Flow Injection Spectrophotometric Method for the Quantitative Determination of Humic Acid (HA) in Treated and Natural Waters

TAHSEEN GHOUS*, AAMIR RASHEED AND MUHAMMAD SIRAJ
Department of Chemistry University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.

(12th December 2009, accepted in revised form 10th February 2010)

Summary: A double channel flow injection (FI) spectrophotometric method was established for humic acid (HA) determination in milligram levels in treated and natural waters. FI method was developed for the determination of humic acid by injecting 100 µL of humic acid into a carrier stream of sodium citrate phosphate buffer (0.05 M, pH 7.0) followed by the injection of 50 µL toluidine blue (TB) solution. Both reagents were allowed to mix at T-junction and passed through a 25 cm reaction coil tubing before passing through the flow through cell (10 mm light path, 30 µL volume). Change in absorbance was measured at 630 nm. The method was calibrated with standard solutions of HA from 0.5-60 mgL^{-1}.The calibration curve showed two linearity trends one ranging 0.5-20 mgL^{-1} and the other ranging 20-50 mgL^{-1}. The detection limit was 0.5 mgL^{-1} of HA, and relative standard deviation (rsd) of 5 replicate measurements was 0.294%.The developed method was successfully applied for the HA determination in water samples from drinking water plants, rivers, lakes and streams. The sampling rate was more than 80 samples h^{-1}. The method proves to be simple, rapid, feasible and reproducible.

Introduction

Humic substances (HS) are major components of natural organic matter (NOM) which are the most widespread and universal natural nonliving organic materials in terrestrial and aquatic environments [1]. Humic substances (HS) are formed by secondary synthesis reactions (humification) during the decay process and transformation of biomolecules originating from dead organisms and microbial activity. Lignin and its transformation products, as well as polyphenols and derived polymers from lower plants and microorganisms, are important starting materials in this process and provide aromatic building blocks of high physicochemical stability. Humic substances comprise a physically heterogeneous mixture of biogenic, relatively high-molecular-mass compounds with mixed aliphatic and aromatic natures [2]. Based on solubility in acids and alkalis, they can be divided into three main fractions: humic acid (HA), which is soluble in alkali and insoluble in acid; fulvic acid (FA), which is soluble in both alkali and acid; and humin, which is insoluble in both alkali and acid [3, 4]. NOM is usually found in natural waters at concentration levels of 2-15 mgL^{-1} [5]. Humic acid (HA) is one of the major components of humic substances (HS) in natural waters since NOM constitutes about 70% of humic acid [6]. The molecule of humic acid (HA) is considered to have a number of discrete sites which bind to protons and metal ions. These sites corresponds to carboxylic (-COO^-), phenolic (-OH) and quinolic functional groups [2, 7, 8]. A model structure of humic acid is presented in Fig. 1a as adopted from Stevenson [2].

Humic substances adversely affect the quality of drinking water since they impart colour in water and serve as precursors for the formation of chlorinated carcinogenic compounds like trihalo-methans. They also have complexing properties that include association with toxic elements and micro-pollutants [3, 9]. Many terms such as chelation, complexation, absorption, etc. are used to describe nature of these metalo-organic complexes. Because of such interactions with metal ions and other organic compounds, humic acid plays an important role in mobility and transport of such toxic substances [10]. Humic substances tend to selectively block adsorption sites on activated carbon and prevent adsorpotion of micropollutants thus reducing water treatment performance [11]. Presences of HS also control viral counts in natural waters [12].

Thus, it is essential to develop a simple, economical and sensitive method for establishing humic acid (HA) concentration in water samples. Such studies might also be useful for elucidating the true nature of such complexes. Various studies including mass spectrometry, fluorescence

*To whom all correspondence should be addressed.

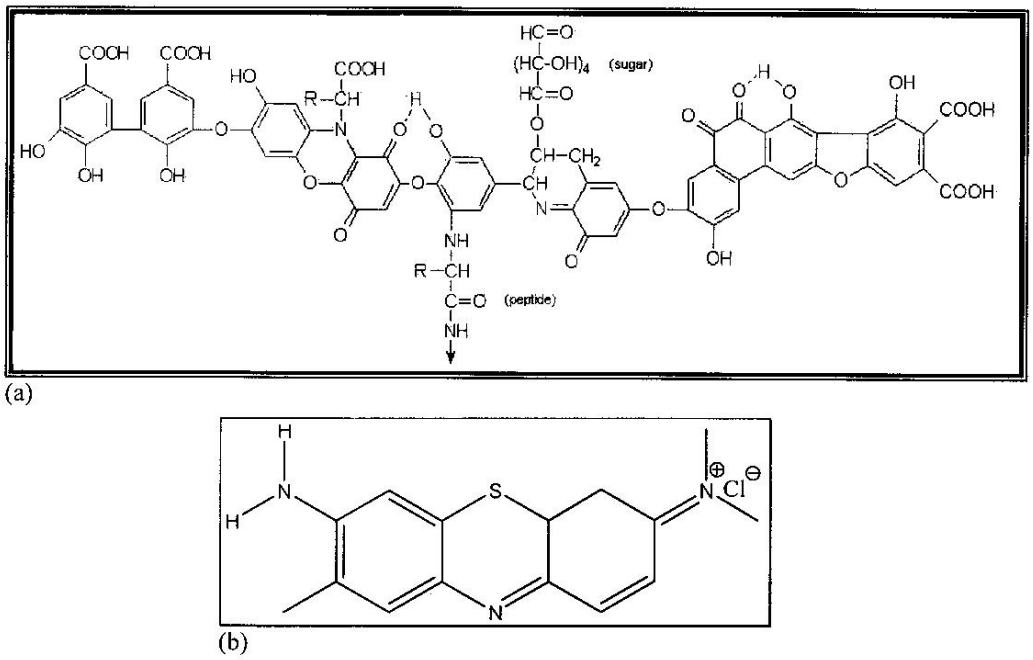

Fig. 1: (a) Structural formula for humic acid adopted from Stevenson [2]. (b) Structural formula for toluidine blue (TB).

spectroscopy, infrared spectroscopy and nuclear magnetic resonance spectroscopy have provided information about structural and functional properties of HS [13]. However, room for rapid, sensitive and simple methods for the determination of HS is still vacant. Various methods including chemiluminescence, fluorescence spectroscopy, FI coupled chemiluminescence for the determination of humic substances are described [14-16]. Most of these studies are based on complex instrumentation and interference from species existing in natural water are significant. Recently, a simple manual spectrophotometric method using TB as a spectrophotomtric probe is described [13]. The method is based on the interaction of TB and HA. At neutral pH many negatively charged functional groups are present on the surface of HA while TB is positively charged. Therefore, these species bind with each other quickly through electrostatic force of attraction. Structural formula for TB is illustrated in Fig. 1b.

In the described procedure interferences like proteins and polysaccharides are eliminated by ultra filtration of water samples. The most interesting feature of this method is that naturally occurring ions do not significantly interfere in the study. So there is no need for long purification process. By this method 0.5-40 mgL^{-1} of HA can be determined. Therefore, the system is sensitive and simple but time consuming and seems slow for routine sample analysis in busy laboratories. In an attempt to improve the sample throughput rate and to reduce waste generation this study describes flow injection (FI) method for the determination of HA which could prove to be rapid, reproducible and environment friendly for routine analysis in busy laboratories.

River water samples were collected from two major rivers of the area namely, Neelum and Jhelum at Muzafarabad. Jhelum rises from a spring at Verinag situated at the foot of the Pir Panjal in the south-eastern part of the valley of Kashmir and enters Pakistan through a deep narrow gorge. The Kishenganga (Neelum) river, the largest tributary of the Jhelum, joins it near Muzaffarabad (MZD).

Results and Discussion

Effect of Flow Rate

Flow rate is an important parameter in flow injection analysis. For, slow chemical reactions

decrease in flow rate gives more time to the reactants to react, while continuously flowing through the reaction coil tubing thus increasing the sensitivity, or otherwise increase in flow rate increases sample through-put rate without loosing sensitivity. Effect of flow rate on change in absorbance was observed with change in flow rate from 1.5-6.0 mL min^{-1}. There, was a continuous increase in change in absorbance from 1.5-5 mL min^{-1}. Essentially, TB concentration is a key factor which influences the binding of HA with TB. Dilution at slow flow rate decreased TB concentration, resulting in less possible binding of TB and HA which consequently, decreased change in absorbance. However, at flow rate above 4.0 $mLmin^{-1}$ the carrier flow became more turbulent and injection time between two injections was decreased which has reduced the reproducibility. Pumping velocity 4.0 $mLmin^{-1}$ was selected as a compromise between sensitivity and reproducibility. Results are presented in Fig. 2.

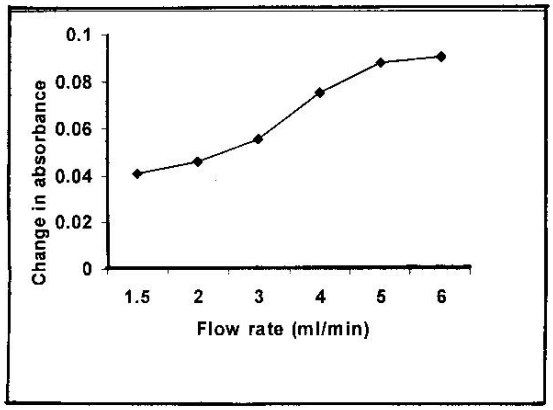

Fig. 2: Effect of flow rate on change in absorbance. TB 0.15 mM, HA 10 mgL^{-1}, TB loop size 50 µL, HA loop size 100 µL.

Effect of Incubation Time

In flow injection methods stopped flow technique is used to increase the yield of reaction product in order to increase the sensitivity of the system. In this study effect of incubation time on change in absorbance was studied by stopping the flow just after the mixing of the reactants at T-point from 30 sec: to 6.0 min. TB 0.075 mM (50 µL) and HA 10 mgL^{-1} (100 µL) were used in this study. Fig. 3 indicates that there was an increase in change in absorbance with increase in incubation time form

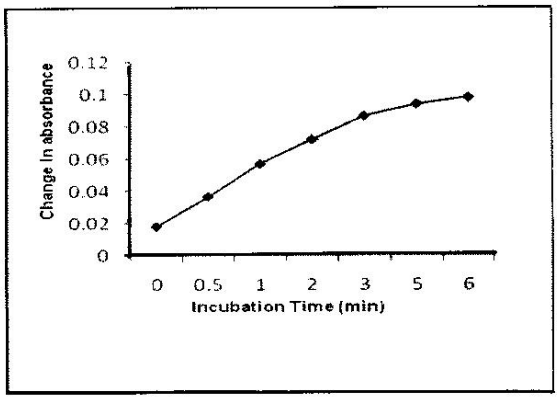

Fig. 3: Effect of incubation time on change in absorbance. TB 0.075 mM, HA10 mgL^{-1}, TB loop size 50 µL and HA loop size 100 µL.

30 s to five min and after that there was no increase. However, TB itself has shown decrease in absorbance up to one min incubation time without HA which may be due to the interaction of TB with (COO^-) ion present in citrate buffer. From one to five min incubation time TB itself did not show further decrease in absorbance and in that time period change in absorbance with incubation time due to humic acid was considerable. The results designate that sensitivity could be increased with increase in incubation time. As the sensitivity without incubation time covered the concentration range of dissolved HA present in natural waters further work was carried out at zero incubation time. This has helped to increase sample throughput rate.

Effect of TB Concentration

Effect of change in TB concentration on change in absorbance in the presence of 5 mgL^{-1} of humic acid was determined. Concentration of TB was an important factor to study as number of binding sites increase with increase in TB concentration. Different concentrations of TB (0.07-0.2 mMolar) were injected (50 µL) both in the presence and absence of humic acid and change in absorbance was measured. The results are presented in Fig. 4. As can be seen change in absorbance increased with increase in TB concentration. TB concentration (0.175 mMolar) was selected for further work where maximum change in absorbance was observed. Concentration lower than the studied range was not

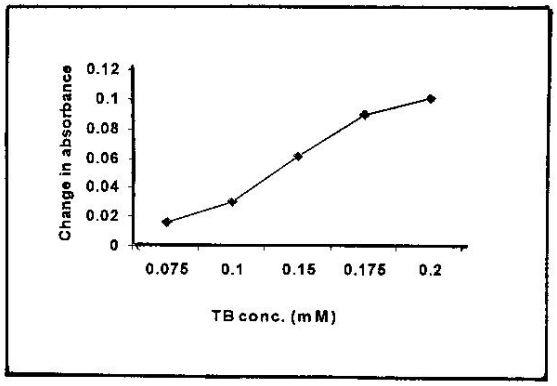

Fig. 4: Effect of TB concentration on change in absorbance. Zero incubation time, HA 10 mgL^{-1}.

used as the low conc. of TB showed very low absorbance even in the absence of HA. Further higher TB concentrations were not studied because solubility was a problem.

Effect of pH

The quantity of negatively charged functional groups on HA increases with increasing pH. Thus, HA molecule is able to bind with a basic dye like; TB, especially, at neutral or alkaline pH which is positively charged at neutral pH [13]. In order to study the effect of pH on the complexing ability of TB with HA, sodium citrate phosphate buffer of different pH (6.5-8.5) was used as a carrier buffer. Results presented in Fig. 5 show that there was a decrease in change in absorbance while increasing pH from neutral to alkaline side. Which indicate that increasing pH though increased negative charge on HA but decreased the positive charge on TB. Similarly, decrease in change in absorbance at pH 6.5 could be due to decrease in positive charge on TB and negative charge on HA. The maximum change in absorbance was observed at pH 7.0.which was selected for further work.

Effect of Reaction Coil Tubing

The length of reaction coil has a significant effect in analytical procedures in FI. In this study reaction coils of variable length (25-45 cm) were tightly coiled on a solid cylinder in order to avoid dispersion. Increase in reaction coil did not bring any significant decrease in change in absorbance but slow down the procedure resulting in decrease in sample

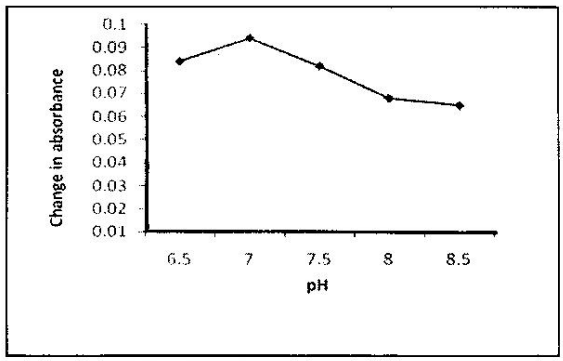

Fig. 5: Effect of pH on change in absorbance. Zero incubation time, TB 0.175 mM, HA 10 mgL^{-1}

through-put rate. Since increasing reaction coil tubing has not increased sensitivity of the studied range 25 cm was selected for further work. Results are tabulated in Table-1.

Analytical Performance

The variables studied and optimum values found for HA determination are listed in Table-1. Under the selected conditions calibration graph was plotted which was linear in a wide concentration range with different trends in linearity. Two working calibration graph were established (0.5-20, 20-50 mgL^{-1}). For both linear ranges (0.5-20, 20-50 mgL^{-1}) the correlation coefficient was 0.996 and 0.998, respectively (n = 8 and 6). Results are given in Table-2. The proposed method using these optimum values gives a relative standard deviation (for five injections of 0.5 mgL^{-1}) of 0.294 %. The limit of detection defined as the concentration of the analyte giving a signal (Y) equal to the blank (Yb) signal plus three standard deviations (sd) of the blank (Y-Yb = 3Sd) was 0.5 mgL^{-1}. The sample throughput rate was more than 80 samples h^{-1}.

Table-1: Parameters studied and selected reaction conditions.

S. No.	Parameters studied	Range studied	Optimum Conditions	Selected conditions
1.	Flow rate, mL min^{-1}	1.5-6	5.0	4.0
2.	Incubation time (min)	0-10	5.0	1.0 min
3.	TB Conc. (mM)	0.075-0.2	0.175	0.175
4.	pH	7.0-8.5	7.0	7.0
5.	Reaction coil tubing (cm)	25-45	25	25

Sample Analysis

River water samples were collected from two major rivers of the area namely, Neelum and

Table-2: Analytical performance under selected conditions.

S. No.	Concentration range studied	Linear range	Correlation coefficient	Correlation points
1	0.5-60 mM	0.5-20	0.991	n=8
2		20-50	0.998	n=6

Jhelum at Muzafarabad. Spring water samples were collected from Muzaffarabad (MZD), stream and treated (collected from drinking water plants; DWP) water samples were collected from different sectors of Islamabad (ISB). Another water sample was collected from Rawal lake (ISB). All water samples were filtered through ultrafilitration syring filters (pore size 0.2 μm) to remove possible proteins and polysaccharides before injection. The results presented in Table-3 shows that most of the tested samples have very low level of HA concentration except few stream samples collected from Islamabad (ISB). Results are consistent with a simple manual UV method described by Mamba [6]. In this method absorbance of HA is directly recorded at 256 nm. The method was established and calibrated on flow injection method before sample analysis. Comparative results of two studies showed that the developed method is reliable and sensitive.

Table-3: Water samples studied under selected conditions.

Source	Water Sample	HA conc.mgL^{-1} | 4 | ± SD (n=5)	HA conc.mgL^{-1} by proposed method ± SD (n=5)
River	Neelum (MZD)	0.821 ± 0.012	1.021 ± 0.051
	Jhelum (MZD)	1.142 ± 0.023	1.240 ± 0.045
Lake	Rawal lake (ISB)	0.428 ± 0.035	0.547 ± 0.052
Spring	Domail (MZD)	0.251 ± 0.024	Not detected
	Nalochi (MZD)	0.285 ± 0.012	Not detected
	Bala Pir (MZD)	1.714 ± 0.011	1.459 ± 0.045
Streams	Sayed pur village (ISMB)	8.821 ± 0.031	7.335 ± 0.054
	G7/1 (ISB)	3.178 ± 0.024	1.751 ± 0.004
	G9/4 (ISB)	1.928 ± 0.025	1.642 ± 0.034
	G10/1 (ISB)	1.821 ± 0.035	1.715 ± 0.054
	G6/Aabpara (ISB)	0.964 ± 0.026	1.058 ± 0.034
	G10/1 (ISB)	1.071 ± 0.034	1.642 ± 0.045
	F7/1 (ISB)	0.535 ± 0.036	0.656 ± 0.046
DWP	G11/3 (ISB)	0.255 ± 0.037	Not detected
	F11/4 (ISB)	0.345 ± 0.026	Not detected

Experimental

Reagents

Humic acid sodium salt analytical grade was purchased from Sigma-Aldrich and was purified prior to the formation of stock solution. Purification was carried out by the procedure described by Sheng [13]. A reasonable amount of HA was dissolved in 0.1 M NaOH. The solution was filtered and pH was adjusted at 1.0 by using 37% HCl. Humic acid was filtered out and washed two to three times with HCl. The precipitates were dried in an oven at 37 °C for 12 h. Stock solution was prepared by dissolving 100 mg of humic acid in 100 mL sodium citrate–phosphate buffer (0.05 M, pH 7.0) containing EDTA (0.05 M). TB was purchased from Merck-Germany and 0.2 mM TB solution was prepared by adding 5.0 mg of TB in 100 mL of distilled deionized water. Stock solution was kept in dark. Sodium citrate–phosphate buffer (0.05 M, pH 7.0) containing EDTA (0.05 M) was used as a carrier.

Flow Injection Manifold and Procedure

A double channel flow injection manifold used for the determination of humic acid is shown in Fig. 6. The peristaltic pump for carrier stream propulsion was made by Ismatec and two rotatry injection valves (Rheodyne rotary) were used for injection of specific volume of the reagents. The manifold tubing was 0.5 mm i.d. PTFE (tefelon). The volume of sample injection loop was 100 μL and that for TB was 50 μL. Sodium citrate phosphate buffer pH 7.0 was used as a carrier to keep TB at neutral pH and EDTA was added in the carrier buffer to chelate out interfering metal ions form water samples.

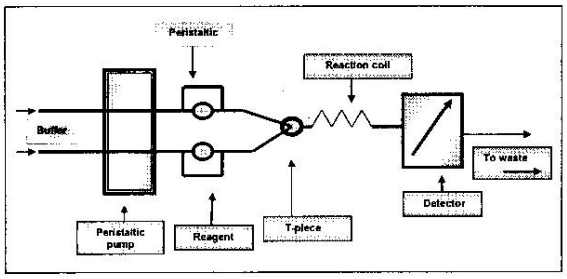

Fig. 6: Flow injection manifold used in the study.

Humic acid was injected first into the carrier stream followed by the injection of toludine blue. The reagents were merged at T-junction passing through a 25 cm reaction coil tubing and allowed to passed through flow through cell (30 μL, 100 mm i.d.) and change in absorbance was recorded at 630 nm using UV spectrophotometer (Shimadzu 1700).

Conclusions

For routine chemical analysis, flow injection analysis FIA always remained a method of choice because of its simplicity, reliability, reproducibility,

and economy. It offers a wide range of applications such as environmental pollution, agriculture, pharmaceutical and clinical analysis. We have exploited this technique for the determination of HA in natural and treated water samples. The results of the study show that the described method is simple, feasible and reliable for routine HA determination in water samples. Compared to the manual method adopted for this study the FI developed method was found more sensitive, efficient, economical and environment friendly with less waste generation. Sensitivity of the system could be further improved by incubating reactants for short time.

Acknowledgements

The corresponding author acknowledges Higher Education Commission of Pakistan for major and University of Azad Jammu and Kashmir for partial funding for the project.

References

1 K. T. Steffen, A. Hatakka and M. Hofrichter, *Applied and Environmental Microbiology,* **68** 3442 (2002).
2 F. J. Stevenson, Humus chemistry. Genesis, composition, reactions, 2nd ed. John Wiley & Sons, New York (1994).
3 J. C. A. de- Wuilloud, R. G. Wuilloud, B. B. Sadi and J. A. Caruso, *The Analyst,* **128**, 453 (2003).
4 N. Senesi and E. Loffredo, Soil humic substances, in: M. Hofrichter and A. Steinbuchel (ed.) Biopolymers. Lignin, Humic Substances and Coal, Vol. 1. Wiley-VCH, Weinheim, Germany, pp. 301 (2001).
5 C. Hepplewhite, G. Newcombe, and D. R. U. Knappe, *Water Science Technology,* **49**, 257 (2004).
6 B. B. Mamba, R. W. Krause, T. J. Malefetse, S. P. Sithole and T. I. Nkambule, Water S. A., **35**, 117 (2009).
7 C. Plaza, N Senesi, A. Polo and G. Brunetti, *Environmental Science Technology*, **39**, 7141 (2005).
8 G. Palladino, D. Ferri, C. Manfred and E. Vasca, *Analytica Chimica Acta*, **582**, 164 (2007).
9 E. Yamada, T. Ozeki and M. Kimura, Analytical Science, **14**, 327 (1998).
10 M. Hiraide, *Analytical Science*, **3**, 453 (1992).
11 K. Ebie, F. Li, Y. Azuma, A. Yuasa and T. Hagishita, *Water Research*, **35**, 167 (2001).
12 A. M. Anesio, C. Hollas, W. Granéli and J. Laybourn-Parry, *Applied Environmental Microbiology*, **70**, 4848 (2004).
13 G. P. Sheng, M. L. Zhang, H. Q. Yu, *Analytica Chimica Acta*, **592**, 162 (2007).
14 J. Michalowski, P. Halaburda and A. Kojlo, *Analytica Chimica Acta*, **438**, 143 (2001).
15 X-Hu, M. Kitano, *et. al., Bunseki Kagaku*, **43**, 1077 (1994)
16 J. J. Mobed, S. L. Hemmingsen, J. L. Autry and L. B. Mcgown, *Environmental Science Technology*, **30**, 3061 (1996).