ELSEVIER

Dyes and Pigments 53 (2002) 267–275

DYES and PIGMENTS

www.elsevier.com/locate/dyepig

Correspondence

Ion-pair high performance liquid chromatographic study of the hydrolysis behaviour of reactive fluorotriazinic dyes

A. Zotou[a], I. Eleftheriadis[b], M. Heli[b], S. Pegiadou[b,*]

[a]*Laboratory of Analytical Chemistry, Department of Chemistry, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece*
[b]*Laboratory of Organic Chemical Technology, Department of Chemistry, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece*

Received 5 December 2001; received in revised form 7 January 2002; accepted 8 February 2002

Abstract

The separation, simultaneous determination in mixture (1:1) and the hydrolysis kinetics of two fluorotriazinic reactive dyes was studied using reversed-phase ion-pair high performance liquid chromatography (HPLC). The hydrolysis pathway was followed in dyeing conditions, both in the presence and in the absence of cotton. The exhaustion values $E\%$ were determined spectrophotometrically in the dyebath, while the total fixation ($T\%$) was calculated by measuring the K/S values spectrophotometrically on dyed specimens, at time intervals of 80, 100 and 120 min. In conclusion, this study has shown that the dyeing process was completed within 10 min after the addition of alkali. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Ion-pair HPLC; Reactive dyes; Fluorotriazinic reactive dyes; Hydrolysis of reactive dyes

1. Introduction

Synthetic dyes and pigments are widely used for textile applications, cosmetics, pharmaceutical formulations, artistic purposes, printing inks and foods [1,2]. Therefore, the quantitative determination and the satisfactory separation of their mixtures, are of great interest to the analytical chemist.

During the last decades, high performance liquid chromatography (HPLC) has proved to be a very successful technique for the separation of dyes, of their bacterial metabolites in waste water [3] and of their intermediates [4,5]. The analysis of those intermediates or precursors in the process of synthesis and the by-products or metabolites, is of vital importance due to their toxic or carcinogenic properties.

Colorants of several classes used as food and drink additives, have been separated and determined by HPLC [5,6]. In cosmetic applications many publications have been reported [7–9], using mostly reversed phase ion-pair liquid chromatography.

Recently, for better separations of azo- and other dyes, the sophisticated combination of liquid chromatography–mass spectrometry was successfully applied, using different techniques, such as thermospray HPLC–TSP–MS [10–12], particle beam electron impact ionization, HPLC–PB–MS [10], electrospray HPLC–ESP–MS [10], atmospheric pressure ionization HPLC–API–MS [13], FAB etc.

---

* Corresponding author.
E-mail address: sopegiad@chem.auth.gr (S. Pegiadou).

PII: S0143-7208(02)00019-0

Among all dyestuffs, the reactive ones mostly used for cotton dyeing, have been very popular since the early 1970s, not only for their high exhaustion (*E*%) and total fixation (*T*%) values, but also for their great wash fastness properties [14–17]. This is attributed to the covalent bond between the dye molecules and the –OH groups of cotton fibre [14,15,17,18] which takes place in alkaline media.

However, the reactive dyes, either vinylsulphone [14–16] or mono halide triazinyl [17,18] possess a disadvantage, which is the simultaneous and competitive hydrolysis reaction by the nucleophilic displacement of the halide ion, in the alkaline medium of the dyebath. Since the hydrolysed dye can not be fixed on fibre via covalent bond, this hydrolysis reaction is of great importance to the dyeing industry, for cost and environmental purposes as well. HPLC has been used for many very years in the study of hydrolysis of reactive textile dyes [14–19].

In this paper, the hydrolysis kinetics of two reactive fluorotriazinic dyes (Cibacron Yellow F-4G and Cibacron Blue F-R) separately and in a 1:1 mixture, in the presence as well as in the absence of cotton, was studied on a laboratory scale using ion-pair reversed phase HPLC.

Optimum separation conditions for these dyes and their hydrolysis products were achieved using a Nucleosil 100–5, C18, 150×4.6 mm I.D. column and an acetonitrile–0.05 M ammonium acetate (47:53 v/v) mobile phase with 1 mM cetyltrimethyl ammonium bromide (CTAB) as ion pairing agent. From the *E*% and *T*% values, measured in the dyebath and on the samples respectively, at time intervals of 80, 100 and 120 min, and from the results of hydrolysis measured by HPLC, it was concluded that the whole procedure of dyeing could be completed within a considerably shorter time (almost 40 min) than that normally used.

2. Experimental

2.1. Reagents and materials

All solvents used for HPLC analysis were of HPLC grade purchased from Riedel Häen AG (Hannover, Germany). The cetyltrimethylammonium bromide (CTAB) ion-pairing agent was obtained from Sigma-Aldrich Chemical Company (St. Louis, USA). Ammonium acetate, sodium chloride and hydrochloric acid were of analytical reagent grade and were obtained from Merck (Darmstadt, Germany). The sodium carbonate was obtained from Fluka Chemie AG (Switzerland).

The aqueous part of the mobile phase was filtered, prior to use, through 0.2 μm membrane filters obtained from Schleicher & Schuell GmbH (Dassel, Germany), using a glass vacuum solvent-filtration apparatus obtained from Alltech Associates Inc. (Deerfield, IL, USA) and the dye solutions were filtered through 0.2 μm membrane Schleicher & Schuell filters using a stainless steel filter holder fitted to a syringe.

The fluorotriazinic dyes studied were Cibacron Yellow F-4G and Cibacron Blue F-R, both supplied by Ciba and were used without any purification.

The fabrics used were greek bleached woven cotton fabrics, 10×15 cm (3g).

2.2. Chromatographic conditions

The reversed phase ion-pair HPLC separation of the dyes was achieved on a Nucleosil 100–5, C18, 150×4.6 mm I.D. analytical column, obtained from Macherey-Nagel GmbH & Co.KG (Duren, Germany). The elution was performed in the isocratic mode with a mobile phase consisting of a 47:53 v/v mixture of acetonitile and 0.05 M ammonium acetate buffer containing 1 mM CTAB, at a flow rate of 0.8 ml/min when each dye was eluted separately and of 0.6 ml/min when the dyes were eluted simultaneously from their mixture. Peak areas were used for quantitation.

The two reactive dyes, eluted from the column, were detected at ambient temperatures, by their UV absorbance at 275 nm, where they both absorb satisfactorily in the UV region, as revealed by their spectra. No common λ_{max} appeared in the visible region of their spectra. The HPLC detector was set at 0.002AUFS.

The ion-pairing agent was loaded on the column after a 72 h continuous passage of the mobile phase through the column. After equilibration, the column was washed only with double-deionized water and filled with acetonitrile at the end of each working day. Lengthy washings with strong solvents can wash out the ion-pairing agent and were, therefore, avoided.

2.3. Study of the hydrolysis kinetics of the dyes by HPLC

The hydrolysis of each dye separately and in mixture was studied under dyeing conditions, both in the presence and in the absence of fabric samples, at 120 min which is the proper time for a typical dyeing procedure, as follows.

2.4. Hydrolysis study in the absence of fabric

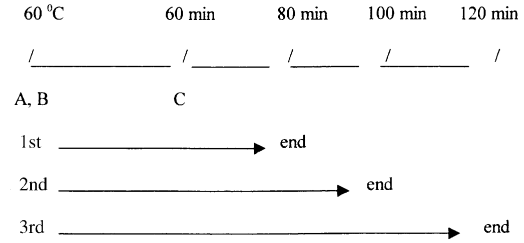
The study was performed in nine 150-ml beakers, each filled with 90-ml dyeing bath, with the quantity of dye corresponding to depth of shade 2% o.w.f. The appropriate quantity of sodium chloride (according to dyeing procedure described below) was added with stirring to each beaker and the beakers were thermostatted in a waterbath at 60 °C for 60 min. A 15-ml portion of the solutions was withdrawn from three beakers, from the first immediately after addition of sodium chloride to the dyebath, from the second at 20 min and from the third at 60 min after addition of sodium chloride and analyzed by HPLC, after a suitable dilution. At 60 min, the appropriate quantity of sodium carbonate (according to dyeing procedure) was added with stirring to each of the remaining six beakers and afterwards every 10 min, i.e. at 70, 80, 90, 100, 110 and 120 min, a 15-ml portion of the solutions was withdrawn from each of the six beakers, from the first at 70 min, from the second at 80 min etc., neutralized with concentrated hydrochloric acid until pH 6.5 and analyzed by HPLC, after dilution. In each case, the final concentration of the solutions after dilution was 6 mg/l and the solutions were filtered through 0.2 μm filters before injection onto the HPLC column. The above procedure was repeated for each dye singly and in mixture at a ratio of 1:1. Three replicate measurements were made for each solution and the mean values of the reported peak areas were used for quantitation.

2.5. Hydrolysis study in the presence of fabric

The study was performed in nine dyeing tubes using the dyeing machine, under identical conditions to those described above, with the only difference that 3 g of cotton fabric was added to every tube.

2.6. Dyeing procedure

The dyeing was carried out isothermally at 60 °C in a Zeltex Vistacolor laboratory- scale dyeing machine. A 30:1 liquor ratio using 3 g cotton pieces was used and 45 g/l sodium chloride and 5 g/l sodium carbonate for depth of shade 2% o.w.f. were added to each dyebath. Three dyeing procedures of total time 80, 100 and 120 min were performed, which are represented schematically as follows:



A: 0.6 g / l dye

B: 45 g / l NaCl

C: 5 g / l Na_2CO_3

After dyeing, the specimens were cut into two equal portions: one of them was left for control and the other one was washed in a soap solution (2 g/l standard soap Cibapon-R) in a liquor ratio 1:30, for 15 min at boiling temperature. The K/S values were measured on both pieces.

Dye exhaustion of cotton E% was measured by sampling the dyebath before dyeing as well as at the end of the time, that is, at 80, 100 and 120 min. The absorbance of the diluted dye solution was measured at the wavelength of maximum absorption ($\lambda_{max}=610.5$ nm for the blue dye and $\lambda_{max}=421.2$ nm for the yellow one) using a Shimadzu 2101 PC UV-visible spectrophotometer.

Table 1
Regression line equations

Dye	Equation[a] $Y=(a+S_a)+(b+S_b)X$	Correlation coefficient
Cibacron Yellow F-4G	$Y=(-1.595\pm11.40)\times10^3+(15.04\pm0.296)\times10^4\ X$	0.9990
Cibacron Blue F-R	$Y=(-2.753\pm6.174)\times10^3+(11.14\pm0.063)\times10^4\ X$	0.9999
Cibacron Yellow in 1:1 mixture with Blue	$Y=(-17.18\pm18.38)\times10^3+(19.48\pm0.434)\times10^4\ X$	0.9988
Cibacron Blue in 1:1 mixture with Yellow	$Y=(-24.39\pm18.73)\times10^3+(14.93\pm0.251)\times10^4\ X$	0.9993

[a] Y = peak area, X = concentration in mg/l, a = intercept, b = slope, S_a and S_b = standard deviation of intercept and slope, respectively.

2.7. Measurements of dyed fabrics

The reflectance values and the corresponding CIE L^*, a^*, b^*, C^* and h coordinates and K/S values (at the appropriate λ_{max} for each dye, i.e. at 620 and 440 nm for the blue and yellow respectively) of the dyed samples were measured using a reflectance spectrophotometer Macbeth CE 3000, interfaced to a digital PC under illuminant D using a 10° standard observer with component included and specular component excluded. Each fabric sample was folded to obtain a fourfold thickness of fabric. Four measurements were made for each sample and the average value was calculated.

All dyeing experiments and measurements of $E\%$ and K/S were performed three times with satisfactory reproducibility and the mean values are given.

3. Results and discussion

Calibration plots of the peak area versus standard concentration were constructed for each dye, using the least square method [16]. Owing to the good linearity of the plots it was possible to carry out a quantitative evaluation of both dyes, either in the hydrolysed, or non-hydrolysed form. The linear regression calibration equations with their correlation coefficients for the fresh non-hydrolysed dyes, both separately and in their 1:1 mixture, are given in Table 1. The two forms of dyes, hydrolysed and non-hydrolysed were separated satisfactorily as can be seen from their retention times, or the capacity factors $k' = t_R - t_o / t_o$, where t_R = the retention time of the dye, t_o = retention time of solvent, which are shown in Table 2.

When the two dyes were studied in mixture, the hydrolysed Blue co-eluted with the non-hydrolysed Yellow, therefore, the flow rate was decreased from 0.8 to 0.6 ml/min in order to obtain complete separation. The hydrolysis reaction of a mono-fluorotriazinic dye in alkaline medium is represented in Scheme 1.

All samples of dyes, when used both in the presence and in the absence of the substrate, were made in solutions using sodium chloride, in a concentration which is normaly used in a dyeing process, as given in the experimental section. That was of great advantage, because as has been proved by Chaytor and Heal [1] the presence of inorganic electrolyte plays an important role in the reproducibility of the retention indice. In Figs. 1–3 the chromatograms of both dyes, separately, and in their 1:1 (w/w) mixture, are presented.

We can see that after the addition of alkali, as the time passes, the peaks of the hydrolysed dyes

Table 2
Retention times and capacity factors of the dyes, Blue F-R and Yellow F4-G, separate and in mixture (1:1 w/w)

Dyes	Retention time (min)	Capacity factor (k')
Blue	4.70	1.41
Hydrolysed Blue	3.44	0.76
Yellow	3.32	0.71
Hydrolysed Yellow	2.38	0.23
Blue in mixture (m)	7.54	1.86
Hydrolysed Blue (m)	5.07	0.93
Yellow in mixture (m)	4.57	0.73
Hydrolysed Yellow (m)	3.16	0.20

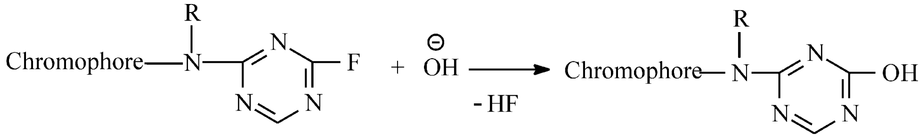
increase stepwise until the end of dyeing process, while the peaks of initial dyes (non-hydrolysed) decrease and finally disappear.

The conversion of the dyes to their hydrolysed forms with time, is clearly shown in Figs. 4 and 5 for both dyes and their mixture, in the absence and in the presence of the substrate respectively.

In the absence of the substrate as shown in Fig. 4, the behaviour of the dyes is different. The yellow dye is hydrolysed completely within 90 min, while the blue one is hydrolysed stepwise from 60 min until the end of time (120 min).

This difference between the two dyes is also shown in Table 3, where the values of hydrolysis pseudo rate constants k [19] are given, according to Eq. (1)

$$\ln C_o/C_t = kt \tag{1}$$



Scheme 1.

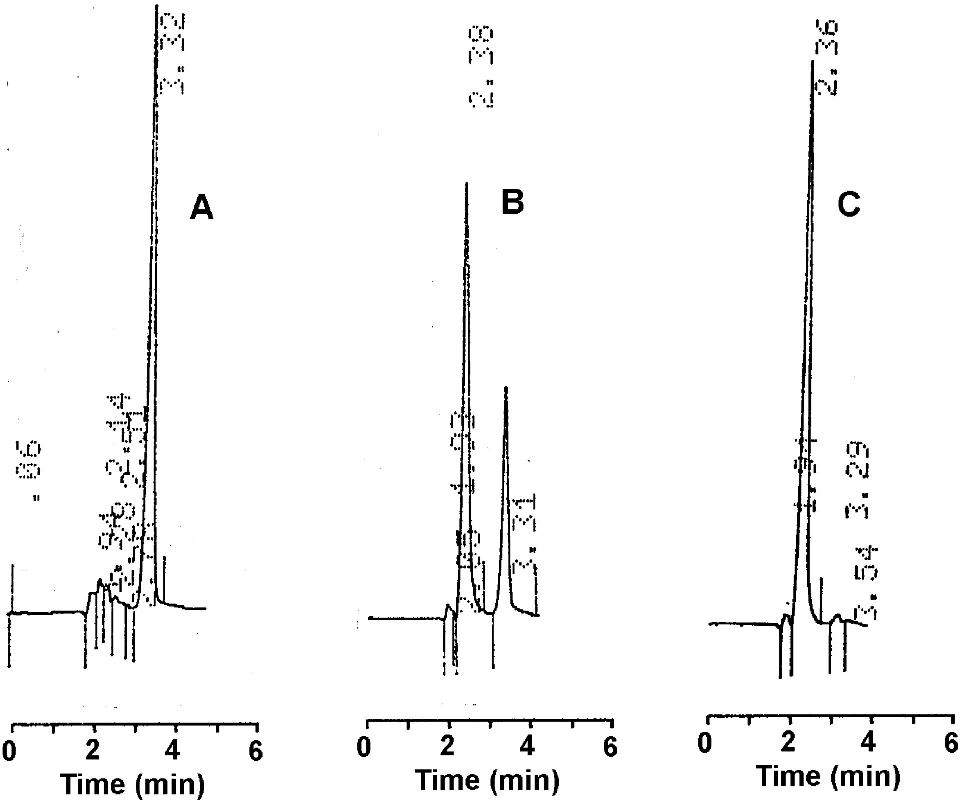


Fig. 1. Chromatograms of cibacron yellow: (A) 40 min before addition of Na_2CO_3, (B) 10 min after addition of Na_2CO_3 and (C) 60 min after addition of Na_2CO_3; flow rate = 0.8 ml/min, other conditions as described in Experimental. The peak at 3.32 min in (A) and the same peak (smaller in size) at 3.31 min in (B) are both attributed to the functional group (-F); the additional peak at 2.38 min in (B) and the same peak (bigger in size) at 2.36 min in (C) are attributed to the hydrolyzed part of the functional group (–OH).

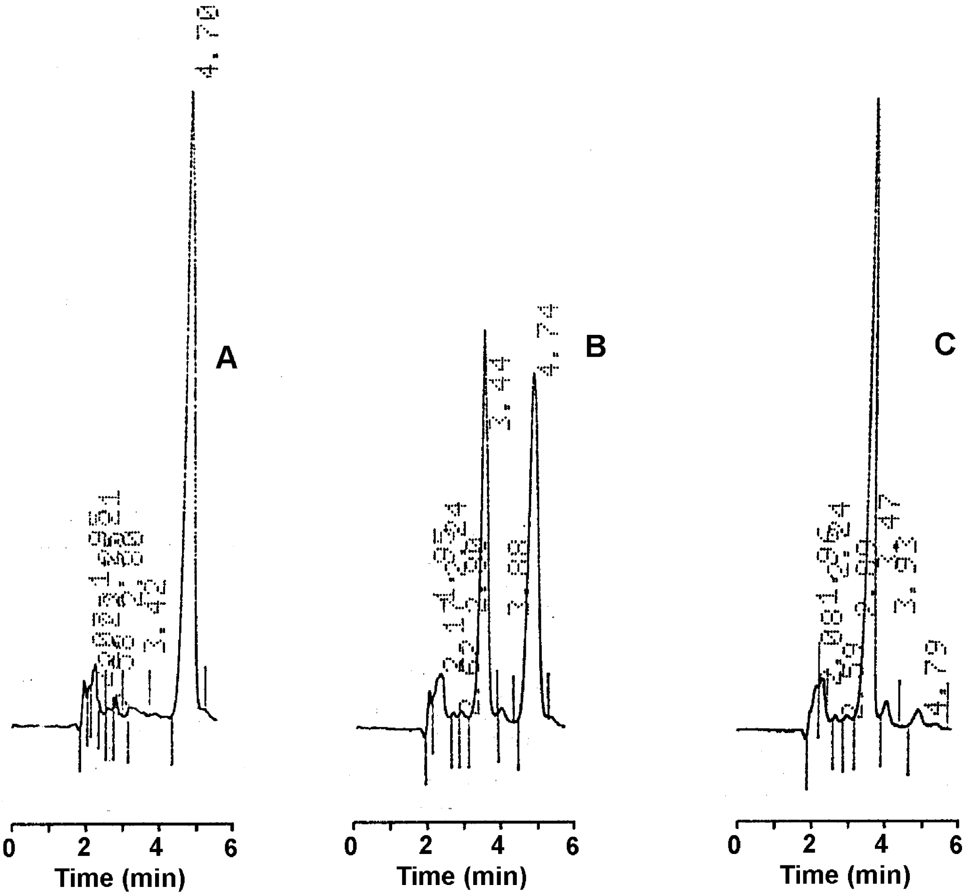


Fig. 2. Chromatograms of cibacron blue : (A) 40 min before addition of Na_2CO_3, (B) 10 min after addition of Na_2CO_3 and (C) 60 min after addition of Na_2CO_3; flow rate = 0.8 ml/min; other conditions as described in Experimental. The peak at 4.70 min in (A) and the same peak (smaller in size) at 4.74 min in (B) are both attributed to the functional group (-F); the additional peak at 3.44 min in (B) and the same peak (bigger in size) at 3.47 min in (C) are attributed to the hydrolyzed part of the functional group (–OH).

where t = time, C_o and C_t are initial dye concentration and dye concentration at time t respectively, as calculated by HPLC. On plotting C_o/C_t against t, a straight line is obtained, where the slope is the pseudo-rate constant k. One can see that the hydrolysis rate of the yellow dye is higher than that of the blue one.

However, when both dyes are in mixture, they behave almost identically and are hydrolysed regularly with time, as shown in Fig. 4 and Table 3.

On the other hand, in the presence of substrate (Fig. 5), both dyes behave similarly, and are dramatically converted (almost 100%) to the hydrolysed form, within 10 min after the addition of alkali, both when studied separately as well as in their mixture.

The behaviour of both dyes in the presence of substrate, can be explained by the absorption process onto the substrate, during which the fixation reaction takes place through the covalent bond as shown in Scheme 2.

That difference between the hydrolysis behaviour of the dyes in the absence and in the presence of the substrate, and mainly the rapid hydrolysis (within 10 min) in the presence of the substrate, led us to perform three dyeing procedures of total time 80, 100 and 120 min (as described in the experimental section) and afterwords

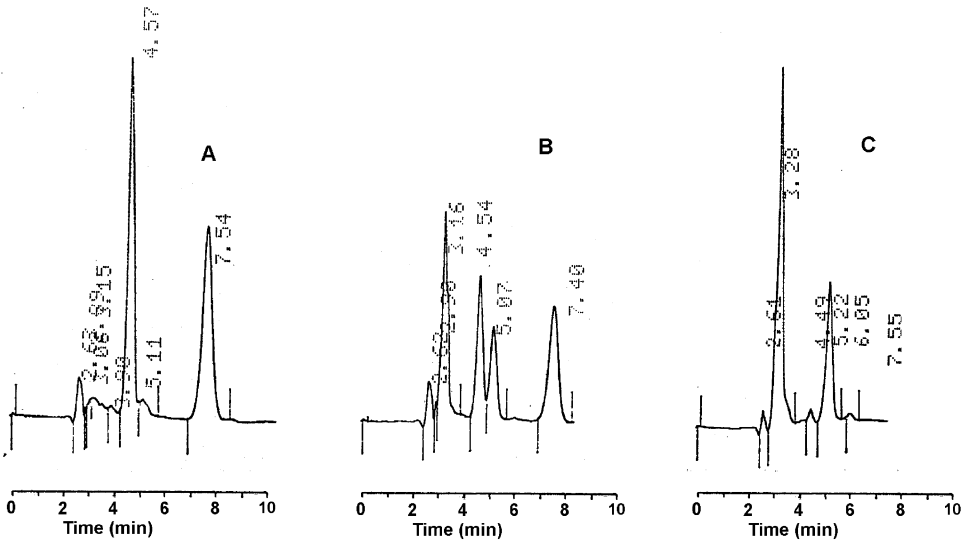


Fig. 3. Chromatograms of a 1:1 dye-mixture: (A) 40 min before addition of Na_2CO_3, (B) 10 min after addition of Na_2CO_3 and (C) 60 min after addition of Na_2CO_3; flow rate = 0.6 ml/min, other conditions as described in Experimental. The peak at 4.57 min in (A) and the same peak (smaller in size) at 4.54 min in (B) are both attributed to the functional group of cibacron yellow (-F); the peak at 7.54 min in (A) and the same peak (smaller in size) at 7.40 min in (B) are both attributed to the functional group (-F) of cibacron blue; the peaks appearing at 3.16 min in (B) and 3.28 min in (C) are attributed to the hydrolyzed part of the functional group of cibacron yellow; the peaks at 5.07 min in (B) and 5.22 min in (C) are attributed to the hydrolyzed part of the functional group of cibacron blue.

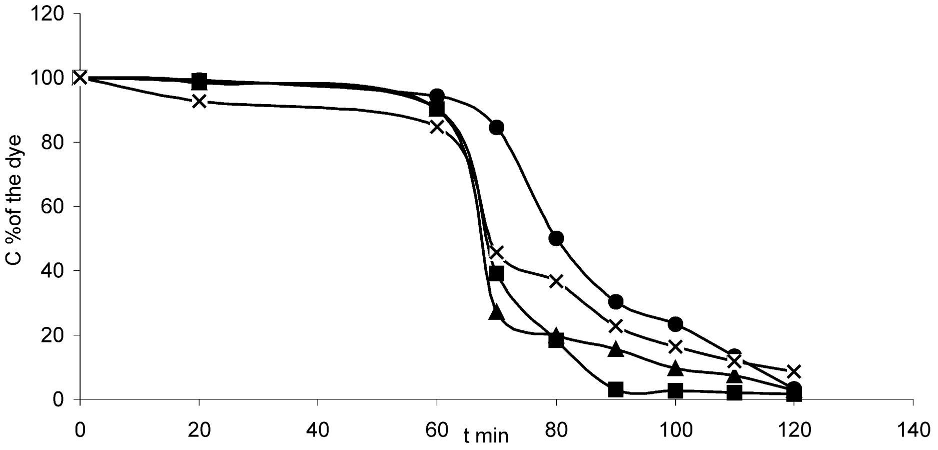


Fig. 4. The hydrolysis kinetics of the two dyes separately and in 1:1 mixture, in the absence of a substrate. ● Blue F-R, ■ Yellow F4-G, × Blue F-R in the mixture, ▲ Yellow F4-G in the mixture.

measure the colour strength K/S of dyed samples, the total fixation $T\%$ and the exhaustion $E\%$, according to the following equations [20, 21].

Percentage dyebath exhaustion $E\%$ was calculated using Eq. (2).

$$E\% = \frac{A_o - A_d}{Ao} \cdot 100\% \tag{2}$$

where A_o and A_d is the absorbance of dye solution before and after dyeing respectively.

The colour strength of the dyed samples was determined as the K/S value calculated from the sample reflectance (R) according to the Kubelka–Munk Eq. (3):

$$K/S = (1 - R)^2/2R \tag{3}$$

The extent of dye fixation of the reactive dyes on cotton was determined by measuring the K/S values of the dyed samples before and after soaping, from which the extent of dye fixation was calculated using Eqs. (4) and (5):

$$F\% = \frac{K/S_2}{K/S_1}\cdot 100\% \tag{4}$$

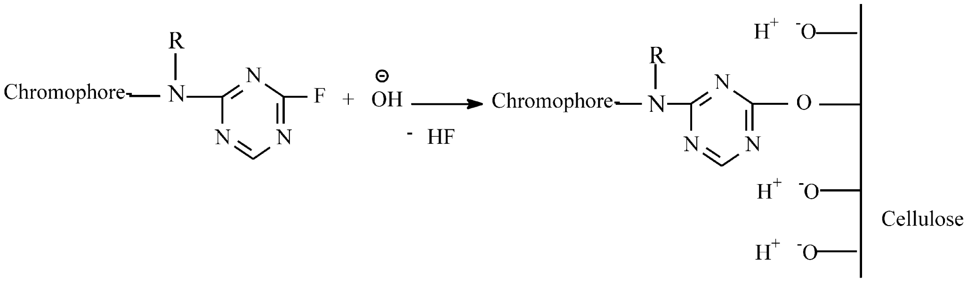
$$T\% = \frac{K/S_2}{K/S_1}\cdot E\% \tag{5}$$

where $F\%$ = degree of fixation of adsorbed dye and $T\%$ = total dye fixed, K/S_1 and K/S_2 are the values of dyed samples before and after soaping respectively.

Table 3
Hydrolysis rate constants (k) of the dyes in the absence of substrate

Dyes	Hydrolysis rate (k)
Blue	4.622×10^{-2}
Yellow	7.698×10^{-2}
Blue in mixture	3.423×10^{-2}
Yellow in mixture	3.260×10^{-2}

As shown in Table 4, the $E\%$, $T\%$ and K/S values are almost equal at all times, for both dyes, which means that the dyeing time was completed within ten minutes after the addition of alkali,



Scheme 2.

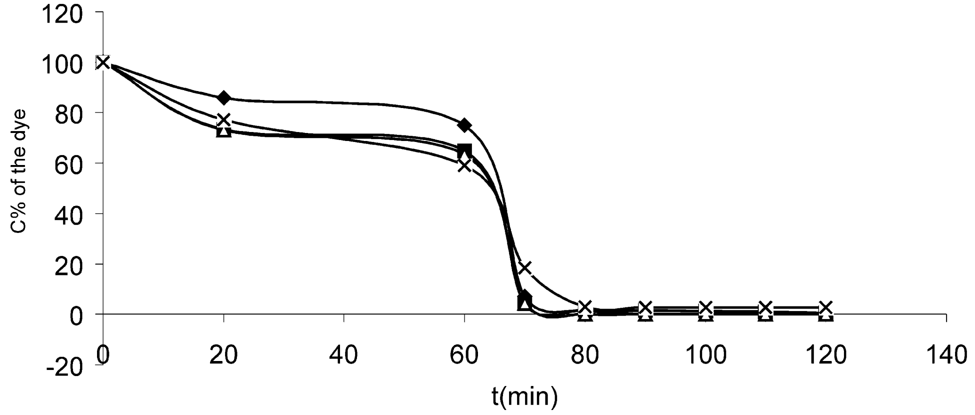


Fig. 5. The hydrolysis kinetics of the two dyes separately and in 1:1 mixture, in presence of a substrate. ◆ Blue F-R, ■ Yellow F4-G, △ Blue F-R in the mixture, × Yellow F4-G in the mixture.

Table 4
E%, K/S values and T% with time for the cotton samples dyed with the reactive dyes blue F-R and yellow F4G

Time (min)	E%		K/S				T%	
	Blue	Yellow	Blue		Yellow		Blue	Yellow
			Before soaping	After soaping	Before soaping	After soaping		
80	75.6	35.2	9.3	5.5	7.4	5.0	44.7	23.8
100	76.3	35.4	9.8	5.8	7.5	5.1	45.1	24.0
120	76.4	35.9	9.8	5.8	7.8	5.3	45.2	24.4

in other words, the whole process could be completed within 80 min instead of 120 min, which is normally used.

References

[1] Chaytor JP, Heal RL. Separation of synthetic dyes by high-performance liquid chromatography on 3-μm columns. Journal of Chromatography 1986;368:450–5.

[2] Fischer C-H. Trace analysis of phtalocyanine pigments by high performance liquid chromatography. Journal of Chromatography 1992;592:261–4.

[3] Grossenbacher H, Thurnheer T, Zurrer D, Cook AM. Determination of sulphonated azo dyestuffs and their bacterial metabolites by high-performance liquid chromatography. Journal of Chromatography 1986;360:219–23.

[4] Zou H, Zhang Y, Wen X, Lu P. Separation of phenylamine- and napthylamine-sulphonic acids by reversed-phase high-performance liquid chromatography. Journal of Chromatography 1990;523:247–55.

[5] Ayyangar R, Bhide SR. Separation of 1-napthylamine from the five known impurities and sub-ppm level detection and quantitation of 2-napthylamine by normal phase high-performance liquid chromatography. Journal of Chromatography 1989;464:201–7.

[6] Hann JT, Gilkison IS. Gradient liquid chromatographic method for the simultaneous determination of sweeteners, preservatives and colours in soft drinks. Journal of Chromatography 1987;395:317–22.

[7] Gennaro MC, Bertolo PL, Marengo E. Determination of aromatic amines at trace levels by ion interaction reagent reversed phase high-performance liquid chromatography, analysis of hair dyes and other water-soluble dyes. Journal of Chromatography 1990;518:149–56.

[8] Gagliardi L, Cavazzuti G, Amato A, Basili A. Identification of cosmetic dyes by ion-pair reversed-phase high-performance liquid chromatography. Journal of Chromatography 1990;523:247–55.

[9] Gagliardi L, Amato A, Cavazzuti G. Identification of xanthene dyes in lipsticks by reversed-phase high-performance liquid chromatography. Journal of Chromatography 1988;448:296–300.

[10] Straub R, Voyksner RD, Keever JT. Thermospray, particle beam and electro spray liquid chromatography-mass spectrometry of azo dyes. Journal of Chromatography 1992;627:173–86.

[11] Yinon J. Analysis of dyes extracted from textile fibers by thermospray high-performance liquid chromatography-mass spectrometry. Journal of Chromatography 1991;586: 73–84.

[12] Mclean MA, Freas RB. Enhanced analysis of sulfonated azo dyes using liquid chromatography/thermospray mass spectrometry. Anal Chem 1989;61:2054–8.

[13] Bruins AP, Weidolf LOG, Henion JD. Determination of sulfonated Azo dyes by liquid chromatography/atmospheric pressure ionization mass spectrometry. Anal Chem 1987;59:2647–52.

[14] Guo LN, Petit-Ramel M, Gauthier R, Chabert B, Jacquet A. Interaction of vinylsulphone reactive dyes with cellulosic fabrics. Part 1-dyeing mechanism, fibre characterisation and effects of alkaline electrolytes. JSDC 1993;109: 213–8.

[15] Wen J, Zhenghua Z, Kongchang C. Study of the hydrolysis kinetics of vinylsulfonyl reactive dye-fiber bond. Dyes and Pigments 1989;10:217–37.

[16] Zhenghua Z, Kongchang C, Ronggeng Y. A study of the hydrolytic stabilities of *n*- and *iso*-propyl ethers of some vinylsulfone reactive dyes. Dyes and Pigments 1990;14: 157–63.

[17] Oxspring DA, O'Kane E, Marchant R, Smyth WF. The separation and determination of reactive textile dyes by capillary electrophoresis and high performance liquid chromatography. Analytical Methods and Instrumentation 1993;1:196–202.

[18] Sire JM, Majer H. Untersuchungen von Reactivfarbstoffen durch Hochleistungs-Fluessigchromatographie. Textilveredlung 1980;15:407–12.

[19] Lee WJ, Kim JP. The rate of hydrolysis of temporarily solubilised disperse dyes. JSDC 1999;115:270–3.

[20] Eleftheriadis IC, Pegiadou-Koemtzopoulou SA, Kehayoglou AH. Colorimetric evaluation of the levelness of modified and unmodified cotton fabrics dyed with direct dyes with and without nonionic surfactants. JSDC 1998;114:130–2.

[21] Lei XP, Lewis DM. The dyeing behaviour of cotton modified with Chloropropionyl chloride and related compounds. Dyes and Pigments 1991;16:273–89.