EFFECTS OF THE ETHANOL CONCENTRATIONS OF THE SAMPLE AND CARRIER ON SAMPLE DISPERSION IN FLOW INJECTION ANALYSIS – II [+]

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ABSTRACT

The effects of the variation of the ethanol concentration in both sample and carrier streams on sample dispersion in a straight single tube flow injection spectrophotometric system, not involving a chemical reaction, are described. Rhodamine B and bromothymol blue were used as the dyes. The results show that the relative ethanol concentrations of the carrier/sample solutions have significant effects on the dispersion coefficient, *D*, the peak height, and the peak-width (sampling frequency), as well as on the noise level. When the ethanol concentration of the sample is less than that of the carrier, the signal is higher but noise may be introduced. When the ethanol concentration of the sample is larger than that of the carrier, the signal is smaller and the noise is avoided. The case where the ethanol concentration of the sample is equal to that of the carrier is the optimum combination, and achieves higher signal sensitivity (i.e. peak height) and effectively avoids both peak broadening and noise.

KEY WORDS: flow injection analysis, dispersion, ethanol, rhodamine B, bromothymol blue, spectrophotometry

+ For paper I, see reference 24.

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INTRODUCTION

The design of the flow system depends primarily on the type of the analysis required, as this dictates the type of dispersion of the sample zone which should be created [1]. In practice the amount of the sample dispersion is controlled by altering the manifold design in flow injection analysis (FIA) [2]. In order to design systems with various dispersion, the influence of the sample volume, tube radius, tube length, flow rate, linear velocity and reactor volume all have to be considered [1]. Other factors, such as temperature [3], flow cell [4,5], and molecular diffusion of the solute [6], as sources of dispersion, also need to be considered in a FIA system. Organic solvents have received numerous applications in FIA to improve the performance or study the dispersion behaviour [7-15].

The choice of the solvent in ultraviolet and visible spectroscopy depends on an adequate solubility of the substance to be measured. Ethanol is miscible with water in any proportion and is an important solvent for UV-VIS spectroscopy. It was observed that in FIA introducing aqueous samples into a system with relatively high ethanol concentration usually produces spurious signals because of refractive index changes. This effect can be avoided by introducing the sample into an aqueous stream subsequently mixed with a reagent stream [15].

Rhodamine B is easily soluble in water, ethanol and cellosolve to give a pink-red solution with strong yellow fluorescence. In a polar solvent such as alcohol, acetone or water, the rhodamine B solution shows an intense violet colour [16]. This reagent has been used in flow injection spectrophotometric [17, 18] and spectrofluorimetric [19] analysis. Bromothymol blue dissolves readily in methanol, ethanol and dilute aqueous alkaline hydroxide solutions, but is only slightly soluble in pure water and benzene. A 0.1 % solution of the indicator acid in 20 % ethanol or a 0.04 % aqueous solution of the sodium salt is used as indicator solution [20]. Bromothymol blue has been used in FIA as an indicator for base-acid titrations and for studies of sample dispersion [1, 2, 21-23].

In our previous paper [24] the effects of the ethanol only in the carrier or in the sample on the sample dispersion, rhodamine B as the tracer, were investigated in a straight tube FIA spectrophotometric system. The dispersion behaviour, such as the dispersion coefficient, D, values and the peak height, as well as the peak width for the rhodamine B dye, is influenced by the ethanol compositions of the carrier and of the sample. A significant noise was observed when the ethanol

concentration of the carrier is more than about 40 % (v/v). In the present paper, employing different fractions of ethanol simultaneously in the carrier and in the sample solvent are investigated in detail for the rhodamine B dye in comparison with bromothymol blue dye. By choosing the sample/carrier ethanol combination the higher sensitivity can be achieved and noise is avoided in the FIA spectrophotometry for both rhodamine B and bromothymol blue dye systems.

EXPERIMENTAL

Reagents

All reagents were of analytical grade. 96 % Ethanol (Merck) and distilled and further deionised water was used in the preparation of the sample solutions and of the carrier streams.

The preparation of the standard solution of rhodamine B was described in our previous paper [24].

The bromothymol blue stock solution (0.4 %) was prepared by dissolving 0.8000 g of bromothymol blue (Merck) in 40 ml of 96% ethanol, diluting to 200 ml with deionised water. This stock solution was further diluted before use with deionised water and/or ethanol in a volume ratio of 1:100.

All solutions were degassed by a water pump prior to use in order to avoid the effect of dissolved air.

Apparatus: A Zeiss PM 2D spectrophotometer, with a glass flow-through cell of 10 mm optical path and 70 μl of volume, was operated at 553 nm for the rhodamine B system and at 432 nm for the bromothymol blue system. The steady-state absorbance of the dye solution was measured with the spectrophotometer, by means of a 10 mm glass cuvette. The absorption spectra of the dye solutions were measured with a diode-array spectrophotometer (HP 8452A) against the respective solvent blank using the glass cuvette. A peristaltic pump (ISM 726B, ISMATEC, Switzerland) was used to deliver the solutions. A polytetrafluoroethylene (PTFE) rotary sampling valve [25] fitted with a 50 μl sample loop (0.9 mm i.d.) was employed for sample introduction into the FIA system. The sampling valve was connected to the detector via a 50 cm length of 0.9 mm i.d. polyethylene straight tube. The absorbance was continuously monitored on a chart recorder (Perkin-Elmer, Japan).

Procedure: The single line FIA manifold used in the present work is shown in Fig. 1. Injection of a dye as a tracer into a water or ethanol solution carrier, and spectro-

photometric measurement and recording of the dispersed sample zone were used to test the effects of the ethanol concentrations both in the sample and in the carrier. A sample volume of 50 μl was injected in all cases. After the sample (1.0×10^{-5} mol/l of rhodamine B or 4×10^{-3} % of bromothymol blue solutions with various fractions of ethanol) was injected, the sampling valve was not returned to the reload position until the maximum absorbance value (peak) for the previous sample had been reached. The flow rates of the carrier and of the sample streams were 1.6 ml/min, which is different from that (1.44 ml/min) in the previous paper [24] due to use of new pump tubes. Fixing the ethanol solution composition of the carrier, the effect of the sample with the same dye concentration but containing different fractions of ethanol were determined one by one. Every sample was injected successively in triplicate. When the determinations of a carrier solution series was completed another composition of the carrier was used in the next series of determinations.

In the calculation of the dispersion coefficient, D $(=H^{o}/H^{max})$, the respective steady-state absorbance, measured in usual spectrophotometry (10 mm cuvette) and deionised water as the blank, is used as H^{o} that depends on the ethanol concentration of the sample solution. H^{max} is the average value of the FIA peak heights of every sample. All experiments were done at room temperature.

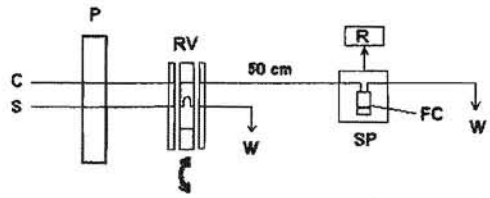

Fig. 1. The single line flow system.
C = carrier; S = sample; P = peristaltic pump; RV = rotary valve; SP = spectrophotometer; R = recorder; FC = flow cell; W = waste.

RESULTS AND DISCUSSION

1. Effect of ethanol on the absorption spectra and steady-state absorbance

The ethanol content affects the absorption spectra of the rhodamine B solutions and thus its steady-state absorbance at the fixed wavelength of 553 nm [24]. In comparison with the rhodamine B solutions the ethanol contents have a similar effect on the bromothymol blue solutions. The λ_{max} of the bromothymol blue solutions shifts about 10 nm to shorter wavelengths, from 432 nm, as the ethanol content increases. However the shape of absorption spectra is different and the absorption peak of bromothymol blue is wider than that of rhodamine B. The wavelengths were fixed at 553 and 432 nm, respectively, with respect to the rhodamine B

and bromothymol blue systems.

In usual spectrophotometric measurement the absorbance is linear up to 1.2×10^{-5} mol/l for the rhodamine B solutions and up to 5.6×10^{-3} % (higher concentrations not tested) for the bromothymol blue solutions. The concentrations of 1×10^{-5} mol/l for rhodamine B and of 4×10^{-3} % for bromothymol blue were selected in the present work.

Using above wavelengths and dye concentrations, the effect of the ethanol content on the steady-state absorbance was studied for the two dyes, and the results show that the ethanol concentration in the dye solutions influences the steady-state absorbance due to the shift of λ_{max}. However, the effects of the ethanol content on the steady-state absorbance for the bromothymol blue solutions are less than those for the rhodamine B solutions because of the difference of the absorption spectra shape.

2. Rhodamine B System

Keeping the dye concentration of the sample constant (1.0×10^{-5} mol/l), the effects of various fractions of ethanol/water in the carrier and in the sample on the response were examined for the rhodamine B system, and the recorded curves are shown in Fig. 2. The results show that the amounts of ethanol in the carrier and in the sample have a significant influence on the peak height, the peak width, and dispersion coefficient, as well as on the noise. Their effects will be respectively discussed in the following paragraphs.

2.1 Effect of the Ethanol Content on the Peak Height and the Peak Width for the Rhodamine B System

It is obvious that the absorbance or the peak height relates to the ethanol contents of the sample and of the carrier (Fig. 3). When the ethanol content of the rhodamine B sample is less than that of the carrier, higher peak heights are obtained, but noise is introduced with higher carrier ethanol concentration (>40 %). When the ethanol content of the sample is higher than that of the carrier, the signals are smaller and remain almost constant (except for the case of water as the carrier). The case where the sample and the carrier have the same ethanol concentrations is the optimum combination to obtain higher sensitivity without both peak broadening and noise. Under the optimum carrier/sample combination without noise (20% / 20%), the sensitivity can be improved 1.4 times with respect to the pure water system.

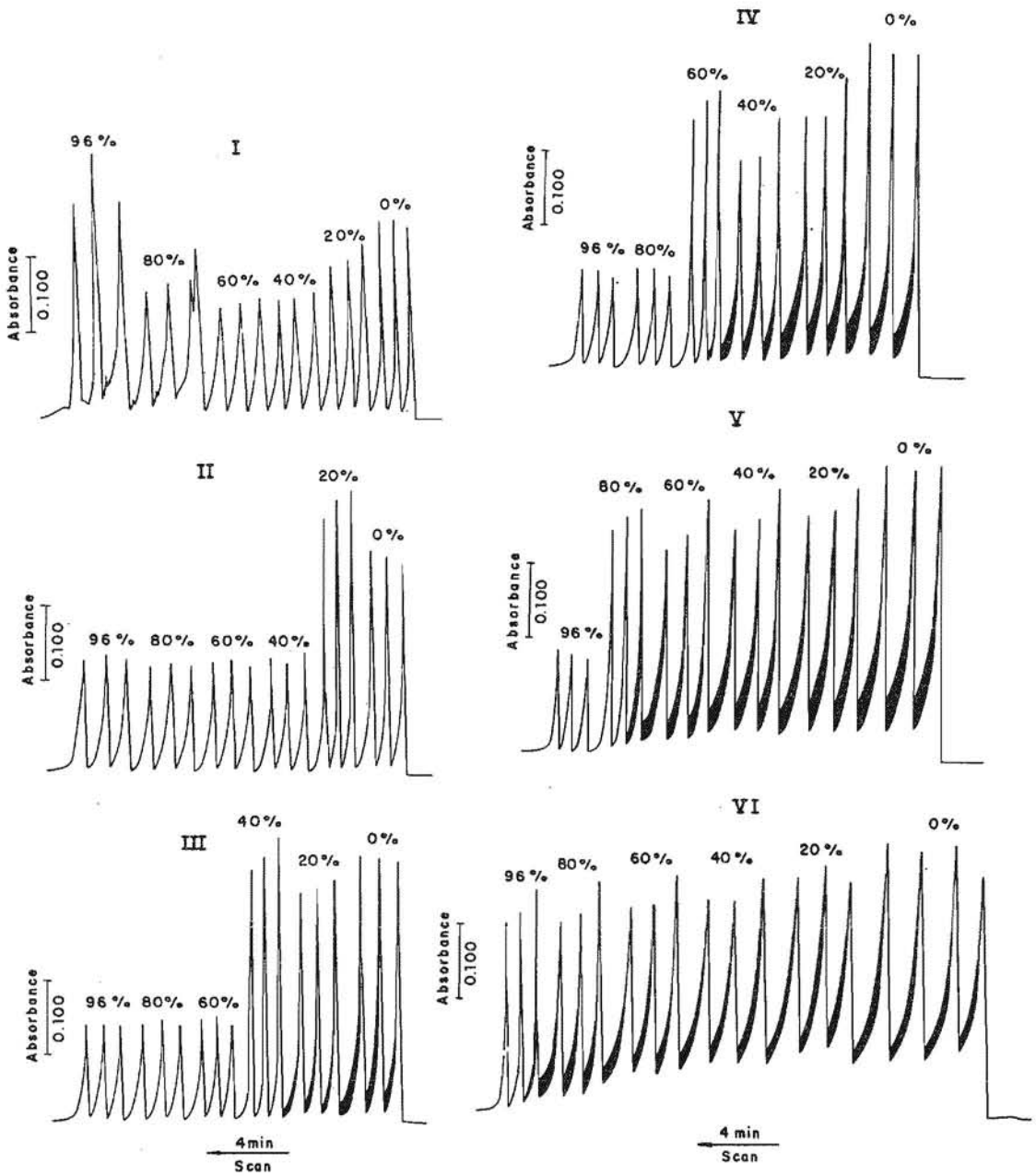

Fig. 2. Recorded graphs for the rhodamine B system.
The carrier solutions were deionised water containing different percentages of ethanol. Roman numerals I, II, III, IV, V and VI represent carrier ethanol concentrations of 0 %, 20 %, 40 %, 60 %, 80 % and 96 % (v/v). The percentages above the curves are the sample ethanol concentrations in % (v/v). The concentration of rhodamine B in the sample was 1.0×10^{-5} mol/l.

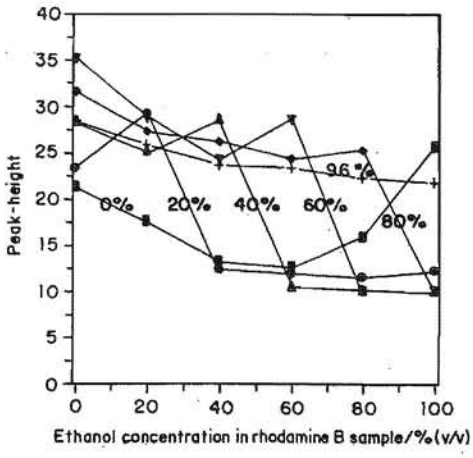

Fig. 3. Peak heights as a function of the ethanol concentration of the carrier and of the sample stream for the rhodamine B system.
Each datum point was the average reading of three measurements. The percentages on the curves represent carrier ethanol concentrations. Peak height in units of chart division. The experimental conditions are the same as in Fig. 2.

The ethanol contents of the carrier and of the sample also influence the peak shape and its width and, thus, the analytical frequency. The peak width qualitatively increases as the difference of the ethanol concentration between the carrier and the sample increases (see Fig. 2). Thus it is possible to enhance the sensitivity without producing peak broadening by choosing the ethanol fractions of the sample and the carrier.

2.2 Effect of the Ethanol Concentration on the Sample Dispersion Coefficient for the Rhodamine B System

The ethanol concentrations of the carrier and/or of the sample have a significant influence on the dispersion coefficient (Fig. 4). When the ethanol concentration of the sample is equal to or smaller to that of the carrier, the dispersion coefficient D is smaller and nearly constant, in the range of low dispersion (D=1-3). When the ethanol concentration of the sample is larger than that of the carrier, the D values are larger, in the range of medium dispersion (D=3-10). These indicate that rhodamine B sample solution with relatively higher ethanol concentration is easily dispersed (or diluted) in a carrier with relatively lower ethanol concentration.

The ethanol concentrations in the rhodamine B solutions affect the wavelength of maximum absorbance of the absorption spectra and therefore the steady-state absorbance at fixed wavelength. These result in a small difference of the D values when the steady-state absorbance relative to the ethanol content respectively used as the H^o or only the steady-state absorbance of the water solution used as the H^o in calculating the D values. However, as previously mentioned for the rhodamine B system [24], the shift of the wavelength of maximum absorbance and the variation of the steady-state absorbance are not the main reason for the changes of the dispersion coefficient.

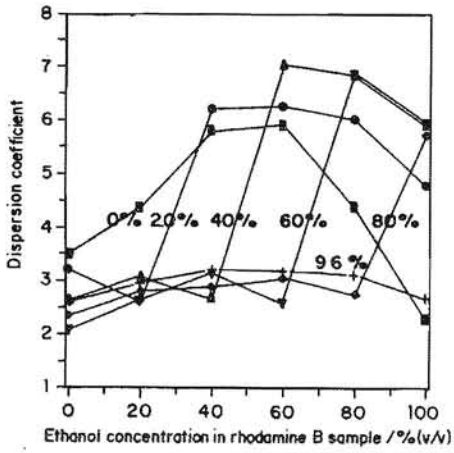

Fig. 4. Influence of the ethanol composition of the carrier and of the sample on the dispersion coefficient for the rhodamine B system.
The percentages on the curves represent carrier ethanol concentrations. The experimental conditions are the same as in Fig. 2.

2.3 Effect on the Noise in the Rhodamine B System

Another influence of the ethanol solvent introduced in the flow injection spectrophotometric system is the noise. The noise is determined by the carrier ethanol concentrations as well as the relative ethanol contents between the sample solution and the carrier. When the carrier ethanol concentration is less than 20 % (v/v), there is no noise observed, even through a dye solution in 96 % ethanol is used as the sample stream. When the carrier ethanol concentration is larger than 40 % (v/v), noise may be introduced, depending on the sample ethanol concentrations: the case where the ethanol concentration of the sample is less than that of the carrier produces a significant noise; in the other case, where the ethanol concentration of the sample is equal to or higher than that of the carrier, there is no noise observed.

3. Bromothymol Blue System

From the studies of the rhodamine B system, it is possible to improve the analytical characteristics by means of the optimum combination of carrier/sample ethanol contents in flow injection spectrophotometric analysis. The calibration solutions must be carefully matched to the sample with respect to the ethanol contents. The ethanol composition of the carrier is preferably the same as the sample. In order to test the above conclusions, bromothymol blue was selected to further investigate the effects of ethanol on dispersion in flow injection spectrophotometric analysis. The results for the bromothymol blue system are respectively shown in Fig. 5 to Fig. 7. It can be seen that the effects of the ethanol concentrations of the sample and of the carrier on the peak height, on the peak width, and on the dispersion coefficient, as well as on the noise, are similar for both the bromothymol blue and the rhodamine B systems.

The reproducibility of the peak height

shown in Fig. 2 (and Fig. 5) is not good, because there is the absorption of the dyes on the internal walls of the pump tubes and of the connecting tubes. The variation of the ethanol concentration in carrier and in sample results in the change of absorption equilibrium and cause the fluctuation of the dye concentration. Thus the peak height change mainly in the first sample peak. In Fig. 5 the interval time of sampling is longer than that in Fig. 2, and the reproducibility is better. Thus it is possible that the reproducibility is further improved after a sufficient equilibrium time between the streams and the tubes.

Compared Fig. 7 with Fig. 4, the tendencies of the D values for the rhodamine B and bromothymol blue, relative to the ethanol concentrations, are similar, although the dispersion coefficient D values for rhodamine B and bromothymol blue do have some differences. An explanation of this variation can be found by considering the different rates of diffusion of different solutes (rhodamine B and bromothymol blue) in solution.

The variation of the ethanol content produces changes in the physical properties of the solution, such as the density, the index of refraction, and the viscosity, as well as the fluidity, etc. [26], which can affect the dispersion behaviour of the solute in FIA. Brooks et al. [27] observed that the dispersion coefficient increases with both an increase in carrier stream viscosity and as the difference in viscosity between the injected sample and the carrier stream increases.

The refractive index (RI) effect is inherent in FIA when colourimetric detection is used, by virtue of the incomplete mixing of sample and reagent and the formation of concentration gradients. This RI effect is superimposed on the absorbance peak of the sample and may alter the shape and the height of the peak. Silfwerbrand-lindh et al. [15] observed that introducing aqueous samples into a relatively high ethanol concentration in a FIA system usually produces spurious signals because of the refractive index change.

The refractive indices of ethanol/water solutions are related to the ethanol content [26]. The RI increases as ethanol concentrations increase, up to 80.0 % (by wt.) of ethanol, then decreases after 80.0 % of ethanol. The difference of the refractive indices between the sample and the carrier is given in Table 1. It can be seen that for the two dye systems the noise is not completely relative to the difference of the RI, but depends on the relative ethanol concentrations between the sample and the carrier.

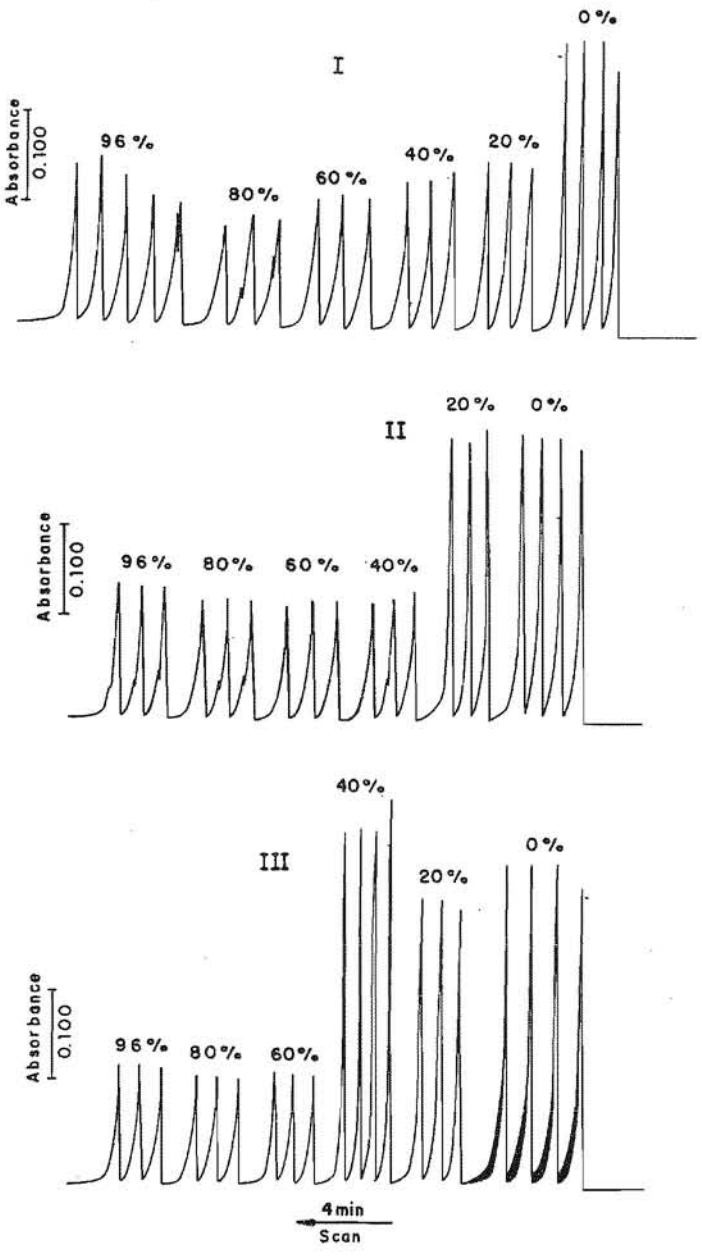

Fig. 5. Recorded graphs for the bromothymol blue system.
The conditions and symbols as in Fig. 2 except the dye is bromothymol blue in 4×10^{-3} %.

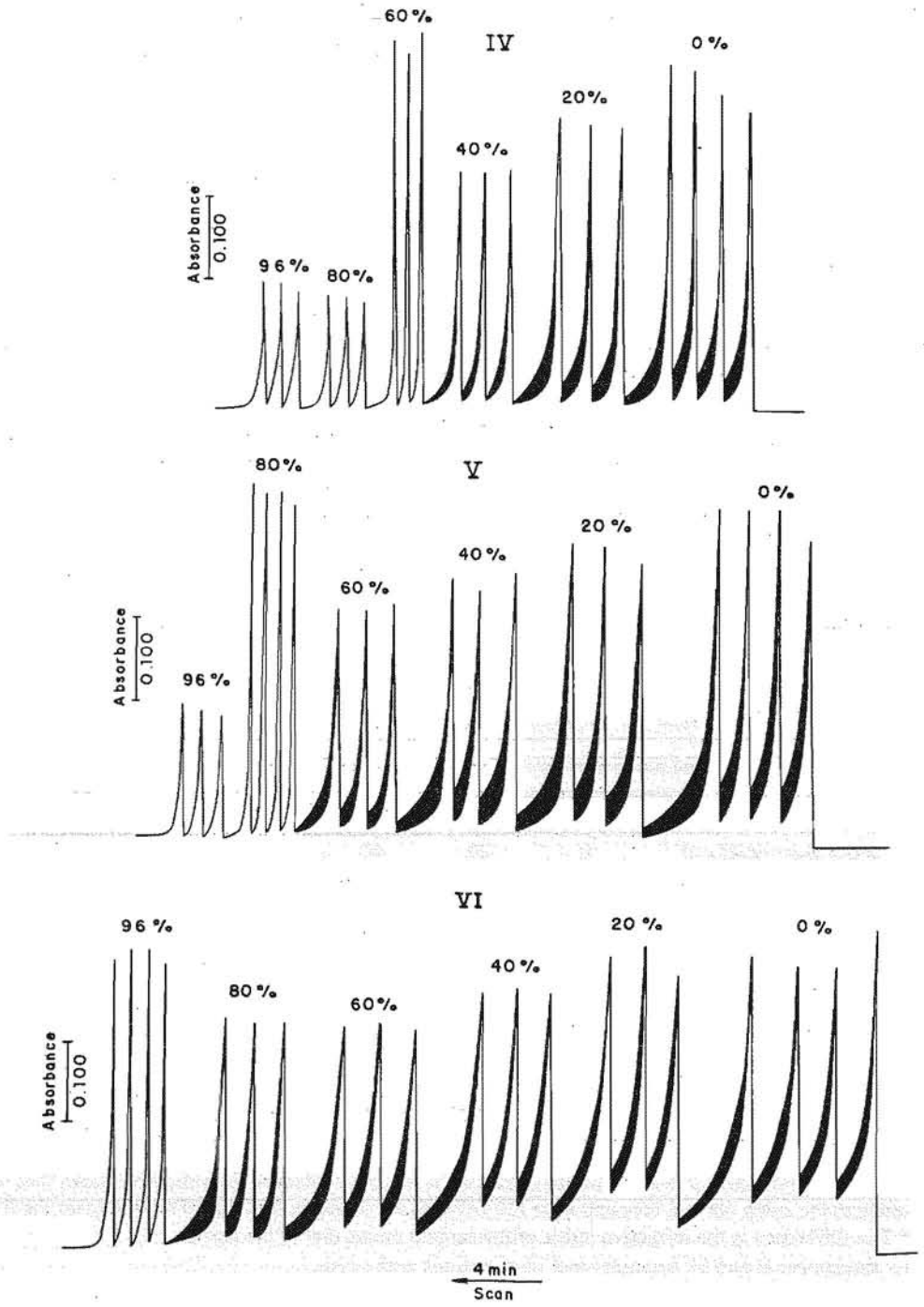

Fig. 5. Recorded graphs for the bromothymol blue system (continued).

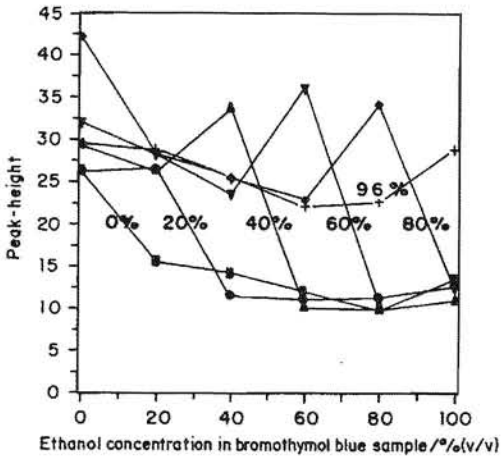

Fig. 6. Peak heights as a function of the ethanol composition of the carrier and of the sample stream for the bromothymol blue system.
Each datum point was the average reading of three measurements. The percentages represent carrier ethanol concentrations. Peak height in units of chart division. The experimental conditions are the same as in Fig. 5.

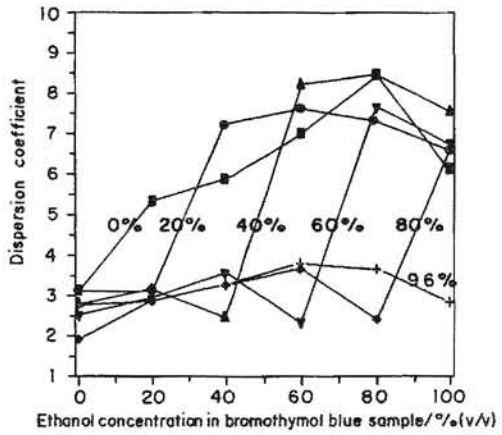

Fig. 7. Influence of the ethanol composition of the carrier and of the sample on the dispersion coefficient for the bromothymol blue system.
The percentages on the curves represent carrier ethanol concentrations. The experimental conditions are the same as in Fig. 5.

Table 1 Differences of the refractive indices between the sample and the carrier for various ethanol combinations +

Difference of RI×10⁴ *		Carrier / % (v/v)					
		0	20	40	60	80	96
Sample / % (v/v)	0	0	-112	-223 ⊕∅	-291 ⊕∅	-325 ⊕∅	-312 ⊕∅
	20	**112**	0	-111 ⊕	-179 ⊕∅	-213 ⊕∅	-200 ⊕∅
	40	**223**	**111**	0	-68 ⊕∅	-102 ⊕∅	-89 ⊕∅
	60	**291**	**179**	**68**	0	-34 ⊕∅	-21 ⊕∅
	80	**325**	**213**	**102**	**34**	0	**13** ⊕∅
	96	**312**	**200**	**89**	**21**	-13	0

+ Tables of refractive index versus concentration were used to deduce the refractive index that would relate to the using ethanol concentration [26,28] without consideration the effect of dye on the RI.
* The difference is the refractive index of the sample minus that of the carrier.
⊕: rhodamine B and ∅: bromothymol blue systems with noise.

In summary, similar results were obtained with the two dye systems as the tracer, i.e., rhodamine B and bromothymol blue. The ethanol concentrations in both the sample and the carrier have a significant influence on the dispersion behaviour, such as the peak height (sensitivity), the peak width (analytical frequency), the dispersion coefficient, D, values, and the noise in a flow injection spectrophotometric system. The effects depend on the relative concentrations of ethanol between the sample and the carrier. It is possible to improve the analytical characteristics in flow injection spectrophotometric analysis by means of a combination of the sample/carrier ethanol contents. When the ethanol concentration of the sample is equal to that of the carrier, higher sensitivity and a narrower peak-width (higher analytical frequency) are achieved, without introduction of noise.

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