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Matrix Selection for the Analysis of Hydrophilic Dyes by Fast Atom Bombardment Mass Spectrometry

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ABSTRACT

This paper summarizes and compares the negative ion fast atom bombardment (FAB) mass spectra of azo and anthraquinone acid, and direct dyes using thioglycerol, glycerol, diethanolamine and 3-nitrobenzyl alcohol as FAB matrices. The results show thioglycerol to be the best general matrix and the one to try first for mono-, di- and trisulfonated dyes having a molecular weight in the range m/z 300–700. Thioglycerol facilitated the formation of molecular ion species ($M^{\overline{\cdot}}$, $[M-H]^-$ and $[M-Na]^-$), as well as abundant fragment ions, due to cleavage of azo linkages or carbon–nitrogen bonds adjacent to the azo linkages. Diethanolamine and 3-nitrobenzyl alcohol were effective, but less so, in this molecular-weight range. For sulfonated dyes having a molecular weight of 700–900, glycerol, 3-nitrobenzyl alcohol and thioglycerol occasionally gave good results. However, no matrix investigated consistently afforded abundant sample ions for dyes having a molecular weight above 900.

INTRODUCTION

In the 20 years since it was found[1] that dyes containing a sodium sulfonate group could be analyzed by mass spectrometry provided that this group was suitably derivatized, several papers have been published[2–7] in which the focus is the generation of molecular-weight information on underivatized, involatile hydrophilic dyestuffs containing cationic or anionic sites. It is readily apparent from those studies that fast atom bombardment (FAB) mass spectrometry has become an effective ionization technique for studying

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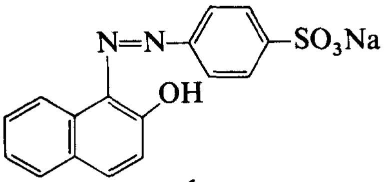
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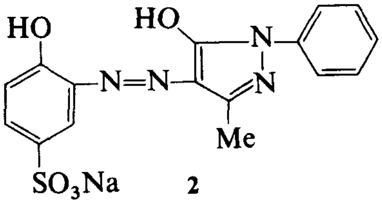
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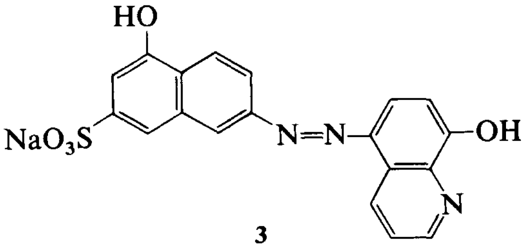
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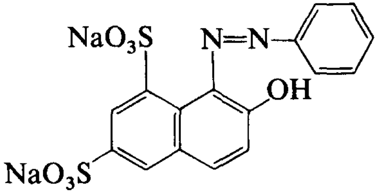
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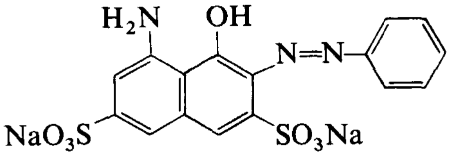
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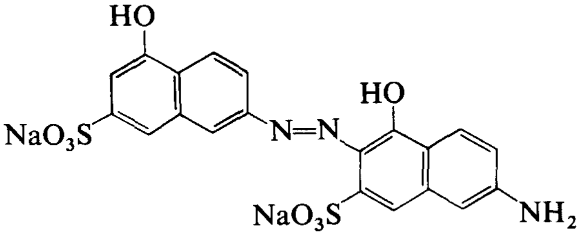
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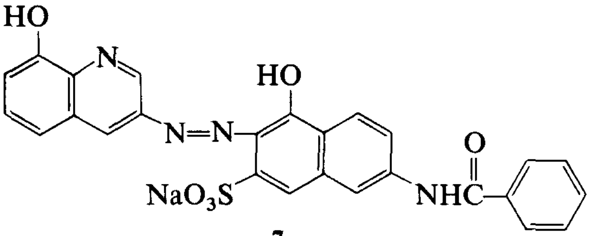
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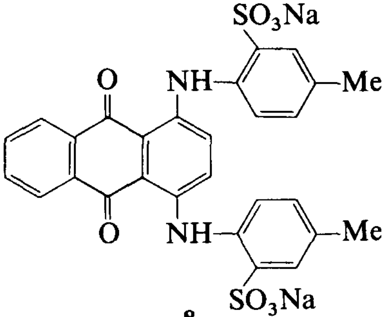
7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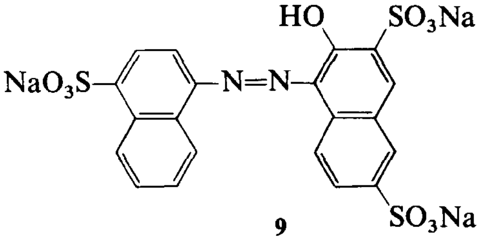
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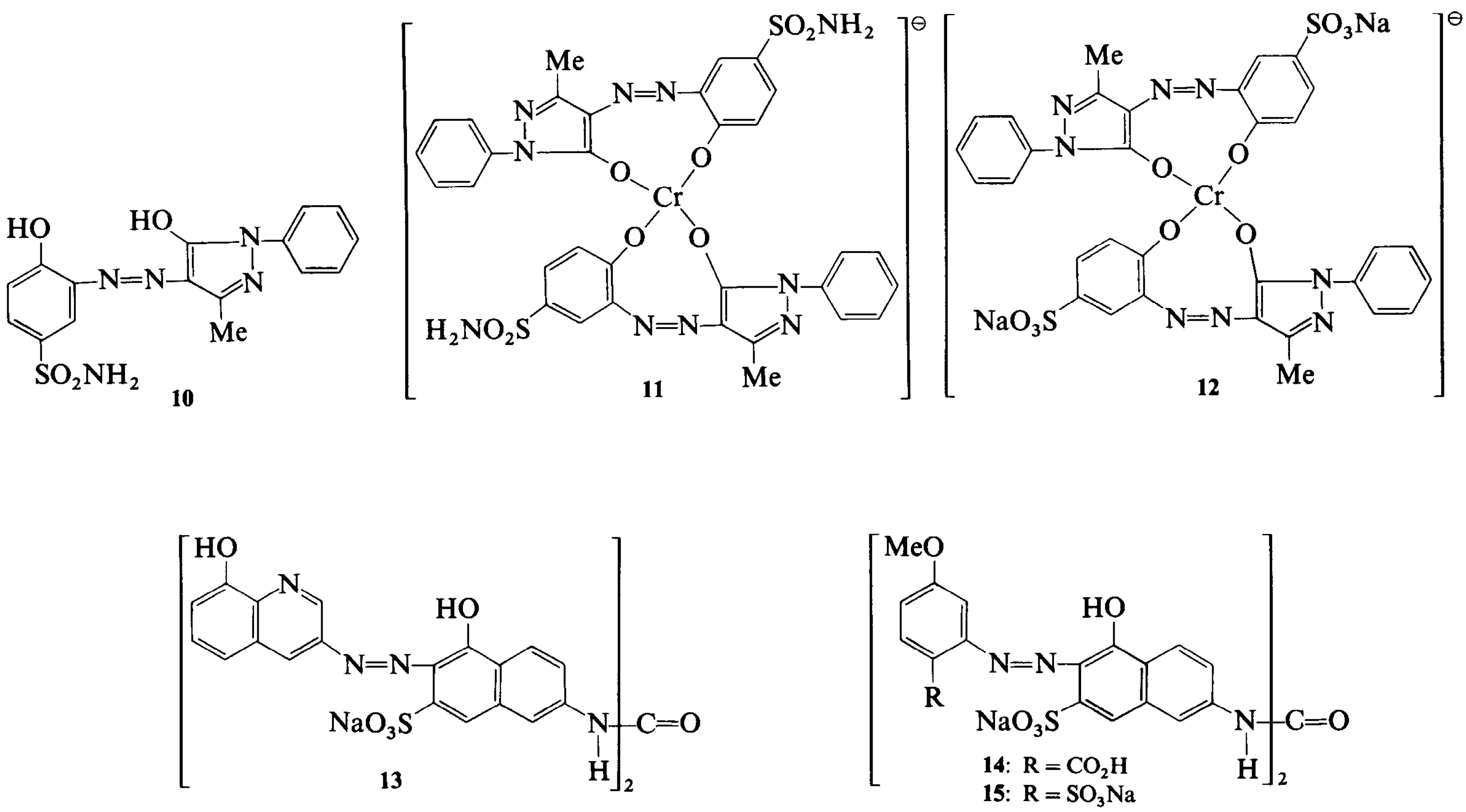
9



10

11

12

13

14: R = CO_2H
15: R = SO_3Na

Fig. 1. Dyes analyzed in this study.

sulfonated dyes. It is interesting to note, however, that the literature in this area contains few examples of studies[6-10] involving matrices other than glycerol, even though compounds such as diethanolamine,[11] 3-nitrobenzyl alcohol[12,13] and thioglycerol[14] have proven effective in the analysis of other classes of organic compounds.

Criteria for the selection of FAB matrices have been published in separate reviews,[15,16] and the physical and chemical properties of several common matrices have been tabulated to assist in their rational selection.[17] However, different models have been proposed to describe the mechanism of FAB ionization, and experimental evidence has been reported to support each of them.[18] Therefore it is probable that FAB ionization occurs by several different mechanisms, and their relative importance depends upon the chemical properties of the analyte and matrix.[18]

The aim of this investigation was to record the FAB mass spectra of some sulfonated dyes using four different matrices, and to evaluate the spectra for possible relationships between dye structure and matrix selection. The structures of the dyes used in this study are shown in Fig. 1.

Because most of the dyes analyzed in this study were sodium salts of strong organic acids, it was anticipated that molecular-weight information would be obtained most easily by detecting $[M-Na]^-$ ions in negative ion FAB mass spectra. In order to facilitate the formation of preformed ions such as $[M-Na]^-$ in solution, protic matrices were selected because of their high dielectric constant and ability to solvate ionic compounds. Glycerol was used as a standard, because it has been the most commonly used protic FAB matrix. Since all of the analytes contained bulky aromatic groups, an aromatic alcohol, 3-nitrobenzyl alcohol, was investigated as a matrix that should solubilize aromatic samples and still promote charge separation through hydrogen bonding.

In a protic matrix some exchange of Na^+ for H^+ could also occur, and this would reduce the abundance of $[M-Na]^-$ ions. For this reason diethanolamine was selected as a FAB matrix that would deprotonate sample molecules in which a sodium cation had been exchanged for a proton. For comparison, samples were also analyzed using the acidic matrix, thioglycerol.

RESULTS AND DISCUSSION

Three types of molecular ion species, $M^{-\cdot}$, $[M-H]^-$ and $[M-Na]^-$, were observed in the negative ion FAB mass spectra of most of the 15 dyes. Only the J-acid-urea-based disazo dyes, **13**, **14** and **15**, formed no molecular ion species. Because dyes **10** and **11** contained no sodium ions, $M^{-\cdot}$ and

$[M-H]^-$ ions were detected in lieu of $[M-Na]^-$ ions. The chromium complex, **11** (Acid Orange 60), also formed a hydride adduct, $[M+H]^-$, which is similar to hydride adducts of other group VIB complexes observed in these laboratories using negative ion FAB mass spectrometry.[19] Only dye **12**, another chromium complex, formed molecular ion radicals in which a sodium ion had been exchanged for a proton. The molecular ion species and the relative abundances for all 15 dyes are shown in Table 1.

As expected, $[M-Na]^-$ ions were the most abundant molecular ion species for sodium salts of sulfonated dyes. However, the matrix diethanolamine did not facilitate the formation of the most abundant $[M-Na]^-$ ions, as was predicted in the rationale for matrix selection discussed in the Introduction. If dye solubility in the matrix were the limiting factor, then 3-nitrobenzyl alcohol should have been the superior FAB matrix. Although 3-nitrobenzyl alcohol was a suitable matrix for many of the dyes in this study and was probably the second best overall, it was never the optimum matrix for any of the dyes.

Although thioglycerol and glycerol have similar structures, thioglycerol was the most effective matrix and glycerol was the least effective. Comparing the physical properties of these two compounds, thioglycerol is more acidic, more volatile and has a lower viscosity.[17] Because increased volatility and low viscosity tend to promote transport processes at the liquid–vacuum interface,[20] thioglycerol would be expected to enhance sample desorption. However, matrices for negative ion analysis are typically good proton acceptors like diethanolamine,[18] and thioglycerol is usually used in positive ion analysis, because it is a good proton donor.[21] Nevertheless, thioglycerol was an effective matrix for these negative ion FAB analyses in spite of its acidity, perhaps because the present sulfonated dyes are weak proton acceptors. It is clear from the data in Table 1 that glycerol, the most often used matrix, was the least effective matrix, among the four, for generating molecular-weight information on the dyes analyzed. Interestingly, glycerol did give the best (most intense) spectrum of the important premetalized dye, Acid Orange 60 (**11**). When the corresponding sulfonic acid analog of **11** (cf. **12**) was examined, however, glycerol gave primarily $[M-Na]^-$ ions. In only three cases (**13**–**15**) did 3-nitrobenzyl alcohol fail to give at least one significant reproducible peak in the molecular-weight region. Like thioglycerol, 3-nitrobenzyl alcohol afforded spectra of a wide variety of structural types (i.e. mono-, di- and trisulfonated azo dyes, metalized dyes, disulfonated anthraquinone and dyes having a molecular weight in the range m/z 300–800). Table 2 summarizes the relative effectiveness of thioglycerol, diethanolamine, 3-nitrobenzyl alcohol and glycerol in generating ions in the molecular-weight region. It is clear from these data that thioglycerol is the matrix to employ first when analyzing sulfonated

TABLE 1

Relative Abundances[b] of Molecular Ion Species in the FAB Mass Spectra of Dyes **1–15**[a]

Dye	*Assignment*	*m/z*	*Thioglycerol*	*Glycerol*	*Diethanolamine*	*3-Nitrobenzyl alcohol*
1	$M^{-\cdot}$	350	28	1·9	14	8·2
	$[M-H]^-$	349	67	3·1	10	16
	$[M-Na]^-$	327	100	47	90	100
2	$M^{-\cdot}$	396	1·6	—	—	1·0
	$[M-H]^-$	395	5·2	1·2	—	1·6
	$[M-Na]^-$	373	21	1·2	1·7	22
3	$M^{-\cdot}$	417	1·4	—	1·5	—
	$[M-H]^-$	416	—	—	1·1	—
	$[M-Na]^-$	394	5·0	—	2·6	4·6
4	$M^{-\cdot}$	452	4·6	—	—	2·1
	$[M-H^-]$	451	5·6	—	—	1·5
	$[M-Na]^-$	429	48	1·2	2·2	51
5	$M^{-\cdot}$	467	4·8	—	2·3	—
	$[M-H^-]$	466	11	—	2·6	2·3
	$[M-Na]^-$	444	32	—	26	29
6	$M^{-\cdot}$	533	4·1	—	1·3	—
	$[M-H]^-$	532	1·8	—	1·2	—
	$[M-Na]^-$	510	3·7	—	7·3	4·2
7	$M^{-\cdot}$	536	7	—	—	—
	$[M-H]^-$	535	10	—	—	—
	$[M-Na]^-$	513	1·0	—	—	6·5
8	$M^{-\cdot}$	622	25	3·8	2·7	2·5
	$[M-H]^-$	621	22	2·0	1·0	1·8
	$[M-Na]^-$	599	67	29	4·3	9·1
	$[M-2Na+H]^-$	577	10	27	—	—
9	$M^{-\cdot}$	604	1·2	—	—	—
	$[M-H]^-$	603	1·0	—	—	—
	$[M-Na]^-$	581	10	—	—	4·8
10	$M^{-\cdot}$	373	18	—	13	—
	$[M-H]^-$	372	63	—	11	1·5
11	$M^{-\cdot}$	795	17	48	19	21
	$[M-H]^-$	794	29	76	23	37
	$[M+H]^-$	796	9·1	27	12	11
12	$M^{-\cdot}$	840	4·6	0·3	—	0·5
	$[M-Na+H]^+$	818	17	1·0	—	1·7
	$[M-2Na+2H]^+$	796	23	2·1	3·5	1·4

[a] The spectra of dyes **13**, **14** and **15** did not contain peaks in the molecular-weight region.

[b] Ion abundances are expressed relative to the base peak in each mass spectrum, *m/z* 107 (thioglycerol), *m/z* 183 (glycerol), *m/z* 209 (diethanolamine) or *m/z* 152 (3-nitrobenzyl alcohol), unless specified otherwise.

TABLE 2

Order of Sample Ion Abundances in the Molecular-Weight Region as a Function of Compound Molecular Weight and Matrix[a]

Molecular weight	*Number of —SO_3Na or —SO_2NH_2 groups*		
	1	*2*	*3*
300–400	**1** (*m/z* 350) THIO > DEA ≈ 3-NBA > GLY		
	2 (*m/z* 396) THIO > 3-NBA > DEA, GLY		
	10 (*m/z* 373) THIO > DEA > GLY > 3-NBA		
400–500	**3** (*m/z* 417) THIO ≈ DEA ≈ 3-NBA > GLY	**4** (*m/z* 452) THIO > 3-NBA > GLY > DEA	
		5 (*m/z* 467) THIO > 3-NBA > DEA > GLY	
500–600	**7** (*m/z* 536) THIO > 3-NBA > GLY ≈ DEA	**6** (*m/z* 533) THIO > DEA > 3-NBA > GLY	
600–700		**8** (*m/z* 622) THIO > GLY > DEA ≈ 3-NBA	**9** (*m/z* 604) THIO > 3-NBA > GLY ≈ DEA
700–800		**11** (*m/z* 740) GLY > 3-NBA > THIO ≈ DEA	
800–900		**12** (*m/z* 840) THIO > GLY ≈ 3-NBA ≈ DEA	

[a] THIO = thioglycerol, DEA = diethanolamine, 3-NBA = 3-nitrobenzyl alcohol, GLY = glycerol.

dyes. Its effectiveness seems to diminish, however, as the molecular weight of the dye exceeds 800. Figures 2 and 3 provide some representative spectra.

For those dyes giving sample ions of low relative abundance in the molecular-weight range (cf. **2**, **3**, **6**, **7**, **9**), a study of dye fragmentation as a function of matrix selection was conducted. The idea was to determine whether molecular fragmentation contributed to the low abundance of these ions. The results of this comparison indicate that very little cleavage of the azo linkage and the two adjacent carbon–nitrogen bonds occurred when 3-nitrobenzyl alcohol was the matrix. Slightly more cleavage at these points occurred when glycerol or diethanolamine was the matrix used; but extensive cleavage of the azo bond and the adjacent carbon–nitrogen bonds occurred when thioglycerol was the matrix. Table 3 shows the fragmentation patterns and most abundant fragment ions for the simple sulfonated azo dyes obtained using thioglycerol as the matrix. In some of these mass spectra fragmentation was accompanied by transfer of one or two hydrogens to the terminal nitrogen of ions designated as **b** or **e** in Table 3. Because ions showing hydrogen transfer were usually less abundant, they are not shown in Table 3. It would appear from this part of the study that fragmentation does contribute to the decreased intensity of the molecular ion peaks in the spectra of these dyes, especially when thioglycerol is the matrix.

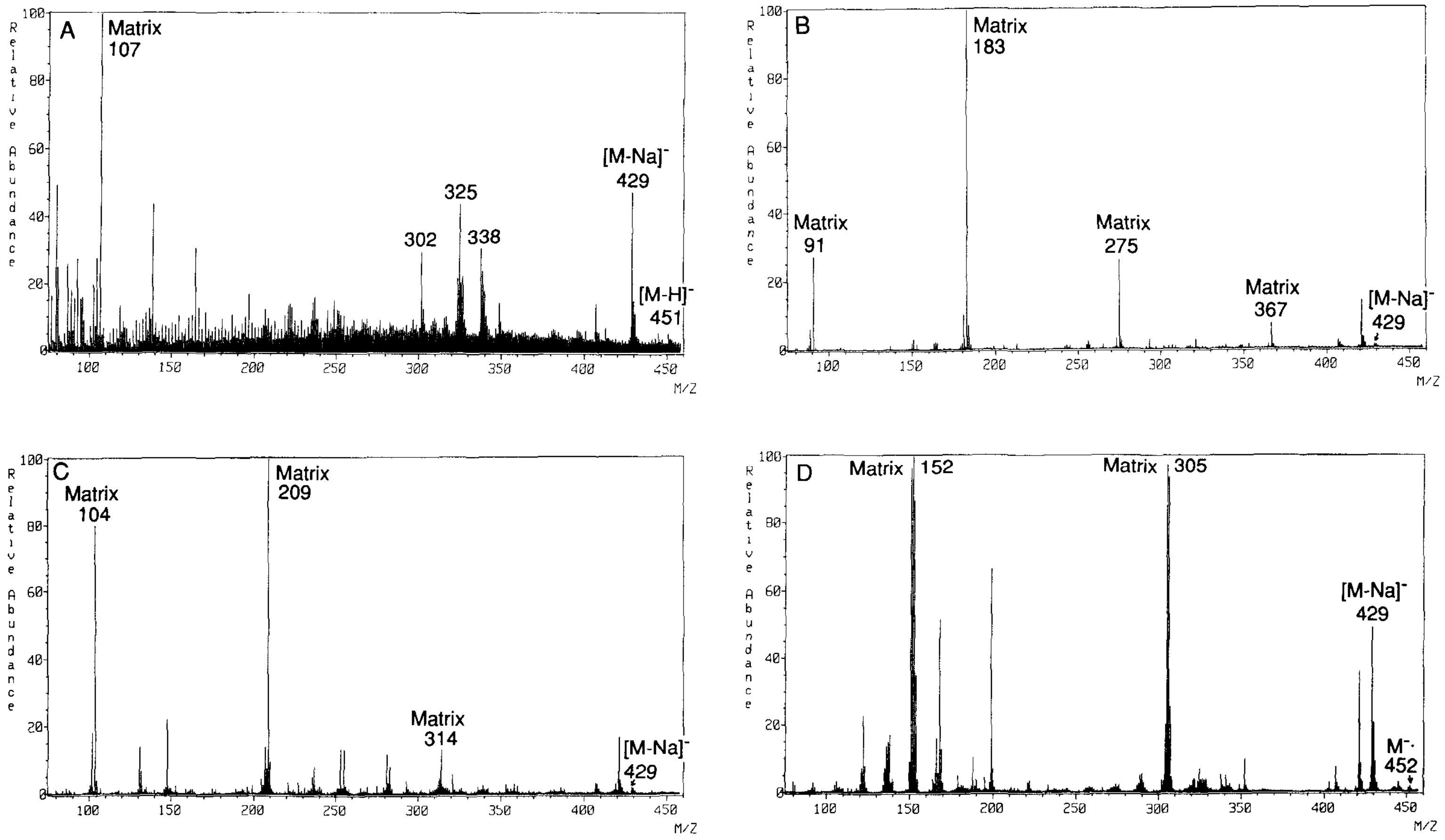


Fig. 2. FAB mass spectra of dye **4** using thioglycerol (A), glycerol (B), diethanolamine (C) and 3-nitrobenzyl alcohol (D) as the matrix.

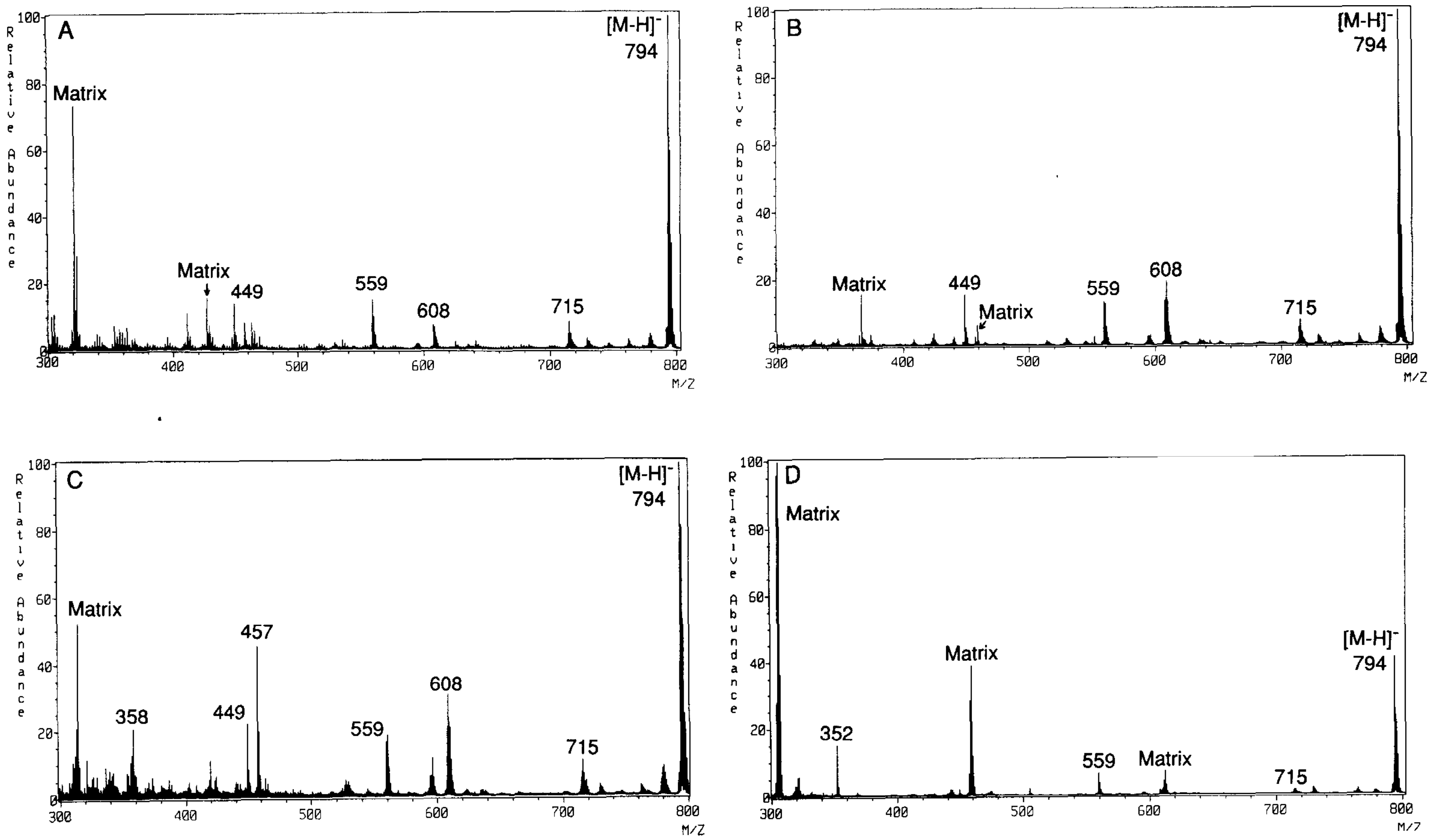
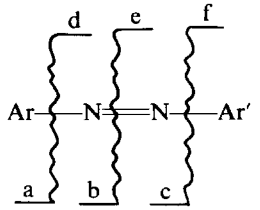


Fig. 3. FAB mass spectra of dye **11** using thioglycerol (A), glycerol (B), diethanolamine (C) and 3-nitrobenzyl alcohol (D) as the matrix.

TABLE 3

Fragmentation of Sulfonated Azo Dyes Using Thioglycerol as the FAB Matrix[a]



Dye	**a**	**b**	**c**	**d**	**e**	**f**
1	143 (46)[b]	157 (14)	171 (89)	—	170 (11)	156 (38)
2	172 (3·1)	186 (2·3)	—	—	187 (4·1)	173 (2·2)
3	—	—	250 (13)	—	—	—
4	324 (22)	338 (31)	—	105 (28)	91 (16)	77 (17)
5	339 (68)	353 (26)	—	×[c]	×	×
6	—	—	250 (24)	265 (5·7)	251 (100)	237 (24)
7	144 (7·7)	—	172 (7·6)	369 (4·9)	355 (12)	341 (14)
9	206 (6·9)	220 (14)	234 (6·4)	352 (2·3)	338 (7·8)	324 (4·7)
10	172 (18)	186 (19)	—	—	178 (6·3)	173 (20)

[a] Where possible, fragment ions have eliminated one sodium cation.

[b] The relative abundance for each *m/z* value is shown in parentheses and has been normalized to the thioglycerol matrix ion at *m/z* 107.

[c] × = not determined.

EXPERIMENTAL

The 15 dyes were either synthesized in these laboratories[9,10] or purchased from Aldrich Chemical Company (Milwaukee, WI, USA). The dyes were purified by recrystallization or chromatography.

Mass spectra were obtained using a JEOL (Tokyo, Japan) HX110HF double focusing mass spectrometer equipped with a DA-5000 data system, and combined EI, CI, DCI and FAB ionization sources. The resolving power was 1000 and the accelerating voltage 10 keV for all measurements. Approximately 1–2 μg of each dye dissolved in DMSO was mixed with 1 μl of liquid matrix on the direct insertion probe sample stage. Samples were bombarded by xenon fast atoms at 6 kV.

CONCLUSIONS

The results of this investigation indicate that thioglycerol is the matrix of choice for the FAB mass spectrometric analysis of sulfonated synthetic dyes having a molecular weight less than 800. In most cases this matrix facilitated the formation of abundant molecular ion species. $M^{\bar{\cdot}}$, $[M-H]^-$, $[M-Na]^-$ and, at the same time, useful structural information was obtained through fragmentation. Interestingly, glycerol, the matrix used most often in the FAB analysis of dyes, was the least useful matrix of the four evaluated.

ACKNOWLEDGEMENT

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