ngafif Akhmad et al. Optimasi natrium alginate dan kalsium klorida ($CaCL_2$) sebagai agen sambung silang nanopartikel ekstrak etanol daun katuk (sauropus androgynous (L) Merr).B I M F I Volume 2 no 1.Oktober 2020- Desember 2020

[15]. ardikasari et al. Microencapsulation of Mefenamic Acid Microcapsules With Chitosan and Sodium Alginate as Polymer Using Ionic Gelation Method. Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal) 2020; 6 (2): 192 – 203. DOI:10.22487/j24428744.2020.v6.i2.14589.

[16]. Syed, A A, Rizvi, Ayman, M Saleh, 2018, Applications of nanoparticle systems in drug delivery technology Saudi Pharm J 2018 Jan;26(1):64-70. doi: 10.1016/j.jsps.2017.10.012.

[17]. Rençber S, Aydın Köse F, Karavana SY, 2020, Dexamethasone loaded PLGA nanoparticles for potential local treatment of oral precancerous lesions. Pharm Dev Technol. 2020 Feb;25(2):149-158. doi: 10.1080/10837450.2019.1673407.

[18]. Banerjee, A, Qi, Jianping, Gogoi, R, Wong, J, Mitragotri, S, 2016, Role of nanoparticle size, shape and surface chemistry in oral drug delivery, J Control Release 2016 Sep 28;238:176-185. doi: 10.1016/j.jconrel.2016.07.051.

[19]. Mir, M., Ahmed, N., Rehman, Ur., 2017, Recent applications of PLGA based nanostructures in drug delivery, Colloids Surf B Biointerfaces 2017 Nov 1;159:217-231. doi: 10.1016/j.colsurfb.2017.07.038. Epub 2017 Jul 28.

[20]. Ding D, Zhu Q. Recent advances of PLGA micronanoparticles for the delivery of biomacromolecular therapeutics., 2017, Mater Sci Eng C Mater Biol Appl. 2018 Nov 1;92:1041-1060. doi: 10.1016/j.msec.2017.12.036. Epub 2018 Jan 5.PMID: 30184728

[21]. Mardiyanto, 2013. Investigation Of Nanoaprticute Formulation Intended For Caffeine Delivery To Hair Foliccles.Dissertation.Dr.Rer.Nat.Faculty III.Chemical,Pharmacy, And Biological.University Searbrucken.Germany

[22]. Kumar, A., & Dixit, C. K. (2017). Methods for characterization of nanoparticles. Advances in Nanomedicine for the Delivery of Therapeutic Nucleic Acids, 43–58. doi:10.1016/b978-0-08-100557-6.00003-1

[23]. Mast Jan et al.. 2020.Characterization of nanomaterials by transmission electron miscroscopy : Measurement procedures.Chapter 2.1.2.https://doi.org/10.1016/B978-0-12-814182-3.00004-3

[24]. Hodali, H. A., Rawajfeh, R. S., & Allababdeh, N. A. (2016). Caffeine loading into micro- and nanoparticles of mesoporous silicate materials: in vitro release kinetics. Journal of Dispersion Science and Technology, 38(9), 1342–1347. doi:10.1080/01932691.2016.1239540

[25]. Hoshyar, N., Gray, S., Han, H., & Bao, G. (2016). The Effect Of Nanoparticle Size On In Vivo Pharmacokinetics And Cellular Interaction. Nanomedicine, 11(6), 673–692. Doi:10.2217/Nnm.16.5

[26]. Alsabri, S. G. Mari, W. O. Younes, S. Alsadawi, M. A. Oroszi, T. L., 2017. Review Article : Kinetic and Dynamic Description of Caffeine. Journal of Caffeine Research. Ohio : Mary Ann Liebert, Inc. 1-7.

[27]. Algul et al.., 2018. Preformulation, Characterization, and In Vitro Release Studies of Caffeine-Loaded Solid Lipid Nanoparticles. J. Cosmet. Sci., 69, 165–173.