CHAPTER IV

RESULTS AND DISCUSSION

4.1 Factors affecting the sample preparation of biodiesel

The preparation of emulsified biodiesel samples can be influenced by various factors. These factors were therefore studied for the physical and analytical evaluations of the prepared samples via inductively coupled plasma atomic emission spectroscopy (ICP-AES).

4.1.1 Types of surfactants

The stability of emulsions greatly depends on the types of surfactants. The surfactants used in this study were Tween 20, Triton X-100, and Span 80. All of these were non-ionic surfactants having the hydrophile-lipophile balance (HLB) values of 16.7, 13.5 and 8.3, respectively. The higher values have more hydrophilicity (water soluble, oil-in-water), while the lower values have more lipophilicity (oil soluble, water-in-oil). Each of surfactant types was fixed at 1.0 mL. Moreover, the effect of the addition of 1.0 mL conc. nitric acid along with each surfactant was also evaluated. From Figure 4.1, the physical appearance of Span 80 was clearly more heterogeneous than that of Triton X-100 and Tween 20. Span 80 has the lowest HLB and thus the emulsion could not be formed as an oil-in-water system. Meanwhile, both Triton X-100 and Tween 20 proved to be potential candidates as surfactant for the emulsified biodiesel samples.
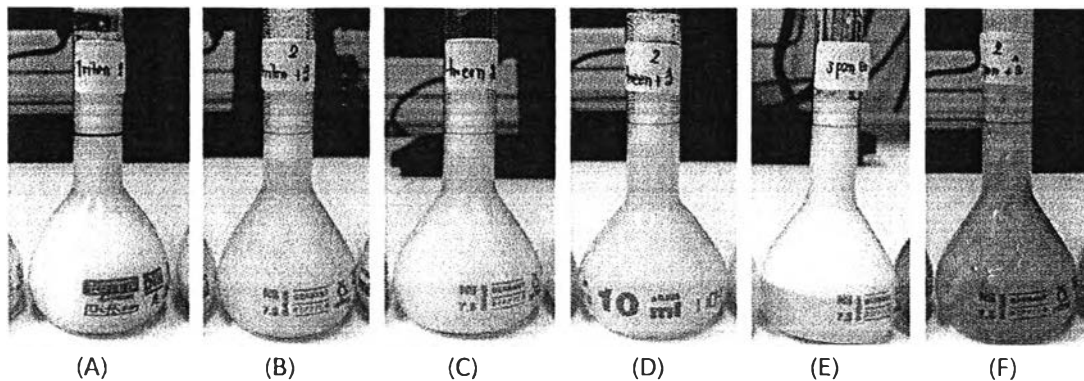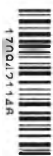
Figure 4.1 Physical appearances of the emulsions prepared with different types of surfactants ; (A) Triton X-100, (B) Triton X-100 with HNO_3, (C) Tween 20, (D) Tween 20 with HNO_3, (E) Span 80, (F) Span 80 with HNO_3

The experiment results (Figure 4.2) showed that Triton X-100 gave longer stability than Tween 20. Because Tween 20 has high HLB values, the emulsion form were less stable. In the meantime, the sample prepared with Tween 20 is more viscous than that using Triton X-100 as indicated by the longer traveling time in the viscosity measurement apparatus. Therefore, Tween 20 was not as an ideal surfactant to form emulsion in oil-in-water system, while Triton X-100 (lower HLB value) provided emulsion which remains stable for about 5 min. Triton X-100 was, hence, selected as a suitable surfactant for further experiments. The analytical evaluation of samples with Triton X-100 were subsequently evaluated by ICP-AES.
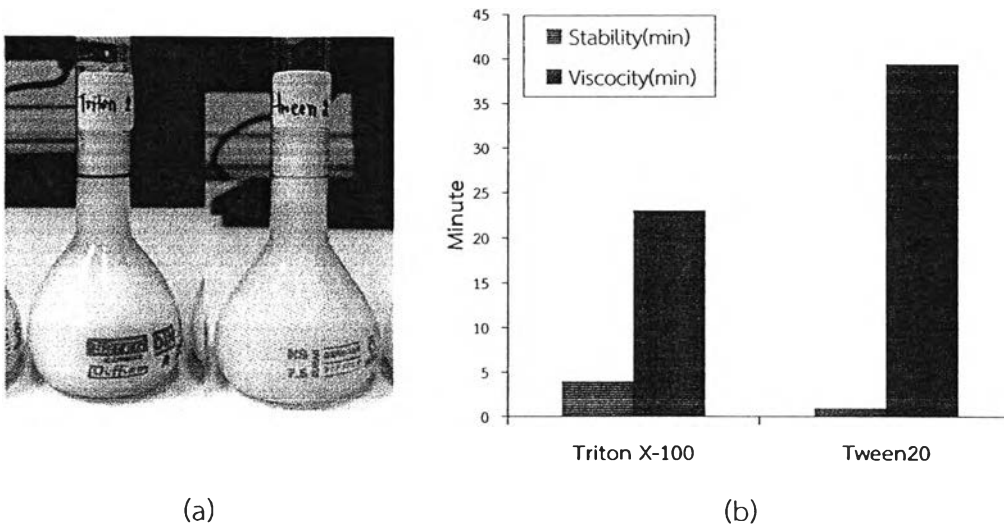
(a)   (b)

Figure 4.2 (a) Physical appearances of the emulsified biodiesel samples (b) Time of stability and viscosity

Normally without any modification, a simple oil-water mixture sample would yield a drastically low emission signal as a result of the incomplete vaporization/ionization and the diminished transportation efficiency of the more viscous oil sample. As clearly seen in Figure 4.3, however, the emission intensities obtained from all of the emulsified samples studied were improved significantly to the levels close to those of the aqueous standard of the same concentration indicating that a much more complete excitation was attained. Triton X-100 was the surfactant that yielded the overall intensity closest to that of the aqueous standard and thus was chosen as a model surfactant for the preparation of oil-in-water (o/w) emulsion for the following studies.
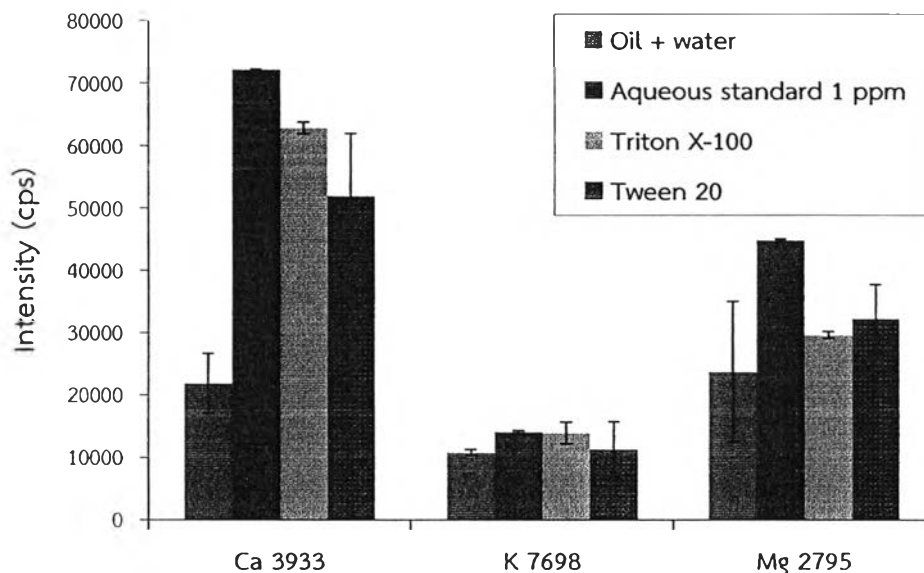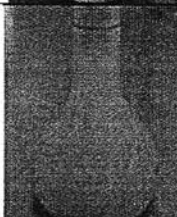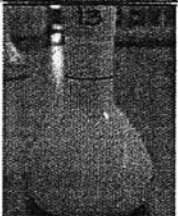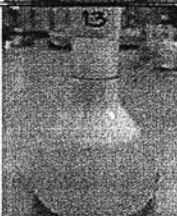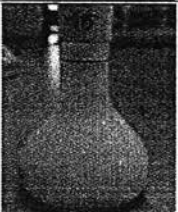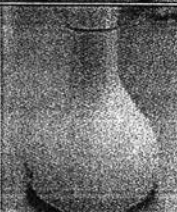
Figure 4.3 Intensity of different types of surfactant

4.1.2 The amount of surfactant

The blending ratios of Triton X-100 were studied in the range of 2.5-50% v/v. Table 4.1 and Figure 4.4 demonstrated that using the amount of surfactant over 20%v/v produced highly viscous samples which are virtually impossible for the determination by ICP-AES. Because the surfactant itself is highly viscous, lower amount of surfactant are necessarily used even though the stability are substantially reduced. The amounts of surfactant at 2.5, 5, and 10% yielded somewhat compromised viscosity and stability of the emulsion in which the homogeneity was sustained for longer than 3 minutes. Therefore, these ratios were further investigated for their analytical evaluation by ICP-AES.

Table 4.1 The physical appearances of the emulsified samples prepared with various ratios to surfactant.

No.	Amount of oil (% v/v)	Amount of surfactant (% v/v)	Physical Appearance (Before)	Stability (min)	Physical appearance (After phase separation)
1	10	50		NA (extremely viscous)	
2	10	30		NA (very viscous)	
3	10	20		NA (viscous)	
4	10	10		3	
5	10	5		3	
6	10	2.5		3	
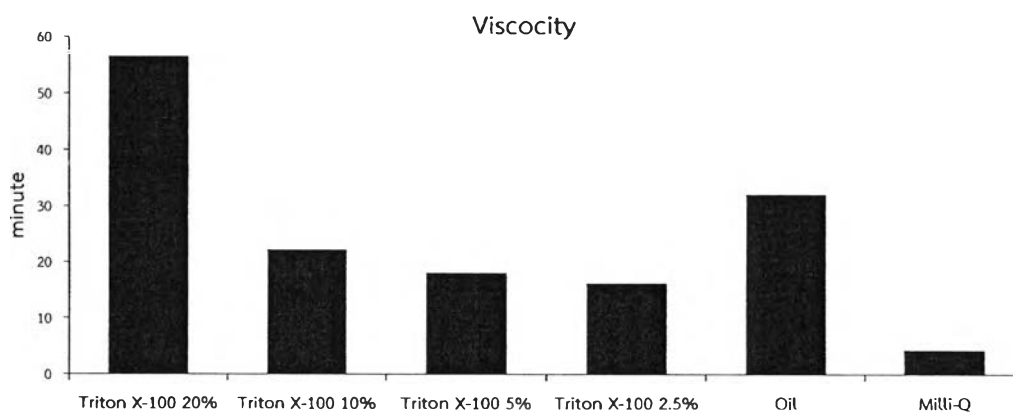
Figure 4.4 Relative viscosity as pertained to the dropping time

The amount of Triton X-100 was evaluated in the range of 2.5 and 10% v/v by ICP-AES. It was found that the optimum condition was established with 10% Triton X-100, as shown in Figure 4.5 because this ratio demonstrated the highest and clostest intensity to that of the aqueous standard at 1 mg/L. Thus, 10% v/v of Triton X-100 was considered the optimal amount of surfactant used for the preparation of biodiesel emulsions in the following experiments.
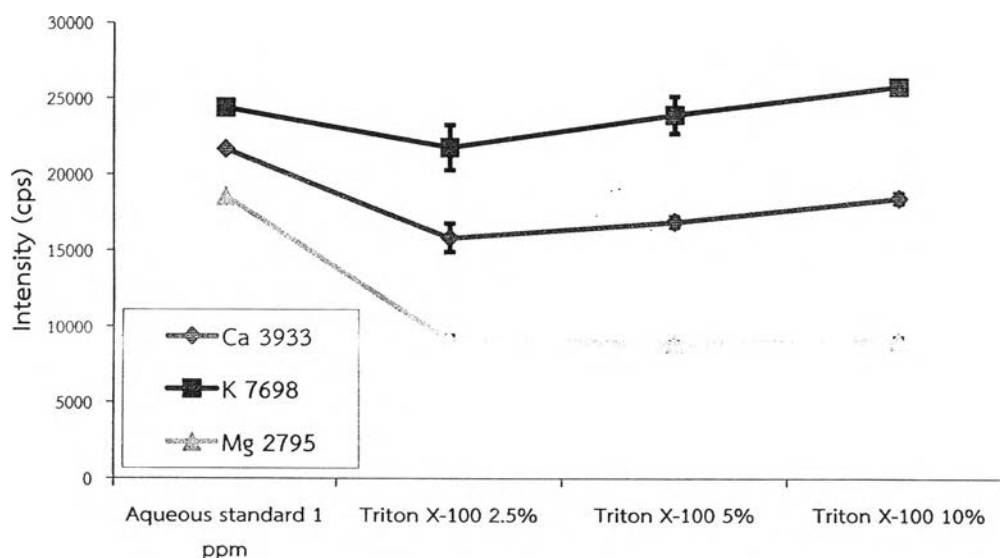
Figure 4.5 Emission intensity of Ca, K, and Mg of the emulsions prepared with 2.5%, 5%, and 10% v/v of Triton X-100

4.1.3 Method of agitation/homogenization

As the emulsion consists of two immiscible liquids, a method of agitation is normally required to obtain the homogeneity of the emulsified samples at least for the duration of analysis [36]. Various means of agitation or homogenization were assessed in this experiment. The physical observation revealed that using ultrasonic probe result in the best emulsion in term of stability which sustained for more than 1 hr. Nonetheless, this method was encountered with certain difficulties in which the homogenization needed to be executed individually for each sample. In addition, a laborious cleaning must be performed between each sample which could potentially introduce contaminants to the samples as well. Meanwhile, the emulsion obtained by manual agitation was able to maintain the homogeneity for at least 3 min which is surprisingly longer than that formed by mechanical agitation. Lastly, the use of ultrasonic bath as a tool for agitation was unsuccessful in producing the homogeneity of samples. Based on these finding, manual and mechanical agitations were chosen and subject to analytical evaluation by ICP-AES.

Figure 4.6 displayed emission intensity of the selected elements from the emulsified samples prepared by manual agitation at various time (10 sec, 3 min, 10 min) and mechanical agitation at 3 and 10 min. the results of all tested conditions were not significantly different. Hence, for the ease of operation, the manual agitation for 3 min was chosen for further experiments.
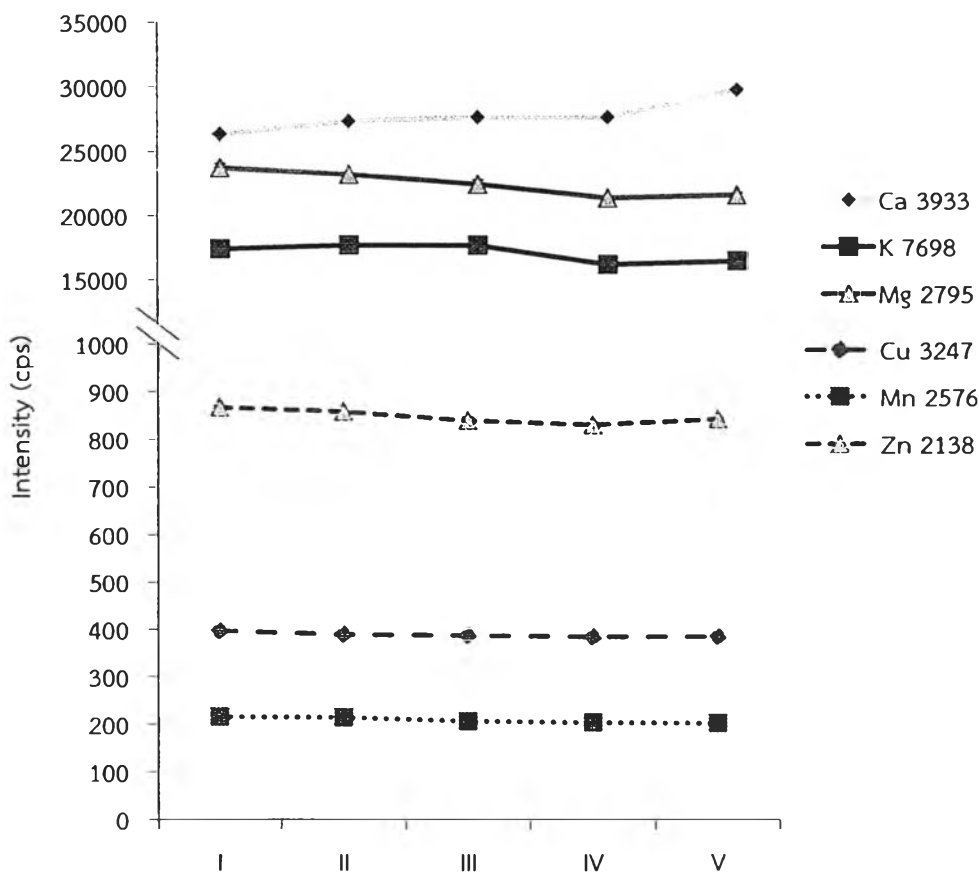
Figure 4.6 ICP-AES emission intensity of elements obtained from various agitation methods; I: manual agitation 10 sec, II: manual agitation 3 min, III: manual agitation 10 min, IV: mechanical agitation 3 min, V: mechanical agitation 10 min.

4.1.4 Effect of co-solvent

All samples prepared by this emulsification method were considerably unstable and the phase separation could take place after a certain time [2]. It was reported in the previous studies that the addition of certain organic solvents, such as propanol, butanol, and pentanol as a co-solvent could result in a microemulsion which become homogeneous and stable over much longer period of time. Generally, alcohols from C3 to C8, which could be readily dispersed in water and oil, were used as co-solvents to obtain the so-called "detergentless microemulsion" [2]. In addition, the co-solvent can reduce the viscosity of the solution, hence, the effect of various co-solvents was investigated in this experiment.

With regard to the effect of co-solvents on the sample preparation, 10% v/v of methanol, ethanol, propanol, and butanol were added as co-solvents into the emulsified samples. By using methanol, ethanol and propanol, homogeneous emulsions were obtained as shown in Figure 4.7 (a), (b), and (c), respectively. Moreover, with butanol used as a co-solvent, a clear and transparent solution-like microemulsion was obtained (Figure 4.7 (d)).

(a) Methanol 10 %

(b) Ethanol 10 %

(c) Propanol 10 %

(d) Butanol 10 %
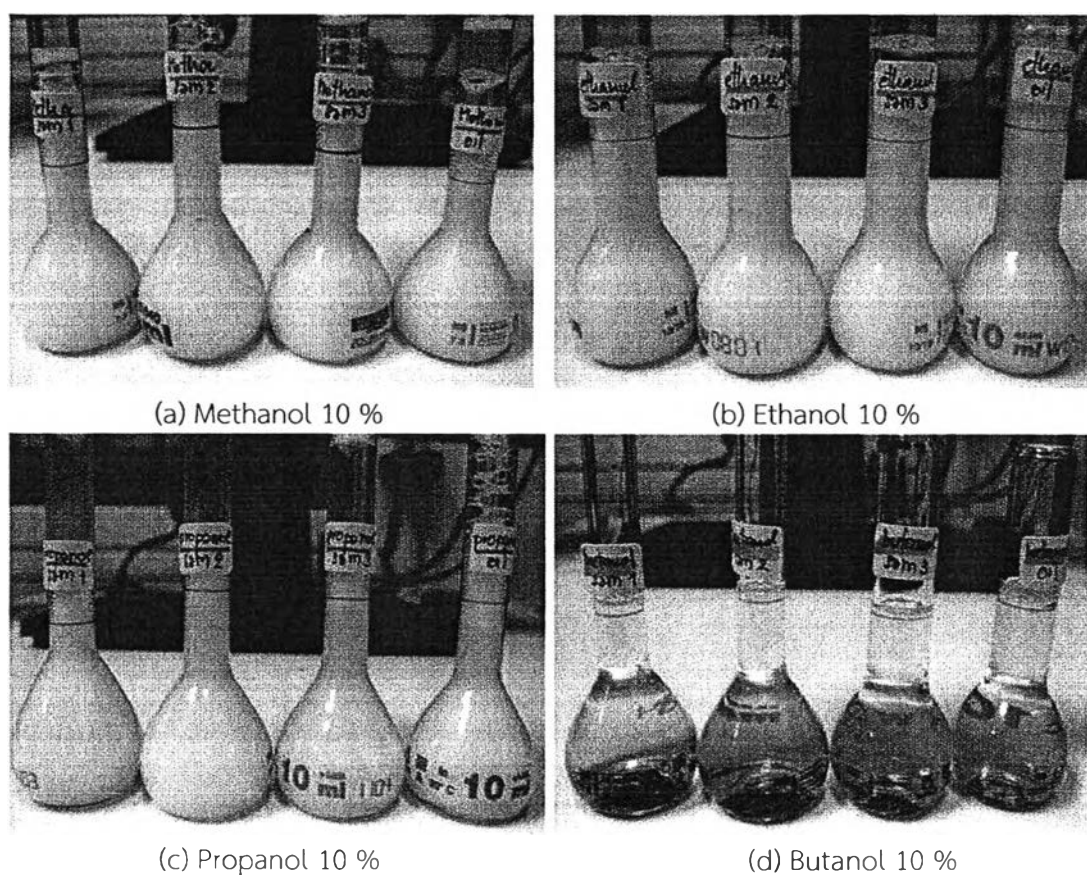
Figure 4.7 Physical appearances of emulsions prepared by using different types of co-solvents

The stability of emulsified samples with and without co-solvents was also investigated. The addition of methanol, ethanol, and propanol as co-solvents give similar results in term of stability which are better than that using Triton X-100 alone, while the one using butanol as co-solvent produced a clear and transparent emulsified sample with the highest stability. This microemulsion was stable for more

than 60 min without any sign of phase separation or inhomogeneity. Besides its appearance, the emulsion prepared with butanol as co-solvent also provided the highest emission intensity which were closely similar to those obtained from aqueous as shown in Figure 4.8.

Meanwhile, the emulsified sample prepared with propanol as co-solvent proved to be a decent combination with regard to both sensitivity and stability. Similar observations have been previously reported by a number of researchers [11, 21, 37]. Therefore, both propanol and butanol were further investigated and compared for their performances as co-solvent in the following experiment.
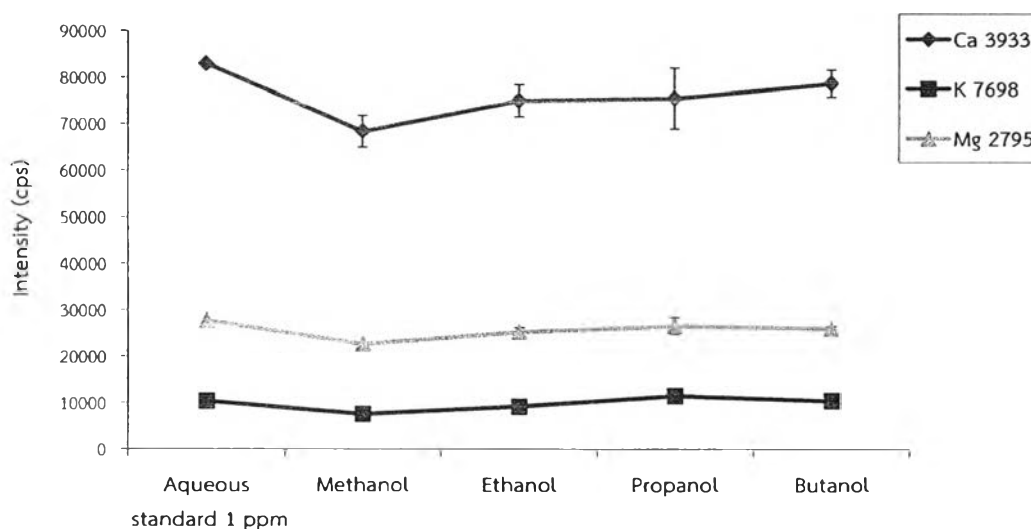
Figure 4.8 Emission intensity of emulsified samples formed with different types of co-solvent

4.1.5 Amount of co-solvent

Various ratios of the two co-solvents, propanol and butanol, in the range of 0-10% v/v were studied. The stability measurement in Figure 4.9 showed that the addition of co-solvent clearly provided better stability than the one without it. The stability of emulsions were likely to increase accordingly with the amount of co-solvent used for both type. However, the sample prepared with butanol provided drastically better stability than those using propanol. The homogeneity of the emulsified sample prepared with 10% butanol was sustained for more than 60

minutes. It was thus presumed that this mixture was formed as a microemulsion system as butanol thoroughly intervene the micelle system and then create a new equilibrium of the microemulsion system between oil, water, surfactant and butanol. Moreover, this sample also produced the highest emission signal for nearly all detected elements and very close to these of aqueous standard under the same condition as shown in Figure 4.10. Hence, 10% v/v butanol was selected as co-solvent for further experiments.

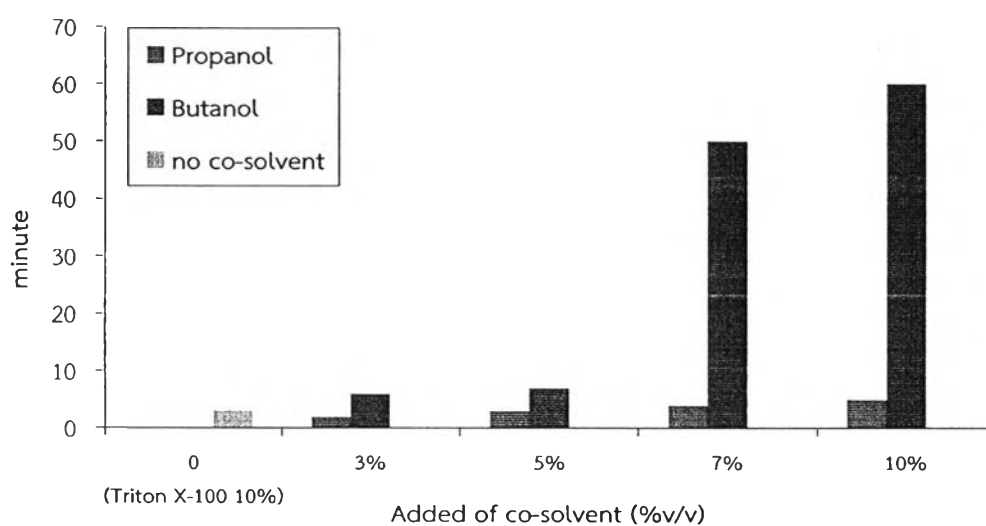Figure 4.9 Stability of the sample prepared with propanol and butanol as co-solvent at different ratios
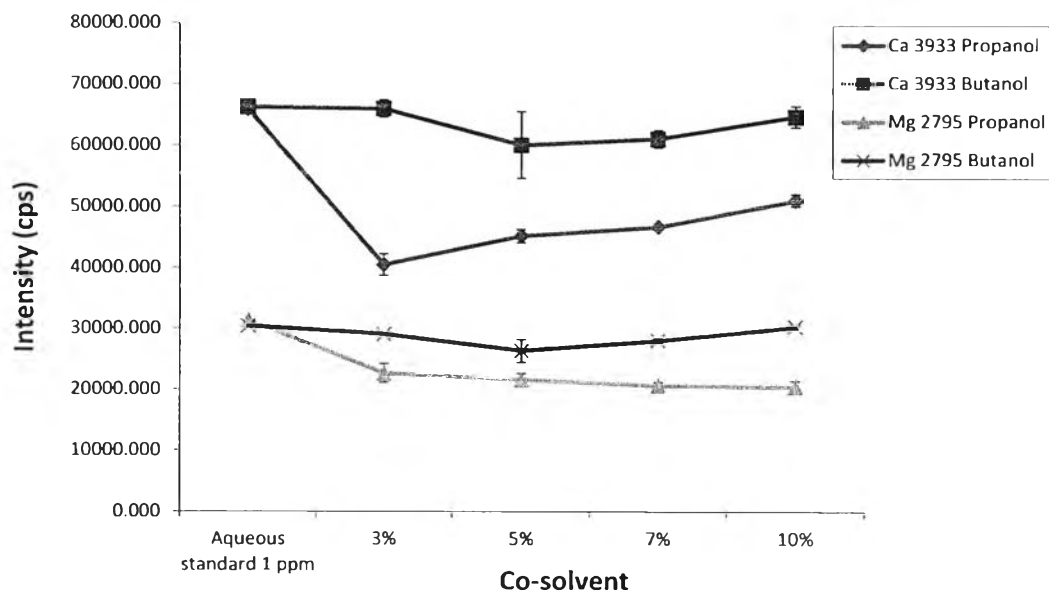
Figure 4.10 Emission intensity of the samples prepared with different ratios of as co-solvents

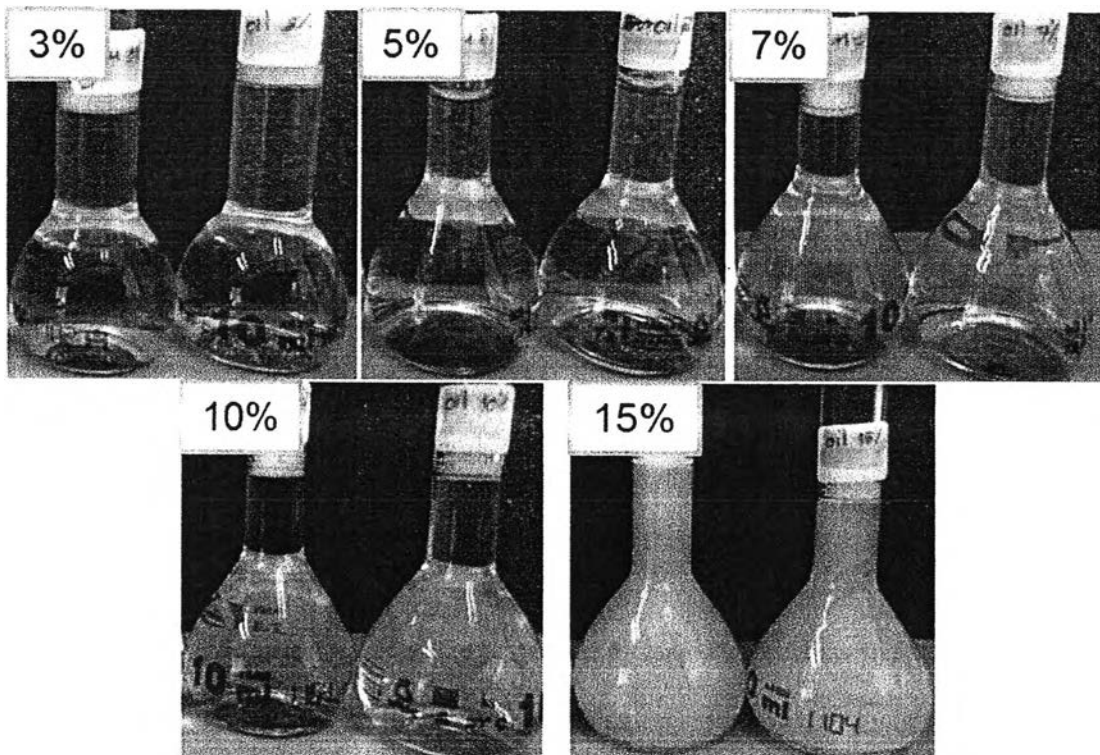### 4.1.6 Amount of biodiesel

Figure 4.11 Physical appearances of the emulsified samples containing various amounts of biodiesel

The amount of biodiesel was studied to determine the acceptable range of oil sample that can be introduced into the ICP without any adverse effect. Figure 4.11 showed that the incorporation of biodiesel in the vicinity of 3-10% v/v resulted in similarly clear and homogeneous emulsions which remained stable for over an hour while their viscosity were only slightly increased as compared to those using the surfactant alone. On the other hand, the addition of 15% v/v biodiesel produced poorer results with regard to both homogeneity and stability of the obtained emulsion. Therefore, the blending amount of biodiesel in the range of 0-10% were initially deemed appropriate and subject to further analysis by ICP-AES.

Figure 4.12 displayed the emission intensities of the selected elements (1 mg/L each) obtained from the emulsions prepared with different amounts of biodiesel from 3-10% v/v compared with those of 1 mg/L aqueous standard solution. It was found that no significant differences were observed in term of emission intensity from all sample investigated which were consistent with the physical appearances observed earlier. These results clearly suggested that the introduction of oil sample within the range of 0-10% v/v did not affect the analytical evaluation by ICP-AES and thus were deemed appropriate for such analysis. Since the contaminant elements are generally present at lower concentrations in the biodiesel, using less amount of sample (i.e. less analytes) may have a negative import on the accuracy and precision of analysis and hence the upper end of sample volume is more preferable. Therefore, the ratio of 10% v/v of biodiesel was chosen as a model for the following experiments.
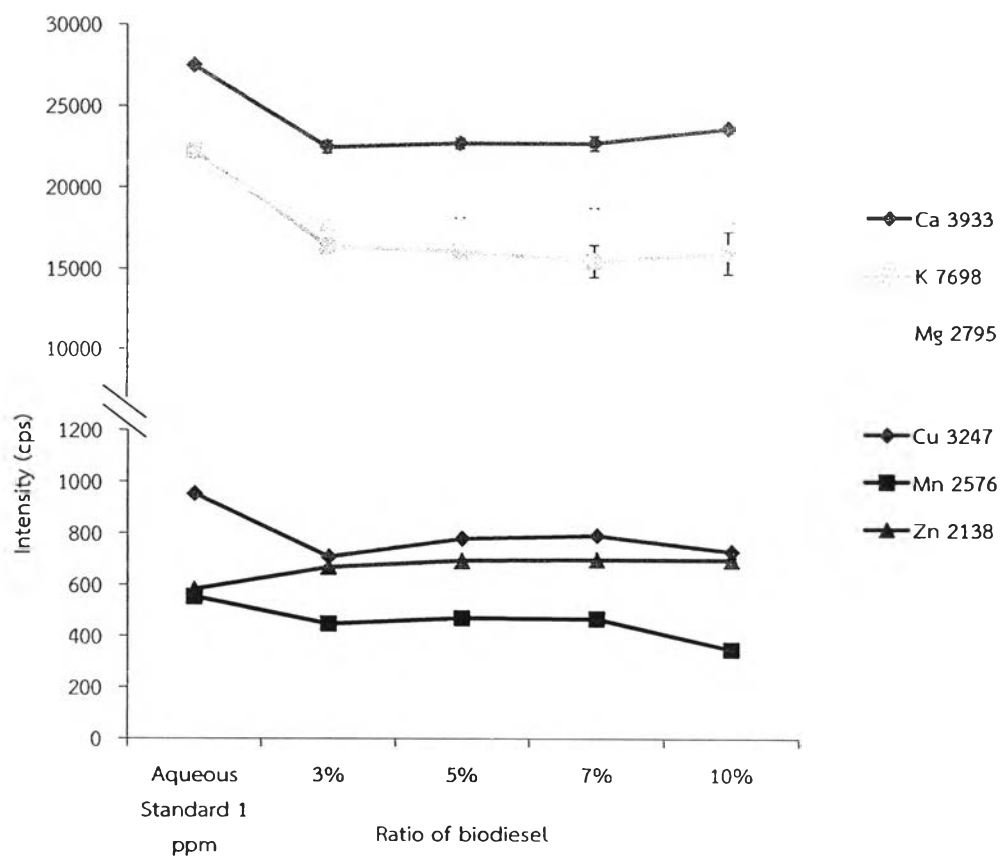
Figure 4.12 Emission intensities of elements from samples prepared with various amount of biodiesel

All of the parameters optimized for the preparation of biodiesel sample were summarized in Table 4.2. This sample was used to further optimize the operating conditions for ICP-AES analysis.

Table **4.2** The condition of the sample preparation

Parameters of sample preparation	Suitable condition
Type of surfactant	Triton X-100
Method of agitation/homogenization	Manual agitation
Amount of surfactant	10% (1 mL)
Type of co-solvent	Butanol
Amount of co-solvent	10% (1 mL)
Amount of biodiesel	10% (1 mL)

4.2 The optimal conditions for ICP-AES analysis of biodiesel

4.2.1 RF Power

The RF power was firstly optimized as it is pertained great influences towards the plasma robustness as well as the excitation performances. From the results shown in Figure 4.13, the intensity of all elements progressively increased as the RF power was tuned up from 1000 to 1300 watts. It was evident from this result that higher RF power would produce more robust and energetic plasma which in turn would enable a more complete combustion and excitation of organometallic elements in the oil samples. However, in the long run the use of such extreme condition could possibly deteriorate or even damage the equipment before its lifetime. Although the emission intensities of most elements continue to gradually increase, the intensities of K, Cu and Mn begin to stabilize at the RF power of 1200 W. Therefore the RF power of 1200 W was selected as an optimal condition for further experiments.
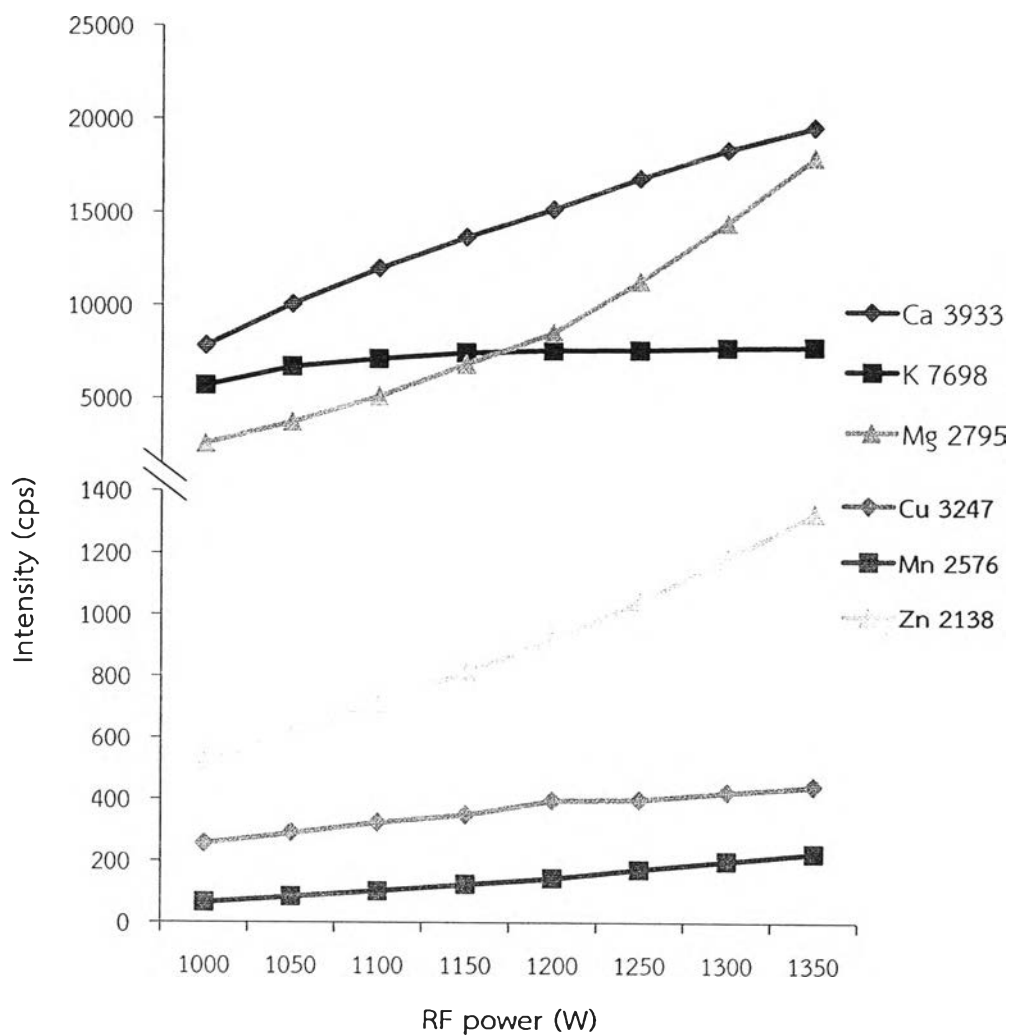
Figure 4.13 Emission intensities of elements obtained at various RF powers

4.2.2 Nebulizer gas flow rates

The nebulizer gas flow rates in the range of 0.3-0.8 L/min were optimized for ICP-AES analysis. Figure 4.14 showed that the intensity of most elements tended to increase with decreasing flow rates with the maximum obtained in the vicinity of 0.45-0.50 L/min for most elements. It was known that the sample transit time through the plasma was mainly controlled by the nebulizer gas flow rate and thus the efficiency of atomization/ionization of these elements are in turn governed by this flow rate as well. The residence time of analytes existing in the plasma was increased at lower nebulizer gas flow rates leading to better excitation efficiency and higher emission intensity as a result of longer exposure to the plasma. Conversely, higher flow rate decreased the residence time of analytes in the plasma [38] leading to poorer performance of the plasma.
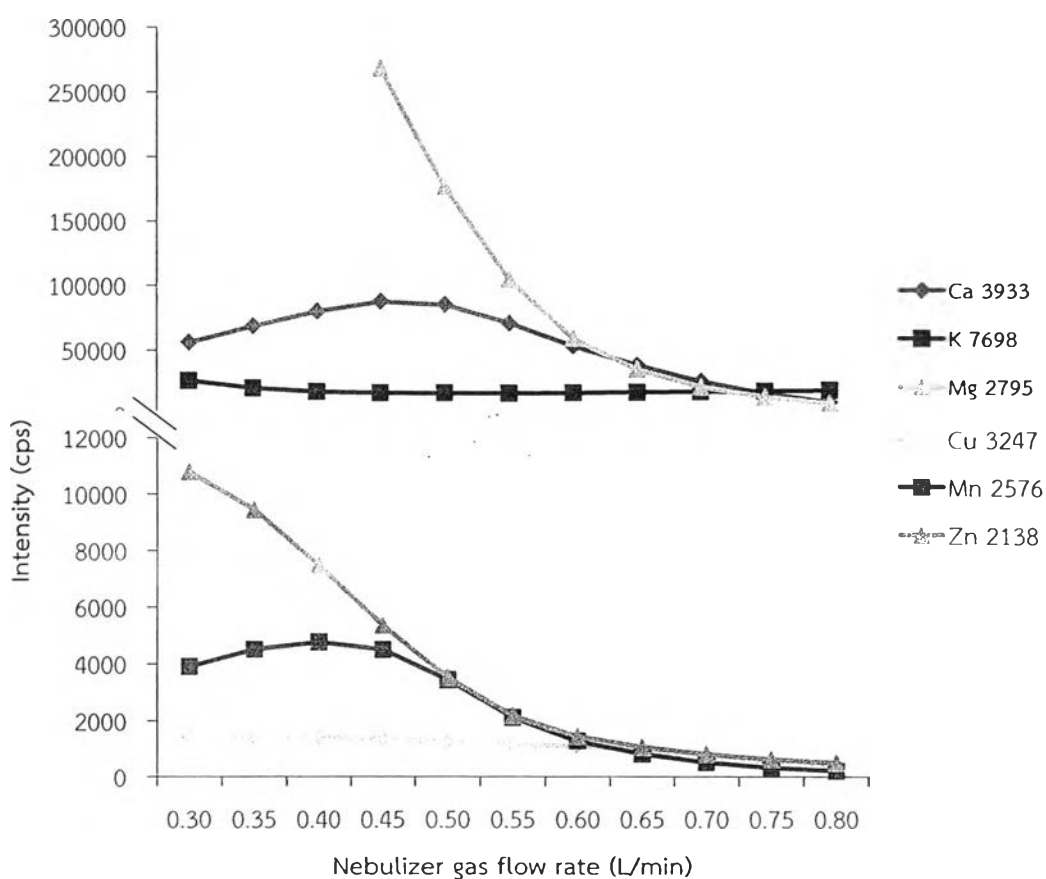
Figure 4.14 Emission intensities of selected elements under various nebulizer gas flow rates

The effect of nebulizer gas flow was also studied by a comparison of the observed emission intensity between the emulsified samples and aqueous standard solutions at the same concentration of 1 mg/L. One of the goals of this work is to enable the use of aqueous standard solutions for calibration of elements in biodiesel, therefore the resulting intensity of analytes obtained from both emulsified samples and aqueous standard solutions should be similar. As a result, the condition that favors the oil samples might not be ideal for fulfilling such objective. Based on the observations in Figure 4.15 and 4.16, the analysis performed with higher flow rates produced analytical readings that are more closely similar for both samples and standards. Despite a significant loss of sensitivity, the nebulizer gas flow rate of 0.7 L/min was chosen to satisfy the above mentioned calibration purpose.

(a)

Ca 3933

(b)

K 7698

(c)
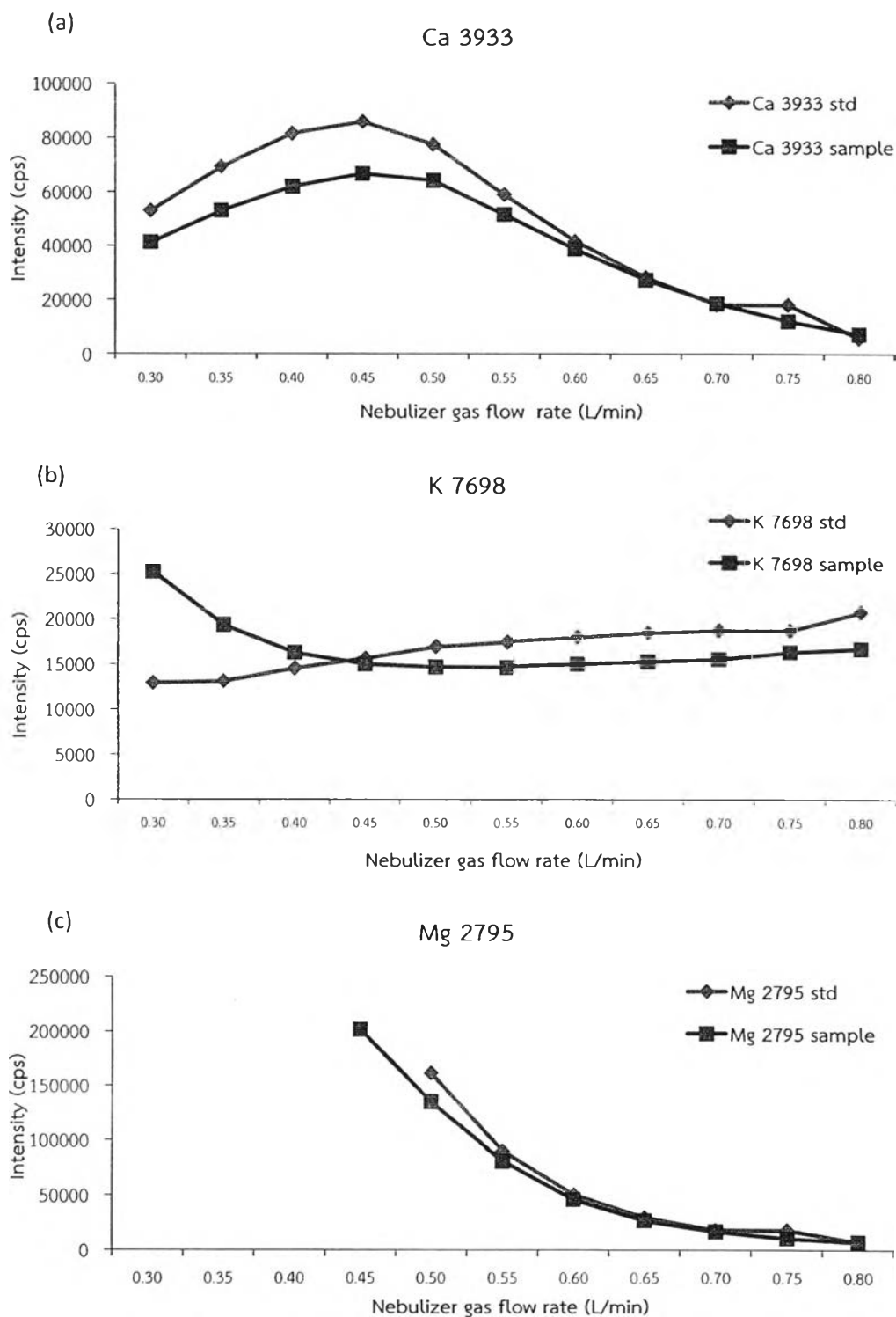
Mg 2795

Figure 4.15 Emission intensities of (a) Ca 3933, (b) K 7698, and (c) Mg 2795 obtained under various nebulizer gas flow rates

(a)

Cu 2247

(b)

Mn 2576

(c)
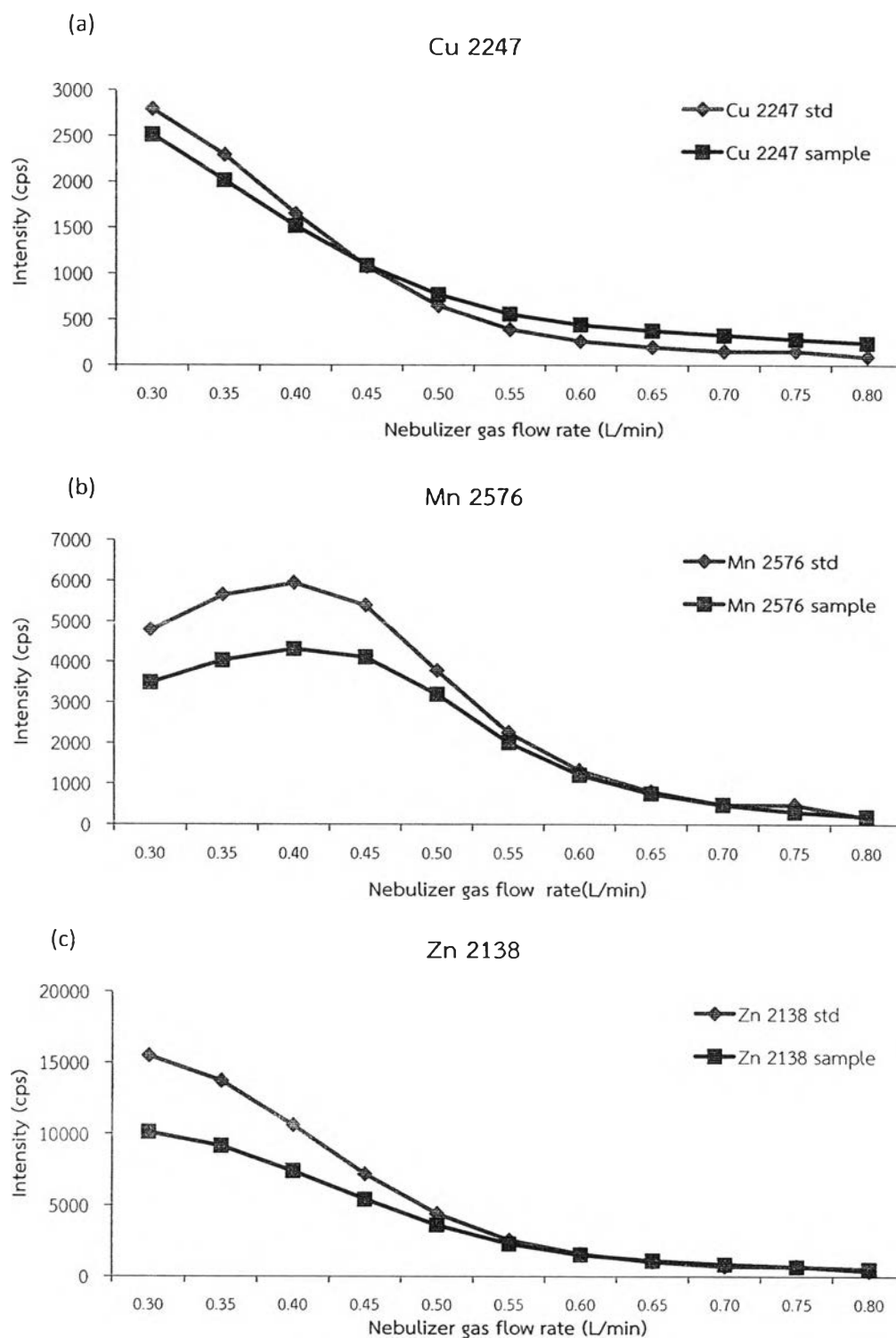
Zn 2138

Figure 4.16 Emission intensities of (a) Cu 2247, (b) Mn 2576, and (c) Zn 2138 obtained under various nebulizer gas flow rates

4.2.3 Sample uptake rate

Sample uptake rate were optimized in the range of 30-100 rpm. Experiment results from Figure 4.17 showed that the intensity of all elements did not significantly change with the increase of the uptake rate. The sample uptake rate of 50 rpm was chosen for simplicity because this sample uptake rate is a default parameter of the ICP-AES.

Figure 4.17 Emission intensities of elements obtained at various sample uptake rates

The optimal operating condition for ICP-AES analysis of biodiesel were summarized in Table 4.3.

Table 4.3 The optimal conditions of ICP-AES analysis of the emulsified biodiesel samples

Parameters of ICP-AES	Suitable condition
RF power	1200 W
Nebulizer gas flow	70 L/min
Sample uptake rate	50 rpm

4.3 Method validation (accuracy, precision, linearity)

4.3.1 Accuracy and precision

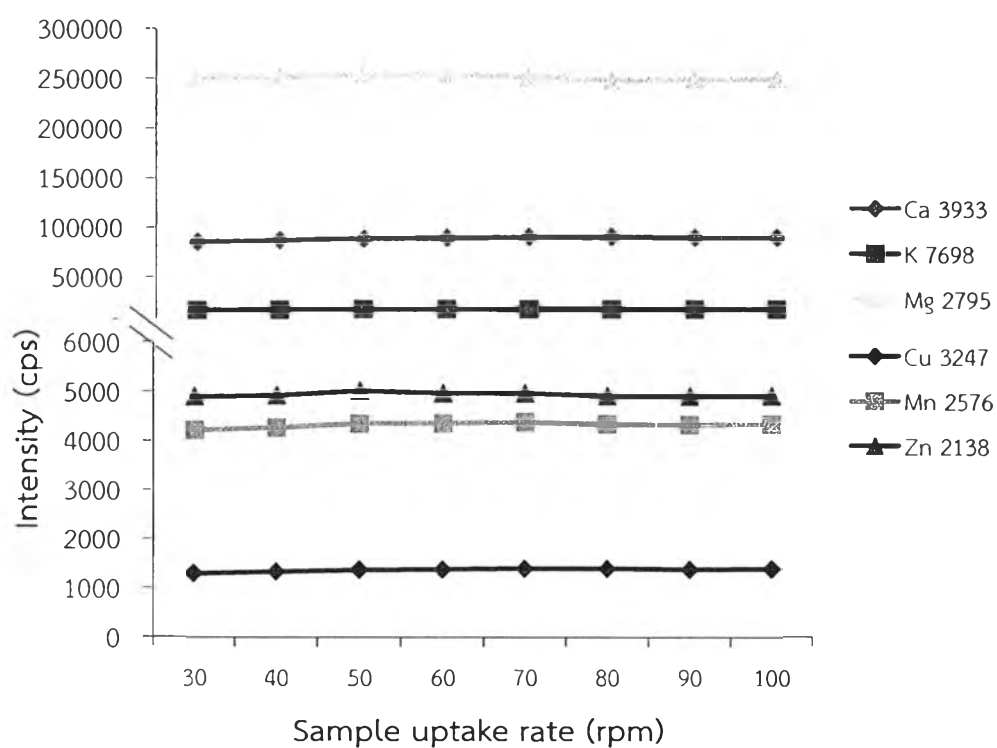The biodiesel samples spiked with organometallic additive elements at 0.2 and 1.0 mg/L were prepared by the developed emulsification method and analyzed by ICP-AES. The accuracy and precision of the analysis were evaluated in terms of recovery (%recovery) and relative standard deviation (%RSD) for the determination of each element in the emulsified samples. The percentage of recovery and relative standard deviation were calculated by using equation 4.1 and 4.2.

$$\%Recovery = \frac{N_{bs} - N_b}{N_s} \times 100 \qquad (4.1)$$

Where N_{bs} = the concentration of elements determined in a spiked sample (mg/L)

N_b = the concentration of elements determined in the unspiked sample (mg/L)

N_s = actual spike concentration (mg/L) [39]

$$\%RSD = \frac{SD}{X} \times 100 \qquad (4.2)$$

Where   SD   = standard deviation of results

X   = mean value of results

The recovery percentage obtained for each element are listed in Table 4.4 while their %RSD are reported in Table 4.5. The recoveries obtained at both concentration levels are of no significant differences with each element fare

differently, for example, Ca provide the recoveries of 98.32 and 99.22% while K only yielded 83.32 and 82.93% for 0.2 and 1.0 mg/L level, respectively. However, the overall recovery performance for all studied elements are within the range of 82.93-102.41% which are well within the acceptable range under the guideline of method validation (Table 4.6)

Meanwhile, the precision, represented by %RSD of analysis (n=3), obtained for the analysis are very similar to those obtained from routine analysis of conventional aqueous samples. The %RSD valves obtained for both 0.2 and 1.0 mg/L levels once again are in compliance with the guideline in Table 4.6.

Table 4.4 Recovery percentages of the additive elements from ICP-AES analysis

Elements	% recovery	
	0.2 mg/L	1.0 mg/L
Ca 3933	98.32	99.22
K 7698	83.32	82.93
Mg 2795	102.41	91.43
Cu 3247	86.59	84.03
Mn 2576	90.24	88.87
Zn 2138	90.19	84.03

Table 4.5 Relative standard deviation of ICP-AES analysis of emulsified biodiesel samples

Elements	% RSD (n=3)	
	0.2 mg/L	1.0 mg/L
Ca 3933	0.36	0.44
K 7698	0.31	0.86
Mg 2795	1.94	1.58
Cu 3247	0.09	0.42
Mn 2576	2.71	0.55
Zn 2138	2.32	0.23

Table 4.6 Acceptable recovery and precision of an analytical method as a function of the analyte concentration. Acceptable values according to AOAC International [40]

Unit	Mean recovery, %	RSD, %
100%	98-102	1.3
10%	98-102	2.8
1%	97-103	2.7
0.1%	95-105	3.7
100 mg/L	90-107	5.3
10 mg/L	80-110	7.3
1 mg/L	80-110	11
100 ppb	80-110	15
10 ppb	65-115	21
1 ppb	40-120	30

4.3.2 Linearity

Aqueous standard solution containing elements in a concentration range of 0.01-50 mg/L were analyzed by the proposed method. These standards were matrix matched with the same surfactant and co-solvent and treated under the same optimal conditions as applied to the biodiesel samples and then analyzed by ICP-

AES. The calibration curves were constructed for each element and its correlation coefficient are reported in Table 4.7.

Table 4.7 Linear range and correlation coefficients of additive elements

Linearity				
0.01-10 mg/L			0.01-50 mg/L	
Elements	R^2		Elements	R^2
Ca 3933	0.9998		Cu 3247	0.9999
K 7698	0.9998		Mn 2576	1.0000
Mg 2795	0.9974		Zn 2138	0.9982

The linear range of the elements can be classified into two groups. The first group of elements comprising Ca, K, and Mg showed the linearity in the range of 0.01-10 mg/L. For the other group which comprised of Cu, Mn, and Zn, the linear ranges were extended further to 50 mg/L, presumable because the ionization and excitation of these elements were not as effective as the alkali and alkaline earth metals in the first group.

4.3.3 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated from equation 4.3 and 4.4.

$$LOD = \frac{(y_B + 3SD)}{Slope} \quad (4.3)$$

$$LOQ = \frac{(y_B + 10SD)}{Slope} \quad (4.4)$$

Where y_B = mean value of intensity of the blank

SD   = standard deviation of intensity of the blank

Slope   = linearity slope of the calibration curve

The LOD and LOQ were calculated using ten repeated measurements of the method blanks. The results are shown in Table 4.8.

Table 4.8 The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method

Reagent blank	LOD (mg/L)	LOQ (mg/L)
Ca 3933	0.007	0.010
K 7698	0.003	0.020
Mg 2795	0.005	0.008
Cu 3247	0.010	0.016
Mn 2576	0.003	0.011
Zn 2138	0.003	0.006