Chapter IV

Results and Discussion

Part I: Preparation and Determination of Modified Starches

A. Preparation of Modified Starches

The modified starches in this study were prepared by three different methods of carboxymethylation reaction using various native starches. The reaction is an etherification reaction with monochloroacetic acid in presence of sodium hydroxide. This method was suggested by Filbert (1952). Ornanong (1996) prepared and evaluated sodium carboxymethyl starch as suspending agent in three different degrees of substitution (DS) based on Filbert's method (Filbert, 1952).The suspending property of modified starches was evaluated in term of viscosity, ease of redispersion and sedimentation volume. As a result, modified glutinous rice starch (MGS), modified rice starch (MRS) and modified tapioca starch (MTS) with DS of 0.16, 0.26 and 0.38, respectively were qualified for further study. Thus, the modified starches in this study were prepared from three native starches using three different conditions of carboxymethylation. Modified glutinous rice starch (MGS) produced by Method I as predicted degree of substitution was 0.16, modified rice starch (MRS) produced by Method II as predicted degree of substitution was 0.26 and modified tapioca starch (MTS) produced by Method III as predicted degree of substitution was 0.38.

Since Filbert's methods and conditions of reaction did not give the modified starch with required DS. It was found that slight change in the reaction conditions (e.g. time interval for adjust to pH) or amount of materials used (e.g. sodium hydroxide, monochloroacetic acid) during modification process affected the DS of modified starches. The conditions and amount of materials used in the preparation of modified starches and presented in Table 7, the following modified starches were made to obtain required products. Modified glutinous rice starch and modified rice starch with DS 0.16 and 0.26, respectively, were successfully prepared by using the

Method I and Method II, respectively. Modified tapioca starch with DS 0.38 was synthesized by Method III with the reaction time decreasing from two hours to 90 minutes.

Table 7 Comparison of the conditions and amount of materials used in the preparation of modified starches (different 3 DS)

Parameter	Method I	Method II	Method III
Amount of materials			
Solvent	MeOH 254 ml	EtOH 286 ml	EtOH 286 ml
Monochloroacetic acid	27.6 g	29.2 g	29.2 g
Starch	GS* 109 g	RS* 102 g	TS* 102 g
Sodium hydroxide	50%, 110 g	97%, 38.4 g	97%, 38.4 g
Reaction Conditions			
Temperature (^{0}C)	60	50	50
Time (minutes)	60	20	90

GS* = Glutinous rice starch, RS* = Rice starch, TS* = Tapioca starch

In the presence of strong base, the carboxymethyl substitution reaction mechanism is undoubtedly S_N2 (substitution nucleophilic bimolecular). This means the formulation of an intermediate complex. The starchate nucleophile was followed by the reaction with a nucleophilic agent (Roberts, 1965). The etherification of starch with sodium chloroacetate in aqueous sodium hydroxide might take place according to the reaction as shown in Figure 11.

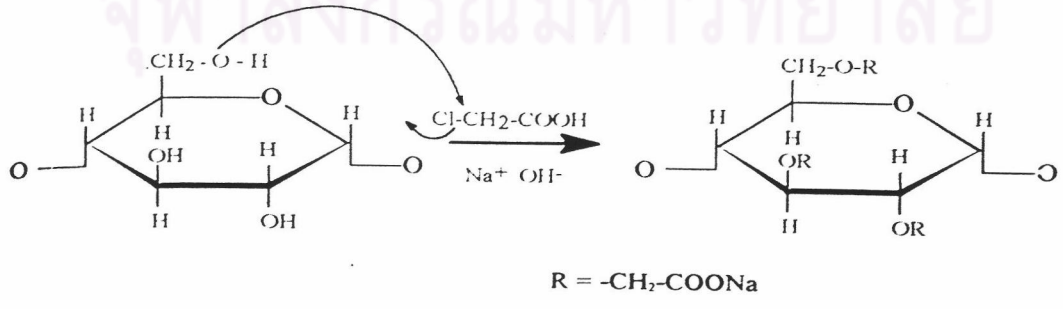


Figure 11 Mechanism of carboxymethyl substitution reaction in the preparation of sodium carboxymethyl starch (Rutenberg, 1980)

Monochloroacetic acid or sodium chloroacetate which was used as etherifying agent was a strong electrophile, since it had chlorine as a good leaving group. There were three reacting positions on one starch unit, i.e. those three –OH group. The number of –OH group substituted with $–OCH_2$-COONa or $–OCH_2COOH$, was determined as the DS of the modified starches. The possible reacting position of each anhydroglucose unit was the primary hydroxyl group on carbon atom 6 and the secondary –OH groups on carbon atom 2 and 3, however, carboxymethylation occurred preferentially at the secondary –OH groups (Hofreiter, 1987; Radley, 1968; Roberts, 1965).

B. Determination of Degree of Substitution (DS)

The degree of substitution of three modified starches and Ultrasperse®2000 (UT) was determined by acid/basic titration and residues on ignition experiment (USP 24). The calculated DS were 0.16, 0.26 and 0.38 for modified glutinous rice starch (MGS), modified rice starch (MRS) and modified tapioca starch (MTS) produced by method I, II and III, respectively, as shown in Table 8. Ultrasperse®2000 was a commercial modified starch that modified from waxy maize, DS determined by acid/basic titration was found to be 0.10. The method of calculation is presented in Appendix A. The DS indicates the average number of hydroxyl groups per anhydroglucose unit which were substituted by carboxymethyl groups. Thus, if all three hydroxyls were substituted, the DS is 3. Most of available modified starches had low DS values, about or more than 0.1, which would represent on average 1 substitute group per every 10 anhydroglucose unit (Wurzberg, 1986)

Summation of degree of acid carboxymethyl substitution (A) and degrees of sodium carboxyl substitution (S) which was calculated from acid/basic titration and residue on ignition experiments (USP 24). Theoretically, the DS value obtained from this calculation represented the carbonyl groups in both acid and salt forms. However, S value contributed to most DS value because the reaction took place in basic condition. One advantage of modified starches prepared by this method was that their salt form improved solubility in water (Mishra, Jain, and Agrawal, 1990).

Table 8 Calculation of degree of substitution of modified starches

Modified starch	DS (Predicted)	M	C	A	S	DS (Calculated)	Average DS from calculated (SD)
MGS1	0.16	0.16641	5.39030	0.02899	0.13226	0.16125	0.16509 (0.00350)
MGS2		0.15021	5.81012	0.02627	0.14315	0.16942	
MGS3		0.11458	6.00517	0.02004	0.14797	0.16802	
MGS4		0.13620	5.71003	0.02377	0.14039	0.16416	
MGS5		0.15060	5.55114	0.02626	0.13634	0.16260	
MRS1	0.26	0.25826	8.19852	0.04685	0.20950	0.25634	0.26124 (0.00428)
MRS2		0.25212	8.26446	0.04575	0.21127	0.25703	
MRS3		0.29477	8.16612	0.05357	0.20908	0.26265	
MRS4		0.30508	8.18137	0.05550	0.20965	0.26515	
MRS5		0.28716	8.30036	0.05226	0.21277	0.26503	
MTS1	0.38	0.37562	11.93529	0.07208	0.32266	0.39474	0.38607 (0.00716)
MTS2		0.27523	12.40227	0.05278	0.33506	0.38784	
MTS3		0.28505	11.98392	0.05440	0.32217	0.37657	
MTS4		0.37807	11.76763	0.07241	0.31747	0.38987	
MTS5		0.30370	12.00627	0.05805	0.32328	0.38133	
UT1	-	0.20597	2.82830	0.03488	0.06747	0.10235	0.10217 (0.00104)
UT2		0.22357	2.72628	0.03786	0.06503	0.10288	
UT3		0.23782	2.64514	0.04027	0.06309	0.10335	
UT4		0.20653	2.79153	0.03496	0.06657	0.10153	
UT5		0.22220	2.65110	0.03759	0.06317	0.10076	

M = Number of milliequivalent of base required to neutralize 1 gram of modified starch

C = Residue on ignition (%)

A = Degree of acid carboxymethyl substitution

S = Degree of sodium carboxymethyl substitution

A+S = Degree of substitution (DS)

MGS = Modified glutinous rice starch

MRS = Modified rice starch

MTS = Modified tapioca starch, UT = Ultrasperse®2000

C. Detection of Carboxymethyl Substitution in Modified Starches

Fourier transform Infrared spectrometer (FTIR) was easily used to detect the carboxymethyl groups in the obtained modified starches (Van, 1976). As illustrated in Figures 12-15, the infrared spectra of native and modified starch were compared. The carboxymethyl substitution reaction was confirmed by a presence of carbonyl

group (C=O) in infrared spectroscopy. The C=O peak appeared as strong broad peak at 1,600 – 1,650 cm^{-1}. The C-O stretching of COH and COC groups was appeared at 1100-1300 cm^{-1} (Van, 1976). For carbonyl group (C=O), the spectra of various native and modified starches were at the same wave number. However, comparing the IR spectrograms between native and modified starches, the carbonyl peak was clearly more intend. Thus, it could be concluded that there was a substitution of carboxymethyl groups in the starch molecules. As illustrated in Figure 15, the same pattern of IR spectrograms of modified starches and Ultrasperse®2000 (UT) was observed. Therefore, this is an indicated that was carboxymethyl starch.

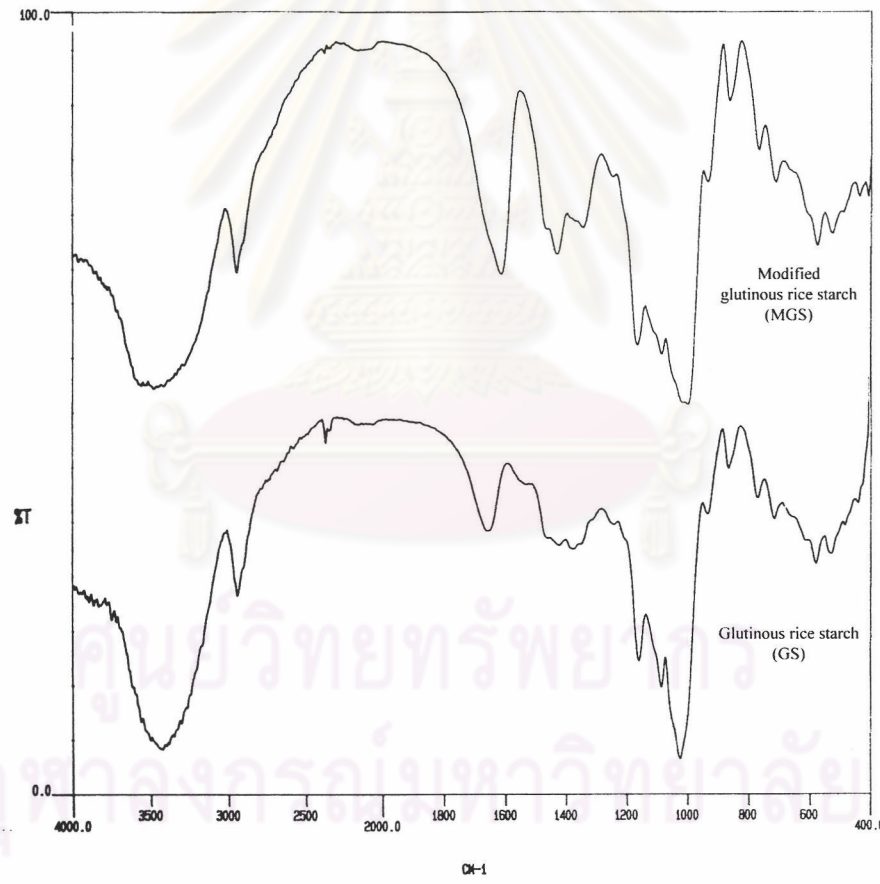


Figure 12 Comparison of the infrared spectra between native glutinous rice starch (GS) and modified glutinous rice starch (MGS)

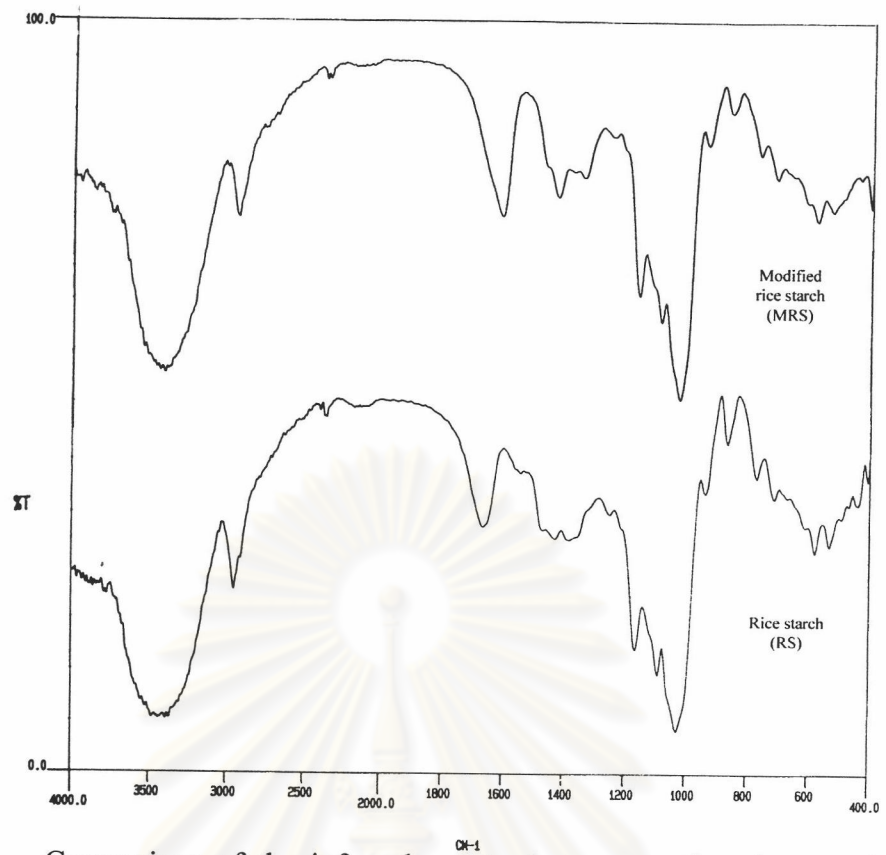


Figure 13 Comparison of the infrared spectra between native rice starch (RS) and modified rice starch (MRS)

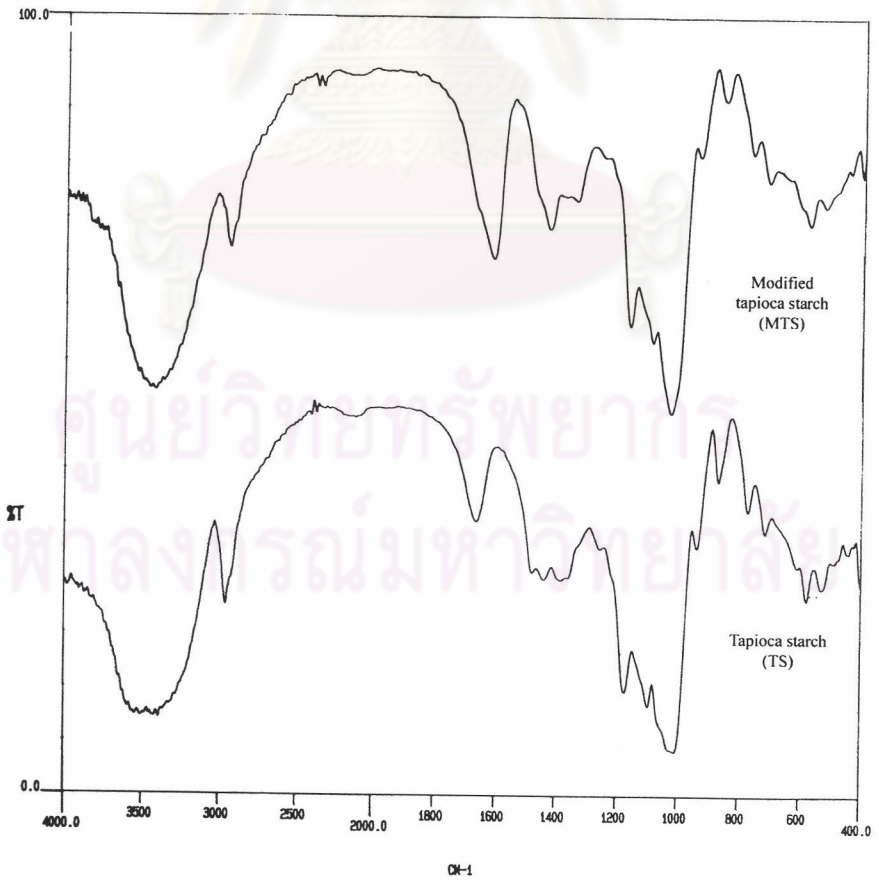


Figure 14 Comparison of the infrared spectra between native tapioca starch (TS) and modified tapioca starch (MTS)

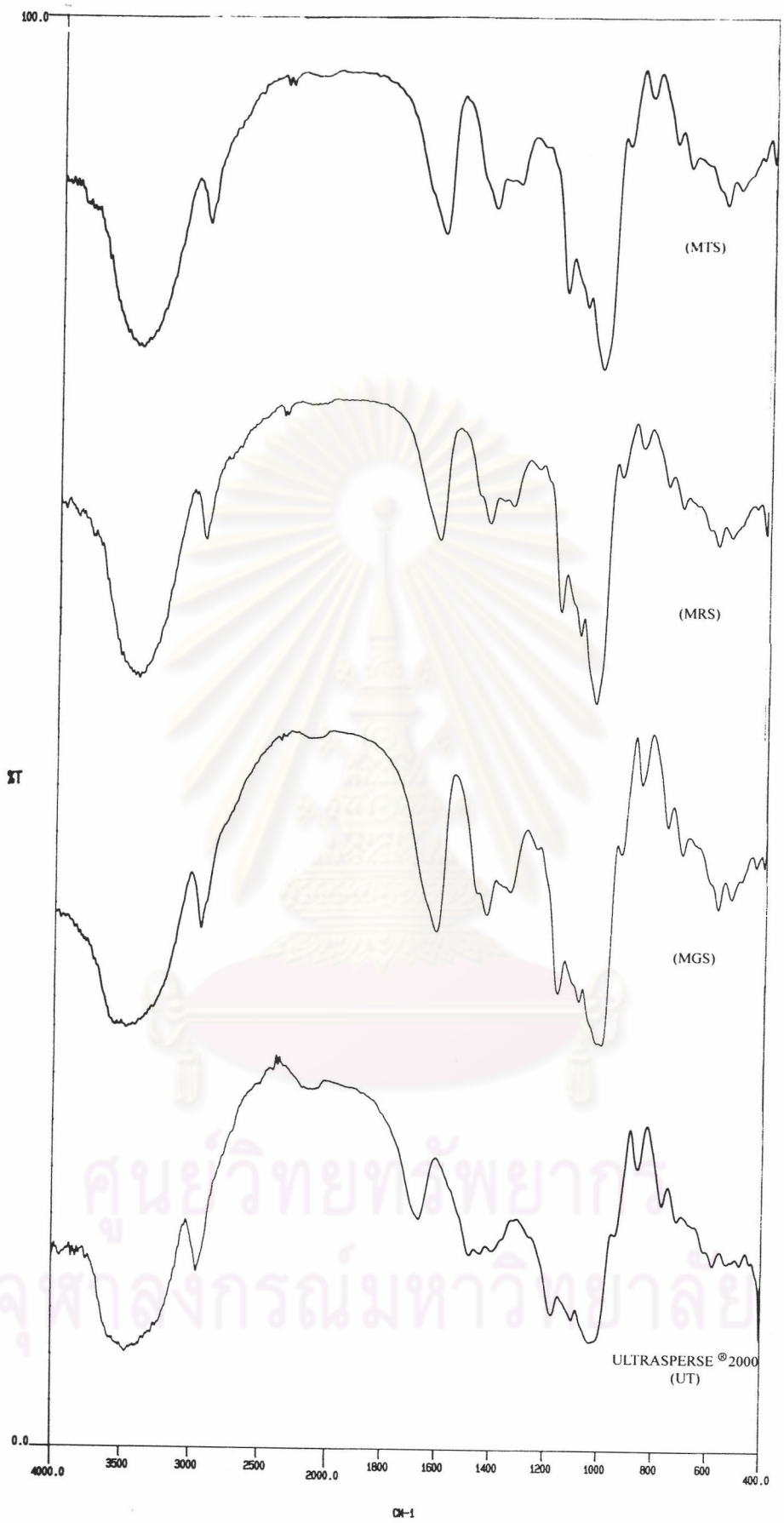


Figure 15 Comparison of the infrared spectra between modified glutinous rice starch (MGS), modified rice starch (MRS), and modified tapioca starch (MTS) and Ultrasperse®2000 (UT)

D. Determination of Reconstitution Time

Reconstitution time was defined as the number of time interval required for receiving complete homogenous dispersion of Ultrasperse®2000 and each of modified starch after water was added (Table 9). For all suspending agents, an increase in suspending agent concentration resulted in an increase in the reconstitution time. The reconstitution times of all modified starches were longer than of Ultrasperse®2000. This result was probably attributed to the fine and then the higher surface area of modified starch powder. When water was added to pure modified starch, it was very fast to have direct contact at surface area, fast to swell and covered some powders inside. So, it was expected that deposit had tendency to form lump when used pure modified starches powders at high concentrations. Ultrasperse®2000 could easily reconstitute with water because its particle was granule (Figure 16), therefore it had surface area lower than modified starches. The swelling rate of Ultrasperse®2000 was slower than modified starches after contacting with water. Thus in this studies, it could be concluded that Ultrasperse®2000 was easily reconstituted with water when compared with all other pure modified starches.

Table 9 Reconstitution time of pure MGS, MRS, MTS and UT

Suspending agent	%w/v	Time interval (times) Average (SD)
MGS DS 0.16	1	9.67 (1.53)
	2	12.37 (0.58)
	3	Lump*
MRS DS 0.26	1	5.30 (0.58)
	2	8.67 (0.58)
	3	17.33 (1.53)
MTS DS 0.38	1	6.33 (0.58)
	2	9.33 (0.58)
	3	9.33 (0.58)
UT DS 0.10	1	1.67 (0.58)
	2	2.00 (0.00)
	3	3.00 (0.00)

*Lump. = After shaking vigorously more than 20 times, the deposit was dispersed non-homogeneous.

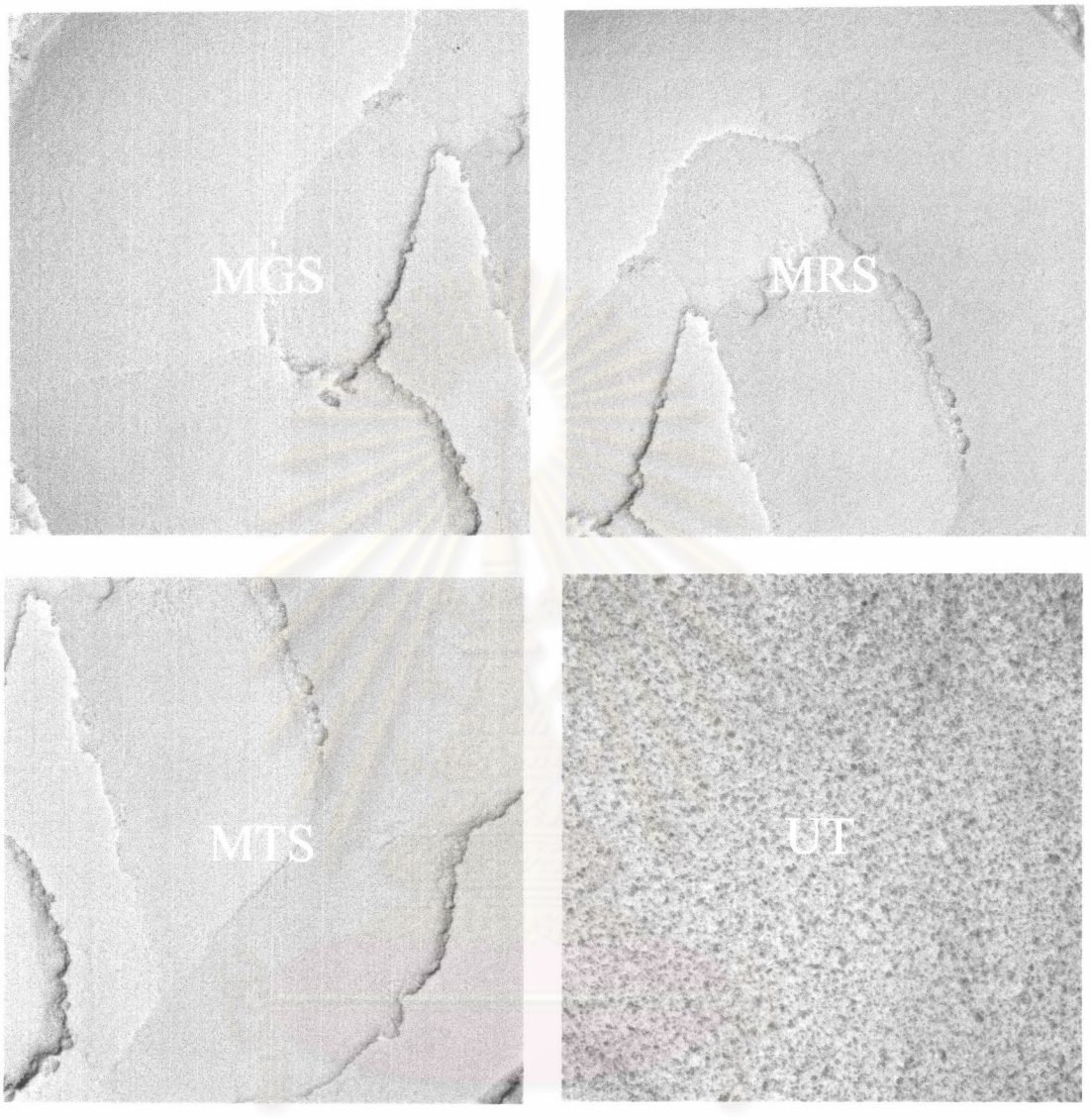


Figure 16 Comparisons of modified starches (MGS, MRS and MTS) as fine powder particle and small granule of commercial starch (Ultrasperse®2000)

E. Viscosity Measurement of Pure Dispersions

Since the dispersion of each modified starch (MGS, MRS and MTS) and Ultrasperse®2000 (UT) gave the maximum viscosity without lump formation at the concentration of 3.0 % and 4.0 % w/v, respectively. Therefore, the viscosities of each modified starch (MGS, MRS and MTS), and Ultrasperse®2000 (UT) dispersions were measured at the maximum concentration of 3.0 % and 4.0 % w/v, respectively.

The viscosities of each modified starch (MGS, MRS, and MTS) at dispersions the concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % w/v and Ultrasperse®2000 dispersion at the concentration of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 % w/v are shown in Figure 17. The viscosity of all suspending agent increased in accordance with increasing of concentration used.

Average apparent viscosity of each pure dispersion at the different concentration was presented in Table 10. The viscosities of pure dispersions were determined by Brookfield viscometer. Viscosities of modified starches and Ultrasperse®2000 at the concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0 % w/v could be ranked in decreasing order as follows; MGS > MRS > MTS > UT. Nevertheless, at the concentration of 0.5 %w/v concentration, the ranked order is MGS > MTS > MRS > UT.

As illustrated in Figure 17, an increase in viscosity of each dispersion occurred when increasing the concentration of suspending agent. Moreover, the results clearly indicated that MGS dispersion, MRS dispersion and MTS dispersion possessed higher viscosity than UT dispersion. The MGS and MRS concentrations had dramatically effected on viscosity. At the concentration between 0.5 % w/v and 3.0 % w/v, the concentration of UT had little effect on viscosity. However, an increase in concentration form 3.0 % w/v to 4.0 % w/v, exhibited a marked increase in viscosity.

MGS had highest viscosity value among selected modified starches. This result suggested that amylose/amylopectin ratio of starch might play an important role in determining the viscosity of modified starch product. Native glutinous rice is sticky because it contains low content of amylose (0 - 5%) while high amylose content in native corn starch (22 - 28 %) made its modified starch inappropriate as suspending agent. This is in agreement with the study of Schwartz and Zelinskie (1978) which reported that the binding property of starch was due to amylopectin

fraction while the disintegrant property was due to amylose fraction (Ornanong Suwannapakul, 1996).

Amylose content in native glutinous rice starch, native rice starch and native tapioca starch were 0 - 5%, 16 - 17% and 17 - 22%, respectively (Hullinger, 1960). The viscosities of MGS, MRS and MTS dispersions were 435.07 - 2861.57, 63.34 - 1421.69 and 107.34 – 404.56 cps. Therefore, the viscosities of modified starches could be ranked in decreasing order as follow; MGS > MRS > MTS.

Table 10 Viscosity of pure dispersion at various concentrations

Concentration (% w/v)	Apparent viscosity(cps) Average (SD)			
	MGS	MRS	MTS	UT
0.5	435.07 (22.27)	63.34 (10.21)	107.34 (11.47)	4.47 (2.11)
1.0	537.13 (47.54)	136.58 (17.52)	115.52 (14.69)	6.00 (0.25)
1.5	784.62 (39.36)	219.45 (21.96)	138.56 (29.74)	9.17 (1.33)
2.0	1052.45 (51.12)	468.35 (44.33)	178.02 (31.21)	12.47 (3.47)
2.5	1820.80 (74.21)	981.94 (55.87)	275.57 (33.58)	53.69 (11.17)
3.0	2861.57 (87.66)	1421.69 (69.12)	404.56 (25.69)	87.82 (19.36)
4.0	-	-	-	537.75 (58.31)

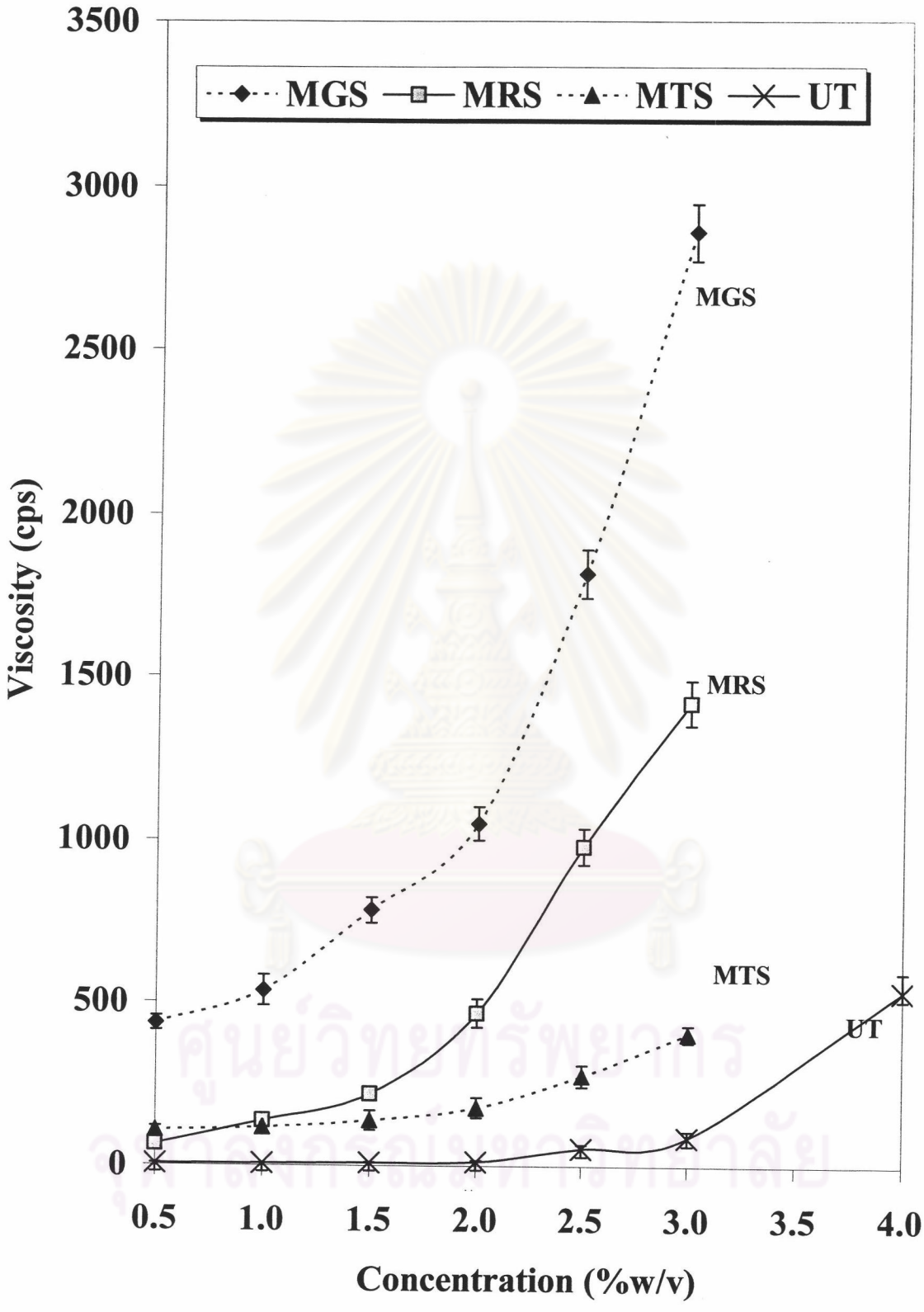


Figure 17 Comparative viscosity among concentration of modified starches and Ultrasperse®2000

F. Rheological Studies of Pure Dispersions

In this study, the rheological behaviors of the suspensions were determined by Brookfield viscometer, using small sample SC4 - 31 spindle at 0 - 10 rpm and gave result in term of viscosity (cps). The result was reported in term of viscosity (cps). Thus in this study, viscosity was transformed form shear rate by using suitable equation. The rheogram was plotted between shear rate and shear stress (Appendix B). As illustrated in Figures 19-25, all pure suspending agents exhibited pseudoplastic flow curve. The pseudoplastic flow curves, the down-curve could be displaced with regard to the up-curve, with shear-thinning system and down-curve was frequently dispersed to right of the up-curve. The phenomenon was known as Thixotropy (Martin, 1993).

Thixotropic value of pure dispersions was measured from area of hysteresis, and the comparative values shown in Figure 18. In this study, the very low viscosity of UT dispersions was observed UT at the concentration of 1.0 % and 2.0 % w/v (Table 10 and Figure 16). The measurement of viscosity using the same condition (type of spindle, up and down shear rate cycles) could not be detected. Therefore, the measurement of viscosity was out of limited. Then, the rheological behavior of UT at concentration of 1.0 % and 2.0 % w/v could not be detected and explained in term of thixotropic value. The thixotropic value could be ranked in decreasing orders as followed; at 1.0 % and 2.0 % concentrations (w/v), MGS > MRS > MTS, and for 3.0 % concentration (w/v), MGS > MRS > MTS > UT. The results implied that MGS, at any concentration used, possessed the highest thixotropic value among all suspending agents employed in this study. The other two modified starches, MTS and MRS, also showed high thixotropic value and much higher than UT at the same condition. Whereas, the thixotropic quantity of 4.0 % concentration (w/v) of UT was similar to MGS at 3.0 % concentration (w/v).

Thixotropic value was desirable characteristic in liquid pharmaceutical systems, including suspension. In this study, thixotropy values of pure suspending dispersion were compared. This could be clearly indicated that MGS, MRS, MTS and UT were pseudoplastic materials and could be applicable as suspending agent (Schramm, 1981).

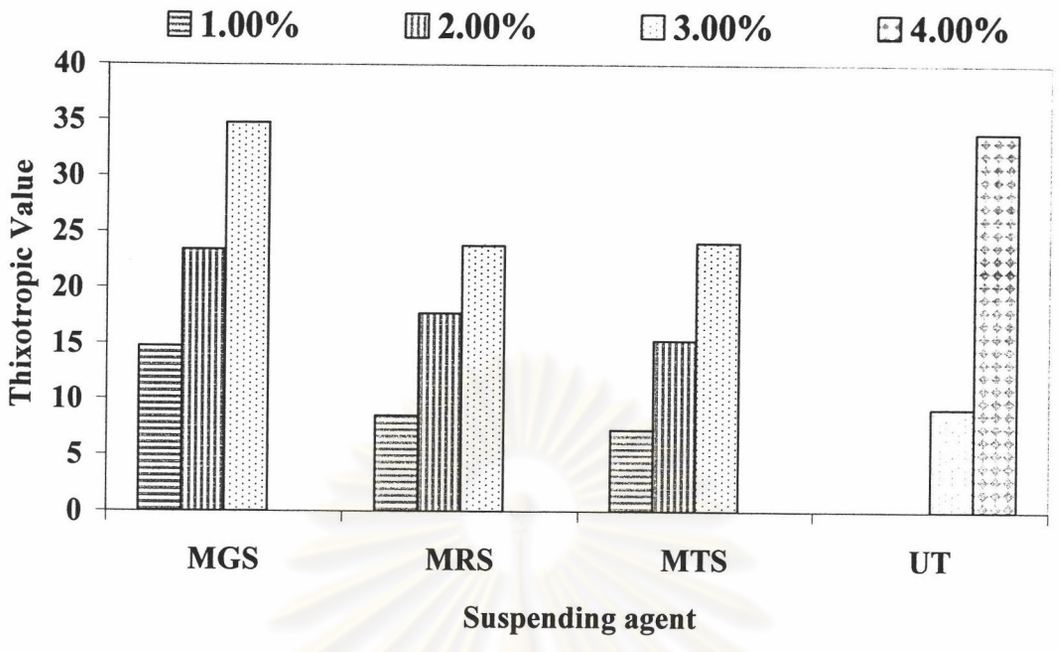


Figure 18 Comparative thixotropic value of pure dispersion as using various types

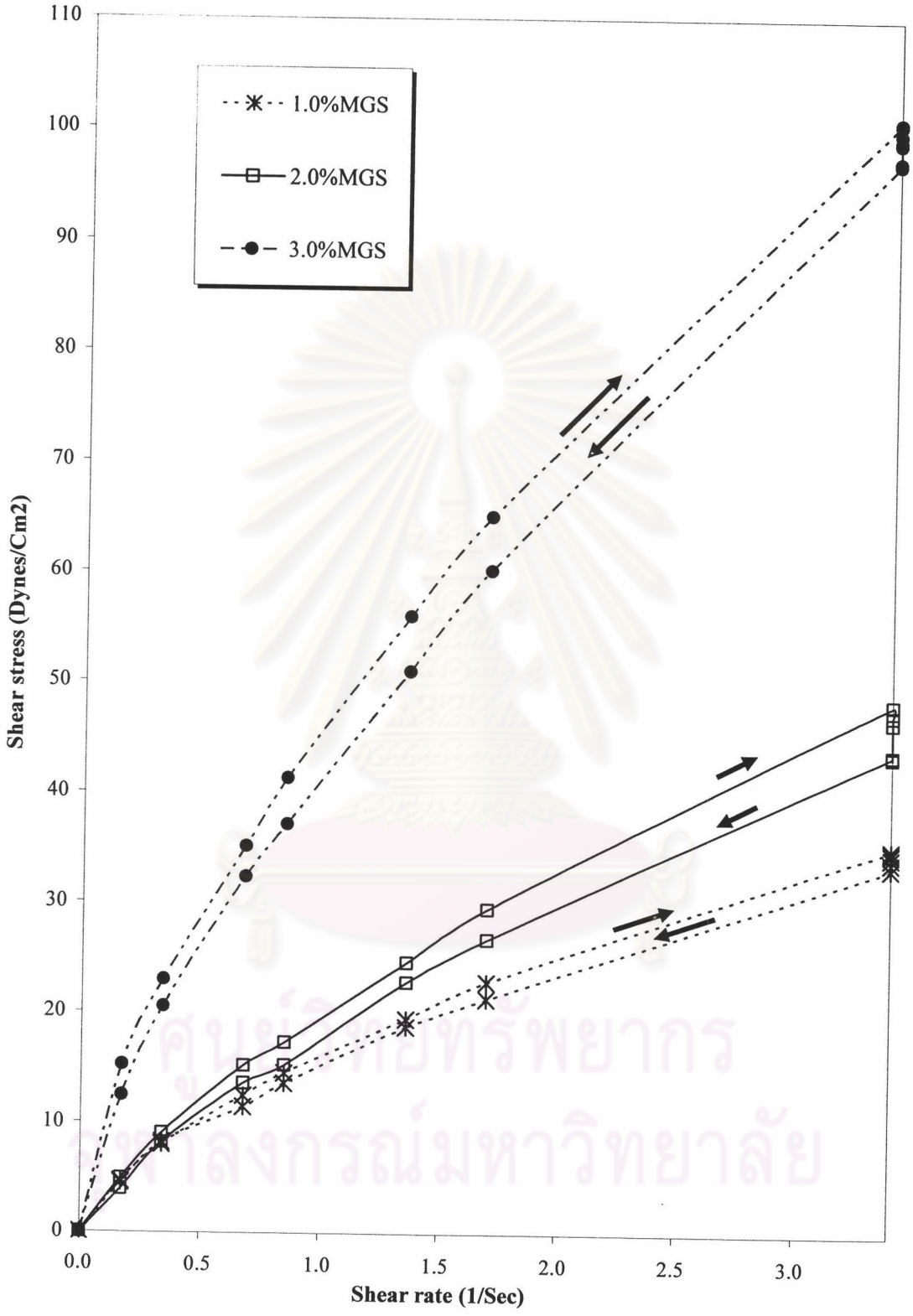


Figure 19 Flow curves of pure modified glutinous rice starches (MGS) dispersion at concentration of 1.0 %, 2.0 % and 3.0% w/v

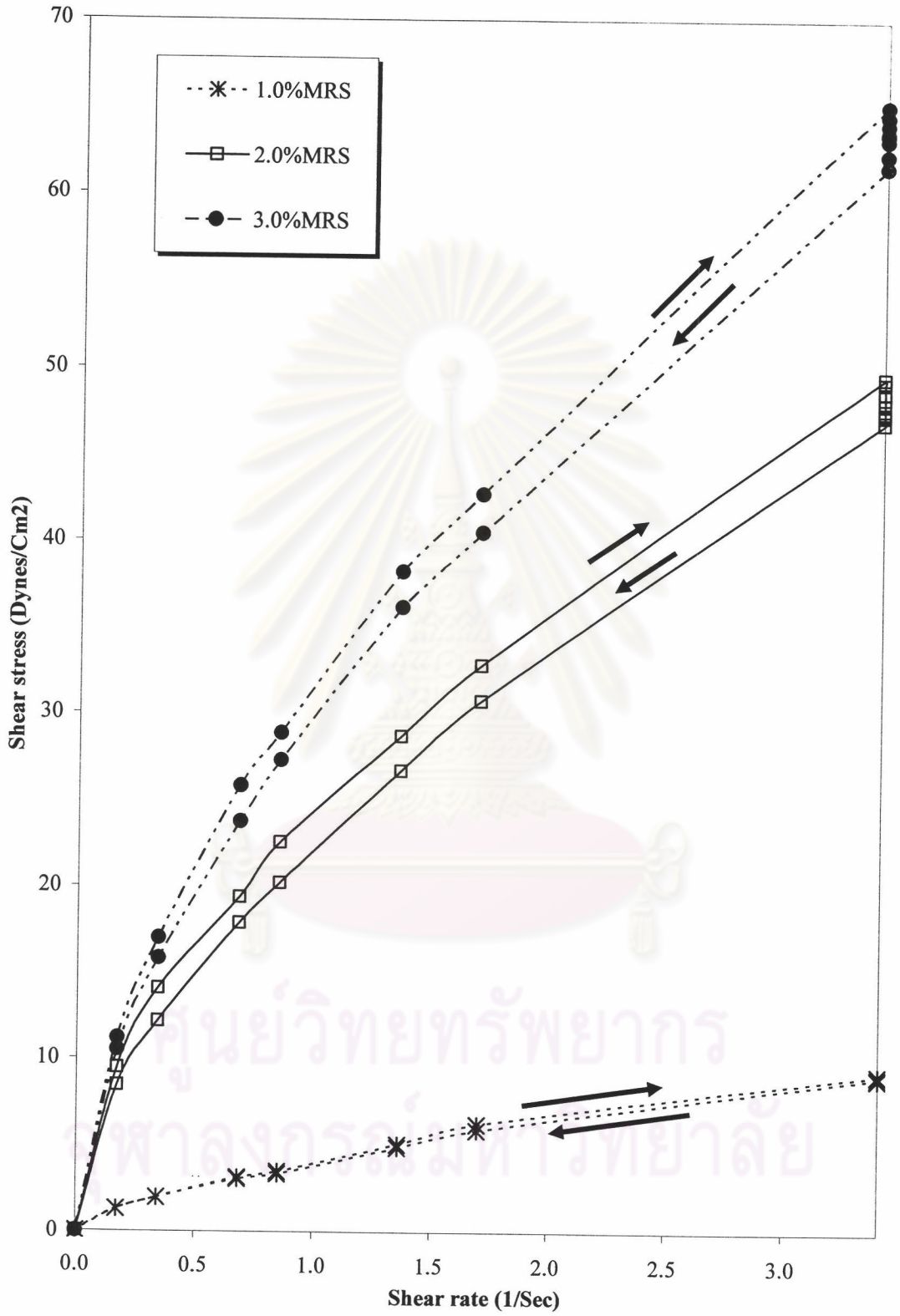


Figure 20 Flow curves of pure modified rice starches (MRS) dispersion at concentration of 1.0 %, 2.0 % and 3.0% w/v

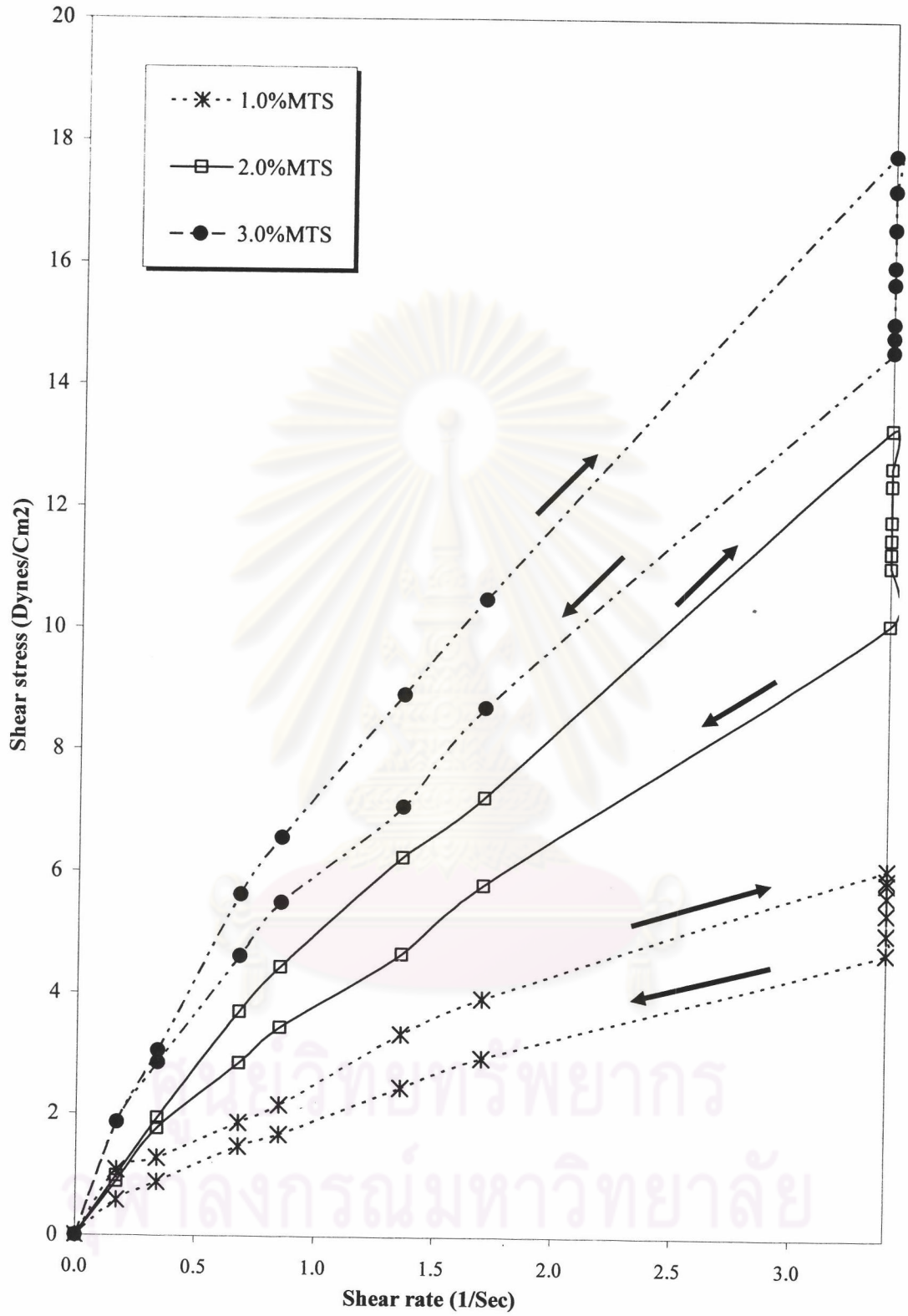


Figure 21 Flow curves of pure modified tapioca starches (MTS) dispersions at concentration of 1.0 %, 2.0 % and 3.0% w/v

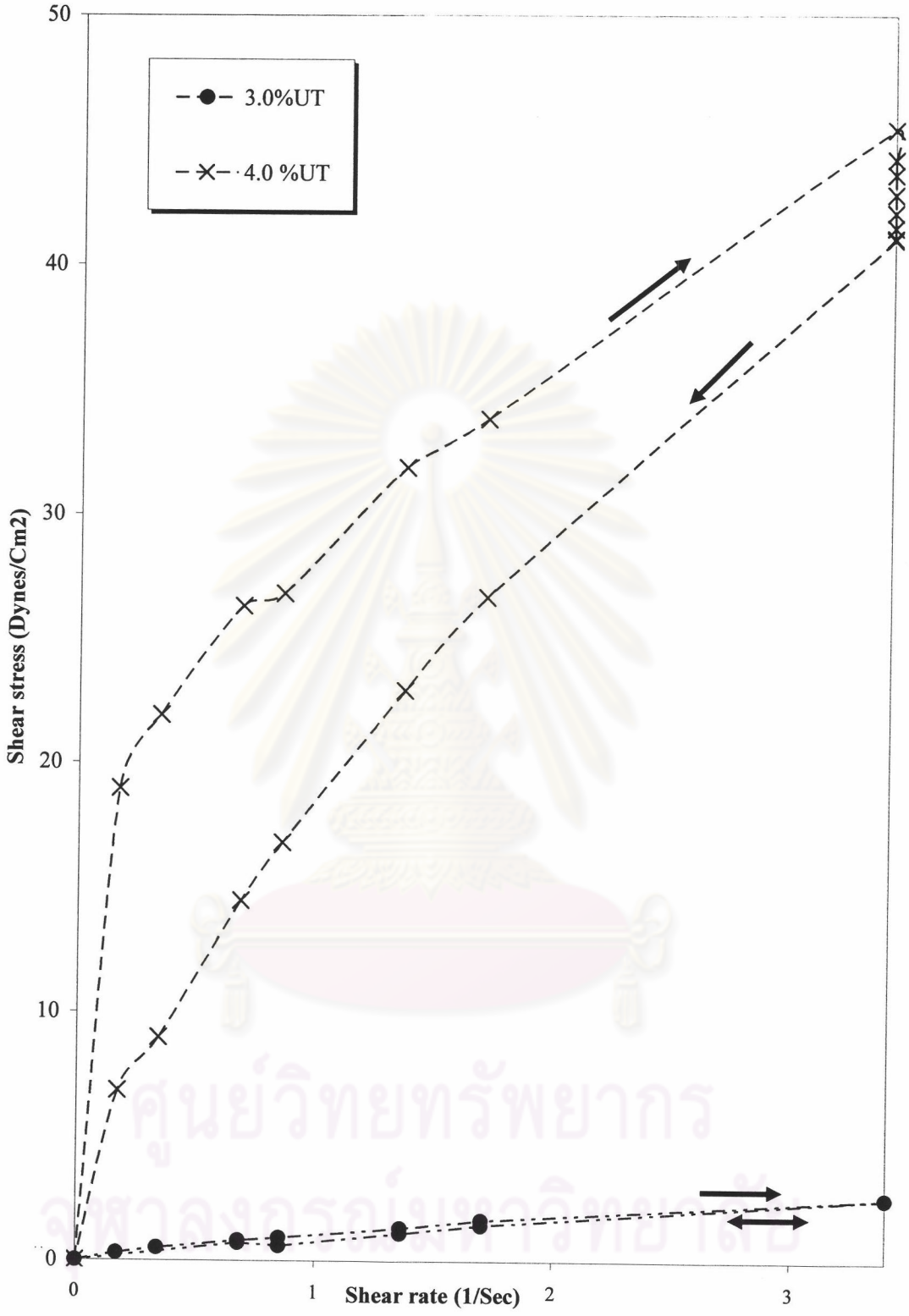


Figure 22 Flow curves of pure Ultrasperse®2000 (UT) dispersion at concentration of 3.0 % and 4.0% w/v

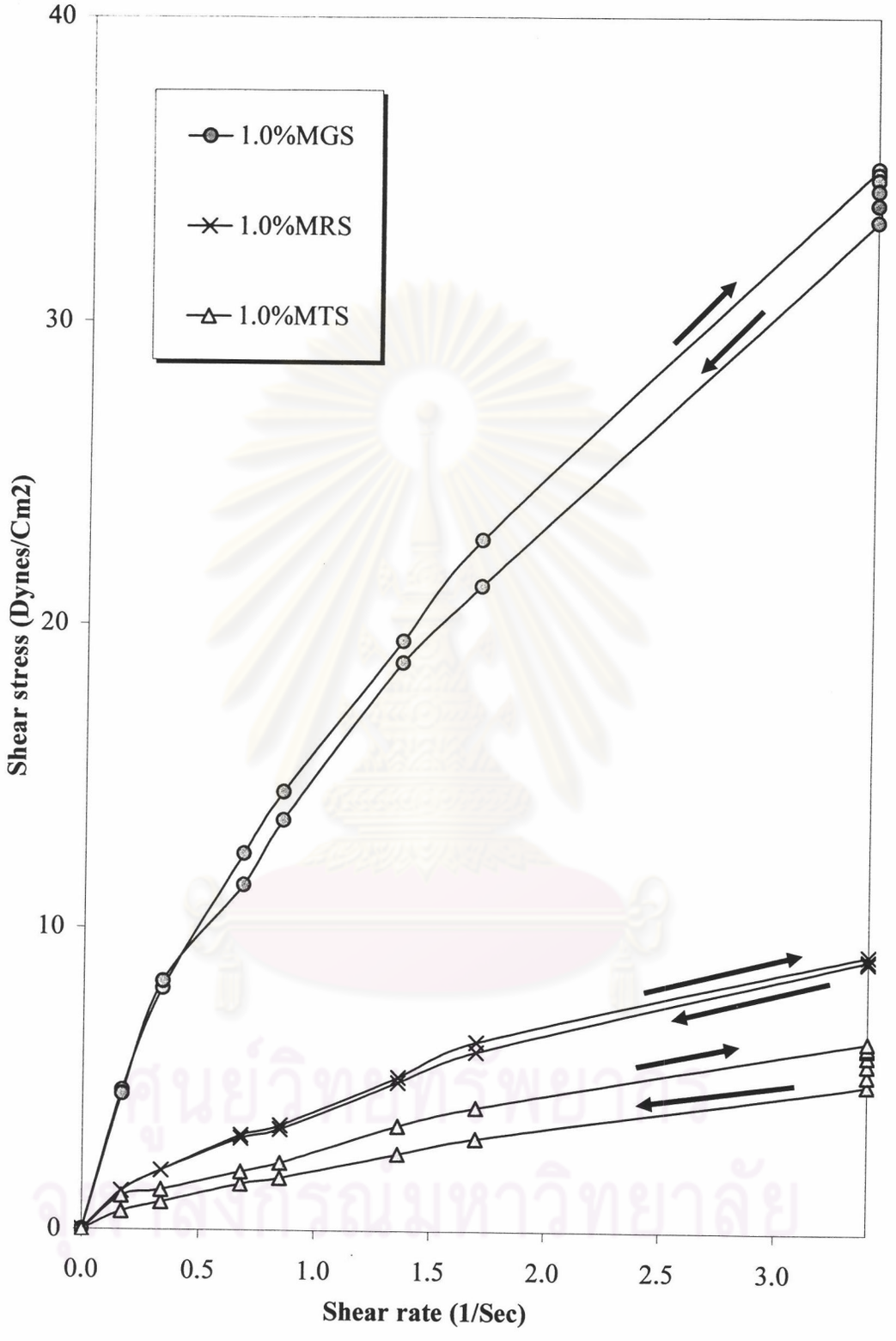


Figure 23 Comparative flow curves of each pure dispersion at concentration of 1.0 % w/v as using various types

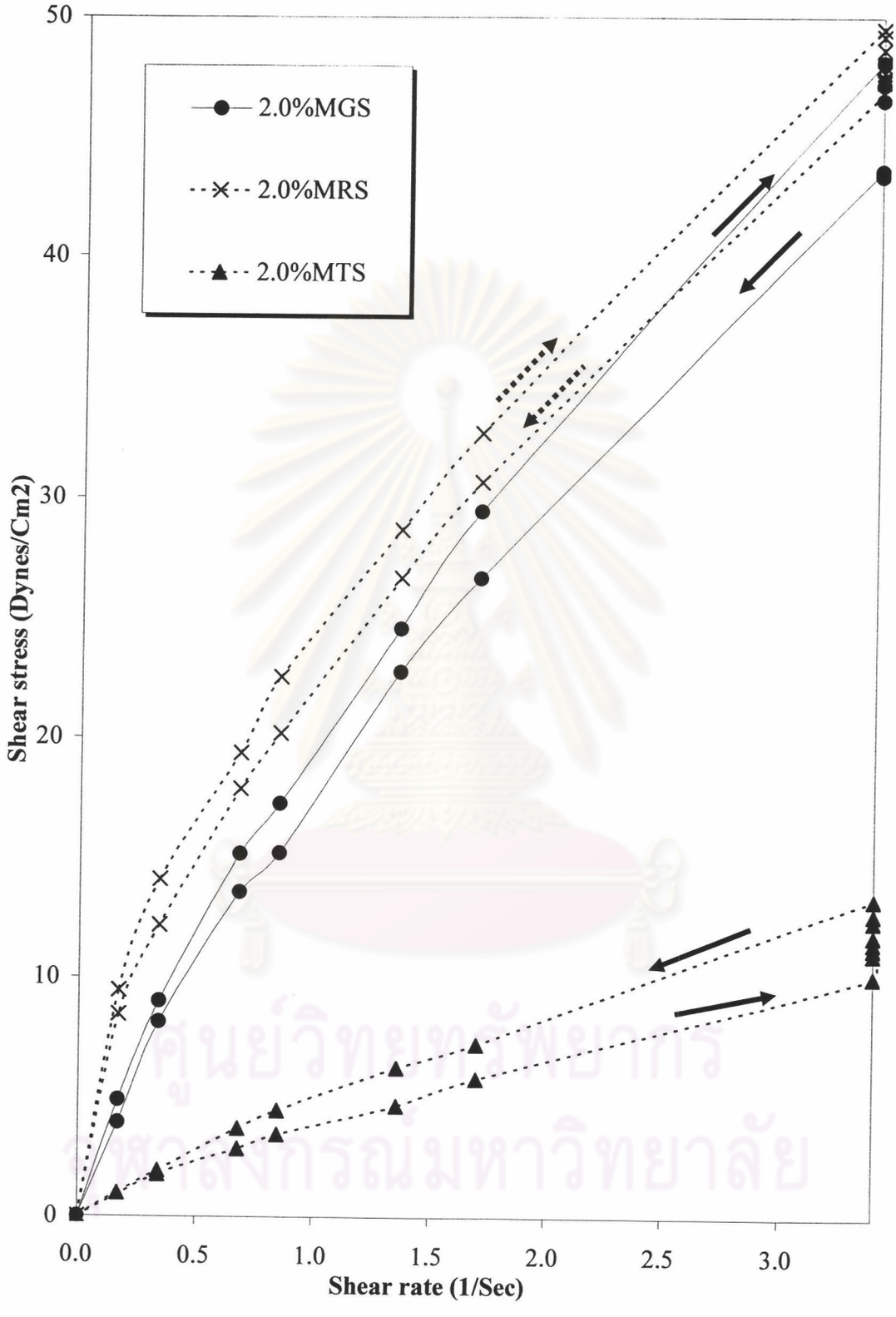


Figure 24 Comparative flow curves of pure dispersion at concentration of 2.0 % w/v as using various types

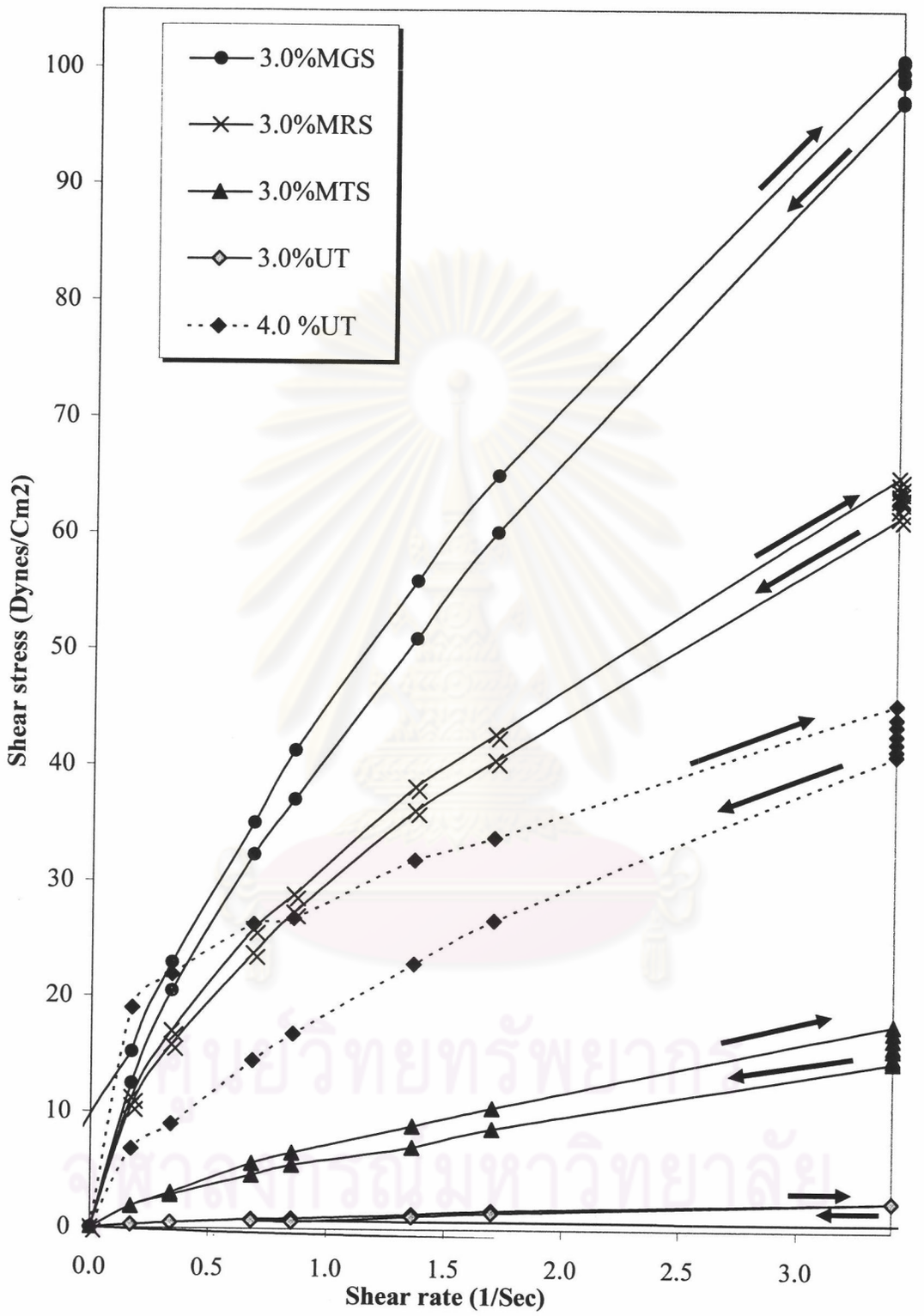


Figure 25 Comparative flow curves of pure starch dispersion at concentration of 3.0 % w/v as using various types and Ultrasperse®2000 dispersion at concentration of 3.0 and 4.0 % w/v

Part II: Evaluation of Modified Starch as Suspending Agent in Calcium Carbonate Suspension

The used of modified starches as suspending agent in calcium carbonate suspension was evaluated by determination of reconstitution time, sedimentation volume, redispersibility and viscosity. These studies were used as the guide for selected suitable concentrations of modified starches and Ultrasperse®2000 which would to be employed as suspending agent in dry syrup formulation.

A. Determination of Reconstitution Time

The comparative reconstitution time of reconstituted calcium carbonate suspension is shown in Table 11. Reconstitution times of all formulations were less than 13.00 times. Nevertheless, the lump formation was observed with MGS at the concentration of 3.0 % w/v. An increase in suspending agent concentration exhibited an increase in reconstitution time. The reconstitution time could be ranked from lower to higher value as following order: at concentration of 1.0 % and 2.0 % w/v, UT < MRS < MTS < MGS , and at concentration of 3.0 %, UT < MTS < MRS < MGS. These results indicated that UT, at any concentration used, possessed the lowest reconstitution time among all suspending agents employed in this study. On the other hand the vice versa was observed with MGS. At any concentration used MGS, possessed that highest reconstitution time among all suspending agents employed in this study and it also exhibited "lump" at the concentration of 3.0 % w/v.

When the reconstitution times of pure dispersions and calcium carbonate reconstituted suspension with the same type of suspending agent were compared, the reconstitution times of pure dispersions was higher than those of calcium carbonate suspension. The result pointed out that the water did not direct contact to each of modified starch in calcium carbonate suspension and then it was slowly swollen and had low opportunity to cover some powders inside.

In conclusion, the result clearly indicated that UT was easier to disperse after reconstituting with water than MGS, MRS and MTS.

Table 11 Reconstitution time of reconstituted calcium carbonate suspension

Suspending agent	%	Time interval (times) Average (SD)
MGS DS 0.16	1	8.50 (0.87)
	2	10.50 (0.87)
	3	Lump*
MRS DS 0.26	1	4.67 (0.76)
	2	7.67 (0.29)
	3	12.00 (0.50)
MTS DS 0.38	1	5.00 (0.00)
	2	8.17 (0.29)
	3	10.00 (0.50)
UT DS 0.10	1	1.17 (0.29)
	2	2.17 (0.29)
	3	2.50 (0.00)
	4	6.00 (1.00)

*Lump = after the shaking vigorously more than 20 times, the deposit was dispersed non-homogeneous.

B. Determination of Sedimentation Volume

The comparative sedimentation volumes (Hu/Ho) of calcium carbonate suspension containing each of modified starches (MGS, MRS and MTS) and UT were shown in Appendix C3 and Figures 26 - 29. As presented in Figure 26, sedimentation volumes of calcium carbonate reconstituted suspensions containing MGS at the concentration of 1.0 % and 2.0 % w/v decreased after keeping between 5 - 8 days, but at concentration of 3.0 % w/v sedimentation volume decreased slowly as the time. However, sedimentation volumes at all concentration after keeping for 14 days were similar. The calcium carbonate suspension containing MRS gave the similar decreasing pattern of sedimentation volume after keeping for 1-14 days (Figure 27).

As presented in Figure 28, the calcium carbonate suspension containing MTS as suspending agent had high sedimentation volumes after storage but the supernatant did not clear. This result indicated that the incomplete sedimentation when stored for 14 days was occurred (Appendix C; Figure C1). As display in Figure 29, sedimentation volumes of calcium carbonate suspension with UT as suspending agent at all concentrations decreased slowly. Although, sedimentation volumes of MGS, MRS, MTS and UT containing calcium carbonate suspension were increased in accordance with the increasing of concentration used, but for after keeping for 7 days were 2.0 % > 3.0 % > 1.0 % w/v of concentration (Figure 30). This result might be caused by the incomplete sedimentation.

In summary, sedimentation volumes of calcium carbonate suspension with different each of modified starches and various concentrations after keeping for 14 days could be ranked in increasing as follow; 1.0 % MRS < 1.0 % MGS < 2.0 % MGS < 3.0 % MRS < 3.0 % MGS, 2.0 % MRS < 1.0 % UT < 2.0 % UT < 3.0 % UT < 1.0 % MTS < 4.0 % UT < 2.0 % MTS < 3.0 % MTS. Moreover, sedimentation volumes of dispersions with MGS, MRS, and UT increased in accordance with the increasing of concentration used and similar calcium carbonate suspensions when keeping for 7 days (Figures 30 - 31).

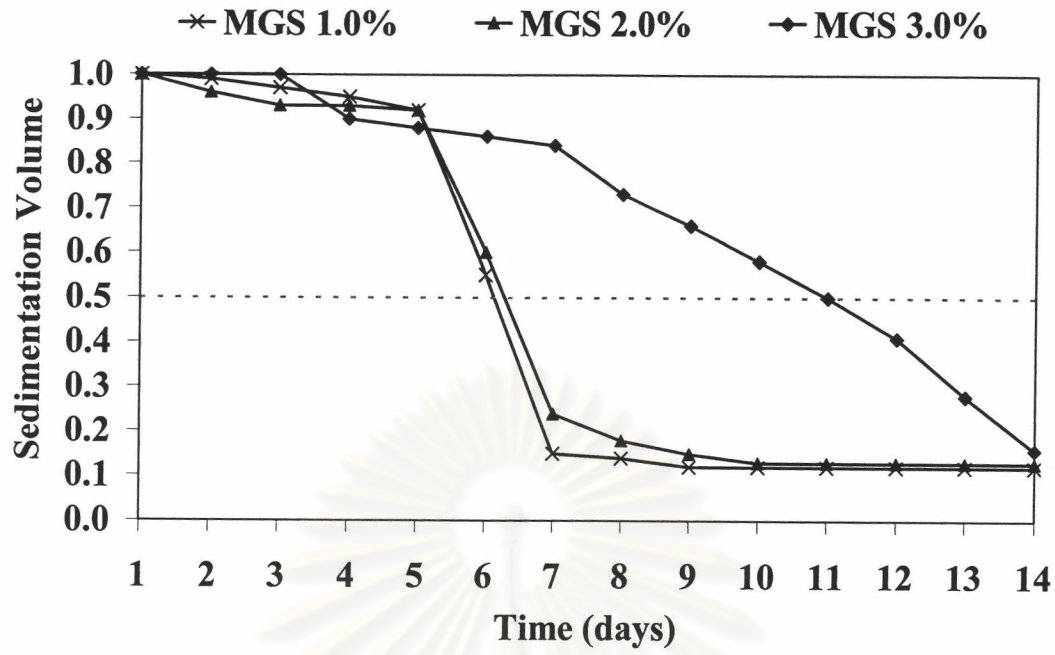


Figure 26 Sedimentation volumes of calcium carbonate suspension containing modified glutinous rice starch (MGS) as suspending agent

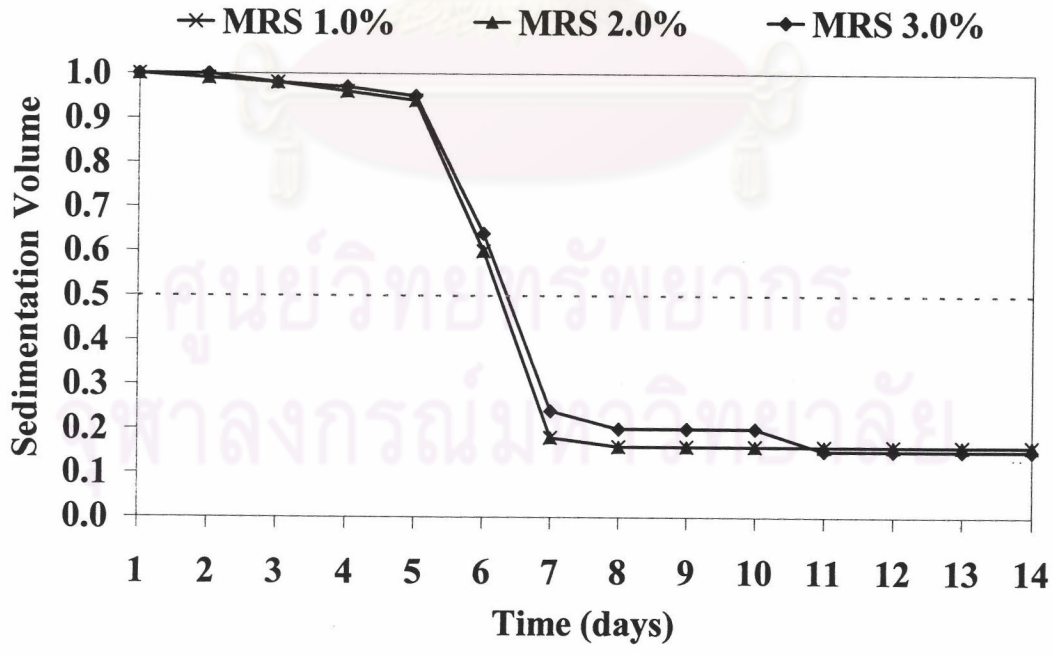


Figure 27 Sedimentation volumes of calcium carbonate suspension containing modified rice starch (MRS) as suspending agent

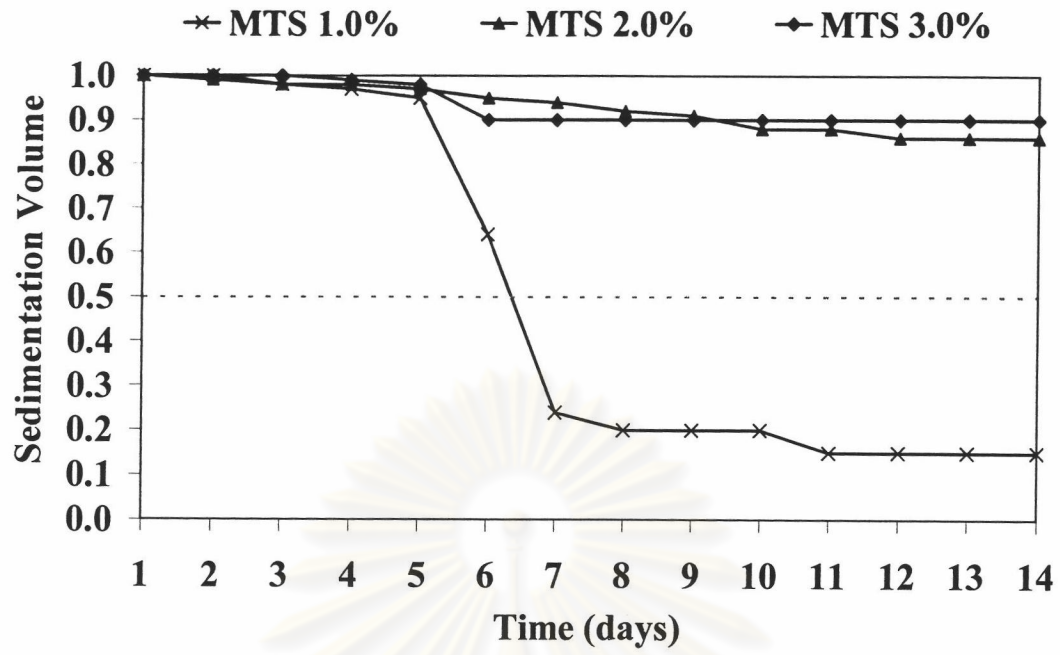


Figure 28 Sedimentation volumes of calcium carbonate suspension containing modified tapioca starch (MTS) as suspending agent

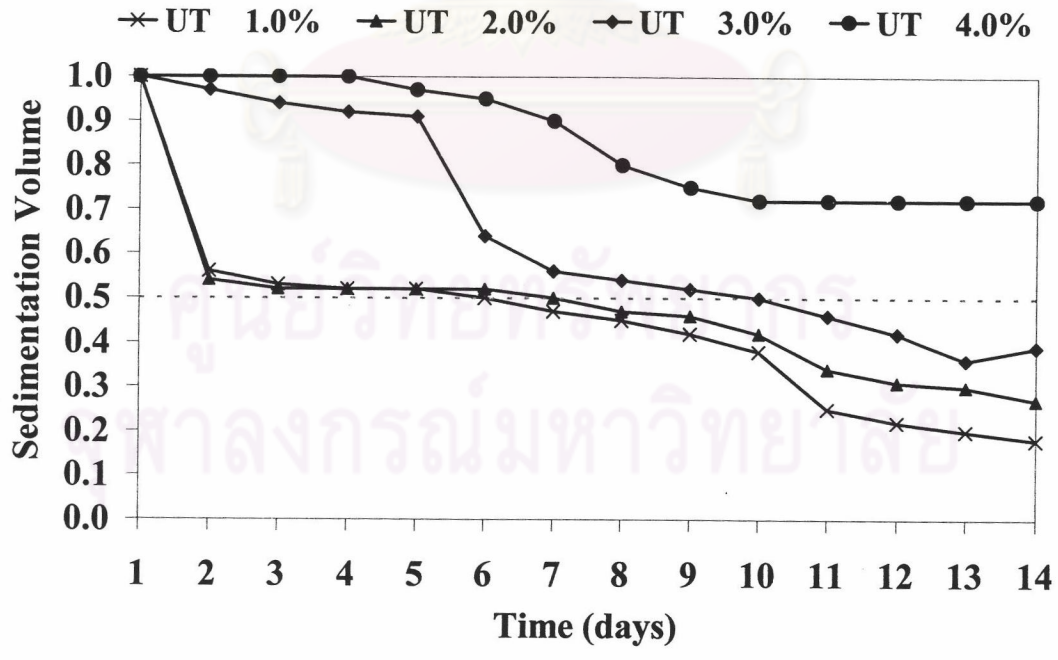


Figure 29 Sedimentation volumes of calcium carbonate suspension containing Ultrasperse®2000 (UT) as suspending agent

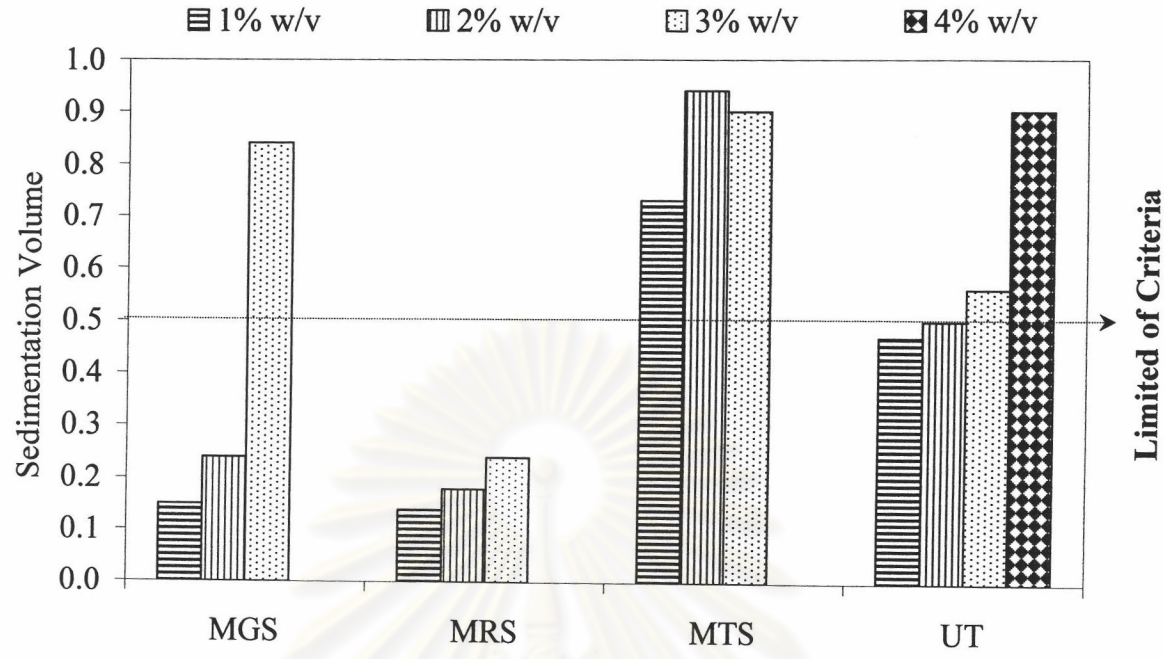


Figure 30 Comparative sedimentation volumes of calcium carbonate suspension containing of MGS, MRS, MTS and UT after storage 7 days

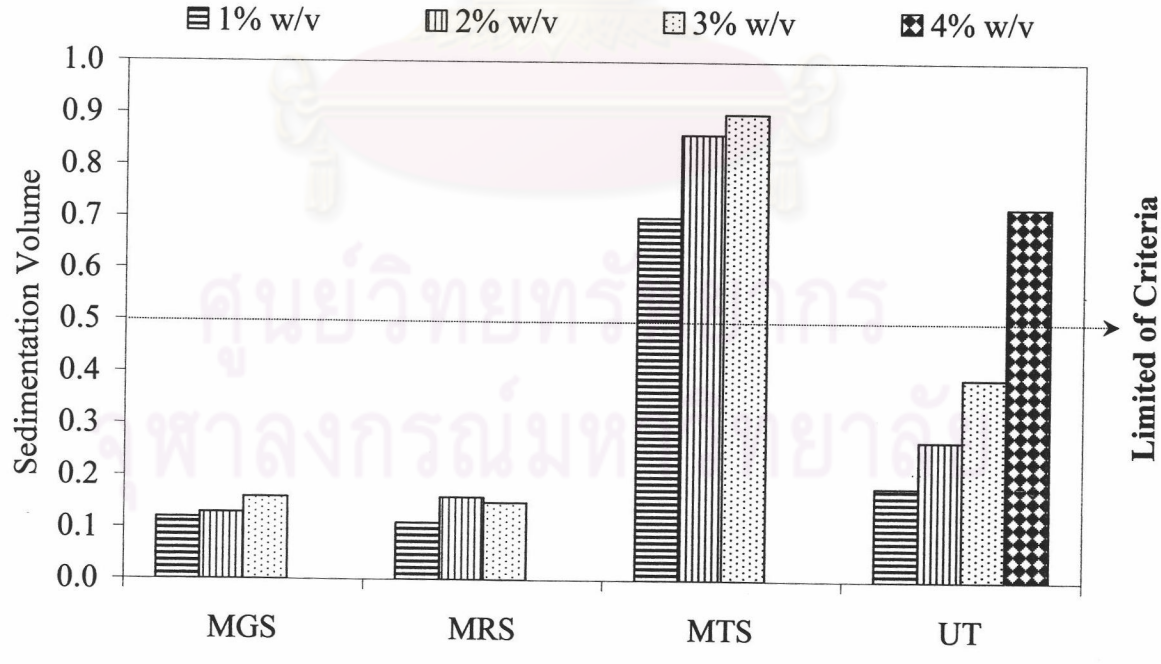


Figure 31 Comparative sedimentation volumes of calcium carbonate suspension containing of MGS, MRS, MTS and UT after storage 14 days

C. Determination of Redispersibility

The number of inversion required for dispersing each suspension was determined. The redispersibility of calcium carbonate suspension containing each of modified starches (MGS, MRS and MTS) and Ultrasperse®2000 as suspending agent was evaluated (Appendix C; Table C2 and Figures 32 – 33). For the better of the redispersibility, the less number of inversion was indicated. The results could be interpreted as following; the lowest number of inversion was required for MGS at concentration of 2.0 % w/v. In addition, after keeping for 14 days, the number of inversion for MGS at concentrations of 1.0 % and 3.0 % w/v were similar and higher than that MGS at the concentration of 2.0 % w/v.

The redispersibility of calcium carbonate suspension containing MRS as suspending agent at all concentrations after keeping for 7 days increased in accordance with the increasing of concentration used (Figure 32). The redispersibility of calcium carbonate suspension containing MRS as suspending agent at the concentration of 1.0% and 2.0 % w/v after keeping for 14 days was higher than those were kept for 7 days. On the contrary, MGS at the concentration of 2.0 % w/v illustrated vice versa result. The deviated result might be explained that some masses of sample after keeping for 7 days deposited at the top of test tube and obstructed when the test tube was inversed.

Calcium carbonate suspension contained MTS as suspending agent, the less number of inversions was required for storage. A decrease in number of inversion for storage 7 days was occurred when increasing the suspending agent concentration. After keeping for 14 days, the number of inversion of MTS containing suspension at concentration of 2.0 % w/v was lowest.

Calcium carbonate suspension containing UT as suspending agent, the number of inversion could be ranked from lower to higher value as following order: concentration of 3.0 % , 4.0 % < 1.0 % < 2.0 % w/v at 7 days storage and 4.0 % < 1.0 % < 3.0 % < 2.0 % at 14 days storage. The redispersibility of calcium carbonate suspension containing UT as suspending agent at all concentrations after keeping for 7 days increased in accordance with the increasing storage time.

The obtained results revealed that, among different types of modified starch, calcium carbonate suspension containing MTS required lowest number of inversion to regain dispersion. This result implied that MTS provided good redispersibility of

none of these suspension required vigorous tube shaking to regain dispersion in all cases.

According to the results, in this study, there was an inconsistency in relationship between concentration of suspending agent used in calcium carbonate suspension and the redispersibility. It was unclear whether higher concentration of suspending agent provided better redispersibility. In this study, in consideration of redispersibility, MTS and UT were suitable for using as suspending agent in calcium carbonate suspension.

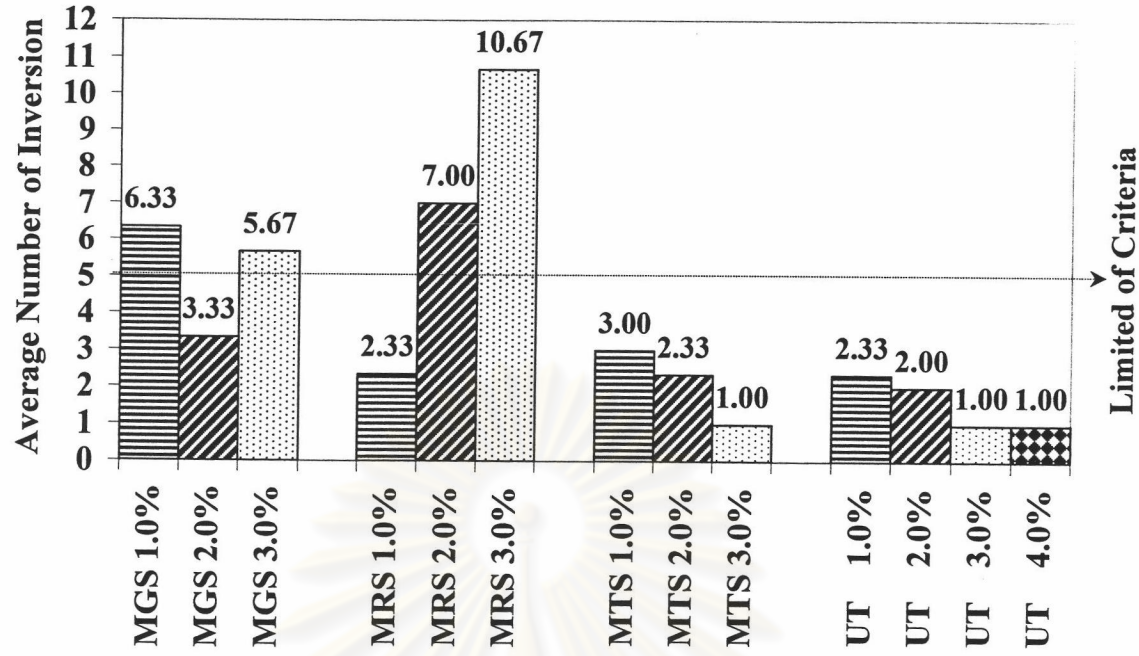


Figure 32 Redispersibility of calcium carbonate suspension containing modified starches and Ultrasperse®2000 as suspending agents after keeping for 7 days

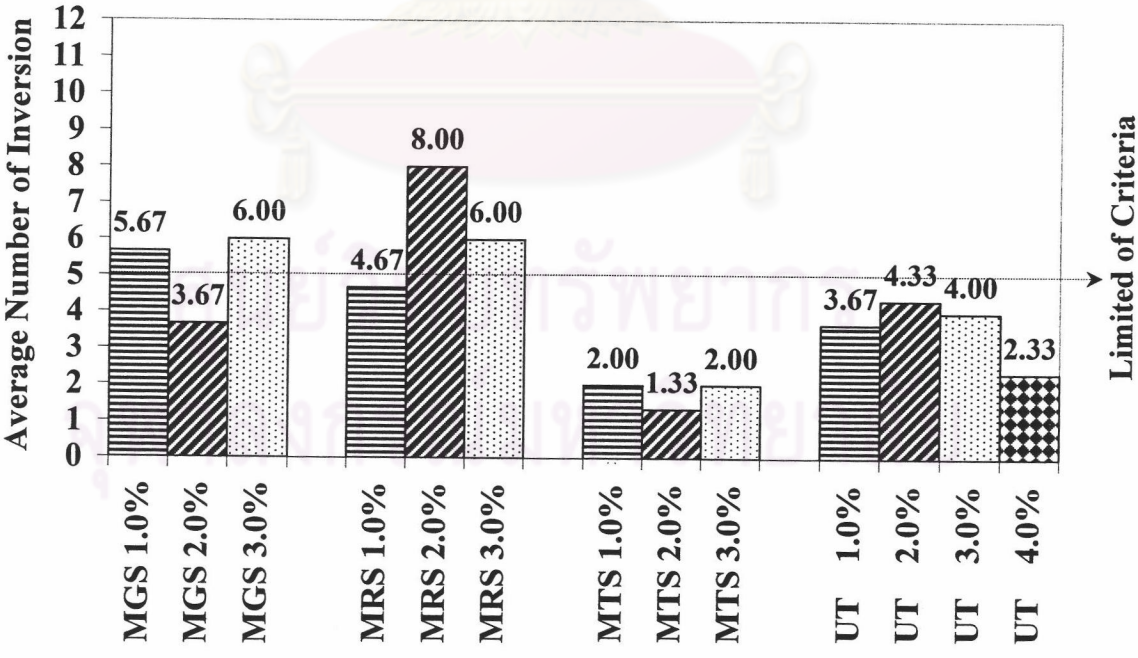


Figure 33 Redispersibility of calcium carbonate suspension containing modified starches and Ultrasperse®2000 as suspending agents after keeping for 14 days

D. Viscosity Measurement of Calcium Carbonate Suspension

The average apparent viscosities of each modified starch (MGS, MRS and MTS) at the concentrations of 1.0 %, 2.0 % and 3.0 % w/v and Ultrasperse®2000 at concentrations of 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v was shown in Appendix C; Table C4 and Figure 34. For all suspending agents, an increase in viscosity of calcium carbonate suspension occurred with increasing suspending agent concentration.

The viscosity of calcium carbonate suspension was determined by Brookfield viscometer. Comparative viscosity between Ultrasperse®2000 and modified starches at the concentrations of 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v could be ranked in following order; MGS > MRS > MTS > UT. The viscosity of each suspending agent increased when the concentration was increased and the results indicated that MGS, MRS and MTS possessed higher viscosity than UT. The reason for this finding might be due to the same that of pure dispersions. In addition, the viscosity of calcium carbonate suspension was little lower than that of pure dispersions.

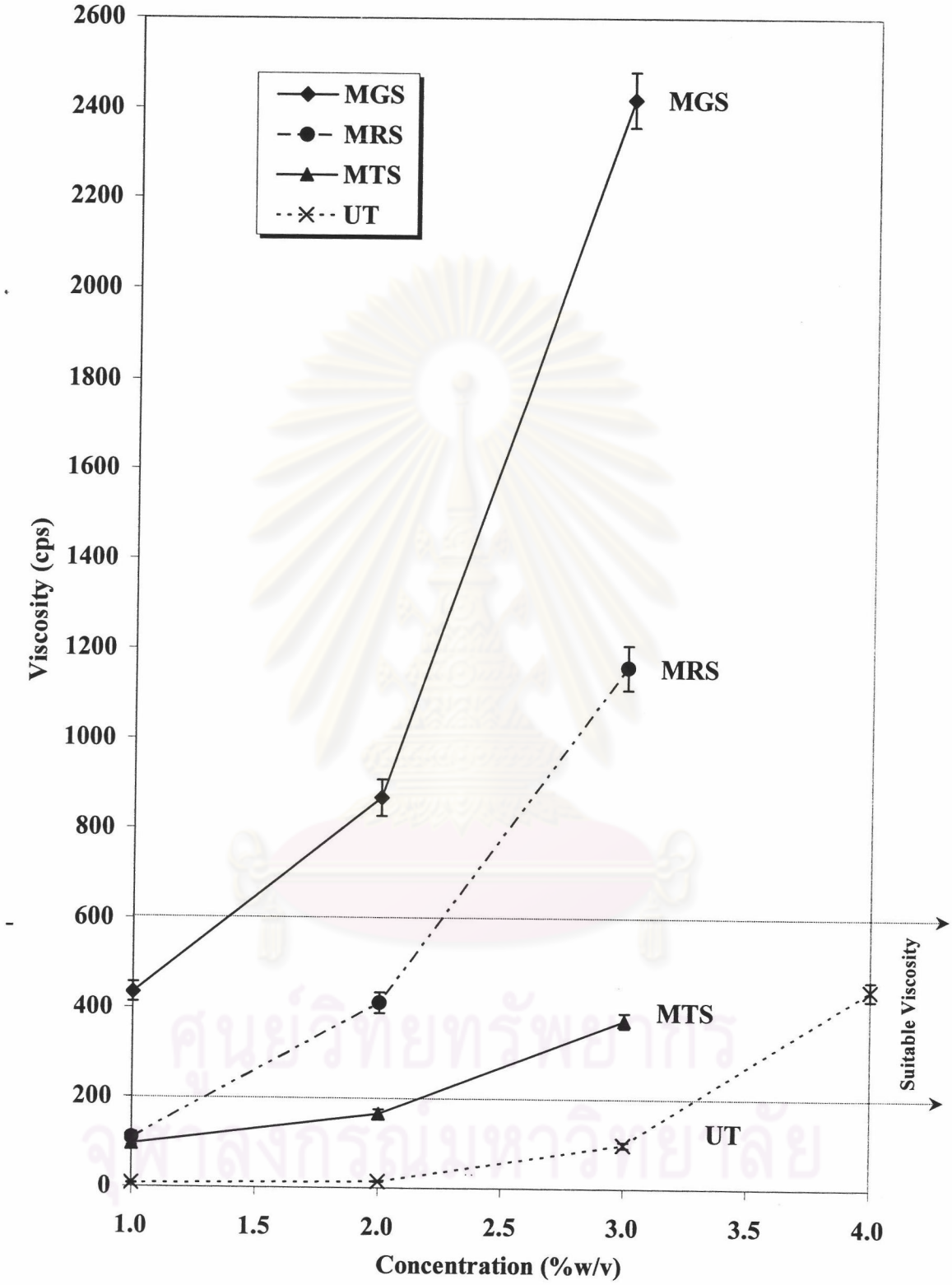


Figure 34 Comparative apparent viscosities of suspending agent at various concentrations when using in calcium carbonate suspension

E. Selected Concentration of Suspending Agent for Application in Dry Syrup

Preliminary selection of modified starches was made by using the results obtained from property study i.e.; viscosity, sedimentation volumes, reconstitution time and ease of redispersibility of calcium carbonate suspension containing modified starches as suspending agent. In each property evaluation, a score of 0, 1, 2 or 3 was used. The concentration of each modified starches that showed the best result was given score of 3 while the one that possessed the poorest result was given score of 0. The selection of modified starch of each type was made based on the obtained score number of four evaluated properties.

For reconstitution time, the lowest number of times interval shown the best value. The reconstitution time of a good calcium carbonate suspension was lower than five times and could be score of 3. The score number for reconstitution time of all dispersions is shown in Table 12.

For sedimentation volume, the highest number is 1.0 and the score number for sedimentation volume of all dispersions after keeping for 7 and 14 days is shown in Table 13.

For redispersibility, the lowest of number of inversion required for dispersing each suspension was showed the best value. The redispersibility of good calcium carbonate suspension was lower than five times and could be score of 3. The score number for redispersibility of all dispersions is shown in Table 14.

For viscosity, suitable viscosity for good calcium carbonate suspensions was 200 – 600 cps and the score number for viscosity of all dispersions is shown in Table 15.

Table 12 The score number for reconstitution time of all dispersions

Reconstitution time (times)	Score number
≤ 5	3
> 5 - 10	2
> 10 - 20	1
> 20	0

Table 13 The score number for sedimentation volume of all dispersions

Sedimentation volume		Score number
7^{th} day	14^{th} day	
≥ 0.5	≥ 0.5	3
≥ 0.5	< 0.5	2
< 0.5	< 0.5	1

Table 14 The score number for redispersibility of all dispersions

Redispersibility (times)		Score number
7^{th} day	14^{th} day	
≥ 5	≥ 5	3
≥ 5	< 5	2
< 5	< 5	1

Table 15 The score number for viscosity of all dispersions

Viscosity (cps)	Score number
< 100	0
100 - < 200	1
200 - 600	3
> 600 - 700	1
> 700	0

The total score number are shown in Tables 16 - 19 for calcium carbonate suspension that containing each of modified starches (MGS, MRS, and MTS) at concentrations of 1.0 %, 2.0 % and 3.0 % w/v. and UT at concentrations of 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v.

Therefore, summary of scoring number of each type of suspending agent in decreasing order was as following: MGS at the concentrations of 1.0 % > 3.0 % > 2.0 % w/v, MRS at the concentrations of 2.0 % > 1.0 % > 3.0 % w/v, MTS at the concentrations of 3.0 % > 2.0 %, 1.0 % w/v and UT at the concentrations of 4.0 % > 3.0 %, 2.0 % > 1.0 % w/v.

In summary, MGS, MRS, MTS and UT that selected for further studies were those with concentrations of 1.0 %, 2.0 %, 3.0 % and 4.0 %w/v, respectively. Selected concentration of each modified starch was applied to be used as suspending agent in dry syrup formulation.

Table 16 Score number of modified glutinous rice starch

Parameter	Score number		
	1.0 % w/v	2.0 % w/v	3.0 % w/v
Reconstitution time	2	1	0
Sedimentation volumes	1	1	2
Ease of redispersibility	3	1	3
Viscosity	3	0	0
Total	9	3	5

Table 17 Score number of modified rice starch

Parameter	Score number		
	1.0 % w/v	2.0 % w/v	3.0 % w/v
Reconstitution time	3	2	2
Sedimentation volumes	1	1	1
Ease of redispersibility	2	2	3
Viscosity	1	3	0
Total	7	8	6

Table 18 Score number of modified tapioca starch

Parameter	Score number		
	1.0 % w/v	2.0 % w/v	3.0 % w/v
Reconstitution time	3	2	1
Sedimentation volumes	3	3	3
Ease of redispersibility	1	1	1
Viscosity	0	1	3
Total	7	7	8

Table 19 Score number of Ultrasperse®2000

Parameter	Score number			
	1.0 % w/v	2.0 % w/v	3.0 % w/v	4.0 % w/v
Reconstitution time	3	3	3	2
Sedimentation volumes	1	2	2	3
Ease of redispersibility	1	1	1	1
Viscosity	0	0	0	3
Total	5	6	6	9

Part III: Application of Selected Modified Starches in Dry Syrup Formulation

In this study, two drugs as amoxicillin trihydrate and cephalexin monohydrate were used in model dry syrup. Assay of drug was used high preferment liquid chromatography (HPLC) method. The result of assay model drug was followed.

Assay of Drugs

1. Assay of Amoxicillin Trihydrate (USP 24)

The content of amoxicillin trihydrate was assayed by HPLC method that described under the monograph of amoxicillin and amoxicillin for oral suspensions in USP 24. A representative HPLC chromatogram of amoxicillin trihydrate indicated the peak of amoxicillin trihydrate at the retention time of 3.108 (Appendix D; Figure D1). Peak areas of amoxicillin trihydrate obtained from HPLC analysis of accurate various concentration of amoxicillin trihydrate solution were demonstrated in Appendix D; Table D8. The Beer's law plot between the concentrations of amoxicillin trihydrate and peak areas revealed excellent linearity with correlation coefficient of 0.9999 and slope of 11,794 as shown in Appendix D; Figure D3.

2 Assay of Cephalexin Monohydrate

The content of cephalexin monohydrate was determined by HPLC method when using pyrazinamide as an internal standard. The representative HPLC chromatogram showed the peaks of pyrazinamide and cephalexin at the retention times of 3.418 and 4.681 min, respectively (Appendix D; Figure D2). The peak area ratios of cephalexin monohydrate to pyrazinamide obtained from HPLC analysis of various standard aqueous solution of cephalexin monohydrated are shown in Appendix D; Table D9. The calibration curve plotted between the concentration of cephalexin monohydrate and the peak area ratio of cephalexin monohydrated to pyrazinamide revealed excellent linearity with correlation coefficient of 0.9999 and slope of 0.0521 (Appendix; Figure D4).

For method validation and system suitable of drugs are show in Appendix D.

After the preliminary evaluation of Ultrasperse® 2000 and modified starches, it was found that different type of modified starch exhibited the best properties as suspending agent at different percentages in formula. The suspending agent concentrations that gave property for each suspending agent were 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v for MGS, MRS, MTS and UT, respectively. Since, pH of the formulation affected the stability of the drugs. Then, suitable buffer used in formula to keep pH was required. However, buffer is a salt which might be incompatible with modified starches and then caused a decrease in viscosity of the formulation. Thus, it was necessary to study the effect of buffer concentration on content of drugs and viscosity of model dry syrup after reconstitution.

A. Development of Model Dry Syrup Formulation

After the preliminary evaluation of Ultrasperse® 2000 and modified starches, it was found that different type of modified starch exhibited the best properties as suspending agent at different percentages in formula. The suspending agent concentrations that gave property for each suspending agent were 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v for MGS, MRS, MTS and UT, respectively. Since, pH of the formulation affected the stability of the drugs. Then, suitable buffer used in formula to keep pH was required. However, buffer is a salt which might be incompatible with modified starches and then caused a decrease in viscosity of the formulation. Thus, it was necessary to study the effect of buffer concentration on content of drugs and viscosity of model dry syrup after reconstitution.

1. Effect of Buffer Concentration on Content of Drugs

Suitable pH buffer concentration for amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup followed the pharmaceutical requirement and pH-rate profile of active ingredients.

Amoxicillin trihydrate is stable at pH 5.0 - 7.0 as shown in Figure 35 (Tsuji et al, 1978). The degradation rate is determined quantitatively as a function of pH. In the pH range studied of 0.30 - 10.50, the degradation of amoxicillin follows pseudo-

first-order kinetics to give type of pH-rate profile as those of ampicillin and cyclacillin (Tsuji et.al., 1978). For cephalexin monohydrate, it is stable at pH 2.0 - 5.0 which is presented in Figure 36 (Yamana and Tsuji, 1976). According to USP 24, the required pH for Amoxicillin for Oral Suspension is between 5.0 and 7.5; and that for Cephalexin for Oral Suspension is between 3.0 and 6.0. In this study, the pH values of amoxicillin trihydrate and cephalexin monohydrate dry syrup were controlled at 6.0 and 4.5, respectively. Also Appendix E; Tables 1E - 2E and Figures 37 show the remaining percentage of amoxicillin trihydrate when keeping at room temperature or in refrigerator (8.0 ± 1 ºC) for 14 days, when using citrate buffer with different concentrations for controlled pH of the formulation. The lowest buffer concentration which could stabilize the amoxicillin trihydrate dry syrup was as 0.05 molar. On the contras, the fast degradation was occurred with amoxicillin trihydrate dry syrup without buffer. The remaining percentages of amoxicillin trihydrate after keeping for 14 days at room temperature and in refrigerator (8.0 ± 1 ºC) were 59.78 and 82.14, respectively.

The pH of cephalexin monohydrate dry syrup controlled by citrate buffer at the same concentrations of those controlling pH of the amoxicillin trihydrate dry syrup. This study found that the lowest concentration of citrate buffer that could stabilize which was strolled at both room temperature and in refrigerator (8.0 ± 1 ºC) was 0.05 molar. Drug content of dry syrup with 0.05 molar citric buffer were 98.96 – 100.20 % when keeping at room temperature for 14 days (Appendix E; Tables 3E-4E and Figures 38).

On the other hand, the fast degradation was occurred with cephalexin monohydrate dry syrup without buffer. The remaining percentages of cephalexin monohydrate after keeping at room temperature and in refrigerator (8.0 ± 1 ºC) for 14 days were 87.63 % and 95.26 %, respectively. This result implied that buffer at the concentration of 0.05 molar could stabilize cephalexin monohydrate dry syrup which kept at room temperature and in refrigerator (8.0 ± 1 ºC).

However, when cephalexin monohydrate dry syrup was reconstituted with deionized water and kept at room temperature, the cephalexin monohydrate dry syrup was not stable. While the vice versa result was occurred when that was kept in refrigerator (8.0 ± 1 ºC) for 14 days. Consequently, citrate buffer at the concentration of 0.05 molar was chosen for using as stabilizer in both amoxicillin trihydrate and cephalexin monohydrate dry syrup.

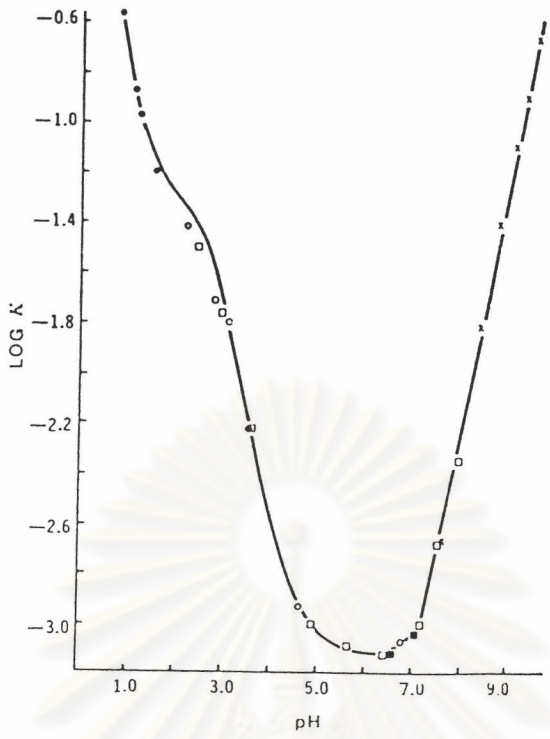


Figure 35 pH rate profile for amoxicillin in the pH range studied of 0.30-10.50 (Tsuji et al, 1978)

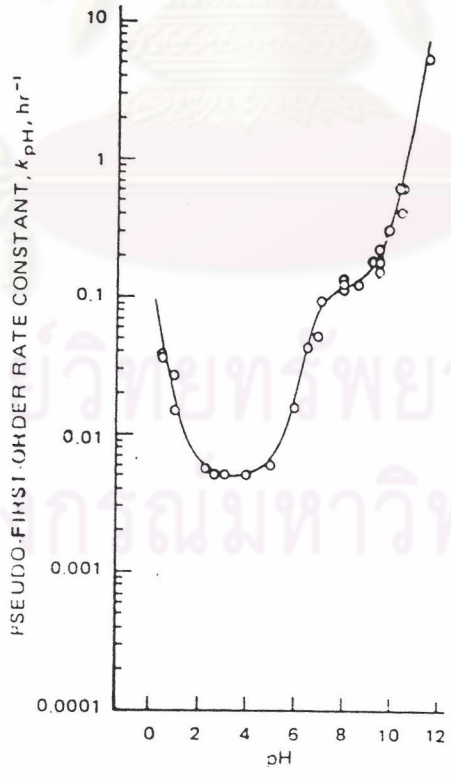


Figure 36 pH rate profile for cephalexin at 35 °C (Yamana and Tsuji, 1976)

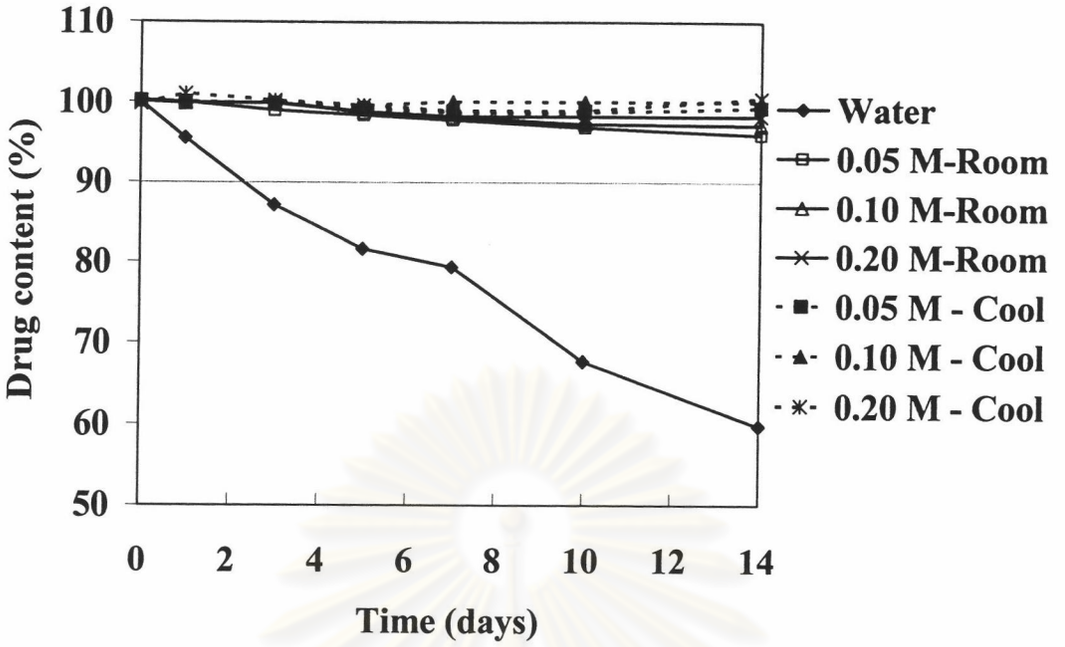


Figure 37 Comparison of drug content of amoxicillin trihydrate in citrate buffer pH 6.0, kept at room temperature and in refrigerator for 14 days at different buffer concentrations

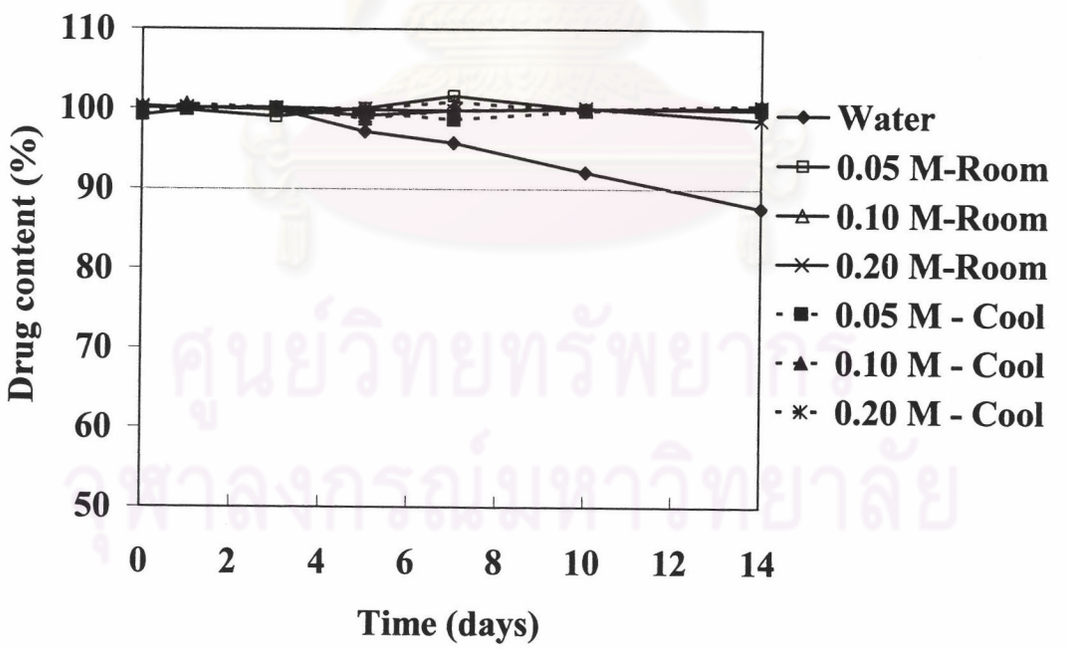


Figure 38 Comparison of drug content of cephalexin monohydrate in citrate buffer pH 4.5, kept in room temperature and in refrigerator for 14 days at different buffer concentrations

3. Effect of Suspending Agent (MGS, MRS, MTS and UT) and Buffer Concentrations on Viscosity

Sodium citrate is salt in citrate buffer system which might affect the viscosity for modified starches and Ultrasperse®2000 dispersions. Composition of citrate buffer pH 4.5 and 6.0 at different concentrations is shown in Table 20.

The effect of pH buffer and concentration on the viscosity of MGS, MRS, MTS and UT dispersions at various suspending agent concentrations is shown in Figure 39 – 44.

For both pH 4.5 and 6.0, the influence of buffer concentration on viscosity of MGS, MRS, MTS and UT dispersions could be noted. At the same buffer concentration, the dependence of viscosity with respect to the pH of modified starches and Ultrasperse®2000 dispersions was reported. The pH value of 6.0 had more effect on viscosity of modified starches and Ultrasperse®2000 dispersions than pH value of 4.5. Comparison of effect of buffer concentration on viscosity of modified starches and Ultrasperse® 2000 are shown in Figures 39 - 44.

The important criteria used for selecting the concentration of citrate buffer were the capability to stabilize the drugs in the formulation when kept at both room temperature and in refrigerator for 14 days. In this study, the lowest buffer concentration was required. An increase in buffer concentration resulted a decrease in viscosity of modified starches and Ultrasperse®2000 dispersions.

According to previous study (PartII), the suitable concentration of each suspending agent that gave appropriate viscosity of dispersion (200 -600 cps) without using buffer solution was reported. Based on calcium carbonate reconstituted suspension, the suspending agent concentrations were 1.0 %, 2.0 %, 3.0 % and 4.0 % w/v, respectively.

The concentrations of MGS, MRS, MTS and UT used to remain the viscosity of (200-600 cps) amoxicillin trihydrate dry syrup containing 0.05 molar citrate buffer pH 6.0 were 2.5 %, 3.0 % ,3.0 % and 4.0 % w/v, respectively (Figure 43). For MRS dispersion, the concentrations of 2.5 % and 3.0 % w/v were within the required range of viscosity. Since, the viscosity of dry syrup might decrease when the other excipient were used in the formulation. Therefore, the higher concentration (3.0 % w/v) was chosen.

Similarly, the concentrations of MGS, MRS, MTS and UT that gave the appropriate viscosity of cephalexin monohydrate reconstituted suspension containing 0.05 molar citrate buffer pH 4.5 were 2.5 %, 2.5 %, 3.0 % and 4.0 % w/v, respectively (Figure 44).

Table 20 Composition of citrate buffer pH 4.5 and 6.0 at different concentration

Molar	pH 4.5 (g/100 ml)		pH 6.0 (g/100 ml)	
	Acid*	Salt**	Acid*	Salt**
0.05 M	0.4274	0.8161	0.1104	1.3014
0.10 M	0.8548	1.6323	0.2209	2.6028
0.20 M	1.7095	3.2645	0.4418	5.2056

* Acid is anhydrous citric acid

** Salt is sodium citrate dihydrate

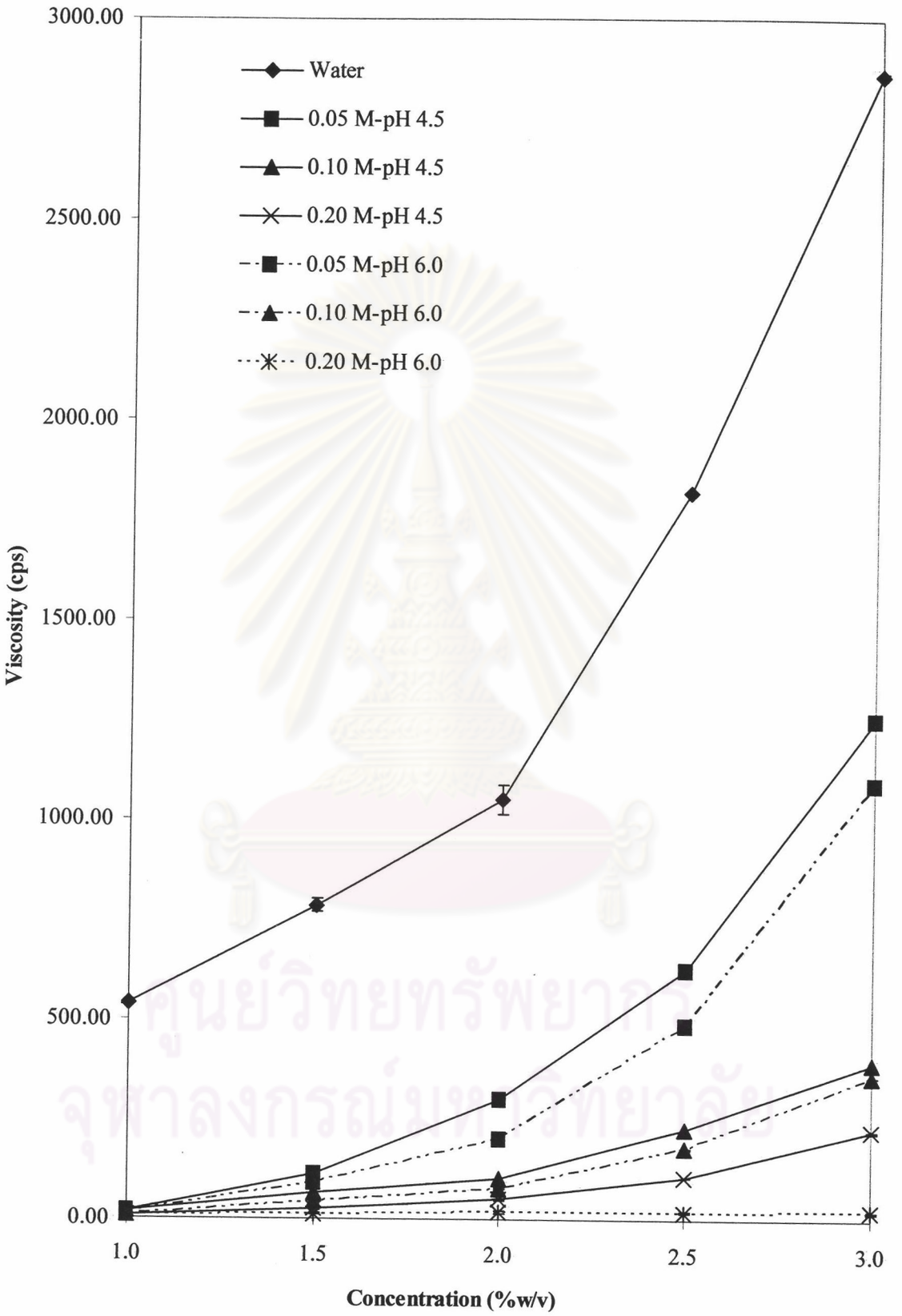


Figure 39 Comparison of viscosity of MGS dispersion in various citrate buffers concentrations at pH value 4.5 and 6.0

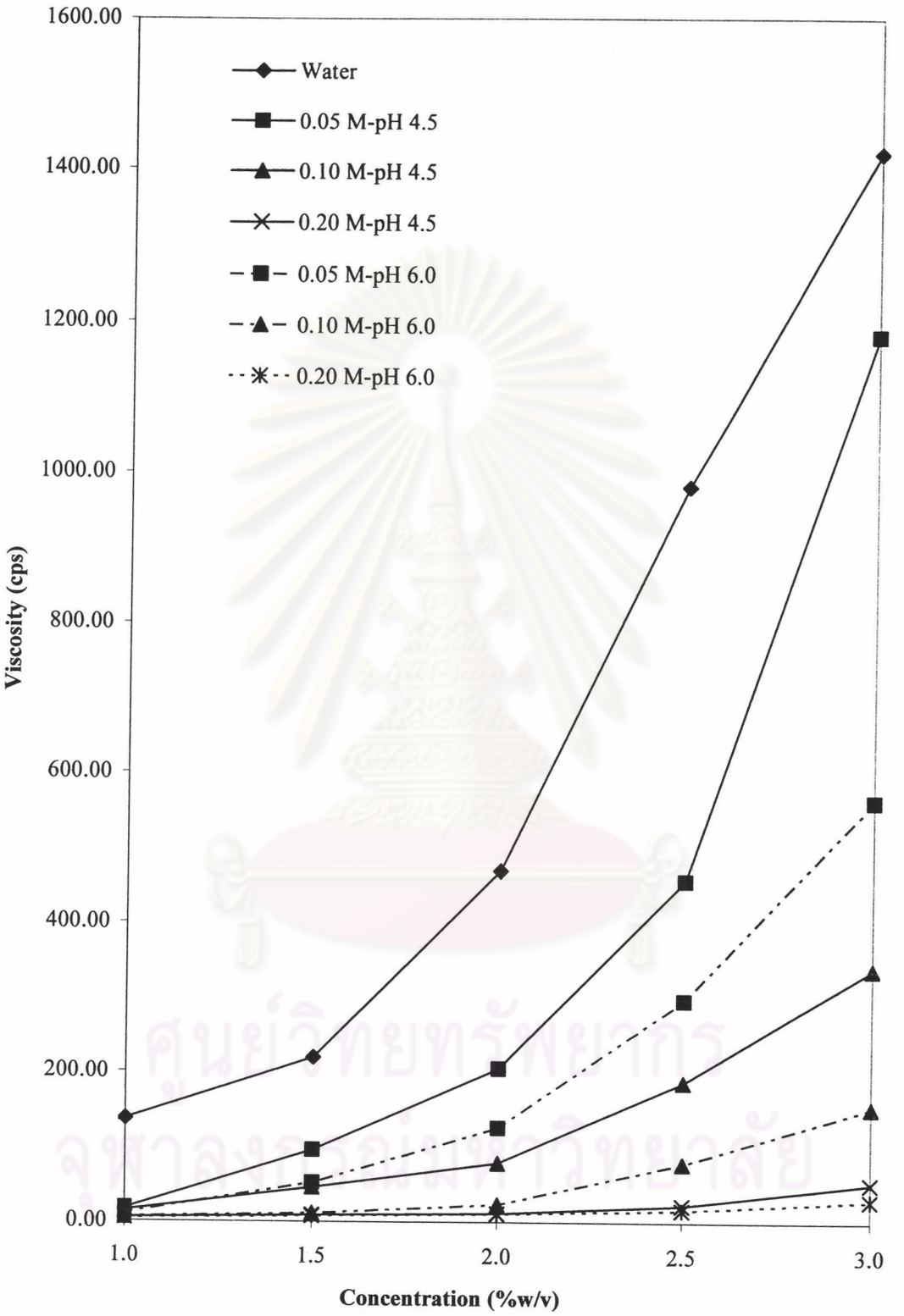


Figure 40 Comparison of viscosity of MRS dispersion in various citrate buffers concentrations at pH value 4.5 and 6.0

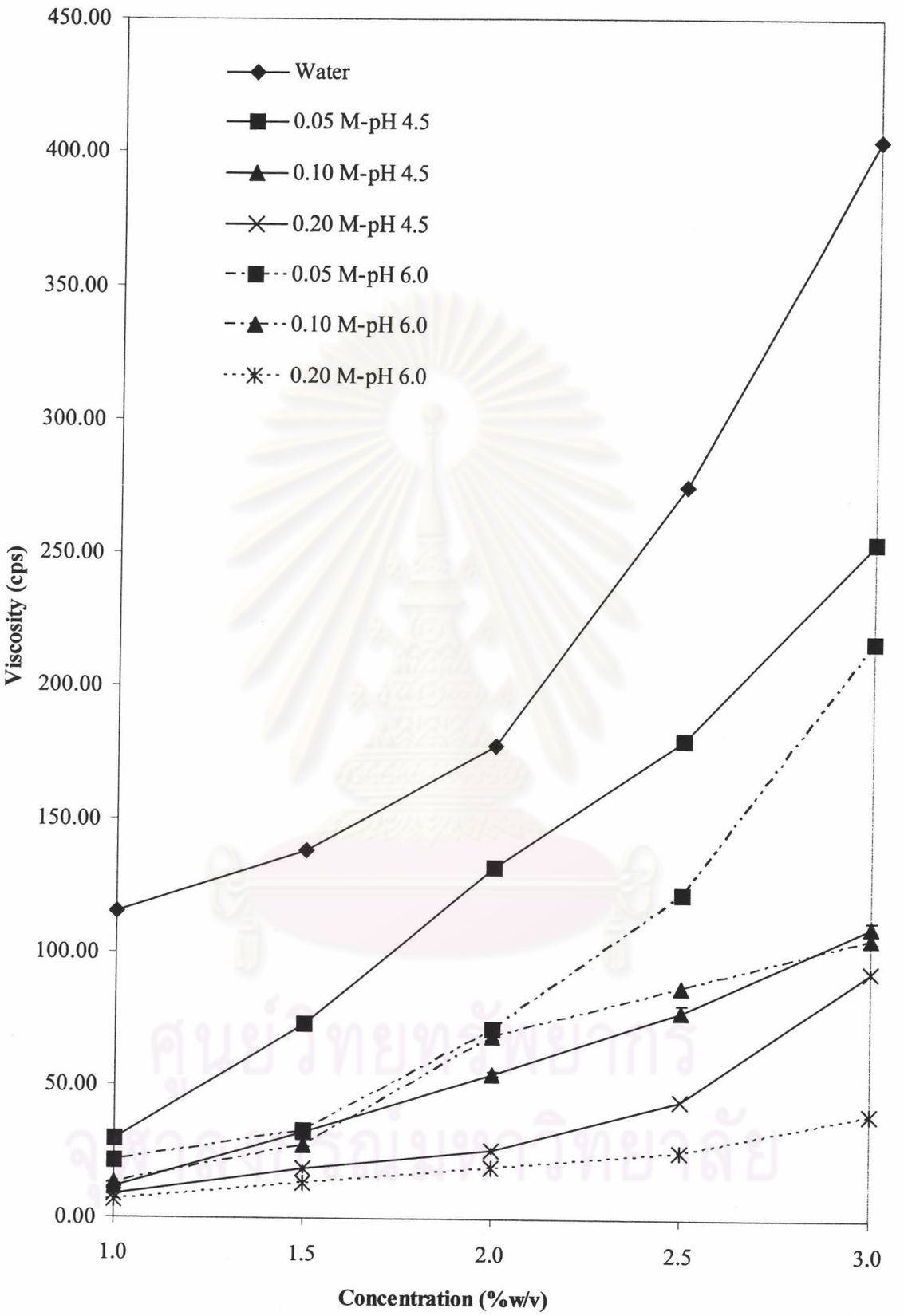


Figure 41 Comparison of viscosity of MTS dispersion in various citrate buffers concentrations at pH value 4.5 and 6.0

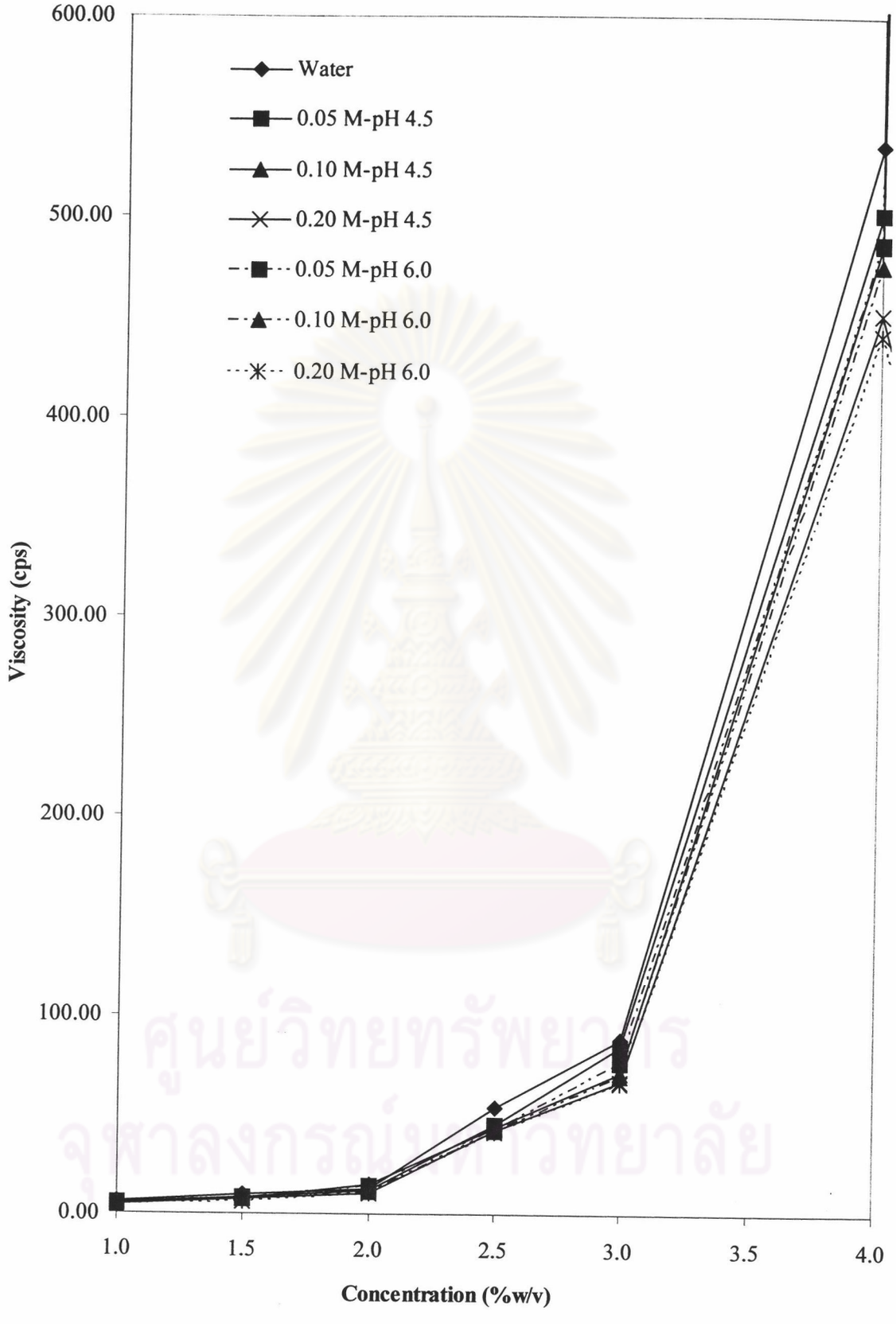


Figure 42 Comparison of viscositiy of UT dispersion in various citrate buffers concentrations at pH value 4.5 and 6.0

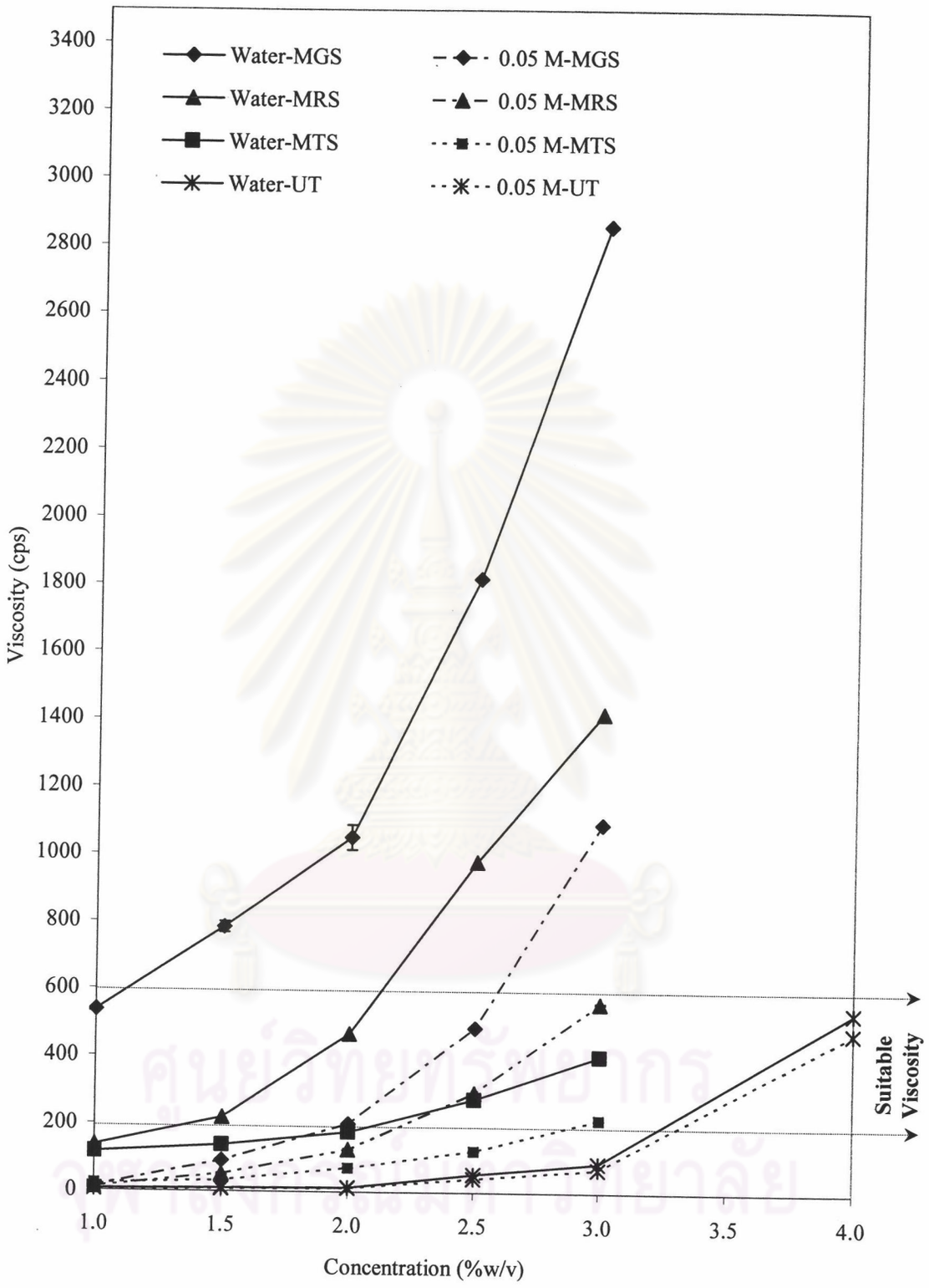


Figure 43 Comparison of viscosity of MGS, MRS, MTS and UT in water and 0.05 molar citrate buffers pH 6.0

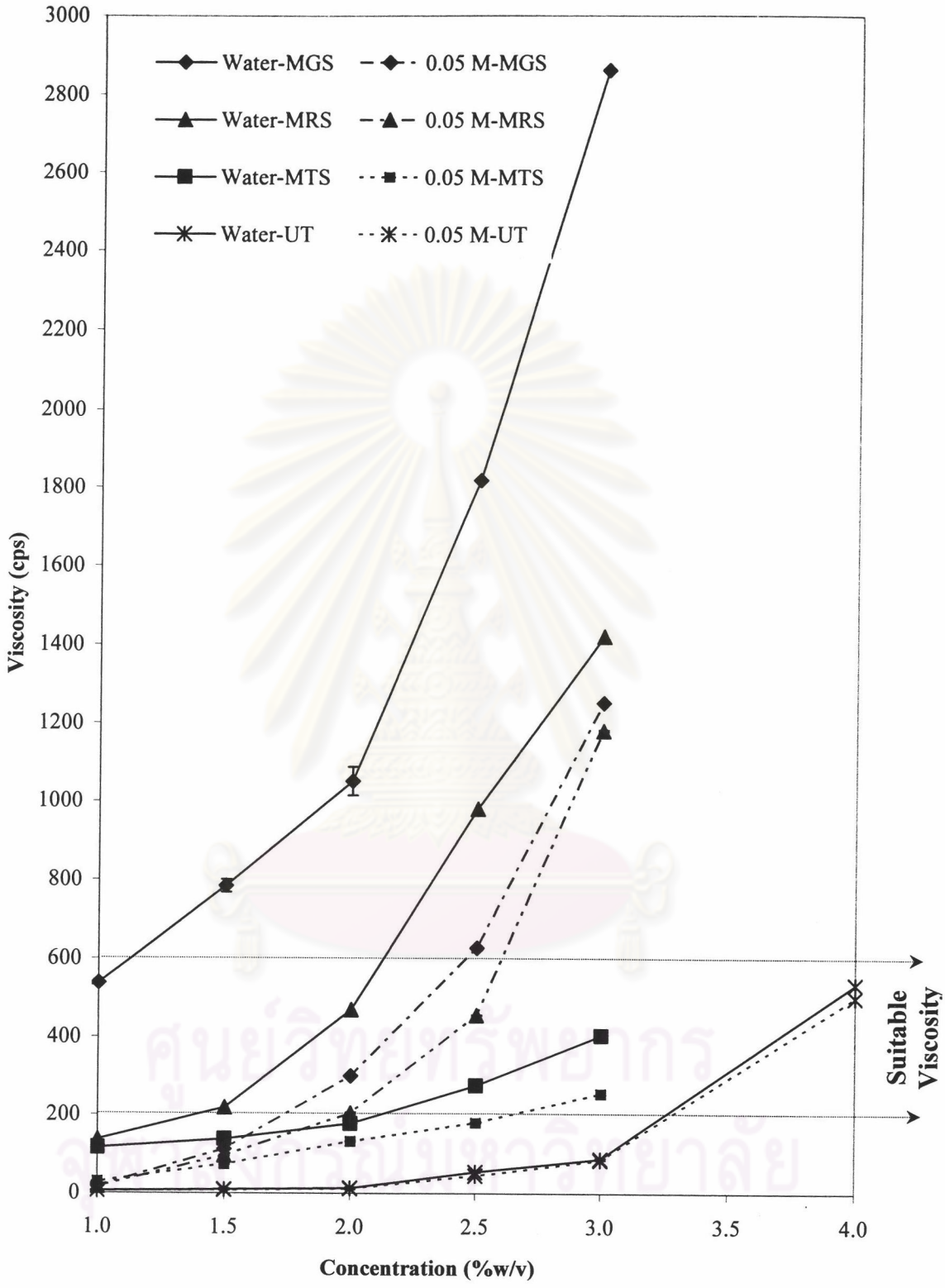


Figure 44 Comparison of viscosity of MGS, MRS, MTS and UT in water and 0.05 molar citrate buffers pH 4.5

3. Formulation of Model Dry Syrup

The main objected of this stuffy was to develop a stable dry syrup. However, amoxicillin trihydrate was not having good taste and odors. Thus the formulation design, the good taste and odors should develop contemporaneously. The non active ingredients of model dry syrup (amoxicillin trihydrate and cephalexin monohydrate at concentration of 125 mg/5 ml) were sweetener, preservative, buffer, anticaking agent and bulking agent. Methyl paraben (MP) and propyl paraben (PP) were used as preservative. Citrate buffer was used to control pH. Icing sugar was used as bulking agent and a sweetening agent. Aerosil®, at the concentration of 0.5 - 1.0 % w/v, was used as anticaking agent.

3.1 Amoxicillin Trihydrate Dry Syrup

Since amoxicillin trihydrate had bitter taste and characteristic odor, suitable flavoring agent and sweetener were selected. Sweetening agents were compared among of aspartame, saccharin sodium, acelsulfame potassium and sodium cyclamate at concentration of 0.02 - 2.0 %, 0.02 - 0.05 %, 0.05 - 0.7 % and 0.01 - 0.2 % w/v, respectively. The taste and appearance of selected formulations were observed. It was found that acelsulfame potassium at concentration of 0.5 % w/v and aspartame at concentration of 2.0 % w/v might be used. However, aspartame were not stable, thus acelsulfame potassium was consider. Furthermore, compatibility of flavoring and coloring agent should consider. Orange flavor powder and sunset yellow were used in amoxicillin trihydrate dry syrup formulation at concentration of 2.0 % and 0.005 % w/v, respectively. Then, the good appearance of formulation was occurred. In conclusion, the formulations of amoxicillin trihydrate dry syrup was as following.

Amoxicillin trihydrate	2.5	g
Citrate buffer pH 6.0	0.05	% w/v
Suspending agent	*a	% w/v
Aerosil®200	0.50	% w/v
Acelsulfame potassium	0.50	% w/v
Orange flavor powder	2.0	% w/v
Sunset yellow	0.005	% w/v
Methyl paraben (MP)	0.18	% w/v
Propyl paraben (PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = Percentage of each suspending agent (MGS, MRS, MTS and UT)

According to preliminary study, the percentage of suspending agents used in formulation of cephalexin monohydrate dry syrup was different. Modified glutinous rice starch (MGS), modified rice starch (MRS), modified tapioca starch (MTS) and Ultrasperse®2000 (UT) which used as suspending agent were 2.5 %, 3.0 %, 3.0 % and 4.0 % w/v, respectively

3.2 Cephalexin Monohydrate Dry Syrup

The development of cephalexin monohydrate dry syrup was influenced by bitter taste, characteristic odor, color, and flavor which to satisfy taste and appearances. Sweetening agents in formulation were compared as among saccharin sodium, acelsulfame potassium and sodium cyclamate at concentration of 0.02 - 4.0%, 0.05 - 1.0 % and 0.01 - 0.5 % w/v. Acceptable when using acelsulfame potassium at concentration of 0.08 % w/v was selected. Further, compatibility of flavoring and coloring agent was selected. Raspberry flavor powder and ponceua 4R was used in cephalexin monohydrate dry syrup formulation at the concentration of 0.50 % and 0.004 % w/v, respectively. Then, the good appearance of formulation was observed. In conclusion, the formulation of cephalexin monohydrate dry syrup was as following.

Cephalexin monohydrate	2.5	g
Citrate buffer pH 6.0	0.05	% w/v
Suspending agent	*a	% w/v
Aerosil®200	1.0	% w/v
Acelsulfame potassium	0.08	% w/v
Raspberry flavor powder	0.50	% w/v
Ponceua 4R	0.004	% w/v
Methyl paraben(MP)	0.18	% w/v
Propyl paraben(PP)	0.02	% w/v
Icing sugar qs to	30	g
Purified Water qs ad	100	ml

*a = Percentage of each suspending agent (MGS, MRS, MTS and UT)

According to preliminary study, the percentage of suspending agents used in formulation of cephalexin monohydrate dry syrup was different. Modified glutinous rice starch (MGS), modified rice starch (MRS), modified tapioca starch (MTS) and Ultrasperse®2000 (UT) which used as suspending agent were 2.5 %, 2.5 %, 3.0 % and 4.0 % w/v, respectively.

4. Evaluation of the Formulation

4.1 Dry Powder

4.1.1 Appearance Model Dry Syrup

Amoxicillin trihydrate dry syrup was slightly orange powder with orange scent and cephalexin monohydrate dry syrup was light pink powder with raspberry scent (Figure 45). In both cases, particle of formulation could be easily manipulated into container and able to sustained cake formation.

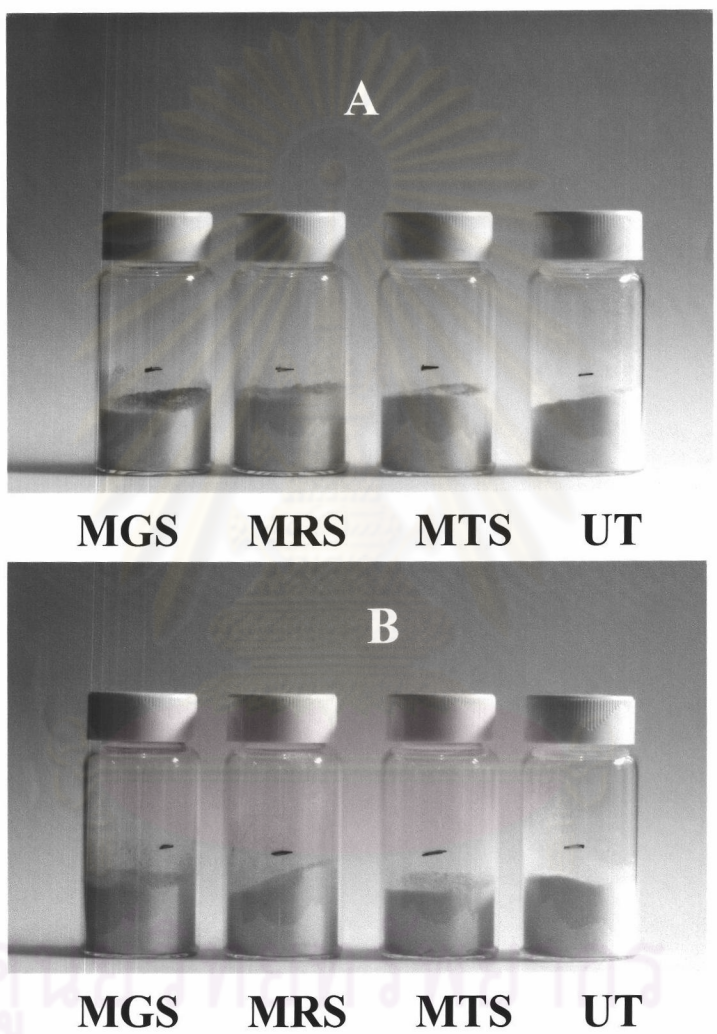


Figure 45 Photographs of freshly prepared model dry syrup using different suspending agents

Key (A) Amoxicillin trihydrate dry syrup

(B) Cephalexin monohydrate dry syrup

4.1.2 Content of Drugs in Model Dry Syrup

Content uniformity of amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup formulations were shown promising. The dry powder was sampling at three different locations of each formulation. The percent labeled amount of dry syrup ranged from 100.36 to 101.10 (0.14-0.42) and that of cephalexin monohydrate dry syrup was between 99.96 - 101.63 (0.03 - 0.51). The standard deviations (SD) of percentage labeled amount for both formulations were less than 0.6 (Tables 21).

Since the acceptable limit of percentage labeled amount conforming to USP 24 is in the range of 80 -120 %, the percentage labeled amount of both formulations passed the specification.

4.1.3 Determination of Water Content in Model Dry Syrup

The water content of both formulations in both formulations is presented in Table 22. The percentage of water content in amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup were 2.00 - 2.93 % w/w and 0.99 - 1.61 % w/w, respectively. The result indicated that the percentage of water content of all formulations were with in the USP 24 acceptable limit.

The water content of both preliminary model dry syrups when using MTS as suspending agent was high than of MGS, MTS and UT, respectively. Therefore, the water content might not pass the USP 24 signification when the formulation was kept longer and hence the stability was decreased.

Table 21 Drug content of amoxicillin trihydrate and cephalexin monohydrate dry syrup using different suspending agents

Suspending agent	Drug content (%) Average (SD)	
	Amoxicillin trihydrate	Cephalexin monohydrate
MGS	101.14 (0.14)	101.63 (0.30)
MRS	100.36 (0.21)	99.96 (0.47)
MTS	100.96 (0.17)	100.36 (0.36)
UT	101.03 (0.42)	100.42 (0.51)

Table 22 Percentage of water content in freshly prepared amoxicillin trihydrate and cephalexin monohydrate dry syrup using different types of suspending agent

Suspending agent	Water content (% w/w) Average (SD)	
	Amoxicillin trihydrate	Cephalexin monohydrate
MGS	2.05	0.99
MRS	2.00	1.07
MTS	2.93	1.61
UT	2.30	1.29

4.2 Reconstitution of Model Dry Syrup

4.2.1 Physical Property Determinations of Model Reconstituted Suspension

Freshly prepared of amoxicillin trihydrate reconstituted suspension was reddish yellow homogeneous dispersion with orange scent and sweet taste. The pH of sample was between 5.98 ± 6.04 (Table 23) and the standard deviation was less than 0.07. After storing the samples in room temperature and in refrigerator (8.0 ± 1 °C) for 7 and 14 days, samples became flocculated and could be redispersed after shaking. The appearance of suspension after keeping for 14 days was no changed (Figure 46).

Freshly prepared of cephalexin monohydrate reconstituted suspension was pink homogeneous dispersion with raspberry scent and sweet. The pH of samples was between 4.53 ± 0.04 (Table 24) and the standard deviation was less than 0.07. After storage in room temperature and refrigerator (8.0 ± 1 °C) for 7 and14 days, the result showed no change in appearance and similar as amoxicillin trihydrate suspension (Figure 45).

Moreover, the results in term of pH were presented stable, all of formulation were within allowanced range of standard (USP 24; pH of Amoxicillin for Oral Suspension was between 5.0 and 7.5 and Cephalexin for Oral Suspensions was between 3.0 and 6.0).

4.2.2 Determination of Reconstitution Time

Reconstitution times of all reconstituted model dry syrup were less than five times (Table 24). Reconstitution time of amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup could be concluded from higher to lower value as following: UT > MTS > MGS > MRS and UT > MRS > MGS > MTS, respectively.

In addition, good formulation in previously study (calcium carbonate suspension) was found the limited of reconstitution time less than 5 minutes and results from formulation design studies were within allowanced ranged.

However, unsuitable characteristic of reconstituted suspension using MTS as suspending agent was observed. When the dry syrup reconstituted by water and shaken, many bubbles were occurred and they remained even after 14 days (Figures 47 - 48).

Table 23 The pH value of freshly prepared amoxicillin trihydrate and cephalexin monohydrate reconstituted suspensions using various suspending agents

Suspending agent	pH Average (SD)	
	Amoxicillin trihydrate	Cephalexin monohydrate
MGS	5.98 (0.06)	4.55 (0.06)
MRS	6.01 (0.02)	4.53 (0.04)
MTS	6.03 (0.03)	4.51 (0.01)
UT	6.04 (0.07)	4.52 (0.04)

Table 24 Reconstitution time of amoxicillin trihydrate and cephalexin monohydrate reconstituted suspensions using various suspending agents

Suspending agent	Reconstitution time (times) Average (SD)	
	Amoxicillin trihydrate	Cephalexin monohydrate
MGS	3.33 (0.58)	3.50 (0.50)
MRS	2.67 (0.29)	3.67 (0.29)
MTS	3.50 (0.00)	2.83 (0.29)
UT	4.83 (0.29)	4.00 (0.50)

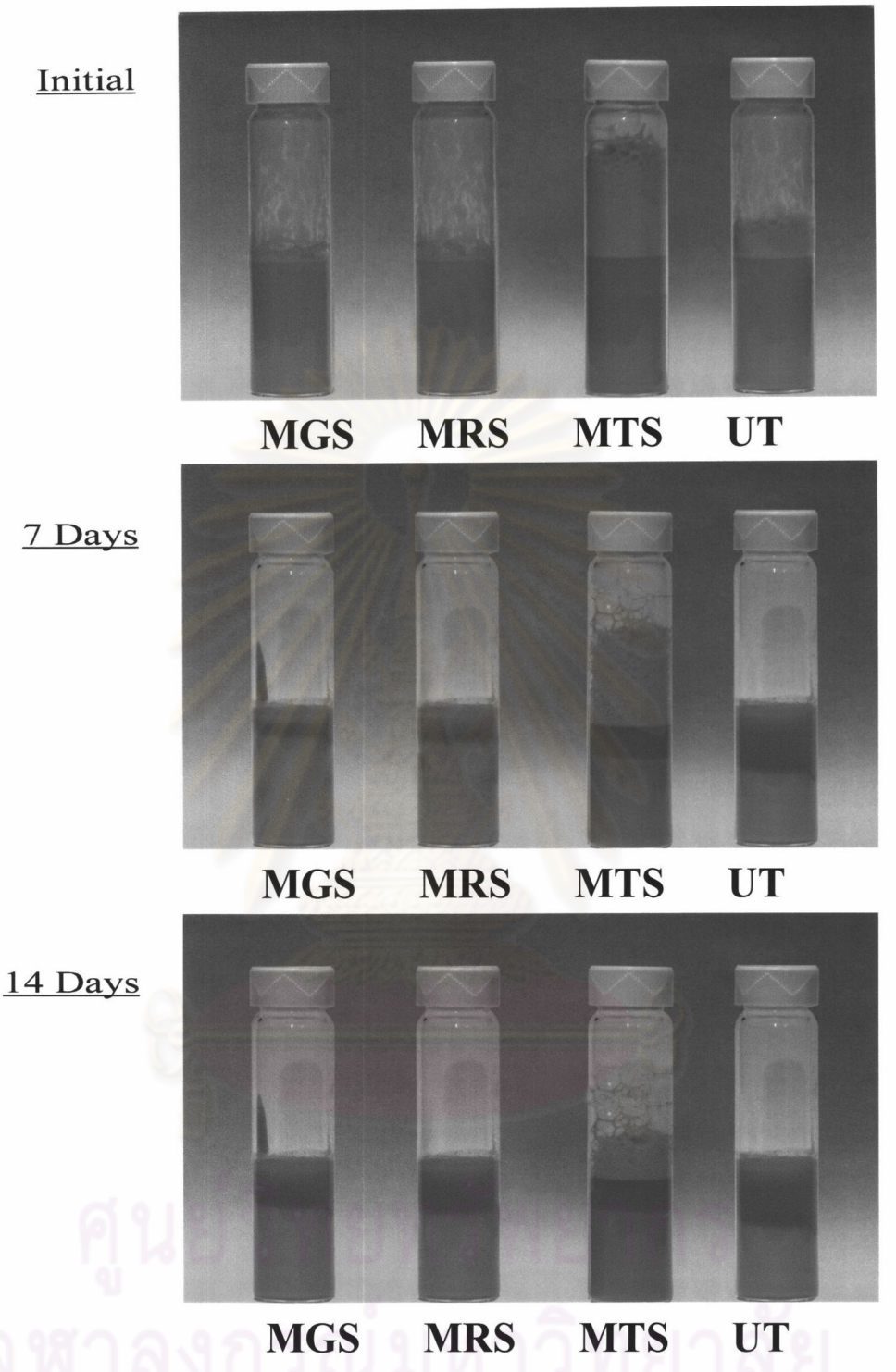


Figure 46 Comparison of amoxicillin trihydrate reconstituted suspension using different suspending agents when kept in refrigerator (8.0 ± 1 °C) for 7 and 14 days

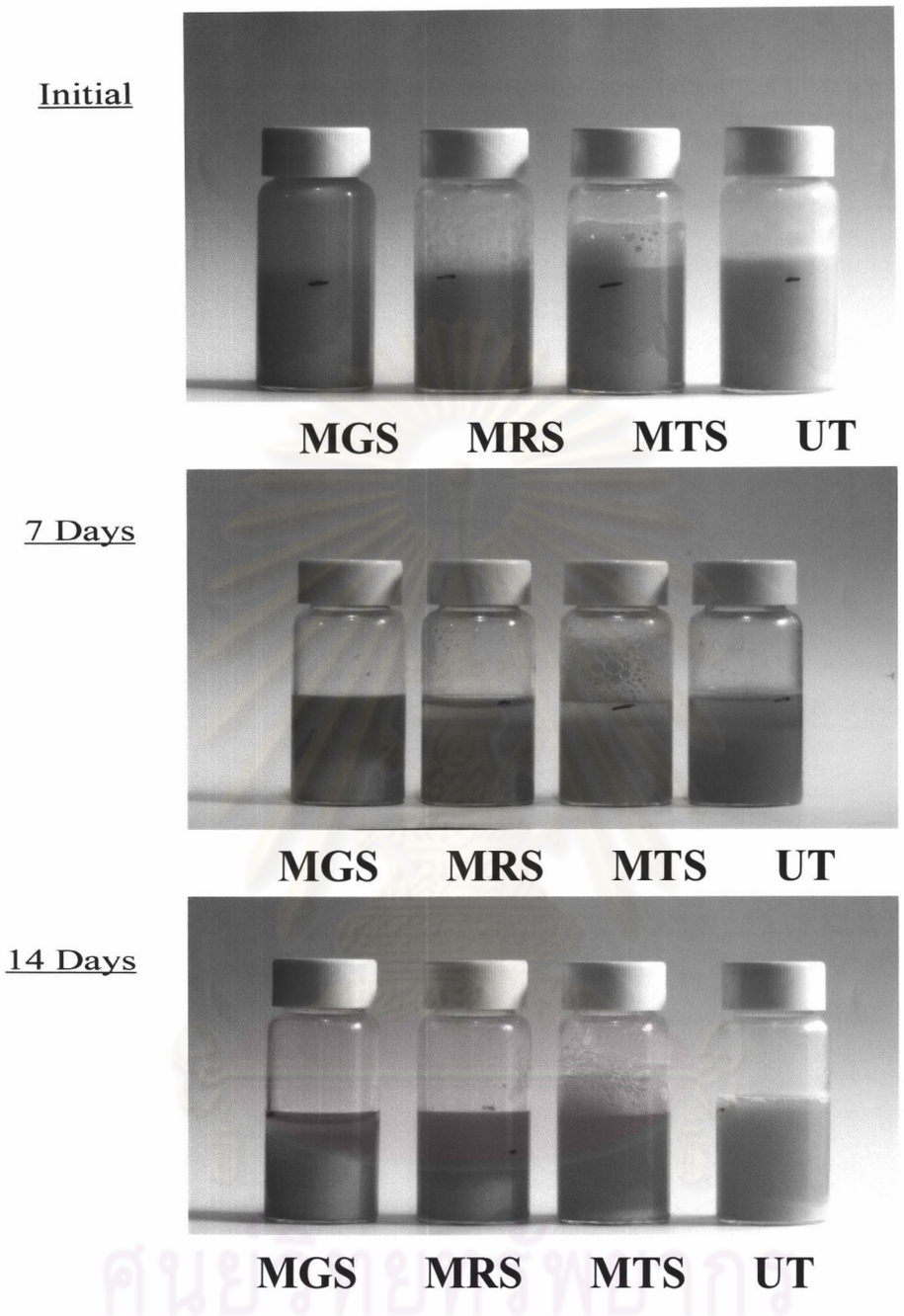


Figure 47 Comparison of cephalexin monohydrate reconstituted suspension using different suspending agents when kept in refrigerator (8.0 ± 1 °C) for 7 and 14 days

4.2.3 Viscosity Measurement of Model Reconstituted Suspension

The average apparent viscosity of amoxicillin trihydrate and cephalexin monohydrate reconstituted suspensions reassure at initial and after 14 days storage are presented in Table 25 and Figures 48 - 49. Initial viscosities of all formulations were range in between 160.75 - 621.23 cps which is considered suitable for formulation. And after storage for 14 days viscosity values were slightly increased but in the range of 203.31 - 574.56 cps. Moreover, the decreasing order of viscosity was as follow: MGS > MRS >UT >MTS for cephalexin monohydrate reconstituted suspensions and MGS > UT > MRS > MTS for amoxicillin trihydrate reconstituted suspensions.

However, the viscosity of reconstituted dry syrup was nearly still in 200- 600 cps range and showed good appearance. The results from formulation design studies were approved.

Table 25 Apparent viscosity of amoxicillin trihydrate and cephalexin monohydrate reconstituted suspensions which freshly prepared and kept for 14 days in refrigerator (8.0 ± 1 °C) , using various suspending agent

	Suspending agent	Apparent viscosity (cps) Average (SD)	
		Amoxicillin trihydrate	Cephalexin monohydrate
Initial	MGS	429.58 (5.99)	564.81 (12.46)
	MRS	621.23 (17.43)	401.23 (10.35)
	MTS	160.75 (5.90)	203.31 (10.19)
	UT	442.68 (12.88)	422.55 (9.80)
14 days in refrigeration	MGS	564.81 (12.46)	574.56 (9.33)
	MRS	401.23 (10.35)	416.44 (9.15)
	MTS	203.31 (10.19)	217.28 (2.30)
	UT	422.55 (9.80)	430.21 (2.99)

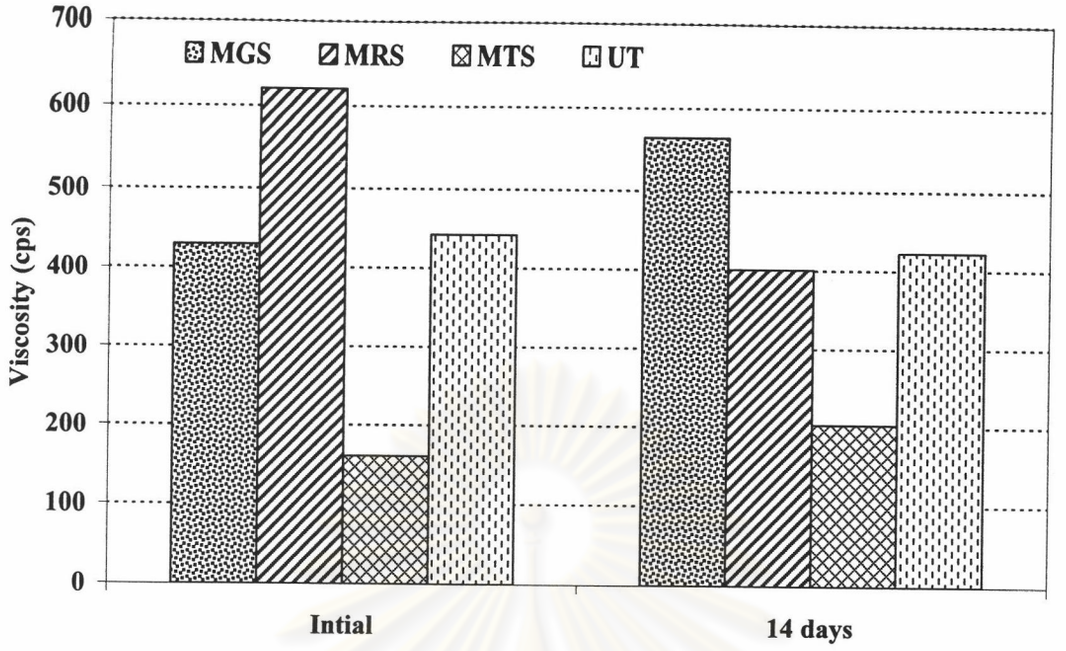


Figure 48 Comparative apparent viscosity of amoxicillin trihydrate reconstituted suspension using various f suspending agents

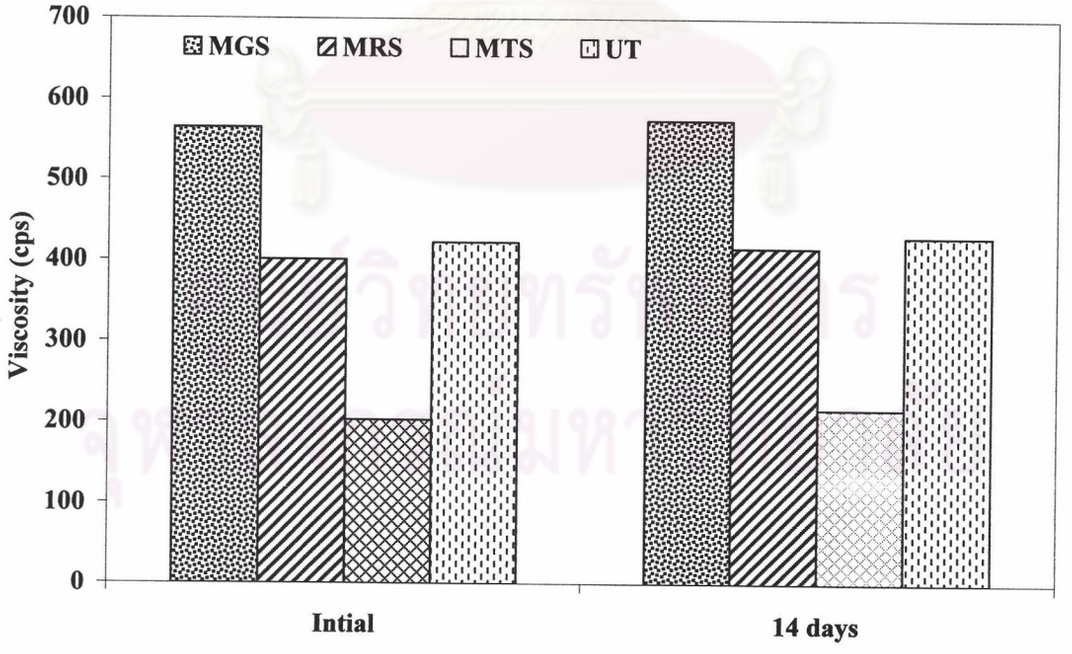


Figure 49 Comparative apparent viscosity of cephalexin monohydrate reconstituted suspension using various suspending agents

4.2.4 Redispersibility of Model Reconstituted Suspensions

Numbers of inversion required to disperse amoxicillin trihydrate reconstituted suspension and cephalexin monohydrate suspension for 7 days and 14 days storage are shown in Figures 50-51.

The result showed the sample used modified starch as suspending agent required 2.00 – 4.33 times of inversion at 7 days storage samples and 3.33 - 6.33 times of inversion for 14 days storage samples. The result was indicated when increased storage time, the redispersibility of model reconstituted suspensions was increased. However, the redispersibility of good reconstituted dry syrup was less than 5 time and the results from formulation design were within allowanced ranged.

4.2.5 Stability of Drugs in Model Reconstituted Suspensions

Content of drug in model reconstituted suspension were presented in term of percentage of the labeled amount after keeping in room temperature and refrigerator (8.0 ± 1 °C), as shown in Tables 26-29. It was found that the content of drugs in all model reconstituted suspension when using different types as suspending agent at suitable pH and viscosity were varies in acceptable ranges.

They were stable at room temperature and in refrigerator (8.0 ± 1 °C) until 14 days. For amoxicillin trihydrate reconstituted suspension, percentage of the labeled amount were 95.77 - 102.31 % and 98.36 -101.55 % when kept at room and in refrigerator (8.0 ± 1 °C), respectively. Cephalexin monohydrate reconstituted suspension, has percentage of the labeled amount were 96.35 – 101.47 % and 99.54 – 101.99 % when kept at room and in refrigerator (8.0 ± 1 °C), respectively. The standard deviation (SD) in all sample not more than 5.0.

In conclusion, the suitable suspending agents in preliminary model dry syrup which were selected for stability study such as MGS, MRS and UT. They had low reconstitution times (2.67 – 4.83 times) and number of inversion for redispersibility property (2.0 - 7.0 times). Moreover, water content and percentage of the labeled amount were in the accepted criteria of USP 24. In addition s, all selected formulations had appropriate viscosity. But MTS was not selected because when the dry syrup reconstituted by water and shaken, many bubbles were occurred contained high water content.

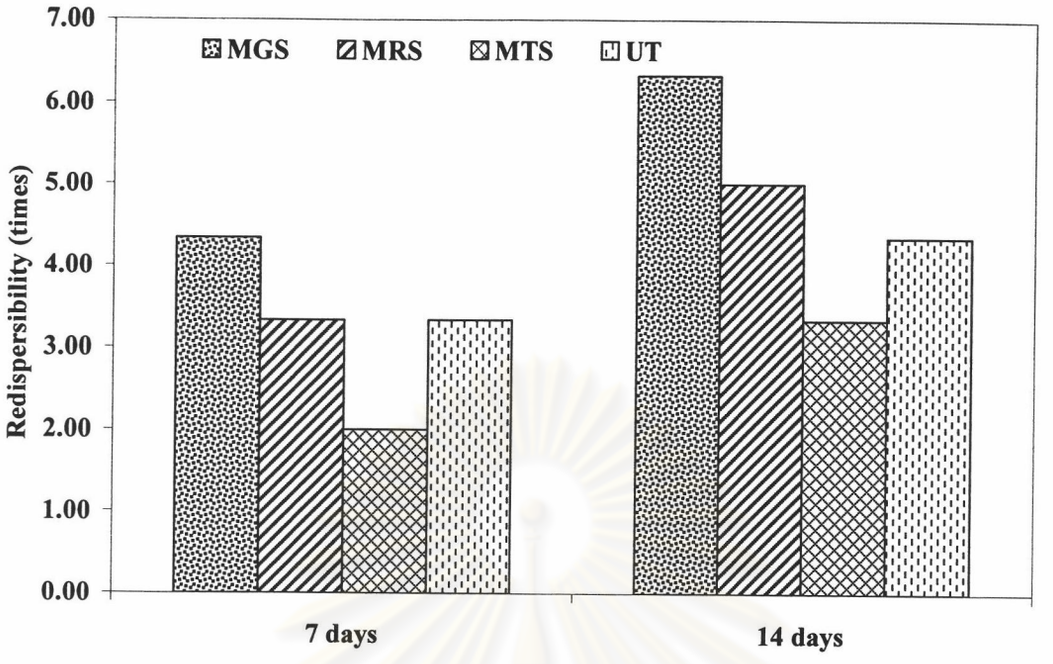


Figure 50 Comparison of average number of inversion required for amoxicillin trihydrate suspension using various suspending agents

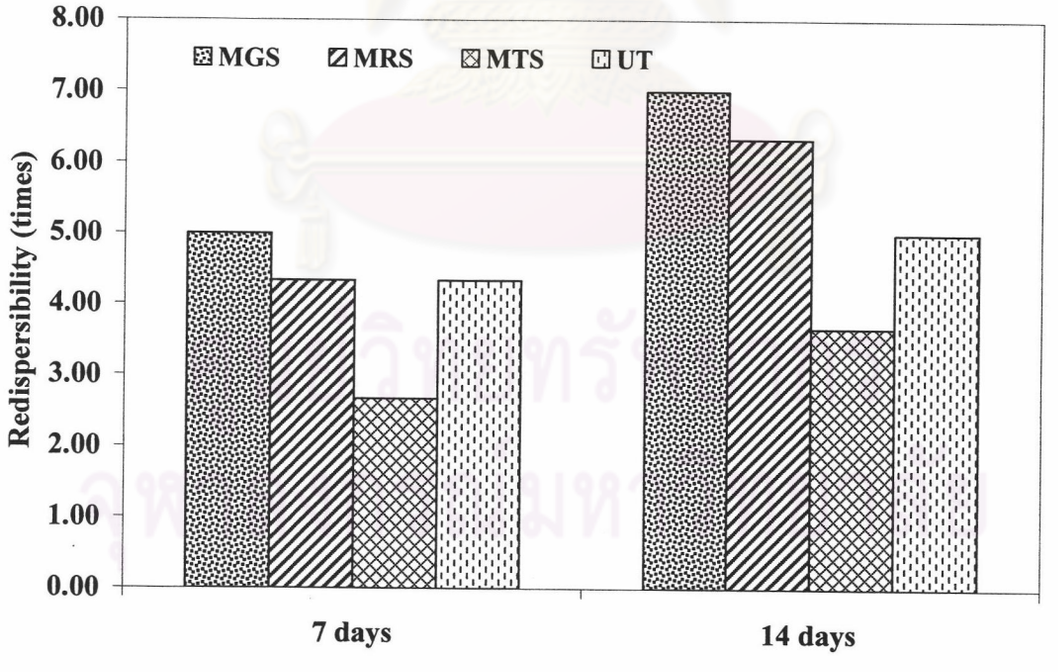


Figure 51 Comparison of average number of inversions required for cephalexin monohydrate suspension using various suspending agents

Table 26 Percentage of remaining drug of amoxicillin trihydrate reconstituted suspensions using different suspending agent, kept at room temperature for 14 days

Time	Drug content (%) Average (SD)			
	MGS	MRS	MTS	UT
0 day	102.31 (0.24)	100.23 (1.44)	101.01 (1.41)	99.89 (4.54)
1 day	101.25 (0.34)	99.86 (2.35)	100.84 (2.47)	100.01 (2.21)
3 days	101.36 (0.60)	99.12 (1.47)	100.27 (0.65)	98.74 (2.27)
5 days	99.78 (0.04)	99.01 (1.21)	99.15 (0.71)	98.01 (2.94)
7 days	98.40 (1.05)	98.00 (2.17)	98.73 (0.97)	97.89 (1.31)
10 days	97.14 (0.47)	97.36 (2.09)	97.47 (1.09)	97.06 (1.67)
14 days	96.35 (0.98)	95.77 (0.71)	95.91 (0.11)	96.55 (0.94)

Table 27 Percentage of remaining drug of amoxicillin trihydrate reconstituted suspensions using different suspending agent, kept in refrigerator (8.0 ± 1 °C) for 14 days

Time	Drug content (%) Average (SD)			
	MGS	MRS	MTS	UT
0 day	100.02 (1.25)	100.11 (1.14)	101.45 (3.41)	101.12 (1.21)
1 day	100.10 (0.87)	100.23 (2.37)	101.02 (1.47)	101.54 (2.17)
3 days	100.23 (1.77)	99.84 (1.98)	101.55 (1.15)	100.43 (0.79)
5 days	99.78 (2.69)	98.36 (2.99)	99.47 (2.21)	100.25 (2.11)
7 days	100.36 (1.19)	99.74 (3.44)	100.89 (0.67)	99.01 (3.31)
10 days	99.63 (3.37)	100.09 (0.74)	100.01 (2.09)	99.73 (2.67)
14 days	100.06 (1.98)	99.77 (1.71)	99.14 (3.11)	99.41 (1.14)

Table 28 Percentage of remaining drug of cephalexin monohydrate reconstituted suspensions using different suspending agent, kept at room temperature for 14 days

Time	Drug content (%) Average (SD)			
	MGS	MRS	MTS	UT
0 day	100.15 (0.24)	100.47 (1.44)	101.25 (1.41)	101.17 (4.54)
1 day	100.23 (0.34)	100.01 (2.35)	101.47 (2.47)	101.36 (2.21)
3 days	99.84 (0.60)	100.54 (1.47)	100.64 (0.65)	100.94 (2.27)
5 days	99.94 (0.04)	99.76 (1.21)	100.53 (0.71)	100.48 (3.14)
7 days	100.06 (1.05)	100.09 (2.17)	99.98 (0.97)	101.01 (1.31)
10 days	100.23 (0.47)	99.74 (2.09)	100.06 (1.09)	100.23 (1.67)
14 days	96.35 (0.98)	100.11 (0.71)	99.97 (0.11)	100.06 (0.94)

Table 29 Percentage of remaining drug of cephalexin monohydrate reconstituted suspensions using different suspending agent, kept in refrigerator (8.0 ± 1 °C) for 14 days

Times	Drug content (%) Average (SD)			
	MGS	MRS	MTS	UT
0 day	100.02 (1.25)	99.81 (1.14)	101.23 (3.41)	99.96 (1.21)
1 day	101.47 (0.87)	99.63 (2.37)	101.47 (1.47)	99.74 (2.17)
3 days	100.36 (1.77)	100.05 (1.98)	101.63 (1.15)	100.06 (0.79)
5 days	99.54 (2.69)	100.23 (2.99)	101.58 (2.21)	100.47 (2.11)
7 days	100.09 (1.19)	100.48 (3.44)	100.96 (0.67)	100.58 (3.31)
10 days	101.07 (3.37)	100.96 (0.74)	100.87 (2.09)	101.05 (2.67)
14 days	100.45 (1.98)	101.33 (1.71)	101.99 (3.11)	100.22 (1.14)

B. Stability Studied of Selected Dry Syrup Formulation

Stability of selected amoxicillin trihydrate and cephalexin monohydrate dry syrup formulations when stored at room temperature (CR) and 45 ºC, 75% RH (CS) for 4 months were determined. Stability was expressed as physical stability (appearance of powder and suspension), reconstitution time, and redispersibility, water content and chemical stability of model drugs (percentage of drug remaining after each storage condition).

1. Dry Powder

1.1 Appearances of Selected Dry Syrup

The powder of all models dry syrup did not form cake was obtained. Based on the appearances of each month of amoxicillin trihydrate dry syrup, the color of sample was slightly orange powder with orange scent when kept at room temperature and 45 ºC, 75% RH for 4 months (Table 30 and Figure 52)

In addition, the appearances of each month of cephalexin monohydrate dry syrup was slightly pink with raspberry scent and appearance did not change after keeping at room temperature and 45 ºC at 75% RH. In both cases particle of dry syrup formulation could be easily manipulated into container and able to sustain forming hard cake. (Table 31 and Figure 53)

Table 30 Appearance of amoxicillin trihydrate dry syrup in stability study

Time (months)	Room temperature (all formulation)			45 °C, 75 % RH (all formulation)		
	Powder	Color	Odor	Powder	Color	Odor
Initial	did not form cake	Slightly orange	Orange	did not form cake	Slightly orange	Orange
1	did not form cake	Slightly orange	Orange	did not form cake	Slightly orange	Orange
2	did not form cake	Slightly orange	Orange	did not form cake	Slightly orange	Orange
3	did not form cake	Slightly orange	Orange	did not form cake	Slightly orange	Orange
4	did not form cake	Slightly orange	Orange	did not form cake	Slightly orange	Orange

Table 31 Appearance of cephalexin monohydrate dry syrup in stability study

Time (months)	Room temperature (all formulation)			45 °C, 75 % RH (all formulation)		
	Powder	Color	Odor	Powder	Color	Odor
Initial	did not form cake	Slightly pink	Raspberry	did not form cake	Slightly pink	Raspberry
1	did not form cake	Slightly pink	Raspberry	did not form cake	Slightly pink	Raspberry
2	did not form cake	Slightly pink	Raspberry	did not form cake	Slightly pink	Raspberry
3	did not form cake	Slightly pink	Raspberry	did not form cake	Slightly pink	Raspberry
4	did not form cake	Slightly pink	Raspberry	did not form cake	Slightly pink	Raspberry

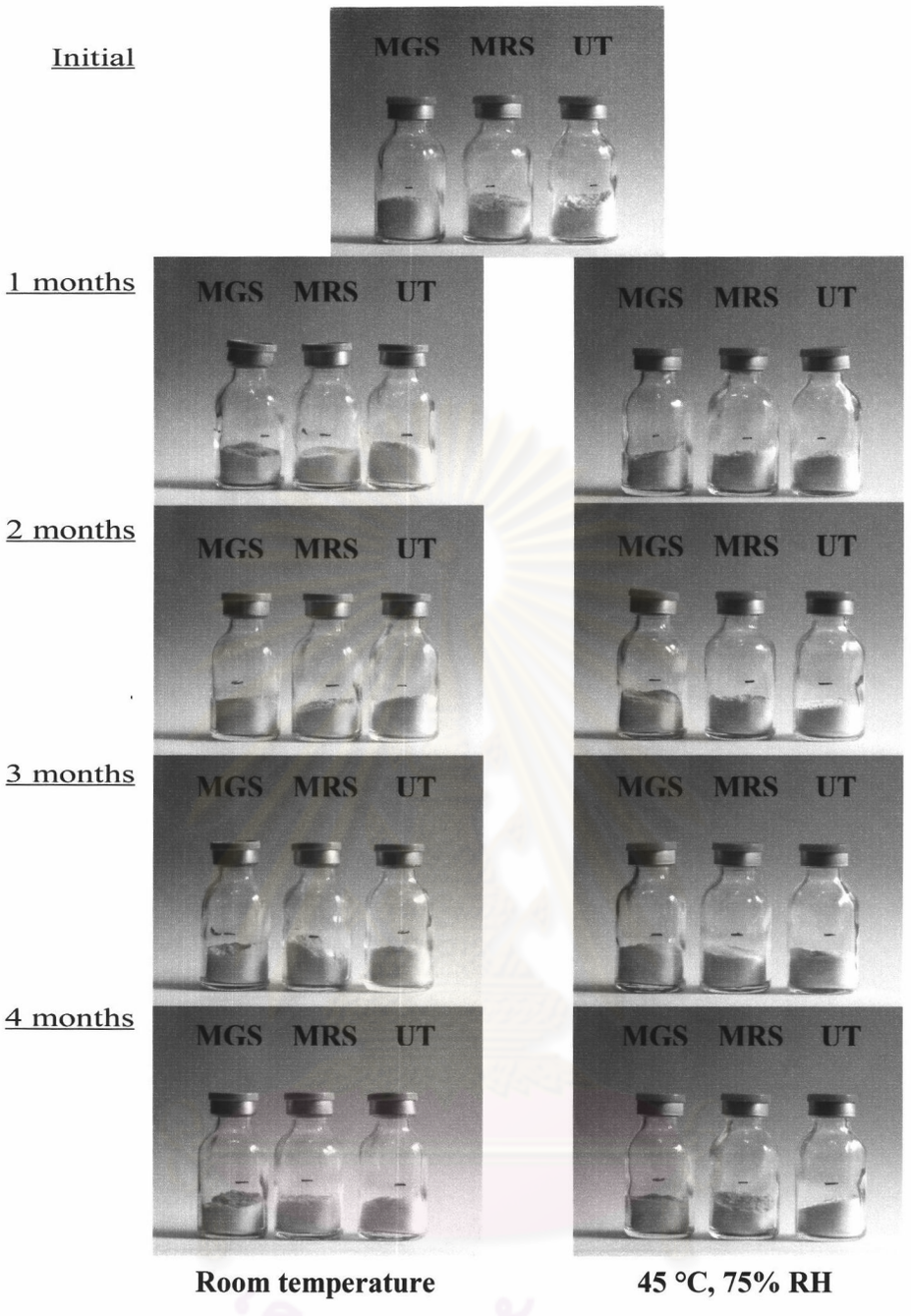


Figure 52 Comparison of amoxicillin trihydrate dry syrup using different suspending agent, when kept at room temperature and 45 °C ,75% RH for 4 months

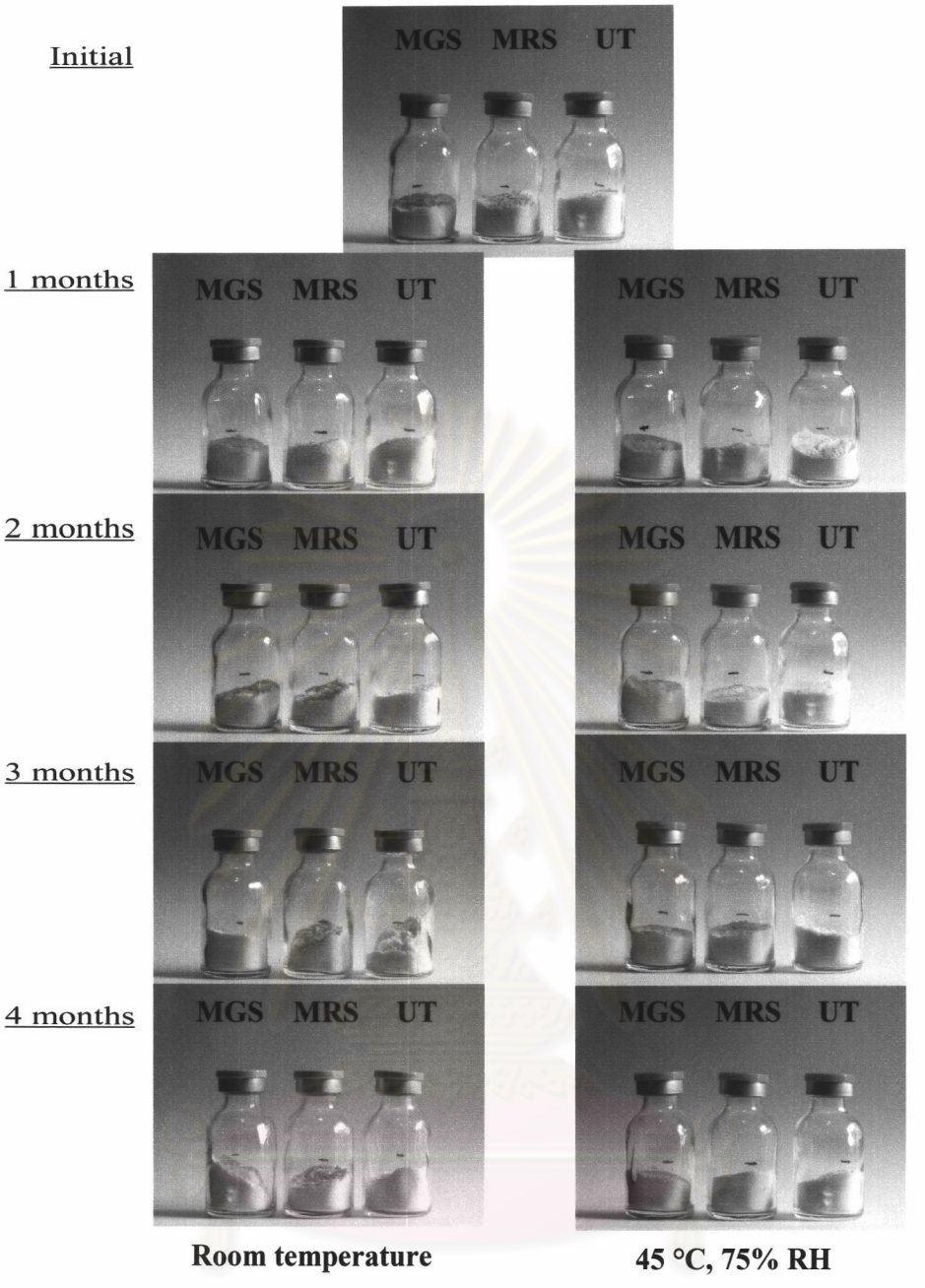


Figure 53 Comparison of cephalexin monohydrate dry syrup using different suspending agent, when kept at room temperature and 45 °C ,75% RH for 4 months

1.2 Content Uniformity of Model Drugs in Selected Dry Syrup

After stability study, uniformity of amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup formulations showed promising quality. Measurement at three different locations of each powder formulation showed the percentage of the labeled amount within allowanced ranged of pharmacopeias (USP 24). Amoxicillin trihydrate dry syrup had the percentage of the labeled amount of 96.41 – 107.14 and cephalexin monohydrate dry syrup had the percentage of the labeled amount of 109.86 – 99.46. The standard deviation (SD) based on the percentage of labeled amount for both formulations were less than 4.0. (Tables 32- 33)

From the statistical results (Appendix H: Tables H25-H45), the content of amoxicillin trihydrate and cephalexin monohydrated dry syrup decreased in accordance with increased storage times. Therefore, content of amoxicillin trihydrate and cephalexin monohydrate dry syrup formulation slightly decrease after keeping at room temperature (CR) and 45 ºC, 75% RH (CS) for 4 mounts. In addition, the content of drugs when kept at 45 ºC, 75% RH was found reduced faster than kept at room temperature. From the stability testing results, it could be concluded that all formulas were stable.

1.3 Determination of Water Content in Selected Dry Syrup

The water content was measured by Karl Fisher method and presented in Tables 34 - 35. The water content of amoxicillin trihydrate dry syrup formulations with different suspending agents as MGS, MRS and UT when kept at room temperature for 4 months were 2.01 - 2.53 %, 2.10 – 2.67 % and 2.29 -2.67 w/w, respectively and after keeping at 45 ºC, 75% RH for 4 months were 2.01 - 2.68 %, 2.10 – 2.76 % and 2.29 - 2.89 % w/w, respectively. The water content of cephalexin monohydrate dry syrups using different suspending agents as MGS, MRS and UT when kept at room temperature for 4 months were 0.94 - 1.21 %, 1.05 - 1.33 % and 1.18 - 1.47 % w/w, respectively and after keeping at 45 ºC, 75% RH for 4 months were 0.94 - 1.40 % w/w, 1.05 -1.56 % w/w and 1.18-1.71 w/w, respectively.

The results showed that the tendency of water content increased in accordance with increasing of storage times. Form the stability testing results, the water content of model drugs formulation was within allowanced ranged of pharmacopeias (USP 24; Water content of Amoxicillin for Oral Suspension and Cephalexin for Oral Suspensions was not more than 3.0 % and 2.0%, respectively).

Table 32 Drug content of selected amoxicillin trihydrate dry syrup using different suspending agents when kept at room condition and 45 °C, 75 % RH for 4 months

	Time (months)	Drug content (%) Average (SD)		
		MGS	MTS	UT
Room temperature	Initial	105.19 (1.09)	107.29 (0.96)	106.56 (2.46)
	1	104.24 (2.72)	105.69 (1.18)	104.06 (1.52)
	2	103.49 (0.82)	102.58 (1.27)	101.24 (1.06)
	3	102.19 (0.60)	100.39 (2.98)	100.26 (1.59)
	4	100.78 (0.98)	98.64 (3.40)	98.82 (1.97)
45 °C, 75 % RH	Initial	105.19 (1.09)	107.29 (0.96)	106.90 (3.00)
	1	102.57 (2.36)	104.66 (1.53)	102.24 (2.93)
	2	100.13 (1.80)	102.17 (1.13)	101.24 (1.06)
	3	99.55 (0.92)	99.22 (1.82)	97.31 (1.16)
	4	98.39 (1.14)	96.66 (0.33)	96.41 (1.13)

Table 33 Drug content of selected cephalexin monohydrate dry syrup using different suspending agents when kept at room condition and 45 °C, 75 % RH for 4 months

	Time (months)	Drug content (%) Average (SD)		
		MGS	MTS	UT
Room temperature	Initial	109.86 (2.41)	105.61 (1.52)	105.28 (1.05)
	1	107.83 (1.89)	105.06 (1.18)	103.44 (1.46)
	2	105.12 (1.37)	103.94 (0.66)	102.98 (1.49)
	3	104.39 (1.41)	102.65 (2.20)	100.79 (1.40)
	4	104.02 (0.46)	100.00 (0.30)	99.59 (0.44)
45 °C, 75 % RH	Initial	109.86 (2.41)	105.61 (1.52)	105.28 (1.05)
	1	106.50 (2.79)	105.69 (1.18)	102.13 (2.86)
	2	103.57 (1.22)	103.58 (2.64)	101.15 (1.18)
	3	102.15 (1.70)	102.98 (2.11)	99.62 (2.41)
	4	102.29 (2.75)	99.98 (2.25)	99.46 (1.73)

Table 34 The water content (%w/w) for amoxicillin trihydrate dry syrup using different suspending agents, kept at room temperature and 45 °C, 75 % RH for 4 months

	Suspending agent	Water content (%w/w)				
		Initial	1 month	2 months	3 months	4 months
Room temperature	MGS	2.01	2.07	2.11	2.34	2.53
	MRS	2.10	2.29	2.33	2.40	2.67
	UT	2.29	2.37	2.44	2.49	2.67
45 °C, 75 % RH	MGS	2.01	2.19	2.24	2.39	2.68
	MRS	2.10	2.30	2.51	2.70	2.76
	UT	2.29	2.41	2.60	2.81	2.89

Table 35 The water content (%w/w) for cephalexin monohydrate dry syrup with different suspending agents, kept at room temperature and 45 °C, 75 % RH for 4 months

	Suspending agent	Water content (%w/w)				
		Initial	1 month	2 months	3 months	4 months
Room temperature	MGS	0.94	0.98	1.07	1.15	1.21
	MRS	1.05	1.12	1.19	1.27	1.33
	UT	1.18	1.29	1.32	1.37	1.47
45 °C, 75 % RH	MGS	0.94	1.04	1.17	1.27	1.40
	MRS	1.05	1.20	1.29	1.47	1.56
	UT	1.18	1.31	1.39	1.52	1.71

2 Reconstitution Selected Model Dry Syrup

2.1 Physical Property Determinations of Selected Reconstituted Suspension

Freshly prepared of amoxicillin trihydrate reconstituted suspension when kept at room temperature and 45 ºC, 75% RH for 4 months were orange homogeneous dispersion with orange scent and sweet flavor as same as initial preparation (Table 36 and Figure 54). The pH of samples was between 5.98 ± 6.08 (Table 38) and the standard deviation (SD) was less than 0.1. Appearance of amoxicillin trihydrate reconstituted suspension after storage at room temperature and refrigerator (8.0 ± 1 ºC) for 7 and 14 days, therefore was became flocculated but could be redispersed after shaking, altogether found that no changed in odor or physical appearance of all sample was observed (Figure 55).

Freshly prepared of cephalexin monohydrate reconstituted suspension kept at both conditions was pink homogeneous dispersion with raspberry scent and sweet flavor (Table 37 and Figure 56). The pH of reconstituted suspension was between 4.49 ± 4.55 (Table 39). After storage at room temperature and refrigerator (8.0 ± 1 ºC) for and 14 days, no changed in odor or physical appearance of all sample was observed (Figure 57).

No significant different pH was observed for dry syrup when kept at room temperature and 45 ºC, 75% RH for 4 months (Appendix H; Tables 1H-H24). Unless pH of amoxicillin trihydrate reconstituted suspension containing MRS as suspending agent when kept at 45 ºC, 75% RH was significant difference observed at 2 and 4 months (P-value = 0.025). But it could be error from reconstitution by water because sample bottom of MRS formulation was found unclear calibrated mark, and then added mistake volume was possible and might be observed slightly changed in pH. Therefore, the results in term of pH were presented stable, all of formulation were within allowanced range of standard (USP 24; pH of amoxicillin for oral suspension was between 5.0 and 7.5 and cephalexin for oral suspensions was between 3.0 and 6.0).

Table 36 Appearance of amoxicillin trihydrate reconstituted suspension in stability study

Time (months)	Room temperature (all formulation)			45 °C, 75 % RH (all formulation)		
	Suspensions	Color	Odor	Suspensions	Color	Odor
Initial	Homogenous dispersion	Orange	Orange	Homogenous dispersion	Orange	Orange
1	Homogenous dispersion	Orange	Orange	Homogenous dispersion	Orange	Orange
2	Homogenous dispersion	Orange	Orange	Homogenous dispersion	Orange	Orange
3	Homogenous dispersion	Orange	Orange	Homogenous dispersion	Orange	Orange
4	Homogenous dispersion	Orange	Orange	Homogenous dispersion	Orange	Orange

Table 37 Appearance of cephalexin monohydrate reconstituted suspension in stability study

Time (months)	Room temperature (all formulation)			45 °C, 75 % RH (all formulation)		
	Suspensions	Color	Odor	Suspensions	Color	Odor
Initial	Homogenous dispersion	Pink	Raspberry	Homogenous dispersion	Pink	Raspberry
1	Homogenous dispersion	Pink	Raspberry	Homogenous dispersion	Pink	Raspberry
2	Homogenous dispersion	Pink	Raspberry	Homogenous dispersion	Pink	Raspberry
3	Homogenous dispersion	Pink	Raspberry	Homogenous dispersion	Pink	Raspberry
4	Homogenous dispersion	Pink	Raspberry	Homogenous dispersion	Pink	Raspberry

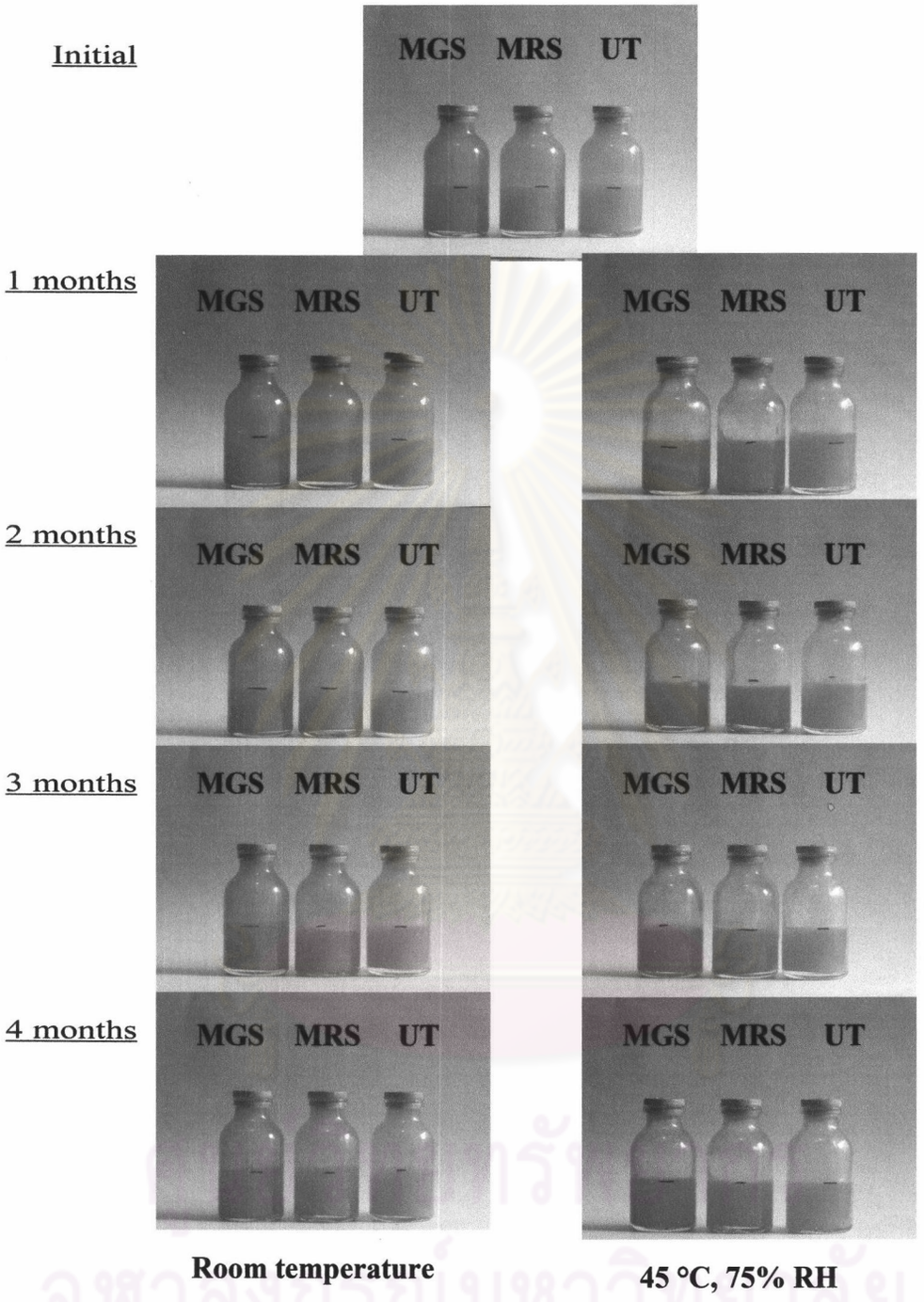


Figure 54 Photography of initial amoxicillin trihydrate reconstituted suspension using different suspending agent, when dry syrup formulation kept at room temperature and 45 °C, 75% RH for 4 months

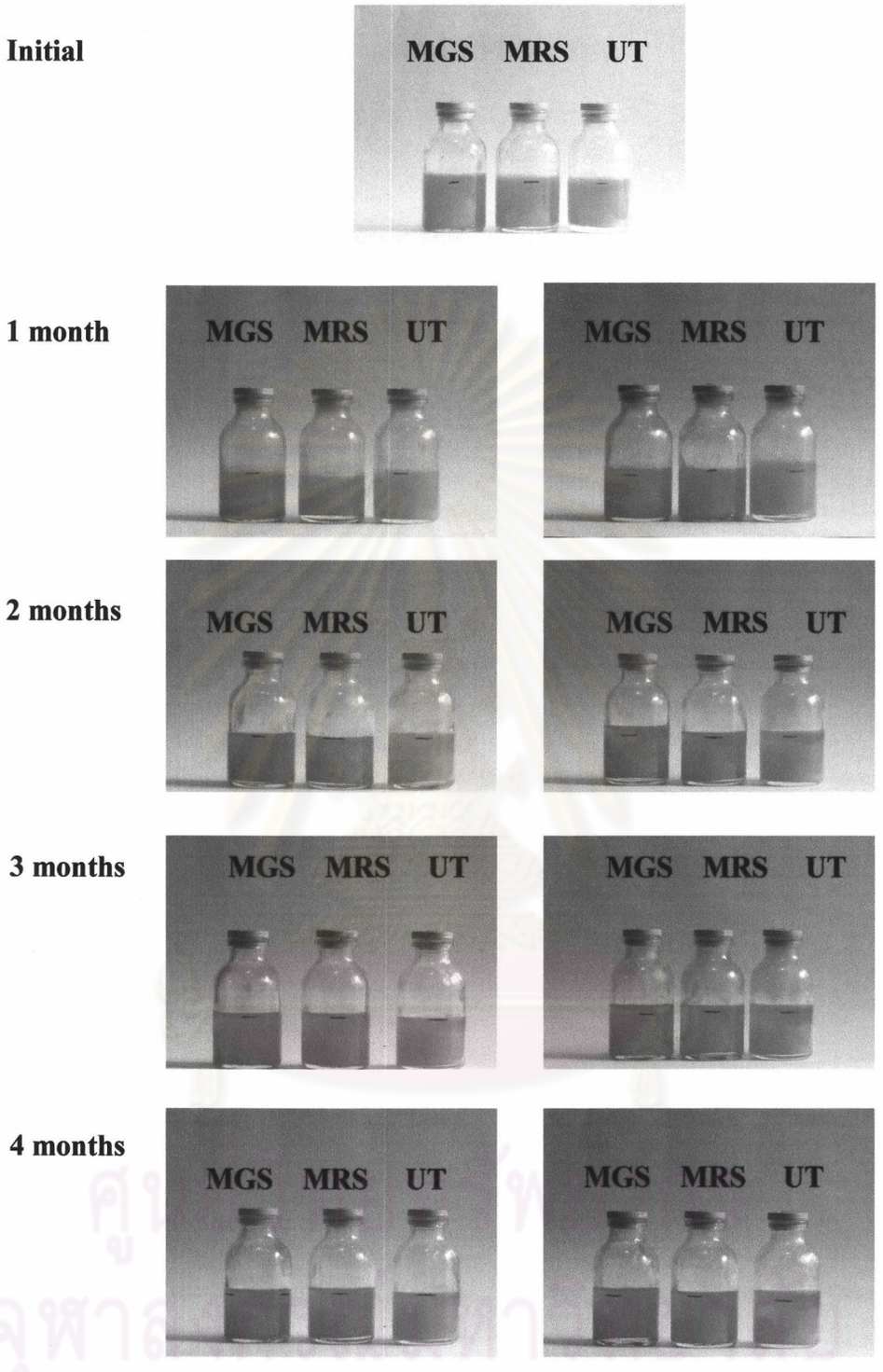


Figure 55 Photography of amoxicillin trihydrate reconstituted suspension using different suspending agent as kept at refrigerator (8.0 ± 1 °C) for 14 days, when dry syrup formulation kept at room temperature and 45 °C, 75% RH for 4 months

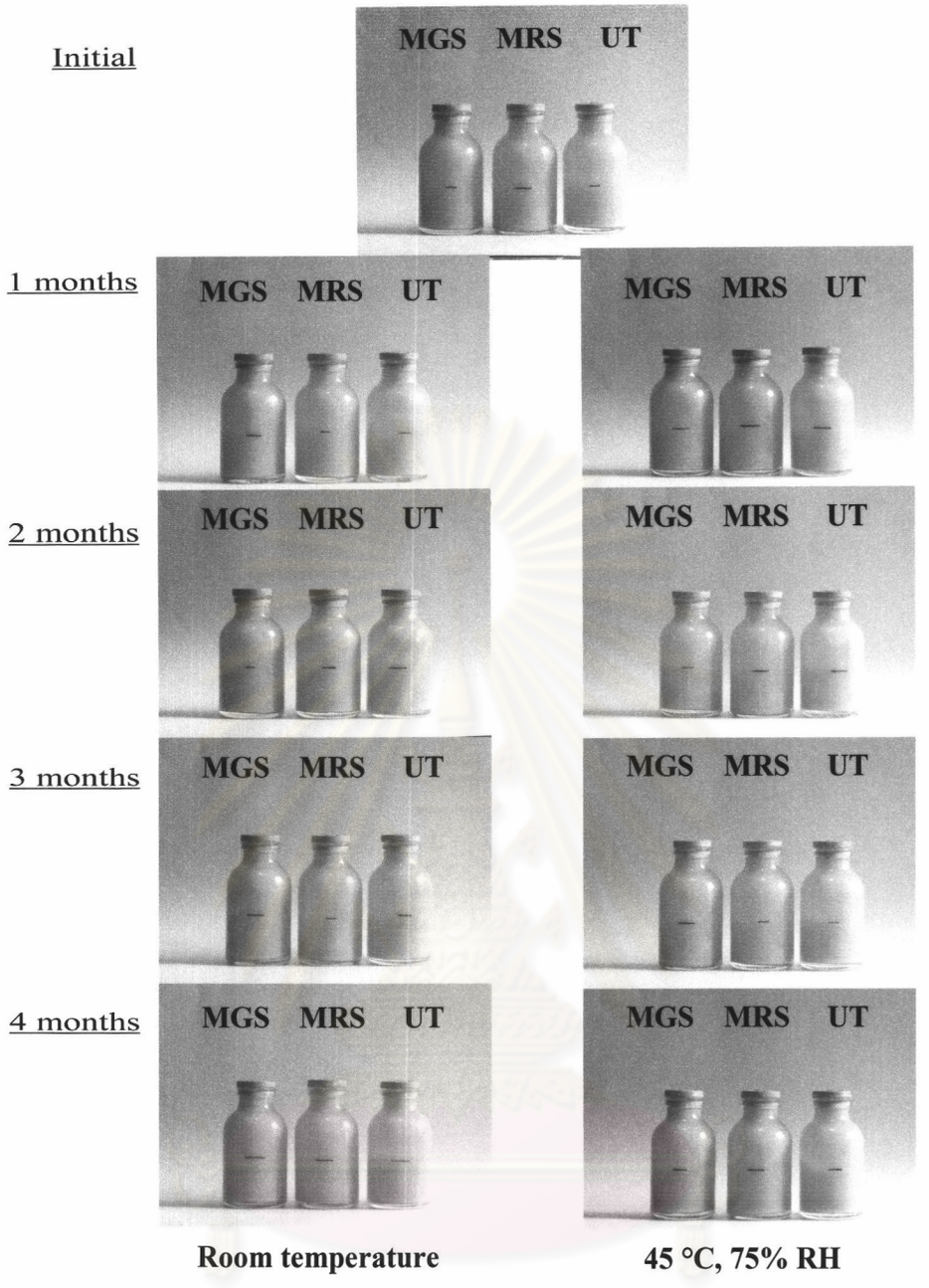


Figure 56 Photography of initial cephalexin monohydrate reconstituted suspension using different suspending agent ,when dry syrup formulation kept at room temperature and 45 °C, 75% RH for 4 months

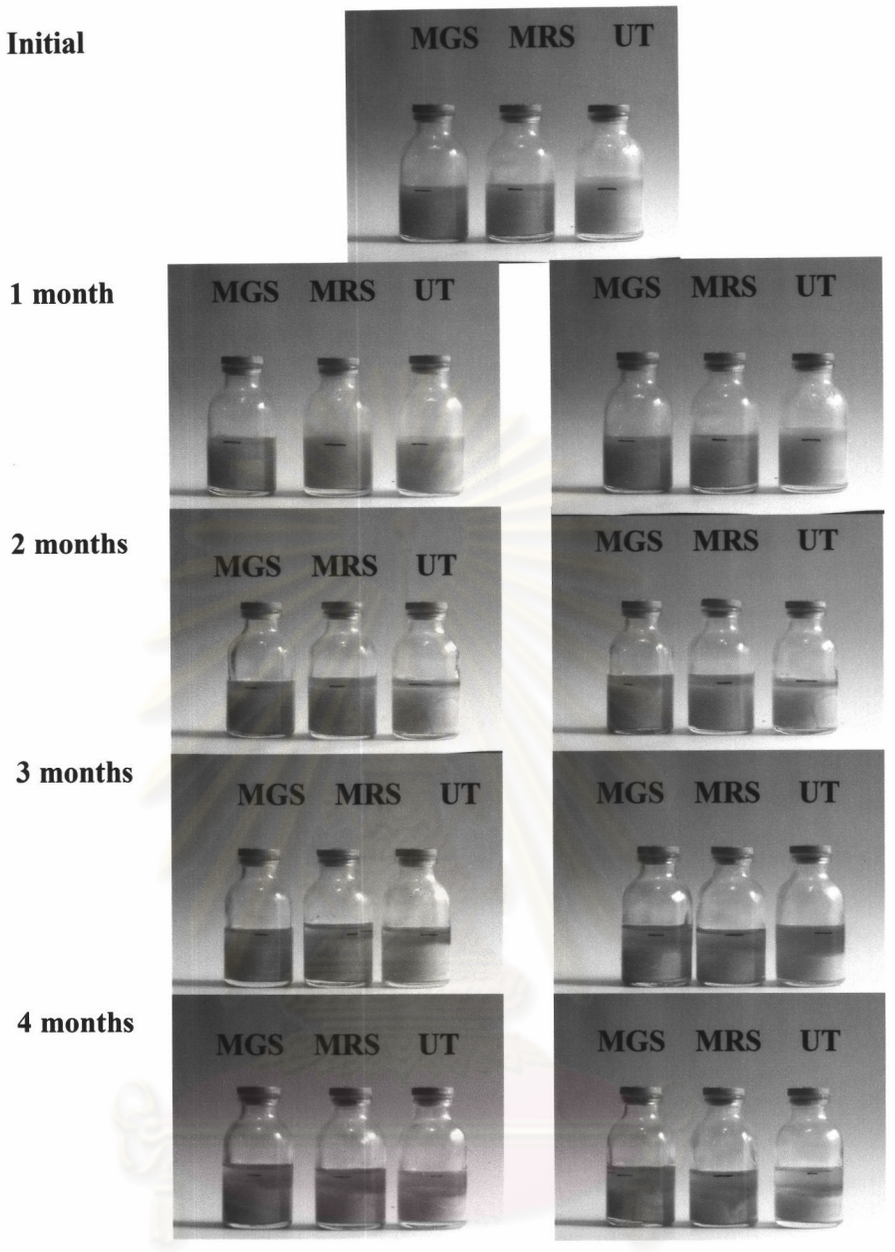


Figure 57 Comparison of cephalexin monohydrate reconstituted suspension using different suspending agent after keeping at refrigerator (8 ± 1 °C) for 14 days, when dry syrup formulation was kept at room temperature and 45 °C, 75% RH for 4 months

Table 38 pH of selected amoxicillin trihydrate reconstituted suspension using different suspending agents, when dry syrup formulation was kept at room temperature and 45 °C, 75% RH for 4 months

	Suspending agent	pH (SD)				
		Initial	1 month	2 months	3 months	4 months
CR*	MGS	6.05 (0.05)	6.02 (0.02)	6.00 (0.04)	6.02 (0.02)	6.02 (0.04)
	MRS	6.02 (0.05)	6.05 (0.03)	5.98 (0.01)	6.04 (0.06)	6.05 (0.06)
	UT	6.06 (0.07)	6.04 (0.05)	6.02 (0.02)	6.03 (0.07)	6.01 (0.08)
CS**	MGS	5.98 (0.01)	6.05 (0.04)	6.03 (0.07)	6.04 (0.01)	6.05 (0.03)
	MRS	5.98 (0.01)	6.05 (0.04)	5.97 (0.03)	6.05 (0.03)	6.07 (0.03)
	UT	6.03 (0.02)	6.02 (0.05)	6.04 (0.05)	6.08 (0.01)	6.01 (0.08)

* CR = Room temperature, CS** = 45 °C, 75% RH

Table 39 pH of selected cephalexin monohydrate reconstituted suspension using difference suspending agents, when dry syrup formulation was kept at room temperature and 45 °C, 75% RH for 4 months

	Suspending agent	pH (SD)				
		Initial	1 month	2 months	3 months	4 months
CR*	MGS	4.55 (0.05)	4.55 (0.07)	4.53 (0.03)	4.51 (0.01)	4.53 (0.06)
	MRS	4.54 (0.05)	4.49 (0.01)	4.49 (0.02)	4.54 (0.04)	4.51 (0.02)
	UT	4.52 (0.03)	4.53 (0.03)	4.49 (0.01)	4.51 (0.02)	4.50 (0.06)
CS**	MGS	4.55 (0.05)	4.55 (0.07)	4.49 (0.02)	4.51 (0.01)	4.53 (0.06)
	MRS	4.54 (0.05)	4.49 (0.01)	4.49 (0.01)	4.54 (0.04)	4.51 (0.02)
	UT	4.52 (0.03)	4.53 (0.03)	4.54 (0.03)	4.51 (0.02)	4.50 (0.06)

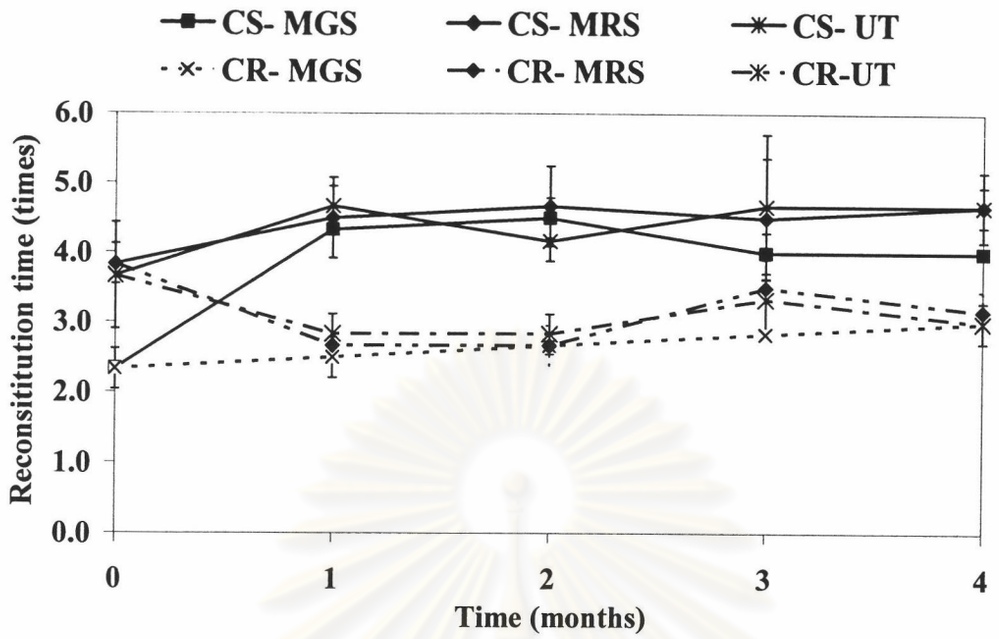
* CR = Room temperature, CS** = 45 °C, 75% RH

2.2 Determination of Reconstitution time

Reconstitution time of amoxicillin trihydrate and cephalexin monohydrate reconstituted suspensions was less than 5 times (Tables 40 - 41 and Figures 58 - 59). After keeping at room condition, the reconstitution time of all reconstitution suspensions was lower than stored at 45 ºC, 75% RH for 4 months.

The reconstitution time of all amoxicillin trihydrate reconstituted suspensions using MGS as suspending agent were no significant difference when kept at room temperature and 45 ºC, 75% RH for 4 months except amoxicillin trihydrate reconstituted containing MRS and MGS kept at 45 ºC, 75% RH (P-value < 0.000 and 0.005, respectively and see Appendix H; Tables H97 - H108).

Reconstitution time of cephalexin monohydrate reconstituted suspensions was significant difference when kept at room temperature and 45 ºC, 75% RH (Appendix H; Tables H109 - H120). The results were clearly indicated that storage time was effected on reconstitution times. However, good reconstituted suspension in previously study was found the limited of reconstitution time less than 5 times and results from stability studies were within allowanced ranged.



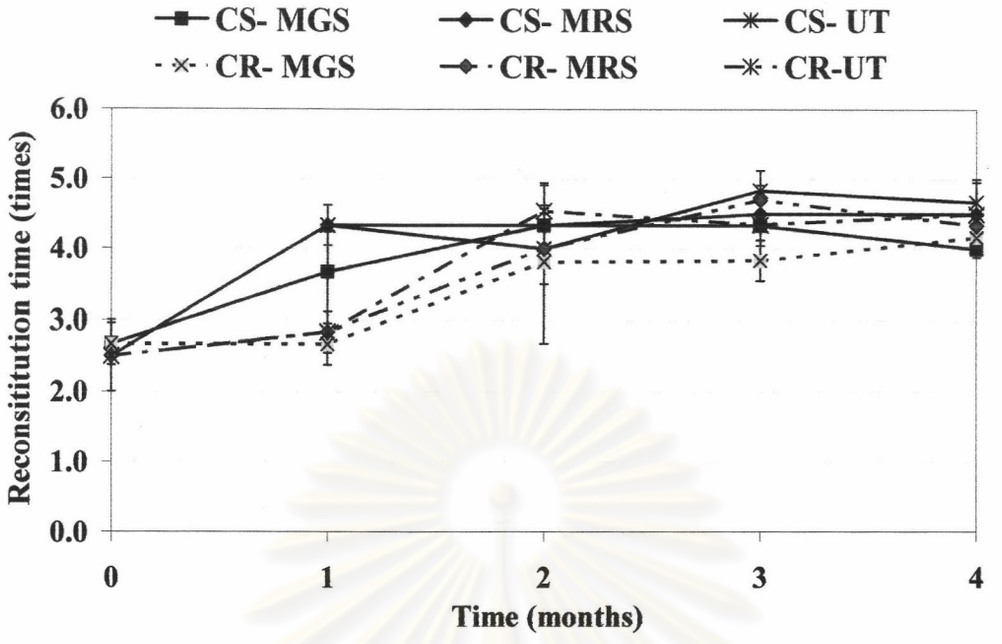
* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 58 Comparison of reconstitution time of amoxicillin trihydrate reconstituted suspension as different suspending agent when kept at room temperature and 45 °C, 75 % RH for 4 months

Table 40 Reconstitution time of amoxicillin trihydrate reconstituted suspension as different suspending agent when kept at room temperature and 45 °C, 75 % RH for 4 months

	Suspending agent	Reconstitution time (times) Average (SD)				
		Initial	1 month	2 months	3 months	4 months
CR*	MGS	2.33 (0.29)	2.50 (0.00)	2.67 (0.29)	2.83 (0.29)	3.00 (0.00)
	MRS	3.83 (0.29)	2.67 (0.58)	2.67 (0.58)	3.50 (0.87)	3.17 (0.29)
	UT	3.67 (0.76)	2.83 (0.29)	2.83 (0.29)	3.33 (1.04)	3.00 (0.50)
CS**	MGS	2.33 (0.29)	4.33 (0.29)	4.50 (0.00)	4.00 (0.00)	4.00 (0.00)
	MRS	3.83 (0.29)	4.50 (0.00)	4.67 (0.29)	4.50 (0.00)	4.67 (0.29)
	UT	3.67 (0.76)	4.67 (0.29)	4.17 (0.29)	4.67 (0.58)	4.67 (0.29)

* CR = Room temperature, CS** = 45 °C, 75% RH



* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 59 Comparison of reconstitution time of cephalexin monohydrate reconstituted suspension using different suspending agent when kept at room temperature and 45 °C, 75 % RH for 4 months

Table 41 Reconstitution time of cephalexin monohydrate reconstituted suspension using different suspending agent when kept at room temperature and 45 °C, 75 % RH for 4 months

	Suspending agent	Reconstitution time (times) Average (SD)				
		Initial	1 month	2 months	3 months	4 months
CR*	MGS	2.67 (0.29)	2.67 (0.29)	3.81 (1.13)	3.83 (0.29)	4.17 (0.29)
	MRS	2.50 (0.50)	2.83 (0.29)	4.00 (0.86)	4.70 (0.26)	4.34 (0.29)
	UT	2.50 (0.50)	2.83 (0.29)	4.54 (0.03)	4.35 (0.30)	4.50 (0.50)
CS**	MGS	2.67 (0.29)	3.67 (0.58)	4.33 (0.29)	4.33 (0.58)	4.00 (0.00)
	MRS	2.50 (0.50)	4.33 (0.29)	4.33 (0.58)	4.50 (0.00)	4.50 (0.00)
	UT	2.50 (0.50)	4.33 (0.29)	4.00 (0.50)	4.83 (0.29)	4.67 (0.29)

* CR = Room temperature, CS** = 45 °C, 75% RH

2.3 Viscosity Measurement of Selected Reconstituted Suspension

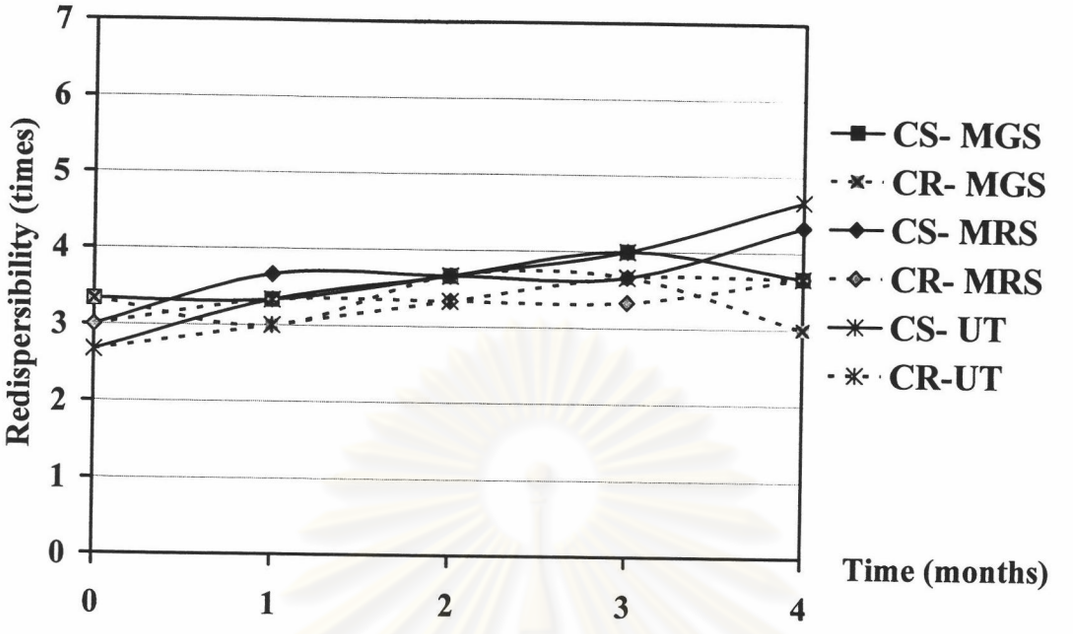
The average apparent viscosity after keeping at room temperature and 45 °C, 75% RH for 4 months was observed. The results were presented in Figures 60-61 and Appendix G; Tables 1G-2G .The viscosities of all formulations were 365.91 - 623.83 cps which considered suitable for reconstituted suspensions and after storage at both condition for 4 months, the viscosity were statistically significance deceased in accordance with increasing storage times (approximated decreasing values was 40 cps).

It was clearly indicated that storage times was effected on viscosity. However, the viscosity of reconstituted dry syrup was still in 200-600 cps range and showed good appearance and results from stability studies were within allowanced ranged. (Appendix H ; Tables H73-H96).

2.4 Redispersibility of Selected Reconstituted Suspension

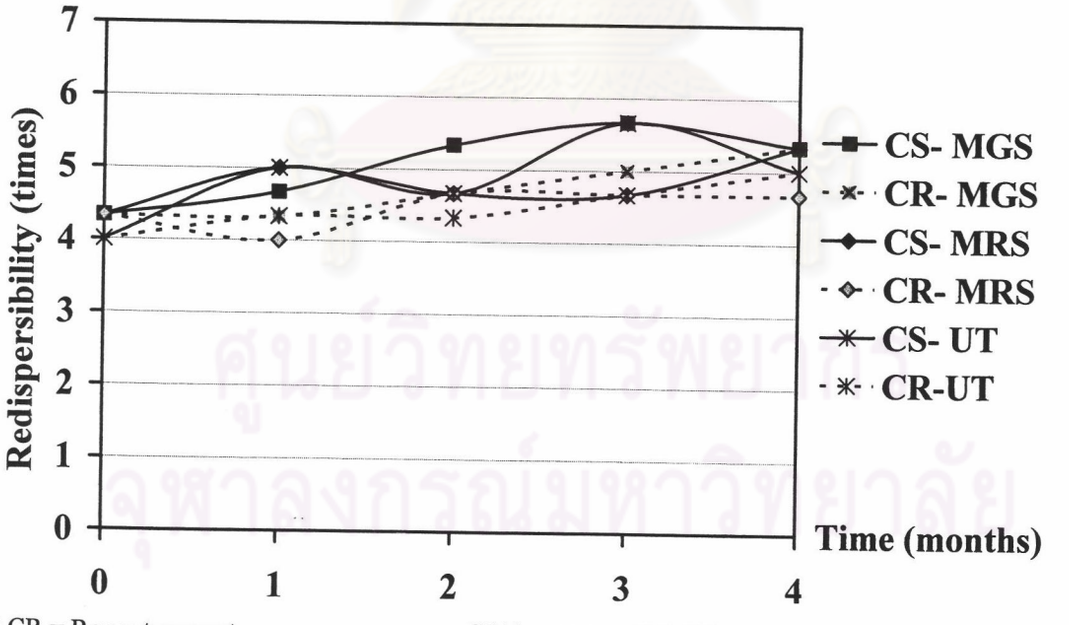
Numbers of inversion required to disperse suspension formulation after storage 14 days was shown in Figures 62-63 and Appendix G; Tables 3G-4G. The results showed that the amoxicillin trihydrate and cephalexin monohydrate reconstituted suspension after keeping for 4 months at room temperature and 45 °C, 75% RH was required to re-suspension of 3.00-3.67 and 5.00-5.33, respectively.

The effect of storage times on redispersibility was no significant difference when kept all dry syrup at room temperature and 45 °C, 75% RH for 4 months (Appendix H; Tables H49-H71) except amoxicillin trihydrate reconstituted suspension containing UT as suspending agent (P value < 0.047, see Appendix H; Tables H54-H72). Therefore it could be concluded that storage times was not effected on redispersibility. However, the redispersibility of good reconstituted dry syrup was less than 5 time and the results from stability studies were within allowanced ranged.



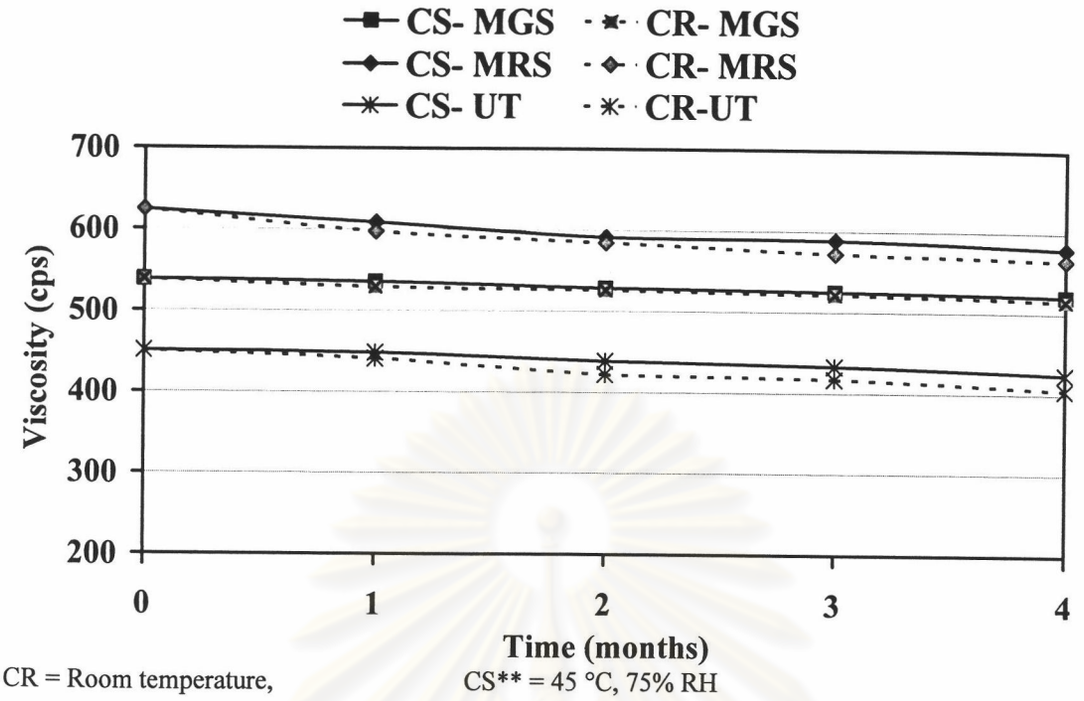
* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 62 Comparison of average number of inversion of amoxicillin trihydrate suspension as different suspending agents, kept at room temperature and 45 °C, 75 % RH for 4 months.



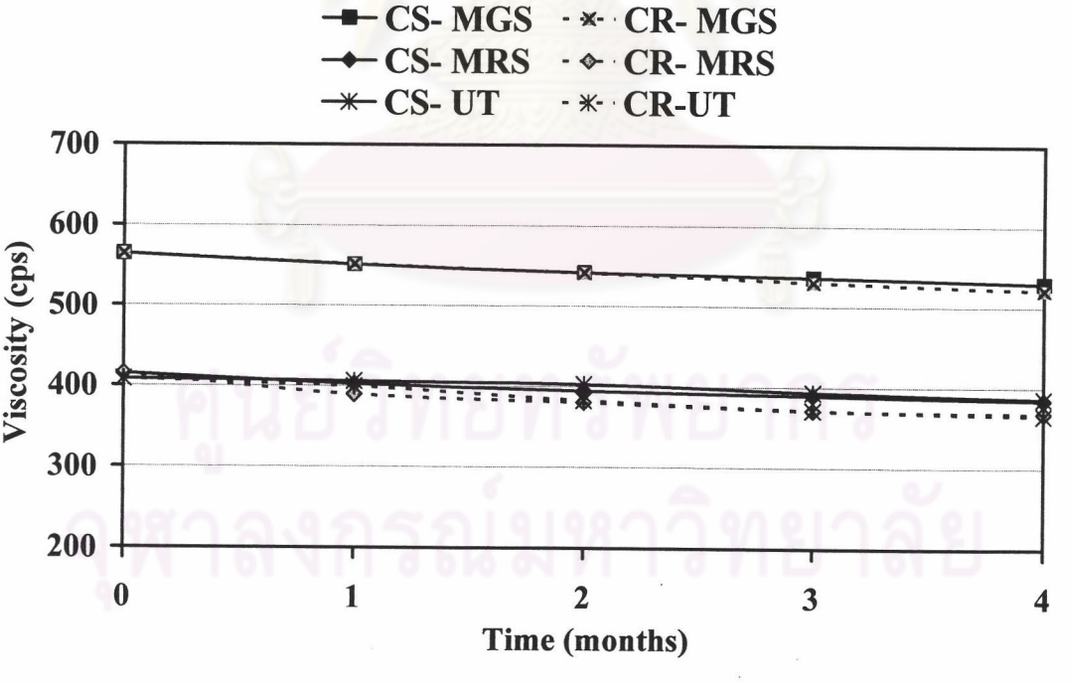
* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 63 Comparison of average number of inversion of cephalexin monohydrate suspension as different suspending agents, kept at room temperature and 45 °C, 75 % RH for 4 months



* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 60 Comparison of apparent viscosity of amoxicillin trihydrate reconstituted suspension using different suspending agents, kept at room temperature and 45 °C, 75% RH for 4 months



* CR = Room temperature, CS** = 45 °C, 75% RH

Figure 61 Comparison of apparent viscosity of cephalexin monohydrate reconstituted suspension using different suspending agents when kept at room temperature and 45 °C, 75% RH for 4 months

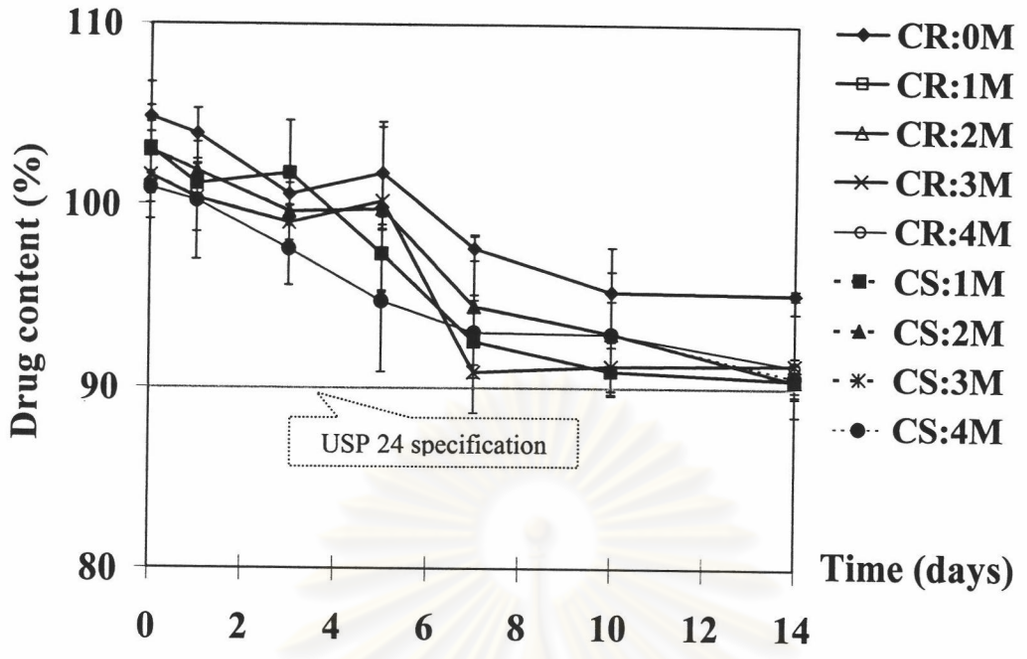
2.5 Content of Model Drugs in Selected Suspension.

The temperature used for testing drug stabilizing of reconstituted suspension were room temperature and in refrigerator (8.0 ± 1 C°) for 14 days.

For cephalexin monohydrate reconstituted suspension kept at room temperature and in refrigerator (8.0 ± 1 C°) for 14 days ,when dry syrup were stored at room temperature (CR) and 45 °C, 75% RH (CS) for 4 months. The percentages of drug contents were within the USP 24 specification for all different types of suspending agent (Appendix G; Tables G9 - G12 and Figures70 - 75).

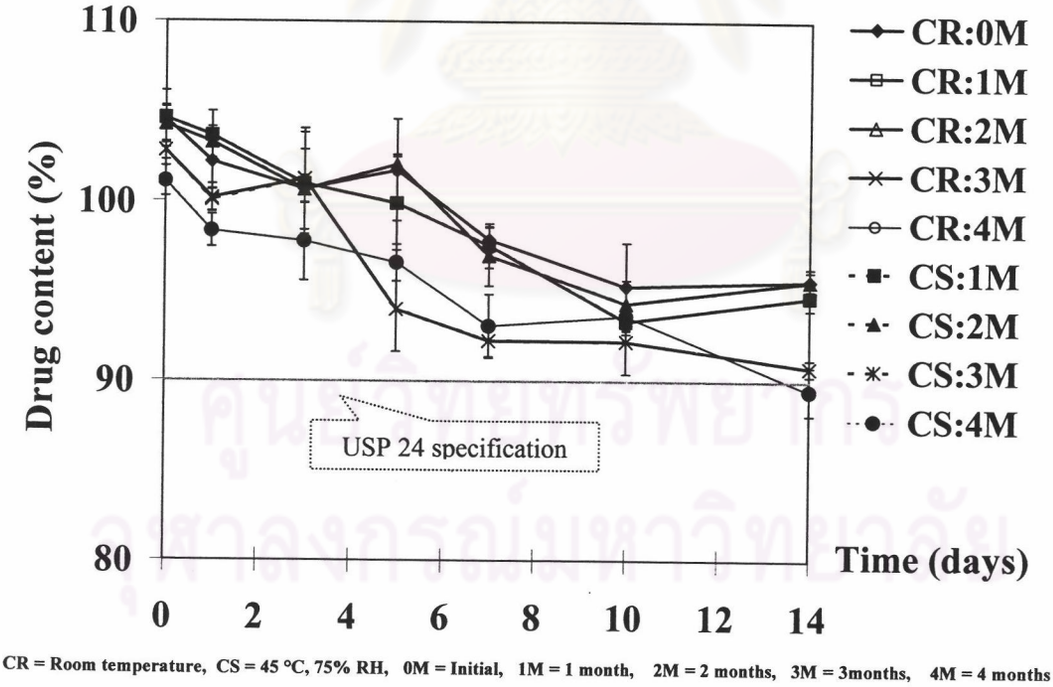
For amoxicillin trihydrate reconstituted suspension kept in refrigerator (8.0 ± 1 C°) for 14 days ,when dry syrup were stored at room temperature (CR) and 45 °C, 75% RH (CS) for 4 months. The percentage of drug contents was within the USP 24 specification for all different types of suspending agent (Appendix G; Tables G5 and G8 and Figures 67 - 69). For amoxicillin trihydrate reconstituted suspension kept at room temperature for 14 days , The percentage of drug contents was within the USP 24 specification for all different types of suspending agent except following; dry syrup using MRS as suspending agent after keeping at room temperature for 4 months, dry syrup using UT as suspending agent after keeping at room temperature for 3 and 4 months, dry syrup using MRS as suspending agent after keeping at 45 °C, 75% RH for 4 months and dry syrup using UT as suspending agent after keeping at 45 °C, 75% RH for 4 months (Appendix G; Tables G5 – G8 and Figures 64-66). The percentage of drug content were little lower than 90 % and did not pass the USP specification. However, the percentages of drug contents of reconstituted suspension were near the margin limit of USP 24 specification. The error might be caused by the sampling error and high standard deviation value. In this results, could conclude that amoxicillin trihydrate dry syrup and cephalexin monohydrate dry syrup after reconstitution and then kept at room temperature and in refrigerator (8.0 ± 1 C°) for 14 days was stable. In addition, the model dry syrup stored at room temperature and 45 °C, 75% RH for 4 month.

For stability study, it could be concluding that storage times were not effected on drug stability in both dry powder and reconstituted suspensions. The result indicated from dry powder when kept at room temperature (CR) and 45 °C, 75% RH (CS) for 4 months were evaluated every month. And evaluation for each of storage month, the reconstituted suspensions when room temperature and in refrigerator (8.0 ± 1 C°) for 14 days for 4 months.



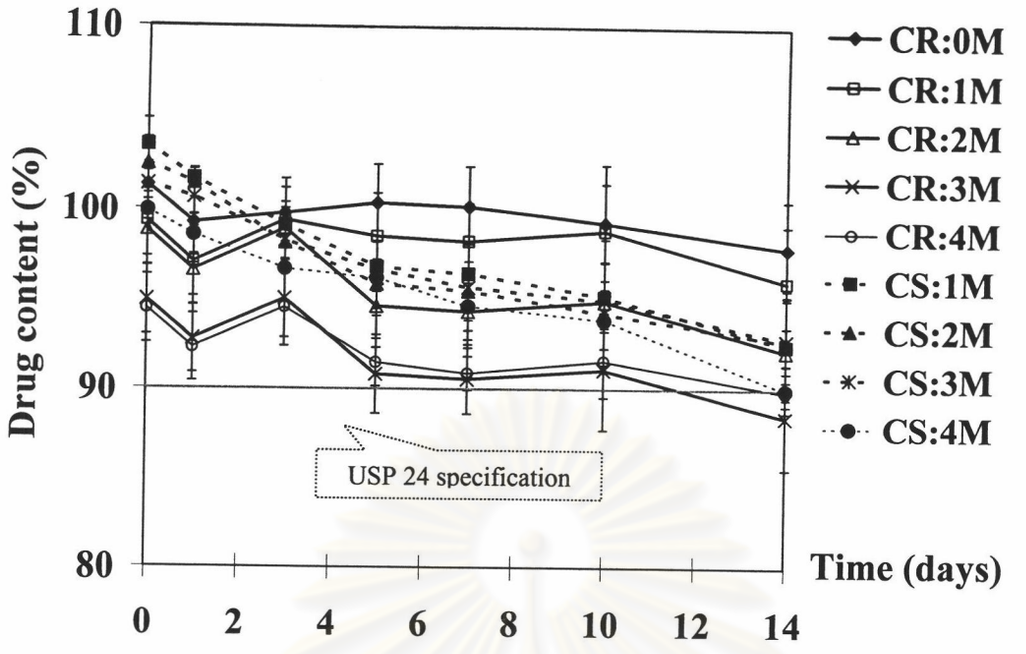
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 64 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using MGS as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



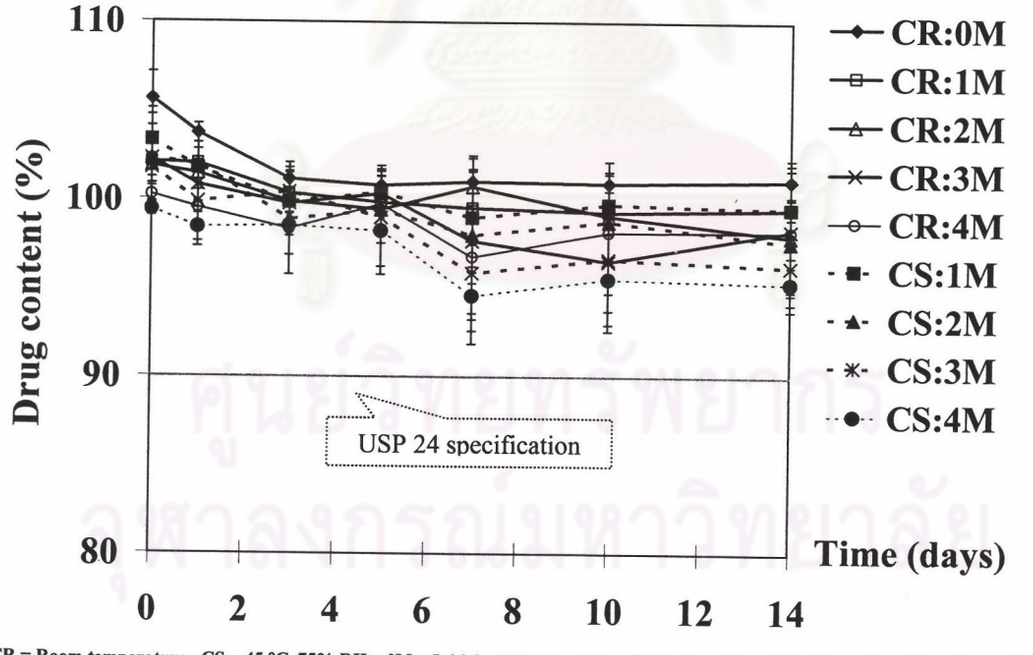
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 65 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using MRS as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



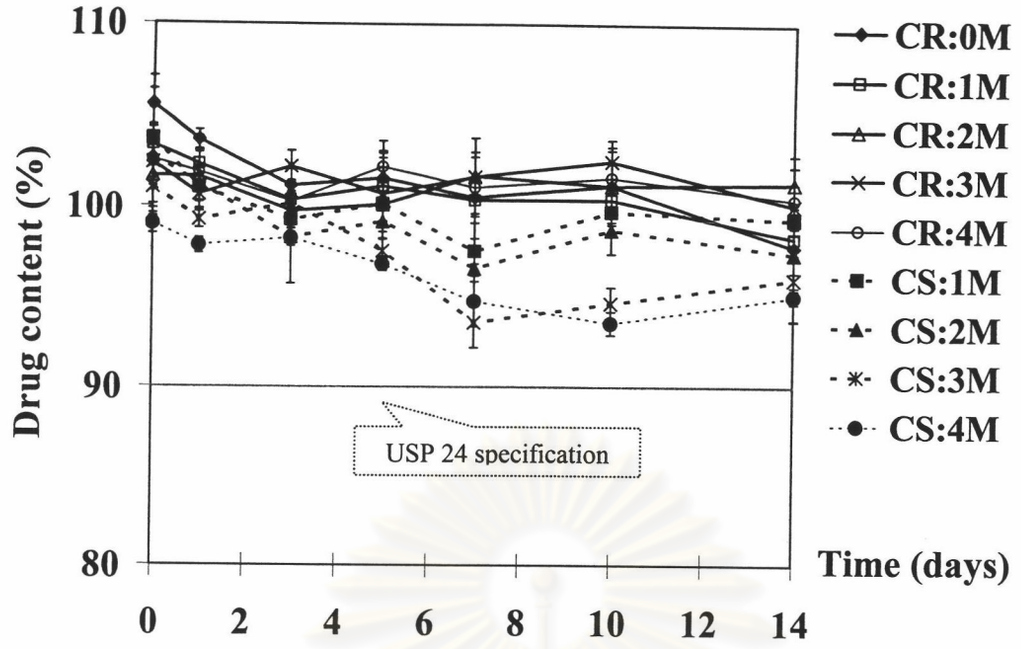
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 66 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using UT as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



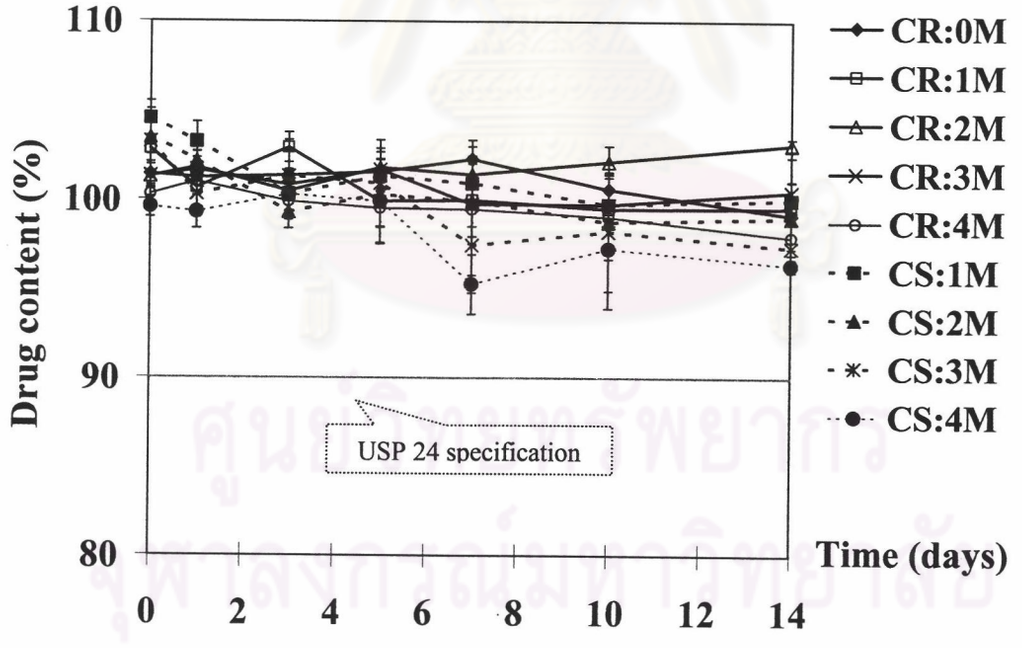
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 67 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using MGS as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



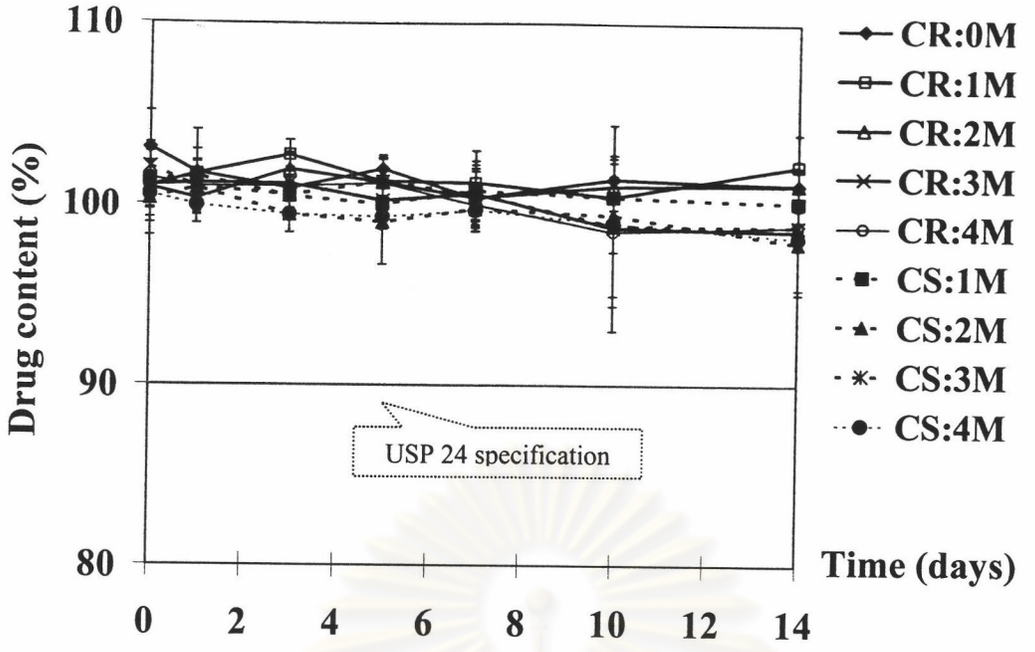
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 68 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using MRS as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



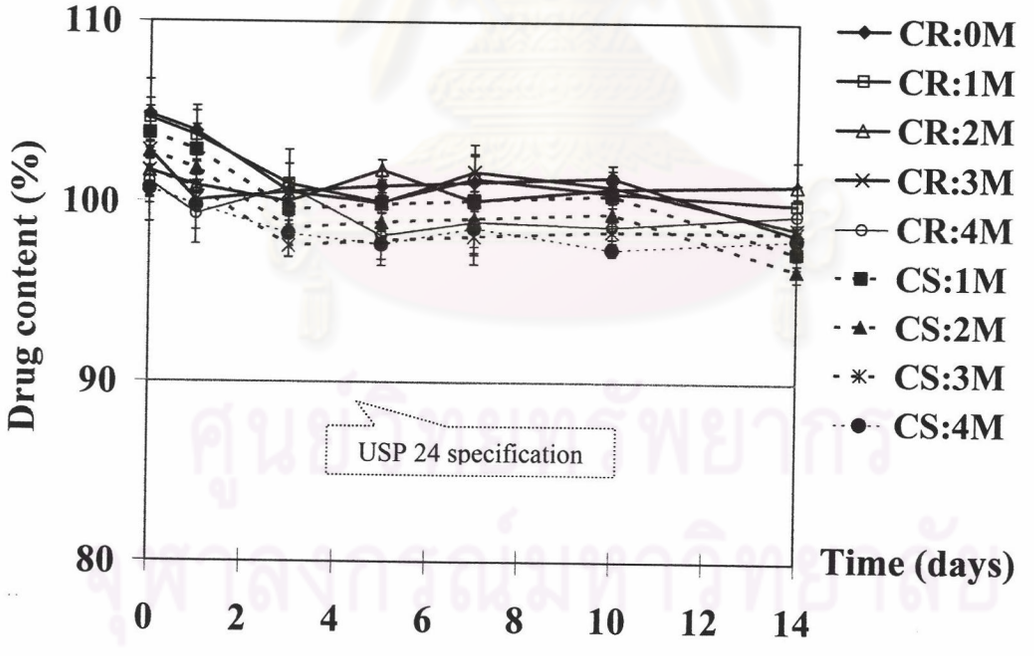
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 69 Comparison of drug content of amoxicillin trihydrate reconstituted suspension using UT as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



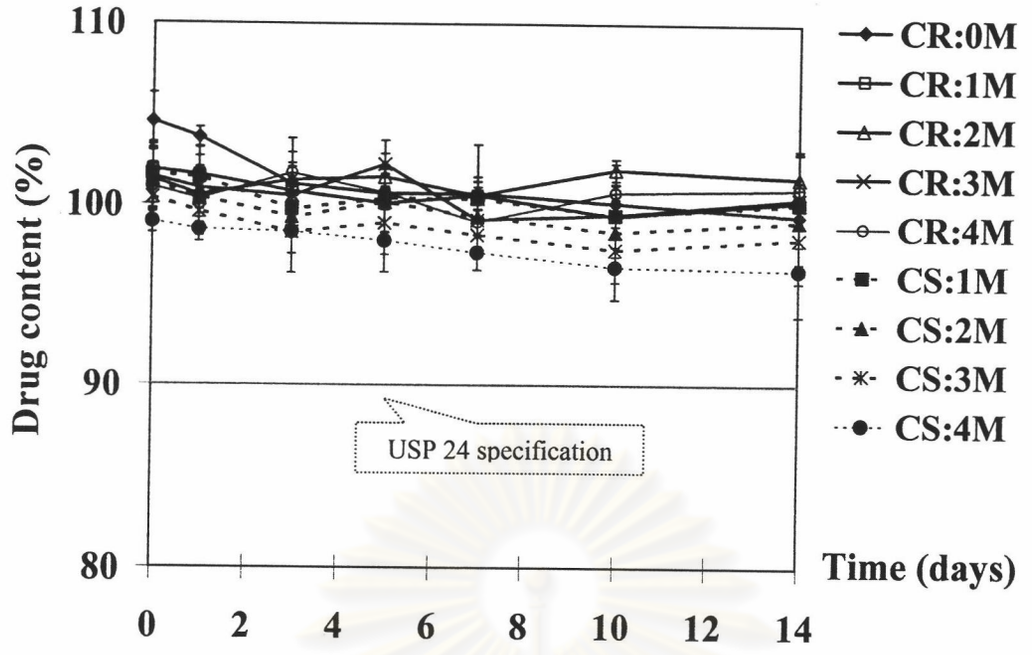
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 70 Comparison of drug content of cephalexin monohydrate reconstituted suspension using MGS as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



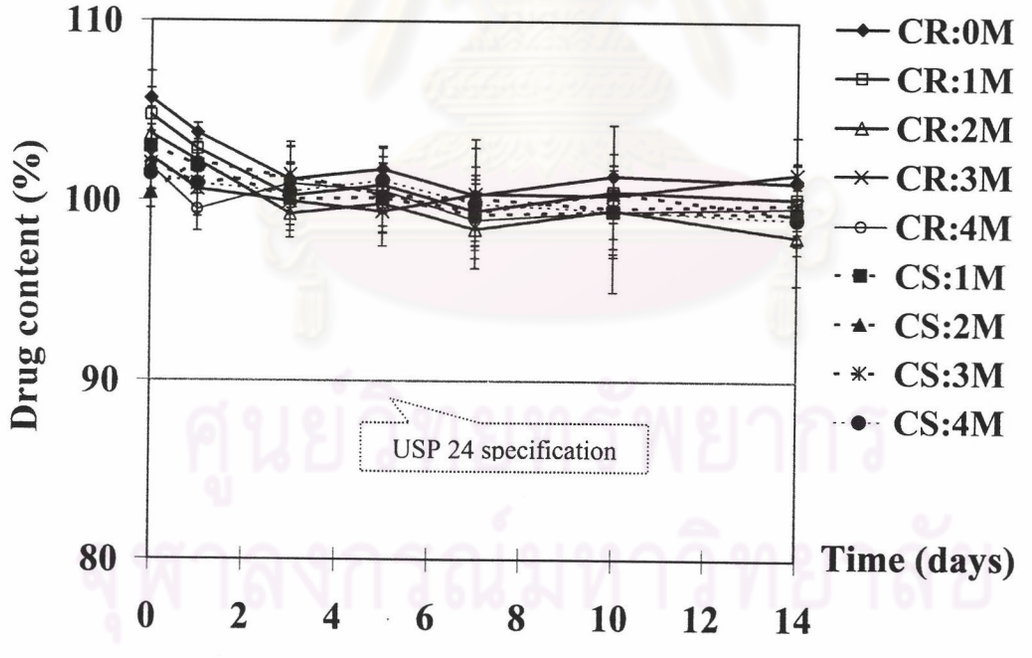
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 71 Comparison of drug content of cephalexin monohydrate reconstituted suspension using MRS as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



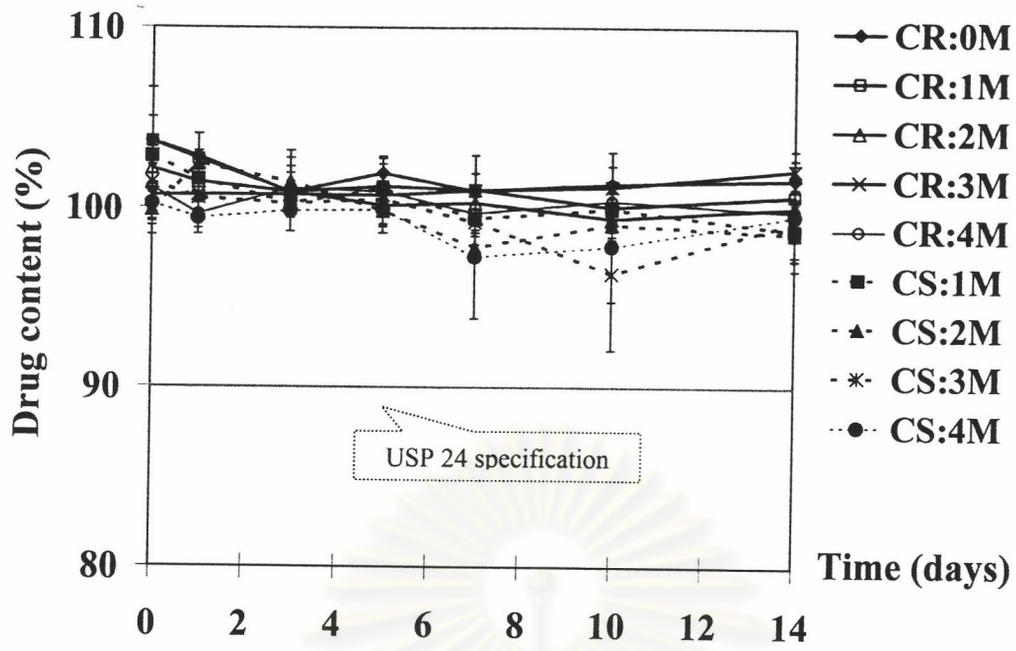
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 72 Comparison of drug content of cephalexin monohydrate reconstituted suspension using UT as suspending agent after storage at room temperature for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



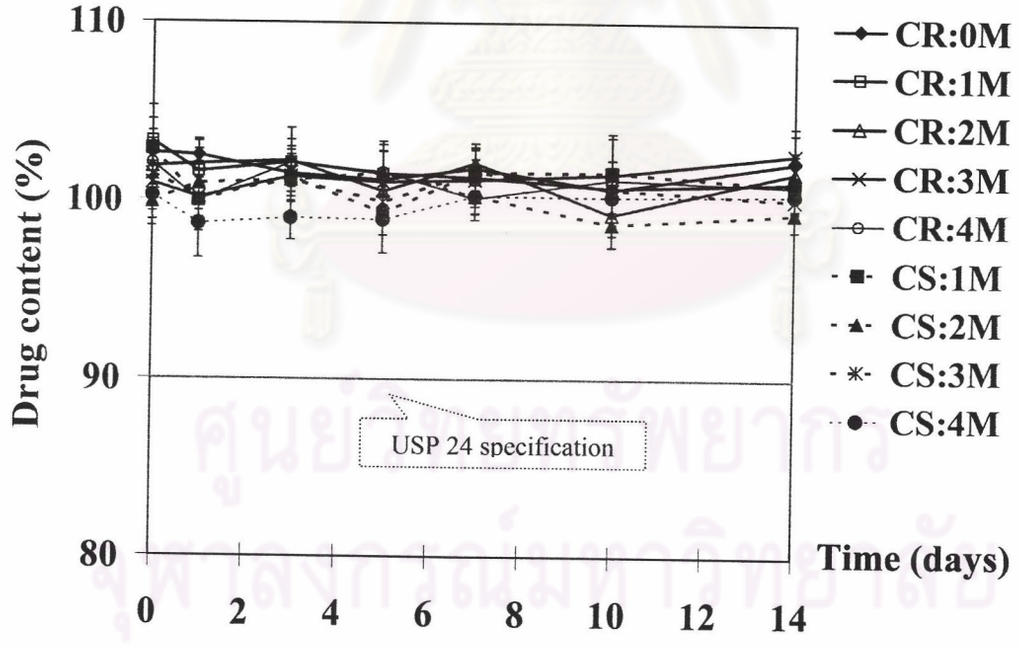
* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 73 Comparison of drug content of cephalexin monohydrate reconstituted suspension using MGS as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 74 Comparison of drug content of cephalexin monohydrate reconstituted suspension using MRS as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)



* CR = Room temperature, CS = 45 °C, 75% RH, 0M = Initial, 1M = 1 month, 2M = 2 months, 3M = 3months, 4M = 4 months

Figure 75 Comparison of drug content of cephalexin monohydrate reconstituted suspension using UT as suspending agent after storage at refrigerator (8.0 ± 1 °C) for 14 days (Dry powder was kept at room temperature and 45 °C, 75% RH for 4 months.)