

# Gelatin Carrier For Metformin Drug

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**Abstract**— During the past few decades there has been an increasing interest in the development of biodegradable micro/nanoparticles as drug delivery devices. Previous studies have reported that some existing additives are expensive and have poor degradability. The use of edible-based biodegradable additive remains attractive because of their abundance in nature and cost-effective. Biodegradable micro and nano-particulate additive is proposed. Selected local additive produced locally is incorporated with Metformin drug. Characterization, physical study and biological study was attempted, such as determination, morphological and rheological test, drug loading capabilities, biodegradation, toxicity and in vitro studies. The focus of the study is to create local biodegradable micro- and nano-sized particulate additive with Metformin loading formulation theory for drug delivery system. Apart from that, formulation theory created purposely aims to support the Malaysia's NKEA in healthcare so that local generic manufacturing for export can be increases.

**Keywords**-component; Biodegradable, micro/nanoparticles, drug delivery devices, drug carrier, Metformin drug, Diabetes, cost-effective, natural-based, locally available

## Introduction

Diabetes among young children were almost unheard fifteen years ago. But recent study done on by Journal of the American Medical Association proved that there are about an increase to at least 3700 of diabetes type 2 cases among youth in the United States alone (Erika Gebel, 2017). This evidence urge doctors all around the world to find solution to solve this problem..

Researcher from Diabetes In Youth Organization, released statistical data that there was an increment by 21% on diabetic patient among children aged 10 to 19 years old within years 2001 until 2009. The data was not included young patient from Native Americans, Asian/Pacific and African American. Increment on Diabetes type 2 sufferers from young girl is more compared to boy sufferer. The diabetic patient number released, excluded patient age under 10, because it is difficult for people under age 10 are rarely suffer from diabetes type 2, but it is possible. People aged between 19 to 21 are favorably to get diabetes type 1. In addition, it is statistically proved that 1 in 3000 young people between age 10 to 19 suffer from diabetes type 2 and 6 case in 3000 people between age 1 to 19 is suffer from either diabetes type 1 or diabetes type 2. This number kept growing from years to years. Another shocking study done on African American shows that 40 percent of them suffer from type 1 diabetes, and 55 percent of them suffer from type 2 diabetes. While the remaining 5 percent are free from diabetes.

According to Jong Koi Chong, (2013), which is the president for Obesity Prevention Council, said that an alarming 3.6 million adults and children are estimated to be affected by diabetes in Malaysia and 3 million people in the UK have now been diagnosed with diabetes.

The possible known cause for diabetes among young children is overweight. Sometimes, people don't expect young kids to suffer from diabetes because some of them are overweight and live in healthy without having any diabetes problem. Obesity is not the only cause. Problems in pancreas that stop producing enough insulin to control blood glucose in children is also the main culprit to this problem.

To tackle this, numerous of drug such as Metformin is introduced to cured the production of insulin in body. But Metformin made by pharmaceutical industry was not kid-friendly, with it bitter taste, and most of them are non-syrup form, which can be easily and favorably swallowed by children. This research study aims to creates a formulation theory that can turns those Metformin tablet into forms that kids will love to eat, which is in gel form. Gelatin, chitosan, starch are among carriers that suitable for metformin drug delivery. Numbers of experiments were done to creates this formulation so that a natural-based-cost-effective- gelatin can be developed from material that can be obtained locally.

## METHODOLOGY

### A. Raw Materials

The chemicals that have been used in this experiment is concentrated 2M Hydrochloric Acid, HCl (37%, 36.46 g/mol) obtained from Fisher Scientific (M) Sdn Bhd. Sodium Hydroxide, NaOH used to obtained alkaline solution, and distilled water. Raw materials used were 20 g Agarose sticks obtained from Giant supermarket, and Metformin HCl BP 500mg tablet strip obtained from CCM Berhad. Other materials used is aluminium wrap 20 g Agarose sticks bought from Giant supermarket was cut into small pieces to facilitate the agar stick dissolves into heated water. Agar sticks were cut approximately into 3cm in size then, weighed using weighing scale and was placed inside a beaker. Metformin HCl BP 500mg tablet was crushed into fine powder using white mortar pestle and weighed and placed inside petri dish.

Hydrochloric Acid, HCl (37%, 36.46 g/mol) was used for preparation of solutions of different pH. To obtained 2M HCl, 167 mL of concentrated HCl is carefully poured from the bottle container into beaker. Poured HCl was diluted with 1L of distilled water. 2M 50 mL of HCl obtained is

transferred into 1L beaker for later usage. Beaker was covered with aluminium wrap to avoid the solvent from vaporise into air.

20 g of sodium hydroxide, NaOH pellet are weighed and placed inside a beaker. 10mL of distilled water is added into the beaker to melt the NaOH pellet and turns it into sodium hydroxide solution.

### B. Apparatus and Equipment

Apparatus used throughout this study were 50mL round bottom flask, 100mL measuring cylinder 50 mL measuring cylinder, 10 mL measuring cylinder, 1 L beaker, dropper, white mortar pestle, spatula, petri dish, dropper, cutting scissors and screw capped reagent glass bottle. Equipment used are Mettler Toledo pH meter, Corning PC420D hot plate heater, and Sartorius weighing scale..

### C. Experimental Setup

*Effect of Solid Loading and Particle Mass* - The experiment started with investigating the effect of solid loading and particle mass on dispersion of colloids particles, to optimize the suspension time. The experiment was carried out in five 50 mL round bottom flasks labeled flask 1, flask 2, flask 3, flask 4 and flask 5, which each flask is filled with 50 mL of distilled water as liquid dispersion medium. Each 250 mL Erlenmeyer flask was added with different amount of Metformin dosage. Five different Metformin dosage were used. Those five were 0.25g, 0.5g, 1.0g, 1.25g, and 1.5g. Metformin tablet were crushed into fine powder using white mortar pestle, weighed and placed on petri dishes. 0.25g, 0.5g, 1.0g, 1.25g, and 1.5g weighed metformin fine powder were poured into flask 1, flask 2, flask 3, flask 4 and flask 5 respectively. Each flask was covered with aluminum foil to prevent overflow of Metformin powder. Each flask was then swirl gently for 20 times and then the metformin powder were left dispersed and suspended within distilled water by itself. Each samples were analyzed and compared to determine the optimum metformin dosage that takes the longer time to settle down. The optimum suspension time is chosen from metformin dosage with slowest settling time.

*Effect of Different pH of Dispersed Medium* - The experiment was conducted by using the optimum metformin dosage obtained from the previous experiment. Solutions of different pH were prepared. 500mL Distilled water was poured into beaker. pH meter used were calibrated first by placing the probe inside distilled water. The pH reading is calibrated until constant pH 7 is achieved. 2M HCl solution prepared is used to achieve the desired pH. Hydrochloric acid is added into 500mL distilled water using dropper, drop by drop until the pH meter reading reach pH 1. 50mL Solution of pH 1 is transferred into first flask. The procedures are repeated for other acidic solution of pH 2 until pH 6. 50mL of distilled water of pH 7 is poured into 7th flask. For alkaline solution of pH 8 until pH 14, same procedure repeated with NaOH solution prepared in section 3.2.2 is added into distilled water to increase the pH meter reading and turns it into alkaline solution. Alkaline solution of different pH were poured into flask 8 until flask 14.

Fourteen 50 mL round bottom flasks labeled Flask 1, Flask 2, Flask 3, Flask 4, Flask 5, Flask 6, Flask 7, Flask 8,

Flask 9, Flask 10, Flask 11, Flask 12, Flask 13 and Flask 14 which each flask is filled with 50 mL of solvent of different pH, from acidic pH 1 to pH 14 alkaline. Metformin tablet were crushed into fine powder using white mortar pestle, weighed, and poured into each flasks. Amount of Metformin powder dosage poured is obtained from the previous experiment. Each flask was then swirl gently for 20 times and then the metformin powder were left dispersed and suspended within distilled water by itself. Samples in each flask were analyzed and compared to determine the optimum solution pH that takes the longer time to settle down. The optimum suspension time is chosen from pH of solution with slowest settling time. The behavior of the colloids within the medium is observed for every 15 minutes interval. Colloids with longer suspension time, slowest settling time, and colloids were uniformly distributed throughout the medium is chosen as the optimum pH to be used for next experiment.

*Effect of Different Gelatin Dosage* - The experiment was conducted by using the optimum metformin dosage obtained from the experiment 1 and optimum pH results from experiment 2. Experiment 3 was conducted to study the effect of viscosity of the emulsifier, by manipulating the concentration of gelatin. Agarose gelatin stick are cut into small pieces and weighed.

200mL of distilled water was poured into the beaker. Hot plate heater is turn on and the temperature was set to 100 °C. Beaker with distilled water is placed on top of hot top plate, and left heated until the water boils. 2g of agarose sticks was added into boiling water. The mixture solution is stirred using spatula until all agarose pieces are dissolves in water. Once all the agarose stick dissolves, and uniform mixture solution is obtained, heating plate is turned off. 50mL of the prepared mixture solution was transferred into beaker. The mixture solution was left cooled at ambient temperature until the gelatin medium starts to thicken. Optimum dosage of crushed metformin powder obtained from experiment 1 is poured into the solution. The mixture of gelatin and Metformin colloids were stirred. The mixture was left to solidified and cooled

Procedures above were repeated for concentration of 3g agarose gelatin. The behavior of the colloids and dispersion of colloids within the medium is observed for both gelatin concentrations. Gelatin with uniform distribution of colloid suspension is chosen as the best concentration to be used for formulation of natural-based metformin drug carrier.

### D. Transmission Electron Microscopy (TEM) Analysis

Colloidal behavior study are the only one that has been done has to be done using Transmission Electron Microscopy (TEM). The morphology and distribution of dispersed Metformin microparticles throughout the solution was characterized by JEM-1254 ESX/V Hitachi Transmission Electron Microscope. Observation and images resulted from the analysis were as displayed below. The distribution of colloids was observed at different scale. Number of images was taken at different spot of the sample

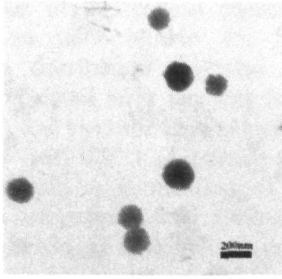


Fig 1: Metformin Colloids Distribution Images At 200nm Scale.

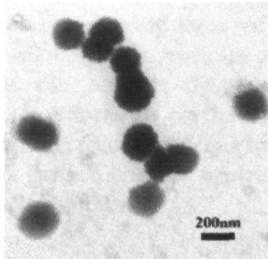


Fig 2: Larger Metformin Colloids Observed at Different Spot

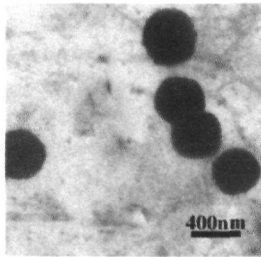


Fig 3: Metformin Colloids Distribution Images At 400nm Scale

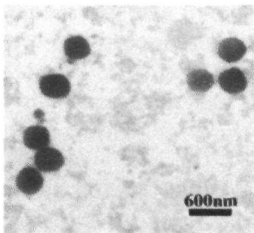


Fig 4: Metformin Colloids Distribution Images At 600nm Scale

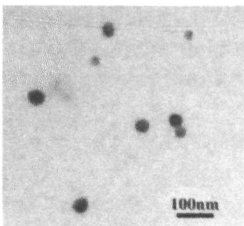


Fig 5: Metformin Colloids Distribution Images At 100nm Scale

## I. RESULT AND DISCUSSION

From the experiment conducted, metformin colloids not only slowly settle down after constant stirring, but after few minutes, the settled colloids start to be suspended back and float at the surface of distilled water. Although dispersion of colloids behaviour is dominated by body force in terms of particle

mass, but it is only applicable to large particles, where increase in particle mass takes shorter time for the particle to sediment.

For small size particles like crushed metformin powder, this theory is not valid. This is because, it was observed that Metformin powder with largest mass, 1.5g takes the longest time to sediment. This is occur due to small particles behaviour in liquid is controlled by surface force such as van Der waals and Electrical double layer force instead of body force.

### A. Effect of Solid Loading and Particle Mass

Trends showed above prove that metformin with the highest mass takes the shortest time to suspend within the liquid solution. This situation occurs might be due to large numbers of particles that accumulate surface charge creating the greatest repulsion force between the particles that allows it to turn into suspension within short period of time. This phenomenon is related to this equation mentioned in chapter 2. Where increase in negatively charged on the surface causing positive force created (repulsion) within the particles.

$$F = -\frac{dV}{dD}$$

0.5 g metformin powder dosage sedimented in distilled water requires 12 minutes 17 seconds to totally resuspended back and float at the surface. 0.75 g metformin powder dosage sedimented in distilled water requires 11 minutes 32 seconds to totally resuspended back and float at the surface. 1.0 g metformin powder dosage sedimented in distilled water requires 10 minutes 3 seconds to totally resuspended back and float at the surface. 0.5 g metformin powder dosage sedimented in distilled water requires 12 minutes 17 seconds to totally resuspended back and float at the surface. 1.25 g metformin powder dosage sedimented in distilled water requires 9 minutes 25 seconds to totally resuspended back and float at the surface. 1.5 g metformin powder dosage sedimented in distilled water requires 8 minutes 7 seconds to totally resuspended back and float at the surface.

From the retrieved data, it was proved that 1.5 g is the optimum dosage to creates a stable suspension within 50 mL liquid medium

### B. Effect of Different pH of Dispersed Medium

Selected optimum dosage from experiment 1 is hired for this experiment 2. Metformin of 1.5 g dosage are test in different pH of solution. Solution of pH 1 until pH 14 were prepared. Solution of pH 12 prepared have a little bit yellowish color. It was observed that, among of the solution, uniform dispersion of metformin colloid is favorable in pH 12. All the metformin particles poured, sediment in the solution, with no metformin powder leftover floating at the surface.

Behavior of the colloid particles were observed from minute zero until minute 45. Sedimentation time and suspension distribution are also observed. Table below describes in detail what happens inside the solution at t=0, t=15, t=30, and t=45 for each pH solution. It can be observed that below pH IEP 12.4, strong attraction point between particle causing agglomeration of particles and force the colloids to sediments at final. Colloid agglomerate the most at pH 1 and the size of agglomerates reduced when reaching IEP as surface force is getting neutral

Amount of agglomerate sediment also decrease when reaching pH 12. Above pH 12, the strong interparticle repulsion observed with most of colloids suspends at the surface of solution. The particles repulsion is the strongest at pH 14 that the colloids dispersed and turns the solution cloudy. Surface force of particle and repulsion is reduced at pH 13 as surface charge reaches neutral. Meanwhile at pH 12, the colloids surface become neutral with no charge making it soluble in the solution.

C. Effect of Different Gelatin Dosage

The experiment was conducted by using the optimum metformin dosage obtained from the experiment 1 and optimum pH results from experiment 2. Experiment 3 was conducted to study the effect of viscosity of different gelatin viscosity on suspension dispersion in medium, by manipulating the concentration of gelatin added. Agarose gelatin of 1g and 2g is added into different beaker. Gelatin medium of 1g concentration was proved to allow metformin colloids to sediment faster (31 minute) than in high concentration gelatin. 2g of agarose added is considered too thick and viscous and take over 43 minutes for the colloids to well dispersed and become stable suspension once the gelatin solidified. Besides, thick gelatin cause most of the colloids are grouped at top because it can't penetrate deeper since the gelatin get solid earlier

For case of colloid particles sediment in viscous fluid under force of gravity, the colloid is said to reach terminal velocity or settling velocity when all force exerting on particles is equilibrated and dv/dt in equation 4. is equal to zero. Besides, the velocity of the settling colloids is directly related to viscosity of the fluid medium and drag force acting on it. This can be proved through Stoke's equation

$$F_d = 6\pi\eta Rv_s$$

Thus, this indicates that drag force acting on the colloids that resist the particles from settled is increases as the viscosity of the liquid increases. That's why metformin colloids tends to accumulate at top section of gelatine medium

proven based on the data shown on Figure 5. Absorbance values when ultrasonic cleaner is used, with optimum solvent ratio obtained from previous parameter test, shows that there is slightly higher than other method used which is at 3.220.. Higher absorbance value means high concentration of coloring matter in extracted dye. Sample of extracted dye with

Metformin Dosage(g)	Distilled Water Volume (mL)	Colloids Settling Time (second)	Time Taken For Colloids To Float <Minute)
0.50	50	5	12.28
0.75	50	9	11.53
1.00	50	33	10.05
1.25	50	38	9.42
1.50	50	41	8.12

Table 1: Effect of Solid Loading and Particle Mass on Particle Dispersion and Suspension in Water medium

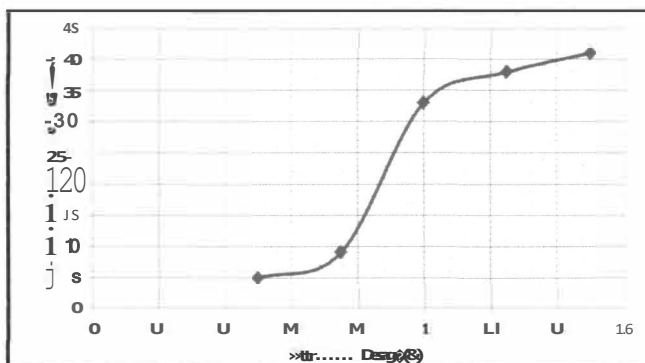


Figure 6: Graph of Colloids Settling Time Versus Metformin Dosage

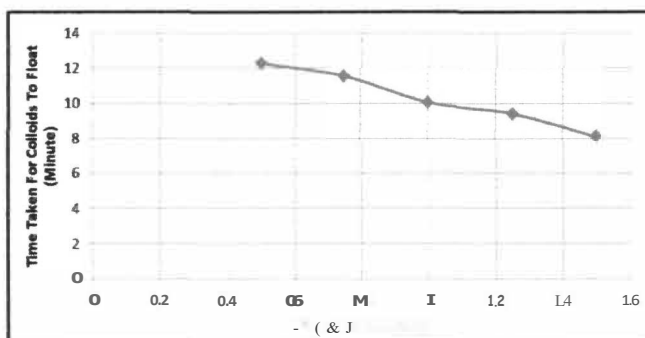


Figure 7: Graph of Colloids Suspension Time Versus Metformin Dosage

Metformin Dosage (g)	Mass Gelatin Added (g)	Time Taken To Settle And Suspend (minute)
0.5	1	31
0.5	2	43

Table 2: Time Taken For Metformin Colloid To Settle For Different Gelatin Concentration

## II. CONCLUSION

The development of natural-based drug carrier which is cost effective, locally available, and biodegradable has been taken into consideration in the present study. Based on the study, it can be concluded that Agarose gelatin is approved to be one of the material suitable to be used as an additive in the making of metformin drug. Besides it is approved that surface forces does dominate the behavior of metformin particles within the medium. This inter particle forces can be controlled by manipulating the particle concentration, pH of the medium, and viscosity of the gelatin itself. By varying the parameters, optimum dosage that is capable of maintaining stable colloid suspension within the gelatin is 1.5g, optimum pH for uniform distribution of colloid throughout the medium is pH above than pH isoelectric point of Metformin which is above 12.4. For viscosity of the gelatin, the gelatin cannot be too thick, and amount of agarose added must be in moderate amount. Gelatin that is too viscous can impact drag force onto the molecule that may resist the movement of metformin particle throughout the medium. Uniform suspension cannot be created if it was too viscous.

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