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ORIGINAL ARTICLE

Application of Partial Least Square (PLS) Analysis on Fluorescence Data of 8-Anilinonaphthalene-1-Sulfonic Acid, a Polarity Dye, for Monitoring Water Adulteration in Ethanol Fuel

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Abstract Fluorescence characteristic of 8-anilinonaphthalene-1-sulfonic acid (ANS) in ethanol-water mixture in combination with partial least square (PLS) analysis was used to propose a simple and sensitive analytical procedure for monitoring the adulteration of ethanol by water. The proposed analytical procedure was found to be capable of detecting even small adulteration level of ethanol by water. The robustness of the procedure is evident from the statistical parameters such as square of correlation coefficient (R^2), root mean square of calibration (RMSEC) and root mean square of prediction (RMSEP) that were found to be well with in the acceptable limits.

Keyword Ethanol · Water · ANS · 8-anilinonaphthalene-1-sulfonic acid · PLS-analysis · Fuel-adulteration

Introduction

In recent years, ethanol has been considered as an alternate for petroleum based fuels such as gasoline and diesel [1–7]. Ethanol is a renewable source of energy that can be fermented and extracted from the natural products [1–7]. In most parts of the world, ethanol is being used as ethanol-gasoline and ethanol-diesel blends [1–10]. However, ethanol has also been successfully used as standalone fuels in internal combustion (IC) engines without much adjustment in the configuration of vehicle engines [11]. Use of ethanol as fuel can significantly reduce the emission of certain hazardous chemicals in the environment [1–11].

Despite these advantages, use of ethanol as a fuel in IC engines has certain problems. One such issue is the solubility of ethanol and water in all proportions. In fact, it is difficult to observe any differences between ethanol, water and ethanol-water mixtures. Thus, there is always a temptation for adulterating the ethanol fuel with water. In order to ensure the quality of ethanol as a fuel it is essential that water adulteration in ethanol should be carefully monitored for the following reasons:

(i) Most of the components of IC engines are prone to degradation when they come in contact of water [1, 2, 4, 7, 12].
(ii) Presence of water in ethanol causes the phase separation in diesel-ethanol and gasoline-ethanol blends limiting their utility as efficient fuels [1, 2, 4, 7–12].

In recent years, ethanol based fuel cell, known as direct ethanol fuel cell (DEFC) has been considered as an alternate to IC engines because of its high-energy efficiency [13, 14]. DEFC involves oxidation of ethanol in presence of oxygen ($C_2H_5OH+3O_2 \rightarrow 2\ CO_2+3H_2O$) [13, 14]. The presence of water in ethanol can significantly reduce efficiency of DEFC for a given volume of fuel. Therefore, monitoring of water content in ethanol sample is also important to ensure maximum efficiency of DEFC.

8-anilinonaphthalene-1-sulfonic acid also known as ANS, molecular structure shown in Fig. 1, is a fluorescence polarity probe [15, 16]. It has been used to study various biological systems [16–21]. ANS shows enhancement of intensity with a blue shift of fluorescence maxima while going from a polar to non-polar system [15–24]. In ethanol-water mixture, water is more polar than ethanol. Therefore, ANS would show a decrease in fluorescence

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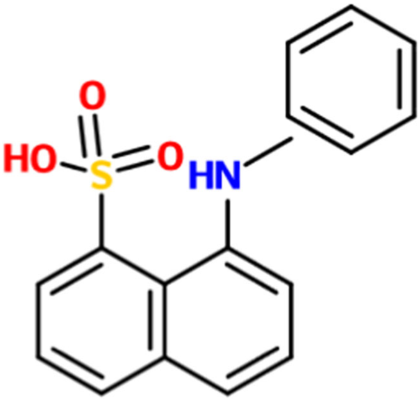

Fig. 1 Molecular formula of 8-anilinonaphthalene-1-sulfonic acid (ANS)

intensity with increase of water percentage in ethanol. In literature, the loss of fluorescence of ANS in water is attributed to quenching process arising due to electron transfer from water to ANS molecule [15–24] The polarity-sensing characteristic of ANS can be explored for monitoring the adulteration of water in ethanol fuel.

The objective of the present work is to show that ANS, a polarity probe, in combination of partial least square (PLS) analysis [25–27] can be used for developing a simple, sensitive and fast analytical procedure for monitoring the adulteration of water in ethanol fuels. In literature, Karl-Fischer [28], electrochemistry [11], ultrasonic parameters [29], time-domain reflectrometry [30], capacitive micro-sensor [31], infrared (IR) [32], Fourier transform infrared (FTIR) [33] and NMR [33] based methods are also available for the analysis of water in ethanol fuels. However, the proposed procedure over the reported methods has the advantage of providing a very simple, fast and user-friendly way without involving any laborious step for monitoring the water adulteration in ethanol.

Material and Methods

Chemicals and Sample Preparation

Ethanol was procured from local vendors from Chennai (India). ANS was purchased from Sigma-Aldrich. Water was distilled three times using KMnO4 and NaOH. Ground water was collected from the well of IIT-Madras campus (Chennai) and it is not suitable for drinking purpose. Calibration set (C1) of eleven samples wherein ethanol is adulterated by water to different extent was prepared. Amounts of ethanol and water in these samples are summarised in Table 1. Two validations sets one made using the tripled distilled water (V1) and other made using ground water (V2) were also made. Amounts of ethanol and water in these samples of both the validation sets are summarized in Table 2. An aliquot of 10^{-3} M was prepared by dissolving 15 mg of ANS in 50 mL of dry-ethanol. 0.1 mL of the aliquot was added to 5 mL of each of the 21 samples. The concentration of ANS in ethanol-water mixture was found to be optimum in the range of ~$(0.7–3)\times10^{-5}$ M. At concentrations higher than this inner filter effects will significantly affect the fluorescence ANS. At very low concentration, it will be difficult to measure the fluorescence of ANS, in particular in ethanol samples with high amount of water adulteration and it is mainly due to quenching of ANS fluorescence due to electron transfer from water to ANS molecule. The calibration sets were made in triplicates to verify the reproducibility of the outcome of proposed procedure. The other two calibration sets were labelled as C2 and C3. Two validations using triple distilled water and ground water were prepared also prepared for each of these two calibration sets C2 and C3.

Table 1 Ethanol and water % in various samples of calibrations set of water adulterated ethanol fuel

Sample	Ethanol (mL)	Water (mL)	Water % (V/V)
1	5.00	0.00	0.00
2	4.75	0.25	5.00
3	4.50	0.50	10.00
4	4.25	0.75	15.00
5	4.00	1.00	20.00
6	3.75	1.25	25.00
7	3.50	1.50	30.00
8	3.25	1.75	35.00
9	3.00	2.00	40.00
10	2.75	2.25	45.00
11	2.50	2.50	50.00

Table 2 Ethanol and water % in various samples of validation set of water adulterated ethanol fuel

Sample	Ethanol	water	Water % (V/V)
Validation set V1			
1	4.875	0.125	2.5
2	4.625	0.375	7.5
3	4.375	0.625	12.5
4	4.125	0.875	17.5
5	3.875	1.125	22.5
Validation set V2			
1	4.875	0.125	2.5
2	4.625	0.375	7.5
3	4.375	0.625	12.5
4	4.125	0.875	17.5
5	3.875	1.125	22.5

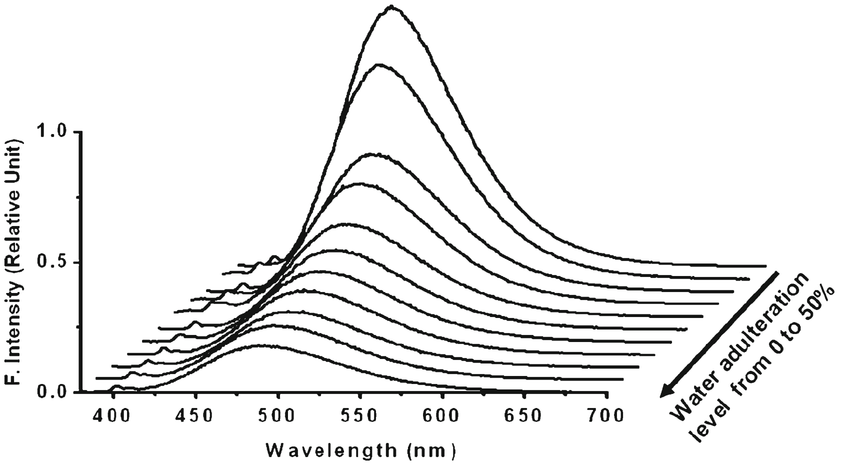

Fig. 2 Fluorescence spectra of ANS in water adulterated ethanol samples

Instrument and Data Acquisition

Fluormax-4 (Horiba Jobin Yvon) spectrofluorometer equipped with Xenon lamp of 150 W was used an excitation source. Excitation and emission band passes were set to 2.5 nm. Fluorescence data were collected by keeping the excitation monchromator at 360 nm (the absorbance maxima of ANS in pure ethanol) and scanning the emission monochromator the wavelength range of 380–700 nm. There was no noticeable background fluorescence; however, it can be taken care by subtracting the blank spectra (i.e., the fluorescence data acquired for the ethanol-water mixture before the addition of ANS) from the ANS fluorescence data set acquired for that particular ethanol-water mixture. The λ_{max} of emission of ANS in both the ethanol samples (spectroscopic-dried and the commercial) were found to be~473. It is characteristic wavelength of emission for ANS in ethanol. It showed that ethanol sample used in the present work is similar to spectroscopic ethanol sample and there is no noticeable difference in their polarities. It further indicates that there is no noticeable difference in the presence of water in commercial ethanol and spectroscopic grade ethanol.

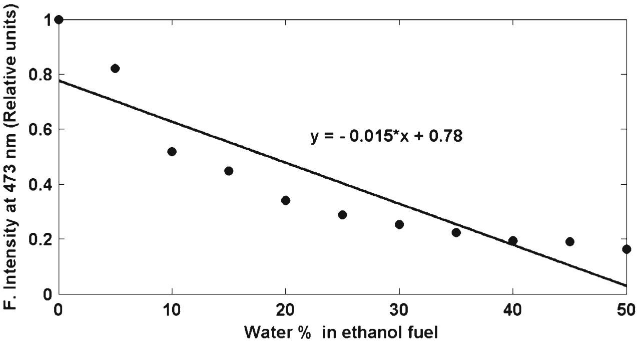

Fig. 3 Linear regression plot between fluorescence intensity of ANS at 473 nm and water % in ethanol fuel

Theory

Partial Least Square (PLS) Analysis

Partial least square (PLS) analysis [25–27] is a chemometric method used for the quantification of the components of our interest. Conventional analytical methods work, only if intensity values are directly proportional to the concentrations of the components of our interest. Whereas, PLS analysis can be safely used to analyse the components even if there is certain amount of nonlinearity between the spectral data set and their concentrations. PLS involves the simultaneous analysis of all the spectral variables therefore a better calibration model can be created [25–27]. Mathematically, PLS involves the simultaneous decomposition of X (independent variables e.g., spectral intensity values etc.) and Y (dependent set of variables e.g., concentration values) block data sets. Decomposition of the data sets are carried out in such a way that PLS model explains maximum variation of the data set and at the same time also achieve maximum correlation between X and Y

Table 3 Variation of Energy Shift (Cm^{-1}) with amount of water in ethanol fuel

Sample	Water % (V/V) in adulterated ethanol fuel	Energy shift Cm^{-1}
1	0	21097.05
2	5	21052.63
3	10	20576.13
4	15	20661.16
5	20	20576.13
6	25	20491.80
7	30	20618.56
8	35	20491.80
9	40	20491.80
10	45	20491.80
11	50	20283.97

block data sets [25–27]. Mathematically, it can be shown using Eqs. 1 and 2

$$\mathbf{X} = \mathbf{TP}^{\mathrm{T}} + \mathbf{E}_{\mathbf{X}} \tag{1}$$

$$\mathbf{Y} = \mathbf{UQ}^{\mathrm{T}} + \mathbf{E}_{\mathbf{Y}} \tag{2}$$

A correlation between X and Y blocks are usually achieved using Eq. 3

$$\mathbf{U} = \mathbf{TB} + \mathbf{E} \tag{3}$$

In the above equations, dimensions of X and Y are I×J and I×N, respectively. T (I×F) and U (I×N) are the score matrices, P (J×F) and Q (N×N) are the loading matrices of X (I×J) and Y (I×F) block. B is the regression matrix of dimension F×N. E_X, E_Y and E are the residual matrices of dimension are I×J, I×N and I×N, respectively. In the above Eqs. 1–3, I and J indicate number of samples and spectral variable, respectively. F is the number of factors used to create PLS model and N is the number of dependent variables (i.e., number of component (e.g., water percentage) whose concentration need to be calibrated). In the present work, the values of I, J, F and N are equal to 11, 321, 2 and 1 respectively. Hence, the dimensions of spectral (X) and concentration data matrices (Y) are 11× 321 and 11×1, respectively.

Statistical Parameters to Evaluate the Robustness o PLS Model

Root mean square error of calibration (RMSEC) [26, 27] and root mean square error of prediction (RMSEP) [26, 27] and square of correlation coefficient (R^2) [34] are statistical parameters that could be used to measure the accuracy of the prediction between the actual and predicted concentration of the components of our interest. RMSEC or RMSEP can be calculated using Eq. 4 and R^2 can be calculated using Eq. 5.

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{I} \left(Y_{act,i}\text{-}Y_{pred,i}\right)^2}{I}} \tag{4}$$

where $Y_{act,i}$ and $Y_{pred,i}$ are the actual and model predicted properties of the i^{th} sample of calibration set, and I is the total number of samples used to create the calibration model. We can obtain RMSEP value if we use Eq. 4 when used for the validation set.

$$R^2 = 1\text{-}\frac{\sum_{i=1}^{I} \left(Y_{act,i}\text{-}Y_{pred,i}\right)^2}{\sum_{i=1}^{I} \left(Y_{act,i}\text{-}Y_{mean}\right)^2} \tag{5}$$

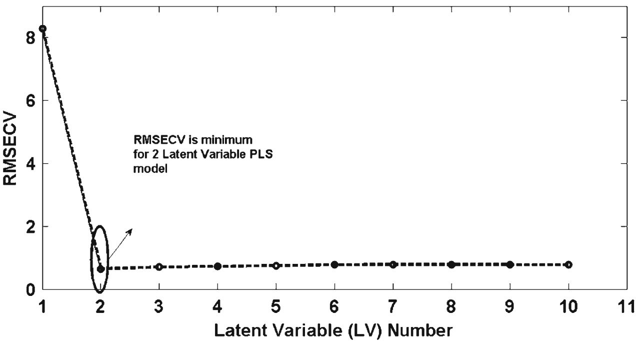

Fig. 4 RMSECV versus number of latent variable plot of PLS model

where Y_{mean} is the mean value of the Y_{act} data set. In principle, a calibration model should have R^2 value close to one and RMSEC and RMSEP value close to zero.

Software Used

PLS-Toolbox 5.0.3 written in MATLAB language was used to carry out PLS analysis.

Results and Discussion

The fluorescence spectra of ANS in water adulterated ethanol samples of calibration set C1 is shown in Fig. 2. As reported in literature, it could be seen that (i) ANS shows maximum fluorescence intensity in ethanol sample at ~473 nm and (ii) fluorescence intensity of ANS decreases with red shift as the amount of water in ethanol-water mixture increases. As discussed earlier, the loss of fluorescence is primarily due to process of charge transfer quenching. A linear regression was carried out between the fluorescence intensity values of ANS at 473 nm against the water percentage. The linear regression plot is shown in Fig. 3. It could be seen that with linear

Table 4 Percentage of variance explained by PLS model of different factors

Factors (or latent variables)	Variance % of X block (spectral data set)	Variance % of Y block (concentration data set)
1	91.97	84.83
2	99.94	99.92
3	99.97	99.95
4	99.98	99.99
5	99.98	100.00
6	99.99	100.00
7	99.99	100.00
8	99.99	100.00
9	99.99	100.00
10	99.99	100.00
11	99.99	100.00

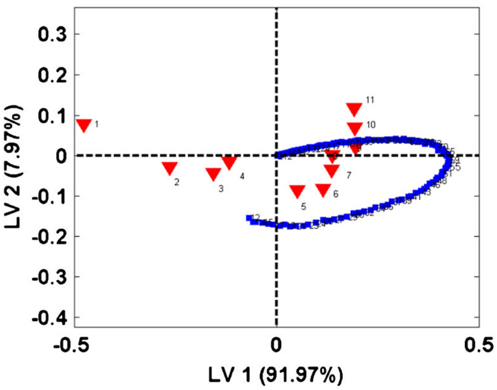

Fig. 5 Biplot of PLS model explaining the relation between sample (▼) and variables (■). Numerical values indicate the sample and variable numbers

regression approach it is difficult to make a calibration model for the analysis of water adulterated ethanol samples. Better correlation between concentration and spectral data set is a must for making a reliable calibration model. The variation of energy shift $(1/\lambda_{max}\ (nm))\times 10^{7}\ cm^{-1}$ can be used for the estimation of water in ethanol-water mixture over entire range. However, this procedure is limited by the fact that variation of emission wavelength (i.e., the energy of transition from excited to ground state) of ANS with amount of water is not sensitive enough to detect small to moderate differences of water concentrations in the ethanol-water mixtures. Variation of energy with water concentration is given in Table 3. Therefore, it makes sense to take all the spectral variables simultaneously and subject them to PLS analysis. As discussed earlier, PLS analysis ensures covariance between spectral (X) and concentration (Y) data matrices and hence improves the robustness and prediction accuracy of the calibration model.

Mean centred spectral data set of calibration set C1 was subjected to PLS analysis. In order to find the optimum number of factors, leave one out cross validation (LOOCV) [26, 27] approach was used, the root mean square error of cross validation (RMSECV) [26, 27] as shown in Fig. 4 was found to be minimum for a two latent variable PLS model. It also correlates well with the fact that there are two solvents of different polarities around the ANS molecule in the calibration sets consisting of water adulterated ethanol samples. Therefore, we preferred two latent variables PLS model to create the calibration model. Using the obtained PLS model, it is possible to explain more than 99.9 % variance of both spectral and concentration data matrices. The percentage of variance captured by the PLS model with different numbers of latent variables (or factors) are summarised in Table 4. It could be observed that PLS models with factors greater than two explained equal amount of variances of the data sets but with added complexity. It is to be noted with the addition of each factor in PLS model the complexity increases which is against the principle of parsimony [26, 27].

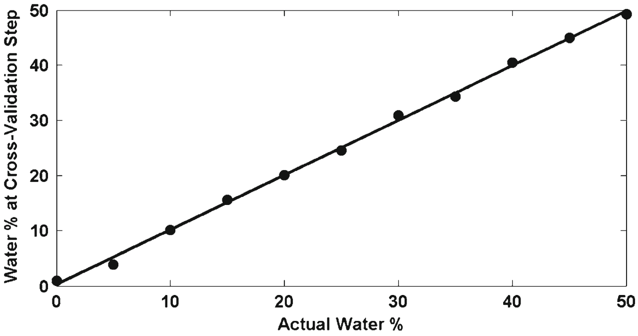

Fig. 6 Actual and PLS predicted water % in ethanol fuel at internal validation (i.e. cross-validation) step

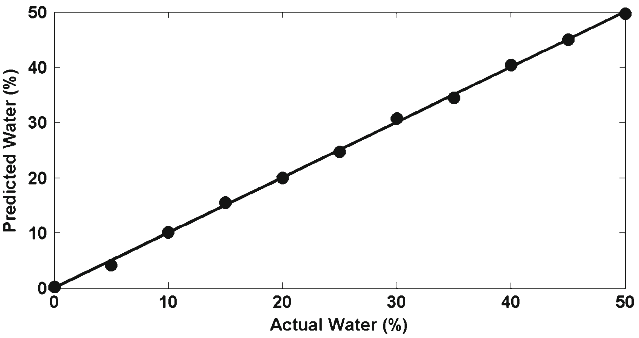

Fig. 7 PLS predicted and actual water % in ethanol fuel

The biplot [26, 27] that allows the study of the correlation of various samples (i.e., score) and the spectral variables (i.e., loading vectors) in a single plot is given in Fig. 5. It could be seen that samples have maximum variation along the first latent variable. It could also be seen that with increase in water concentration LV1 score values of ethanol-water samples vary form the negative to positive values though the variation is not linear. This nonlinearity is in correspondence with the nonlinear variation of ANS fluorescence along the intensity and wavelength (i.e., the energy) axes. It could also be seen that first 100–125 spectral variables are more comparatively more

Table 5 Actual and predicted water percentage in water adulterated ethanol fuel

Sample	Actual water % (V/V)	Predicted water % (V/V)	RMSEP
Validation set V1			
1	2.5	2.73	1.087
2	7.5	6.16	
3	12.5	10.70	
4	17.5	17.98	
5	22.5	23.27	
Validation set V2			
1	2.5	2.95	1.541
2	7.5	7.988	
3	12.5	14.98	
4	17.5	19.01	
5	22.5	24.23	

Table 6 Various statistical parameters of PLS model of C2 and C3 set

Calibration set	Statistical parameters			
	R2	RMSEC	RMSEP for validation set prepared using tripled distilled water	RMSEP for Validation set prepared using ground water
C2	0.999	0.367	1.101	.987
C3	0.999	0.489	1.231	1.467

informative. The predicted water concentration in ethanol-water mixtures in LOOCV approach i.e., 'the internal validation' results of PLS model is shown in Fig. 6. There is close correspondence between the actual and predicted water concentration of the calibration set. Briefly, LOOCV approach involves development of PLS model using all the samples except one and then the model is used to predict the concentration of left one, the procedure repeated so that each sample is left and predicted once.

The actual and predicted concentration of water in adulterated ethanol samples of PLS model is shown in Fig. 7. It could be seen that there is one to one correspondence between the actual and predicted water concentration. It is also evident from RMSEC and R^2 values of 0.46 % and 0.999, respectively. The two validation sets V1 (prepared using tripled distilled water) and V2 (prepared using ground water) each consisting of five samples were subjected to the obtained PLS model. The predicted concentration of water samples in adulterated ethanol samples of validation sets are summarised in Table 5. It is observed that PLS model makes prediction with great amount of accuracy for each of the five samples of both the validation set. It is also evident from small RMSEP values (summarised in Table 5) for both the validation sets.

The entire calibration procedure was repeated on other two calibration sets (C2 and C3) of the triplicates. Various statistical parameters RMSEC, R^2 and RMSEP values of these two models were calculated and summarised in Table 6. The obtained results clearly showed that it is possible to make accurate predictions of water percentage in water adulterated ethanol samples.

As discussed earlier that Karl-Fischer [28], electrochemistry [11], ultrasonic parameters [29], time-domain reflectrometry [30], capacitive micro-sensor [31], infrared (IR) [32], Fourier transform infrared (FTIR) [33] and NMR [33] based methods are available for the analysis of water in ethanol fuels. These methods are also reported to make accurate predictions of water in water-ethanol mixtures. However, the proposed procedure in the current work over the reported methods has the advantage of providing a very simple, fast and user-friendly way without involving any laborious step for monitoring the water adulteration in ethanol fuel. The only care that is required is the preparation of ANS solution and its addition to the samples in the fixed amount in the optimum concentration range. Otherwise, the procedure is robust and sensitive to quantify water adulteration in ethanol fuel.

Conclusions

In the present work, a simple and sensitive analytical procedure was developed for monitoring the water adulteration in ethanol fuel. The proposed procedure involves PLS analysis of the fluorescence data acquired for ANS in ethanol-water samples. The obtained PLS model was also successfully used to determine the amount of ground water adulteration level in ethanol fuel. The robustness of PLS model is evident from small RMSEC and RMSEP values and unit R^2 value.

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