Final Report

The effects of tapioca maltodextrin with different concentration and dextrose equivalent values on the formulation and characteristics of plasma expander

Surapong Chatpun

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Abstract

Project Code : MRG5680044

Project Title : The effects of tapioca maltodextrin with different concentration and dextrose equivalent values on the formulation and characteristics of plasma expander

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Abstract:

Volume replacement is a conventional treatment when blood loss from the body to maintain cellular oxygenation activities. Plasma expanders (PEs) are widely used in the volume replacement such as hydroxyethyl starch which is modified corn starch based PE. Maltodextrin from tapioca starch was selected as a study candidate due to its uncomplicated production process and its solubility at room temperature. The formulations of mixture between tapioca maltodextrin and 0.9% sodium chloride solution were prepared and characterized to investigate the effects of dextrose equivalent (DE) and concentration on the physicochemical properties. Viscosity, colloid osmotic pressure and pH of solutions were compared with clinical used PE (6% HES 130/0.4). Plasma viscosity after dilution blood with each formulation was measured. Storage stability of each formulation was also determined. Morphology of red blood cells (RBCs) was observed using microscopy techniques. The results showed that low DE value led to high retrogradation, turbidity and viscosity but low COP and poor solubility. In contrast, high DE value caused the opposite results relative to low DE values. There was a noticeable effect of DE value on RBCs shape changes. Among the prepared solutions, tapioca maltodextrin with DE6 at 10% w/v concentration had comparable properties with 6% HES 130/0.4. However, to use tapioca maltodextrin as a raw material for plasma expander, some properties have to be considered and optimized efficiently. Further studies on other modified tapioca starches are interesting in order to improve the improper properties of tapioca maltodextrin for plasma expander.

Keywords: plasma expander, tapioca maltodextrin, dextrose equivalent, colloid, viscosity

บทคัดย่อ

เมื่อมีการสูญเสียเลือดจากร่างกายเป็นปริมาณมากจะทำการรักษาโดยการใส่สารเพื่อเพิ่มปริมาตรของเลือด เพื่อรักษากิจกรรมของเซลล์ต่างๆที่มีการใช้ออกซิเจน โดยสารที่ใส่เพื่อเพิ่มปริมาตรของเลือดนั้นจะเรียกว่าสารเพิ่มน้ำเลือด เช่น ไฮดรอกซีเอททิล สตาร์ช (Hydroxyethyl starch, HES) ซึ่งทำมาจากแป้งข้าวโพดดัดแปร ดังนั้นการใช้แป้งดัดแปรชนิดอื่นเพื่อการใช้เป็นสารเพิ่มน้ำเลือดจึงเป็นสิ่งที่น่าสนใจ ในการศึกษานี้แป้งดัดแปรชนิดมอลโตเดคทรินจากแป้งมันสำปะหลังจะเป็นตัวเลือกในการศึกษาเนื่องจากมีกระบวนการผลิตที่ไม่ยุ่งยากและมีความสามารถในการละลายได้ที่อุณหภูมิห้อง งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของ ความเข้มข้นและค่าเทียบเท่าน้ำตาลเดคโทรซของมอลโตเดคทรินจากแป้งมันสำปะหลังต่อลักษณะทางเคมีกายภาพของสารเพิ่มน้ำเลือด โดยลักษณะทางเคมีกายภาพ เช่น ความหนืด ความดันสารแขวนลอย ความเป็นกรดด่าง จะถูกทำการวัดและเปรียบเทียบกับสารเพิ่มน้ำเลือดที่มีใช้ในทางคลินิก คือ 6% HES 130/0.4 นอกจากนี้จะทำการวัดความหนืดของน้ำเลือดเมื่อนำสารเพิ่มน้ำเลือดที่เตรียมขึ้นจากแป้งมันสำปะหลังชนิดมอลโตเดคทรินมาผสมกับเลือด และจะศึกษาผลของระยะเวลาในการเก็บต่อคุณสมบัติของสารเพิ่มน้ำเลือด รวมทั้งการศึกษาผลของสารเพิ่มน้ำเลือดที่เตรียมต่อลักษณะของเม็ดเลือดแดง ผลการศึกษาพบว่ามอลโตเดคทรินที่มีค่าเทียบเท่าน้ำตาลเดคโทรซต่ำจะกลับมาเกิดเป็นเจลได้ง่ายเมื่อวางทิ้งไว้ มีความขุ่นสูง และมีความหนืดสูงกว่ามอลโตเดค ทรินที่มีค่าเทียบ เท่าน้ำตาลเดคโทรซสูง แต่จะมีค่าการละลายที่ด้อยกว่าและมีค่าความดันสารแขวนลอยต่ำกว่า สำหรับผลที่มีต่อลักษณะของเม็ดเลือดแดงนั้นไม่พบผลที่แตกต่างกันอย่างมีนัยสำคัญระหว่างสารเพิ่มน้ำเลือดที่เตรียมขึ้นในแต่ละค่าเทียบเท่าน้ำตาลเดคโทรซ เมื่อเปรียบเทียบคุณสมบัติด้านความหนืดและค่าความดันสารแขวนลอยกับ 6% HES 130/0.4 จะได้ว่าสารเพิ่มน้ำเลือดที่เตรียมจากแป้งมันสำปะหลังชนิดมอลโตเดคทรินที่มีค่าเทียบเท่าน้ำตาลเดคโทรซที่ความเข้มข้น 10% โดยน้ำหนักต่อปริมาตรมีคุณสมบัติที่ใกล้เคียง แต่อย่างไรก็ตามการนำแป้งมันสำปะหลังชนิดมอลโตเดคทรินมาใช้เป็นสารเพิ่มน้ำเลือดจำเป็นจะต้องคำนึงถึงคุณสมบัติที่ยังไม่เหมาะสม เช่น ความสามารถในการละลายที่อุณหภูมิห้อง การคืนตัวเป็นเจล และความขุ่น เป็นต้น ดังนั้นการศึกษาเพื่อนำแป้งมันสำปะหลังชนิดดัดแปรอื่นจึงมีความจำเป็นและท้าทายเพื่อพัฒนาคุณลักษณะที่เหมาะสมเพื่อใช้เป็นสารเพิ่มน้ำเลือดต่อไป

คำสำคัญ สารเพิ่มน้ำเลือด แป้งมันสำปะหลังชนิดมอลโตเดคทริน ค่าเทียบเท่าน้ำตาลเดคโทรซ ความหนืด ความดันสารแขวนลอย

Research problem and its significance

The reduction of the amount of red blood cells in pathological conditions, for example hemorrhagic shock and anemia, lowers tissue oxygenation, leads to anaerobic metabolism and ultimately induces multi-organ dysfunction. In case of severe blood loss, blood or fluid infusions are used to treat volume deficit. Plasma expanders (PEs) are infused fluids which restitute blood volume by expanding the volume of plasma. Two catagories of PEs, crystalloid and colloid, are clinically used. Colloid solutions such as commercial products, Voluven® and Hextend®, have hydroxyethyl corn starch as an important component in an electrolyte solution. Venofundin® and Tetraspan® are potato hydroxyethyl starch-based plasma expanders. Therefore, if it is possible to use other starches such as tapioca starch from cassava plant to be modified for PEs, it will be useful alternative source. Tapioca starch has been introduced in many fields of application such as foods, pharmaceutical products, paper, cosmetics and textiles. Therefore, this will expand the application of tapioca starch into non-food industrial products especially in pharmaceutical and medical products.

Objective of the study

1.To formulate the plasma expander from tapioca maltodextrin.

2.To study the effects of dextrose equivalent (DE) and concentration on the physicochemical and rheological properties and storage stability of tapioca maltodextrin-based plasma expander.

Methodology

In this project, the major investigations will focus on in vitro study to complete the objective of the study.

Tapioca maltodextrin-based solution preparation

Tapioca maltodextrin starch was prepared by enzyme process using α-amylase. Various concentrations of solution (1, 2, 3, 4, 5, 6 and 10 % by weight per volume) were prepared by mixing tapioca maltodextrin with 0.9% sodium chloride solution.

Physicochemical properties measurement

Molecular mass of the tapioca maltodextrin was confirmed by using size exclusion chromatography. pH and turbidity of solutions were measured by pH meter and spectrophotometer, respectively. Viscosity of solutions is measured at a varied shear rate using a cone-plate viscometer. Colloid osmotic pressure of solutions is determined with a membrane colloid osmometer. Aforementioned physicochemical properties of Voluven® (6% HES 130/0.4) were also measured to compare with tapioca maltodextrin-based plasma expanders.

Determination of enzymatic hydrolysis

Solutions of tapioca maltodextrin were added with amylase enzyme to obtain the liquefaction. The mixtures were kept at room temperature for 1 minute for the reaction and then imediately put into boiled water to stop the reaction. 3,5-dinitrosalicylate (DNS) solution was added and boiled for 5 minutes. Distilled water was then added to obtain 6 mL of mixture. The sample was collected to measure the absorbance at 540 nm using a UV/Visible spectrophotometer.

Storage stability

The tapioca maltodextrin in saline solutions were stored at the room temperature and 4^{o}C in the refrigerator for 90 days. The soultions were sampled and tested with interval of 0, 14, 30, 60 and 90 days for the measurement of colloidal osmotic pressure, pH, viscosity.

Red blood cell morphology

Blood was collected from Golden Syrian hamsters. A visualization using microscope was performed to investigate the effect of the tapioca maltodextrin solution on the red blood cell shape when mixing solution with blood sample. Furthermore, scanning electron microscope was used to look at the morphological feature of red blood cells.

Plasma viscosity measurement

Mixtures of blood and tapioca maltodextrin solution were prepared in the 1:1 and 3:1 ratios. Mixtures were then centrifuged to separate plasma and blood cells. Plasma portion was pipetted and measured for viscosity.

Results and Conclusion

This study found that both dextrose equivalent (DE) and concentration had the effects on the physicochemical and rheological properties of tapioca maltodextrin-based plasma expanders. Furthermore, those properties also varied during long duration storage. Our investigation provided the information about the formulations and characteristics of an

alternative plasma expander based on tapioca maltodextrin. This study showed the effects of DE on the physicochemical properties of tapioca maltodextrin solutions. Low DE value led to high retrogradation, turbidity and viscosity but low colloid osmotic pressure and poor solubility. In contrast, high DE value caused the opposite results relative to low DE values. However, to use tapioca maltodextrin as a raw material to be prepared for plasma expander, some properties have to be considered and optimized efficiently. Further studies on other modified tapioca starches are interesting in order to improve the improper properties of tapioca maltodextrin for plasma expander.

Outputs

1. Information of the appropriate formulation of tapioca maltodextrin solution which has similar physicochemical properties to 6% HES 130/0.4.
2. An abstract submitted to the annual conference hosted by Faculty of Medicine, Prince of Songkla University.
3. A manuscript preparation for an international publication.

Expense

Type	Total expense (Baht)
1. Compensation	240,000.00
2. Materials and supplies	114,237.90
3. Expenses	128,009.30
4. Wages and labor	0.00
Total amount	**482,247.20**

Introduction

Plasma expanders (PEs) are hyperoncotic and/or hypertonic solutions that increase plasma volume in the circulatory system by pulling interstitial fluid into vasculature. In case of blood loss, plasma expanders have several advantages comparing to whole blood such as no problem about blood types, lower risk of infections and longer shelf life. Crystalloids and colloids are typical PEs using in treatment of hypovolemic patients. Several studies indicate that colloid infusion is less than crystalloid infusion about two folds.[5, 42] Furthermore, colloids circulate in the circulatory system longer than crystalloids, enhancing volume replacement efficacy.[19, 31] Many colloids are used in clinic such as albumin, gelatins, hydroxyethyl starch and dextrans.[24, 26] However, there is a caution to be concerned in an administration with colloids about anaphylactic reaction.[31, 33] Furthermore, it might affect kidneys function when using in high volume of colloids.[29, 34] On the other view point, not only the colloid osmotic pressure, the viscosity of plasma expander plays an important role in volume replacement and maintenance of functional capillary density during hemorrhagic shock resuscitation and acute hemodilution.[7, 37, 45] Cabrales et al. demonstrated that, during extreme hemodilution, high viscosity plasma expander using a mixture of alginate and dextran70 kDa provided higher arterial blood pressure than low viscosity plasma expanders.[8]

Hydroxyethyl starch (HES) has been made since 1970s and many generations of HES have been developed.[47] HES derived from amylopectin in waxy corn starch has been introduced as an alternative plasma volume expander due to its relatively short intravascular half life of 2-3 hours and low anaphylactoid reactions compared to other colloids. Molecular weight, C2/C6 ratio and degree of substitution in HES are major factors of volume effect, solubility, duration of action and elimination and coagulation.[4, 15, 17, 30] For example, 6% HES 130/0.4 means the concentration of HES is 6% in saline solution and HES molecular weight is 130 kDa with 0.4 molar substitution. Furthermore, this HES has 9:1 of C2/C6 ratio.[47] There was the study on the effects of two different hydroxyethyl starch solutions (6% HES 130/0.4 and 200/0.5) on blood viscosity by using in vitro and ex vivo.[27] Their results showed that HES 130/0.4 might have hemorheological advantages compared to conventional HES 200/0.5 when used in large volume. Several in vivo studies reported that HES has viscosity about 2.1 cP which is significantly lower than normal whole blood. [14, 23] Therefore, the volume extent, coagulation effect and circulating time of HES depend on the chemical and physical characteristics of HES solution.

Most HES plasma expanders using in clinical applications are still based on waxy maize amylopectin such as Voluven, Hespan and Hextend. However, there are recent products of HES plasma expander based on potato starch that challenge to waxy maize HES plasma expander. Recent study by Sommermeyer et al has reported that potato starch-based HES has higher degree of esterification with phosphoric than waxy maize starch.[40] Furthermore, potato starch-based HES has lower degree of branching compared with waxy maize-based HES. However, the pharmacological and clinical equivalence between potato starch-base and waxy maize-base HES plasma expanders still need to be further investigated. More recently, Ahmad and colleagues have presented an alternative source of starch, Assam Bora rice, for plasma expander.[1, 3] They have characterized Assam Bora rice starch in the view of FTIR spectra, degree of branching, osmotic pressure and molecular weight-viscosity relationship to use as plasma expander. There were some works that studied and utilized tapioca starch and its derivatives in several aspects. The physical properties of pregelatinized tapioca starch, a modified tapioca starch, showed that the viscosity was high and decreased after storage.[21] Furthermore, they demonstrated that the viscosity was a treatment dependent. Loksuwan has used acid-modified tapioca starch, native tapioca starch and tapioca maltodextrin as encapsulation material for β-carotine.[20] She reported that an acid- modified tapioca starch had better characteristics than native tapioca starch. But there was no discussion in detail about maltodextrin in this study. Other study by Udomrati et al have investigated the effect of tapioca maltodextrin on the stability of oil-in-water emulsion for the food purpose. They have reported that tapioca maltodextrin with a lower DE inhibited creaming more efficiently than maltodextrin with a higher DE because of higher viscosities.[43] Rocha et al reported that maltodextrin from cassava starch had higher viscosity on cooling at 50^{o}C compared to that from corn starch.[35] Furthermore, they showed that cassava starch was hydrolysed more easily than corn starch and the DE of cassava starch was less than that of corn starch. Therefore, these studies about alternative sources of starch challenge Thai researchers to develop a new plasma expander which is made from the derivative of tapioca starch. Tapioca maltodextrin is a candidate of our study because its water solubility at room temperature and it can be produced in Thailand. Furthermore, tapioca maltodextrin is polysaccharide which is not rejected from body. DE has been related to many physical properties of maltodextrin such as glass transition temperature, hygroscopicity, compressibility, gelation, viscosity and boiling and freezing temperatures.[22] DE value can be determined by many methods such as high performance liquid chromatography (HPLC), titrimetric Lane Eynon method with alkaline

Fehling's solution, near infrared (NIR) calibration model and osmometry.[36, 41] Maltodextrin, a starch hydrolysate product, has widely used in the pharmaceutical and food industry. However, using tapioca maltodextrin as a plasma expander has not been reported in any aspect related to the physicochemical and rheological properties of plasma expander.

This work aimed to study the effects of dextrose equivalent (DE) and concentration on the physicochemical and rheological properties and storage stability of tapioca maltodextrin-based plasma expander. By the outcome of this research, it will provide the information about formulations and characteristics of an alternative plasma expander based on tapioca maltodextrin. Combining this knowledge with the microcirculation study can further extend to a new tapioca starch-based plasma expander that will challenge to a lower-cost plasma expander.

Research Methodology

Tapioca maltodextrin preparation

Tapioca maltodextrin starch was prepared by an enzyme process using α-amylase enzyme, i.e. Spezyme® LT300 (Activity 30000 RAU/g, DuPont™ Genencor Science, USA). In brief, 0.004% (volume/weight, v/w, of dry starch weight) of Spezyme® LT300 was added into 25% starch slurry and agitated for 30 min in boiling water. Then the solution was put into 70°C water bath and 0.016% (v/w of by dry starch weight) of Speczyme® LT300 was added. The solution was incubated for 30 min and then 120 min. Consequently, the enzyme activity was sterminated by putting the solution into boiling water for 30 min. Then, the maltodextrin starch solution was dried using lyophilization technique.

Total sugars and reducing sugars were measured using Phenol sulfuric acid and Somogyi-Nelson methods, respectively. Then, the percentage of the ratio between reducing sugars and total sugars was calculated for dextrose equivalent (DE). Furthermore, a percent of branching was determined using Park-Jonhson's method to obtain the amount of reducing sugars of debranched sample (by hydrolyzing 4 mg/mL starch with 4 unit of Isoamylase (from *Pseudomonas sp*., 1000 U/ml, Megazyme, Ireland) and 2.88 unit of Pullulanase M1 (from *Klebsiella planticola*, 720 U/ml, Megazyme, Ireland) in 0.01 M sodium acetate buffer pH 5.5, at 25°C for overnight) and Phenol sulfuric acid to measure the total sugars. The amount of reducing sugars and total sugars were estimated using glucose as a standard curve and the degree of branching, reported as percentage, was calculated as the following equation.

Percent of branching= Difference of reducing sugars before and after branching enzyme digestion x 100

Total glucose after brancing enzyme digestion

Size exclusion chromatography, equipped with Ultahydrogel 250 column (Water Corporation, MA, USA) at 40°C, using 0.02M Phosphate buffer with 250 mM NaCl as an eluent with the flow rate of 0.3 ml/min, was used to determine an average molecular weight by weight (MW_w)and a molecular weight by number (MW_n), using the dextran of different MW as the standard.

Tapioca maltodextrin solution preparation

Three different dextrose equivalents (DE1, DE6 and DE12) of tapioca maltodextrin were used in this study. Various concentrations of solution (1%, 2%, 3%, 4%, 5%, 6% and 10% by weight per volume) were prepared by mixing tapioca maltodextrin with 0.9% sodium chloride solution. The solutions were stired and heated at 70°C using a hotplate stirer for 15 minutes (M6, Ingenieurbüro CAT, Germany) as shown in Figure 1.

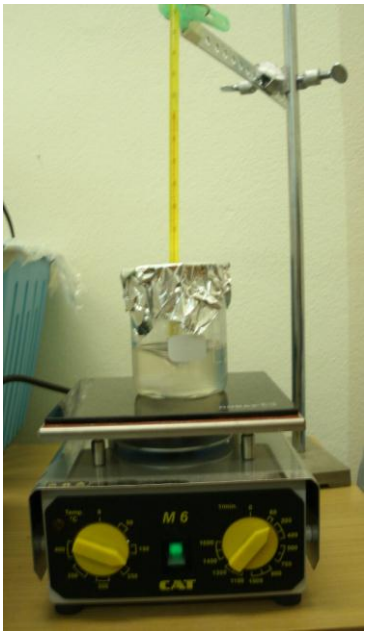


Figure 1 Tapioca maltodextrin solution preparation.

Physicochemical properties measurement

Turbidity and pH of tapioca maltodextrin solutions were measured by a spectrophotometer (Spectroquant ® pharo 300, Merck, Germany) and pH meter (Eutech pH700, Eutech

Instrument, Singapore), respectively. Viscosities of tapioca maltodextrin solutions were measured at a varied shear rate using a cone-plate viscometer (Brookfield DV-II+, Brookfield Engineering Laboratories, USA). Colloid osmotic pressure of tapioca maltodextrin was determined with a membrane colloid osmometer (Osmomat 050, Gonotec, Germany). Aforementioned physicochemical properties of Voluven®, hydroxyethyl starch (HES 130/0.4) were also measured to compare with tapioca maltodextrin solutions.

Determination of enzymatic hydrolysis

Four concentrations (1%, 3%, 6% and 10% w/v) of each DE were selected to determine the enzymatic hydrolysis. Solutions of tapioca maltodextrin were added with amylase enzyme (Sigma Aldrich, Singapore) to obtain the liquefaction. The mixtures were kept at room temperature for 1 minute for the reaction and then imediately put into boiled water to stop the reaction. 3,5-dinitrosalicylate (DNS) (Sigma Aldrich, Singapore) solution was added and boiled for 5 minutes. Distilled water was then added to obtain 6 mL of mixture. The sample was collected to measure the absorbance at 540 nm using a UV/Visible spectrophotometer (Libra S22, Biochrom, UK). HES 130/0.4 was also determined for the enzymatic hydrolysis using the same method as tapioca maltodextrin solutions.

Storage stability

The tapioca maltodextrin in 0.9% sodium chloride solution and HES 130/0.4 were stored at the room temperature and 4^{o}C in the refrigerator for 90 days. The soultions and HES 130/0.4 were sampled and tested with the interval of 0, 2, 4, 8 and 12 weeks for the measurement of colloidal osmotic pressure, pH, viscosity.

Red blood cell morphology and aggregation

Blood was collected from male Golden Syrian hamsters weighted 100-120 g. The protocol for blood collection was approved by the animal ethic committee of Prince of Songkla University (Ethic number 42/2014). Collected blood was mixed with tapioca maltodextrin solutions (1, 3, 6 and 10% concentration) and kept at 4^{o}C in the refrigerator for 1 night and 3 nights. Blood smear was performed on blood mixtures and then a visualization with 200X magnification using a microscope (BX51WIF, Olympus, USA) was carried out to investigate the effect of the tapioca maltodextrin solution on the red blood cell aggregation and morphology. Furthermore, scanning electron microscope (SEM; Quanta400, FEI, USA) was used to look at the 3-dimension morphological feature of red blood cells. These procedures were also performed on a mixture of blood and HES 130/0.4.

Plasma viscosity measurement

Mixtures of blood and tapioca maltodextrin solution were prepared in the ratio of 1:1 and 3:1 to get the hematocrit in a range of 20-35%. Mixtures were then centrifuged to separate plasma and blood cells. Plasma portion was pipetted and measured for viscosity.

Results

As our results, tapioca maltodextrin had the percent of branching less than native tapioca starch as presented in Table 1. Furthermore, tapioca maltodextrin with lower DE tends to have less percent of branching. Tapioca maltodextrin also had lower averaged molecular weight compared with native tapioca starch. The lower DE value is, the higher molecular weight obtains. As the DE value increased, the peak of RI response had shifted from the right to the left as presented in Figure 2 to Figure 4.

The tapioca maltodextrin easily dissolved in 0.9% saline solution when heat was applied, especially the tapioca maltodextrin with DE1 and DE6. Moreover, tapioca maltodextrin with DE12 was able to dissolve easier than other DE values. Figure 5 to Figure 7 show the prepared solutions of tapioca maltodextrin. The solutions had clear color. When the solutions cooled down, the precipitation of starch was observed as shown in Figure 8. The physicochemical properties of tapioca maltodextrin solutions and 6%HES 130/0.4 are presented in Table 2. It was found that the turbidity of solution increased when the concentration was higher for each DE value. Furthermore, at the same concentration, tapioca maltodextrin with higher DE value had lower turbidity than tapioca maltodextrin with lower DE value. However, the tapioca maltodextrin solutions had apparently higher turbidity compared with 6%HES 130/0.4.

Table 1. Properties of native tapioca starch and tapioca maltodextrin

Properties	Native tapioca starch	Tapioca maltodextrin		
		DE1	DE6	DE12
Percent of branching	5.2±0.0	3.7±0.0	4.8±0.2	4.6±0.9
Averaged molecular weight by weight	259,837±4,321	103,399±1,613	21,328±932	6,555±584
Averaged molecular weight by number	47,729±6,238	17,661±918	2,768±321	1,455±47

As presented in Table 2, for acidic and basic properties, it was noticed that the pH value decreased when the concentration of tapioca maltodextrin increased. It was found that 6%HES 130/0.4 was slightly acidic solution compared with tapioca maltodextrin solutions at the same concentration. Furthermore, the higher DE value tapioca maltodextrin is, the lower pH solution achieves.

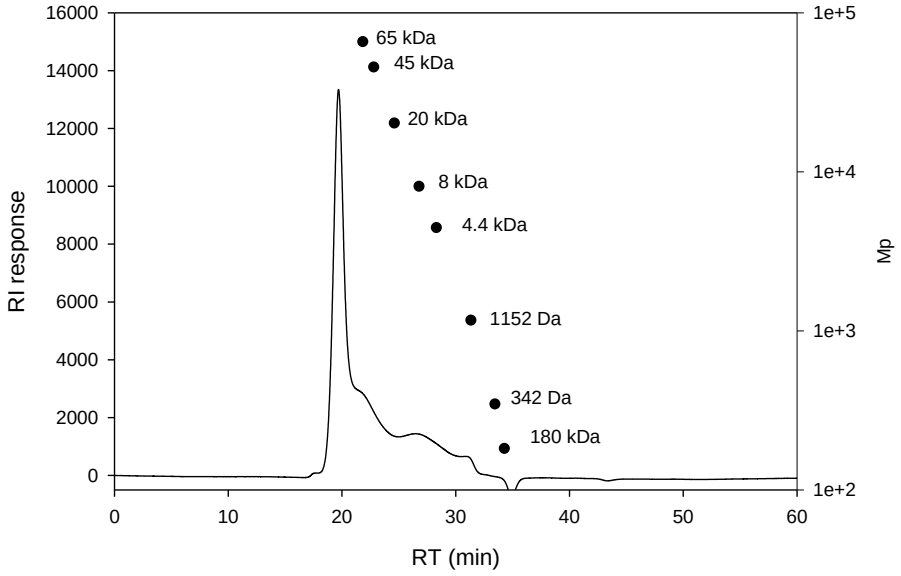


Figure 2 Chromatogram of tapioca maltodextrin with DE1 analyzed with column High Performance Liquid Chromatography (HPSEC).

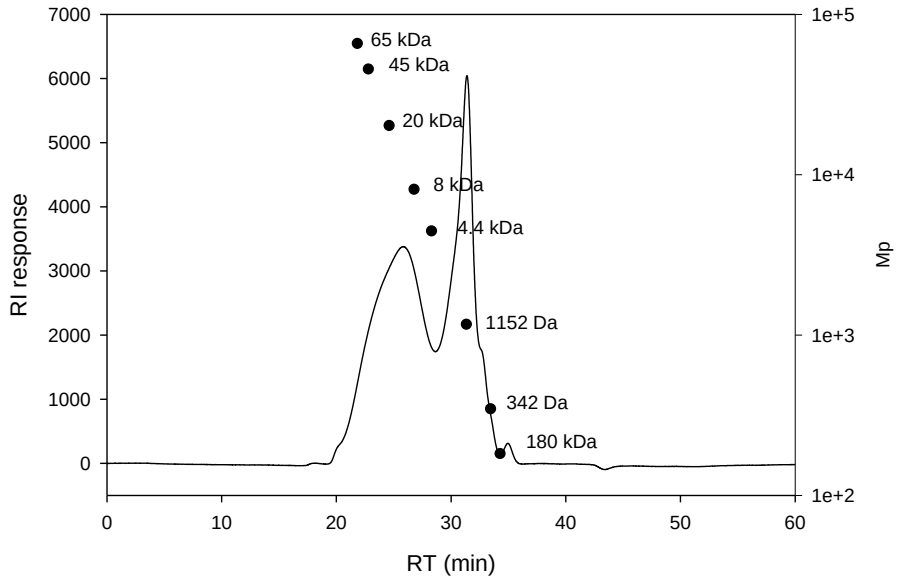


Figure 3 Chromatogram of tapioca maltodextrin with DE6 analyzed with column High Performance Liquid Chromatography (HPSEC).

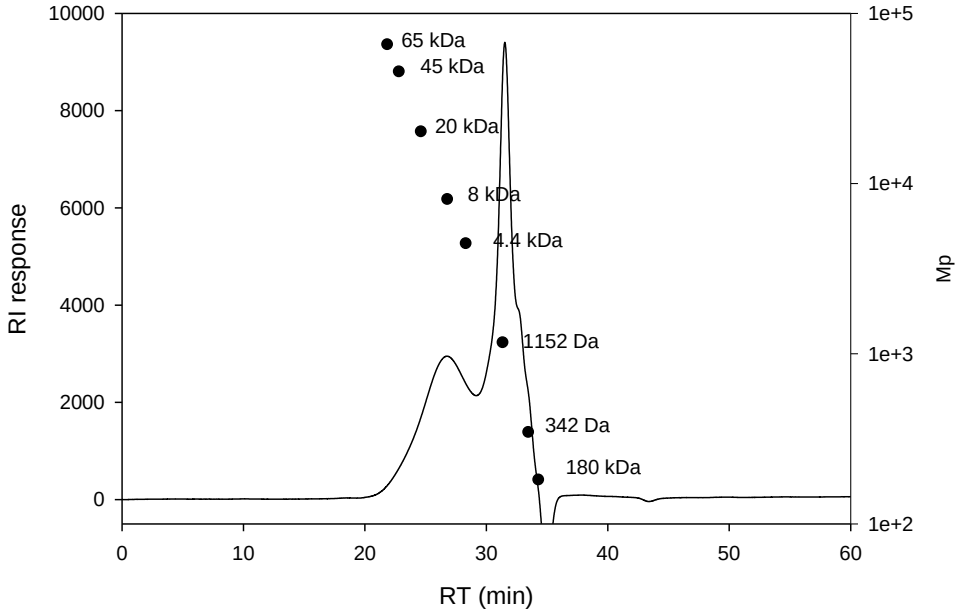


Figure 4 Chromatogram of tapioca maltodextrin with DE12 analyzed with column High Performance Liquid Chromatography (HPSEC).

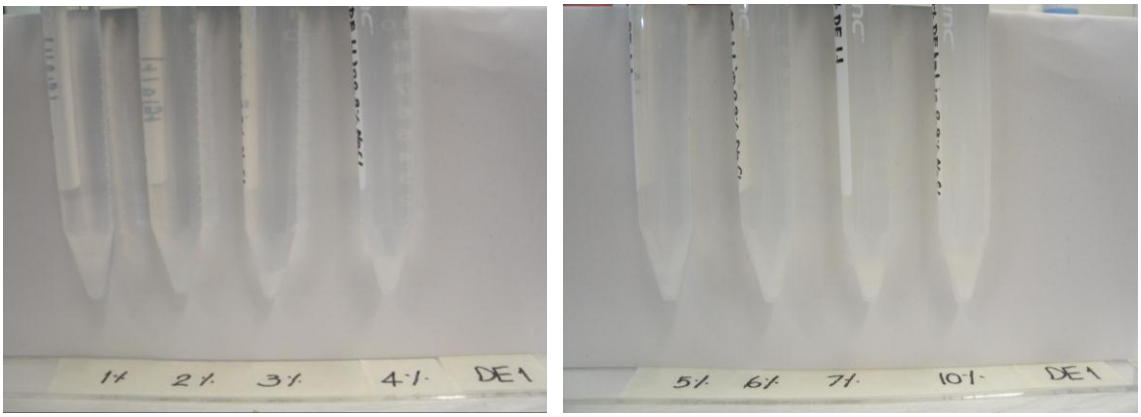


Figure 5 Appearances of tapioca maltodextrin with DE1 at different concentrations (1%, 2%, 3%, 4%, 5%, 6% and 10% w/v).

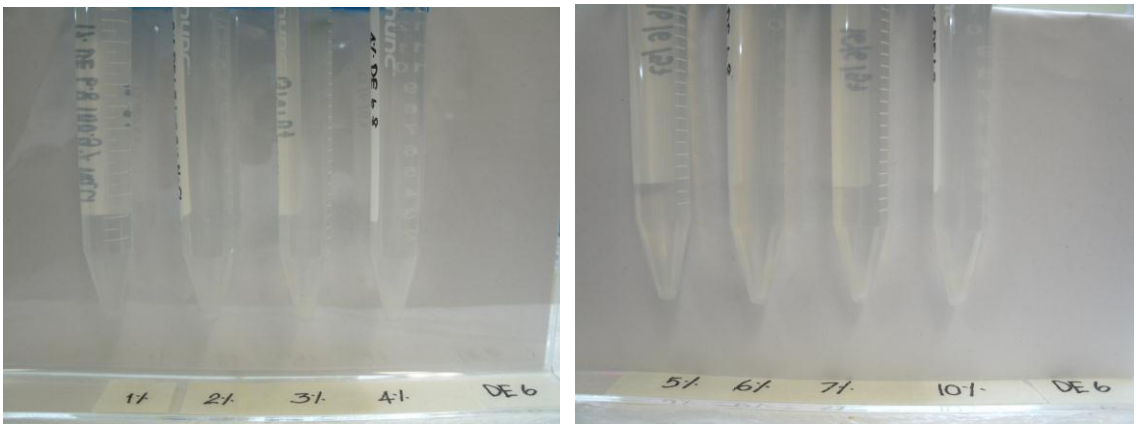


Figure 6 Appearances of tapioca maltodextrin with DE6 at different concentrations (1%, 2%, 3%, 4%, 5%, 6% and 10% w/v).

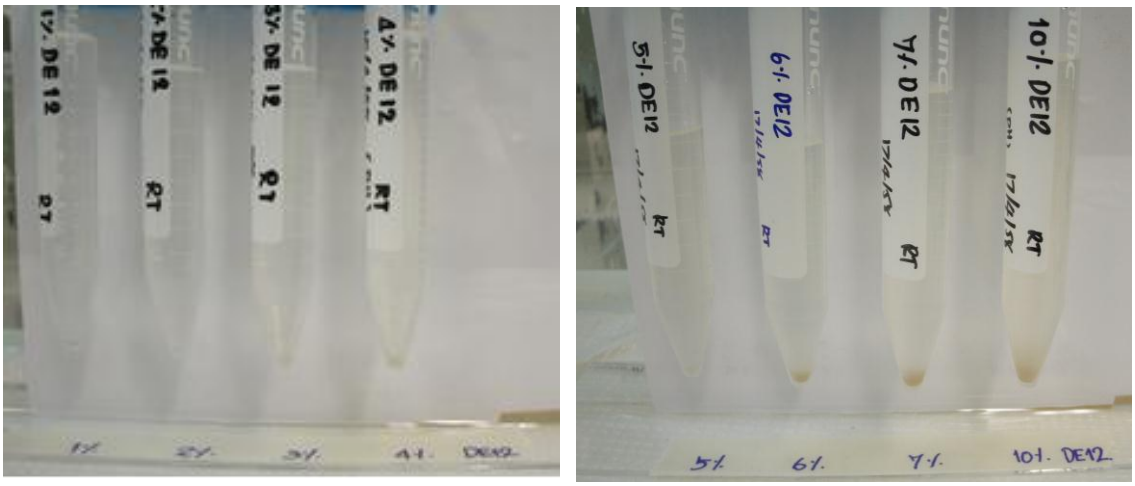


Figure 7 Appearances of tapioca maltodextrin with DE12 at different concentrations (1%, 2%, 3%, 4%, 5%, 6% and 10% w/v).

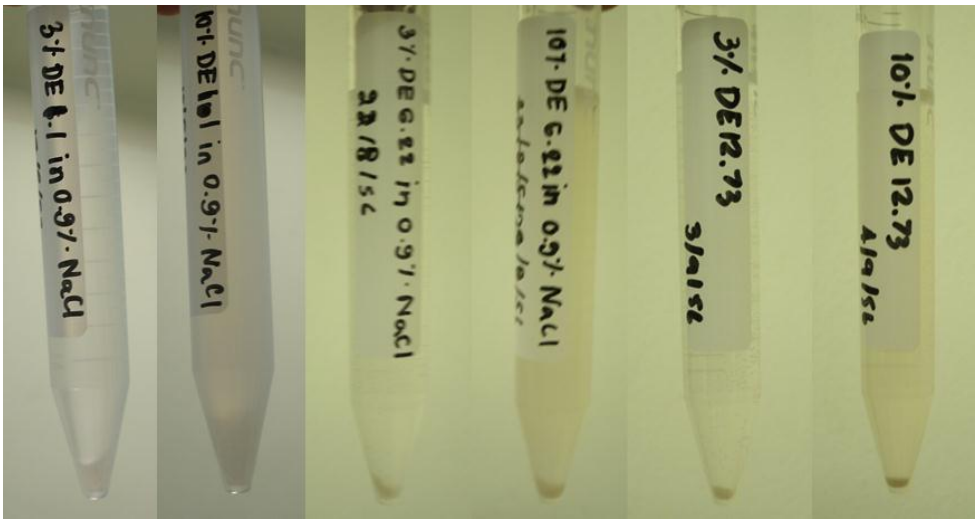


Figure 8 Precipitation of starch in tapioca maltodextrin solutions after cooling down.

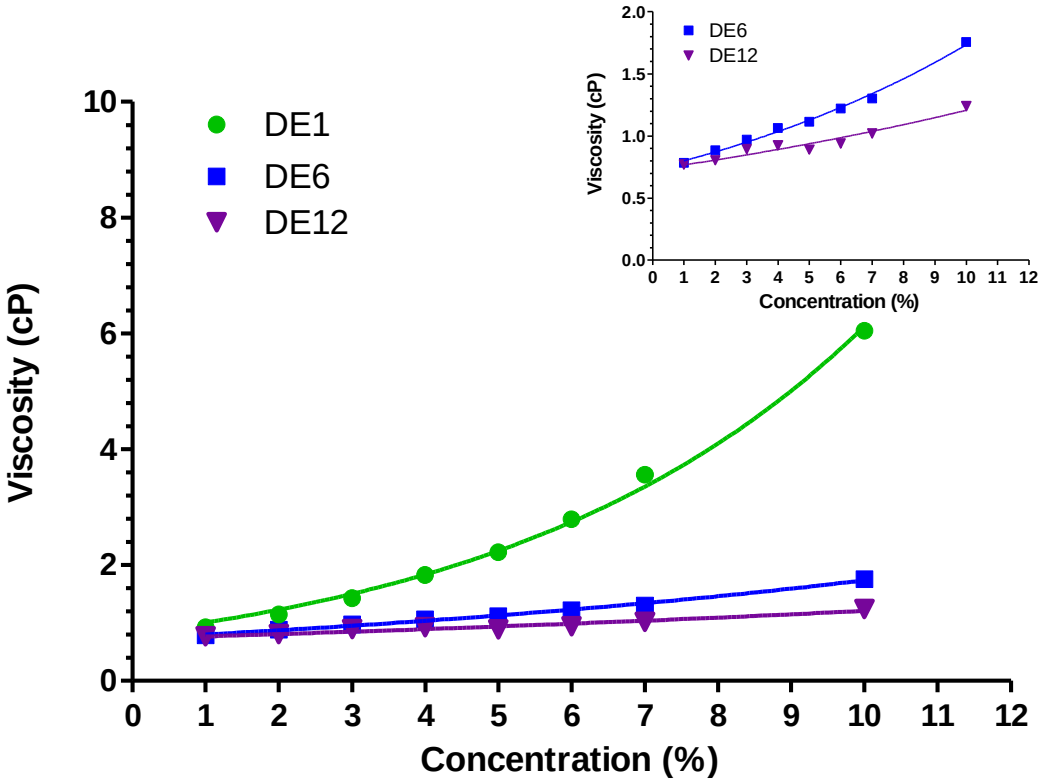
It was clearly demonstrated that an increase of concentration of tapioca maltodextrin elevated colloid osmotic pressure (COP) of the solutions. At the same concentration, solutions of tapioca maltodextrin with DE12 had highest COP compared with other DE values. Moreover, tapioca maltodextrin with DE12 at 10% w/v concentration had COP nearly similar to 6% HES 130/0.4.

As similar as COP, an increase of concentration of tapioca maltodextrin increased solution's viscosity. It was also found that low DE tapioca maltodextrin had higher viscosity compared with tapioca maltodextrin with high DE value. Comparing to 6% HES 130/0.4, there were only 4% w/v DE1 and 10% w/v DE6 which had similar viscosity. It is clearly noticed that the viscosity of tapioca maltodextrin with DE1 was a nonlinear relationship, exponential function, with the concentration as presented in Figure 9. The change in a slope of the viscosity of tapioca maltodextrin with DE1 solutions is greater than other two DE values. Furthermore, the relationships between COP and the concentration of tapioca maltodextrin solutions were all linear relationships (Figure 10). The linear relationship was also observed for pH and the concentration, especially for tapioca maltodextrin with DE1 and DE12 as showed in Figure 11.

With varying shear rate, the viscosity of tapioca maltodextrin solutions was nearly unchanged at the high shear rate (> 200 s^{-1}) while the viscosity increased when shear rate was less than 200 s^{-1}. The relationship between the viscosity and shear rate of tapioca maltodextrin solutions was fitted with the decay exponential relationship which was similar to 6% HES 130/0.4 as shown in Figure 12 to Figure 14.

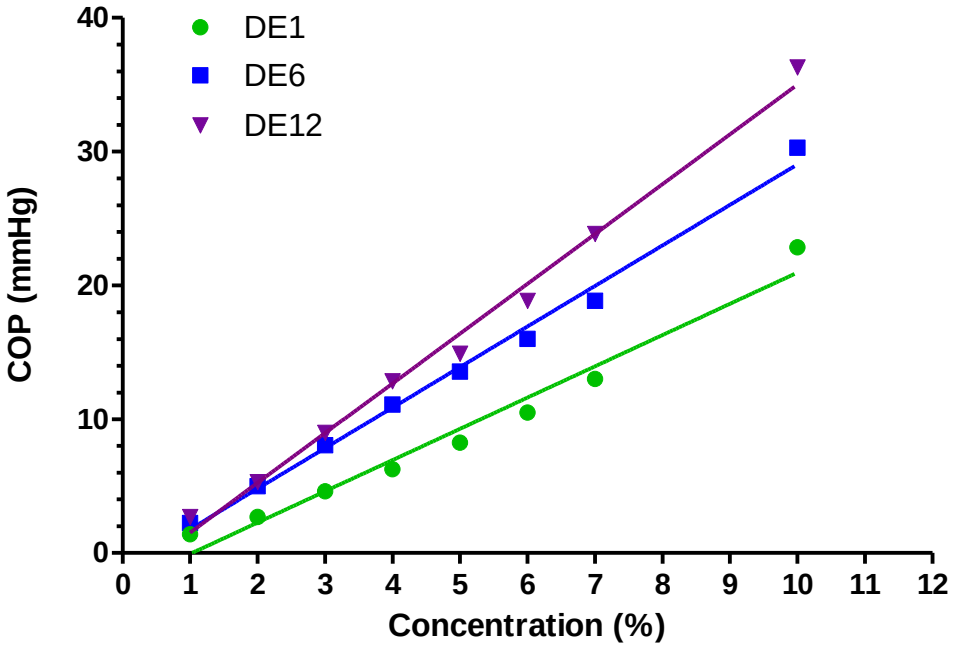
Table 2. Physicochemical properties of 6% HES 130/0.4 and tapioca maltodextrin solutions with different DE values and concentrations

Plasma expander	**Turbidity (NTU)**	**pH**	**COP (mmHg)**	**Viscosity (cP) At shear rate 150 s^{-1}**
Voluven (6%HES 130/0.4)	2	5.36±0.08	37.03±0.79	1.85
1% DE1	>100	6.58±0.02	1.40±0.00	0.93
2% DE1	>100	6.45±0.05	2.70±0.00	1.15
3% DE1	>100	6.46±0.02	4.60±0.00	1.43
4% DE1	>100	6.30±0.02	6.25±0.07	1.83
5% DE1	>100	5.34±0.05	8.25±0.07	2.23
6% DE1	>100	5.23±0.03	10.50±0.14	2.79
7% DE1	>100	5.18±0.02	13.00±0.14	3.56
10% DE1	>100	5.14±0.04	22.85±0.35	6.28
1% DE6	41	6.24±0.06	2.25±0.07	0.79
2% DE6	80	6.34±0.03	5.00±0.14	0.89
3% DE6	95	6.25±0.03	8.05±0.21	0.97
4% DE6	>100	6.15±0.05	11.10±0.28	1.07
5% DE6	>100	6.53±0.06	13.55±0.21	1.12
6% DE6	>100	6.31±0.03	16.00±0.28	1.22
7% DE6	>100	6.23±0.03	18.85±0.07	1.30
10% DE6	>100	6.14±0.04	30.30±0.99	1.76
1% DE12	17	6.79±0.01	2.70±0.28	0.77
2% DE12	33	6.76±0.01	5.30±0.71	0.81
3% DE12	47	6.73±0.01	9.00±1.56	0.90
4% DE12	71	6.66±0.02	12.85±2.05	0.93
5% DE12	85	6.72±0.02	14.90±2.26	0.89
6% DE12	97	6.70±0.01	18.85±2.76	0.94
7% DE12	>100	6.67±0.02	23.85±4.17	1.02
10% DE12	>100	6.68±0.03	36.30±4.67	1.24



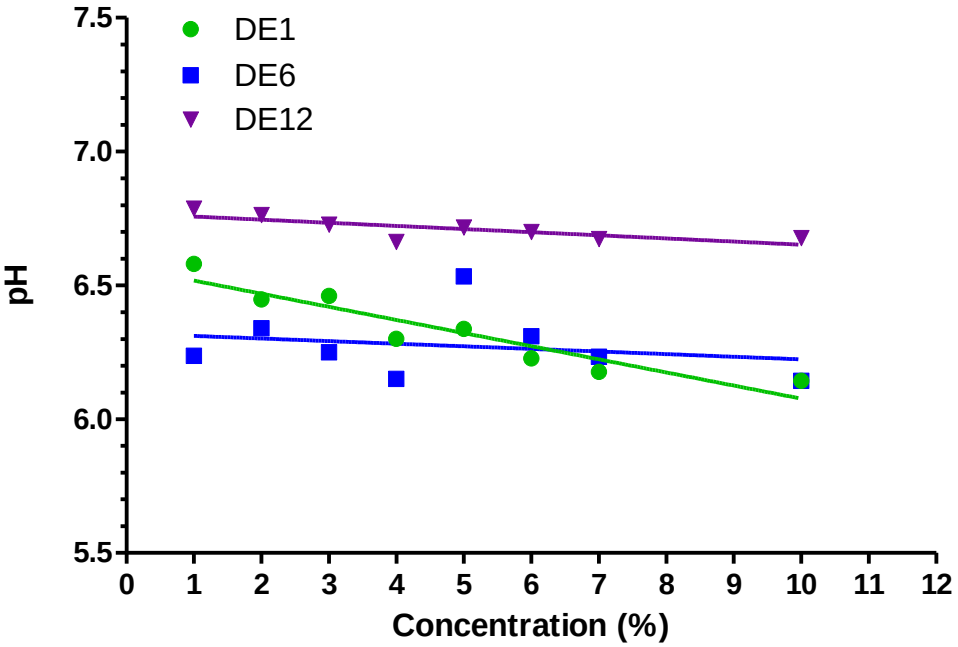
Exponential equation: $Y=Y_0*\exp(kX)$			
Tapioca maltodextrin	Y_0	k	r^2
DE1	0.823	0.201	0.9964
DE6	0.734	0.086	0.9938
DE12	0.729	0.050	0.9380

Figure 9 The relationship between concentration and viscosity of tapioca maltodextrin solutions.



Exponential equation: Y=mX+b			
Tapioca maltodextrin	m	b	r^2
DE1	2.336	-2.402	0.9713
DE6	3.030	-1.254	0.9925
DE12	3.722	-2.209	0.9916

Figure 10 The relationship between concentration and colloid osmotic pressure (COP) of tapioca maltodextrin solutions.



Exponential equation: Y=mX+b			
Tapioca maltodextrin	m	b	r^2
DE1	-0.049	6.567	0.8780
DE6	-0.010	6.321	0.0516
DE12	-0.012	6.769	0.5925

Figure 11 The relationship between concentration and colloid osmotic pressure (COP) of tapioca maltodextrin solutions.

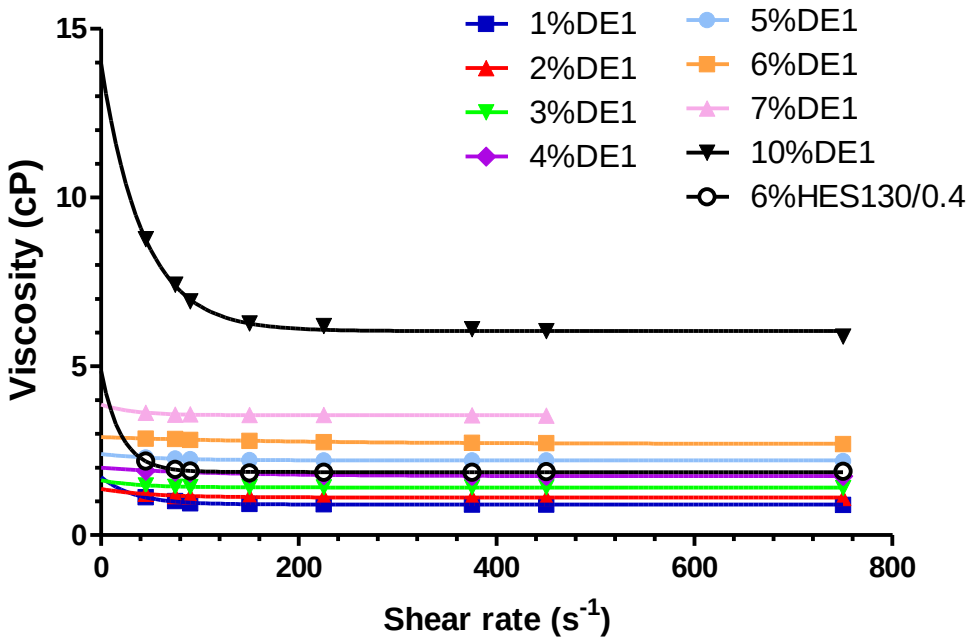


Figure 12 The viscosities of tapioca maltodextrin solutions with DE1 and 6% HES 130/0.4 at different shear rate.

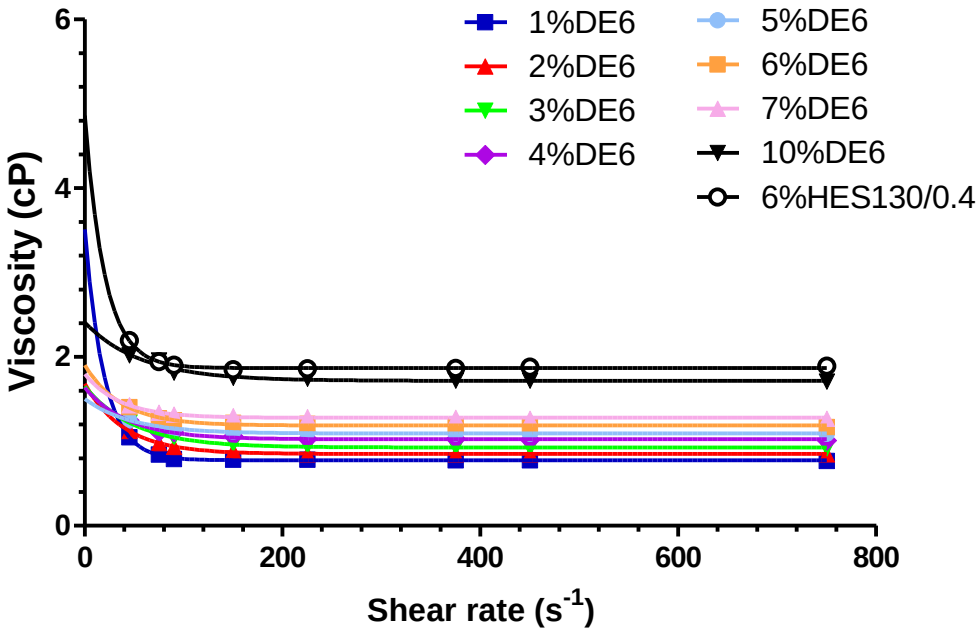


Figure 13 The viscosities of tapioca maltodextrin solutions with DE6 and 6% HES 130/0.4 at different shear rate.

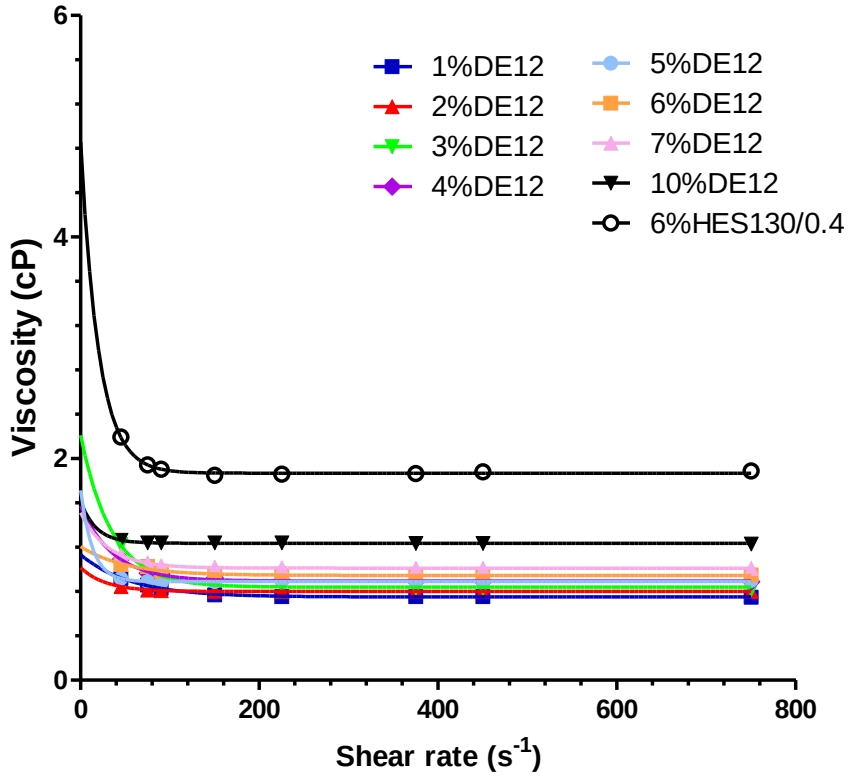


Figure 14 The viscosities of tapioca maltodextrin solutions with DE12 and 6% HES 130/0.4 at different shear rate.

As the enzymatic hydrolysis showed in Figure 15, tapioca maltodextrin with DE12 solutions had the absorbance higher than others whether they were hydrolyzed with lower concentration of enzyme. As expected, the higher concentration of tapioca maltodextrin was, the grater absorbance of solution was obtained. The absorbance of 6% HES 130/0.4 was lowest compared with other tapioca maltodextrin solutions.

For the storage test for the reliability of physical properties, it was found that 10% w/v tapioca maltodextrin with DE1 solution became a gel after 4 week when keeping at room temperature; therefore, its viscosity could not be measured as presented in Figure 16A. Furthermore, the viscosity of 6% and 7% w/v tapioca maltodextrin with DE1 solution increased as the storage time extended to 12 weeks. However, tapioca maltodextrin with DE1 which had the concentration less than 6% w/v seemed unchanged. For tapioca maltodextrin with DE6 solution, at room temperature, all concentration could be measured. Moreover, the viscosities of these solutions were not changed although they were kept for

12 weeks (Figure 17A). For colloid osmotic pressure and pH, at room temperature, tapioca maltodextrin with DE1 solutions showed a decreasing trends of COP and pH when the storage time increased as shown in Figure 18A and Figure 20A, respectively. In case of tapioca maltodextrin with DE6 solutions, the COP seemed unchanged when the concentration was less than 4% w/v (Figure 19A). Furthermore, the pH of tapioca maltodextrin with DE6 solutions showed fluctuation during first four weeks for all concentrations (Figure 21A). However, after the 4^{th} week, the pH of these solutions decreased for the concentration 1% to 5%. In case of the storage at $4^{o}C$, solutions of tapioca maltodextrin with DE1 became gels when the concentration was higher than 4% w/v. The viscosity of tapioca maltodextrin with DE1 greatly increased when the concentration was higher than 3% w/v after 12-week storage as presented in Figure 16B. In addition, 6% HES 130/0.4 had unchanged viscosity after keeping for 12 weeks which was similar to 1% and 2% w/v tapioca maltodextrin with DE1 solutions. For tapioca maltodextrin with DE6 solutions, the viscosity was quite stable for the concentration less than 6% w/v whereas the viscosity of concentration 10% w/v was slightly higher than 6% HES 130/0.4 (Figure 17B). For colloid osmotic pressure, the trends of COP decreased when keeping 12 weeks for both tapioca maltodextrin with DE1 and DE6 solutions as shown in Figure 18B and 19B. Furthermore, it was noticed that COP of 6% HES 130/0.4 and that of 10% w/v tapioca maltodextrin with DE6 solution had a similar trend. For pH, all solutions of tapioca maltodextrin with DE1 decreased greatly after the 8^{th} week (Figure 20B). Unlikely, pH of 6% HES 130/0.4 increased after keeping 8 weeks. In addition, the similar trend of pH which was found in tapioca maltodextrin with DE1 solutions was noticed for 6%, 7% and 10% w/v tapioca maltodextrin with DE6 solutions as shown in Figure 21B. After 12 weeks, it was visually observed that the solutions of tapioca maltodextrin with DE1 had white color and was not transparent for both keeping at room temperature and $4^{o}C$ as presented in Figure 22. On the other hand, the solutions of tapioca maltodextrin with DE6 still had clear color for keeping at room temperature whereas the solutions kept at $4^{o}C$ were not quite transparent (Figure 23). Furthermore, the results of turbidity measurement as mentioned in Table 3 were in consistent with the visual results. The turbidities of tapioca maltodextrin with DE1 and DE6 decreased after keeping at room temperature for 12 weeks.

The morphological changes of red blood cells (RBCs) before and after mixing with tapioca maltodextrin solutions and 6% HES 130/0.4 are presented in Figure 24 to Figure 26. For tapioca maltodextrin with DE1, it was found that, after 24 hours, low concentrations (1% and 3% w/v) did not influence a shape of RBCs. Their shape was still round and did not apparently change from the beginning of mixing. No aggregation of RBCs was also

noticed. On the other hand, after 24 hours, for the moderate to high concentration (6% and 10% w/v), a shape of RBCs was not quite round compared with the beginning of mixing. Furthermore, there was an aggregation of RBCs which could be noticed, especially at the concentration of 10% w/v. For 6% HES 130/0.4, the morphology of RBCs after 24 hours of mixing was still intact and similar to the beginning of mixing. In case of tapioca maltodextrin with DE6, it was noticed that 1% w/v concentration did not alter RBCs shape which was similar to the results observed in the control as displayed in Figure 25. Furthermore, an increase of concentration led to visibly notice the change in RBCs morphology, especially at the concentration of 10% w/v. In addition, for tapioca maltodextrin with DE12, it could not observed significant changes in RBCs morphology either low or high concentration (Figure 26). The shape of RBCs, after 24 hours mixing between blood and tapioca maltodextrin with DE12 solutions, was still round as similar as the shape at the beginning of mixing. The samples of RBCs from the mixtures between blood and tapioca maltodextrin with DE6 solutions were observed with SEM. The SEM images show that tapioca maltodextrin with DE6 solutions altered the shape of RBCs compared with the control sample as shown in Figure 27. However, in every sample, not only normal RBCs but also the echinocytes were observed (Figure 27 to Figure 29). The shape of RBCs was not completely round or biconcave, the cell periphery had changed.

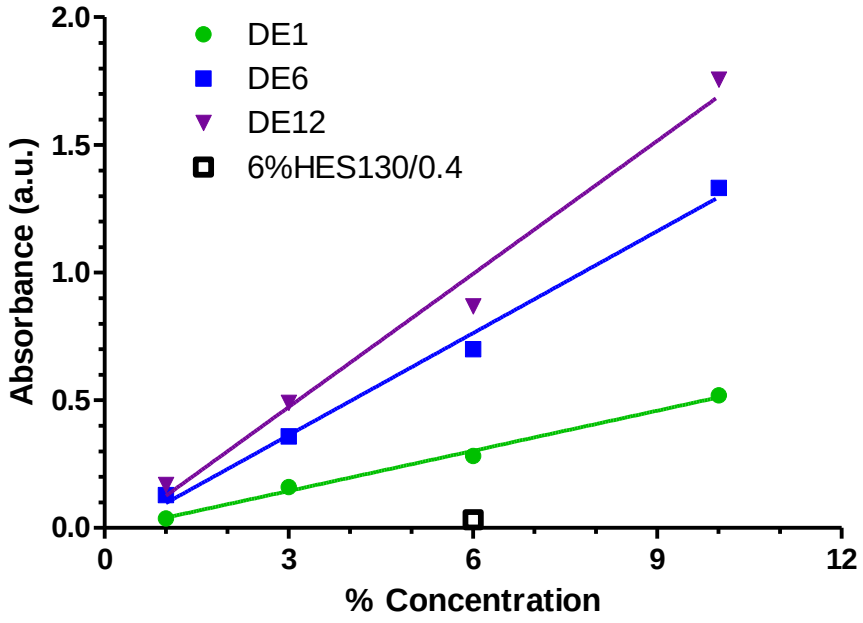
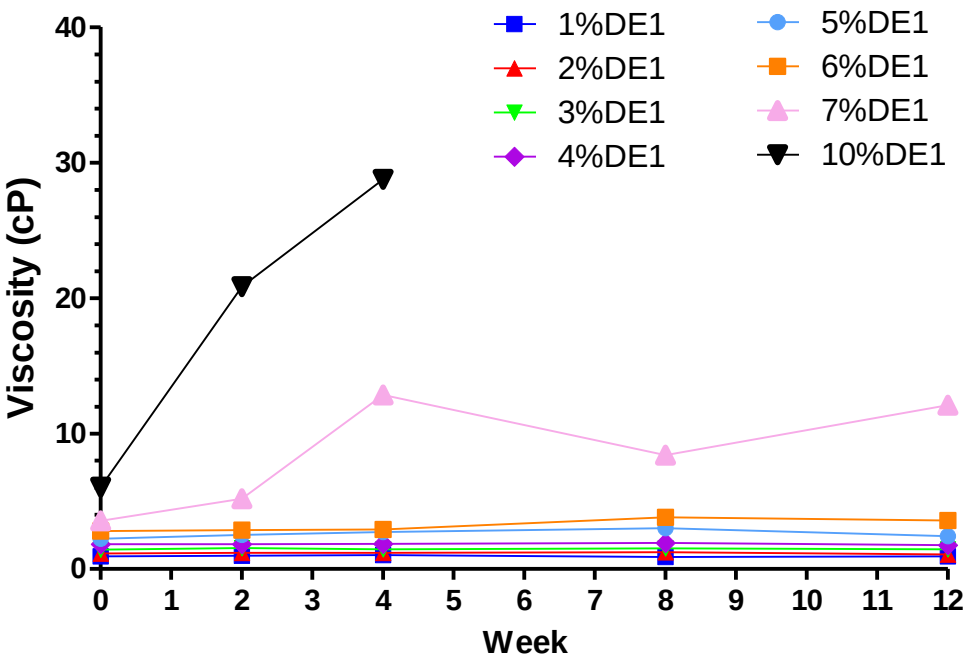
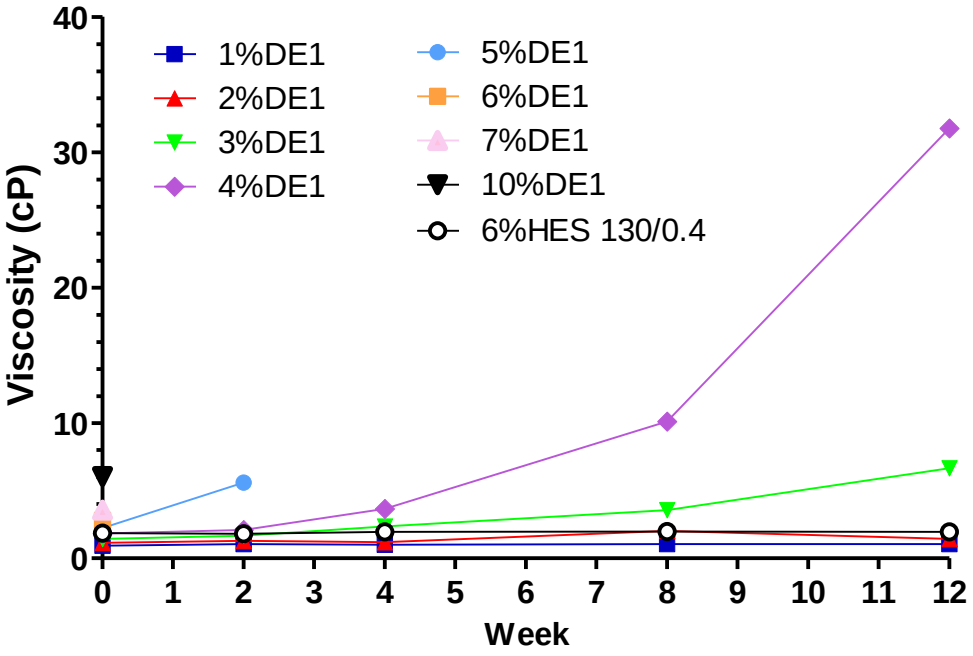


Figure 15 The absorbance of enzymatic hydrolysis of tapioca maltodextrin solutions and 6% HES 130/0.4.

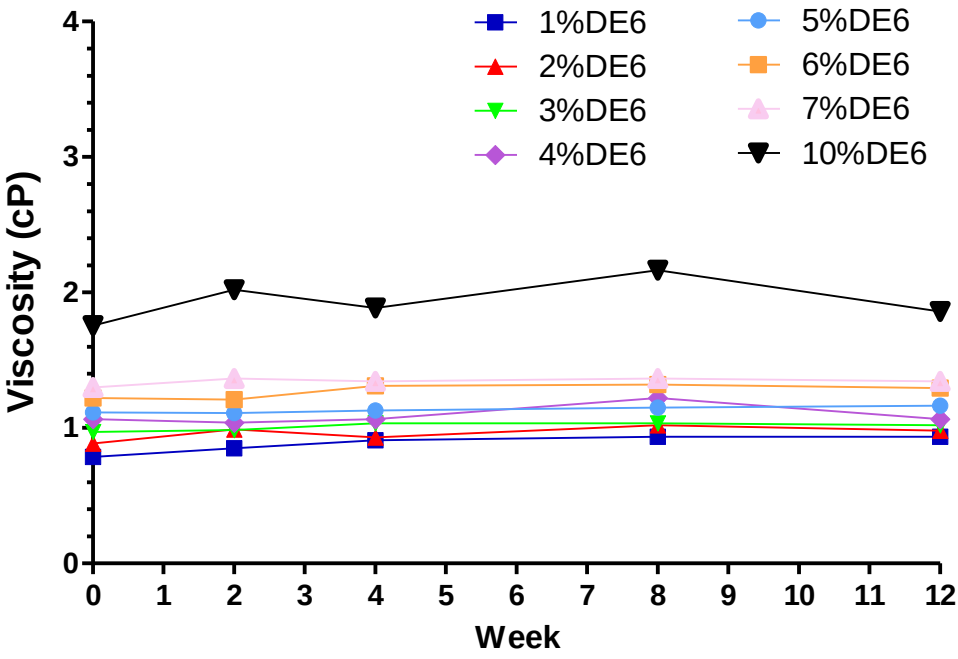


(A)

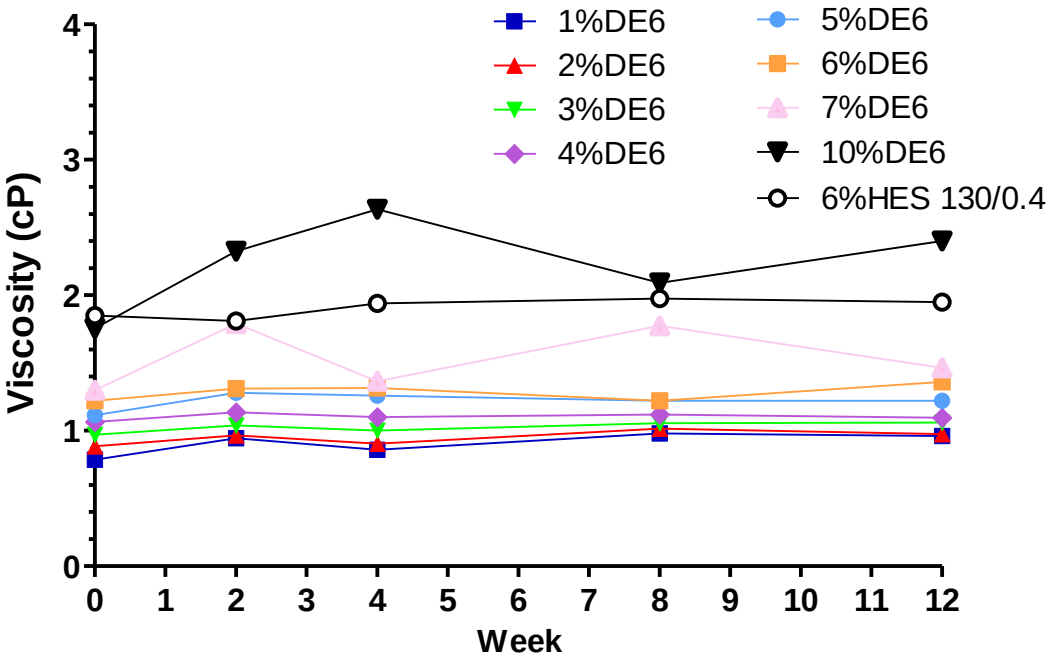


(B)

Figure 16 The viscosities of tapioca maltodextrin with DE1 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26^{o}C) and (B) refrigerated temperature (4^{o}C).

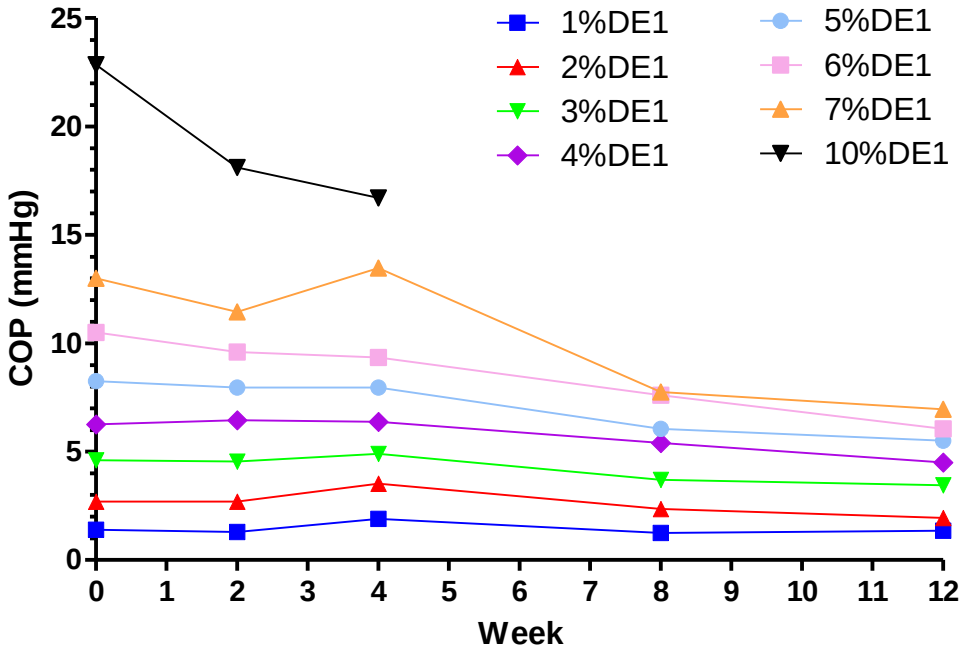


(A)

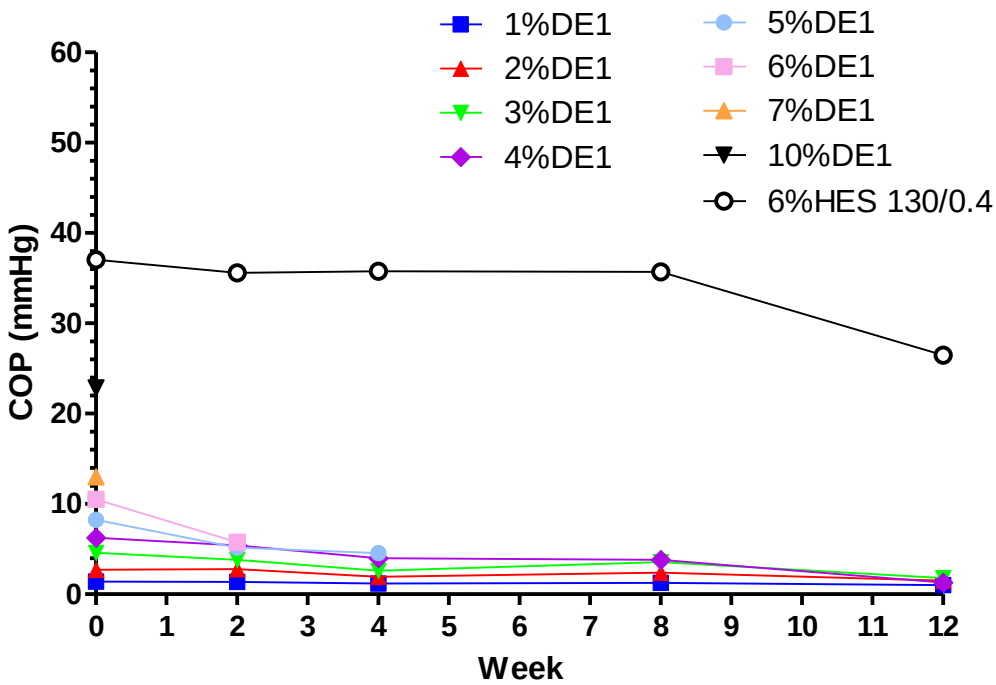


(B)

Figure 17 The viscosities of tapioca maltodextrin with DE6 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26^{o}C) and (B) refrigerated temperature (4^{o}C).

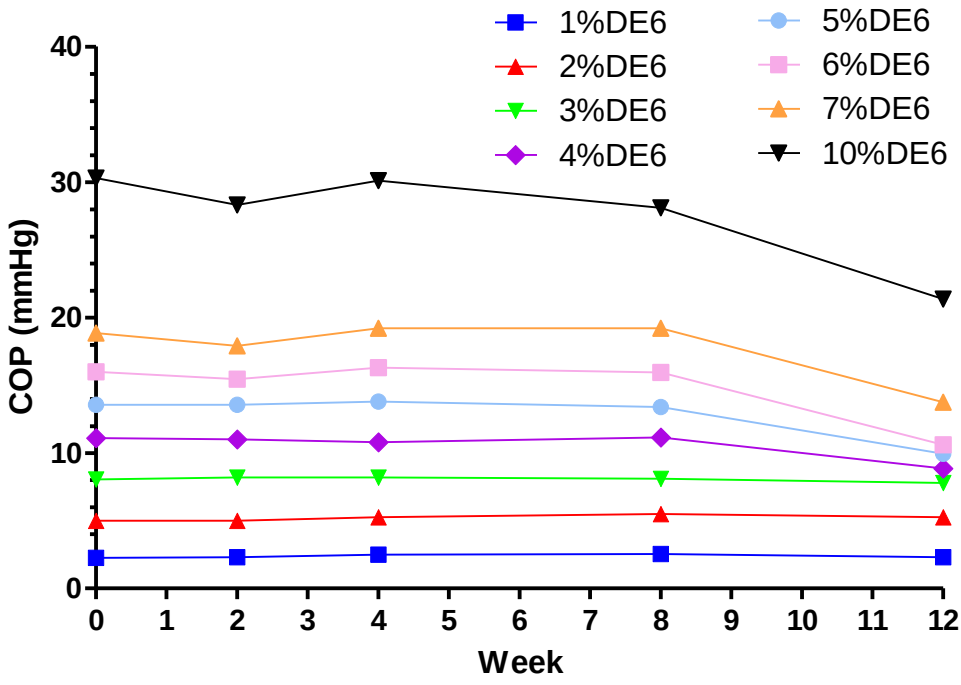


(A)

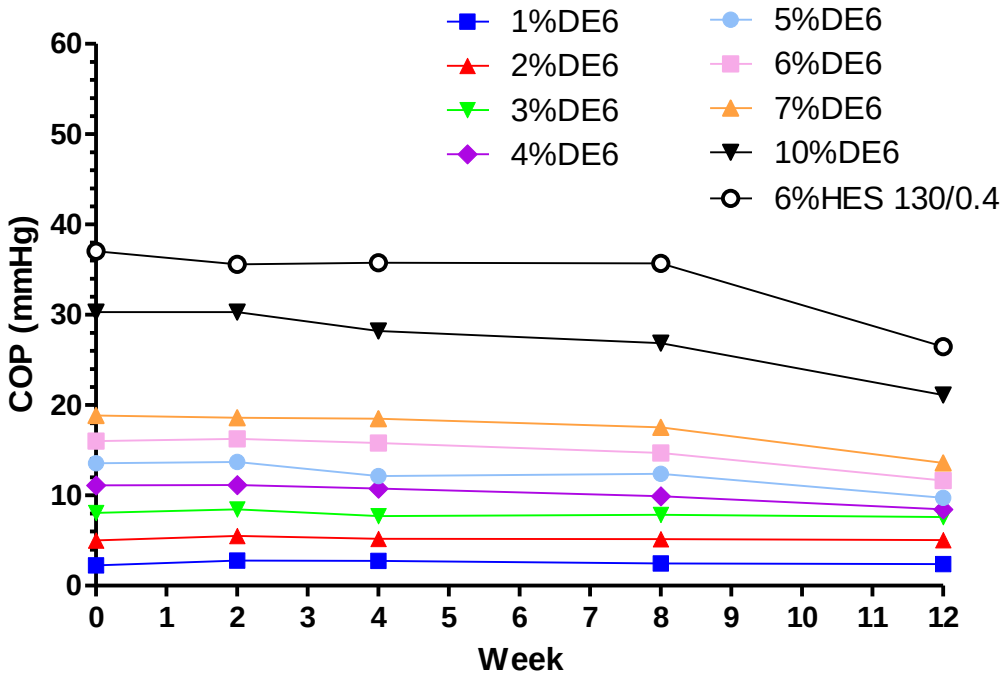


(B)

Figure 18 The colloid osmotic pressure of tapioca maltodextrin with DE1 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26^{o}C) and (B) refrigerated temperature (4^{o}C).

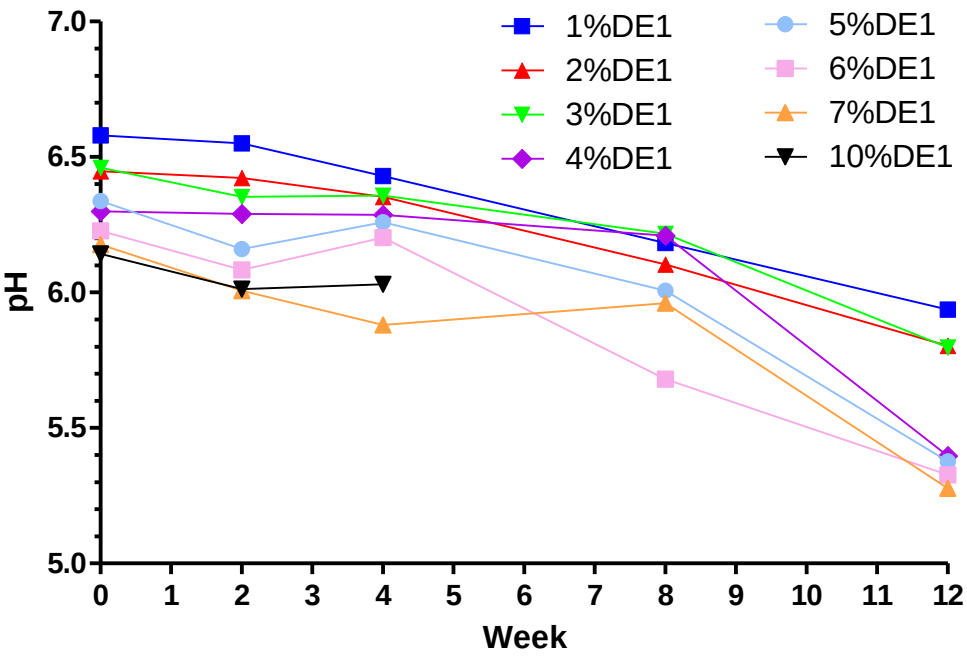


(A)

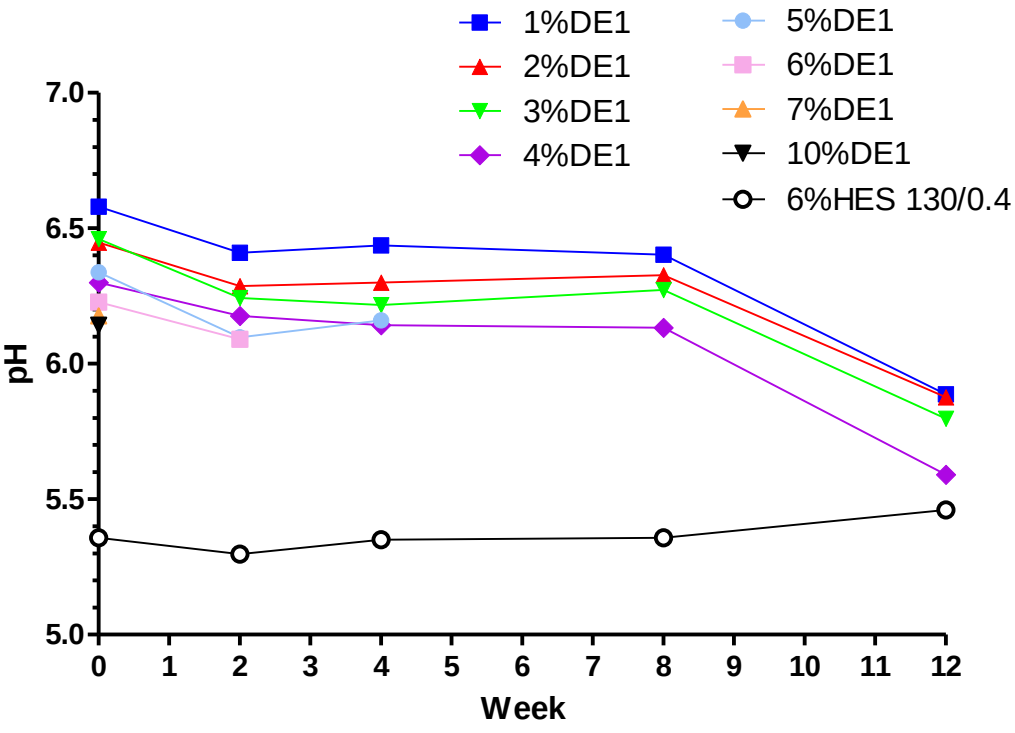


(B)

Figure 19 The colloid osmotic pressure of tapioca maltodextrin with DE6 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26^{o}C) and (B) refrigerated temperature (4^{o}C).

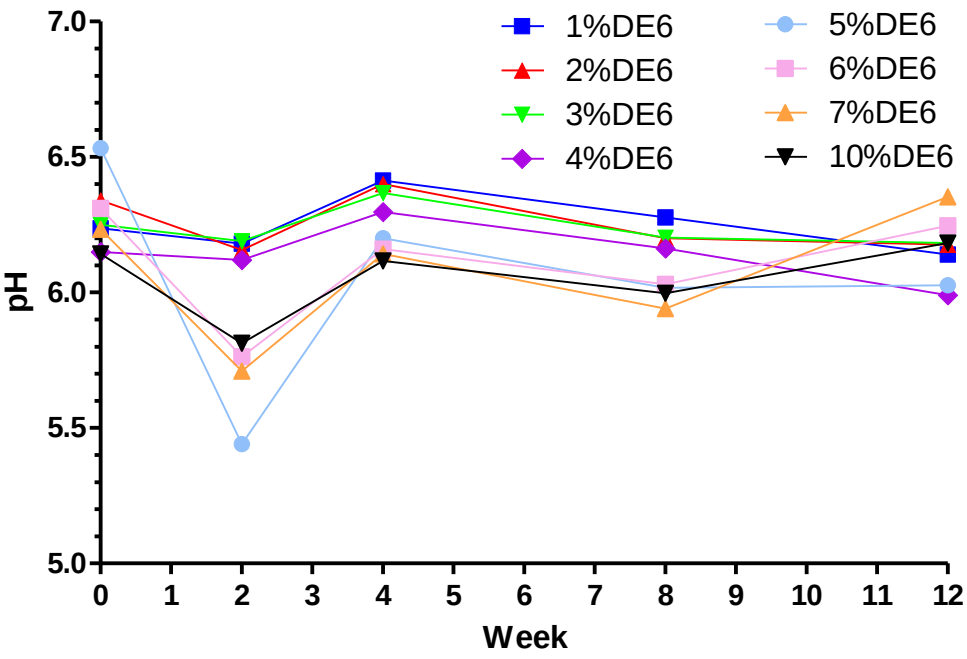


(A)

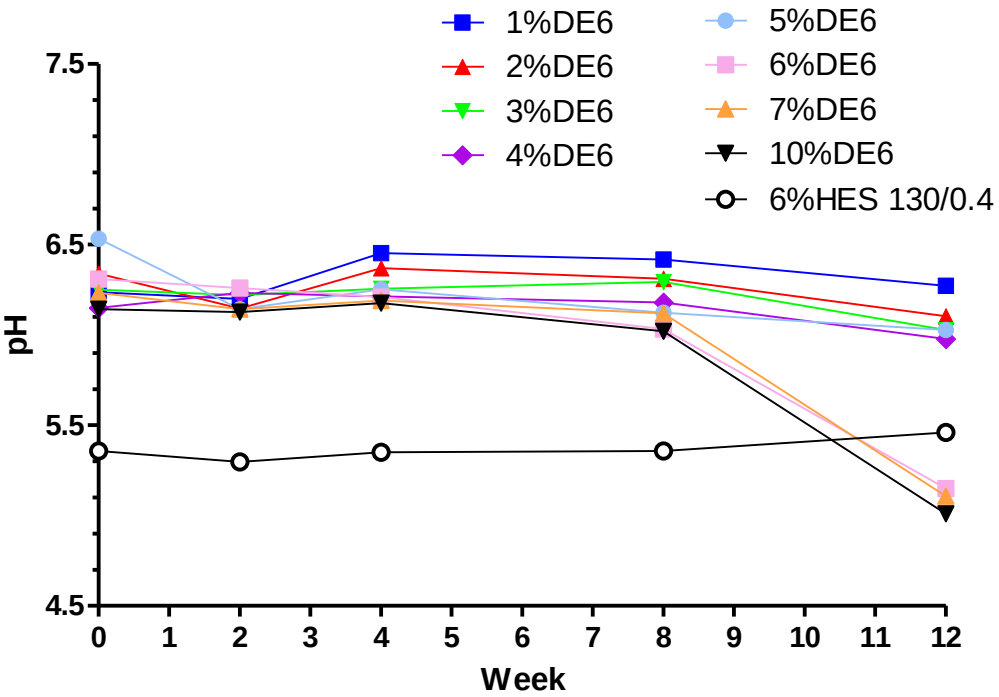


(B)

Figure 20 The pH of tapioca maltodextrin with DE1 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26^{o}C) and (B) refrigerated temperature (4^{o}C).



(A)



(B)

Figure 21 The pH of tapioca maltodextrin with DE6 solutions and 6% HES 130/0.4 for storage duration 12 weeks at (A) room temperature (26°C) and (B) refrigerated temperature (4°C).

Table 3. Turbidity of 6% HES 130/0.4 and tapioca maltodextrin solutions with DE1 and DE6 at different concentrations for day 1 and 12th week storage duration at room temperature and 4^{o}C

Solution	Turbidity (NTU)			
	26°C (room temperature)		4°C	
	day1	W12	day1	W12
6%HES 130/0.4	9	-	9	<1
1%DE1	>100	71	>100	>100
2%DE1	>100	>100	>100	>100
3%DE1	>100	>100	>100	>100
4%DE1	>100	>100	>100	>100
5%DE1	>100	>100	>100	Gel
6%DE1	>100	>100	>100	Gel
7%DE1	>100	>100	>100	Gel
10%DE1	>100	>100	>100	Gel

Solution	Turbidity (NTU)			
	26°C (room temperature)		4°C	
	day1	W12	day1	W12
6%HES 130/0.4	9	-	9	<1
1%DE6	41	24	41	47
2%DE6	80	53	80	79
3%DE6	95	98	95	>100
4%DE6	>100	>100	>100	>100
5%DE6	>100	>100	>100	>100
6%DE6	>100	>100	>100	>100
7%DE6	>100	>100	>100	>100
10%DE6	>100	>100	>100	>100

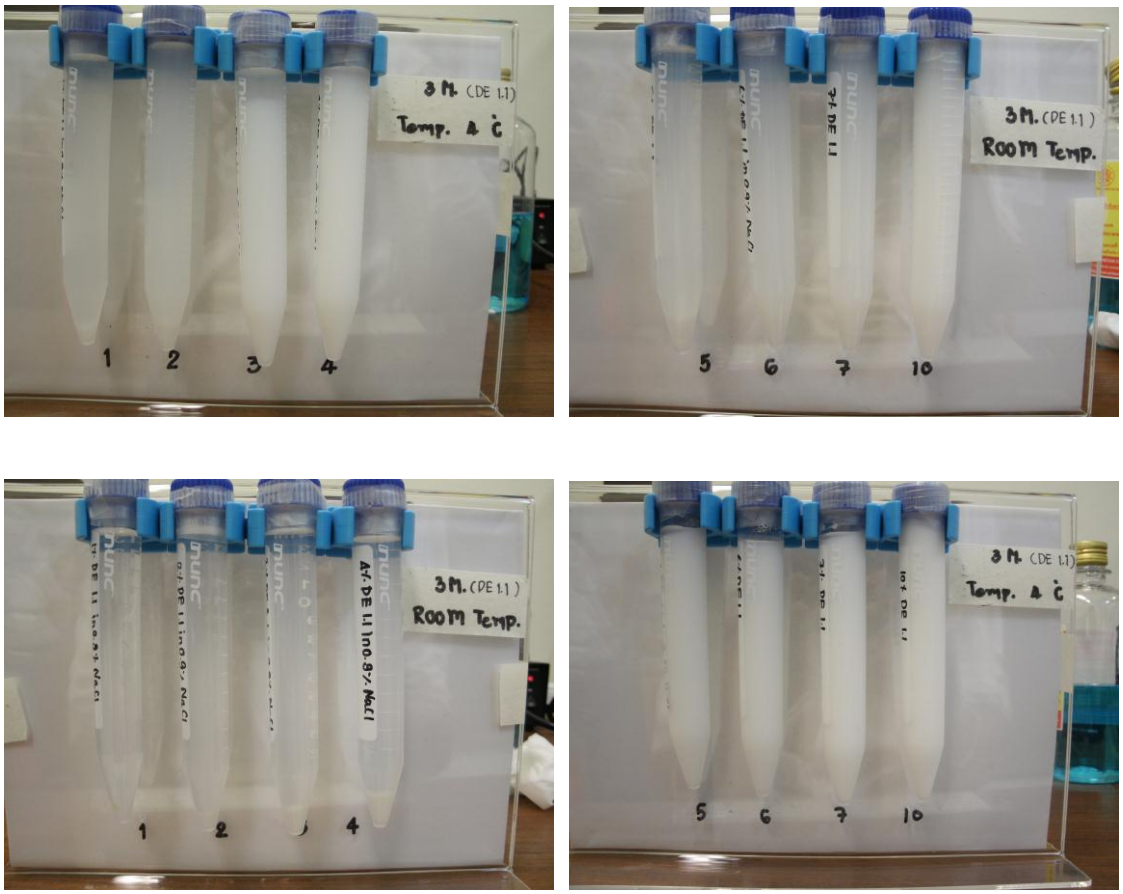


Figure 22 The appearance of tapioca maltodextrin with DE1 solutions on day 1 and 12th week of storage duration at room temperature and 4^{o}C.

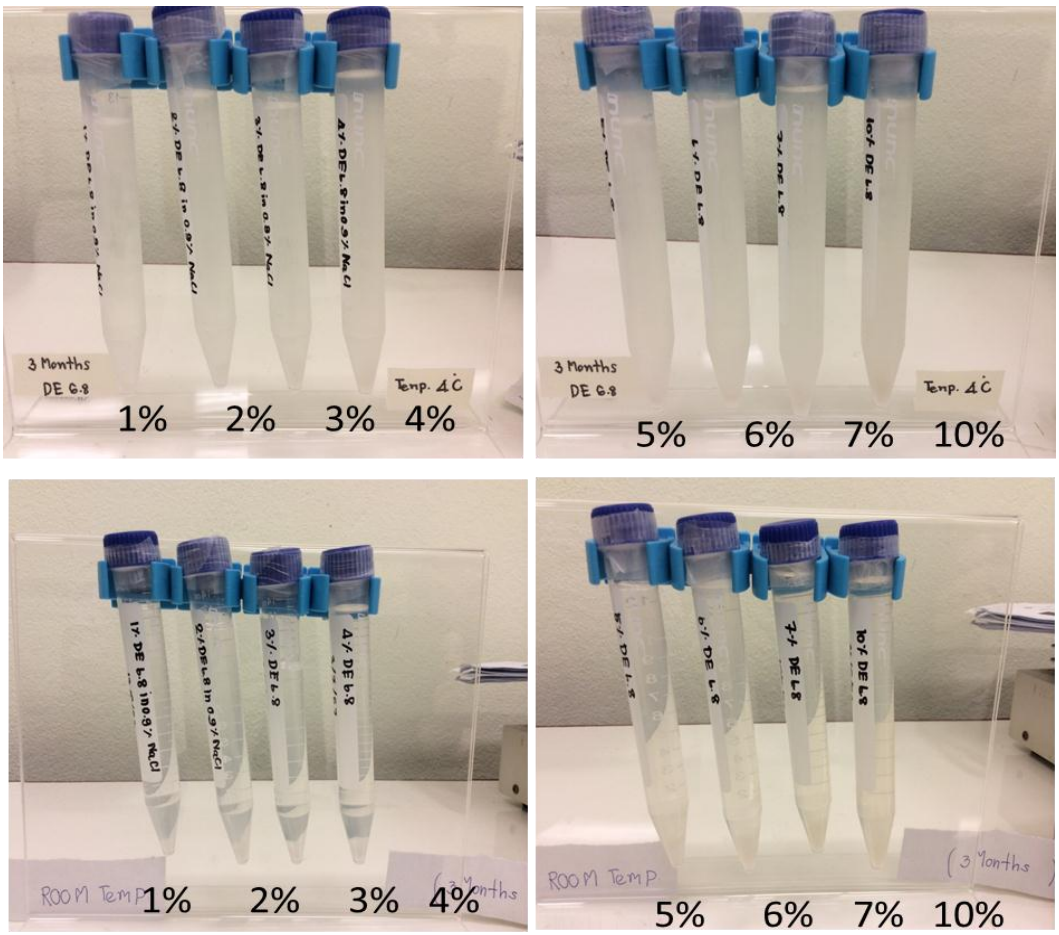


Figure 23 The appearance of tapioca maltodextrin with DE6 solutions on day 1 and 12th week of storage duration at room temperature and 4^{o}C.

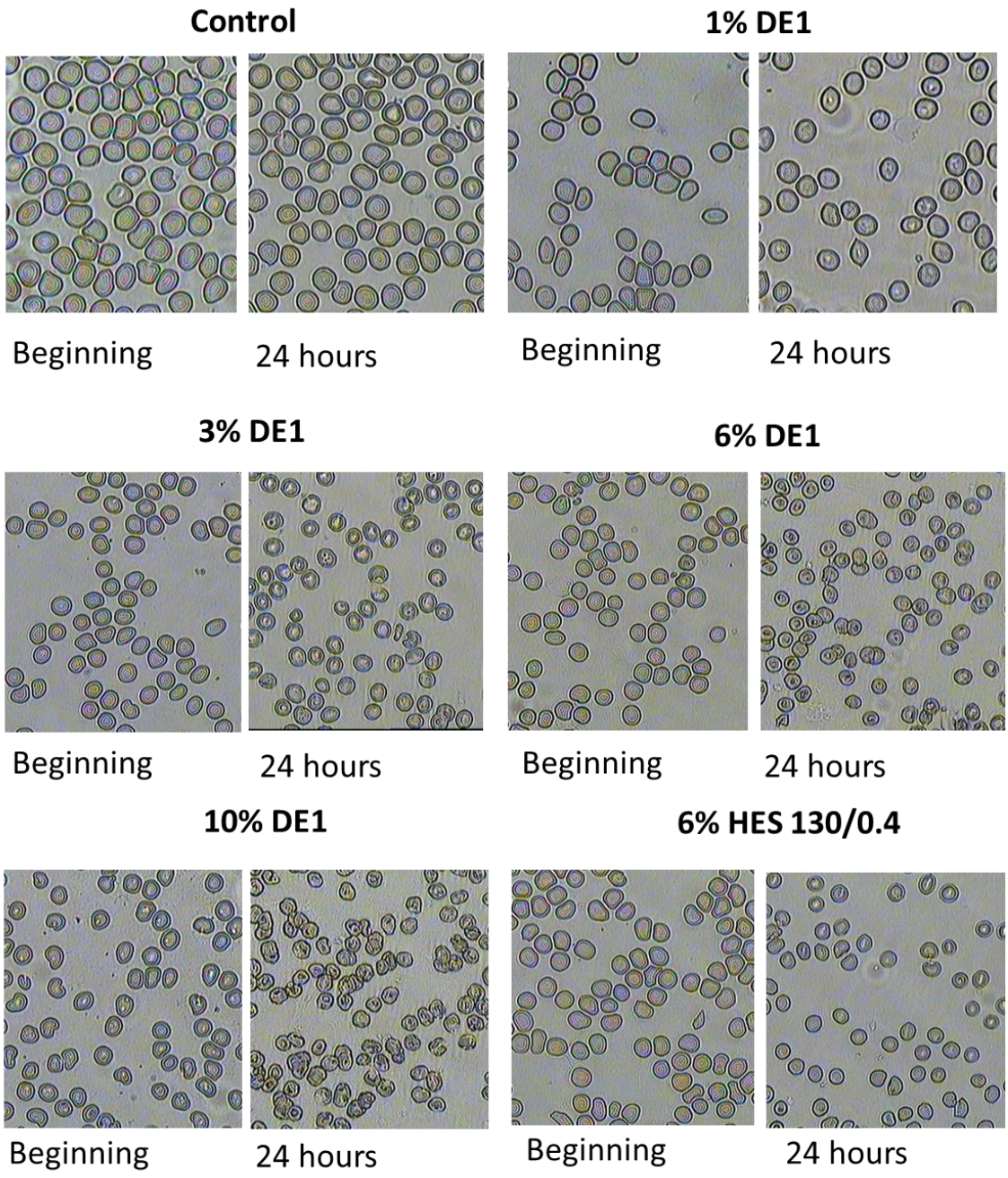


Figure 24 The morphology of red blood cells under intravital microscopy when mixing blood without and with tapioca maltodextrin with DE1 solutions or 6% HES 130/0.4 in 1:1 ratio.

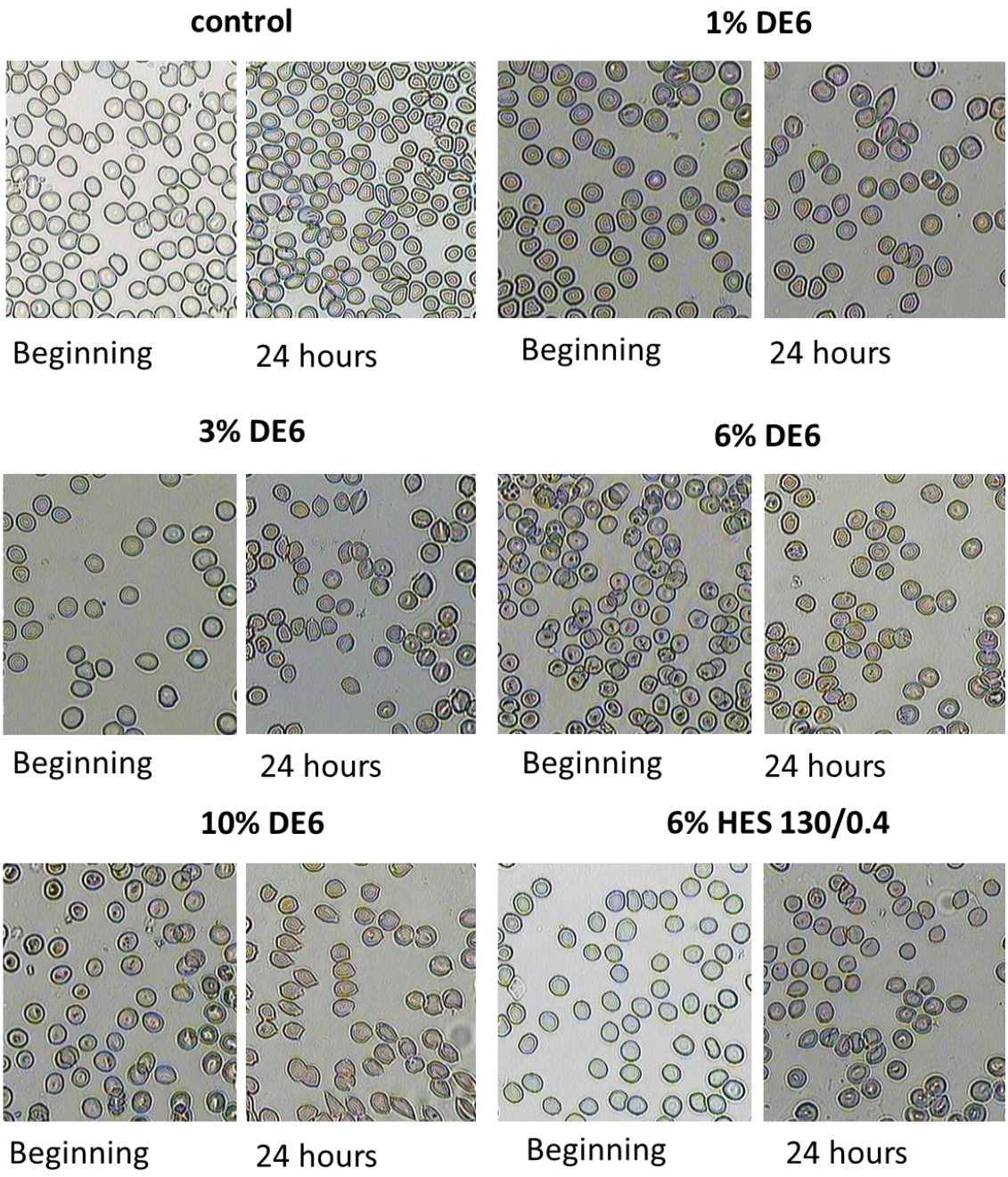


Figure 25 The morphology of red blood cells under intravital microscopy when mixing blood without and with tapioca maltodextrin with DE6 solutions or 6% HES 130/0.4 in 1:1 ratio.

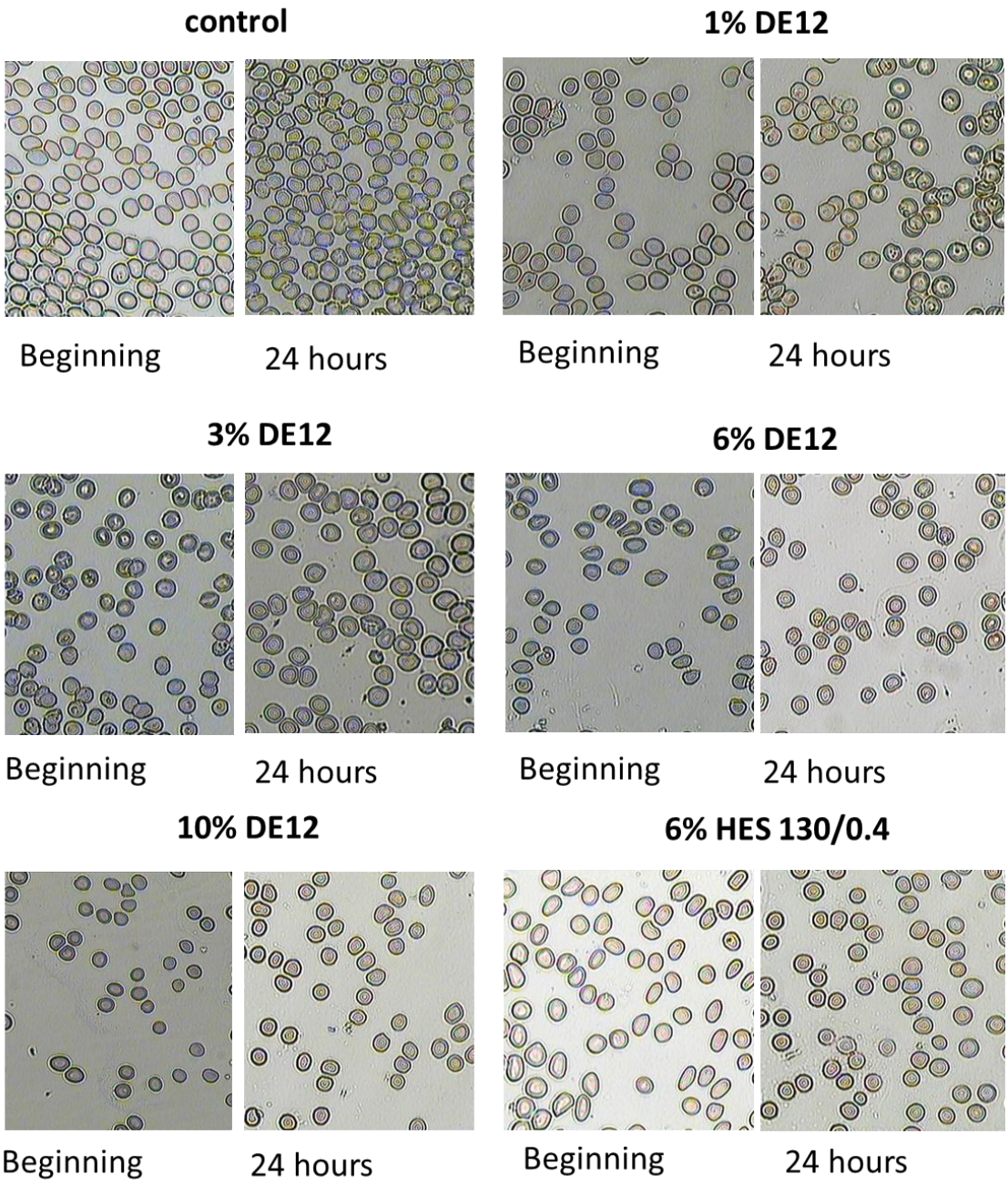
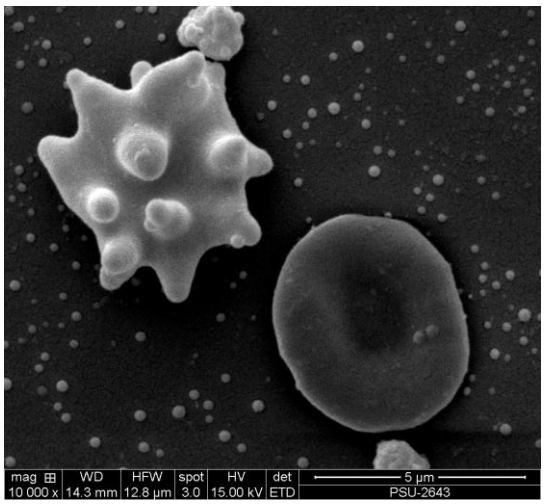
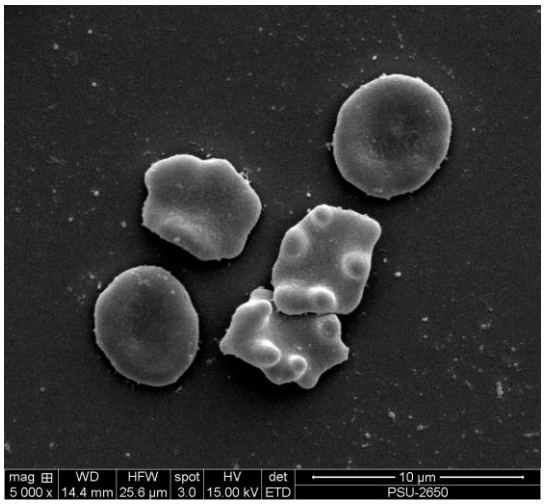


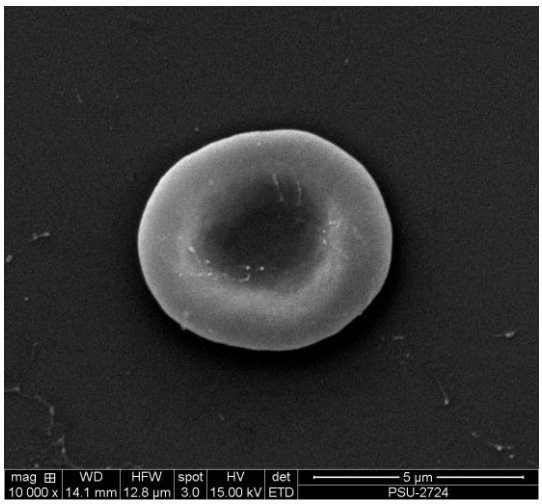
Figure 26 The morphology of red blood cells under intravital microscopy when mixing blood without and with tapioca maltodextrin with DE12 solutions or 6% HES 130/0.4 in 1:1 ratio.



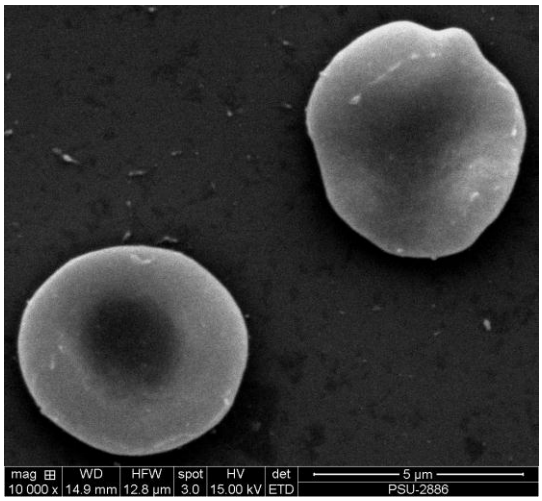
Control



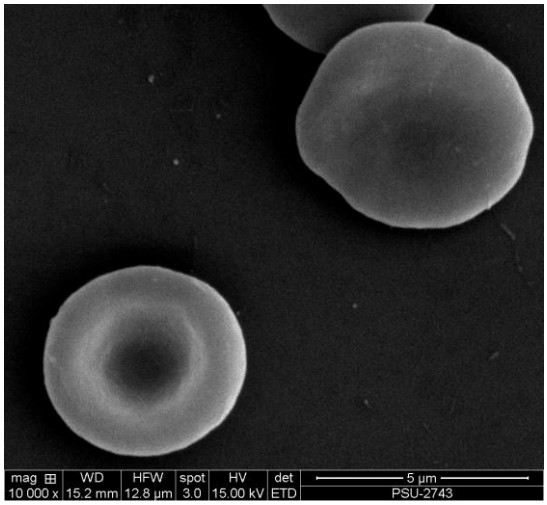
6% HES 130/0.4



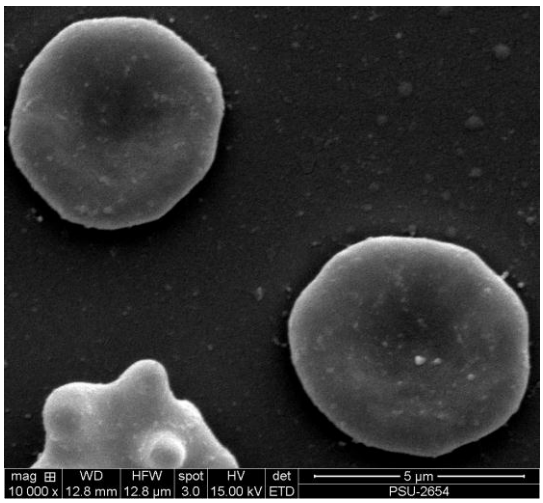
1% DE6



3% DE6

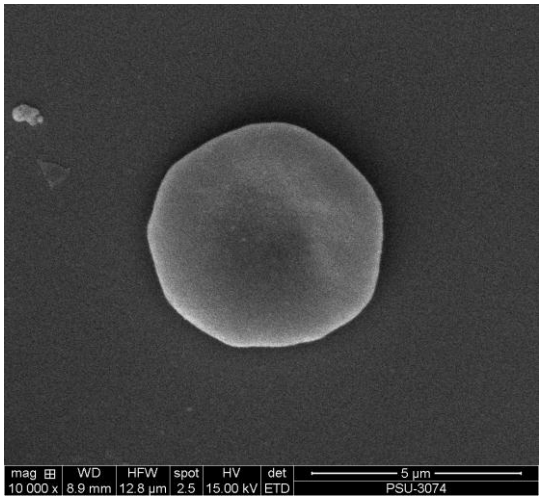


6% DE6

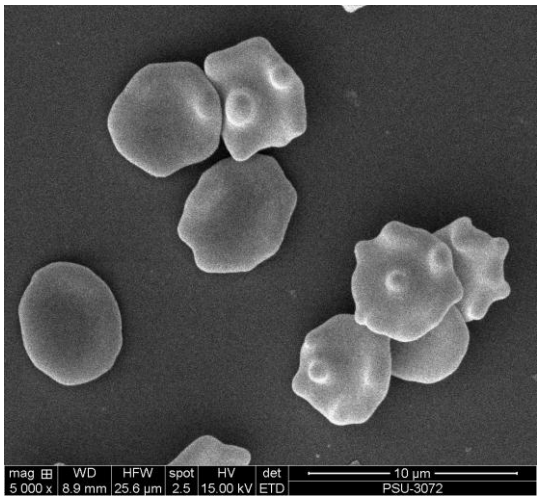


10% DE6

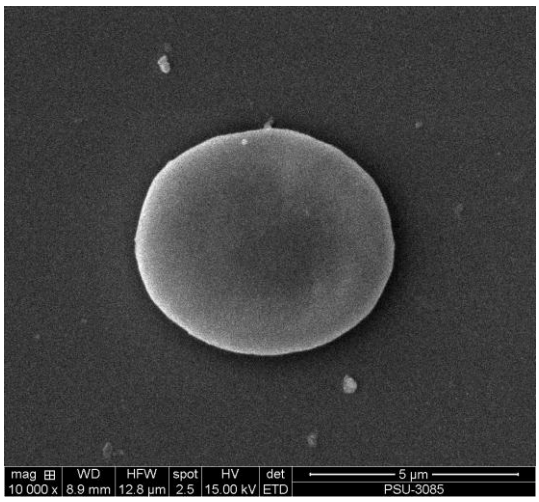
Figure 27 The morphology of red blood cells under scanning electron microscopy when mixing blood without and with tapioca maltodextrin with DE6 solutions or 6% HES 130/0.4 in 1:1 ratio after 24 hours.



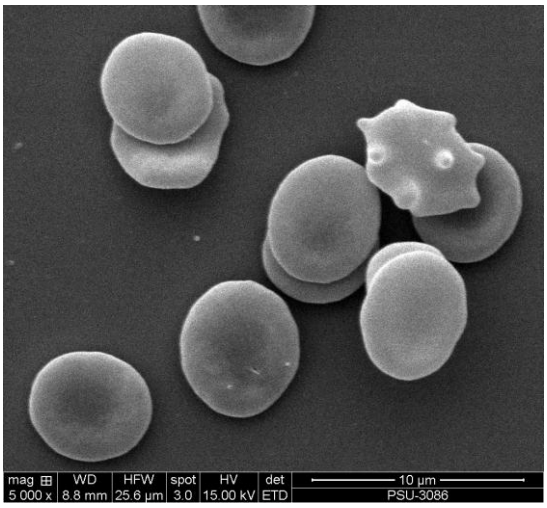
1% DE1



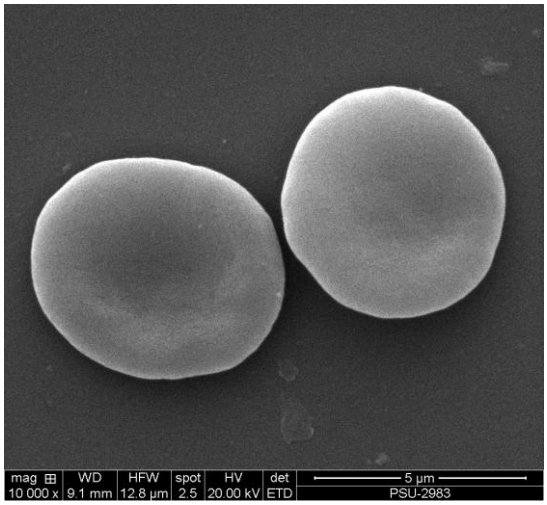
1% DE1



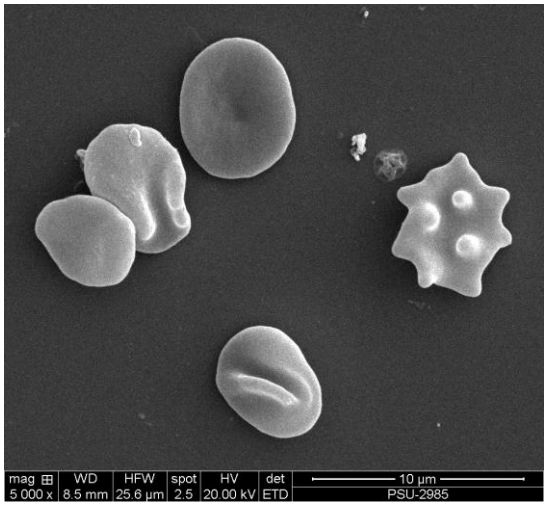
6% DE1



6% DE1

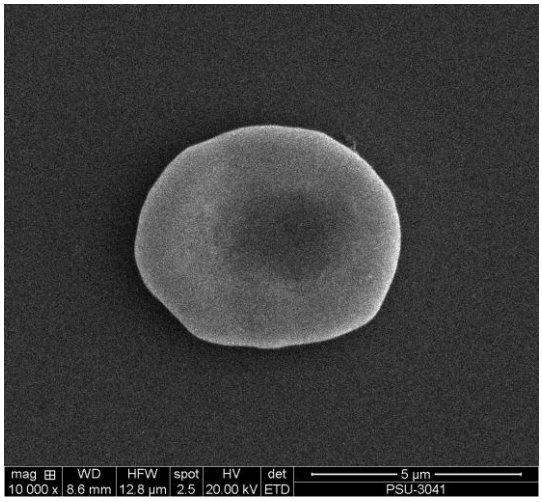


10% DE1

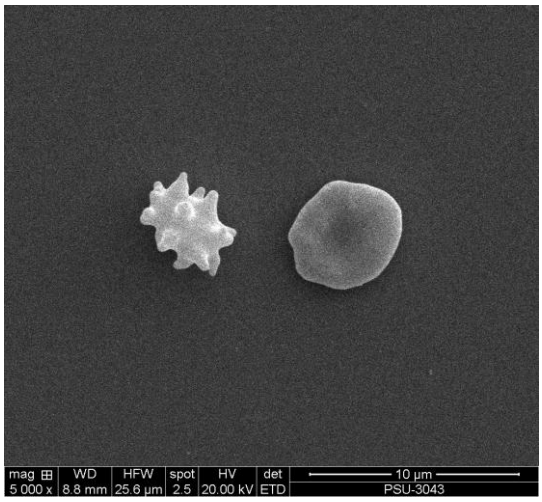


10% DE1

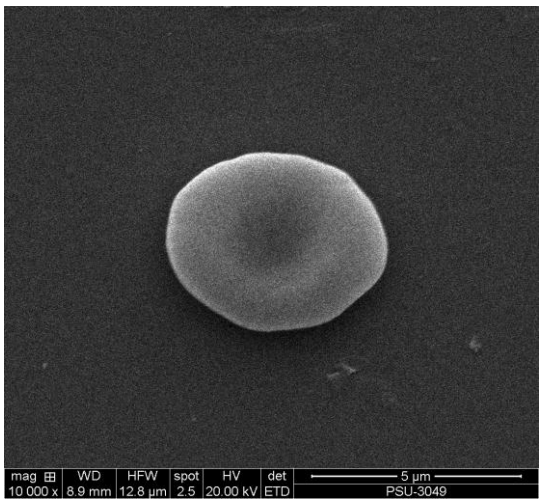
Figure 28 The morphology of red blood cells under scanning electron microscopy when mixing blood without and with tapioca maltodextrin with DE1 solutions in 1:1 ratio after 24 hours.



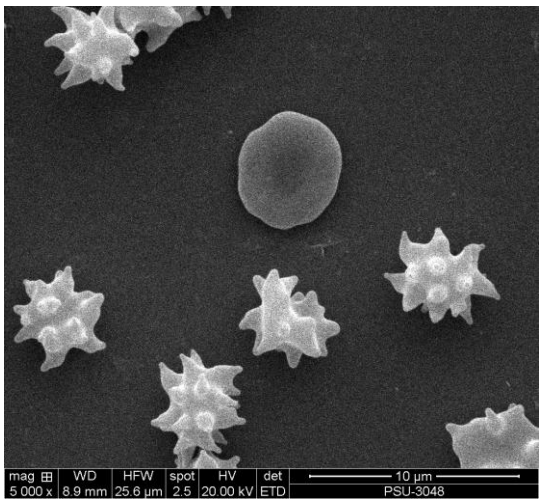
1% DE12



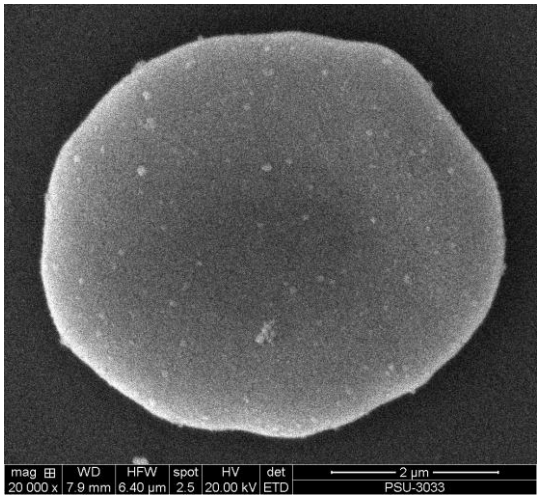
1% DE12



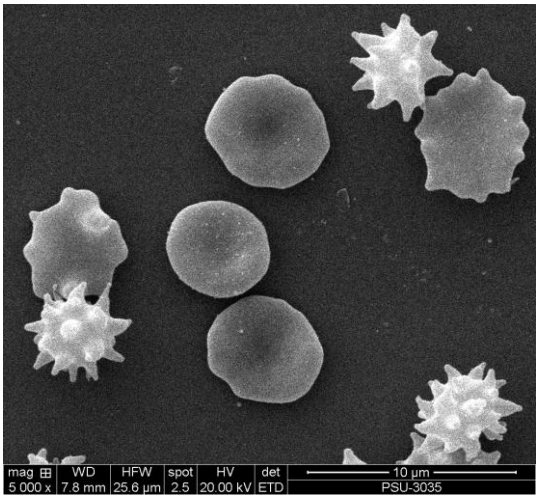
6% DE12



6% DE12



10% DE12



10% DE12

Figure 29 The morphology of red blood cells under scanning electron microscopy when mixing blood without and with tapioca maltodextrin with DE12 solutions in 1:1 ratio after 24 hours.

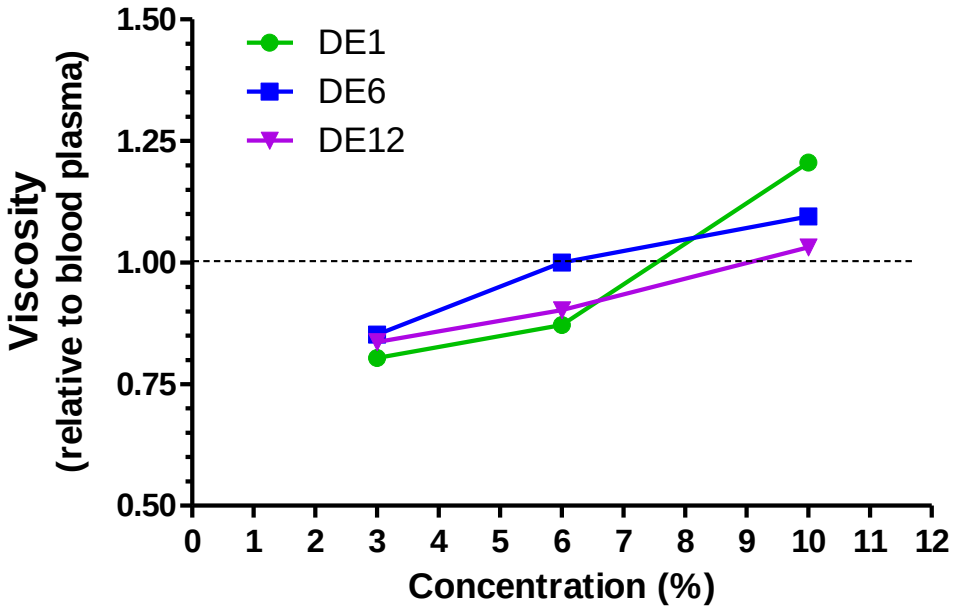


Figure 30 The plasma viscosity after mixing blood and tapioca maltodextrin solutions with 1:1 ratio

Plasma viscosities after mixing between blood and tapioca maltodextrin solutions were determined with two mixing ratios (1:1 and 3:1). The results indicated that the concentration 10% w/v of each DE with the 1:1 mixing ratio could increase plasma viscosity higher than baseline value of plasma viscosity as demonstrated in Figure 30. Otherwise, it was found that tapioca maltodextrin with DE6 provided the highest plasma viscosity compared with other twos when using 3:1 mixing ratio. In addition, at 1:1 mixing ratio, the concentration of tapioca maltodextrin with DE6 higher than 6% w/v tentatively increased higher than blood plasma as shown in Figure 30 whereas the concentration of tapioca maltodextrin with DE1 was higher than 8% w/v.

Discussion

This study found that both dextrose equivalent (DE) and concentration had the effects on the physicochemical and rheological properties of tapioca maltodextrin-based plasma expanders. Furthermore, those properties also varied during long duration storage.

Tapioca maltodextrin is one of modified tapioca starches. Due to the preparation technique of tapioca maltodextrin, the value of DE depends on the amount of enzyme,

temperature and digesting time. Therefore, low DE tapioca maltodextrin has long chains and high molecular weight whereas high DE tapioca maltodextrin has short chains and low molecular weight. Basically, enzyme such as α-amylase is used to hydrolyze α,1-4 glycosidic bond which presents in the inner part of the amylose or amylopectin chains.[44] As the result of enzymatic hydrolysis of starch, glucose or dextrose is an end product. When starch is hydrolyzed, it decreases the degree of polymerization (DP) and reduces molecular weight of starch. Furthermore, DP is inversely proportional to DE.[11]

It is well understand that native starch cannot dissolve at the room temperature. The native starch granules suspend in the solution. Generally, the solubility of starch in the solution increases as the solution's temperature increases.[32] To enhance the solubility, therefore, modified starches are developed. Tapioca maltodextrin is a modified tapioca starch based on enzymatic hydrolysis as aforementioned. As our results, we observed that low DE value (DE1) was difficult to dissolve at room temperature but higher DE values (DE6 and DE12) dissolved easier. However, our experiment increases the temperature of solutions in order to completely insure about the dissolution.

Our study demonstrated that, at the same concentration, lower DE values led to higher turbidity. The principle of nephelometer used in turbidity measurement is the scattering of light due to the amount of particles. Therefore, this indicates low DE solutions contain small solid particles greater than high DE solutions. Moreover, an increase of concentration exactly increases the solution turbidity due to an increased amount of particles. This finding is in agreement with the results reported by Klaochanpong and colleagues.[16] As our results, it can also see that solutions of tapioca maltodextrin with DE1 had higher precipitation of starch particles than other DE solutions.

Furthermore, our study presented that storage duration affected the turbidity of the solutions. These results is similar to the finding in the different corn varieties.[38] Room temperature tended to decrease the turbidity whereas temperature at 4^{o}C tended to increase the turbidity. It was noticed that keeping the solutions at the low temperature such in the refrigerator critically affected the fluidity of the solutions, especially tapioca maltodextrin with DE1. The solutions of tapioca maltodextrin with DE1 at the concentration higher than 4% w/v became a gel but this phenomenon was not observed with the solutions of tapioca maltodextrin with DE6. That means low DE value has a potential to turn solution into gel at the high concentration at refrigerated temperature. Thus, this observation indicated that tapioca maltodextrin with low DE retrograded easier than that with high DE. The retrogradation depends on origin of starch which results in the

difference of amylose and amylopectin contents.[13, 39] The commercial plasma expander, 6% HES 130/0.4, has slightly acidic property (pH 5) and its pH is quite unchanged during storage duration. In our study, we found that both DE value and concentration could influence the pH of solution. In addition, the storage duration and storage temperature had the effect on the pH value of the solutions. Even the pH of tapioca maltodextrin solutions was not lower than the pH of 6% HES 130/0.4, longer storage duration tended to cause the solution to be more acidic.

Colloid osmotic pressure (COP) is important for fluid retention in vascular compartment.[9, 18] Human blood plasma normally has COP about 25 mmHg which is a result from albumin contribution.[28] Therefore, most of plasma expanders have to concern about COP. The COP of 6% HES 130/0.4 is about 37 mmHg which is higher than albumin contained in vasculature. Our study demonstrated that tapioca maltoextrin with low DE had lower COP compared to tapioca maltodextrin with high DE. This might relate to the molecular size of tapioca maltodextrin. Because of the enzymatic digestion in the tapioca maltodextrin preparation, low DE had higher molecular weight. In addition, high concentration of tapioca maltodextrin led to an increase of COP in a linear fashion. It was noticed that storage duration had not much effect on COP change for the low concentration of tapioca maltodextrin solutions. Unlike high concentration of tapioca maltodextrin solutions, the COP tended to decrease as the storage duration decreased.

Our study showed that storage temperature also affected on the viscosity of tapioca maltodextrin solutions, especially low DE. According to retrogradability of starch, starch with high content of amylose, a linear long chain polymer, is easily to be gel when it is cooled.[13] Higher DE value potentially had a stability of viscosity due to short chain polymers. Therefore, tapioca maltoextrin solutions with DE1 and high concentration became gels and their viscosities increased. On the other hand, tapioca maltoextrin with DE6 and DE12 slightly changed in viscosity during the period of storage. Dokic and colleagues reported that intrinsic viscosity of diluted solution of maltodextrin decreased with increasing DE values.[11] Their findings are in agreement with our results. Furthermore, they stated that the intrinsic viscosity of maltodextrin did not depend on the botanical source but it is dependent on molecular properties of samples.

Physiologically, Blood behaves as non-Newtonian fluid while blood plasma and most of plasma expanders behave as Newtonian fluid.[6, 46] Our study observed that tapioca maltodextrin solutions behaved as shear thining at low shear rate (less than 150 s^{-1}) but exhibited as Newtonian fluid when shear rate was greater than 150 s-1. In addition, tapioca maltodextrin with DE12 solutions were most likely Newtonian fluid compared to

other solutions. In general, the viscosity of solution depends on many parameters such as concentration, molecular weight of solute and temperature. Higher concentration of solution is, more viscous solution becomes. Our study presented that viscosity of tapioca maltodextrin solution had an exponential relationship with the concentration for all DE values with a high correlation coefficient (r^2>0.9). Therefore, using tapioca maltodextrin with low DE to prepare for plasma expander should be warned regarding to exponential change of viscosity. The effect of concentration of starch on solution's viscosity was also observed in the study by Bhattacharya et al which showed that the viscosity of 6% w/v starch was higher than 4% w/v starch at different rotational speed for both treated and untreated starch.[3]

As amylose and amylopectin are the major components in most starches, their structure and amount of content have the effect on viscosity. Amylose is a linear long chain polymer of D-glucose units whereas amylopectin is a branched short chain polymer of D-glucose units. Amylose has less content in starch compared with amylopectin by mean of 2-4 folds. For native tapioca starch, amylose has about 16-22% of the content.[10] A waxy corn starch which has 100% amylopectin in the content had lower viscosity compared with Gelose80 which is corn starch that has 20% amylopectin.[48] This indicated that amylose plays a major role on solution viscosity much greater than amylopectin. However, high amylose content can cause retrogradation much easier.

Thus, if we consider the rheological property such viscosity as a major concern for an alternative plasma expander which can be equivalent to 6% HES 130/0.4, the solution of tapioca maltodextrin with DE6 at the concentration of 10% w/v is a good candidate.

The important function of colloid plasma expander is to prolong itself in the circulation for the effect of volume expansion.[12, 18] As starch can be digested with amylase by breakdown the α-1,4 bonds into the small glucose subunits. Therefore, the small molecules can be easily to excrete from the circulatory system by renal function.[25] Our study points that high DE value was easily to be digested which implies the short circulating time if it is administered into circulatory system.

There are two factors which influence the whole blood viscosity; the amount of RBCs and the plasma viscosity. Plasma expander have the direct effect on the plasma viscosity, leading to an increase of blood viscosity.[27] Moreover, plasma expander may have an effect on RBCs by causing an aggregation of RBCs and then blood viscosity is increased. As expected, our study revealed that increased concentration of tapioca maltodextrin elevated plasma viscosity. However, the mixing ratio between blood and plasma expander is an important factor that affects the plasma viscosity. In this study, at mixing ratio 1:1,

the critical concentration that can elevate plasma viscosity higher than normal value was 8% w/v for tapioca maltodextrin with DE1 and DE6 while it was 10% w/v for tapioca maltodextrin with DE12.

As plasma expander is infused into circulatory system, it contacts with circulating cells and molecules such as RBCs, white blood cells, platelets and plasma proteins. Therefore, the effect of plasma expander on blood components is important to investigate. Our study observed the morphology change of RBCs and found that the high concentration of tapioca maltodextrin with DE6 and DE1 altered the shape of RBCs compared with low concentration. This observation was revealed by both staining and SEM methods. Even there were echinocytes (Burr cells) in the observation but they were found in every sample. However, the study by Berezina and colleagues who investigated the influence of storage an RBCs rheological properties showed that there was a transformation of RBCs shape during blood storage.[2]

In summary, our investigation provided the information about the formulations and characteristics of an alternative plasma expander based on tapioca maltodextrin. This study showed the effects of dextrose equivalent (DE) on the physicochemical properties of tapioca maltodextrin solutions. Low DE value led to high retrogradation, turbidity and viscosity but low COP and poor solubility. In contrast, high DE value caused the opposite results relative to low DE values. However, to use tapioca maltodextrin as a raw material to be prepared for plasma expander, some properties have to be considered and optimized efficiently. Further studies on other modified tapioca starches are interesting in order to improve the improper properties of tapioca maltodextrin for plasma expander.

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Appendix

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Rheological Property of Tapioca Maltodextrin Plasma Expander and Its Effect on Morphology of Red Blood Cells

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Introduction: Plasma expanders (PEs) are infused fluids given to increase blood volume in case of blood loss. In clinic, hydroxyethyl starch produced from waxy corn starch is an available plasma expander. Alternatively, other sources of starch might be a potential source for plasma expanders such as tapioca starch and potato starch.

Objective: This work aimed to study rheological property of plasma expander prepared from tapioca maltodextrin, a modified tapioca starch, and to investigate its effects on morphology of red blood cells.

Materials and Methods: Two kinds of tapioca maltodextrin were studied, they were tapioca maltodextrin with dextrose equivalent (DE) 1 and 6. Rheological and physicochemical properties were investigated including viscosity, pH, colloid osmotic pressure and turbidity. Mixture of blood and tapioca maltodextrin plasma expander was sampled and morphology of red blood cells was observed with microscopy technique.

Results: The results showed that the degree of DE and concentration of tapioca maltodextrins affected the rheological and physicochemical properties of plasma expanders. PE prepared from 10% w/v tapioca maltodextrin with DE6 (10% DE6) had nearly similar rheological property to 6% hydroxyethyl starch 130/0.4. It was also found that high concentration of tapioca maltodextrin altered a shape of red blood cells, especially 10% DE6. Stability of viscosity for 10%DE6 plasma expander was in an acceptable range.

Conclusion: Tapioca maltodextrin with DE6 can be a candidate for a source of PEs. However, it is still necessary to continually research and investigate the effects of this novel PE in animal models.

Keywords: Tapioca maltodextrin; Plasma expander; Rheological property; Dextrose equivalent