# TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Investigating the effect of the stabilizer concentrations (1%, 2%, 3%, 4% and 5% PVA (polyvinyl alcohol)) on the surface properties of the nanoparticles and the drug delivery systems that are formed with the nanoparticles of 2% PLGA (poly(lactic-co-glycolic acid)), by solvent evaporation method.

## Biology Extended Essay


**Supervisor** : Fuat İsmet ŞİŞMAN

**Name of student** : Mine Su ERTÜRK

**Candidate number**: D1129056


**Word count** : 3920

## Abstract

Nanotechnology aims to manipulate characteristics of matter at atomic scale in order to improve drug delivery systems. The purpose of this extended essay is to investigate how surface properties of nanoparticles and drug delivery sytems of these molecules are affected of the concentration of the stabilizer, used during the preparation. The research question is "How does changing the concentration of the stabilizer affect the surface properties of the nanoparticles and the drug delivery systems that are formed with the nanoparticles of 2% PLGA (poly(lactic-co-glycolic acid)) and the stabilizer concentrations of 1%, 2%, 3%, 4% and 5% PVA (polyvinyl alcohol), by solvent evaporation method?".

The hypothesis states that there will be significant difference between the means of sizes and zeta potentials of nanoparticles that are produced with varying PVA concentrations. Thus, it will be concluded that drug delivery systems will have variety, as well.

So as to verify the hypothesis, nanoparticles of PLGA are going to be prepared by solvent evaporation method where PVA concentration is going to be the independent variable. Solutions containing PLGA and PVA are mixed and the solvent is evaporated by stirring at high rates in this method. Then, the particles are analysed with a software programme and data of size and zeta potential are compared.

The results illustrated that mean size and zeta potential values of varying concentrations showed significant differences. Therefore, the biocompatibilities of nanoparticles can be altered by changing the stabilizer concentration.

Word count: 238

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# Introduction

I have been interested in nanotechnology and especially nanomedicine since I first saw my brother's scientific journals of The Scientific and Technological Research Council of Turkey, "Bilim ve Teknik", where I read about this new technology. So, I decided to visit a pharmaceutics laboratory and asked a pharmacist who is one of my mother's friends. After my visit to the lab, I started doing research on the topic and learned that nanotechnology promises a wide range of reforms for drug delivery. Thus, I decided doing my research on nanomedicine and especially the use of nanomedicine on drug delivery.

After doing some investigation on the topic and reading articles such as "*Nanotechnology for the biologist*"[1], I found out that nanotechnology includes all research done with the purpose of changing the characteristics of a substance on an atomic scale. On the other hand, nanomedicine deals with the medical applications of nanotechnology such as diagnosing and treating diseases with the help of nanoparticles. Another aim of nanomedicine is the development of drug delivery systems based on nanoparticles.

I learned the importance of drug delivery systems of nanoparticles is that they can escape the reticuloendothelial system (RES), which is a part of the immune system and functions to remove foreign molecules from the body. The time period during which they can stay in the body is much longer than larger molecules since larger molecules are detected easier than nanoparticles. Additionally, it is worth noting that nanoparticles are able to interact with biomolecules on the cell surface because they have similar dimensions.[1]

Another advantage of nanoparticles in drug delivery that caught my attention was the fact that their surface properties can be altered to have desired characteristics as mentioned in "*Nanoparticles – A Review*"[2], "Particle size and surface characteristics of nanoparticles can be easily manipulated..". These desired properties may include the surface charge of the particle, its size or its solubility in different solvents. Surface charge of a nanoparticle is characterized by its zeta potential, where zeta potential indicates the stability of the particle and shows its electrical potential. It is mostly influenced by the composition of the nanoparticle and the medium in which it is dispersed. Nanoparticles having a zeta potential value above (+/-) 30mV have shown to be stable in suspension, as the surface charge prevents aggregation.[2] After reading this article, by VJ Mohanraj[2], about drug delivery systems and nanoparticles, I realised that it is important to have particles that are stable because if the particles aggregate, they will not function properly. As zeta potential demonstrates the degree of repulsion between similarly charged particles; a high zeta potential means that the molecules can resist the repulsion forces; thus can resist aggregation.

In "*Biodegradable polymeric nanoparticles based drug delivery systems*"[3], the article I have examined, I saw that there are many polymers used in drug delivery systems such as PLGA (poly(lactic-co-glycolic acid)), PEG (polyethylene glycol), gelatin and chitosan. PLGA is one of the most commonly used polymers for the preparation of nanoparticles in pharmaceutics. It is an approved polymer being studied for many years as a suitable drug delivery material due to its biocompatibility, biodegradability and non-toxicity.[4] By the usage of this polymer, surface charge, solubility and some other properties of the drug molecule can be changed. The reasons that have affected my decision on polymer choice being PLGA

were that it is not harmful to human body and I am allowed to use this polymer, but not others, because I am a non-professional at the area.

To obtain a nanomolecule of PLGA, I had to choose a method suitable for the characteristics of the polymer and while deciding, had to consider the fact that it should be a method I can use. After some research, I found that solvent evaporation method is the most convenient one in these conditions. In this method, the polymer should be solved with an organic solvent and then added into the aqueous phase with stabilizer. In this experiment, the organic solvent is acetone whereas PVA (polyvinyl alcohol), a water-soluble synthetic polymer, is the stabilizer which are picked from the article "*Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery*"[5]. It is stated in the article "*Nanoparticles – A Review*"[2] that particle size is influenced by the concentration of the stabilizer. Stabilizer is a compound that decreases the surface tension and avoids the aggregation of the nanoparticles.

With all these information, the topic of my research will be comparing the characteristics such as size and zeta potential of blank PLGA nanoparticles which are prepared with different concentrations of water phase (PVA solution) and commenting on their differences in drug delivery systems. Size and zeta potential values are used in the comparison because they indicate the functionality of a nanoparticle as stated earlier. There are many studies with the purpose of developing nanotechnological drugs to overcome barriers in body such as blood-brain barrier (BBB) and to cure diseases in easier ways by altering the characteristics of the devices used. My research question in this study is "How does changing the concentration of the stabilizer affect the surface properties of the nanoparticles and the drug delivery systems that are formed with the nanoparticles of 2% PLGA (poly(lactic-co-glycolic acid)) and the stabilizer concentrations of 1%, 2%, 3%, 4% and 5% PVA (polyvinyl alcohol), by solvent evaporation method?".

## Hypothesis

Nanotechnology is the study of particles on the atomic level, generally dealing with molecules having a size between 1-100 nanometers. The main purpose is to manipulate the surface properties of the substance and to obtain particles with desired characteristics to use in drug delivery.

PLGA is a copolymer that has biocompatibility and biodegradability properties making it used in drug delivery systems. Although its monomers (lactic acid and glycolic acid) have poor solubilities, PLGA can be dissolved by a wide range of common solvents.[4]

The characteristics of nanoparticles can be altered easily by many factors such as the type and concentration of both polymer and stabilizer, stirring speed and the preparation method as stated in the article "*Nanoparticles - A Review*"[2]. With reference to the study "*The influence of technological parameters on the physiochemical properties of blank PLGA nanoparticle*"[3], where it is shown experimentally that the factors listed above affect the properties of nanoparticles, it was hypothesized that "There is a significant difference between the biocompatibilities and drug delivery properties due to the different sizes and zeta potentials of nanoparticles that are prepared with 2% PLGA (poly(lactic-co-glycolic acid)) and varying concentrations (1%, 2%, 3%, 4% and 5%) of PVA (polyvinyl alcohol) by solvent evaporation method."

The characteristics that will be compared in this research are size and zeta-potential values of blank PLGA nanoparticles.

## Method Development and Planning

In order to observe how the concentration of the stabilizer affects the nanoparticle's size, zeta potential values and the biocompatibility of the molecule, I had to conduct an experiment with the chemicals that are most suitable. With this aim in mind, I started searching for articles about nanoparticle preparation. From these articles such as "*Controlled release systems and polymers used in these systems*"[7], I learned that various chemicals can be used to produce nanoparticles to work in the best way by using different methods. After gaining some information on the preparation methods, I talked with a pharmacist in order to decide which chemicals to use and which method to perform during my experiment.

As a non-professional at this area, I had to find chemicals that are not harmful for human body and are allowed for student experiments. Due to this reason, my choices of chemicals were PLGA, PVA and acetone. In this experiment, independent variable was decided to be the concentration of PVA and dependent variable to be the characteristics of the PLGA nanoparticles where acetone was used as the solvent.

I had realized that the preparation method is usually determined according to the desired properties of the final product and the properties of the chemicals being used. The polymer used in the experiment was PLGA, a hydrophobic molecule. According to "*Polymer nanoparticles: Preparation techniques and size-control parameters*"[8], "solvent evaporation method" is the most appropriate and common one with PLGA. Thus, I determined my method to be solvent evaporation, which is preferred when the nanoparticle is being obtained from an early prepared polymer. After deciding on the polymer and the method, I had to choose the stabilizer and the solvent; both of which have important functions in the production of the nanoparticles. Two different organic solvents had been used in solvent evaporation method for years, one of which was toxic. So that, acetone, having much less toxicity, became the solvent of my experiment. The last chemical to be determined was the stabilizer and in this case there weren't any other options than PVA because of my polymer.

Solvent evaporation method is the name given to the process of preparing a nanoparticle from a present polymer. In this method, solution containing the polymer and the stabilizer are mixed and the solvent is evaporated by stirring it at high rates or increasing the temperature.[9] Since the temperature change can affect the characteristics of the nanoparticles, only stirring will be used in this research and temperature will be constant.

Another important thing in developing the method is controlling all the variables that might have an effect on the results. It was stated that particle size is influenced by the type of the stabilizer, homogenizer speed, temperature and polymer concentration.[2] So as to control these variables, same source of chemicals are used in all trials and the type of stabilizer is always PVA. Similarly, all of the devices, such as homogenizer and magnetic stirrer are identical with fixed values of speed for varying concentrations of PVA. For control of temperature, heater of the magnetic stirrer is turned off at all times and the mixtures are kept in the same room, being exposed to identical conditions, whereas during centrifuge, the device is adjusted to 4°C. Moreover, PLGA is used with a constant concentration of 2% throughout the experiment.

Purities of the mixtures are protected by covering the containers with parafilm when they are not being used.

Analysis of the nanoparticles can only be done with technological devices due to the fact that the molecules are too small for observation with naked eye. I didn't know how I could do the analysis hence, I asked a pharmacist to learn that there are machines and software programmes for this purpose. The data obtained from the software programme is going to be used in the comparison of nanoparticle characteristics -zeta potential and size- when concentration of the stabilizer is changing.

At the end of the experiment, photographs of the nanoparticles are going to be taken with Transmission Electron Microscopes (TEM) in TEM laboratories of METU, Turkey. These photographs are going to provide information about relative sizes of particles.

With a view to find chemicals and necessary devices, I asked for help from the pharmacists of Hacettepe University, Turkey. By providing me with a suitable laboratory, they gave me access to the centrifuge, homogenizer, magnetic stirrers, ultrapure water system, zeta-size analyzer and other tools that might be needed. Likewise, the chemicals (PVA, PLGA and acetone) are supplied by the university in necessary amounts.

In order to reduce error sources, the experiment has to be conducted very carefully, following the steps of the method and under the supervision of a professional. I performed my experiment after watching a professional following a similar method so as to avoid mistakes. By this way, I gained some experience and knowledge about what to do first and how to do it. Also, one of the pharmacists working at Hacettepe University observed my experiment and warned me when it was necessary.

I expect that the characteristics of nanoparticles which have varying stabilizer concentrations will be different from each other.

## Materials

100.00mL beaker (15 of them, glass)
200.00mL beaker (5 of them, glass)
10.00mL graduated cylinder (1 of them, glass)
100.00mL graduated cylinder (1 of them, glass)
1000.00mL graduated cylinder (1 of them, glass)
Cuvette (5 of them, plastic)
Pasteur pipette (3 of them, plastic)
5.00mL pipette (2 of them, glass)
10.00mL pipette (2 of them, glass)
Pipette pump (1 of them)
Spatula (1 of them)
Stirring rod (1 of them, glass)
Parafilm
Magnetic stirrer (5 of them)
Magnetic stir bar (5 of 3 cm long, 5 of 1 cm long)
Electronic scale (1 of them)
1.00mL syringe (5 of them)
10.00mL syringe (5 of them)
Homogenizer (1 of them)
Centrifuge (1 of them)
Centrifuge tube (5 of them, plastic)
Ultrapure water (1000.00mL)
Ultrapure water system
Zeta-size analyzer (Zetasizer)
PLGA -poly(lactic-*co*-glycolic acid) (1.000g)
PVA -polyvinyl alcohol (7.500g)
Acetone (50.00mL)
Gloves

## Method

Part 1: Preparation of water phase (1%, 2%, 3%, 4% and 5% PVA solutions)

1. Take 0.500g of PVA in a 100.00mL beaker for 1% PVA solution.
2. Add 50.00mL of ultrapure water into the beaker.
3. Add a 1cm long magnetic stir bar and place the beaker on the magnetic stirrer. Stir PVA and ultrapure water for 3-3.5 hours at 750rpm (rate per minute) until all PVA dissolves in water. Do not use the heater.
4. Repeat steps 1-3 with 1.000g, 1.500g, 2.000g and 2.500g of PVA instead of 0.500g for 2%, 3%, 4% and 5% PVA solutions respectively.

Part 2: Preparation of organic phase (2% PLGA solution)

1. Take 0.200g of PLGA in a 100.00mL beaker.
2. Add 10.00mL of acetone into the beaker.
3. Add a 1cm long magnetic stir bar and place the beaker on the magnetic stirrer. Stir PLGA and acetone for 2 minutes at 750rpm until all PLGA dissolves in acetone. Do not use the heater.
4. Repeat steps 1-3 with the same amount of chemicals for 4 more times.

Part 3: Preparation of nanoparticles

1. Take 20.00mL of 1% PVA in a 300.00mL beaker.
2. Take 10.00mL of 2% PLGA in a 50.00mL beaker.
3. Add a 3cm long magnetic stir bar into the 300.00mL beaker that has 20.00mL of 1% PVA in it. Place the beaker on the magnetic stirrer at 750rpm.
4. Get all of the 2% PLGA solution into a syringe.
5. Quickly pour the organic phase into the water phase. Water phase should be stirred by the magnetic stirrer during the process. Do not use the heater.
6. Stir the mixture for 2 minutes at constant temperature.
7. Repeat the steps 1-6 with 2%, 3%, 4% and 5% PVA instead of 1% PVA solution.
8. Remove the magnetic stir bar from the beaker. Stir the 1% PVA + 2% PLGA mixture with homogenizer at 11000rpm for 2 minutes.
9. Repeat step 8 with 2% PVA + 2% PLGA, 3% PVA + 2% PLGA, 4% PVA + 2% PLGA and 5% PVA + 2% PLGA mixtures instead of 1% PVA + 2% PLGA mixture under the same conditions.
10. Add 30.00mL of ultrapure water into each of the beakers.
11. Place a 3 cm magnetic stir bar in each of the beakers.
12. Cover the beakers with parafilm to protect from impurities that might enter during the stirring process. Open small holes with a needle on the parafilm in order to let the solvent evaporate.
13. Place the beakers on the magnetic stirrers. Stir the mixtures for 20-22 hours at 750rpm and constant temperature to evaporate the solvent. Do not use the heater.
14. After solvent evaporation, remove magnetic stir bars and parafilm from the beakers. Pour the mixtures into centrifuge tubes and label each tube.
15. By weighing the tubes with an electronic scale, equalize the masses of the tubes for proper centrifugation.

16. Place the first tube in the centrifuge and the other tube at its opposite hole. Total weight must be equal, and the system must be in equilibrium.
17. Centrifuge the mixtures for 30 minutes at 13500rpm and 4.00°C.
18. After centrifugation, remove supernatant from the tube. Keep the precipitate.
19. Add 10.00mL ultrapure water into each of the centrifuge tubes.
20. Get all the particles that are on the walls of the tube into the water by scratching the tube's walls with a stirring rod.
21. Repeat the steps 15-19 for removal of clearing water. This time, centrifuge for 10 minutes instead of 30 minutes.
22. Add 10.00mL of ultrapure water into each tube for dilution.

Part 4: Analysis of nanoparticles

1. With a 1.00mL syringe, fill a cuvette quickly with 1% PVA + 2% PLGA solution. There must not be any air bubbles in the cuvette.
2. Place the cuvette inside the zeta-size analyzer.
3. With the Zetasizer Software, measure the sizes and zeta potentials of the particles.
4. Remove the cuvette and repeat the steps 1-3 with 5% PVA + 2% PLGA solution.

Repeat the procedure for 4 more times.

Notes: Always label the containers with concentration values.
Wear gloves at all times for safety.
Always cover the containers with parafilm.
Material list is for one trial only.

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# Data Collection and Processing

| Trial | Concentration of PVA | Concentration of PLGA | Size of nanoparticle (nm±0.1) | Zeta potential of nanoparticle (mV±0.01) | Mass of PVA (g±0.001) | Mass of PLGA (g±0.001) | Volume of Ultrapure Water Used to Dissolve PVA (mL±0.01) | Duration of Magnetic Stirring for Dissolving PVA (hour) | Volume of Acetone Used to Dissolve PLGA (mL±0.01) | Duration of Magnetic Stirring for Dissolving PLGA (min) | Temperature (°C±0.01) | Duration of Magnetic Stirring to Evaporate the Solvent (hour) | Rate of Magnetic Stirring (rpm) | Duration of First Centrifuge (min) | Duration of Second Centrifuge (min) | Rate of Centrifuge (rpm) | Temperature During Centrifuge (°C±0.01) | Duration of Homogenization (min) | Rate of Homogenization (rpm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1% | 2% | 158.4 | -6.31 | 0.500 | 0.200 | 50.00 | 3-3.5 | 10.00 | 2 | 23.50 | 20-22 | 750 | 30 | 10 | 13500 | 4.00 | 2 | 11000 |
| 2 | | | 158.4 | -6.35 | | | | | | | | | | | | | | | |
| 3 | | | 159.3 | -6.45 | | | | | | | | | | | | | | | |
| 4 | | | 157.6 | -6.24 | | | | | | | | | | | | | | | |
| 5 | | | 158.2 | -7.20 | | | | | | | | | | | | | | | |
| 1 | 2% | | 169.6 | -14.10 | 1.000 | | | | | | | | | | | | | | |
| 2 | | | 168.2 | -13.50 | | | | | | | | | | | | | | | |
| 3 | | | 166.7 | -13.60 | | | | | | | | | | | | | | | |
| 4 | | | 166.5 | -13.70 | | | | | | | | | | | | | | | |
| 5 | | | 166.1 | -13.80 | | | | | | | | | | | | | | | |
| 1 | 3% | | 171.3 | -6.17 | 1.500 | | | | | | | | | | | | | | |
| 2 | | | 171.2 | -6.11 | | | | | | | | | | | | | | | |
| 3 | | | 171.6 | -6.61 | | | | | | | | | | | | | | | |
| 4 | | | 170.6 | -6.29 | | | | | | | | | | | | | | | |
| 5 | | | 168.1 | -6.37 | | | | | | | | | | | | | | | |
| 1 | 4% | | 213.1 | -15.90 | 2.000 | | | | | | | | | | | | | | |
| 2 | | | 219.6 | -15.50 | | | | | | | | | | | | | | | |
| 3 | | | 215.5 | -16.10 | | | | | | | | | | | | | | | |
| 4 | | | 214.6 | -16.20 | | | | | | | | | | | | | | | |
| 5 | | | 213.5 | -16.20 | | | | | | | | | | | | | | | |
| 1 | 5% | | 224.4 | -8.23 | 2.500 | | | | | | | | | | | | | | |
| 2 | | | 224.1 | -8.34 | | | | | | | | | | | | | | | |
| 3 | | | 225.0 | -8.16 | | | | | | | | | | | | | | | |
| 4 | | | 224.2 | -8.28 | | | | | | | | | | | | | | | |
| 5 | | | 224.3 | -8.23 | | | | | | | | | | | | | | | |

**Table 1:** shows the sizes and zeta potentials of nanoparticles with respect to their values of PVA concentrations where PLGA concentration is 2%; and the constant variables: mass of PLGA, volume of ultrapure water used to dissolve PVA, duration of magnetic stirring for dissolving PVA, volume of acetone used to dissolve PLGA, duration of magnetic stirring for dissolving PLGA, duration of magnetic stirring to evaporate the solvent, rate of magnetic stirring, duration of first centrifuge, duration of second centrifuge, rate of centrifuge, temperature during centrifuge, duration of homogenization, rate of homogenization.

| For | Size of Nanoparticle | | | | | Zeta Potential of Nanoparticle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Concentration of PVA | 1% | 2% | 3% | 4% | 5% | 1% | 2% | 3% | 4% | 5% |
| Mean | 158.38 | 167.42 | 170.56 | 215.26 | 224.4 | -6.51 | -13.74 | -6.31 | -15.98 | -8.248 |
| Median | 158.4 | 166.7 | 171.2 | 214.6 | 224.3 | -6.35 | -13.7 | -6.29 | -16.1 | -8.23 |
| Mode | 158.4 | - | - | - | - | - | - | - | -16.2 | -8.23 |
| SD | 0.609918 | 1.454991 | 1.422322 | 2.602499 | 0.353553 | 0.393128 | 0.230217 | 0.195959 | 0.294958 | 0.066858 |
| SE | 0.272764 | 0.650692 | 0.636082 | 1.163873 | 0.158114 | 0.175812 | 0.102956 | 0.087636 | 0.131909 | 0.0299 |
| T | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 | 2.7764 |
| 95%CI(SE*T) | 0.757301 | 1.806581 | 1.766017 | 3.231377 | 0.438987 | 0.488126 | 0.285848 | 0.243312 | 0.366232 | 0.083014 |
| 95%CI(Excel) | 0.534607 | 1.275333 | 1.246697 | 2.281149 | 0.309898 | 0.344586 | 0.201791 | 0.171763 | 0.258537 | 0.058603 |

**Table 2:** shows mean, mode, median, standard deviation, standard error, t, 95% confidence interval values for the data of size and zeta potential of nanoparticles with respect to their concentrations of PVA.



Mine Su Erturk
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Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| 1% | 5 | 791.9 | 158.38 | 0.372 |
| 2% | 5 | 837.1 | 167.42 | 2.117 |
| 3% | 5 | 852.8 | 170.56 | 2.023 |
| 4% | 5 | 1076.3 | 215.26 | 6.773 |
| 5% | 5 | 1122 | 224.4 | 0.125 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 18349.67 | 4 | 4587.417 | 2010.262 | 9.7E-26 | 2.866081 |
| Within Groups | 45.64 | 20 | 2.282 | | | |
| Total | 18395.31 | 24 | | | | |

**Table 3:** Single factor Analysis of Variance (ANOVA) statistical calculation for all groups of sizes of nanoparticles.

Anova: Single Factor

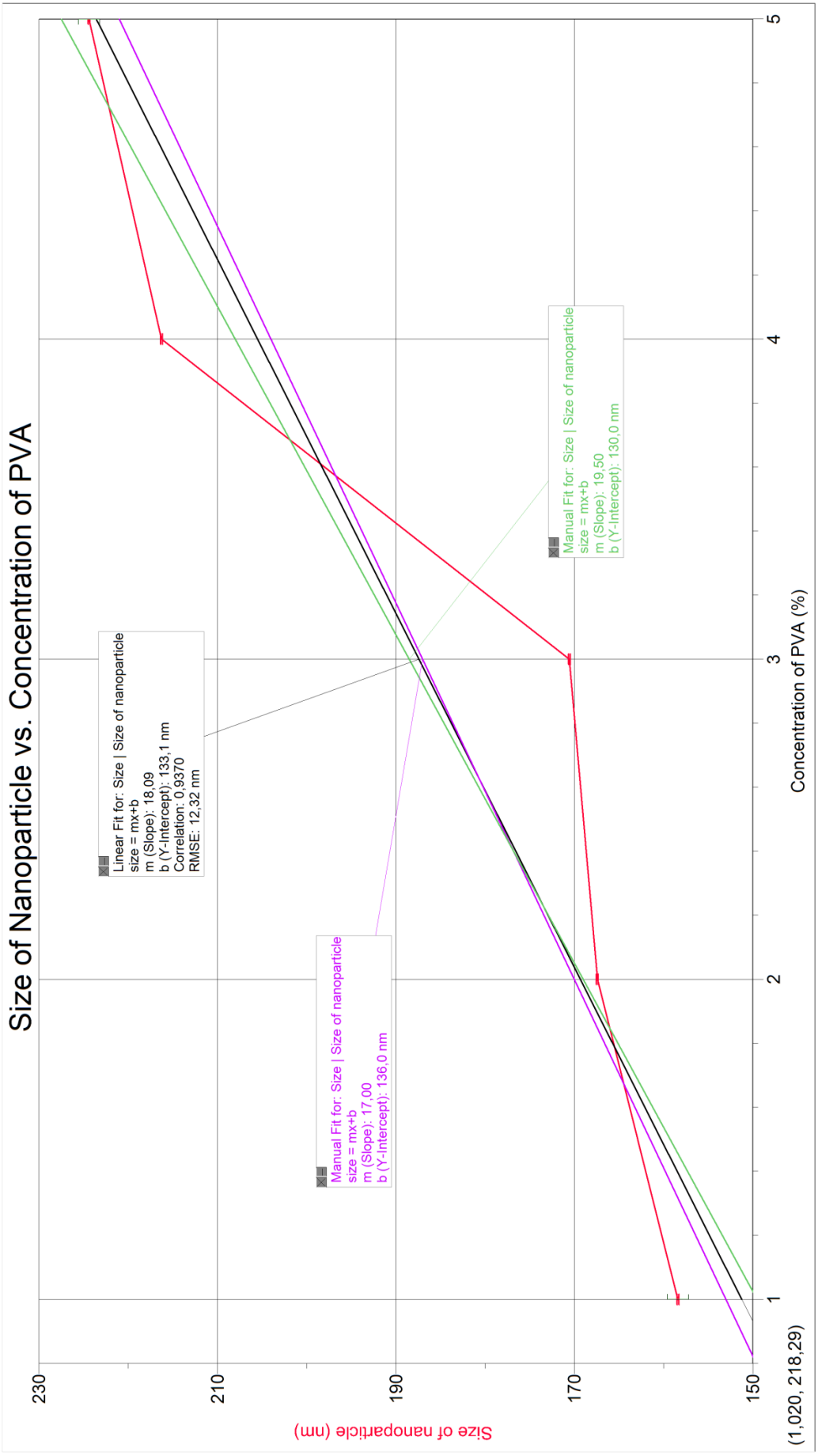
SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| 1% | 5 | -32.55 | -6.51 | 0.15455 |
| 2% | 5 | -68.7 | -13.74 | 0.053 |
| 3% | 5 | -31.55 | -6.31 | 0.0384 |
| 4% | 5 | -79.9 | -15.98 | 0.087 |
| 5% | 5 | -41.24 | -8.248 | 0.00447 |

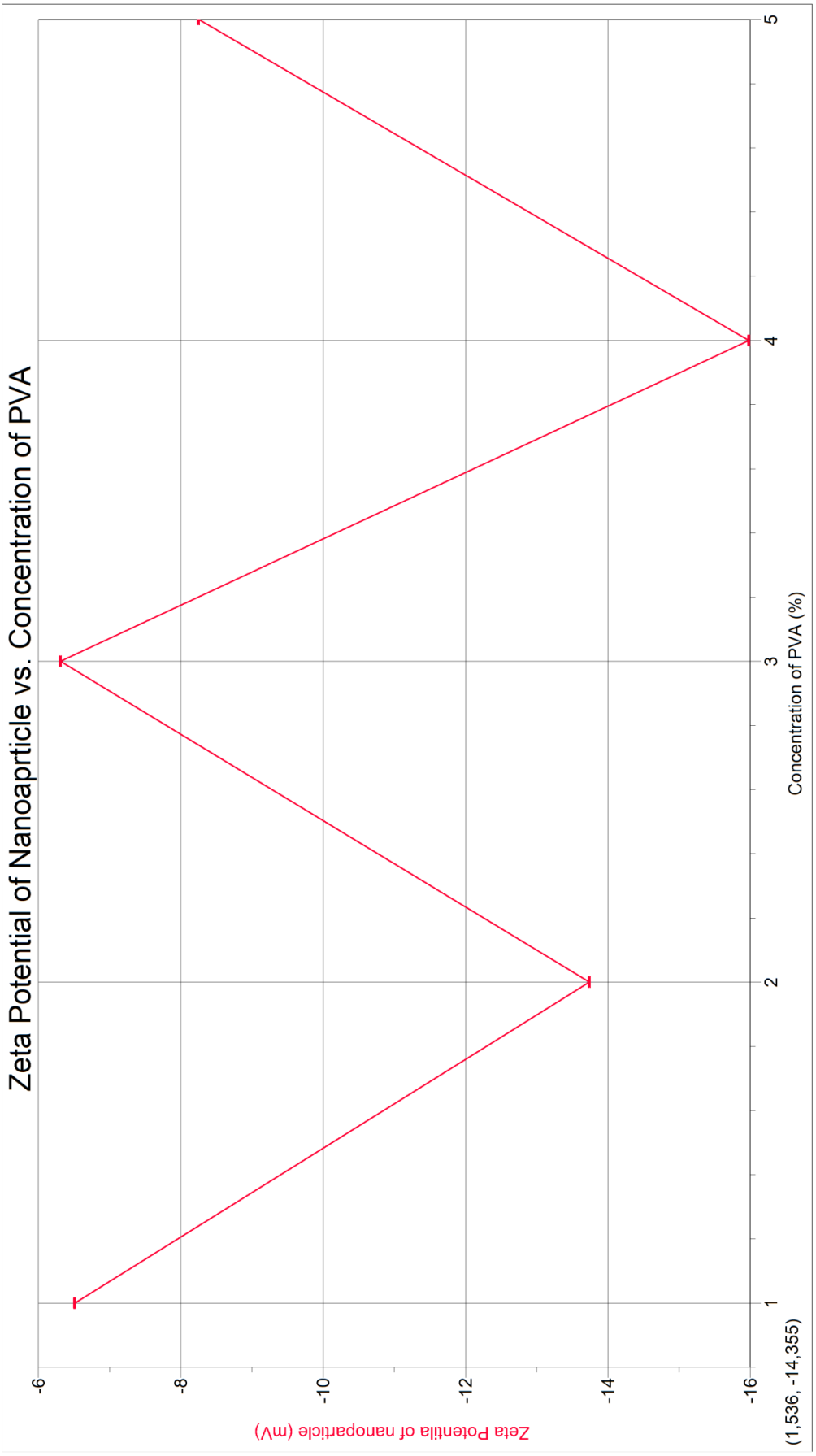
ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 392.4476 | 4 | 98.11189 | 1453.854 | 2.45E-24 | 2.866081 |
| Within Groups | 1.34968 | 20 | 0.067484 | | | |
| Total | 393.7973 | 24 | | | | |

**Table 4:** Single factor Analysis of Variance (ANOVA) statistical calculation for all groups of zeta potential of nanoparticles.




**Graph 1:** shows Size of Nanoparticle vs. Concentration of PVA where red line indicates experimental data, black is the best fit line, and purple and green lines are the most and the least steep lines.




**Graph 2:** shows Zeta Potential of Nanoparticle vs. Concentration of PVA.

## Discussion

In this research, the aim was to observe the effect of the stabilizer concentration on the surface properties of nanoparticles that were prepared with 2% PLGA polymer and varying concentrations (1%, 2%, 3%, 4%, 5%) of PVA. The hypothesis was that there would be significant difference between the sizes and the zeta potentials of the particles due to their stabilizer concentrations, hence expected biocompatibilities of these molecules would be compared.

It was seen that the biggest molecule size belonged to the nanoparticles that were produced from 5% PVA while 4%, 3%, 2% and 1% concentrations followed decreasingly in sizes of nanoparticles. Particles had 158.38, 167.42, 170.56, 215.26 and 224.4 nanometres of average sizes respectively for the concentrations 1%, 2%, 3%, 4% and 5%. From Single Factor Analysis of Variance (ANOVA), P-value for the data of size was found to be $9.7 \times 10^{-26}$.

Similarly, there was also difference between the zeta potentials of the nanoparticles whose stabilizer concentrations were the independent variable. The most negative value (-15.98mV) of zeta potential was that of the particles with 4% PVA whereas the molecules 2%, 5%, 1% and 3% PVA concentrations followed with the average values of -13.74, -8.248, -6.51 and -6.31mV.

P-value for the data of zeta potential was calculated as $2.45 \times 10^{-24}$ from Single Factor ANOVA. Since both P-values were smaller than $\alpha=0.05$, the null hypothesis, "There isn't any significant difference between the biocompatibilities and drug delivery properties due to the different sizes and zeta potentials of nanoparticles that are prepared with 2% PLGA (poly(lactic-co-glycolic acid)) and varying concentrations (1%, 2%, 3%, 4% and 5%) of PVA (polyvinyl alcohol) by solvent evaporation method.", was rejected.

Although the data obtained from the experiment proved that there were significant differences between the characteristics of nanoparticles due to the concentrations of the chemicals used during the preparation, there were some error sources affecting the results. The biggest problem in the set-up was that the zeta potential values of samples were recorded one after another; some of the solutions had to wait until data of the previous concentrations were collected. It is known that aggregation and zeta potential of the molecules are affected of each other; values between +30mV and -30mV are accepted not to be stable. As the particles wait, they begin aggregating and their zeta potentials change. The zeta potentials were altered because of this time difference since aggregation happened during the wait. That's why; the data was not accurate especially for the concentrations 1% and 3% PVA. In order to reduce this error source, recordings can be done with separate devices simultaneously. However, this might introduce another random error source since the analyzer won't be identical for all of the samples.

Moreover, the organic phase was poured into the water phase with a syringe. Even though the addition was successful and at the same rate for all of the solutions in this experiment, it could have been different due to the hardness of pushing the syringe. Not only speed but also pressure variations might have occurred. For a more accurate experiment, it should be made sure that the syringes won't cause speed and pressure differences by being stuck. Syringes that can move easily should be preferred for better results.

Finally, standard deviation and standard error values are shown in the following table for size data of the nanoparticles:

| For | Size of Nanoparticle | | | | |
|---|---|---|---|---|---|
| **Concentration of PVA** | 1% | 2% | 3% | 4% | 5% |
| **SD** | 0.609918 | 1.454991 | 1.422322 | 2.602499 | 0.353553 |
| **SE** | 0.272764 | 0.650692 | 0.636082 | 1.163873 | 0.158114 |

**Table 5:** shows standard deviation and standard error values for the data of size of nanoparticles with respect to their concentrations of PVA.

"Standard deviation shows how much variation exists from the average. A low standard deviation indicates that the data points tend to be very close to the mean."[10] whereas standard error is the standard deviation of the sample-mean estimate of a population mean.[11] Standard deviation values are relatively low for 1% and 5% PVA concentrations yet that of the concentrations 2%, 3% and especially 4% are high. This situation can be explained with the variations caused by the addition with syringe. Uniform addition is important for the formation of similar sized nanoparticles. Thus, there is a systematic error in the experiment which results in inaccuracy and imprecision of size data. Besides, standard error value for 4% PVA concentration is also high, so that there must have been a mistake in the procedure for this solution.

For zeta potential data of the nanoparticles, standard deviation and standard error values are below:

| For | Zeta Potential of Nanoparticle | | | | |
|---|---|---|---|---|---|
| **Concentration of PVA** | 1% | 2% | 3% | 4% | 5% |
| **SD** | 0.393128 | 0.230217 | 0.195959 | 0.294958 | 0.066858 |
| **SE** | 0.175812 | 0.102956 | 0.087636 | 0.131909 | 0.0299 |

**Table 6:** shows standard deviation and standard error values for the data of zeta potential of nanoparticles with respect to their concentrations of PVA.

Considering all of the standard deviation and standard error values are small, it can be concluded that data is precise and not spread out around the mean value for zeta potential.

## Conclusion

My research question in this study was "How does changing the concentration of the stabilizer affect the surface properties of the nanoparticles and the drug delivery systems that are formed with the nanoparticles of 2% PLGA (poly(lactic-co-glycolic acid)) and the stabilizer concentrations of 1%, 2%, 3%, 4% and 5% PVA (polyvinyl alcohol), by solvent evaporation method?". After the experiment, it was observed that there are significant differences between the sizes and zeta potentials of nanoparticles that are produced with varying stabilizer concentrations.

The size values in this study were seen to be directly proportional to the concentrations whereas there were experimental errors affecting the accuracy of zeta potentials. However, the experimental data was still enough to show that there existed differences between the values. The method could be renewed to obtain more accurate results yet the overall experiment was successful.

This research provided experimental data to support that the surface properties of nanoparticles can be altered by changing their stabilizer concentrations. Nanotechnology is a very broad area investigating how characteristics of matter can be manipulated to have desired effects. I decided to narrow down my area of research by only experimenting on the effect of stabilizer concentration on size and zeta potentials of nanoparticles that are prepared by a specific method and with one constant polymer (PLGA). The study can be extended for larger research by changing the preparation method or the chemicals chosen for polymer, stabilizer and solvent.

In the light of this research, it can be said that it is possible to obtain molecules with sizes and zeta potentials that are advantageous in drug delivery systems. Only by changing the concentration of the stabilizer, various nanoparticle characteristics are attained. More can be achieved with different polymers and methods. For instance, the zeta potential can be used to have optimum permeability through membranes or size can be utilized for interaction with biological molecules of similar sizes. Therefore, better drug delivery systems can be built. The importance of this ability to manipulate matter at nano-scales is that diseases might be cured with less amount of drug, barriers in human body can be overcome and drug molecules can be kept in body for longer periods of time and for larger effects. As a conclusion, for bigger developments in medicine, nanotechnology offers great opportunities.

# Appendices

## Appendix 1

Below is the information about "solvent evaporation method" that was used to prepare nanoparticles of the polymer PLGA with stabilizer PVA.

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.[2]

# Appendix 2

Information about the device "Zetasizer", which was utilised in measuring size and zeta potential data, is given in the following pages. The principles of the machine are also included.

## A) Zetasizer Nano ZS

### Measurement type 1:

**Measurement type:** Particle size and molecular size

**Measurement range:** 0.3nm – 10.0 microns* (diameter).

**Measurement principle:** Dynamic Light Scattering

**Minimum sample volume:** 12µL

**Accuracy:** Better than +/-2% on NIST traceable latex standards

**Precision/Repeatability:** Better than +/-2% on NIST traceable latex standards

### Measurement type 2:

**Measurement type:** Zeta potential (and optional Protein Mobility)

**Measurement range:** 3.8nm – 100 microns (diameter)*

**Measurement principle:** Electrophoretic Light Scattering

**Minimum sample volume:** 150µL (20µL using diffusion barrier method)

**Accuracy:** 0.12µm.cm/V.s for aqueous systems using NIST SRM1980 standard reference material

**Sensitivity:** 10mg/mL (BSA).

## B) How size is measured using Dynamic Light Scattering?

The principle of dynamic light scattering is that fine particles and molecules that are in constant random thermal motion, called Brownian motion, diffuse at a speed related to their size, smaller particles diffusing faster than larger particles. (...) To measure the diffusion speed, the speckle pattern produced by illuminating the particles with a laser is observed. The scattering intensity at a specific angle will fluctuate with time, and this is detected using a sensitive avalanche photodiode detector (APD). The intensity changes are analysed with a digital autocorrelator which generates a correlation function. This curve can be analysed to give the size and the size distribution.

## C) How zeta potential is measured?

The charge or zeta potential of particles and molecules is determined by measuring their velocity while they are moving due to electrophoresis. Particles and molecules that have a zeta potential will migrate towards an electrode if a field is applied. The speed they move is proportional to the field strength and their zeta potential. If we know the field strength, we simply measure the speed of movement, using laser Doppler electrophoresis, and then apply established theories to calculate the zeta potential.

## D) Why measurement of zeta potential is useful?

The choices of materials used in a formulation may be restricted by regulations and also have an impact on cost. Knowledge of the zeta potential of particles in a formulation can be used to make logical choices about chemistry of a formulation in order to select the most appropriate materials to provide stability and improve shelf life.

Zeta potential can also be used to study the effect of formulation components on other bulk properties such as viscosity, in order to achieve lower viscosity at higher concentrations for example.[12]

## Appendix 3

Information about the cenrifuge is listed below.

### Universal High Speed Centrifuges

### Z 383 / Z 383 K

For High Speed Performance

- Capacity for 4 x 500 ml at a speed of 5000 rpm
- Accommodate nearly every size tube or bottle from 0.2 ml - 250 ml at high speeds
- Spin many tubes at once (e.g. 48 x 15 ml)
- Many rotors and adapters available for both swing-out and fixed angle rotors as well as microplate rotors
- High speed microrotors up to 17.000 rpm / 26.810 x g
- High speed angle rotors for 85 ml or 50 ml up to 15.000 min-1 / 25.909 x g
- Rotors are easy interchangeable[13]

# Appendix 4

Statistical formulas used during data analysis are written below.

## A) Arithmetic Mean

The arithmetic mean of a set of values is the quantity commonly called "the" mean or the average. Given a set of samples $\{x_i\}$, the arithmetic mean is[14]

$$\bar{x} \equiv \frac{1}{N}\sum_{i=1}^{N} x_i .$$

## B) Median

The word "median" has several different meanings in mathematics all related to the "middle" of mathematical objects.

The statistical median is an order statistic that gives the "middle" value $\tilde{x}$ of a sample. More specifically, it is the value $\tilde{x}$ such that an equal number of samples are less than and greater than the value (for an odd sample size), or the average of the two central values (for an even sample size).[15]

## C) Mode

The most common value obtained in a set of observations.[16]

## D) Standard Deviation

The standard deviation $\sigma$ of a probability distribution is defined as the square root of the variance $\sigma^2$,

$$\sigma = \sqrt{\langle x^2 \rangle - \langle x \rangle^2}$$
$$= \sqrt{\mu_2' - \mu^2},$$

where $\mu = \bar{x} = \langle x \rangle$ is the mean, $\mu_2' = \langle x^2 \rangle$ is the second raw moment, and $\langle x \rangle$ denotes the expectation value of $x$. The variance $\sigma^2$ is therefore equal to the second central moment (i.e., moment about the mean),[17]

## E) Standard Error

The standard error of a sample of sample size $n$ is the sample's standard deviation divided by $\sqrt{n}$. It therefore estimates the standard deviation of the sample mean based on the population mean.

The standard error of an estimate may also be defined as the square root of the estimated error variance $\hat{\sigma}^2$ of the quantity,[18]

$$s_e \equiv \sqrt{\hat{\sigma}^2}$$

### F) Confidence Interval

A confidence interval is an interval in which a measurement or trial falls corresponding to a given probability. Usually, the confidence interval of interest is symmetrically placed around the mean, so a 50% confidence interval for a symmetric probability density function would be the interval $[-a, a]$ such that[19]

$$\frac{1}{2} = \int_{-a}^{a} P(x)\,dx.$$

## Appendix 5

Below are photographs of nanoparticles, having PVA concentrations of 1% and 3%, taken by Transmission Electron Microscopes (TEM) in METU, Ankara, Turkey.

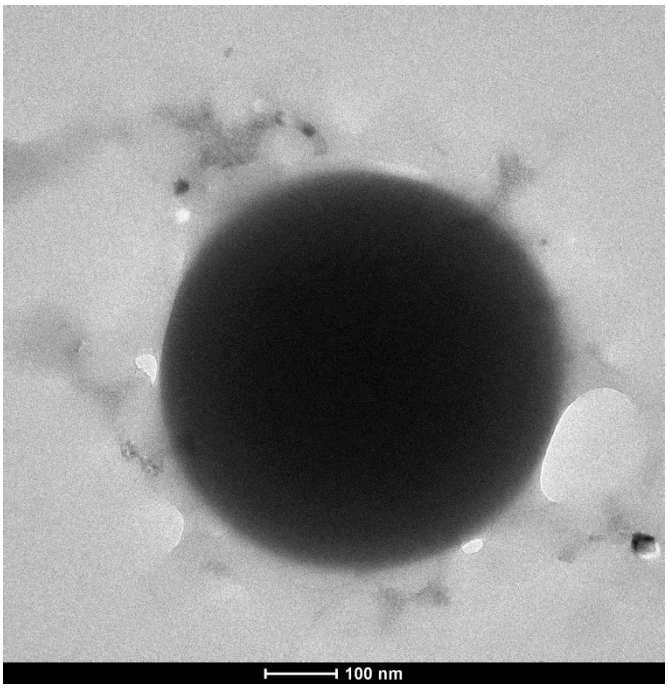



**Image 1:** shows a nanoparticle of 2%PLGA & 1%PVA.

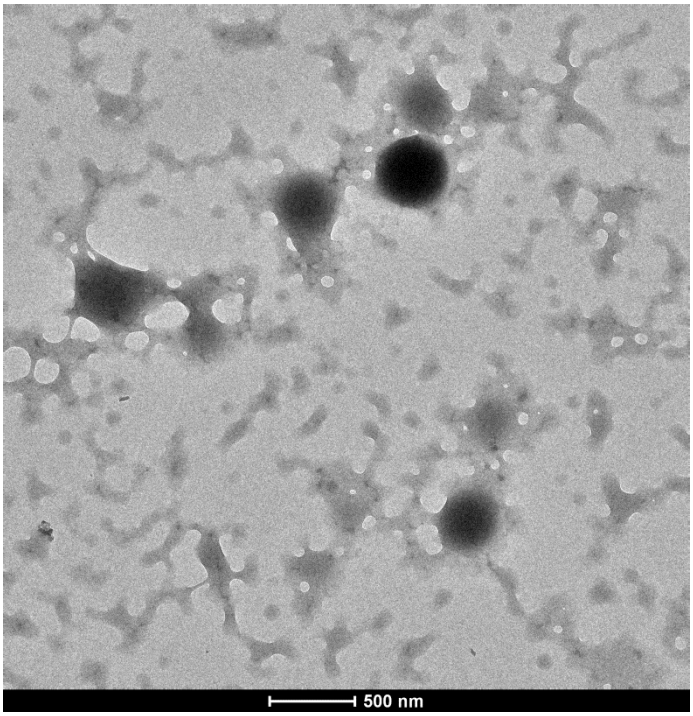



**Image 2:** shows nanoparticles of 2%PLGA & 1%PVA.

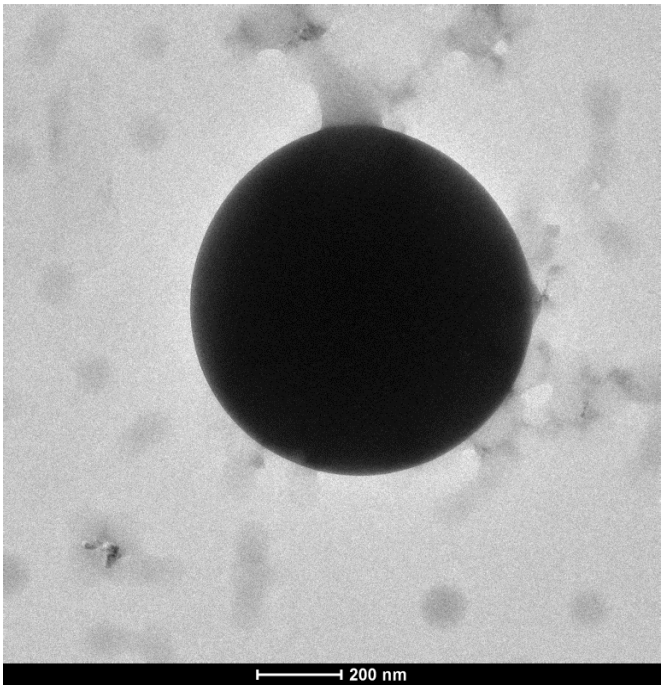



**Image 3:** shows a nanoparticle of 2%PLGA & 1%PVA.

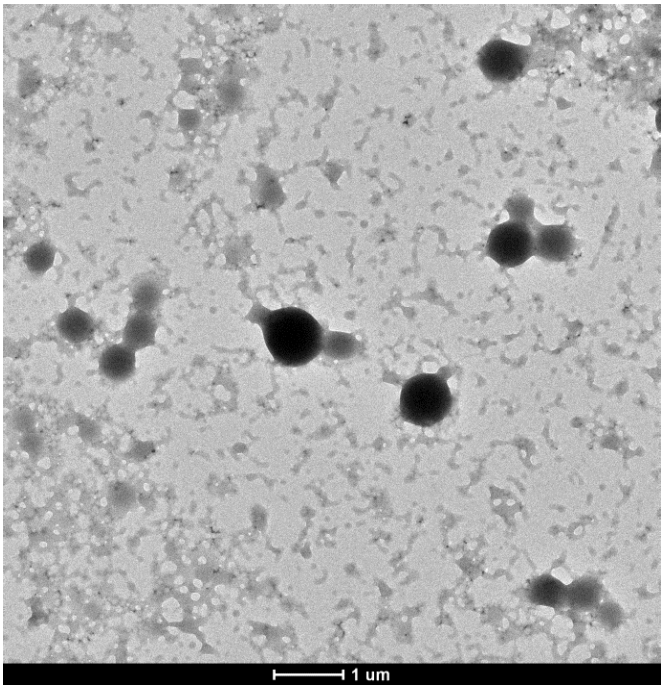



**Image 4:** shows nanoparticles of 2%PLGA & 1%PVA.

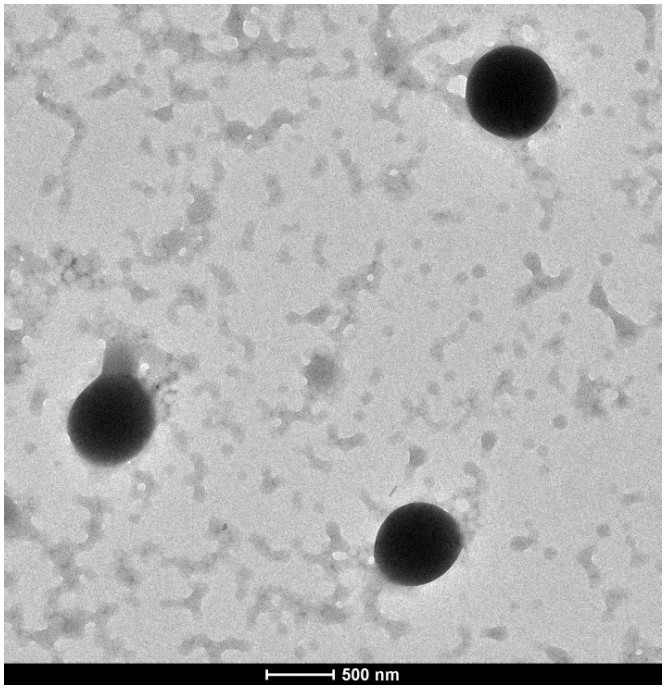



**Image 5:** shows nanoparticles of 2%PLGA & 1%PVA.

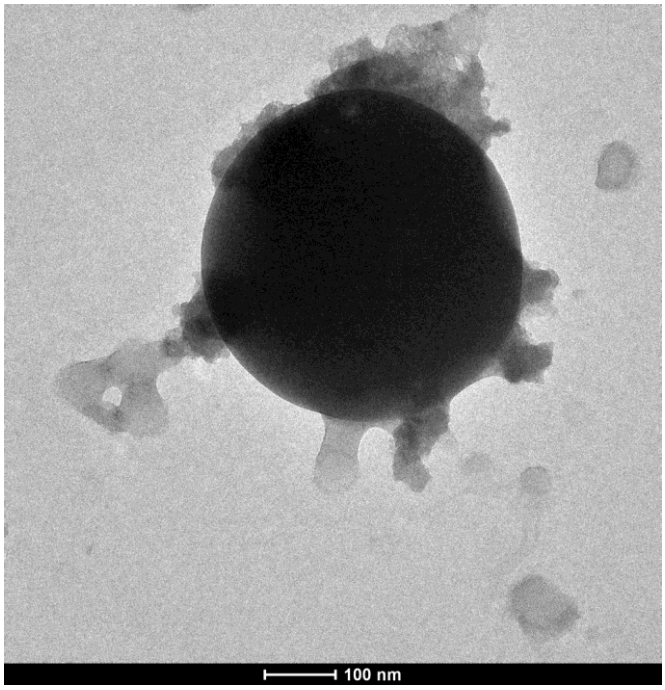



**Image 6:** shows a nanoparticle of 2%PLGA & 3%PVA.

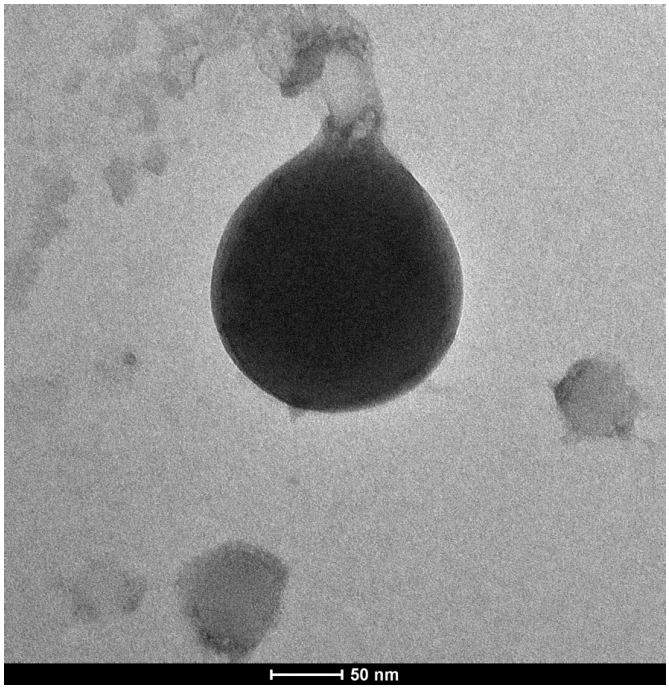



**Image 7:** shows a nanoparticle of 2%PLGA & 3%PVA.

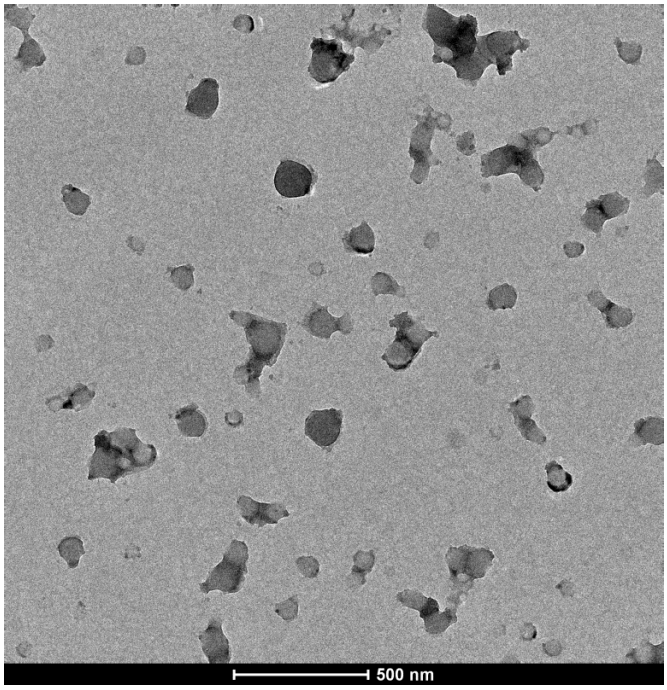



**Image 8:** shows nanoparticles of 2%PLGA & 3%PVA.

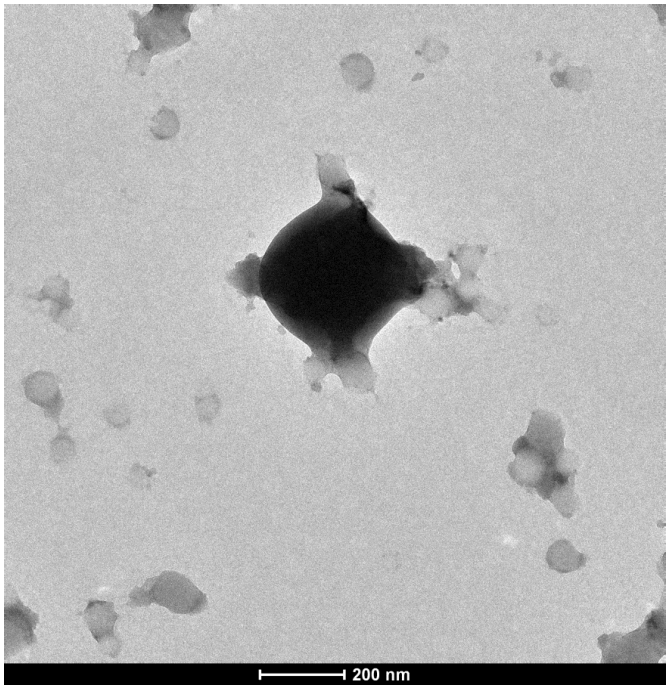



**Image 9:** shows a nanoparticle of 2%PLGA & 3%PVA.

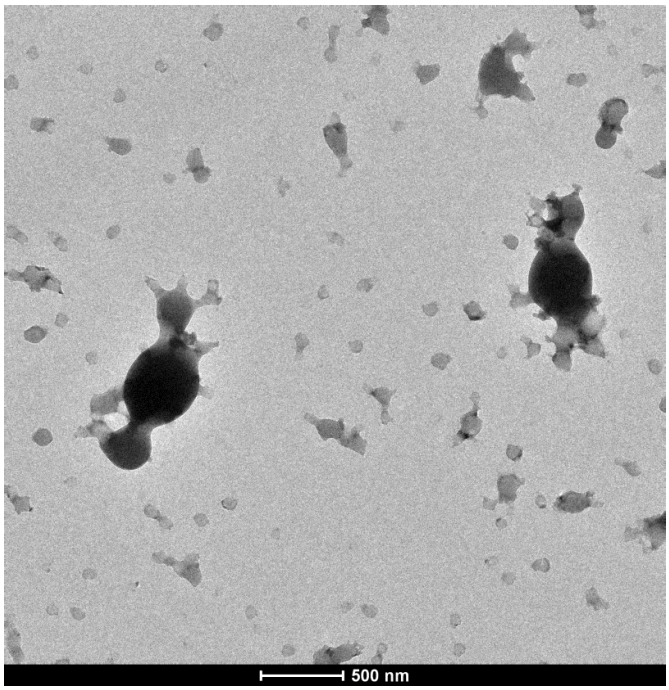



**Image 10:** shows nanoparticles of 2%PLGA & 3%PVA.

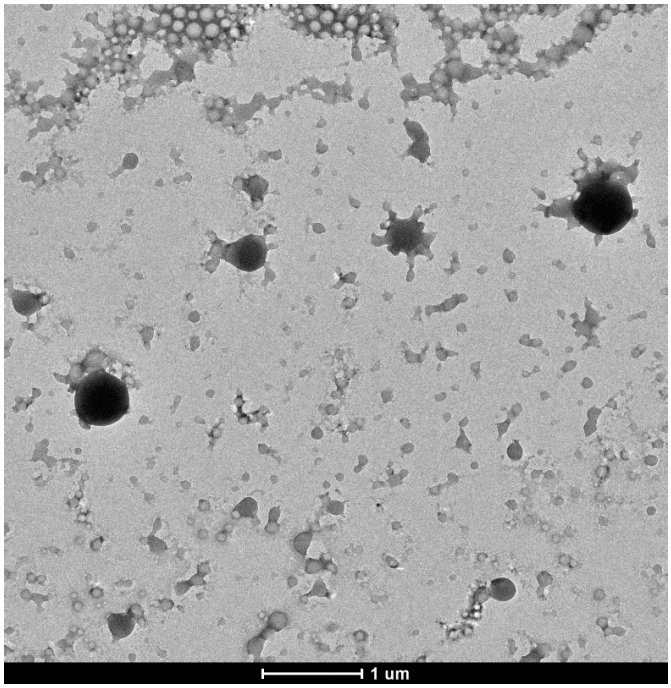



**Image 11:** shows nanoparticles of 2%PLGA & 3%PVA.

## Appendix 6

Following is the chemical structure of PLGA.

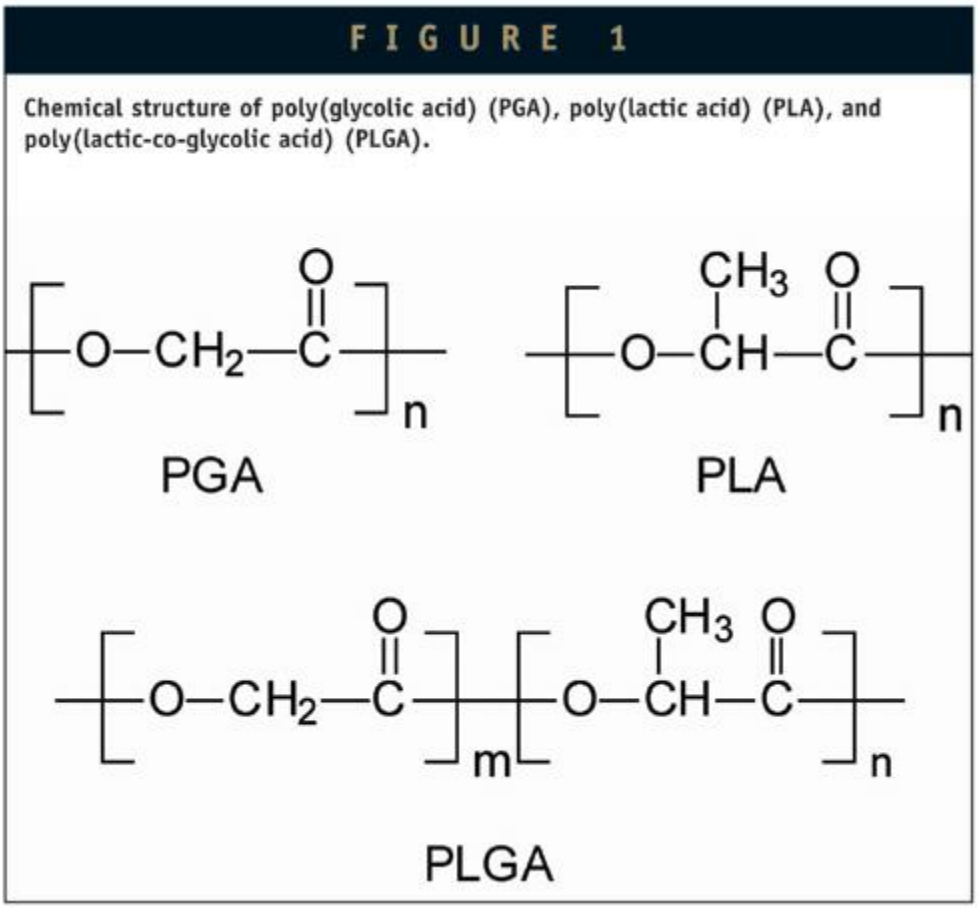



**Image 12:** shows chemical structure of PLGA molecule.[20]

## Appendix 7

Photographs below are taken by me during the preparation of nanoparticles in Hacettepe University, Ankara, Turkey.


**Image 13:** shows nanoparticles of 2%PLGA & 1%PVA being stirred with magnetic stirrer.

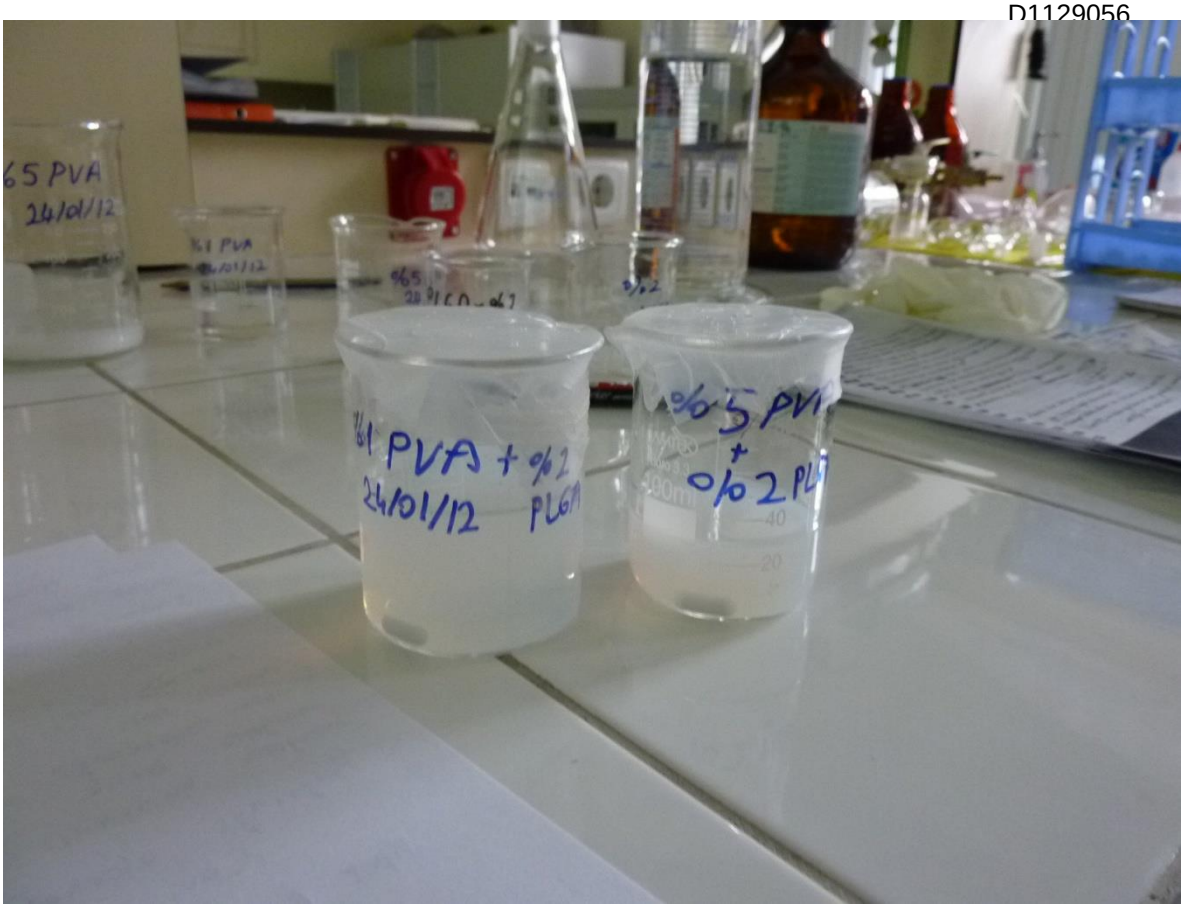



**Image 14:** shows solutions containing nanoparticles of 2%PLGA & 1%PVA and 2%PLGA & 5%PVA.

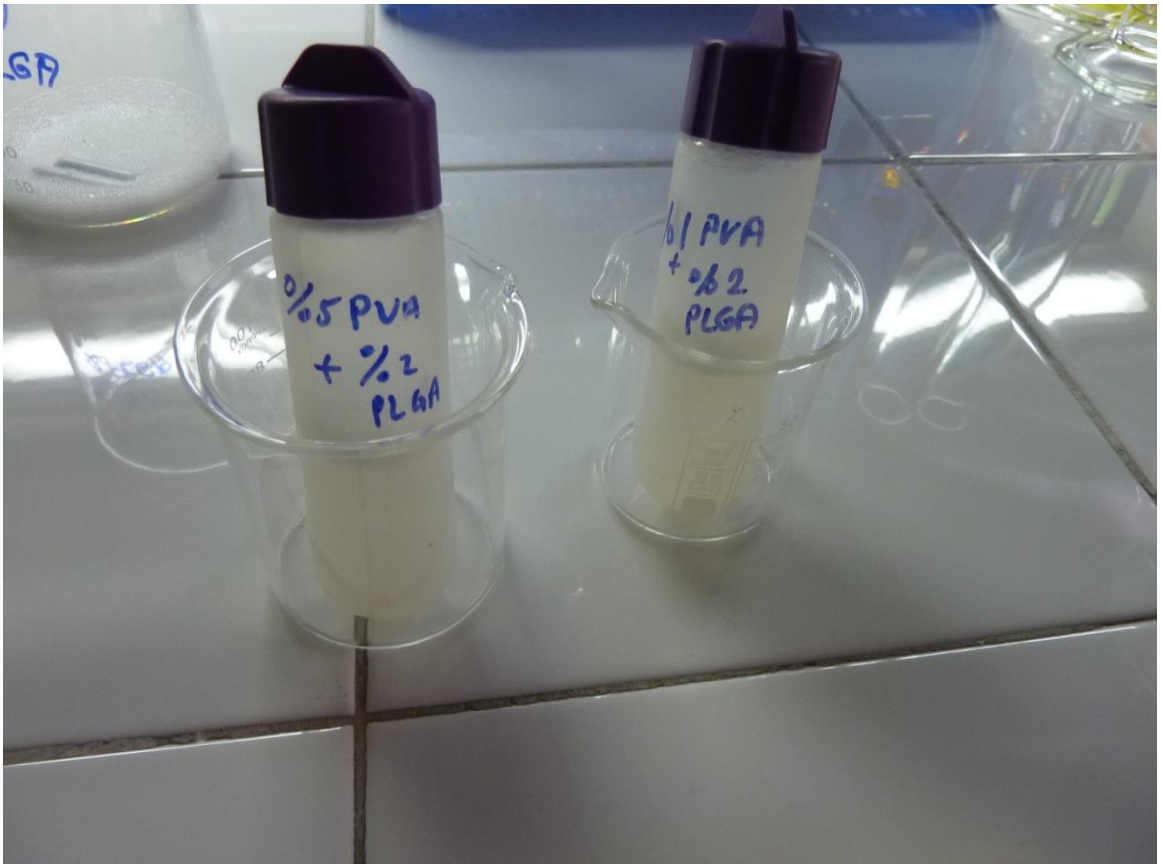



**Image 15:** shows solutions containing nanoparticles of 2%PLGA & 1%PVA and 2%PLGA & 5%PVA in centrifuge tubes.

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