Asian Journal of Chemistry; Vol. 25, No. 4 (2013), 2273-2277

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13840

Determination of Nitrite Using UV Absorption Spectra Based on Multiple Linear Regression

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(*Received*: 4 April 2012; *Accepted*: 22 October 2012) AJC-12329

The concentration of nitrite with potential toxicity is an important parameter within environmental, food, industrial and agricultural systems. To determinate nitrite concentration, the method using UV absorption spectra based on multiple linear regression is employed. Principal absorption wavelengths related closely with nitrite concentration are confirmed through analyzing the absorption spectra between the nitrite and nitrate with similar S/N ratio treatment. Utilizing multiple linear regression, mathematical model between absorbance obtained form ten principal absorption wavelengths and nitrite concentration is established. The result shows nitrite concentration between the predicted values and measured values are well coincident when the pH value of nitrite aqueous solution is more than 5 and the related coefficient (R) is 0.9624 over the concentration range 200-2000 ppm. This mathematical model is applied in detecting nitrite concentration of river water and results imply that the system based on this method may be used for on-line determination of the nitrite concentration in the river water.

Key Words: Nitrite, UV absorption spectra, Multiple linear regression.

INTRODUCTION

The continuous use of nitrite in food and industrial systems as a versatile reagent combined with revelations of their potential toxicity has raised numerous concerns[1,2]. The problems caused by the abuse of nitrite have been widely recognized and, as a result, statutory ordinances with the purpose of controlling their level within environment and food have been imposed in most industrialized countries[3,4]. Therefore, the need for determination of nitrite was undisputed. The techniques used commonly before 2001 for detecting nitrite concentration have been reported by Matthew *et al.*[5], such as energy dispersive X-ray, fluorescence, Griess assay, chemiluminescence, AAS, UV and visible spectra. Spectroscopic methods are most widely used for nitrite determination due to the excellent limit of detection. However, the monitoring solutions based on the absorption spectra described in the Matthew's review typically depended on the reaction between the specific reagents and nitrite for providing a highly chromophore[5]. Nitrite concentration could be detected indirectly through analyzing UV and visible absorption spectra of these chromophores instead of nitrite ion. This indirectly method was also used commonly by most researchers as the basis of some new methods in recent decade[6-8].

For directly analyzing absorption spectra of the nitrite ion to detect its concentration, only a few cases have been reported. Riordan *et al.*[9], indicated that the component of aqueous solution of nitrite depended on the pH value. Above pH 5 the solutions contain > 99 % nitrite ion and when under pH 1.1 contain > 99 % HONO. For pH value more than 5, UV absorption spectra are recorded at different concentrations of nitrite solutions and molar absorption coefficients in the range 270-400 nm are calculated on the basis of chemical analysis technique. According to Beer's law, a plot of absorbance *versus* concentration at 290 and 354 nm is constructed and in the concentration range 0.02-0.06 M had a good linearity[9]. Although this method employed by Riordan *et al.*[9] could determinate nitrite concentration directly, yet the main work in this method focused on chemical analysis rather than UV absorption spectra.

Multivariate statistical methods are frequently used in exploratory data analysis to obtain environmental knowledge. Therefore, the technique combining spectroscopy and multiple linear regression (MLR) was used widely by researchers in previous years[10-12]. Commonly nitrate and nitrite exist simultaneously in different water and wastewater streams and nitrite can be converted into nitrate on one condition[13-15]. So it is necessary to study the absorption spectral characteristics of nitrate and nitrite in order to select suitable wavelength range for effective determination of the nitrite.

In the present work, UV absorption spectra based on multiple linear regression as a repid method for determination of nitrite concentration in aqueous solution is employed. In this method nitrite concentration is detected directly through analyzing UV absorption spectra of nitrite ion without adding any other special regents reacted with nitrite.

EXPERIMENTAL

The experimental instrument setups used for measuring absorbance and pH value of nitrite aqueous solution are shown in Fig. 1. UV absorbance spectra were recorded with an AvaSpec-2408-USB2 fiber optic spectrometer. This is a single channel instrument capable of acquiring spectra from 200-1100 nm at a resolution of 2.4 nm. The signal-to-noise radio associated with such spectrometer is 200:1. The power and spectra data transmission to AvaSoft Basic software can be implemented by a high speed USB interface cable connected to PC. In addition, the spectrometer has a standard SMA fiber optic entrance connector to connect with the fiber. The Integration time and Average of AvaSoft Basic software is setup 30 ms and 100, respectively when the absorbance is recorded in all experiments. Light source (Avalight-DHS) utilizes two light sources: a deuterium arc lamp in the UV range (consistent in the 215-400 nm region) and a halogen lamp in the visible spectrum (consistent in the 360-1500 nm region) and this two light sources can be turned on-off separately. In the present experiment only the deuterium arc lamp is turn on and needs to be warmed up for 0.5 h before it goes to normal testing operation. The transmission dip probe with variable path length (FDP-7UV200-2-VAR, Avantes) is used to obtain online absorbance measurements in aqueous solutions. The probe has an optical path length, which can be set by the user in a range from 0.5-20 mm and is therefore extremely versatile for high- or low-absorption fluids. *Via* a standard SMA905 connector light produced by light source is coupled into a fiber bundle, consisting of 6 fibers and carried to the probe end, where it crosses a user-defined physical gap of 0.25-10 mm and reflects against a diffuse white material. The back reflected light returns into a 7th fiber. This fiber transfers the data to the output SMA905 connector, which can be coupled to a spectrometer. The optical path length is adjusted to 10 mm in all experiments. The pH values of the aqueous solutions are measured to within ± 0.02 of a pH unit by an Istek pH sensor which consists of a gel-filled Ag-AgCl electrode. This pH sensor can realize temperature compensation automatically through using an integrated temperature sensor while measuring. According to instruction manual of the pH sensor, calibration was performed before measuring and the electrode was clearly rinse in distilled water to eliminate the effect caused by the buffer solution used for calibration.

Solution samples: All reagents used are of analytical-reagent grade. Distilled water obtained from a Milli-Q water purifier is used for preparing all solutions and diluting them. Distilled solution of sodium nitrate and sodium nitrite is prepared, respectively to observe absorbance at the different wavelengths. Nitrite standard solution: a stock solution (2000 mg L^{-1}) was prepared by dissolving 0.4 g of sodium nitrite in distilled water to give a 200 mL solution. The samples with varying concentrations of sodium nitrite are prepared by diluting accurately a stock solution.

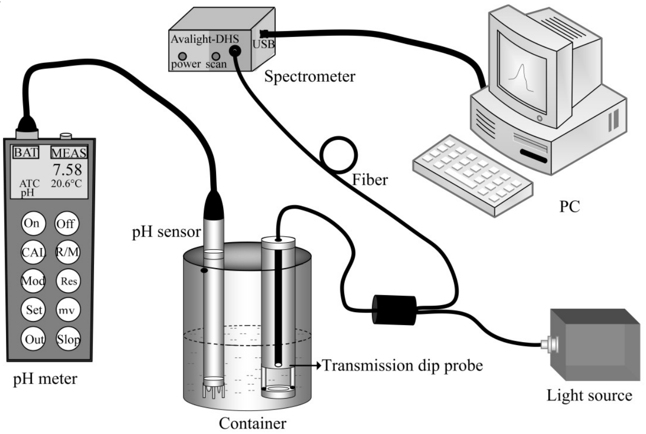

Fig. 1. Experimental instrumental setups for UV absorption spectrum and pH value measurement of the sample solutions

Methodology: The spectra data from the UV absorption of nitrite samples are multivariate, so multivariate methods such as multiple linear regression (MLR) were used to establish mathematics model between the absorbance at different wavelengths and nitrite concentration. Multiple linear regression is a highly flexible system for examining the relationship of a collection of independent variables (or predictors) to a single dependent variable (or criterion). To establish the mathematics model a multivariate data matrix containing concentration data of 14 nitrite samples occupying two-thirds of the total samples and absorbance data at 10 wavelengths corresponding to every kind of sample is used. Concentration and absorbance data of other six nitrite samples are used for mathematics model verification. The ten wavelengths are selected according to the correlation with the nitrite concentration. The broadband absorption spectrum for each nitrite sample is smoothed with the use of four-point moving average algorithm to improve the signal-to-noise ration. All broadband absorption spectra are preprocessed by baseline correction before using for mathematics model.

RESULTS AND DISCUSSION

Spectral characteristics of nitrate and nitrite sample: The pH values of all sample solutions are in range of 7.21-7.95 through real-time measurement and obviously these pH values are greater than 5. Fig. 2 illustrates the measured UV broadband absorption spectra for sodium nitrate sample, sodium nitrite sample and composite sample, respectively. By comparing the spectra of the nitrate and nitrite, it is observed that they show different absorbance characteristics. The absorption spectrum of sodium nitrate sample has an absorption peak at 300 nm while the absorption spectrum of sodium nitrite sample has an absorption peak at 354 nm. The absorption spectrum of composite sample of these two samples with the same concentration indicates that the absorbance of sodium nitrite at the 340-400 nm is almost unaffected by sodium nitrate. In order to find the most suitable range of wavelength, also the ratio of effective signal, absorbance of sodium nitrite sample and unnecessary signal, absorbance of sodium nitrate sample, is shown in Fig. 3, as a similar one of S/N ratio curve. It can be observed that the suitable range to determine nitrite concentration is 340-370 nm.

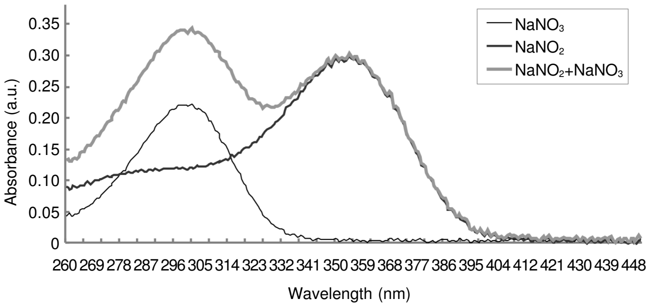

Fig. 2. Absorption spectra of sodium nitrate sample, sodium nitrite sample and composite sample of these two samples with the same concentration.

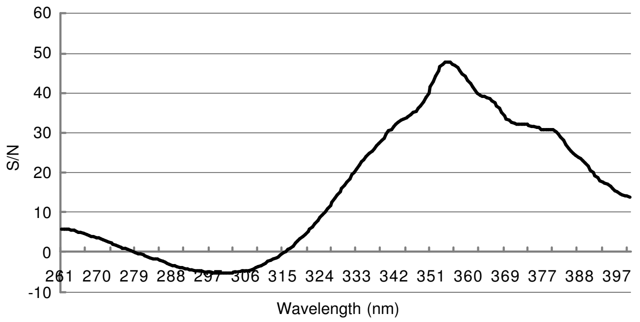

Fig. 3. Signal to noise ratio in composite sample solution of sodium nitrate and sodium nitrite

Model establishment and verification: In order to obtain the absorbance data with varying concentrations of sodium nitrite, a prepared stock solution (2000 mg L^{-1}) of sodium nitrite is diluted accurately. In the present experiment, 20 absorption spectra corresponding to each kind of concentration obtained through diluting gradually are recorded by the AvaSoft Basic software with 30 ms Integration time and 100 average. These absorption spectra are preprocessed by smoothing and baseline correction before used. These twenty spectra are divided into two groups. One group including fourteen absorption spectra selected according to statistical analysis method is used for establishing multiple linear regression model, the other is used for model verification. Fig. 4 illustrates 14 absorption spectra at the different concentrations of sodium nitrite. The absorbance as shown in Fig. 4 tend to be linear change in the wavelength range of 320-370 nm. This phenomenon implied the absorbance is proportional to the concentration of substance to be determined when path length and molar absorption coefficient at different wavelengths are confirmed, as described by Lambert-Beer law[16-18].

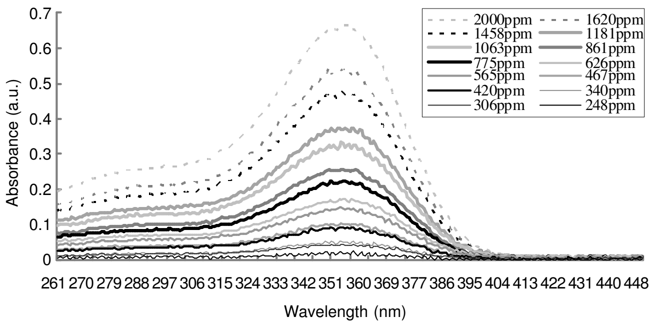

Fig. 4. Absorption spectra selected according to statistical analysis method at the different concentrations of sodium nitrite samples

On the basis of above analysis, corresponding to 14 concentrations of sodium nitrite samples, the absorbance at 10 wavelengths which sodium nitrite has the strongest absorption relative to other wavelengths is shown in Table-1. First row and first column in Table-1 is 10 wavelengths and 14 concentrations (dependent variables), others are absorbance (independent variable). The mathematic model is established through analyzing these data (Table-1) by making use of multiple linear regression method and the correlation coefficient of the established model reaches up to 0.9997.

Absorbance corresponding to other six concentrations for model verification is shown in Table-2. Through plugging these verification absorbance data into the established multiple linear regression model, six predicted concentration values are obtained. The correlation between predicted values and measured values is shown in Fig. 5. It shows that the correlation coefficient is 0.9624 and the slope of correlation line is close to 1.0. It is realized that the predicted values obtained from the established multiple linear regression model show good agreement with measured values in the range of 200-2000 ppm. Therefore, it is possible to measure the concentration of nitrite effectively in the mixed solution by the present method.

Apply the established multiple linear regression model to the river water: In order to verify whether the present method is suitable for determination of nitrite concentration

TABLE-1
ABSORBANCE AT 10 WAVELENGTHS SELECTED ON THE BASIS OF ABOVE ANALYSIS AND 14 CONCENTRATIONS FOR ESTABLISHING MULTIPLE LINEAR REGRESSION MODEL

	352.5	353.1	353.7	354.3	354.9	355.5	356.0	356.6	357.2	357.8
2000	0.652	0.657	0.666	0.668	0.664	0.665	0.664	0.653	0.651	0.653
1620	0.529	0.529	0.538	0.540	0.531	0.531	0.537	0.528	0.518	0.525
1458	0.533	0.541	0.549	0.546	0.542	0.544	0.544	0.539	0.537	0.538
1181	0.447	0.446	0.440	0.442	0.446	0.442	0.437	0.439	0.443	0.441
1063	0.315	0.324	0.335	0.330	0.321	0.325	0.328	0.321	0.318	0.325
861	0.251	0.253	0.257	0.258	0.253	0.251	0.252	0.251	0.252	0.256
775	0.293	0.290	0.291	0.295	0.297	0.294	0.292	0.294	0.291	0.286
626	0.244	0.243	0.242	0.247	0.248	0.242	0.240	0.246	0.245	0.240
565	0.146	0.150	0.149	0.146	0.145	0.147	0.147	0.144	0.145	0.146
467	0.097	0.103	0.105	0.102	0.100	0.102	0.103	0.099	0.099	0.104
420	0.163	0.163	0.164	0.165	0.165	0.165	0.164	0.164	0.165	0.162
340	0.125	0.125	0.124	0.124	0.126	0.125	0.123	0.124	0.125	0.123
306	0.041	0.043	0.045	0.045	0.044	0.044	0.043	0.042	0.043	0.045
248	0.093	0.096	0.096	0.092	0.093	0.096	0.094	0.092	0.093	0.095

TABLE-2
ABSORBANCE AT 10 WAVELENGTHS SELECTED ON THE BASIS OF ABOVE ANALYSIS AND 6 CONCENTRATIONS FOR MODEL VERIFICATION

	352.5	353.1	353.7	354.3	354.9	355.5	356.0	356.6	357.2	357.8
1312	0.415	0.424	0.426	0.422	0.418	0.420	0.423	0.416	0.415	0.422
957	0.366	0.368	0.372	0.371	0.367	0.366	0.366	0.366	0.365	0.365
697	0.189	0.195	0.193	0.192	0.196	0.195	0.186	0.185	0.195	0.196
518	0.196	0.195	0.192	0.194	0.198	0.194	0.189	0.195	0.199	0.194
378	0.097	0.103	0.105	0.102	0.100	0.102	0.103	0.099	0.099	0.104
275	0.103	0.104	0.102	0.099	0.100	0.102	0.101	0.102	0.103	0.102

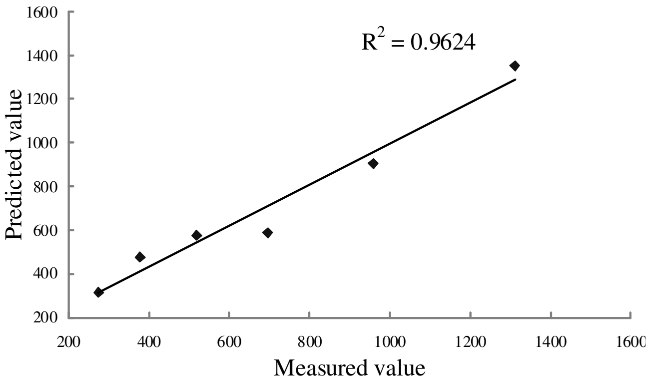

Fig. 5. Correlation between the predicted value and measured value of the sodium nitrite samples obtained from the established multiple linear regression model

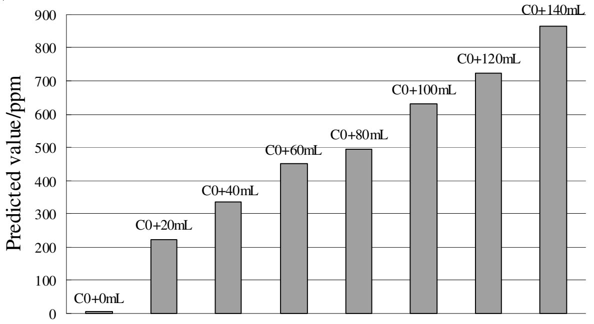

Fig. 6. Predicted values of the nitrite concentration in the river water based on the established multiple linear regression model in the present method

in the river water was sampled in Beijing Chang River and may include the particle matter and dissolved inorganic matter. For evaluating the nitrite concentration of river water, particle matter must be filtered out. Dissolved inorganic matter, iodine, bicarbonate, hydrogen sulfidem *etc.*, generally show very small effect in UV range. Nitrate that may be also included in the water samples has no effect on the absorbance at 10 wavelengths selected in the multiple linear regression model for nitrite determination. In the experiment, the total solution volume corresponding to every sample is added up to 200 mL. Clarified river water is divided into two parts, one as the pure river water is put into 100 mL distilled water and C_0 is obtained through plugging the absorbance of this sample into the established multiple linear regression model. The other is prepared by dissolving unknown qualitative sodium nitrite. 20, 40, 60, 80, 100, 120, 140 mL of later part are taken out and added into 180, 160, 140, 120, 100, 80, 60 mL distilled water, respectively. Predicted values of the nitrite concentration in the river water is shown in Fig. 6. The measured values are not provided because of lack of standard detecting instruments for nitrite concentration. It is observed that the concentration of nitrite for pure river water is very low and as the accumulation of the nitrite in the river water the predicted concentrations tend to be linear change. This confirms the effectiveness of the present method to estimate nitrite concentration in the river water.

Conclution

In this study, considering the possibility of simultaneous existence of nitrate and nitrite, the UV absorption spectral characteristics of nitrate and nitrite have been investigated. Since the influence of nitrate is large in particular range, the suitable wavelength range is selected for determination of nitrite concentration. In order to obtain the concentration of nitrite from the absorbance data at selected wavelengths, the multiple linear regression analysis is employed. The correlation coefficient between measured values and predicted values obtained from the established multiple linear regression model is 0.9624. In the concentration range of 200-2000 ppm, the predicted values show good agreement with measured values at pH value more than 5. Although the measurement limit is lower than analysis by standard method, nitrite concentration is determinated directly without adding any other special regent reacted with nitrite and measurement limit can also be lowered through increasing the optical path length. In the case of using the present method against the river water, it is shown that the effectiveness to estimate nitrite concentration in the river water. Therefore, this method may be applied in on-line determination of the nitrite concentration present in the river water.

ACKNOWLEDGEMENTS

This work has been financially supported by the Innovation Building Program of Beijing Academy of Agriculture and Forestry Sciences (Grant No. KJCX201102001) and Major Subject of Science and Technology Project of Beijing (Grant No. D111100001011002).

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