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Detection of flow injection analysis with pH gradient by acousto-optic tunable filter based spectrophotometry

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Abstract

The instrumentation development of a spectrophotometer based on an acousto-optic tunable filter (AOTF) and its applications in a flow-injection (FI) system for gradient exploitation is described. This compact, all-solid state spectrophotometer which contains no-moving parts, can be scanned in the visible region (390–650 nm) at a speed of 300 nm/s. The fast scanning capability of the instrument made it possible to monitor the spectral response of an acid–base indicator mixture. The recorded spectra of the indicators were then converted to pH by means of a partial least square (PLS) multivariate calibration technique. With use of the same indicator mixture, the relationship obtained facilitates the determination of pH of any flow-injection pH gradient system which is produced by injecting a strong acid or base over a mixture of buffered solutions with a wide range of pH values. The time versus pH relationship can, therefore, be readily established for any buffer–reagent mixture flowing in the FI system. The FI system, which has been calibrated this way, can then be used to rapidly characterize the behavior of chemical systems as a function of pH. Two examples are demonstrated in this work: the determination of the pK_a of the weak acids and of the complex formation between iron(III) and sulfosalicylic acid.

Keywords: Acousto-optic tunable filter; Flow injection; Multivariate calibration

1. Introduction

The acousto-optic tunable filter (AOTF) is an electronic monochromator. It is based on the acousto-optic interaction in an anisotropic medium [1]. Generally, the filter is fabricated from an anisotropic TeO_2 crystal onto which an array of $LiNbO_3$ piezoelectric transducers are bonded. A radio frequency (rf) signal is applied to the transducers which, in turn, generate an acoustic wave propagating through the TeO_2 crystal. The propagating acoustic wave will diffract incident white light into monochromatic light whose wavelength is dependent on the frequency of the applied rf. Because of the anisotropic properties of the TeO_2, randomly polarized incident white light will be diffracted into two spatially separated and orthogonally polarized diffracted beams. As the two beams are spatially well separated, they can be readily isolated by means of an aperture. Because of the advantages (all solid state, non-moving parts, fast scanning ability, wide

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tuning range, high resolution) the AOTFs have been used to develop a variety of novel instruments which would not have been possible otherwise [1]. They include the UV and visible spectrophotometer, circular dichroism spectropolarimeter [1], multidimensional fluorimeters [2,3], multiwavelength thermal lens spectrophotometer [4] and rapid-scan UV–visible detector for liquid chromatography (LC) [5]. The principles and applications of the AOTFs have been reviewed recently [1].

In addition to the high resolution (< 1 Å) [5] and no mechanical moving parts, the AOTF based spectrophotometer has other characteristics that are particularly useful for flow injection systems, particularly those designed for gradient exploitation [6,7]. Specifically, the rapid scanning rate (μs) that can be achieved with AOTF instruments is essential to FI gradient techniques. It is rather interesting to realize that because the AOTF can be scanned at this speed, the scanning rate of AOTF-based instruments is usually limited by the associated devices used for signal processing including, for example, lock-in amplifiers [5]. Generally, scanning rates as fast as 300 nm/s can be obtained easily with conventional rf generators, PMT and low cost lock-in amplifiers [5]. It is important to point out that in addition to the intrinsically high scanning rate, the AOTFs are also random access devices. Any wavelength can be randomly accessed any time by adjusting the rf generator to the appropriate frequency. Another characteristic of the AOTF spectrophotometer is its ability to work as an electronic modulator. This can be accomplished by enveloping the applied rf signal (usually in the 100 to 200 MHz range) with a low frequency signal (about 50 kHz) [2,3,5]. This will cause the instantaneous power applied to the transducer to suffer a sinusoidal modulation [2,3,5]. As a consequence, the diffracted beam will be amplitude-modulated at that enveloping frequency (i.e., 50 kHz). Conventional techniques to improve signal-to-noise ratio, such as phase lock detection can, therefore, be readily implemented without the need for any mechanical moving part. Finally, the modulating amplitude can be used to control the power of the rf signal applied to the AOTF and, during a scan in a given wavelength range, the amplitude can be appropriately increased to enhance the signal in those regions where the light source and/or the detector sensitivity are low. These characteristics make the AOTF based spectrophotometers ideally suited for applications where a reliable, rapid scanning and low noise figures of merit are necessary.

Flow-injection gradient exploitation has been devised since the early days of that technique [6,7]. The gradient can be generated by using different devices placed in line with the sampling valve and the flow cell. Simple straight tubing [8], single-bead string reactor [9] and mixing chambers [6] are the most commonly used. The gradient can also be generated by controlling the flow rate of one pump in a two peristaltic pumps system [10]. In any of these approaches the concentration profiles generated are reproducible, and were used mainly for cases where a smooth time variable concentration profile is required.

Initially, FI gradients were used to perform FI-titrations [11]. The FI gradients were also utilized to distinguish different types of metal complexes which were generated as a function of the pH of the flowing stream [12]. Calibration of the gradient to establish the concentration versus time profile has also been proposed. Its use in the standard addition has also been reported [13,14].

In addition to being a useful tool for quantitative determinations or to improve quantitative results through standard addition [6,7], the gradient exploitation technique also makes it possible to rapidly obtain information on a variety of chemical systems. For example, it has been used to determine formation constants of various chemical systems [15,16], and to characterize a variety of chemical systems including acidity constants [17] and proton–ligand equilibria [9]. The pH of a flowing solution inside the flow cell of a FI system can be determined by synergistically using an indicator mixture, a spectrophotometric probe and a partial least square (PLS) multivariate calibration procedure [18].

pH is one of the parameters that are frequently used to induce changes on chemical systems. Complexation reactions, enzyme catalysis, inorganic catalysis and many others chemical systems are known to be affected by the environmental pH. It is, therefore, possible to use the flow injection gradient technique to generate the pH gradient in a flow system, and to obtain the pH profile as a function of time. The profile obtained can then be used to determine the pK_a of a variety of indicators, and the structures of complexes which are formed at different pH values. It is evident that this type of study can only be performed if the detection techniques used in the flow injection gradient are capable of rapidly recording entire spectra of indicators as they are flowing through the detector (at different pH values). Diode arrays [19] and a Hadamard transform spectrophotometer [20] have, in fact, been used for the rapid recording spectra of samples in the FI generated concentration gradient systems. Spectrophotometers based on diode arrays detectors have, in addition to their rapid scanning ability, no moving parts in their monochromator system. However, the sensitivity of such multichannel devices is relatively low and, as a consequence, long integration time intervals are necessary if light with low intensity is being monitored. Relatively higher sensitivity can be achieved with devices which were developed recently including the intensified diode arrays and CCD arrays. However, in addition to their high costs, these array based spectrophotometers are serial devices for which the resolution and wavelength range are interdependent. The multiplexed Hadamard spectrophotometer can improve the signal-to-noise ratio but its resolution is rather poor and the fastest instrument reported is still based on mechanical moving masks [20]. Such limitations can be effectively removed, and the sensitivity can be substantially improved if the AOTF is used to develop a spectrophotometric detector for the FI gradient systems. However, in spite of its potentials, to date, the AOTF based spectrophotometer has not been used as a detector in the FI gradient techniques.

The information provided is indeed provocative and clearly indicates that it is possible to use the AOTF based spectrophotometer as a sensitive and rapid detector for flow injection gradient. Such considerations prompted this study which aims to develop the first FI-gradient fast scan spectrophotometric system based on an AOTF. The instrumentation development and its use in the determination of the

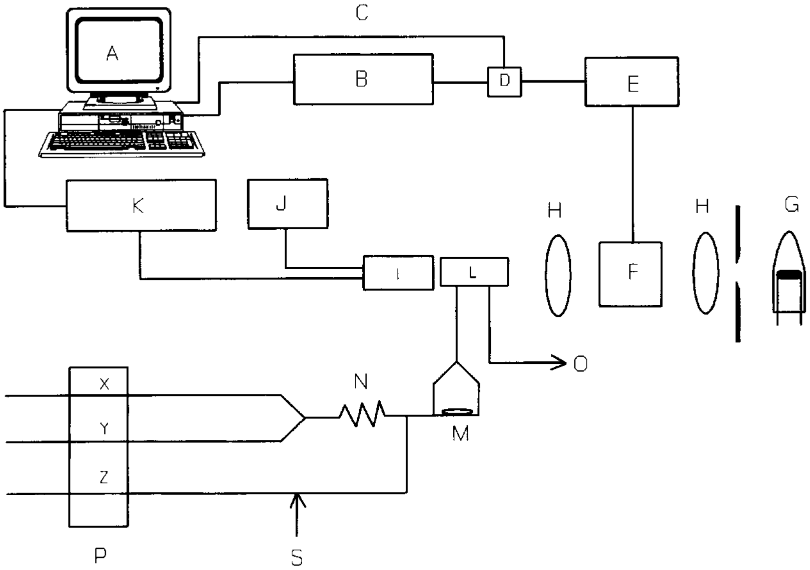

Fig. 1. Schematic diagram of the FI-gradient AOTF system. A, IBM-486 compatible personal micro-computer; B, rf generator; C, 50 kHz signal generated by the DAC compute board; D, mixer; E, rf amplifier; F, TeO3 AOTF; G, light source; H, collimating lens; I, photomultiplier tube; J, high voltage supplier; K, lock-in amplifier; L, flow cell; M, mixing chamber with magnetic stir; N, 50 cm long PTFE coil; O, solutions waste line; P, peristaltic pump; S, sample introduction device. *x*, Indicator acid–base probe mixture or system under investigation; *y*, buffer solution for pH calibration or wide range buffer mixture; *z*, water carrier stream. Flow rates for *x*, *y* and *z* are equal to 1.5 ml/min.

pH versus time profile established in the FI system for buffered solutions will be reported in this paper. It will be demonstrated that this AOTF-based FI-gradient system is particularly useful for the investigation of a variety of systems including the determination of the pKa of weak acids, and the elucidation of structures of complexes formed at different pH values.

2. Experimental

Fig. 1 shows a diagram of the FI-gradient AOTF based spectrophotometric system employed in this work. The spectrophotometer light source was a 250 W, 24 V halogen tungsten lamp (Osram, HLX 64655). The light leaving the lamp housing was collimated onto the noncolinear AOTF fabricated from TeO_2 (Brimrose Model TEAF 040-070H). Because the incident light is unpolarized, in addition to the transmitted beam, there were two diffracted light beams coming out of the AOTF when an rf signal was applied into the filter. The polarizations of the two diffracted beams are orthogonal to each other, and in this work, the diffracted beam having p-polarization was used. This beam, after being collimated by a lens, was incident onto the flow-cell. The light passing through the flow-cell was detected by a photomultiplier tube (PMT, RCA Model 434) which was powered by a high voltage power supply (Hamner Model NV 13). The rf signal applied to the AOTF was supplied by a sweep generator (Wavetek Model 1062) whose sweep rate and amplitude were remotely controlled by a microcomputer. The rf signal was modulated at 50 kHz before being amplified by an rf amplifier (Mini-Circuits Model ZHL 1–2 W). The amplified signal leaving the amplifier was then applied to the AOTF.

The PMT signal which was modulated at 50 KHz was connected to a lock-in amplifier (Stanford Research Systems Model SR510) for demodulating and amplifying. The analogue output of the lock-in was then presented to an ADC (ComputerBoards, Model CIO-DAD08-PGA) board inside a microcomputer (Gateway 2000 Model 4DX2-66MHz). A software was written in C + + language to control the frequency and power and to scan the applied rf signal, to acquire data, to calculate the absorption spectra, and to save the data in ASCII format (for the subsequent analysis using other software packages).

A home-made flow cell constructed from PTFE having 1.5-cm pathlength and an inner volume of 80 μl was used in this work. The gradient chamber was made of acrylic in order to handle acid and alkaline solutions which were introduced in the FI manifold. Solutions were injected into the system by using a sampling device which was made from acrylic and is similar to that previously described [21]. PTFE tubing with 0.8-mm i.d. was used to assemble the FI manifold; other tubings were Tygon. A peristaltic pump (Rainin, Rabbit Miniplus 2) was employed to impel the fluids in the manifold.

To synchronize the introduction of the solution in the FI manifold and the scanning measurements, an optical switch was installed at the base of the sampling device. When the solution is injected a TTL logical level transition is generated and sent to the I/O board to flag the injection instant.

The software resources allow the selection of the wavelength range and the resolution under which spectral data are obtained. The number of spectra and the time interval between scans can also be set by the user. In this work, the wavelength was generally scanned from 400 to 650 nm at 1 nm increment. Usually a delay interval is set before the system starts to acquire spectra. That is useful because data can be acquired after the peak gradient has passed the detection point where the concentration of the injected specimen decreases smoothly. The maximum scan rate obtained for the present instrument is 300 nm/s. Typical settings were 13-s delay time; 2-s time interval between successive scans; and 40 spectra were recorded.

Data collected were treated using commercial software including GRAMS and PLS-PLUS for GRAMS (Galactic Industries, Version 2.1G) and GrafTool for DOS (v. 3.3) and Slide Write (v. 2.1) for graphical display.

2.1. Reagents and solutions

Deionized water was employed throughout. The indicator probe mixture was daily prepared by appropriately diluting from a 10^{-3} M stock solution of the indicators. The final concentration of each indicator in the mixture was as follows: chlorophenol red,

5.0×10^{-6} M; cresol red, 8.0×10^{-6} M; bromocresol green, 8.0×10^{-6} M; bromothymol blue, 2.0×10^{-5} M and methyl orange, 2.0×10^{-5} M.

The buffer solutions employed in the pH multivariate modelling stage were prepared to be 0.02 M of potassium hydrogen phthalate, 0.02 M of potassium phosphate, 0.02 M of acetic acid or 0.02 M of boric acid. The pH of the solutions were appropriately adjusted using HCl or KOH solution, and the final pH values for the buffers were read from a pH-meter (Orion, 501). The two weak acids (methyl red and methyl orange) investigated were used in concentration equal to 1.0×10^{-5} M.

The buffer employed in the weak acid pK_a determination was 0.02 M in each acetic acid, potassium phosphate and potassium hydrogenphthalate. The mixture was adjusted to pH 8 by adding KOH and monitoring the pH in a pH-meter.

The buffer employed for Fe(III)–sulfosalicylic acid complexation study was 0.02 M for potassium phosphate, potassium hydrogenphthalate, boric acid, acetic acid and sulfosalicylic acid. The mixture has its pH adjusted to 9.5 with KOH solution before being used in the FI system. Iron(III) solution was 7.5×10^{-4} M and was freshly prepared by diluting from a daily prepared 1.0×10^{-2} M stock solution. Several drops of concentrated HCl were added to the stock solution to avoid metal hydrolysis.

2.2. Spectrophotometric determination of pH

The instantaneous pH of a solution present inside the flow cell was determined by employing an acid–base indicator mixture and multivariate calibration as previously proposed [18]. During the modelling stage the line y of the FI manifold (see Fig. 1) contained a buffer with a known pH and line *x* contained the indicator mixture solution. Line *z* contained water. A steady-state was created after the solutions were flowed for at least 1.5 min. After this time the absorption spectrum of the flowing mixture was obtained in the 500 to 610 nm range. Eighteen buffers encompassing the pH range from 2 to 10 were employed at this stage to obtain spectral information used in the subsequent PLS multivariate calibration analysis. Fewer number of buffer solutions was required for more restricted pH scanning ranges. The average accuracy of the model was verified to be equal to 0.03 pH units for the entire pH range from 2 to 10.

2.3. Calibration of a pH versus time profile generated in the FI-gradient system

In order to obtain the pH versus time relationship for any buffer solution over which a strong acid or base solution is injected, the same indicator mixture employed in the previous pH multivariate calibration was kept in line *x*. Line *y* contained a buffer mixture. A suitable volume (between 100 to 200 μl) of a strong acid or base solution was injected and the software was set up to obtain the absorption spectra. Forty spectra were recorded, and they were used to calculate the pH values using the PLS model constructed in the calibration step described above. The pH versus time relationship can, therefore, be found for any buffer mixture pumped through line *y* of the FI manifold. Usually three injections were made and data were used to average the pH vs. time curve. The precision obtained for each pH point, expressed as the estimate of the absolute standard deviation for triplicate measurements, was found to be ±0.03 pH units.

2.4. Effect of pH on chemical systems

After the pH versus time profile has been established for the wide range of pH values using the procedure described above, the contents of line *x* of the FI manifold were replaced by a solution containing at least one of the compounds involved in the chemical system under investigation. Line *y* contains the same buffer. The buffer can also contain any reagent and its effect on the pH vs. time profile will be taken into account in the calibration stage.

The solutions were allowed to flow for a certain time to reach steady-state conditions. The same volume of the same acid or base solution injected in the calibration stage was again introduced into the manifold. The same timing set up for data acquisition was also employed. Therefore, the pH at any time (e.g., at the time the spectrum is measured) is known and the behavior of the chemical system as a function of the pH can be verified by following any spectral feature observed during the data acquisition time when the pH was being varied in a buffered medium.

3. Results and discussion

We have successfully used a mixture of indicators as pH probe in the spectrophotometric flow system for the determination of the pH of the flowing solution. pH in the range of 2 to 9.5 can be determined with an overall precision of ±0.03 pH units. These results agree well with the those previously reported [21].

The procedure used to determine the pH versus time profile which was generated in the FI-gradient system can also be used to obtain other types of information such as those shown in Fig. 2. The forty spectra shown in this figure were collected 13 s after injecting a 1.0 M hydrochloric acid over the wide-pH-range phosphate–acetate–phthalate buffer. The spectra of the indicator mixture probe provide the needed information which through the use of the multivariate model can be converted to pH values. A relationship between the pH and time can therefore, be determined and the results obtained are shown in Fig. 3. The goodness of the pH profile obtained in the FI system can be evaluated from this relationship. For instance, it seems that the pH undergoes relatively faster change in the pH range from 3 to 4.5. This observation does not imply that the region is not buffered but rather is due to the fast concentra-

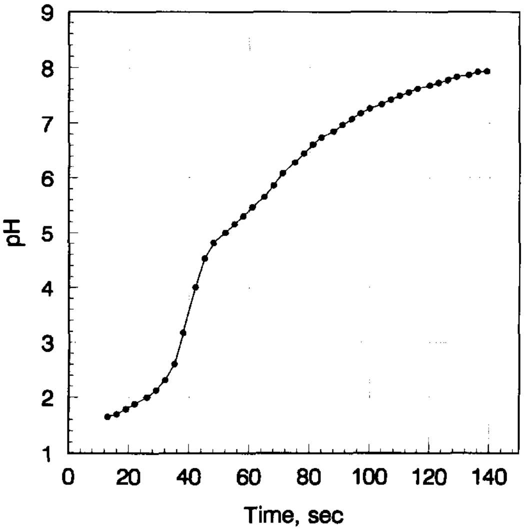

Fig. 3. Relationship between pH and time for the different buffer solutions used in the weak acid studies.

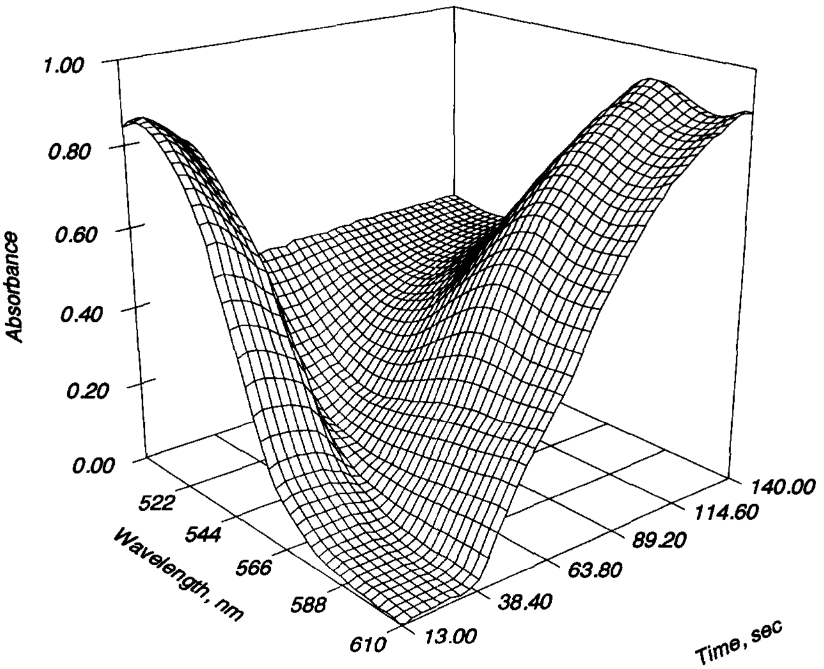

Fig. 2. Spectra collection obtained for the wide pH range of different buffer mixtures used in the determination of pK_a values of weak acids. The spectra are converted to instantaneous pH values by the multivariate calibration procedure.

tion change observed in the first part of a concentration gradient generated by the mixing chamber. The rate of pH change could be slowed down by decreasing the flow rate of either the *z* line or all lines in the FI manifold. Alternatively, the linear buffer proposed by Astrom [22] could be used to obtain a more predictable pH change with time. The presence of strong complexing ligands should be taken into account when selecting the buffer composition because these ligands could affect the behavior of the chemical system under investigation.

The relationship between pH and time makes it possible to determine the instantaneous pH of the mixture flowing through the cell. Therefore, it can be used to study the effect of pH on a variety of chemical systems including the determination of the pK_a of weak acids, and the effect of pH on the complex formation between Fe(II) and sulfosalicylic acid. In such studies, the aforementioned probe indicator mixture in line *x* was replaced by the chemical system under study. As illustrated in Fig. 4, this exchange did not produce any observable change in the pH of the mixture in the FI manifold. This was accomplished because the chemical mixture under studied was used at a very dilute concentration, Alternatively, one or more of the chemicals (of this chemical mixture) was included in the buffer which was used in the calibration of the pH versus time relationship.

3.1. pK_a determination of weak acids

As described in the previous section, the pH versus time relationship makes it possible to determine the instantaneous pH of the detector. Fig. 4 shows forty spectra obtained for methyl red in the pH profile showed in Fig. 3. Due the rapid scan characteristics of the AOTF based spectrophotometer employed in this work, the concentrations of the basic and acidic specimens can be simultaneously monitored.

In Fig. 5 two curves for methyl red and methyl orange were obtained by following the absorption of their acid forms as a function of the pH in the FI-gradient system proposed. The procedure to find the pKa's is similar to that previously described [10].

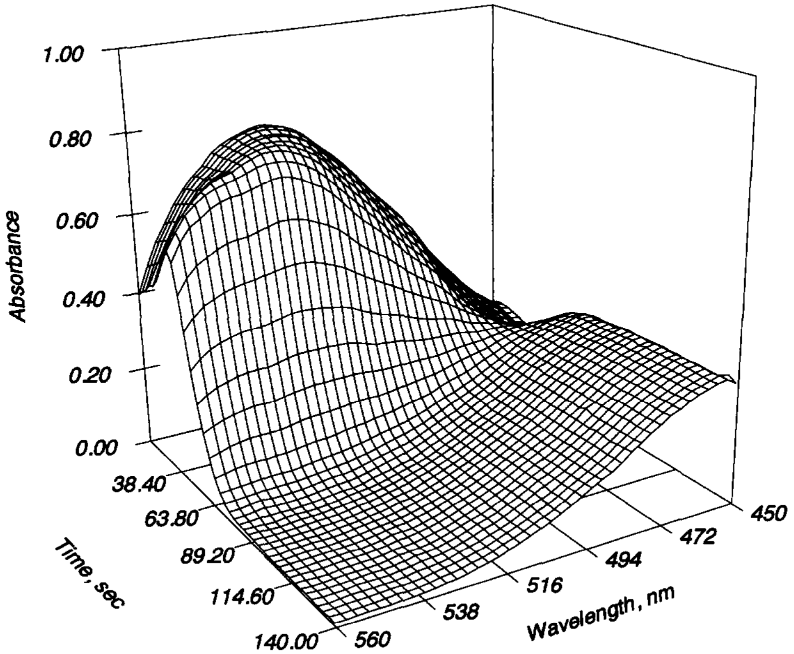

Fig. 4. Spectra of methyl red obtained under the same (FI-gradient) conditions as those used to obtain the relationship between pH and time as shown in Fig. 3.

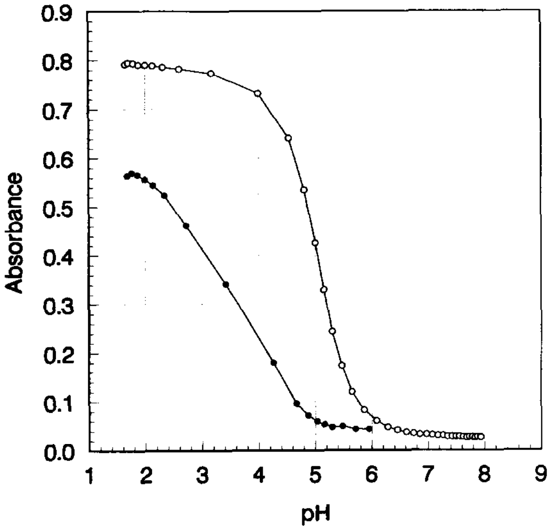

Fig. 5. Plots of absorbance versus time obtained in the FI-gradient system for the acidic forms of methyl red (○) at 525 nm, and methyl orange (●) at 540 nm.

However, the present flow system is simpler because it does not require two peristaltic pumps, and the concentration of the solutions do not need to be accurately known (because the calibration step is used to find out the relationship between pH and time). The pH values where the acid and basic forms are present in equal concentration are the pK_a of the weak acids. The pK_a values for methyl red and methyl orange were found to be 5.05 ± 0.05 and 3.42 ± 0.05, respectively. These values are in good agreement with the reported values of 5.00 and 3.46 [23].

No care was taken during these experiments to control the ionic strength of the flowing solutions but it could be easily done by adding the necessary amount of strong electrolyte to the wide-pH-range buffer solution or to the weak acid solution. Also, the ionic strength could be varied and its effect on the pK_a could be easily observed. The present pK_a determination was facilitated because the acidic and basic form of the weak acids show distinct absorbance spectra in the visible range (as illustrated in Fig. 4 for a set of spectra of methyl red which were obtained during a pH scan experiment). Also, the pK_a values have been showed to be independent of the ionic strength in the range from 0.1 to 0.5 [17]. However, the present methodology can also be used

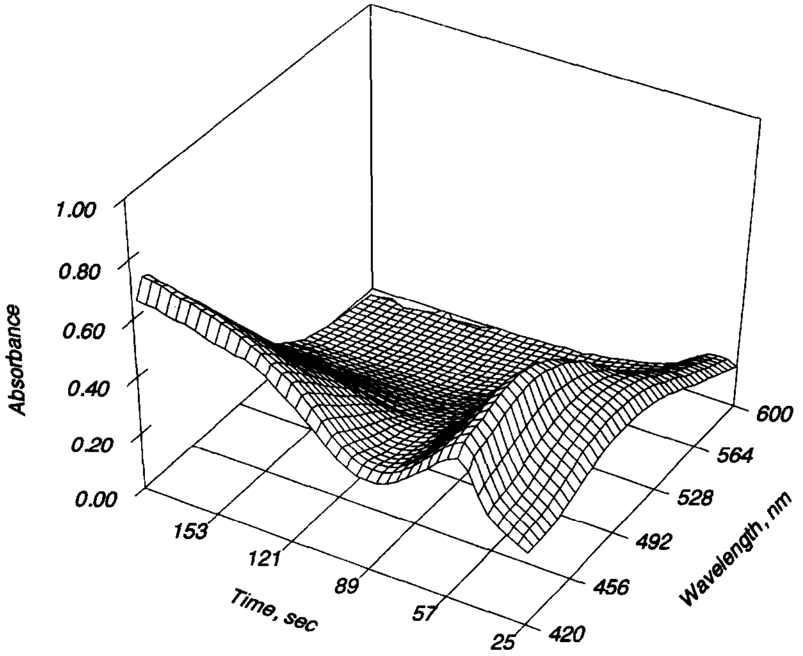

Fig. 6. Spectra collection for the complex formation of Fe(III) and sulfosalicylic acid in the pH profile established in the FI-gradient system.

for other chemical systems even though in such systems the different chemical forms induced by the change in the pH, do not have different absorption spectra in the visible region. This is because special and distinct features of such systems in the UV and/or near-infrared (NIR) regions can be monitored. The AOTF based spectrophotometer is particularly suited for this purpose because the AOTF is known to have a much wider tuning range (compared to grating monochromators). It is, therefore, evident that the AOTF based spectrophotometer used as a detector, substantially extends the number of chemical systems which can be studied by the FI gradient technique.

3.2. Complexation of Fe(III) with sulfosalicylic acid

Iron(III) is known to form stable complexes with the sulfosalicylic acid. In a recent paper [24] the chemistry of this system has been addressed and the existence of a polynuclear Fe_2L_3 specimen have been proposed based on potentiometric results.

It was previously reported that, as the pH reaches values > 2.0, the purple color, which was observed when the mono protonated complexes are mixed with the non-protonated mono complexes, is replaced by the brown color. This brown color is attributed to the formation of the polynuclear complexes. Finally at pH > 7 the yellow color, three ligand complexes dominate [24]. Therefore, this system is suitable to serve as model for the proposed FI-gradient methodology.

Fig. 6 shows a curve of the wavelength versus absorbance versus time obtained for the system including a solution of the Fe(III) complexes and the sulfosalicylic acid in the wide pH range buffer as described in the experimental section. 200 μl of a 1.0 M HCl solution were introduced in the FI system. The amount and the concentration of HCl injected used in this study were selected because under this condition, the concentration of the complexes generated by this injection reached a maximum value at about 25 s after the injection. Accordingly, spectra collection was made after this elapsed time. By use of the evolved factor analysis technique (EFA) [25–27] to analyze the data shown in 6, it is possible to observe not three as previously reported but four different types of complexes which were formed at different pH values (in the pH range studied, which was previously determined by the multivariate model, from 2.0 to 9.5). The results obtained are plotted as the logarithm of the eigenvalues for the first six principal components as a function of pH (Fig. 7). From the figure, it is evident that the first principal component was associated with FeL and/or FeHL complexes (the purple color is due to the mixture of these complexes present at pH < 2). It was not possible, at these low pH values, to differentiate the protonated form (FeHL) and the unprotonated form (FeL) of the complexes. The second component which can be attributed to the Fe_2L_3 complexes began to form at pH around 2.0. The third component arises from pH 3.0 to 4.0 and is due to the FeL_2 complexes. Finally, the fourth component which can be assigned to the FeL_3 complexes was generated at pH > 6. These results are in good agreement with those previously reported [24].

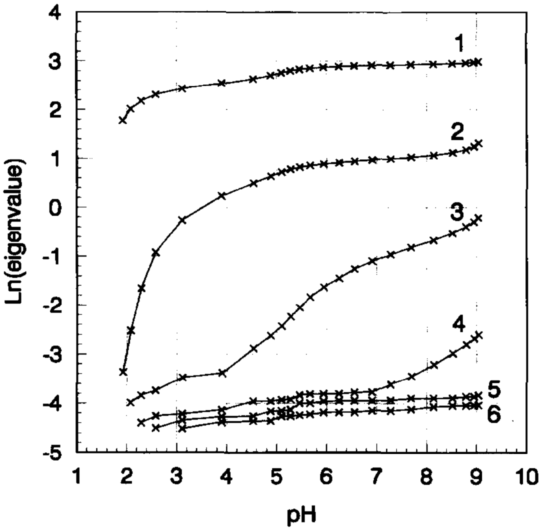

Fig. 7. Plot of logarithm of eigenvalues for the first six principal components (calculated by use of the evolved factor analysis to analyze data shown in Fig. 6) against pH. The first four components (curves 1, 2, 3 and 4) can be attributed to (FeHL and FeL), Fe_2L_3, FeL_2 and FeL_3 complexes, respectively.

4. Conclusions

The AOTF spectrophotometer employed in this work shows exceptional performance when coupled with FI-gradient systems. The absence of moving parts and the random wavelength access of such instruments together with a very rapid scanning capability are certainly the characteristics that will make this instrument of common use in FI systems in the near future.

The present methodology makes it possible to instantaneously and accurately determine the pH of the flow any time. Knowledge on the pH of any specific time is necessary for the study of chemical processes that release or consume protons in the flow.

Reversible and fast chemical systems were investigated in the present study in order to demonstrate the usefulness of the proposed pH-gradient methodology. It should be realized, however, that the system can also be used for kinetic studies as a function of pH. For that objective, the peristaltic pump can be stopped (under computer control) at any instant after the introduction of the acid or base solution. The chemical system under investigation is the solution in the flow cell at that instant. Different stopping times could be used to determine the kinetic behavior of chemical systems as a function of pH. Finally, the spectrophotometric pH calibration methodology could also be used in hyphenated techniques where other physico-chemical properties of the system could be investigated.

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