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Hydrolyzed reactive dyes. Part 1: Analyses via fast atom bombardment and electrospray mass spectrometry

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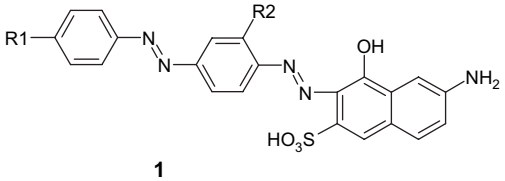
Abstract

This paper provides a summary of results from the use of negative ion fast atom bombardment (FAB) and negative ion electrospray (ES) mass spectrometry to characterize dye structures remaining following the dyeing of cotton with reactive dyes. It was found that the ES method gave peaks of higher relative abundance in the molecular weight region and better signal to noise ratios than the FAB ionization process.

Keywords: Mass spectrometry; Reactive dyes; Hydrolyzed dyes; Electrospray spectra; Fast atom bombardment

1. Introduction

Since its introduction in 1981, FAB mass spectrometry has become a widely used method for the analysis of polar, non-volatile and thermally labile compounds [1]. In this regard, preformed ions, protonated or deprotonated species, molecular ion radicals and cationized species have been observed in FAB mass spectra. Disadvantages of FAB mass spectrometry include the frequent presence of intense signals arising from the matrix employed, and the occasional observation of adducts of analyte and matrix ions. In prior work in our laboratories, negative ion FAB mass spectrometry was found superior to the positive ion mode for the analysis of sulfonated azo dyes such as **1**. The resultant spectra contained molecular ions $[M^{\bullet}]^{\theta}$, pseudomolecular ions $[M - Na]^{\theta}$, and abundant fragment ions arising from cleavage at the azo bonds [2–5]. To a lesser extent, fragmentation also occurred at the carbon–nitrogen linkages adjacent to the azo bonds. All of the fragments detected contained at least one sulfonic acid group or azo group. The authors also found that diethanolamine was a suitable matrix for molecular weight confirmation of hydrophilic disazo dyes.



1

R_1 = H, NEt_2, $N(CH_2CH_2OH)_2$
R_2 = OMe, OEt, OPr, OBu, OCH_2CH_2OH

In other studies, the negative ion FAB mass spectra of azo and anthraquinone acid dyes and direct dyes were recorded using glycerol, thioglycerol, diethanolamine, and 3-nitrobenzyl alcohol as matrices [6]. It was found that thioglycerol was the best overall matrix for the analysis of mono-, di-, and trisulfonated

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dyes having molecular weights in the *m/z* 300–700 range. For sulfonated dyes having a molecular weight of 700–900, glycerol, 3-nitrobenzyl alcohol, and thioglycerol gave better results. Ironically glycerol, which was the matrix used most often in FAB analysis of dyes, was the least useful of the four matrices evaluated.

Electrospray mass spectrometry (ES-MS), developed in 1985, is a soft ionization process that produces abundant sample ions with high sensitivity and little fragmentation [1]. This method gives high sensitivity in the negative ion mode, making it suitable for the analysis of sulfonated azo dyes. ES-MS has been interfaced with capillary zone electrophoresis (CZE) [7] and reversed phase HPLC [8,9] techniques to analyse polysulfonated azo dyes [10]. In the latter study, ES mass spectra of some commercial acid, direct, and metal–complex dyes were recorded. The ES mass spectra of the monosulfonated compounds were very simple, as only an $[M-H]^{\theta}$ ion was observed. However, the spectra of compounds with two or more sulfonic acid groups showed molecular ions with different charges (e.g. $[M-xH]^{x-}$). In addition, adducts with various numbers of sodium cations were observed, including $[M-(x+y)H+yNa]^{x-}$, where the maximum value of x or $(x+y)$ equals the total number of sulfonic acid groups.

The objective of the present work is to examine the utility of FAB and ES-MS for the characterization of colorants remaining in the dyebath following the dyeing of cotton reactive dyes. In comparison with dyes that have been extensively reported in previous papers, reactive dye analysis using MS methods as the analytical tool is relatively new. In addition, while the nature of hydrolyzed dyes is widely accepted, direct analytical evidence for their structures has not been published. Interestingly, in related studies HPLC has been used to separate and quantify hydrolyzed dyes [11,12]; however, in those cases, retention times were employed as a basis for confirming the presence of hydrolyzed reactive dye. In the present study, the commercial and hydrolyzed forms of six reactive dyes were analyzed using negative ion FAB and ES mass spectrometry, the structures of which are shown in Fig. 1. These dyes were selected as representatives of the structural types currently used commercially.

2. Experimental

The six reactive dyes employed in this study were used as received from either Zeneca Colours, Crompton & Knowles DyStar L.P., Rite Industries, Inc., or Ciba Specialty Chemicals. The dyes were applied to 10 g samples of 100% cotton fabric from 2% (owf) dyebaths using an Ahiba Texomat laboratory dyeing machine at a 40:1 liquor ratio [13]. The specific procedures employed are summarized below. Dye remaining in the dyebath after the dyeing step was saved and designated as residual dye.

2.1. Vinylsulfone dyes

The fabric (10 g) was placed in a bath containing water (280 mL) at 25 °C and agitated for 5 min. Dye (10 mL, 2% owf) and NaCl (100 mL, 20–30% w/w) were added, followed by a 10 mL mixture of Na_2CO_3 (5%) and NaOH (1%). The dyebath temperature was raised to 60 °C and held for 30 min. The dyed fabric was removed, rinsed with tap water, soaped 5 min at 70 °C using a nonionic surfactant (2 g/L; AATCC standard detergent), rinsed again with tap water, and air-dried.

2.2. Dichlorotriazine dyes

The fabric (10 g) was placed in a bath containing water (330 mL) at 40 °C and agitated for 5 min. Dye (10 mL, 2% owf) and NaCl (50 mL, 32% w/w) were added, followed by a 10 mL mixture of Na_2CO_3 (4%) and $NaHCO_3$ (4%). The dyebath temperature was held at 40 °C for 30 min and the temperature was raised to 60 °C and held for 40 min. The dyed fabric was removed, rinsed with tap water, soaped 5 min at 70 °C using a nonionic surfactant (2 g/L; AATCC standard detergent), rinsed again with tap water, and air-dried.

2.3. Monochlorotriazine dyes

The fabric (10 g) was placed in a bath containing water (250 mL) at 40 °C and agitated for 5 min. Dye (10 mL, 2% owf) and NaCl (100 mL, 20% w/w) were added, followed by Na_2CO_3 (40 mL, 20%). The dyebath temperature was raised to 60 °C and held for 40 min. The dyed fabric was removed, rinsed with tap water, soaped 5 min at 70 °C using a nonionic surfactant (2 g/L; AATCC standard detergent), rinsed again with tap water, and air-dried.

In separate experiments, the six dyes were converted to the hydrolyzed form using hot NaOH. Commercial reactive dye (1 g) was dissolved in water (100 mL) at 60 °C and the solution was heated to 80 °C with stirring. An aliquot of the dye solution was removed for use in TLC comparisons. NaOH (2 g) was added and stirring was continued for 30–45 min at 80 °C. After the addition of alkali, TLC of the dye solution was recorded every 5 min using flexible plates coated with carboxymethyl cellulose as the stationary phase and MeOH:EtOH:2-PrOH:NH_4OH:EtOAc (1:1:1:1:5) as the mobile phase. During the first 15 min, a new component appeared on the TLC plate having a lower R_f value. This component first increased in intensity and then decreased in intensity over the next 15–30 min as the second product formed. Hydrolysis of all dyes was complete in 30–45 min. The dye so obtained was designated as *hydrolyzed* dye and corresponded (via TLC) to dye in dyebaths following the dyeing of cotton.

Solutions containing hydrolyzed dye and residual dye were acidified to pH 1.5 using concentrated HCl and the resultant solutions were concentrated by rotary evaporation at reduced pressure (5 mm). The dry solid was stirred with DMF (100 mL) and salt was removed by filtration. The filtrate was concentrated by rotary evaporation and the dry solid (dye) was used in MS experiments.

The FAB MS analysis was carried out using a JEOL HX-110 double focusing mass spectrometer employing Nier-Johnson configuration, an acceleration voltage of 10 keV,

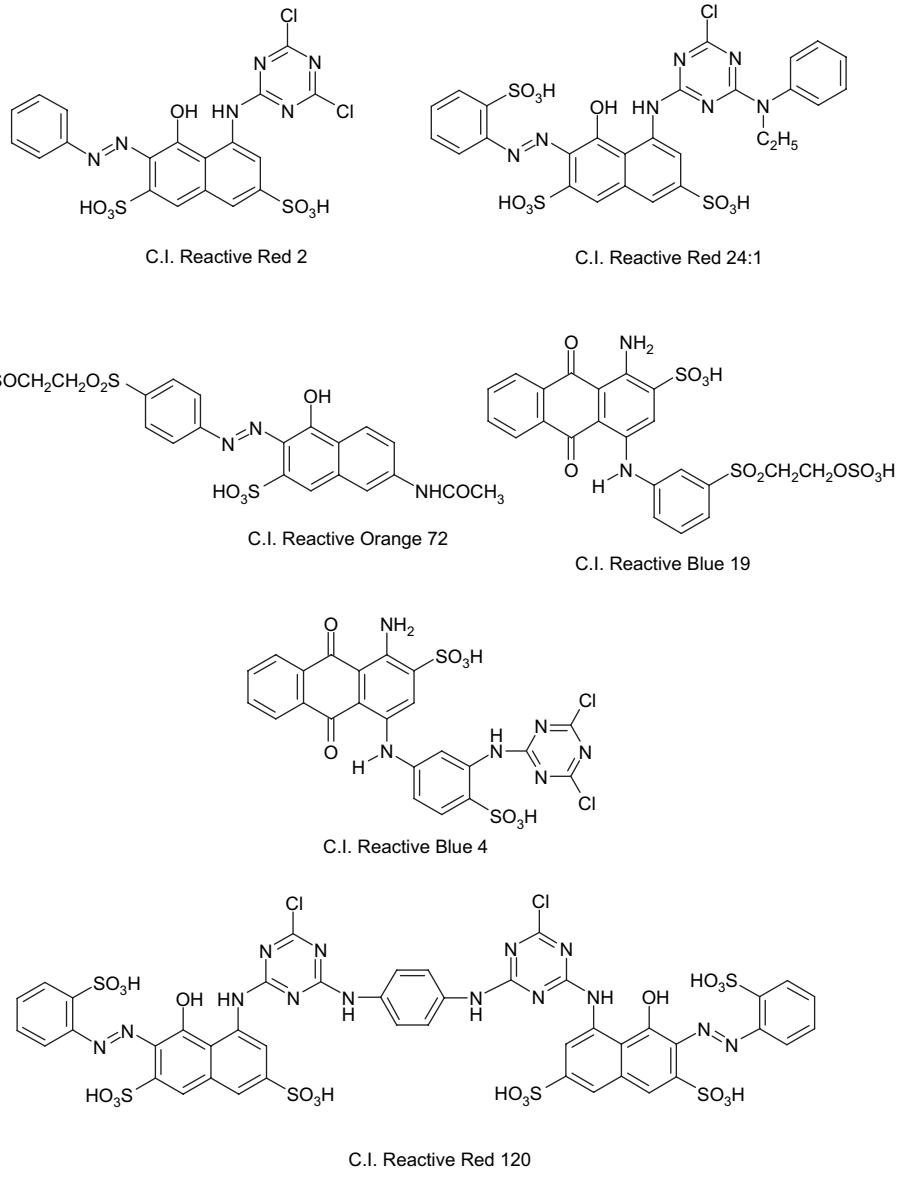


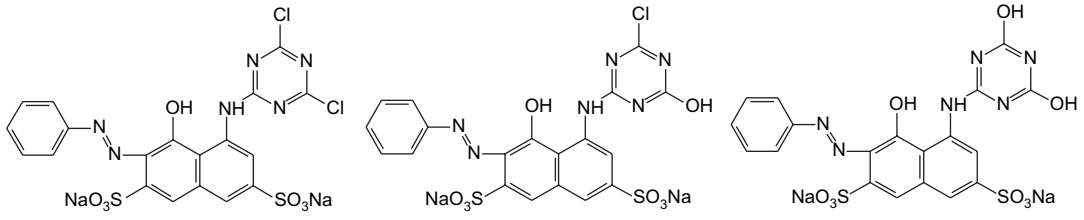
Fig. 1. Structures of reactive dyes employed in this study.

and an FAB gun emitting xenon atoms at 5 keV. Thioglycerol was used as the matrix. Negative ion ES-MS analysis was carried out using an electrospray triple quadrupole mass spectrometer and a solvent comprising 50:50 acetonitrile:water at a flow rate of 10 μL/min.

3. Results and discussion

The initial mass spectrometric analyses of the neutral solutions containing hydrolyzed dye and residual dye did not yield peaks in the expected molecular ion ranges. This was attributed to the presence of the sodium salt form of the dye. Therefore, dye obtained from solutions that had been acidified to pH 1.5 was employed for the mass spectral analyses.

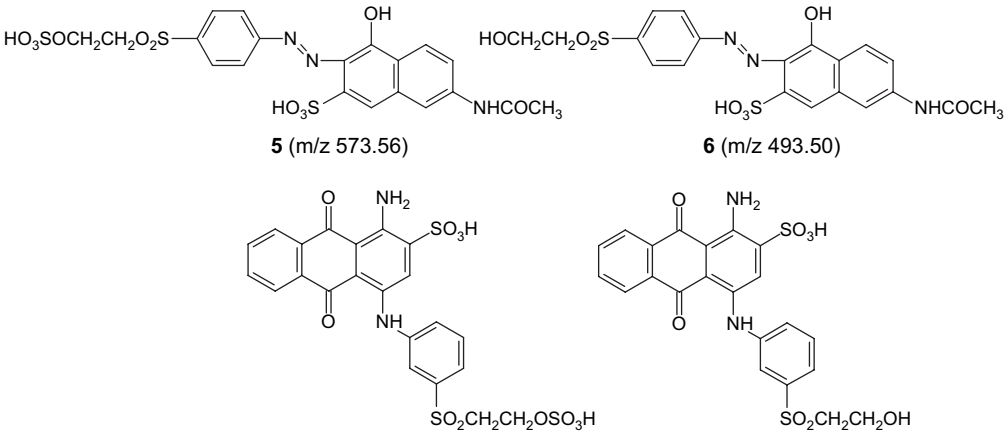
Figs. 2–14 exhibit representative negative ion FAB and negative ion ES spectra of the six dyes that generated in this study. The FAB spectrum for the molecular weight region of hydrolyzed Reactive Red 2 is shown in Fig. 2. The dichlorotriazine form (**2**) has *m/z* 571.36. The potential hydrolyzed products for this dye are monohydrolyzed dye (**3**; *m/z* 552.92) and fully hydrolyzed dye (**4**; *m/z* 534.47). The negative ion FAB spectrum did not contain a peak at *m/z* 552. However, a signal was detected at *m/z* 533.17 which arises from the $[M-H]^{\theta}$ ion. The spectrum also showed a peak at *m/z* 555.17, which arises from the addition of a sodium ion to the deprotonated molecular ion (i.e. $[M-H+Na]^{\theta}$) and a peak at *m/z* 577.14, which arises from the addition of sodium ion to the $[M-H+Na]^{\theta}$ species ion. For the residual dye, the FAB spectrum showed weak signals at *m/z* 533.1, 555.1, and 577.1 (Fig. 3). By contrast, the negative ion ES spectrum contained a strong signal at *m/z* 533.02 (Fig. 4). From these results, it can be seen that both hydrolyzed and residual Reactive Red 2 existed only as the fully hydrolyzed form.



2 (m/z 571.36) **3** (m/z 552.92) **4** (m/z 534.47)

The negative ion ES mass spectrum of hydrolyzed Reactive Orange 72 is shown in Fig. 5. The sulfatoethylsulfone form of dye (**5**) has *m/z* 573.56 and the hydrolyzed dye (**6**) has *m/z* 493.50. Negative ion ES analysis produced a strong signal at *m/z* 492.1, which is the $[M-H]^{\theta}$ form of structure (**6**). The negative ion ES spectrum of residual Reactive Orange 72 also contained a strong signal at *m/z* 492.1 (Fig. 6). These spectra confirm that Reactive Orange 72 is present in the hydrolyzed form in both the hydrolyzed and residual dye solutions.

The negative ion FAB spectrum of hydrolyzed Reactive Blue 4 is shown in Fig. 9. The dichlorotriazine form of the dye (**9**) has *m/z* 637.42, the monohydrolyzed product (**10**) has *m/z* 618.97, and the fully hydrolyzed product (**11**) has *m/z* 600.53. The negative ion FAB spectrum of the hydrolyzed and residual dyes did not contain a peak above *m/z* 618. However, a weak signal was detected at *m/z* 599.18, which corresponds to the $[M-H]^{\theta}$ of the fully hydrolyzed product. In addition, the spectrum contains



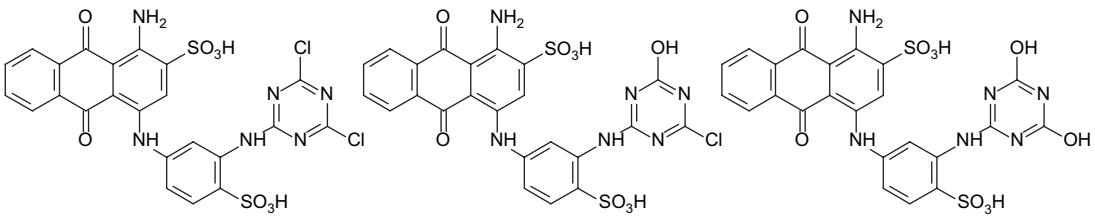
5 (m/z 573.56) **6** (m/z 493.50)

7 (m/z 582.57) **8** (m/z 502.51)

The negative ion FAB spectrum of hydrolyzed Reactive Blue 19 is shown in Fig. 7. The sulfatoethylsulfone form of the dye (**7**) has *m/z* 582.57 while the hydrolyzed dye (**8**) has *m/z* 502.51.

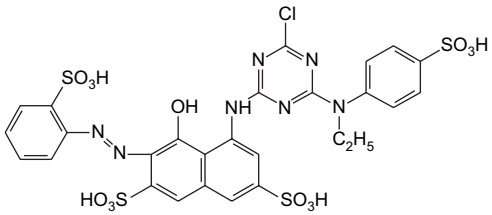
In this case, FAB analysis produced a strong peak at *m/z* 501.1, which is the $[M-H]^{\theta}$ of hydrolyzed Reactive Blue 19. Analysis of the residual dye solution using ES produced a significant peak at *m/z* 501.26 (Fig. 8).

peaks at *m/z* 621.15, the $[M-H+Na]^{\theta}$ species, and at *m/z* 643.17, the $[M-2H+2Na]^{\theta}$ species, both of which arise from the fully hydrolyzed dye. Negative ion electrospray analysis of the residual dye, however, generated a strong peak at *m/z* 599 (Fig. 10) which corresponds to $[M-H]^{\theta}$ for the fully hydrolyzed dye. These results showed that

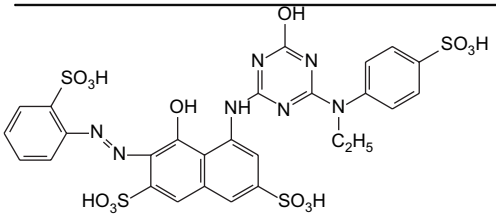


9 (m/z 637.42) **10** (m/z 618.97) **11** (m/z 600.53)

hydrolyzed and residual Reactive Blue 4 exist in the fully hydrolyzed dye.



12 (m/z 816.20)

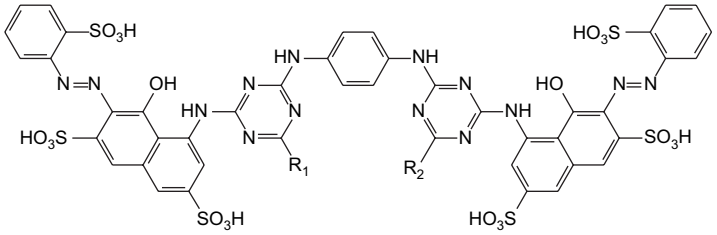


13 (m/z 797.75)

The negative ion FAB spectrum of hydrolyzed Reactive Red 24 is shown in Fig. 11. The monochlorotriazine form of the dye (**12**) has *m/z* 816.2 and the hydrolyzed form (**13**) has *m/z* 797.75. The negative ion FAB spectrum of the hydrolyzed dye did not contain a signal at *m/z* 797.75. However, it did contain strong peaks at *m/z* 716.2, 738.1, and 761 which correspond to $[M - SO_3H]^{\theta}$, $[M - SO_3H - H + Na]^{\theta}$, and $[M - SO_3H - 2H + 2Na]^{\theta}$. For the residual dye, the FAB spectrum contained a very weak signal at *m/z* 761.1 (Fig. 12). The negative ion electrospray spectrum of the residual dye showed peaks at *m/z* 715.96, 737.91, 759.99, and 781.94 (Fig. 13) which correspond to $[M - SO_3H]^{\theta}$, $[M - SO_3H - H + Na]^{\theta}$, $[M - SO_3H - 2H + 2Na]^{\theta}$, and $[M - SO_3H - 3H + 3Na]^{\theta}$, respectively. From these spectra, it can be seen that Reactive Red 24 exists in the hydrolyzed form in both the hydrolyzed and residual dye solutions, and that this dye has a tendency to eliminate a sulfonic acid group. This is consistent with the results of Freeman et al. [5], who reported the elimination of a sulfonate group during FAB mass spectrometric analyses involving certain disazo dyes.

dye (**15**) has *m/z* 1319.62, and the fully hydrolyzed dye (**16**) has *m/z* 1301.17. The negative ion ES spectrum of hydrolyzed Reactive Red 120 did not contain a peak at *m/z* 657.81, which would correspond to the $[M - 2H]^{2\theta}$ peak for the monohydrolyzed product. However, the spectrum did contain an intense peak at *m/z* 649 (Fig. 14) which corresponds to the $[M - 2H]^{2\theta}$ peak for the fully hydrolyzed product. It is typical for large dye molecules to exhibit more intense multiple charged species than singly charged species.

During these studies, we found that the intensity of ES spectra was significantly reduced by even millimolar levels of NaCl, requiring the use of very dilute dye solutions. We also found that the minimum analyte concentration required for FAB experiments was higher than that required for ES analysis, and believe that this contributes to inferior results compared to ES. In hydrolyzed dye solutions which had much less salt, FAB gave good results. However, for the residual dye solutions, the higher amount of salt in the solution made ES analysis the preferred method. Based on mass spectrometry results, it was found that all of the reactive dyes were converted to the fully hydrolyzed form following dyeing of cotton. These results demonstrate that mass spectrometry provides clear evidence of the presence of hydrolyzed species in the reactive dye dyebath.



Bis-monochlorotriazine dye (**14**): R_1, R_2 = Cl (m/z 669; z=2)

Monohydrolyzed dye (**15**): R_1 = OH, R_2 = Cl (m/z 657.81; z=2)

And Fully hydrolyzeddye (**16**): R_1, R_2 = OH (m/z 650.58; z=2)

The negative ion ES spectrum of hydrolyzed Reactive Red 120 is shown in Fig. 14. The bis-monochlorotriazine form of the dye (**14**) has *m/z* 1338.07, the monohydrolyzed

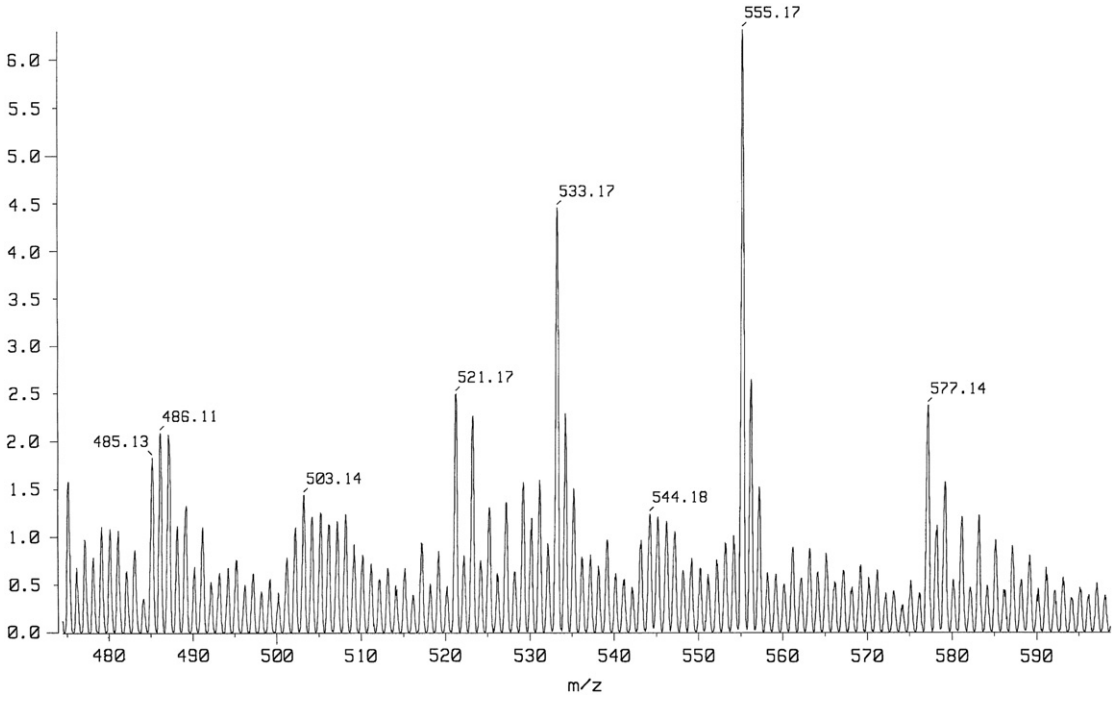


Fig. 2. Negative ion FAB mass spectrum of hydrolyzed C.I. Reactive Red 2.

4. Conclusions

Negative ion FAB and ES mass spectrometric analyses have been used to determine the level of hydrolysis following the dyeing of cotton with reactive dyes. In this regard, negative ion ES analysis was the preferred method, as it always had intense analyte ions in the molecular weight region.

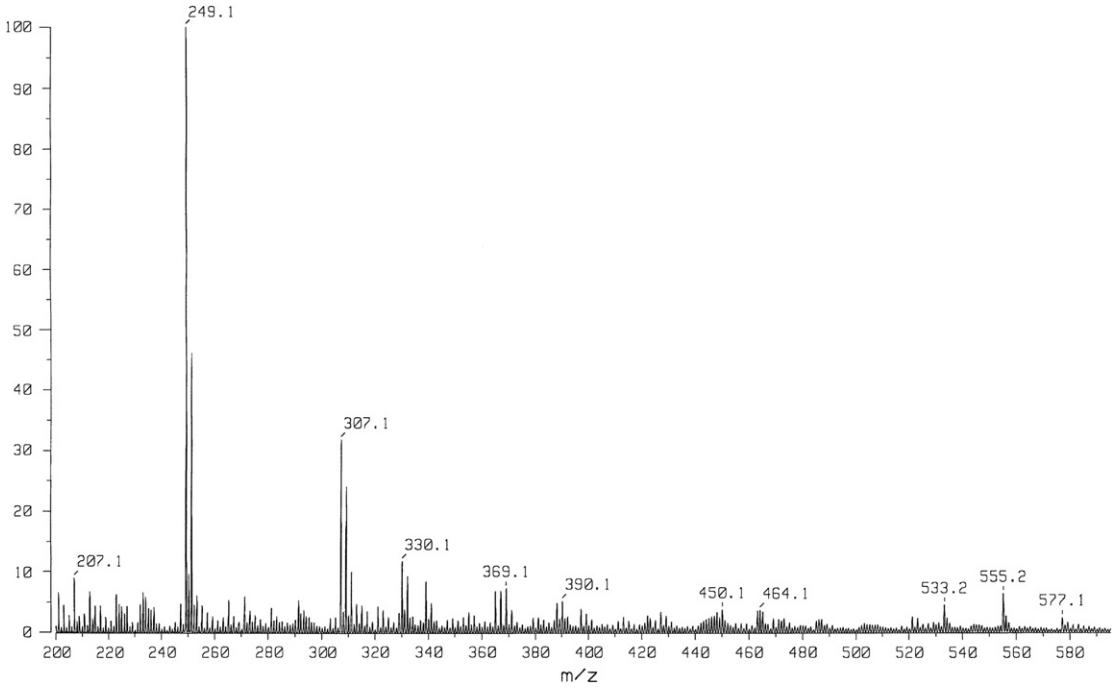


Fig. 3. Negative ion FAB mass spectrum of residual C.I. Reactive Red 2.

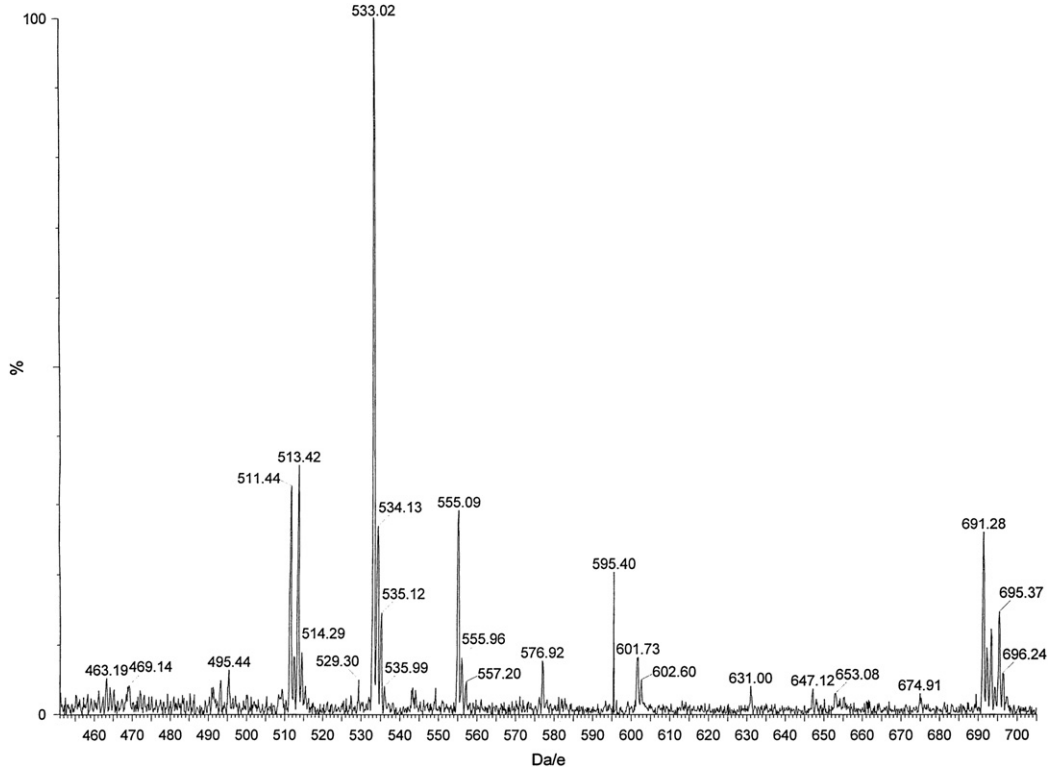


Fig. 4. Negative ion electrospray mass spectrum of residual C.I. Reactive Red 2.

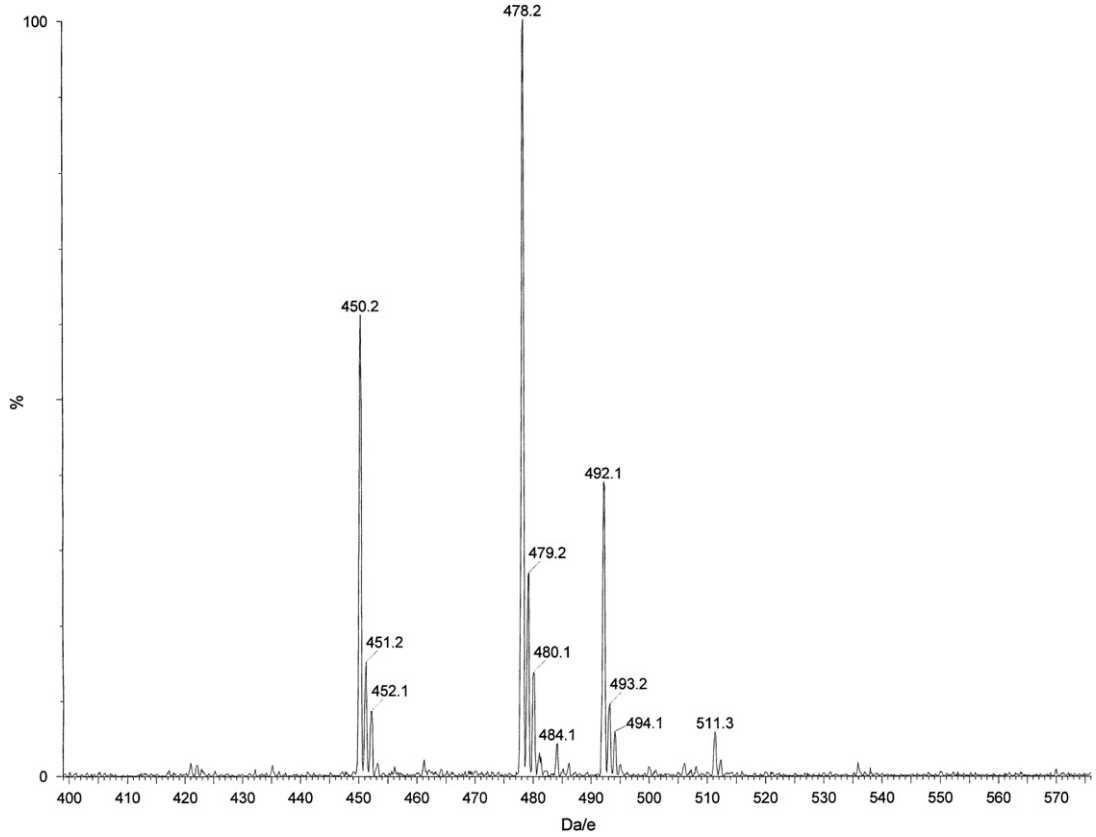


Fig. 5. Negative ion electrospray mass spectrum of hydrolyzed C.I. Reactive Orange 72.

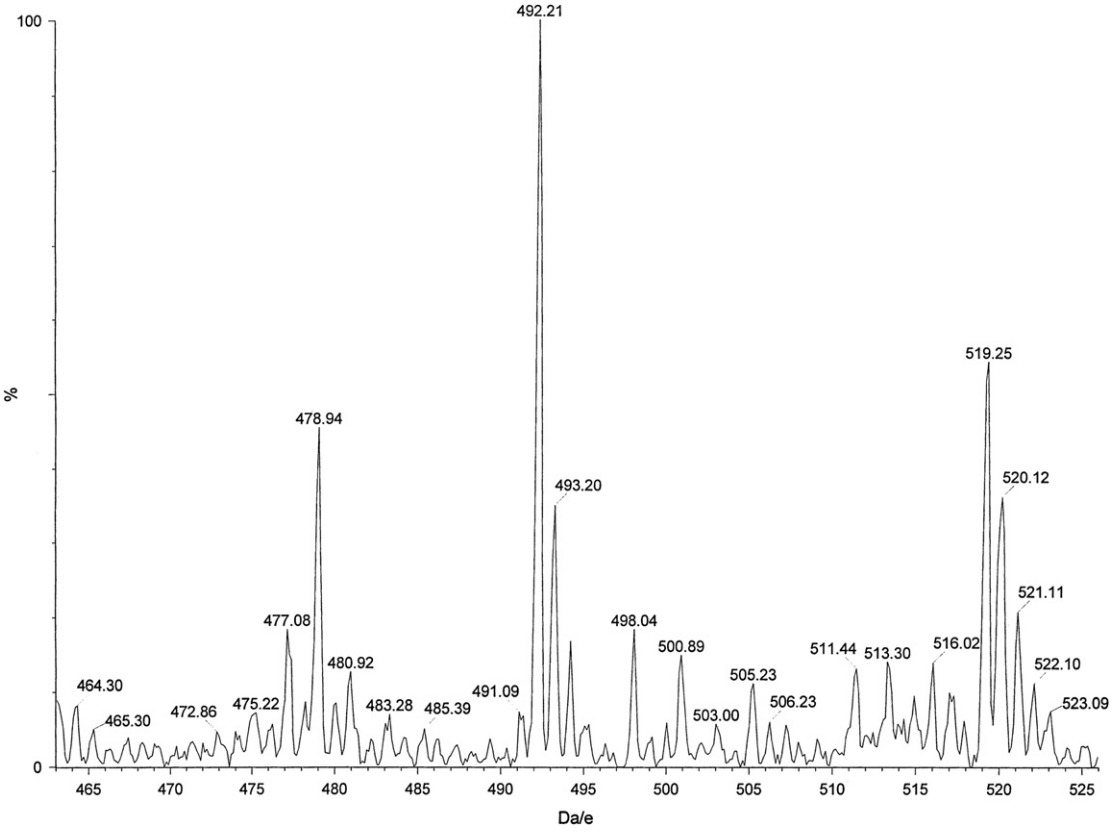


Fig. 6. Negative ion electrospray mass spectrum of residual C.I. Reactive Orange 72.

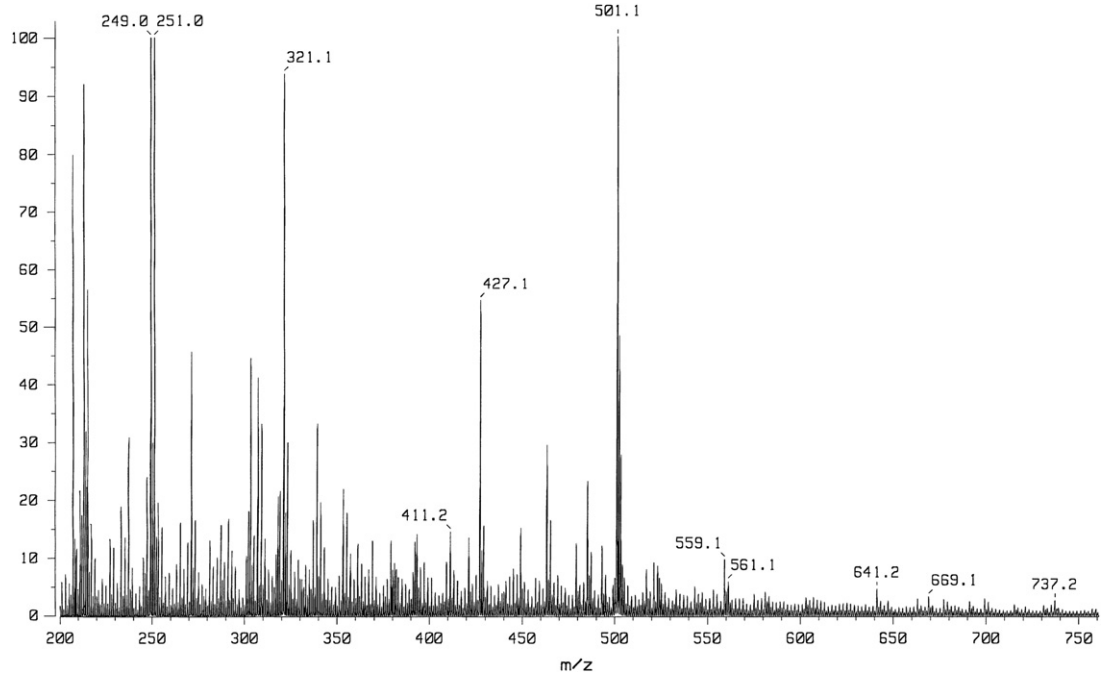


Fig. 7. Negative ion FAB mass spectrum of hydrolyzed C.I. Reactive Blue 19.

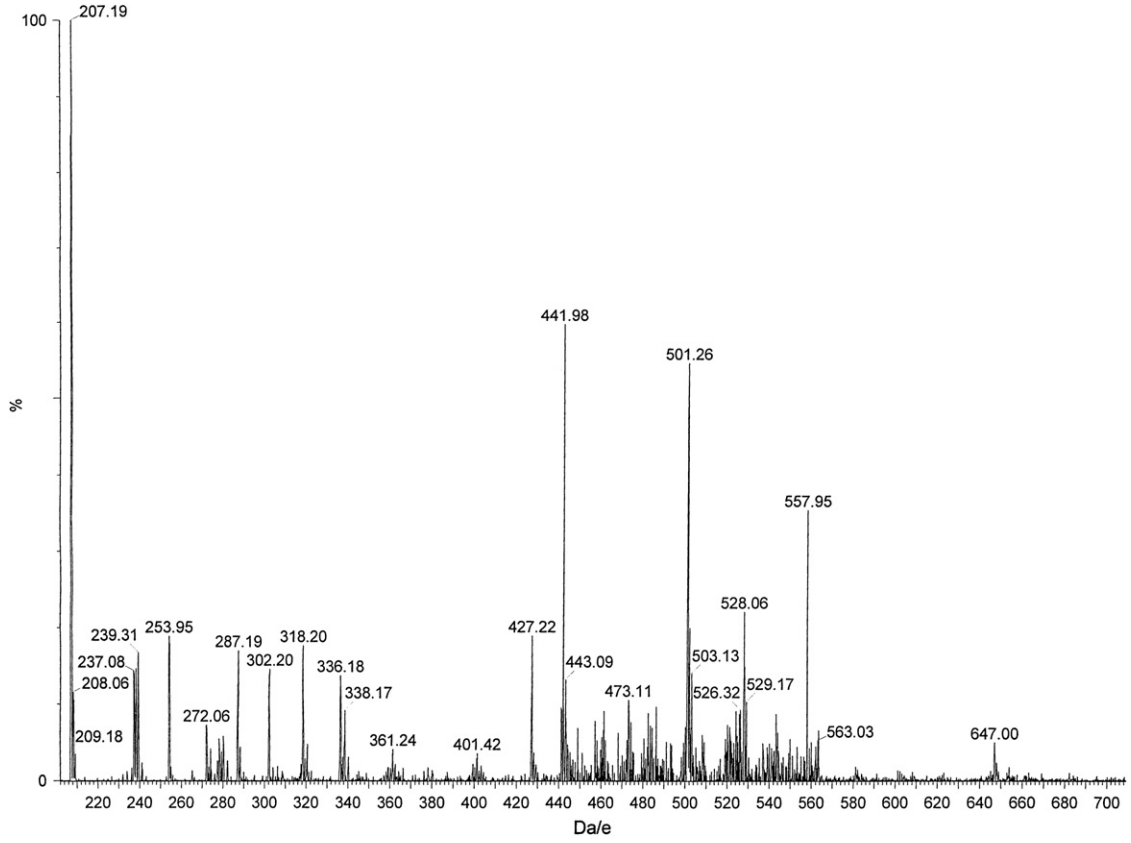


Fig. 8. Negative ion electrospray mass spectrum of residual C.I. Reactive Blue 19.

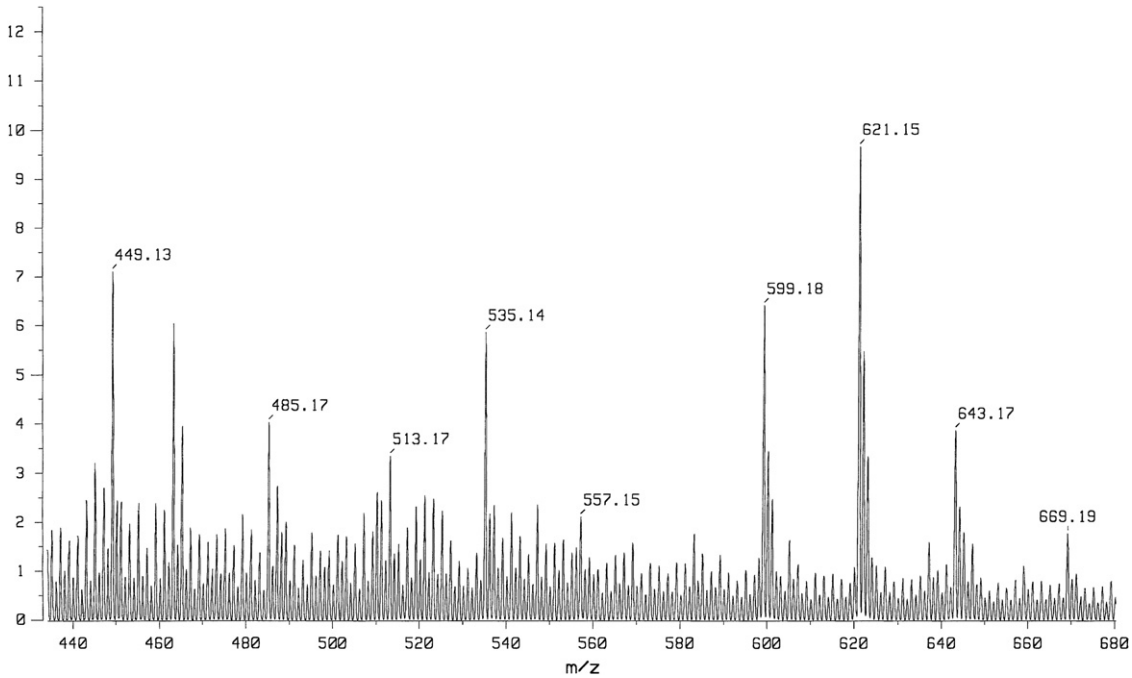


Fig. 9. Negative ion FAB mass spectrum of hydrolyzed C.I. Reactive Blue 4.

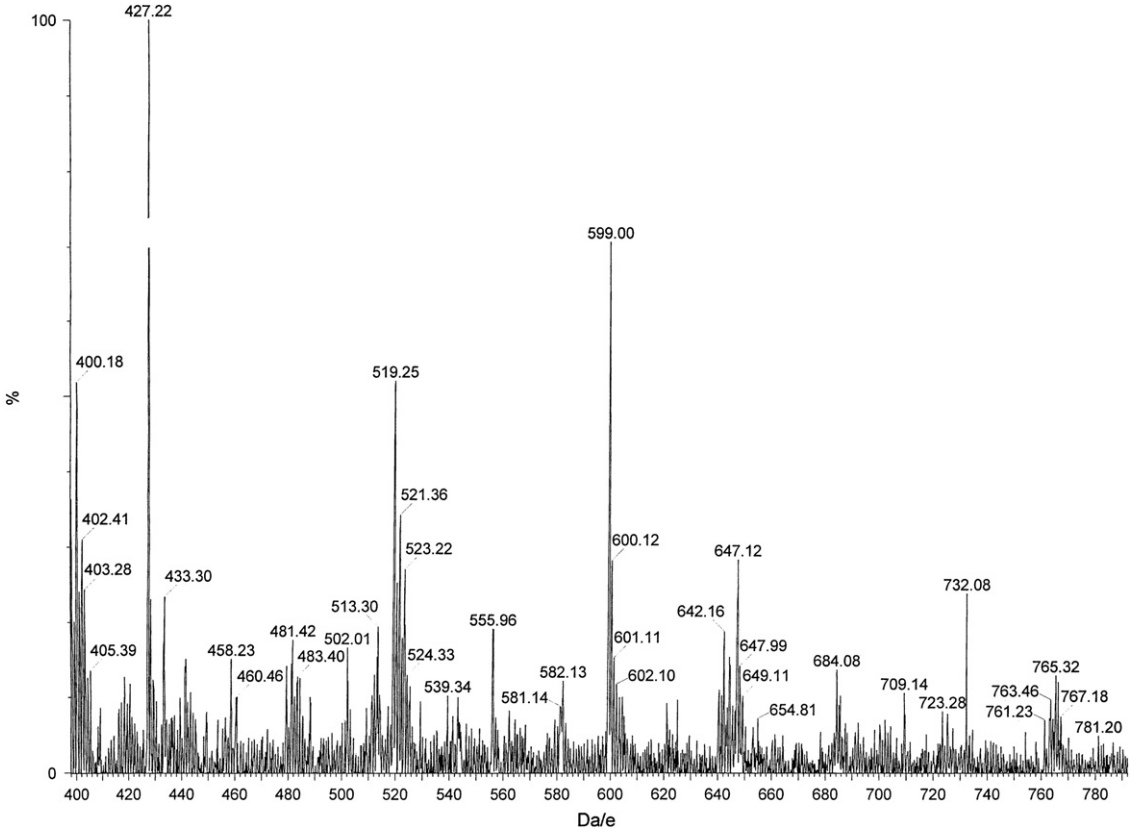


Fig. 10. Negative ion electrospray mass spectrum of residual C.I. Reactive Blue 4.

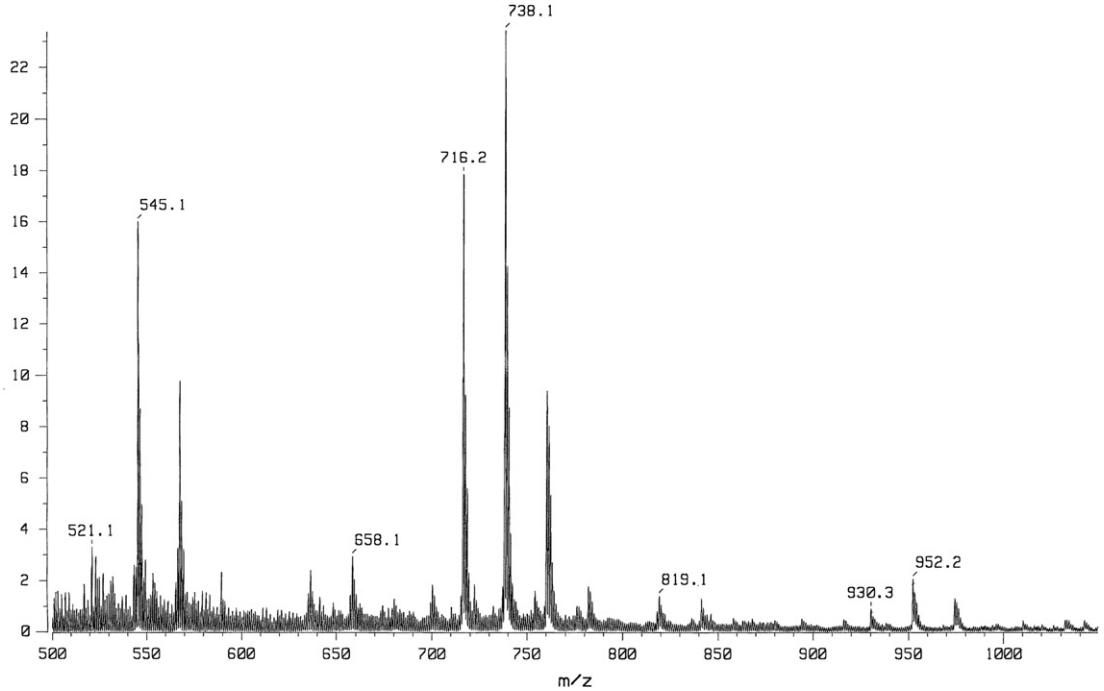


Fig. 11. Negative ion FAB mass spectrum of hydrolyzed C.I. Reactive Red 24.

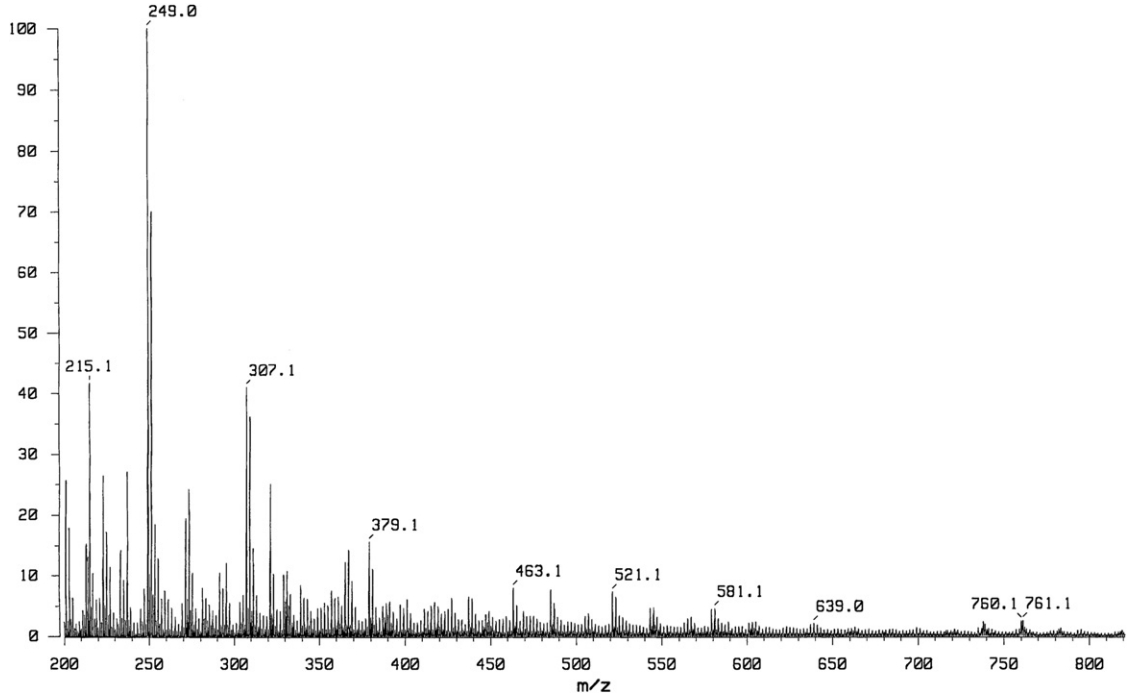


Fig. 12. Negative ion FAB mass spectrum of residual C.I. Reactive Red 24.

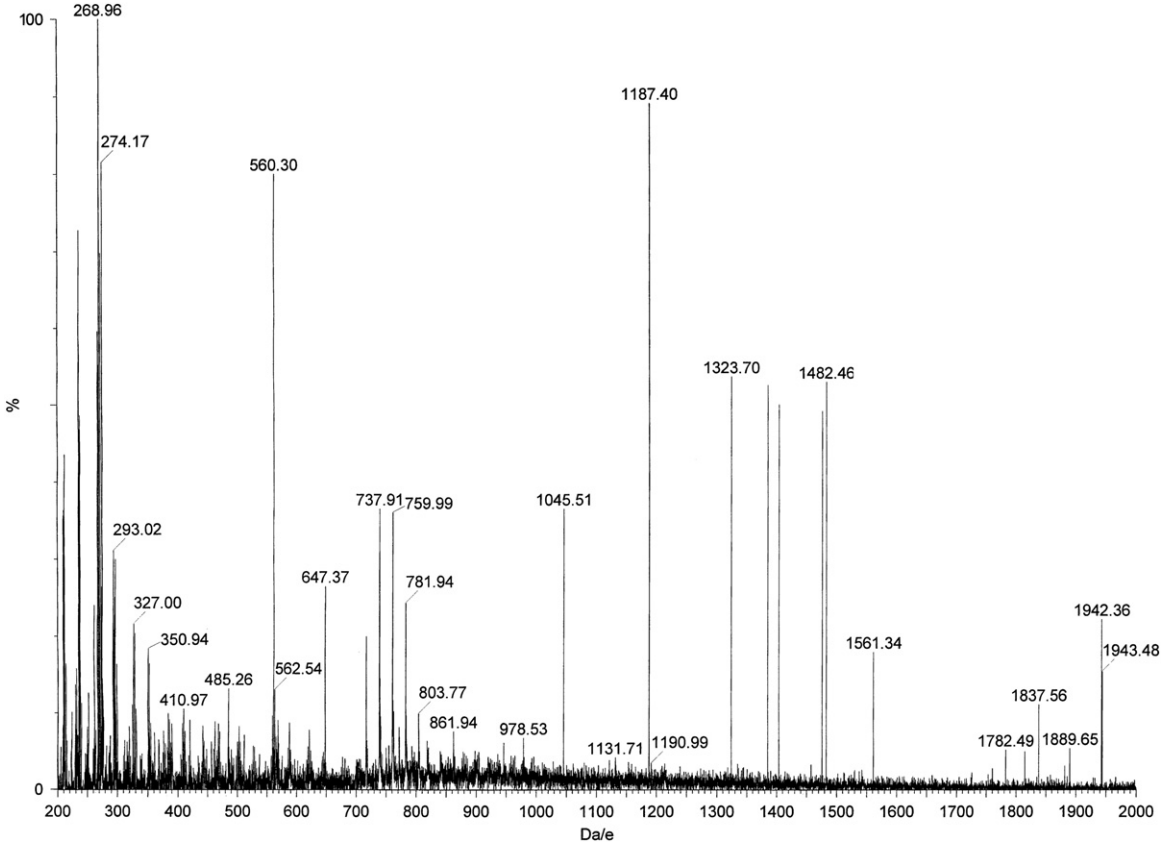


Fig. 13. Negative ion electrospray mass spectrum of residual C.I. Reactive Red 24.

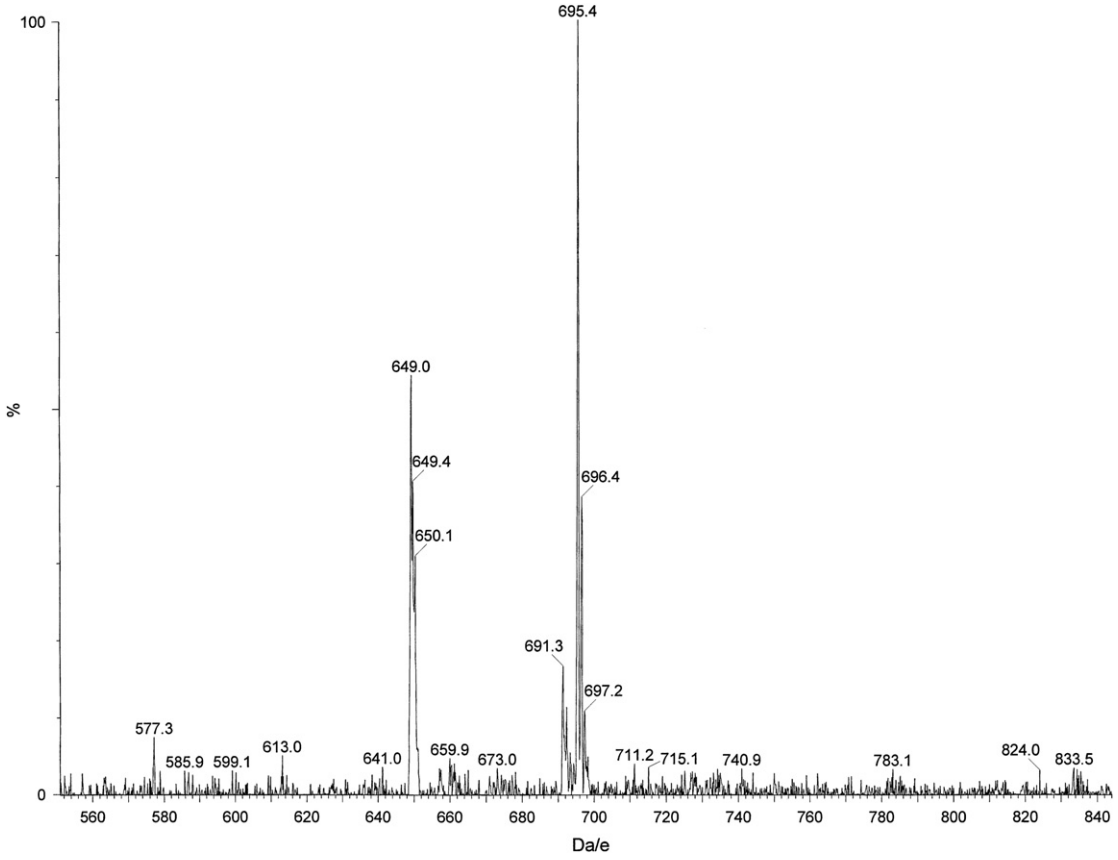


Fig. 14. Negative ion electrospray mass spectrum of hydrolyzed C.I. Reactive Red 120.

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