

**EFFECTS OF ETHANOL CONCENTRATIONS OF THE SAMPLE AND CARRIER
ON THE SAMPLE DISPERSION IN FLOW INJECTION ANALYSIS**

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ABSTRACT

The effects of ethanol concentrations of the sample and carrier on the sample dispersion, in a straight single tube flow injection manifold in which Rhodamine B was used as tracer, are described in comparison with water carrier and aqueous sample not containing ethanol. The fractions of ethanol in the carrier and in the sample influence the sample dispersion coefficient (D), the peak height and peak width. From 0 % (v/v) to about 60 % (v/v) of ethanol in the carrier, D decreases; for higher concentrations of ethanol than 60 %, D remains constant. For the sample solutions, D increases with increasing in the fraction of ethanol up to about 60 %, then decreases for higher ethanol concentrations (> 60 %). The compositions of ethanol in Rhodamine B solutions influence the absorption spectra. The wavelength of maximum absorbance shifts toward shorter wavelengths as increases in ethanol concentrations in Rhodamine B solutions.

INTRODUCTION

The dispersion coefficient D is a key parameter in the flow injection analysis (FIA), that has been defined as the ratio of the concentrations of the sample before and after the dispersion process taken place in the fluid ($D = C^o/C$). It indicates the degree of dilution of the original sample solution when the injected sample is mixed with the carrier stream (reagent) [1]. In conjunction with the knowledge of the residence time T , this parameter D allows comparison of the sample/reagent dilutions and of reaction conditions between FIA and conventional manual procedures.

The factors that influence the sample dispersion in a FIA system are: (a) the manifolds for flow injection analysis [2,3]; (b) the injected sample volume [4]; (c) the tube length and the inner radius [5]; (d) the flow rate [6] and flow rate ratios [2]; (e) the channel geometry [1]; (f) the type of flow cell [7,8]; (g) the temperature [9] and (h) the diffusion coefficient of the solute [6,7,10,11]. There are few papers reported about the effect of organic solvent on the dispersion behaviour in a FIA system [12-14]. Attiyat and Christian studied the use of non-aqueous solvents as carrier in FIA with atomic absorption detection (FIA/AA) [12], and reported the effect of the sample solvent (acetone or ethanol with water) on the FIA/AA signal using the non-aqueous solvent carrier. An 8-fold enhancement of the signal was achieved, compared to aqueous systems, at the optimum carrier-sample solvent combination, due to the fact that organic solvents increase the atomisation efficiency and enhance the FIA/AA signal. Chen et al [13,14] investigated the relationship between the dispersion coefficient and the physical properties or structural parameter of solvents (six types of alcohols) used as carrier in a microwave field, however the effects of the solvent compositions of the carrier and sample on the dispersion coefficient have not been described. It is necessary further to explore the dispersion behaviour of a chromogenic reagent in different compositions of solvent. Because ethanol can be miscible with water in any proportion and is an important solvent for UV-

vis spectroscopy, it is chosen for evaluating the dispersion characteristics in spectrophotometric analysis. The aim of this work is to investigate the influences of ethanol fractions in the sample and carrier on the sample dispersion, and to improve the sensitivity of the peak height in spectrophotometric analysis. Rhodamine B was used as the tracer.

EXPERIMENTAL

Reagents

The standard solution of Rhodamine B was prepared by dissolving 0.3832 g of Rhodamine B (Carlo Erba, Milan, Italy) into 200 ml of distilled and de-ionised water. The concentration of the Rhodamine B stock solution was 4×10^{-3} M. Working solutions of 1.0×10^{-5} M Rhodamine B were prepared by serial dilution of the stock solution with water and/or ethanol. The wavelength of maximum absorbance (λ_{max}) of Rhodamine B aqueous solution is 553 nm.

Absolute ethanol (Merck, Germany) and distilled and de-ionised water were used for the preparation of the various compositions of ethanol/water solutions as the carrier and Rhodamine B/ethanol/water solutions as the sample.

All solutions were degassed by a water pump before in use.

Apparatus

A single-beam spectrophotometer (Zeiss PM 2D, Germany) equipped with a glass flow-through cell (inner volume 70 μl , path length 10 mm) was used to monitor the absorbance at the wavelength of 553 nm. The absorption spectra of Rhodamine B solutions were measured with a diode-array spectrophotometer (HP 8452A) against respective solvent blank using a 10 mm path length glass cell. A peristaltic pump (ISM 726B, ISMATEC, Switzerland) and a

polytetrafluoroethylene (PTFE) injection valve [15] fitted with a 50 μl of sample loop were employed. The injection valve was connected to the detector via a 50 cm length of 0.90 mm i.d. polyethylene tube. Other tubes with 0.68 mm and 1.04 mm i.d. were also used in the study of the effect of the carrier compositions. The absorbance was continuously monitored on a chart recorder (Perkin-Elmer, Japan).

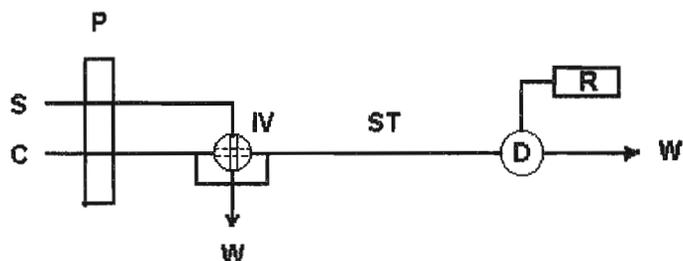


FIG. 1. Manifold for the straight single tube flow injection system

S, sample; *C*, carrier; *P*, peristaltic pump; *IV*, PTFE injection valve; *ST*, polyethylene tube (50 cm); *D*, spectrophotometric detector; *R*, recorder; *W*, waste.

Procedure

A single straight tube FIA manifold depicted in FIG. 1 was used for evaluating the dispersion characteristics of various ethanol compositions in sample and carrier. After the sample (1.0×10^{-5} M of Rhodamine B solution) was injected it was forwarded by the carrier to pass through a 50 cm length straight tube with an inside diameter of 0.90 mm (0.68 or 1.04 mm). Flow rates of the carrier and of the sample stream were 1.44 ml/min. The absorbance was continuously measured at 553 nm. The effects of ethanol contents in the sample and in carrier, on the peak height and on the sample dispersion, were respectively studied. When the effect of the carrier ethanol

concentration was tested, various fractions of ethanol/water solutions were used as the carriers and 1.0×10^{-5} M of Rhodamine B water solution was used as the sample. When the influence of ethanol fraction in the sample was studied, de-ionised water and 1.0×10^{-5} M of Rhodamine B solutions with different compositions of ethanol were used as the carrier and the sample stream, respectively. Every sample was injected three times. When the compositions of the carrier were changed, the peristaltic pump and the recorder were stopped. After this change, the pump and the recorder were turned on. The steady-state absorbance was spectrophotometrically measured by means of a 10 mm glass cell. The peak height was used to calibrate the dispersion coefficient D . All experiments were made at room temperature about 26 °C.

RESULTS AND DISCUSSION

Effect of Ethanol Concentrations in the Carrier

Keeping the sample concentration of Rhodamine B (1.0×10^{-5} M) water solution constant, the effect of various compositions of ethanol/water carrier solutions on the responses was examined using three tubes of different inside diameters (0.68, 0.90 and 1.04 mm), and the recording curves are given in FIG. 2. The amounts of ethanol in the carrier influence not only the peak height but also the dispersion coefficient D (FIG. 3). Different diameters of the tube (ST) connecting the injector to the detector show different dispersion coefficient. The narrower the connecting tubes are, the smaller the D values are. However the changing tendency of the dispersion coefficient D related to ethanol concentration of the carrier is similar for the three tubes with different diameters. The D values decrease with increasing the ethanol concentration in the carrier, in the range from 0 % to 60 % (v/v). After 60 % (v/v) of ethanol, the D values remains almost constant. The behaviour of D in function of the ethanol content in the carrier can be attributed to the viscosity and to the interactions

of the solution with the internal walls of the tubes. The kinematic viscosity reaches a maximum at a composition of 48 % (w/w) ethanol [16]. The ethanol fraction of the carrier also influences the peak width and the base-line noise as well, and the response curves given in FIG. 2 show this effect. Below about 40 % (v/v) of ethanol in the carrier, the base-line is smooth. When ethanol concentration is higher than 40 % (v/v), the noise of the base-line begins to increase. The noise observed in the base-line over 40 % (v/v) ethanol may be caused by the difference in component concentrations of the carrier and sample. The peak width slowly increases as increases the carrier ethanol concentrations. The reasons are not well understood. However, the change of ethanol fractions in carrier does result in the changes of viscosity (or fluidity) [16], which maybe influence the peak width. The peak shape and its width at the base line are important features and relate to the sample throughput, and the sample and reagent consumption. Therefore the analytical rate decreases from about 90 to 60 samples/h by changing from water to 100 % ethanol as carrier. The relative standard deviation of the reproducible sampling for the manifold of 0.68 mm i.d. tube is 1.1 % (n=11) and is similar for the different carrier concentrations (see FIG. 2).

Effect of Ethanol Concentrations in the Sample

The influence of ethanol concentrations of the sample (1.0×10^{-5} M of Rhodamine B solutions) was investigated (FIG. 4) with 0.90 mm i.d. tube when de-ionised water was used as the carrier. It shows that the compositions of ethanol of the sample influence the values of dispersion coefficient D , peak height and peak width. However, the effect of ethanol composition in Rhodamine B sample on the dispersion coefficient (FIG. 4B) is different from that observed for the carrier (FIG. 3). The dispersion coefficient D first increases up to about 60 % (v/v) of ethanol, then decreases after that fraction. It can be observed from the recording curve (Fig. 4A) that the amount

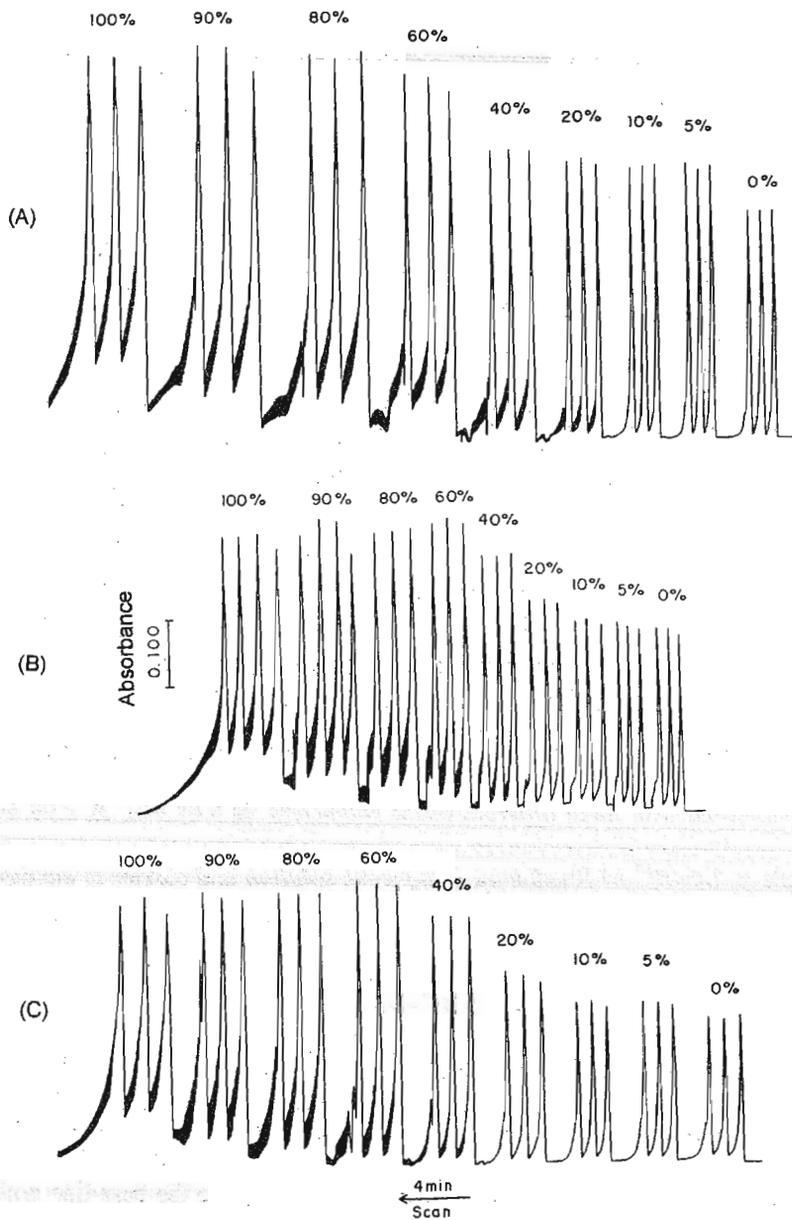


FIG. 2. Effect of the carrier ethanol concentrations on the peak height.

Inside diameters of tubes are (A): 0.68 mm, (B): 0.90 mm and (C): 1.04 mm. The percent values represent the carrier ethanol concentrations. For the B manifold, after the carrier was changed, peristaltic pump was turned on, after a delay of 60 seconds then the recorder was turned on.

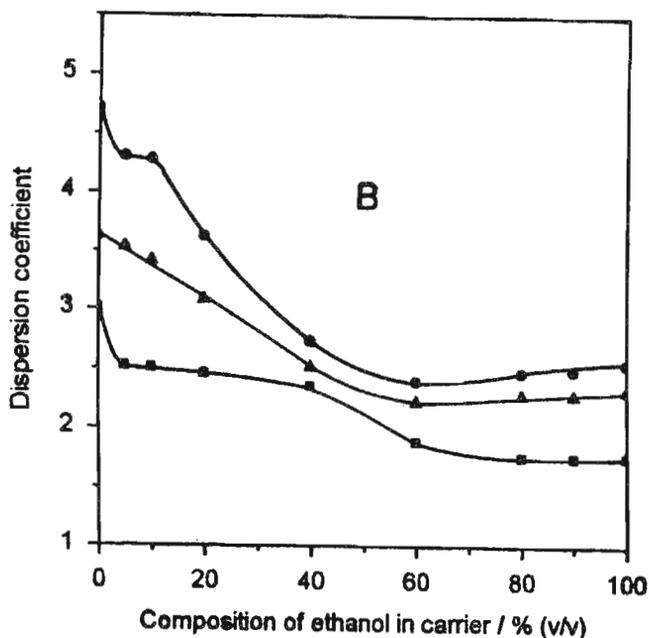


FIG. 3. Influences of the composition of ethanol in carrier on the dispersion coefficient in the straight tube manifold with three different inside diameters. ■ 0.68 mm, ▲ 0.90 mm and ● 1.04 mm i.d. Sample is 1.0×10^{-5} M Rhodamine B aqueous solution and carrier is mixture of water and ethanol.

of ethanol in the sample do not affect the base-line noise. This illustrates that the method of solvent introducing (in sample or in carrier) has an important effect on the base-line noise. That the sample containing ethanol is injected into the water carrier don't produce the base-line noise, and that the sample not containing ethanol is introduced into the ethanol solution carrier can cause the noise after certain ethanol content. The peak width increases a little by changing Rhodamine B water solution to 100 % ethanol solution.

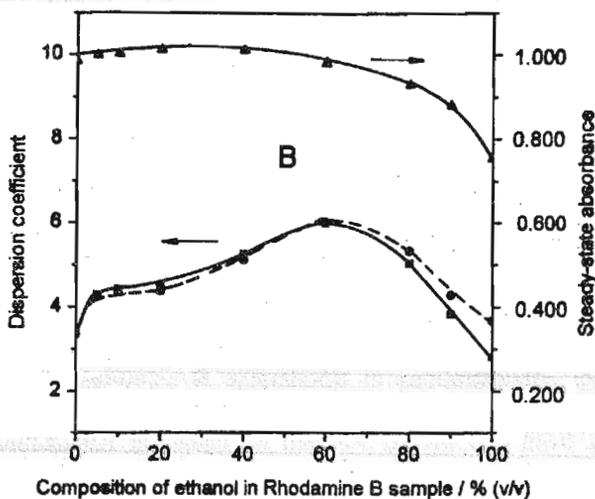
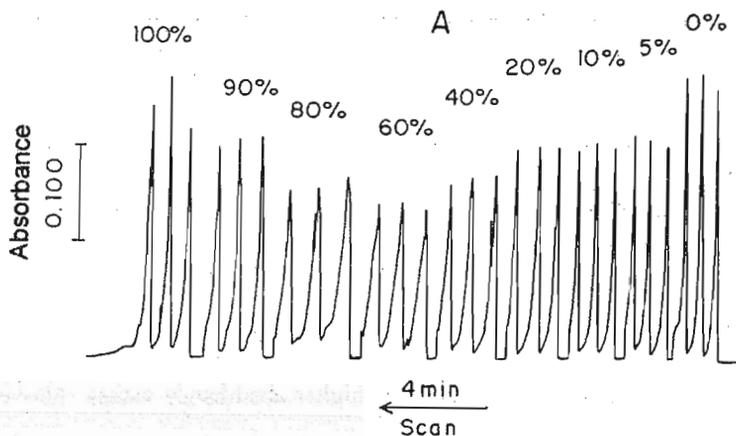


FIG. 4. The effects of the sample ethanol concentrations on the dispersion coefficient in FIA and on the steady-state absorbance at 553 nm.

(A) The recording graph corresponding to (B) in FIA for 0.90 mm i.d. tube. The percent values represent the sample ethanol concentrations;

(B) Solid line ■ and dashed line ● are the dispersion coefficients respectively calibrated from the steady-state absorbance of Rhodamine B solutions with various fractions of ethanol and from the steady-state absorbance of Rhodamine B aqueous solution. ▲ is the steady-state absorbance of 1.0×10^{-5} M Rhodamine B ethanol solutions.

Influence of the Ethanol Concentrations on the Steady-state Signals and the Absorption Spectra

The steady-state absorbances of Rhodamine B solutions with various concentrations of ethanol in common spectrophotometric analysis were measured at 553 nm and the result is given in FIG. 4(B). The ethanol concentrations of the Rhodamine B solutions affect the steady-state absorbance at the wavelength of 553 nm. The Rhodamine B solutions with the ethanol concentrations in the range of 5-40 % (v/v) have the higher absorbance values. Above 40 % (v/v) ethanol the absorbance begins to decrease as increases the ethanol fraction. The absorbance of Rhodamine B 100 % ethanol solution is smallest and equal to about 77 % of that in water at 553 nm.

The absorption spectra of 1.0×10^{-5} M Rhodamine B solutions with different fractions of ethanol were measured (FIG. 5) and show that the fractions of ethanol in Rhodamine B solutions influences the wavelength of maximum absorbance (λ_{\max}). The λ_{\max} shifts to shorter wavelengths as increases the ethanol concentrations in Rhodamine B solutions. The wavelength of maximum absorbance changes from 553 nm to 544 nm by changing Rhodamine B aqueous solution to Rhodamine B 100 % ethanol solution. The shift of λ_{\max} causes the change of steady-state absorbance for Rhodamine B solutions at 553 nm observed in FIG. 4(B). However, the changing tendencies of dispersion coefficient with ethanol content in Rhodamine B sample are similar for either considering the changes of absorbance at 553 nm (solid line in FIG.4B) or not (dashed line in FIG.4B). The λ_{\max} of the absorbing species can be shifted to shorter or longer wavelengths depending on the refractive index, polarity, permittivity or donor power or acid-base properties of the solvent [17]. The refractive index (n) of ethanol aqueous solution changes with the ethanol concentrations. n increases from water up to 80 % (w/w) ethanol; after 80 % (w/w) ethanol it

gradually decreases [16]. In a polar solvent such as ethanol, acetone or water, Rhodamine B exists as the zwitterion R^+ form, giving an intense violet colour [18]. In addition, Rhodamine B exists a series of protonation and dimerization equilibria, in the aqueous solution, that can influence the absorbance spectra of Rhodamine B solutions [18]. These probably are the reasons for the shift of λ_{\max} toward shorter wavelengths (shown in FIG. 5) as increases in ethanol concentrations for Rhodamine B ethanol solutions.

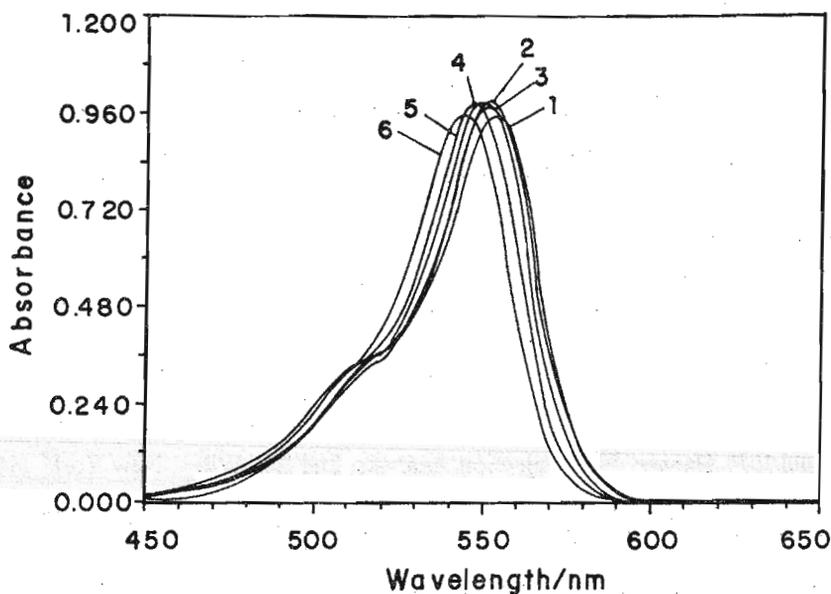


FIG. 5. Absorption spectra of Rhodamine B solutions with different ethanol compositions.

1: water solution; the numbers of 2,3,4,5,6 represent the Rhodamine B solutions ($1.0 \times 10^{-5} M$) with 20 %, 40 %, 60 %, 80 % and 100 % (v/v) ethanol.

CONCLUSIONS

In the straight single tube flow injection manifold, the dispersion behaviour such as dispersion coefficient D , peak height as well as the peak width of Rhodamine B solutions is

influenced by the ethanol compositions of the carrier and the sample. The ethanol compositions in Rhodamine B solutions affect the wavelength of maximum absorbance of the absorption spectra and the steady-state absorbance. It is possible to improve the sensitivity by selecting the ethanol compositions of the sample and/or the carrier. The work of comparison with other dye compounds is doing in order to obtain a general rule.

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REFERENCES

1. J. Ruzicka and E.H. Hansen, *Flow Injection Analysis*, 2nd Ed., Wiley, New York, 1988, p. 299-300 and 23-39.
2. B. Karlberg and G.E. Pacey, *Flow Injection Analysis – A Practical Guide*, Elsevier, Amsterdam, 1989, p. 16-23.
3. Y. Israel and R.M. Barnes, *Anal. Chem.*, 66 (1994) 3937.
4. J. Ruzicka and E.H. Hansen, *Anal. Chim. Acta*, 99 (1978) 37.
5. T. Korenaga, *Anal. Chim. Acta*, 261 (1992) 539.
6. Y. Li and H. Ma, *Talanta*, 42 (1995) 2033.
7. D.C. Stone and J.F. Tyson, *Anal. Chim. Acta*, 179 (1986) 427.
8. D.C. Stone and J.F. Tyson, *Anal. Proc.*, 23 (1986) 23.

9. C.L.M. Stults, A.P. Wade and S.R. Crouch, *Anal. Chim. Acta*, 192 (1987) 301.
10. T. Korenaga, F. Shen, H. Yoshida, T. Takahashi and K.K. Stewart, *Anal. Chim. Acta*, 214 (1988) 97.
11. Y. Israel and R.M. Barnes, *Microchim. Acta*, I (1990) 17.
12. A.S. Attiyat and G.D. Christian, *Anal. Chem.*, 56 (1984) 439.
13. Y. Xu, X. Chen and Z. Hu, *Anal. Chim. Acta*, 292 (1994) 191.
14. X. Chen, Y. Xu and Z. Hu, *Anal. Chim. Acta*, 314 (1995) 213.
15. M. Tubino and F.G. Barros, *Quím. Nova*, 14 (1991) 49.
16. CRC Handbook of Chemistry and Physics, 70th ed., CRC Press, 1989 – 1990, p. 223 and 230.
17. L. Sommer, *Analytical Absorption Spectrophotometry in the Visible and Ultraviolet – The Principles*, Elsevier, Amsterdam, 1989, p. 36.
18. E.B. Sandell and Hiroshi Onishi, *Photometric Determination of Traces of Metals - General Aspects*, 4th ed. of Part I of *Colorimetric Determination of Traces of Metals*, Wiley, New York, 1978, p. 661-669.

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