Preparation and in vitro Evaluation of Acyclovir Suspension

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ABSTRACT:

The suspension is one of the successful oral liquid dosage forms. It has many advantages such as increase the bioavailability of poorly water soluble drugs. Acyclovir is poorly water soluble drug. It is antiviral agent, which is commonly indicated for treatment of wide variety of viral infections; e.g., herpes simplex, varicella zoster in children and adults.

The aim of this study, was preparation of acyclovir suspension dosage form using single ionic type surfactant; sodium stearate was used as suspending and flocculating agent. The flocculating ability of sodium stearate was assessed via measuring the sedimentation volume. Acacia also was added as thickening agent and its ability to impart a more stable successful suspension was evaluated. In this work, the sedimentation volume, content uniformity and viscosity were studied for several formulas containing different ranges of sodium stearate (from 0.5% to 3) in combination with or without acacia at different concentrations (1% to 2%). Sodium stearate was found to be able to give a flocculated system at concentrations higher than 1%. The sedimentation volume (F) was found to be equals to **(F= 1)**, which is an ideal flocculated system; however, the resultant dispersion is thick and very hard to be handled. Also the addition of acacia did not enhance the stability of the dispersed system.

التحضير والتقييم المختبري لمعلق العقار ايسايكلوفير

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الكلمات المفتا□ية: معلق الاسايكلوفير ,ستيارات الصوديوم, الصمغ العربي

الخلاصة:

المعلق هو احد أشكال الدواء الصيدلانية السائلة الناجحة التي يمكن إعطاؤها عن طريق الفم.المعلق يمتلك العديد من المزايا, مثلا زيادة التوافر البيولوجي للأدوية قليلة الذوبان في الماء الأسيكلوفيرهو مادة قليلة الذوبانية في الماء, الأسيكلوفير احد الادوية المضادة للفيروسات الفعالة التي تستخدم في علاج مجموعة واسعة من الالتهابات الفيروسية ؛ على سبيل المثال، الهربس البسيط، والحماق النطاقي. في هذه الدراسة ، تم إعداد الأسيكلوفير على شكل معلق ؛حيت تم استخدام ستيارات الصوديوم كمادة مساعدة. هذه المادة تقوم بحفظ دقائق المادة الفعالة (الاسايكلوفير) بشكل دقائق معلقة بشكل متجانس في الوسط الخارجي, بالإ□افة إلى عملها كمادة مرسبة . وقد تم تقييم قدرتها على تكوين معلق ناجح من خلال قياس ومقارنة حجم الراسب .بعد ذلك تم إ□افة الصمغ العربي (كعامل مثخن) وتم دراسة قدرته على إ□فاء تعليق أكثر استقرارا. في هذا العمل ، تمت دراسة حجم الترسب ، تجانس المحتوى, و اللزوجة لعدة خلطات. هذه الخلطات تحوي تراكيز مختلفة من ستيارات الصوديوم (من 0.5٪ إلى 2 ٪) بوجود او بدون وجود الصمغ العربي (0.1 ٪ إلى 0.2 ٪).لقد وجد في هذا البحث إن ستيارات الصوديوم قادرة على تكوين نظام ترسبي بمقادير أعلى من 1 ٪ . والمثبت من خلال حجم

الراسب (F) ,حيث وجد انه (F = 1)، الذي يدل على انه نظام ترسبي مثالي ؛ إلا إن الخلطة الناتجة من هذه المقادير كانت لزجة جدا, حيث من الصعب التعامل معها أو حتى قياسها. في حين إن الصمغ العربي المضاف بالمقادير المستخدمة في هذا البحث لم تضف أي تغيير في خواص المعلق.

1. INTRODUCTION:

The oral dosage forms represent the most invested area of dosage form manufacturing and design. This area accounts around 75% of all dosage form available in the market. One of the attractive oral dosage forms is oral liquid dosage form. The liquid dosage forms for solid materials are either solution or suspension form. However, not all drugs can be given as solution liquid dosage form because of water insolubility of the drug, instability in water or the bitter test of the drug (1). Generally, Suspension dosage, which is the other form of liquid dosage form, can be defined as finely divided solid particles; i.e., active ingredient(s) which are dispersed in liquid phase which is mostly aqueous or it could be dispersed in a gas; e.g., aerosol. A surfactant might be incorporated to increase the wettability and stability of suspension in most of cases, as well (2).

The suspension system can be categorised depending on the particle size of the dispersed phase, into three main categories: coarse suspensions; colloidal dispersion and nano-dispersion (3). Another classification of suspension is according to the intended route of administration, which is more useful in regarding to the type of excipients, the range of particle size and sterility. In oral suspension, the particles of the drug are mostly dispersed in viscous liquid to which a suitable flavouring agent and sweetener agent are added (3).

Suspension dosage form will deliver the active substance into the GIT as fine particles; therefore it will enhance the bioavailability of hydrophobic drugs, such as Acyclovir. Therefore, it skips the stage of disintegration required in solid dosage form before absorption in the stomach. (1, 3). For these fundamental reasons, the formulation of acyclovir as suspension was chosen.

2. THE GENERAL FEATURES REQUIRED IN SUSPENSION SYSTEM:

Generally the suspension system must have the following features:

- ***Optimum Particle size***

The particle size of the most suspension is ranging from 1-50μm(3). The reduction in particle size enhances both the stability of suspension and the bioavailability of drug. The reduction in particle size reduces sedimentation rate according to Stoke's low($V=gd2\ (\rho_1-\rho_2)/18\eta$ ..... Equation 1):

$V=gd^2\ (\rho_1-\rho_2)/18\eta$ ..... Equation 1

Where V represents the velocity of sedimentation; d= particle diameter;ρ_1 and ρ_2 are the densities of the particles and the dispersion medium, respectively; g= gravity, η= the viscosity.

The settling velocity for the same material in the same medium depends on the particle diameter. Thus, the smaller the diameter is the less the settling velocity and the longer stable suspension. The best suspension is the one which supports the dispersed particles for long enough time until the patient pours the required dose volume after shaking (4). In regarding to the bioavailability, the reduction in particle size increases the effective surface area. Consequently, it increases the surface area in contact with water, and hence enhances the dissolution of insoluble or poor water soluble drug. Therefore, more drug would be available in solution form which is ready for absorption from the GIT, and hence improvement of the bioavailability (1, 3). However, this is not absolutely unlimited because very fine particles tend to form cake layer after settling at the base of the container.

- ***Redispersible Easily and no Cake Formation:***

The suspension should be re-suspended easily by gentle shaking after settling(3). When the particles settle, they might form hard caked layer of the dispersed system. This layer cannot be easily redispersed by gentle shaking and sometime forms very hard cake layer that does not dispersed even with vigorous shaking. That because the particles at the base of the sediment are compressed to each other by the weight of the above particles (3), and the fine particles tend to fill the gabs between each other and arrange in tight fashion. This cake layer is not easily redispersed and renders the suspension as two distinctive separated phases, which causes a stability and a bioavailability issues as the delivered dose would contain little or no actives (4).

- ***Controlled particle size and no crystal growth:***

The particle size distribution must stay constant upon storage. The growth of the crystals or the increase in particle size is the most important physical stability issue for suspension system. Crystal growth occurs upon storage and exacerbated by fluctuation in temperature during storage(3). This also has a consequence on dose to dose accuracy since the particles are not distributed uniformly in the dispersion liquid anymore(4).

- ***Optimum viscosity:***

The ideal suspension should have a high viscosity at no shear (no shaking or at standing position) and flow freely during shaking or pouring. These properties can be obtained from fluids that have pseudoplastic flow and are thixotropic (1).

- ***Palatable, no microbial growth and easily pourable:***

As the targeted population are mostly children, Oral suspension must be palatable. Therefore, flavouring agents, sweeteners, and viscosity enhancers are added.

3. ACYCLOVIR:

It is (2-Amino–1,9–dihydro–9-(2–hydroxyethoxymethyl)-6H-purin–6–one , A white to almost white crystalline powder. It is sparingly to slightly soluble in water; practically insoluble or very slightly soluble in ethanol (5, 6). Its Oral bioavailability is 20% (7).

A liquid suspension formulation of the acyclovir can be developed for children and other Patients who cannot ingest medications in solid form. As excipients, acacia is commonly used as emulsifying agent; stabilizing agent; suspending agent ; and tablet binder(8). Sodium Stearate may find application as a gelling agent for deodorant and fragrance sticks, as a component of vegetable-based bar soaps, as a viscosifier , cleansing agent, emulsifying agent (O/W) (0.5-1.5%), and gelling agent (aqueous) (9).

4. MATERIALS:

Material	Source

Micronized acyclovir	Imported from china
Acacia Gum BP	Chemicals Ltd Poole England
Sodium stearate	Lab Chem Fine Chemicals; Mumbai; India
Hydrochloric Acid (HCl) 35%-38% pure	POCH SA; Poland

Table 1:The materials were used in this work and their sources

5. EQUIPMENT:

Table 2: the equipment was used in this work and their sources

Equipment	**Source**
Power Sonic405	Diahanlabtech,Co. LTD.; Korea
Sensitive electronic balance	Denver instrument
pH meter; pH7110	InoLab, WTW; Germany
Spectrophotometer; Cecil:CE7200	Cecil Instruments Ltd., Milton,Technical Centre, Cambridge CB4 6AZ, England
Brookfield viscometer	Brookfield Engineering Laboratories Inc. Middleboro, MA
electronic Capillary Melting point tester	, Steuart,UK

6. METHODS:

6.1 Identification of Acyclovir:

Determination of Melting point:

The measurement of melting point was performed by capillary melting point method. The procedure was done according USP 2007<741>(10). A sufficient amount of fine acyclovir powder was loaded into a capillary tube, one end of which is sealed, to form a Colum height of 3mm with gentle tapping to pack the powder. The temperature was raised gradually until black ash appeared which considered as the melting point.

6.2 Calibration curve:

6.2.1 Preparation of HCl (0.1N):

10 ml of concentrated HCl (10N) was diluted with water up to 1000ml to produce 0.1N HCl.

6.2.2 Preparation of calibration curve and spectrophotometric scanning:

The calibration curve was prepared by using five standard pointes. A stock solution of 100µg/ml was prepared by weighing 10 mg of acyclovir precisely into a 100-ml volumetric flask and dissolved in freshly prepared HCl (0.1N). Then, further five standard dilutions were made by frequent dilutions of 100µg into (25, 20, 15, 10, 5)µg. A freshly prepared HCl (0.1N) was used for diluting. The concentration was measured at wave length of maximum absorbance.

6.3 Preparation of acyclovir suspension:

For preparation of a 20 ml-suspension, the required amount of sodium stearate was firstly dissolved in 5ml water (part A). Then, 800mg of acyclovir was dispersed in part A using mortar and pestle (the resultant dispersion is part B). For those suspension formulas containing acacia, the required weight of acacia was dissolved in 10 ml water (part C). Then, the resultant solution was mixed with part B. and the volume was completed with water up to 20 ml. The amounts of sodium stearate and acacia in each formula are listed in Table 3.

Table 3: The composition and amounts of each ingredient required to prepare 20 ml of suspension.

Formula Sample	Composition (amount percentage w/v)		
	Acyclovir	**Sodium stearate**	**Acacia**
F0	5%	Nil	Nil
F1	5%	0.5%	Nil
F2	5%	0.75%	Nil
F3	5%	1%	Nil
F4	5%	2%	Nil
F5	5%	3%	Nil
F6*	5%	0.75%	0.5%
F7*	5%	0.75	1%
F8*	5%	0.75	1.5%
F9*	5%	0.75	%2

*** These formulas contain the same amounts of surfactant in Formula (F2); the only modification is the addition of acacia.**

6.4 Tests and Evaluation of suspension:

6.4.1 Measurement of the viscosity:

The rheological studies were done by using Brookfield viscometer at room temperature (30ºC). 20 ml of the freshly prepared formula was placed in a 50 ml plastic container and the viscosity measurement was performed via using the spindle 2 (**Figure**). Different shear rate was selected to assess the viscosity (1.5, 2, 2.5,3 and 4 RPM).

6.4.2 U/v Spectroscopy -Content uniformity:

The content uniformity was performed by using Cecil spectrophotometer CE7200, the solvent media was (0.1N; pH=1) HCl. The content uniformity was assessed according to the USP2007 section <851>(10).Two samples of 5ml of suspension were assayed after gentle shaking for 5 times. Each sample was poured into a 200ml volumetric flask and dissolved in 200ml (0.1N) HCl; the resultant concentration of the solution was supposed to be(1mg=1000□g/ml).Then, 1ml of the resultant dilution was further diluted with (0.1N) HCl up to 100 ml using a 100-ml

volumetric flask to result in a supposed concentration of 10µg/ml. then the resultant solution was filtered to remove any suspended particles of acacia or sodium stearate. Then (3 ml) of the final dilution was measured spectorophotometrically to find out the concentration of acyclovir in the solution.

6.4.3 The sedimentation volume (F):

The sedimentation volume of the freshly prepared formula was performed after vigorous shaking. The suspension was poured into graduated cylinder immediately, and the volume was recorded (V_0) which was 20 ml. The suspension was observed for any change in sediment volume after 24 hrs, and the volume of the sediment was recorded (V_f).

7. RESULTS AND DISCUSSION:

Acyclovir is not a new chemical entity; it has been in medical use for decades. Therefore, no preformulation studies were required to be performed. However, prior formulation information is necessary to help in choosing the assay method, the compatible excipients, the suitable pH, the condition of storage and suitable package.

7.1 Identification of Acyclovir:

7.1.1 Melting point for identification of acyclovir:

The identification of acyclovir as a raw material can be performed by several pharmaceutical analysis techniques, such as IR, DSC study…etc. However, the simplest and straightforward technique for identification is the measuring of the melting point.

For identification of acyclovir, the melting point was measured and compared to the standard values stated in USP2007.Although the value of melting point varies depending on method of measurement, the recorded value in this work was (256-259ºC) which does not differ significantly than the stated values in literatures or pharmacopeia(255ºC) (5).This revealed that the used material was acyclovir and no other impurities were included. The BP 2012 asks for at least two identification tests for example IR and melting point; IR was not accessible. However, the melting point assay can give a clue for purity of acyclovir and its identity.

7.2 Calibration curve:

The calibration curve was made via measuring five points in acidic solvent. Acyclovir is water non soluble; it is an amphiphilic molecule (5); therefore, the acidic media was used to generate ionized species of acyclovir.

The correlation between the absorbance and the concentrations was found to be a leaner relationship (Figure 1). That means any increase in concentration of the solute (acyclovir) increases the absorbance capacity of the solution. This relation between the concentration and the absorbance follows Beer–Lambert law; it was found that the linear equation for absorbance and the concentration is (y=0.0461x) with regression of (0.9985). And the lambda max (λ_{max}) was found to be (257 nm)z by spectrophotometric scanning.

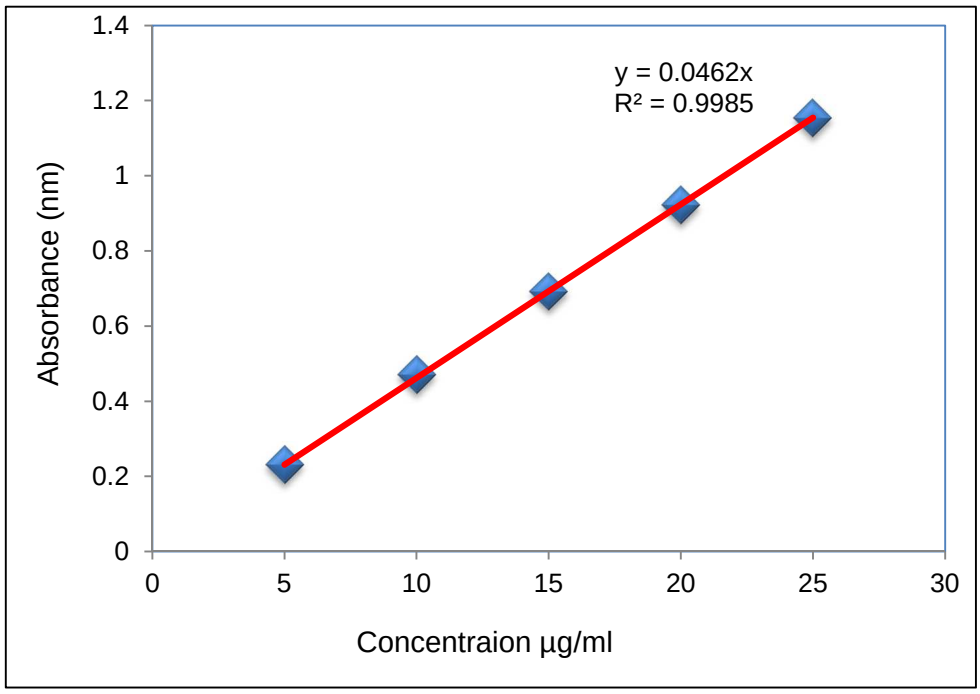

Figure 1: Calibration curve of Acyclovir using spectrophotometer at length of (257nm) in (0.1N)

7.3 Preparation of acyclovir suspension:

The preparation of acyclovir suspension utilized the principal of wetting of acyclovir powder by small amount of water containing the surface active agent, Sodium stearate, and then dispersing the wetted acyclovir in water. The acacia was added at different concentrations as viscosity modifying agent to investigate whether the viscosity might enhance the sedimentation volume or not.

7.4 Tests and Evaluation of suspension:

7.4.1 The content uniformity – spectroscopy Study:

The assay of content uniformity was done to the freshly prepared sample. The recovered concentration was found to be (10.75µg/ml; 10.75*4000(dilution factor)= 43mg/ml OR 215mg/ml). The concentration for each sample was estimated by application of the linear equation (concentration (x) = absorbance(y)/0.0461), which derived from the calibration curve (Figure 1). The USP 2007 sets+/-5% (190-210mg/5ml) as a limit of variation in content uniformity of acyclovir suspension. Thus, the recovered concentration was within the limit stated in the pharmacopeia for the content uniformity. This is the main criterion that USP asks for. The recovered concentration from the commercial suspension (Medvirox®) was (9.95 µg/ml +/- SD(n=2) OR 9.95 µg/ml*2000(dilution factor) =39.8mg/ml OR 199mg/5ml). This experiment on the commercial product was performed for two reasons; to validate the procedure for assessment of the content uniformity test in addition to comparing of the prepared suspension to the commercial one to be as a standard. The recovered concentration from the commercial formula was exactly the same amount which stated on the package.

7.4.2 Dilution factor:

The dilatation factor was calculated as bellow:

Dilution factor (DF) =final volume/ initial »DF1=200/5=40

DF2=100/1=100, thus the final dilution factor is 40*100= 40000

7.4.3 Sedimentation volume (F):

In general, the F value ranges from less than one to greater than one. Less than one when the particles have settled; whereas more than 1 when the particles have swollen(1). In case of F= 1 the flocculation is stable and no clear sediment can be seen. The F value can be used to assess the stability and extent of flocculation; the higher the value of F is the more stable flocculated system and hence is the more stable suspension (3).

Sedimentation volume (F)=V_u/V_0 , where V_u and V_0 represent the volume of the final sediment and the volume of sediment at time zero after vigorous shaking, respectively. The estimated values for F after a 24hrs-trail are listed in Table 4. The sedimentation test (**Error! Reference source not found.**) for freshly prepared

suspension showed a difference in the sedimentation volume (F) among the prepared formulas at different concentration. The sedimentation volume of formula F0 (only acyclovir in water) is (0.2). This value has not been changed by incorporation of the minimum amount of sodium stearate in this work as shown in formula F1 (Table 4). The sedimentation volume was the same and did not change along the range of concentrations of sodium stearate (0.5%, 0.75% and 1%). It was 0.2 for F1, F2 and F3.

F4, F5 had the highest value for the sedimentation volume (F); F=1. Thus the best flocculated system could be achieved via incorporation of sodium stearate at concentrations higher than 2%. However, the resultant dispersion from such high concentration was difficult to deal with. The resultant dispersion was very viscous, sticky and not easily handling. Therefore, in this work, F 2 (sodium stearate=0.75%) was selected to be further modified to get a better flocculated dispersion by introducing another agent, the viscosity modulating agent- acacia (F6, F7, and F8).

The study of sedimentation volume for F6, F7, and F8 revealed a non-significant difference even with the highest concentration of the acacia used in this study. Acacia here was added as a viscosity modifying agent. It might be as a result of the low concentrations of acacia were used in this study, as acacia can bring about suspending activity to the system if the concentration is more than 5%(8).

Table 4: the estimated sedimentation volume after 24hrs for each formula. the composition of each formula is stated in (Table 3)

Formula	Initial volume (ml)	Volume after 24hrs (ml)	Estimated sedimentation volume(F)
F0	20 ml	4 ml	0.2 ml
F1	20 ml	4 ml	0.2
F2	20 ml	4 ml	0.2
F3	20 ml	4	0.2
F4	20 ml	20	1
F5	20 ml	20	1
F6	20 ml	4	0.2
F7	20 ml	4	0.2
F8	20 ml	4	0.2
F9	20 ml	4ml	0.2

7.4.4 Measurement of the Viscosity:

One of the important factors, that should any successful suspension has, is the optimum viscosity, as introduced above. The viscosity was measured at different shear stress; the shear rate was as following:(1.5, 2, 2.5and 3) RPM. The prepared suspension did not show any value for viscosity even for formula F8, which contains the highest concentration of the viscosity modifying agent, acacia. The aim of this experiment was to measure the viscosity and compare the value to viscosity of the commercially available acyclovir suspension (Medvirox®, Medpharma).The rheology study for the prepared formula was inconclusive. In this experiment the recorded values of the viscosity for the Medvirox® suspension are shown in Table 5; whereas, all the prepared formulas at all concentrations revealed no values for viscosity. This might be as a result of the low viscosity of the prepared formula, which might be improved by increasing the concentration of acacia for more than 25% (the minimum concentration of acacia to impart rheological modifications (8)), or by combining more than one viscosity modifying agent, such as tragacanth and / or using other viscosity modifying agents such as carpobol(1, 4).

Table 5: Viscosity study of Medvirox®(the commercial formula)

Shear rate (RPM)	Viscosity(cP)
1.5	299
2	275
2.5	252
3	181

The results form sedimentation volumes of acacia containing formulas go well with the results from the viscosity measurements. The viscosity measurement did not give any value for viscosity of the tested preparations (F1, F2, F3, F6 and F7). Therefore, it could be concluded further increase in concentration of acacia and/ or adding another viscosity enhancing agent might be required to achieve a more stable flocculated system.

Thus, it is not advice able to fabricate a flocculated dispersed system of acyclovir via using sodium stearate alone or in combination with acacia. However, it might be possible to fabricate such system via examine a broader range of concentrations for both acacia and sodium stearate, with further additives, such as rheology modifiers, electrolytes….etc.

8. FURTHER WORK:

The aim of this work is to evaluate the possibility of using sodium stearate as a flocculating agent and a suspending agent to prepare acyclovir suspension in addition to the evaluation of the addition of acacia on the suspension stability; the sedimentation volume was used to assess the flocculating and the suspending ability.

The prepared formula might need further confirmatory tests such as assessment of particle size and crystal growth of particles upon storage, stability test. And accordingly, other additives might be needed to impart more successful formula, such as viscosity modifiers and/ or electrolytes as flocculating agents.

Other tests might be needed, such as dissolution test and microbiological test.

9. CONCLUSION:

In conclusion, Sodium stearate is effective as flocculating and suspending agent as concentrations more than 2%, however, the resultant dispersion is too thick and sticky, which makes the dispersion difficult to handle.

The rheology studies showed no real results; the viscosity study for the prepared formula is inconclusive for all the ranges of concentrations of the suspending agents even when the thickener agent was added along the range of the studied concentrations.

Thus, in light of all the tests that have been performed, it is not advisable to fabricate a flocculated dispersed system of acyclovir via using sodium stearate alone or in combination with acacia at the studies concentrations in this work. However, it might be possible to fabricate such system via examine a broader range of concentrations for both acacia and sodium stearate; with further additives, such as rheology modifiers, electrolytes….etc.

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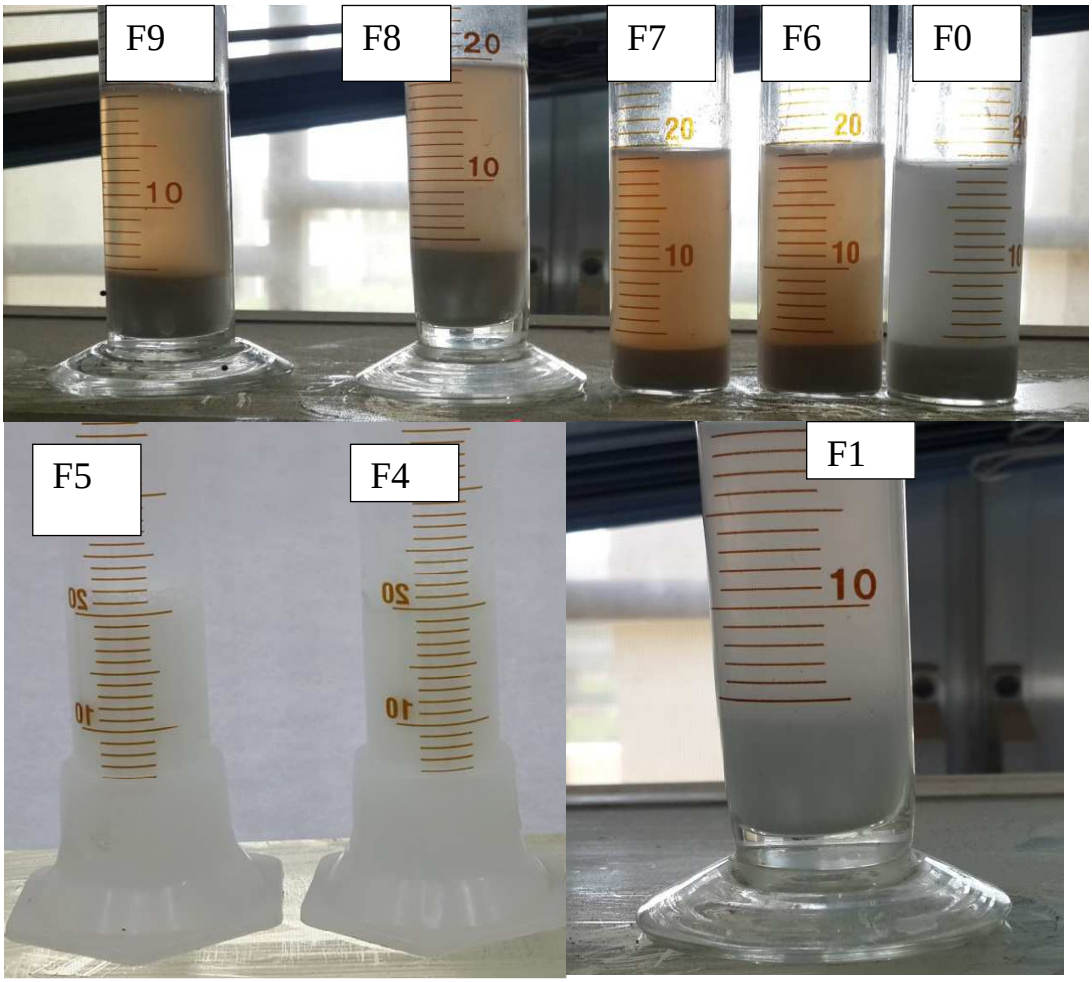

Figure 2: Sedimentation volume study

Figure 3 : spindle 2. the spindle was used to measure the viscosity