

DEVELOPMENT OF NIR METHOD FOR DETERMINATION OF RHEOLOGICAL BEHAVIOR AND DRUG CONTENT OF SEMISOLID CONTAINING DIFFERENT RATIOS OF LIPID TO AQUEOUS PHASE

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ABSTRACT

Objectives: to demonstrate the efficiency of the near infrared spectroscopy for determination of the potency of active ingredient and viscosity of cream formulations containing different ratios of lipid to aqueous phase.

Methods: The drug model used is ibuprofen at a concentration of 0.2% w/w to 0.8% w/w. Formulations containing of different ratios of lipid to aqueous phase (different viscosity) containing 1% w/w ibuprofen were also prepared. An accurately 15 samples of

0.3 40 g cream from each batch were placed in vials and transferred to a sample wheel for analysis using NIR spectroscopy. The resultant spectra for each sample was calculated by OPUS software. The average transmittance of sixteen (16) scans measured at 700 nm – 2500 nm was calculated. A reference UV spectroscopy technique was used to compare the NIR method to the reference method for determination of drug content in the cream. The Brookfield viscometer was used as a reference lab technique to compare viscosity measured using Near-infrared versus the reference method. Calibration curves between NIR and reference lab methods were conducted.

Results: For drug content, the correlation coefficient and the root mean square error of cross validation were 90.67% and 0.138 mg respectively, and for viscosity were 98.04% and 1.4×10^3 CP. A series of model equations were developed using partial least square calibration models to predict drug content and viscosity of cream from NIR data. **Conclusion:** The NIR data for drug content and viscosity were precise and similar to the lab reference method.

Keywords: Near infra-red spectroscopy, Rheological behavior, Drug content.

INTRODUCTION

The quality of the outcome pharmaceutical products depends on two aspects, raw material quality and manufacturing process parameters. Often changes in active ingredient or pharmaceutical ingredients properties can affect product quality [1]. Drug content of semisolid products like ibuprofen cream are currently analyzed by UV spectroscopy and liquid chromatography which are time consuming because they require several steps as extraction, sample preparation, treatment of column and the use of organic solvents [2]. Another important analysis of semisolid includes viscosity measurement since the application and the acceptance of pharmaceutical formulation is dependent on the flow properties of the final product. In general rheological measurement of semisolid are performed 1) to understand the fundamental nature of a system, 2) for quality control of raw materials, manufacturing process parameter, and final product. The process of viscosity measurement using viscometers is time consuming, non cost effectiveness, non accurate and can not predict stability.

During the last years, the Near infra-red spectroscopy has been found to be attractive for the pharmaceutical industry and the quality control because of its advantages. Analysis can be done directly, can be used on-line or in-line during the manufacturing of pharmaceutical products, requires no sample preparation, non-destructive, generate no waste matter, eliminate operator bias, and greatly reduces exposure to potent drug and toxic organic solvents [3]. Near infra-red radiation appears between far-infrared and visible region in the electromagnetic spectrum. The conventional near infra-red region lies between 700 and 2500 nm. Near infra-red spectra arises from absorption bands resulting from overtones and combinations of fundamental near infra-red stretching and bending bonds. They have low molar absorptivity with broad, overlapping peaks. The low absorptivities are the primary usefulness of the method for the analysis of intact dosage forms. These absorbance arises from mainly C-H, O-H, N-H and S-H bonds [4].

There are two different measurements commonly performed in the near infra-red spectral range: transmittance and reflectance.

Transmittance measures the decrease in radiation intensity as function of wave length when radiation is passed through the sample. Conversely, reflectance measures the ratio of the intensity of light reflected from the sample [5].

There are many studies [6, 7, 8, 9, 10] that demonstrate the efficacy and utility of near infra-red as an analytical technique in quality control, manufacturing process, and for final dosage form. Moes et al. evaluate near infra-red spectroscopic imaging as a tool to assess blend uniformity problem in the final product [11]. Blanco et al proposed a method based on near infra-red diffuse reflectance spectroscopy for the analysis of active ingredient in commercial product [12]. They found a model to determine the active ingredient in tablets with high accuracy and precision permitting content uniformity analysis. Other investigators studied the hypothesis that using near infra-red imaging spectroscopy for tablets in blister packs permits identification and determination of multiple tablets composition simultaneously. Sulub et al [13] validated a near infra-red method for on-line identity check of pharmaceutical finished product in order to assure product safety.

Donoso et al. used the near infra-red for quantitative measurement of viscosity and concentration of active ingredient in pharmaceutical gel. The results obtained proved that near infra-red method can be an alternative method for analysis of drug content and viscosity [14]. Schiro et al [15] study the quaternary relaxation in sol-gel encapsulated hemoglobin using NIR and UV spectroscopy.

Kreft et al [16] used near infra-red spectroscopy for quantitative determination of polyvinylpyrrolidone type and they found that the different types of povidone are characterized by their viscosity measured in water. Ajmera et al, [17] use reverse phase high performance liquid chromatography determination of active drugs in commercial tablets and Rant et al [18] used spectrophotometric method for determination of angiotensin-II receptor antagonist in bulk and pharmaceutical dosage forms.

Parfire et al [19] used near infrared spectroscopy for determination of drug content and pharmaceutical property of blends. Kelly et al

[20] used near infrared spectroscopy for monitoring co-crystal of ibuprofen nicotinamide.

The aim of this research is to present an alternative method for measurement of drug content and viscosity as a result of varying the ratios of lipid to aqueous phase of pharmaceutical products such as creams. Also to develop calibration models for prediction of drug content and viscosity from the near infra-red spectra.

MATERIALS AND METHODS

Ibuprofen, US, lot No. 39790, Mova Pharmaceutical Co., Puerto Rico; cetyl alcohol, lot No. 01702 LB, Sigma Aldrich Inc., St. Louis, MO, USA, stearic acid, lot No. 025407, Fisher Scientific, Fair Lawn, New Jersey, USA, glycerin, lot No. 041365, Fisher Scientific, Fair Lawn, New Jersey, USA, mineral oil, USP, lot No. 044608, Fisher Scientific, Fair Lawn, New Jersey, USA, triethanolamine, lot No. 042806, Fisher Scientific, Fair Lawn, New Jersey, USA, sodium phosphate monobasic (lot No. 025346, Fisher Scientific, St. Louis, Fair Lawn, New Jersey, USA), and sodium hydroxide (lot No. 010145, Fisher Scientific, Fair Lawn, New Jersey, USA).

Preparation of the cream formulations

Four cream formulations each of 100 g batch size were prepared containing 0.2% w/w to 0.8% w/w ibuprofen and additional five cream formulations of the same composition but of different ratios of lipid to phase and consequently of different viscosity 6,800 Cp, 15,200 CP, 16,700 CP, 24,000 CP, and 38,400 CP) containing 1% w/w drug and each of 1 Kg batch size were prepared for viscosity study. The composition of the different formulations is shown in Tables 1 and 2.

Formulations containing different drug content were prepared as follows: The oily phase containing stearic acid, cetyl alcohol and mineral oil was transferred to a beaker and the base was allowed to melt and heat to a temperature of 75°C using water bath (Isotemp 128, Fisher Scientific, Fair Lawn, New Jersey, USA). The drug was added to the oily phase and mixed until completely dissolved. The aqueous phase containing distilled water, glycerin and triethanolamine was also heated to 75°C using a water bath.

The aqueous phase was added to the oily phase and mixed using homogenizer (Power Gen 700 homogenizer, model GLH-115, PG 700, Fisher Scientific, Fair Lawn, New Jersey, USA) set at constant stirring speed for 15 minutes until the cream congeals and the consistency increased. The cream was set for 24 hours before analysis.

The same procedure was used for preparation of the different viscosity formulations except that mixing of the two phases was done at higher speed (mixer speed was set at 4.5 instead of 3.5 speed) and mixing was achieved for a period of 45 minutes instead of 15 minutes.

Methodology development of Near Infrared spectroscopy

A single Near Infra-red spectra was run for 15 samples from each batch. Sample positioning is the largest source of measurement variability in near infrared analysis and in order to reduce this error, samples were placed in glass vial. Each vial contains 0.340 g cream and all vials were placed on samples wheel and the Near infra-red spectra were collected using Near infra-red spectrophotometer (model Vector 22/NI, Bruker Optics Inc., Billerica, MA, USA), personal computer and the OPUS Near infra-red spectral analysis software chemometric version. Each spectrum was an average of 16 scans of a resolution over the range of 700 to 2500 nm using the

transmittance mode. The light that was transmitted through the cream sample was measured using a sensitive indium, gallium and arsenide (IN GA AS) external detector positioned above the sample.

Development of calibration models

Partial least square (PLS) regression analysis was used to develop calibration models. The spectra were normalized and the first derivative spectra was computed in order to correct for the base line shifting effect that are common in Near infra-red spectra.

The calibration model for drug content was developed with 40 sample (10 samples from each of the 4 batches prepared), and 50 samples for viscosity (10 samples from each of the 5 batches prepared). As a preliminary test for the model, the software performed a leave-one out cross validation. This step consists of developing a calibration model with all the samples, but one. The sample left out was then predicted by the calibration model. This was done in order to make the calibration model rugged and eliminate the bias that may arise during the prediction of drug content if the data of the cream in the prediction set was were include in the calibration model.

The root mean square error of cross validation was used to describe the results of cross validation. The performance of the Near infra-red calibration model was evaluated with the prediction of an independent validation set comprised of 20 cream samples for drug content (5 sample from each batch of the 4 batches prepared), and 25 samples for viscosity (5 samples from each batch of the 5 batches prepared). The root mean square of error was used to describe the difference observed between the predicted drug content or viscosity and the reference method value.

Data Analysis and statistical evaluation

The pair T-test was used to determine if there is significant difference between the reference method and the Near infra-red method. This test is used for testing the mean difference between paired observation. The p value is used to determine if there is significant difference or not. One way ANOVA analysis as also used to test the hypothesis that the means of several populations are equal. This test requires a response or measurement taken from the units sampled and a factor or discrete variable that is altered systematically. The most important statistic in the analysis of variance table is the p value which indicates if the level means are significantly different from each other. If p is less than or equal to the α level selected, one or more means are significantly different.

RESULTS AND DISCUSSIONS

Tables 1 and 2 show the composition and the viscosity of the different formulations of the pharmaceutical creams.

Table 1: Composition and formulation of pharmaceutical creams

Ingredients	Amount (g)
Ibuprofen	0.2%, 0.4%, 0.6%; 0.8% w/w
Stearic acid	6.00
Cetyl alcohol	3.00
Mineral oil	20.00
Triethanolamine	2.00
Glycerin	15.00
Distilled water Q.S to	100 g

Table 2: Formulation of pharmaceutical cream of different viscosity

Ingredients	Formulation				
	#1	#2	#3	#4	#5
Oleaginous phase					
Cetyl alcohol (g)	33.0	50.0	66.0	83.0	99.0
Stearic acid (g)	66.0	99.0	132.0	165.0	198.0
Mineral oil (g)	220.0	220.0	220.0	220.0	220.0
Ibuprofen (g)	10.	10.0	10.0	10.0	10.0
Aqueous Phase					
Distilled water (g)	600	550	500	450	400
Triethanolamine (g)	22.0	22.0	22.0	22.0	22.0
Glycerin (g)	165.0	165.0	165.0	165.0	165.0

The Near infra-red spectrum exhibit characteristic information of ibuprofen from 0.8 to 1.4 um which is related to the third overtone peak of C-H, second overtone peak of C-H and first overtone peak of C-H combinations as shown in Figure 1. Also, shows that non-linear signal response are predominant in the range of 1.4 um to 2.5 um which is not useful in constructing a calibration model.

Absorbance

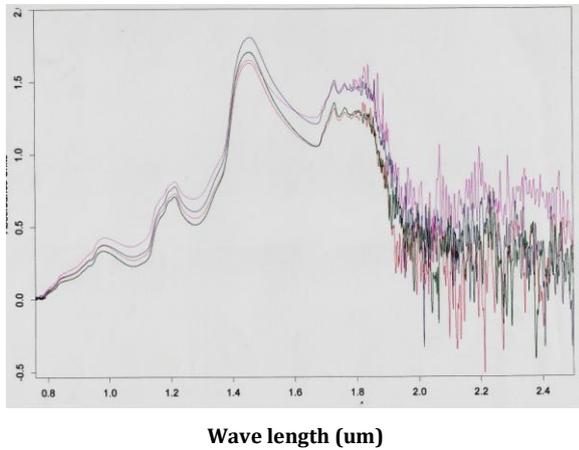


Fig. 1: NIR full spectrum (0.76 μm - 2.5 μm) of ibuprofen pharmaceutical cream at four different concentration levels

Absorbance

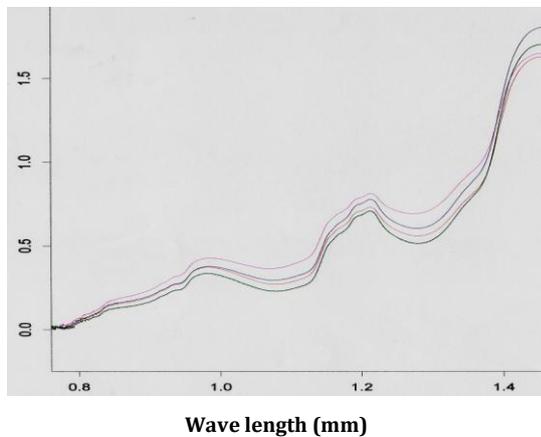


Fig. 2: NIR spectrum (0.76 - 1.4 μm) of ibuprofen pharmaceutical cream at four different concentration levels

Absorbance

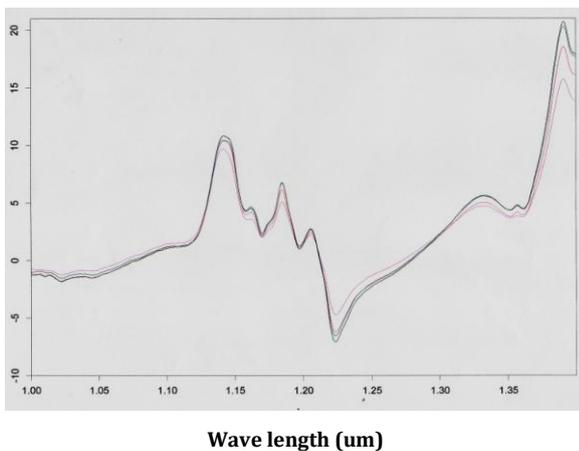


Fig. 3: First derivative transformed spectra in spectral region used to quantify drug content.

The correlation of near infra-red absorption versus the drug concentration levels can be clearly observed in the normalized spectra of ibuprofen cream containing different concentration levels shown in Figure 2. As the concentration of ibuprofen in the cream is increased from 0.2% w/w to 0.8% w/w, the absorbance is increased.

In order to improve spectral features, the first derivative was used as data pre-treatment, transforming the spectra in a spectral region that was used to quantify the drug content. Figure 3 shows the first derivative spectra for the average spectra of the different drug concentration levels.

Table 3 shows the results for the paired T-Test , standard deviation and the standard error mean for both reference method (UV spectroscopy) and Near infra-red spectroscopy for ibuprofen cream containing different drug concentration levels. The pair T-test showed that at a 95% confidence interval there is no significant difference between the two methods. The P value was 0.998 > less than 0.005 indicating no significant difference.

Table 3: Results for the paired t-test and confidence interval for UV and NIR- drug content study

	N	Mean	Standard deviation	Standard error mean
UV	40	1.35735	0.64752	0.10238
NIR	40	1.35739	0.63454	0.10033
Difference	40	-	0.128528	0.020322
		0.000040		

95% confidence interval (CI) for mean difference (-0.41145, 0.041065); T - value = - 0.00 ; P- value =0.998.

Figure 4 shows the NIR and UV versus experimental spectra of ibuprofen cream formulations of the same composition but of different ratios of lipid to aqueous phase and consequently of different viscosity which were obtained over the range of 0.76 to 2.5 um. The spectra (Figure 4) shows that non-linear signal response is predominant in the range of 1.04 um to 2.5 um which was not useful constructing

Absorbance

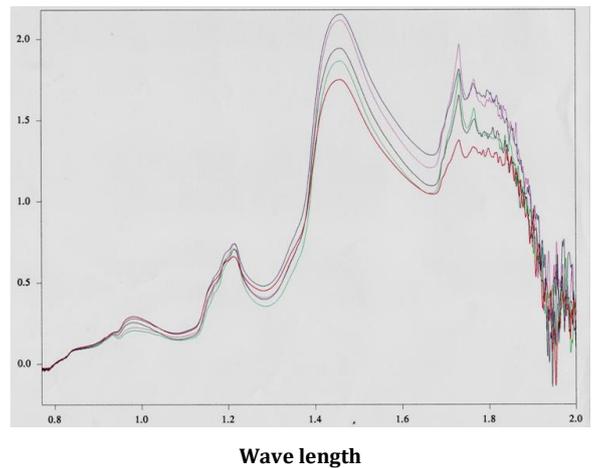
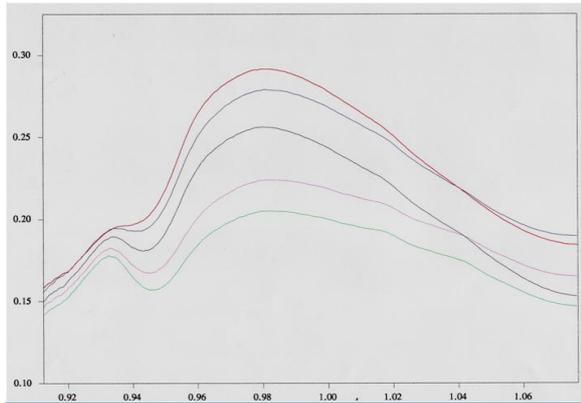


Fig. 4: NIR spectrum (0.76 μm - 2.0 μm) of ibuprofen pharmaceutical cream at five different viscosity levels

Calibration model

The method of normalization was used and the correlation of Near infra-red absorption versus the viscosity levels can be seen in Figure 5. As the viscosity of the cream formulation is increased due to the decrease of amount of distilled water and the increase of the amount of lipid, the absorbance is decreased in the region of 0.90 um to 1.04 um. As the amount of water is increased and the lipid is decreased, the viscosity decreased and the NIR absorbance increased as shown in Figures 5 to 6.

Absorbance

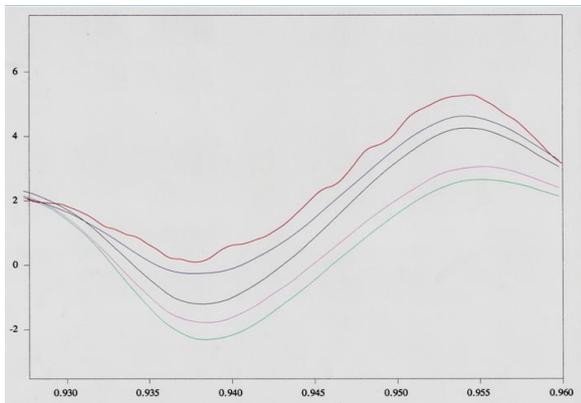


Wave length

Fig. 5: NIR spectrum of ibuprofen pharmaceutical cream at five different viscosity levels (region from 0.90µm to 1.08µm)

Figure 6 shows the first derivative spectra for ibuprofen cream formulations of different viscosity as a result of varying the ratios of lipid to aqueous phase..

Absorbance



Wave length

Fig. 6: First derivative transformed spectra in spectral region used to quantify viscosity.

Table 4 shows the results for the paired T-test, standard deviation and the standard error mean for ibuprofen cream formulations of different viscosity. The pair T-test showed that at a 95% confidence interval, there was no significant difference between the two methods (Brookfield viscometer and Near infra-red spectroscopy).

Table 4: Results for the paired t-test and confidence interval for viscometer and NIR

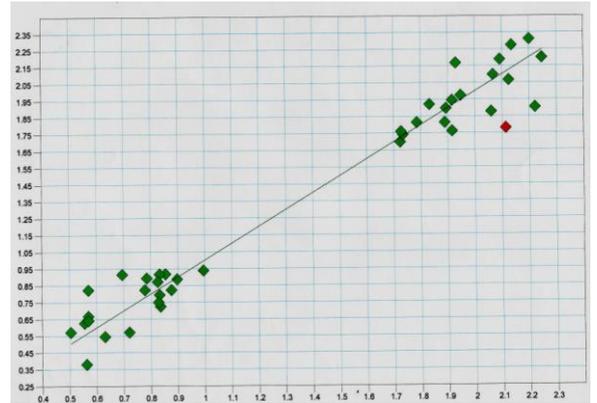
	N	Mean	Standard deviation	Standard error mean
Viscometer	50	20124.0	10718.0	1515.8
NIR	50	20124.7	10661.0	1507.7
Difference	50	-	1105.472576	156.337431
		0.660000		

95% CI for mean difference: (-314.831830, 313.511830); T - value = -0.00; P- value =0.997

The p value was 0.99 was higher than 0.005 indicating no significant difference.

Figure 7 shows the calibration curve of drug content using UV analysis versus drug content using near infrared spectroscopy and using partial least square calibration model. The correlation coefficient (R²) with five factors was 0.9607 and with root mean square error of cross validation of 0.138 mg..

NIR value

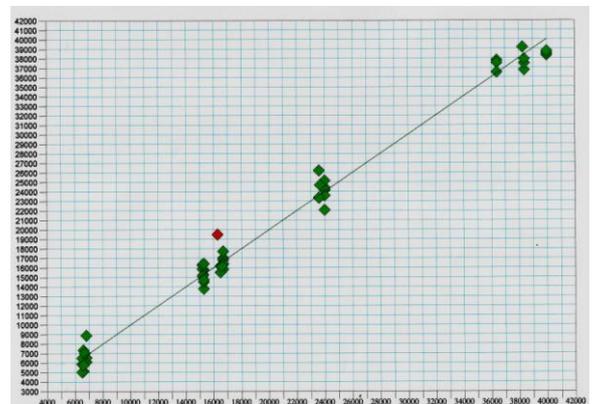


UV analysis values

Fig. 7: PLS linear regression of the calibration model

Figure 8 shows the calibration curve of viscosity as a result of varying the ratios of lipid to aqueous phase using Brookfield viscometer versus viscosity using Near infra-red spectroscopy. The correlation coefficient (R²) was for the linear regression of the partial least square calibration model with three factors was 0.9894 with root mean square error of cross validation of 1.14 X 10³ CP

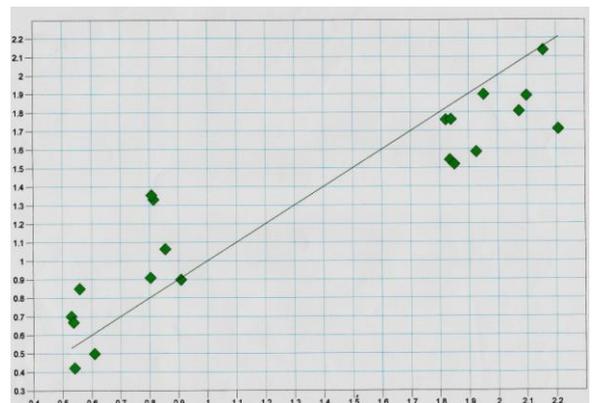
NIR values



Viscometer analysis values (cp)

Fig. 8: NIR spectrum of ibuprofen pharmaceutical cream at five different viscosity levels (region from 0.90µm to 1.08µm)

NIR predicted values (mg)



U.V. analysis values (mg)

Fig. 9: Plot of NIR prediction vs. UV-vis data with samples in validation set

The calibration model was tested and evaluated with the prediction of 20 samples that were not used for construction of the calibration model. The evaluation of the test set validation was based on the value of root mean square error of prediction and its correlation coefficient of a linear regression line between Near infra-red prediction and UV reference values. The linear regression of the samples in the validation set from 0.9997 μm to 1.4003 μm using first derivative and the multiplicative scatter correction as data pre-processing is shown in Figure 9. The correlation coefficient (R^2) for the linear regression of the validation set is 0.8293 with a root mean square error of cross validation of 0.271 mg.

The calibration model was tested and evaluated with the prediction of 25 sample for viscosity study. The linear regression of the samples in the validation set from 0.91987 μm to 0.97009 μm using first derivative and the multiplicative scatter correction as data pre-processing is shown in Figure 10. The correlation coefficient (R^2) for the linear regression of the validation set is 0.9699 with a root mean square error of cross validation of 1.84×10^3 CP

NIR predicted viscosity value

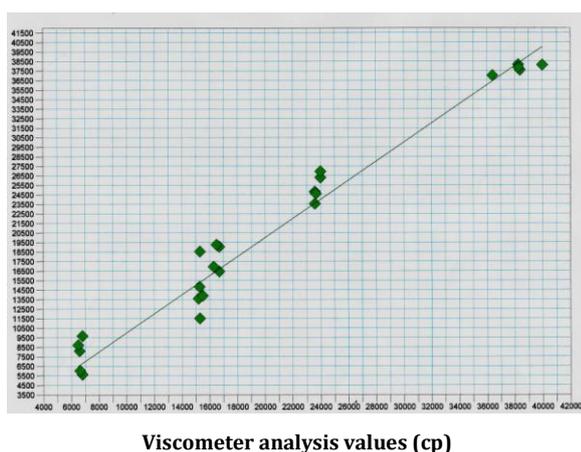


Fig. 10: Plot of NIR prediction vs. viscosity data with samples in validation set

The viscosities obtained by the two methods, Brookfield viscometer and near infrared appear to be similar.

CONCLUSIONS

This research indicated that the proposed near infrared method is reliable, rapid and accurate for direct determination of drug content and viscosity of pharmaceutical cream dosage forms of the same composition but containing different ratios of lipid to aqueous phase. The Calibration models were developed using partial least square regression analysis and Quant 2 Near infra-red software. The predicted values for drug content and viscosity of different formulations of the same composition but containing different ratios of lipid to aqueous phase were similar to the values obtained by using reference methods.

It can be concluded from this research that near infrared method can be used as an alternative non destructive method for quantitative analysis of active ingredient and for viscosity determination of pharmaceutical cream.

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