Short communication

# Determination of 4,4'-diaminostilbene-2,2'-disulfonic acid by thin-layer chromatography and densitometry

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## Abstract

The determination of 4,4'-diaminostilbene-2,2'-disulfonic acid diammonium salt was performed by thin-layer chromatography and densitometry from a reaction mixture which also contained 4,4'-dinitrostilbene-2,2'-disulfonic and 4-amino-4'-nitrostilbene-2,2'-disulfonic acid diammonium salts. The calibration graph and a detailed discussion of the method are reported. The method is rapid, accurate and very useful because degradation of the compound can easily occur in light.

*Keywords:* 4,4'-Diaminostilbene-2,2'-disulfonic acid; Stilbenes; 4,4'-Dinitrostilbene-2,2'-disulfonic acid; 4-Amino-4'-nitrostilbene-2,2'-disulfonic acid

## 1. Introduction

4,4'-Diaminostilbene-2,2'-disulfonic acid (DASS acid) is useful in the preparation of fluorescent brighteners which can be included in paper and textiles to increase the degree of white colour of these materials [1]. Stilbene derivatives are widely used as fluorescent brighteners and the main raw material for obtaining them is DASS acid. The sodium salt of DASS acid absorbs UV radiation at 340 nm and has emission in the visible region (blue). DASS acid can be obtained on an industrial scale from 4,4'-dinitro-stilbene-2,2'-disulfonic acid (DNSS acid) in different ways: by using the Bechamp method [2,3], the catalytic method [4–8] or the electrochemical method [9–17].

DASS acid was obtained in our laboratory by the electroreduction of DNSS acid. Together with the electroreduction of DNSS acid to DASS acid, partial reduction can occur in the reaction process, and 4-amino-4'-nitrostilbene-2,2'-disulfonic acid (ANSS acid) can also be obtained. The structures of DASS, DNSS and ANSS acids are shown in Fig. 1.

The separation of these stilbene derivatives has been performed by paper chromatography [18]. The spots can be eluted from the chromato-
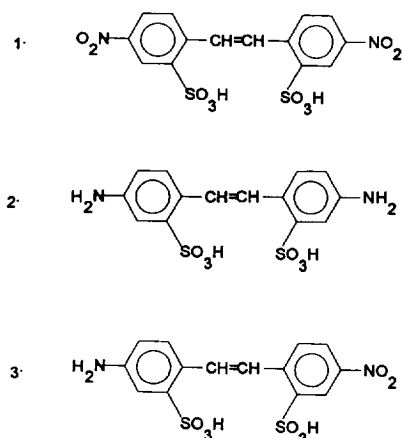
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Fig. 1. Structure of the compounds studied: 1 = 4,4'-dinitro-stilbene-2,2'-disulfonic acid (DNSS acid); 2 = 4,4'-diaminos-tilbene-2,2'-disulfonic acid (DASS acid); 3 = 4-amino-4'-ni-trostilbene-2,2'-disulfonic acid (ANSS acid).

graphic paper and the quantitative analysis performed by spectrophotometry and polarography [19]. The separation of the same compounds has also been performed by ion-pair column liquid chromatography using Bondapak $C_{18}$ as the stationary phase and tetrabutylammonium as positive ion [20].

The purpose of this work was to develop a thin-layer chromatographic method for the separation and quantitative evaluation of DASS acid. Taking into consideration that the reaction mixture can contain DASS, DNSS and ANSS acids, with similar structures, it is difficult to determine the reaction yield and hence there is a necessity for a suitable chromatographic method.

## 2. Experimental

### 2.1. Chemicals and standard solutions

Chloroform and methanol were obtained from Chimopar (Bucharest, Romania) and dimethylformamide (DMF) from Fluka (Buchs, Switzerland). The standard substances for chromatography DNSS acid was obtained from TCI (Japan) and DASS acid from Lancaster (Morecambe, UK); ANSS acid was obtained in our laboratory by the reduction of DNSS acid with $Ti^{3+}$ (previ-

ously formed by electroreduction of $TiOSO_4$) followed by separation of the mixture on a silica gel column using dimethylformamide–chloroform (50:50, v/v) as the eluent. The purity of ANSS acid was checked by thin-layer chromatography and the structure was determined by NMR spectroscopy.

DASS acid is not freely soluble in most solvents because its aromatic structure possesses amino and sulfonyl groups. A $2 \cdot 10^{-3}$ $M$ stock standard solution of DASS diammonium salt in water was prepared by bubbling gaseous $NH_3$ until all of the sulfonic acid groups of DASS acid were neutralized. Working standard solutions were prepared from this stock standard solution with the concentrations ranging from $2 \cdot 10^{-3}$ to $1 \cdot 10^{-4}$ $M$.

### 2.2. Chromatography

Plates precoated with Kieselgel $60F_{254}$ on a plastic support, obtained from Merck (Darmstadt, Germany), were used for thin-layer chromatography. The layer width was 0.2 mm. Samples from the working standard solutions, 4 $\mu$l per spot, were applied to the plates, using calibrated micropipettes, 2 cm from the bottom edge of the plate. The distance between spots was 2 cm. The spots were dried in a gentle stream of cold air.

Elution was carried out using a normal chamber for thin-layer chromatography, protected from light because these substances are sensitive to light. Presaturation of the chromatographic chamber for 2 h is necessary for a good separation.

The reaction mixture can be formed only by DASS, DNSS and ANSS acids. Some binary eluents were tested and the best separation was obtained with $CHCl_3$–DMF (45:55, v/v). After performing elution, the plate was dried in a stream of cold air in the dark.

A Shimadzu CS-9000 dual-wavelength flying spot scanner was used for densitometric evaluation of the plates. The wavelengths of maximum absorbance of DASS and ANSS diammonium salts were obtained form the in situ UV–Vis spectra. UV radiation (345 nm) was used for

scanning for DASS diammonium salt in the first part of the plate and Vis light (545 nm) for ANSS and DNSS diammonium salts in the second part of the plate. The scanner was used in the zig-zag mode with a swing width of 12 mm and a single wavelength (345 or 545 nm).

## 3. Results and discussion

When using electroreduction to obtain DASS acid, the reaction mixture can also contain unreacted DNSS acid and ANSS resulting of a partial reduction of DNSS acid.

The TLC separation of the reaction mixture (DASS, DNSS and ANSS diammonium salts) using the binary eluent $CHCl_3$–DMF (45:55, v/v) is shown in Fig. 2. Densitometry performed with UV and Vis light (345 nm for DASS and 545 nm for ANSS) explains the discontinuous baseline. The $hR_F$ values obtained for DASS, ANSS and DNSS diammonium salts are 56, 85 and 99, respectively. Despite its high $hR_F$ value, ANSS diammonium salt can also be determined by densitometry owing to the good separation from DNSS diammonium salt, which is in the eluent front. The determination of DNSS acid can be performed by polarography. The method is much more sensitive for the determination of DASS than for ANSS acid. DASS and ANSS diammonium salts from the densitogram shown in Fig. 2 have similar concentrations, 0.185 and 0.22

mg/ml, respectively, but the corresponding peak areas are significantly different.

Samples from the working standard solutions were applied to the plate to obtain the calibration graph for DASS diammonium salt. Four spots were applied at each concentration. The preparation of the working standard solutions from the stock standard solution of DASS diammonium salt, the elution and the densitometry must be carried out on the same day. For each concentration, the mean result for four corresponding spot areas (obtained by densitometry) was calculated, and this mean was used to plot the calibration graph. A second-order calibration graph was obtained (Fig. 3).

The accuracy of the method was checked using a series of six samples with known concentrations. These samples were applied to the plate in quadruplicate and then developed. The corresponding concentrations were obtained by densitometry.

A comparison between the two series of concentration values ($y$ and $x$) can be made using a regression line. In the ideal case, the regression line $y = a_0 + a_1 x$ must have an intercept $a_0 = 0$ (i.e. it passes through the origin) and the slope $a_1 = 1$. Owing to the random errors, the values from the two series of concentrations will not be the same and $a_0 \neq 0$ and $a_1 \neq 1$. The differences that occur have to be checked in order to see whether they are statistically significant or not.

The statistical evaluation of the TLC method was performed using Student's $t$-test. First the
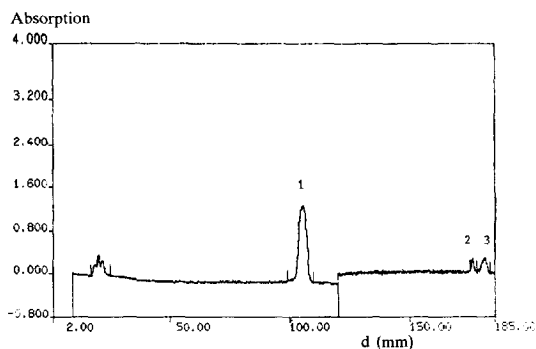


Fig. 2. Chromatogram of diammonium salt reaction mixture. Peaks: 1 = DASS ($hR_F = 56$); 2 = ANSS ($hR_F = 85$); 3 = DNSS ($hR_F = 99$). Eluent system: dimethylformamide–chloroform (55:45, v/v).
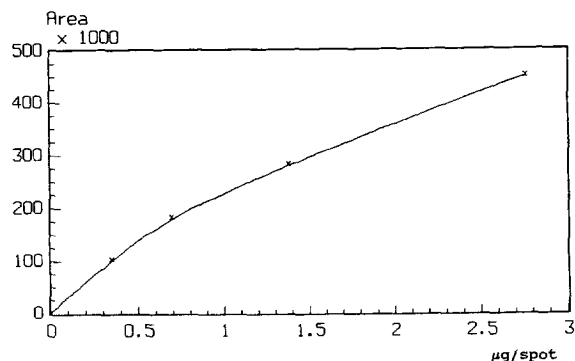


Fig. 3. Second-order calibration graph for DASS diammonium salt.

Table 1
Standard amounts of DASS acid applied to the plate, values determinated by TLC–densitometry and parameters for the regression line and for the $t$-test

| No. | DASS acid ($\mu$g per spot) | | $y = a_0 + a_1 x$ | $t_{n-2, p\%}$ |
|-----|----------|----------|----------|----------|
|     | Standard ($x$) | Recovery ($y$) | | |
| 1 | 0.175 | 0.173 | $a_0 = 0.0126, s_{a0} = 0.0256, t_{a0} = 0.492;$ | $t_{4, 90\%} = 2.132$ |
| 2 | 0.340 | 0.330 | $a_1 = 0.9229, s_{a1} = 0.1267, t_{a1} = 0.609$ | |
| 3 | 0.462 | 0.466 | | |
| 4 | 0.518 | 0.481 | | |
| 5 | 0.699 | 0.562 | | |
| 6 | 0.770 | 0.799 | | |

All spots were applied in quadruplicate and the mean areas were used.

values $t_{a0}$ and $t_{a1}$ were calculated, where $t_{a0} = |a_0|/s_{a0}$, $t_{a1} = |1 - a_1|/s_{a1}$ and $s_{a0}$ and $s_{a1}$ are standard deviations [21]. The tabulated $t$ values for $n - 2 = 4$ degrees of freedom and a probability $p = 90\%$ were compared with the calculated $t$ values and the results are given in Table 1. The value $t_{4;90\%} = 2.132$ is greater than the calculated value for the intercept $t_{a0} = 0.492$ and for the slope $t_{a1} = 0.609$. It can be concluded that the two series of concentration values are not statistically different and consequently the proposed TLC method is accurate.

The precision of the method was checked for a series of four DASS sample solutions with known concentrations. Nine spots (4 $\mu$l per spot) were applied for each concentration on the same plate. The standard deviation, $s$, and the $t$-test are shown in Table 2. All the calculated $t$ values

are smaller than the tabulated value for $n - 1 = 8$ degrees of freedom and a probability $p = 90\%$.

In order to verify the sensitivity of the method, a calibration graph was also constructed for ANSS diammonium salt. Four spots were applied on the plate for each concentration and the eluent system was the same as previously. The mean areas obtained by densitometry were used to plot the linear calibration curve $y = 412.54 + 16084.89x$ within the ANSS diammonium salt concentration range 0.22–1.76 $\mu$g per spot.

Using the described thin-layer chromatographic method, the limits of detection obtained were 0.1 $\mu$g per spot for ANSS acid and 0.03 $\mu$g per spot for DNSS acid. The sensitivity of the method is high and impurities, such ANSS acid, have no influence on its accuracy and precision, at any level of concentration.

Table 2
Precision of determination of DASS diammonium salt by the TLC–densitometric method

| DASS applied to plate ($\mu$g per spot), $\mu_0$ | DASS mean value for nine determinations ($\mu$g per spot), $x$ | Standard deviation, $s$ | $t_{calc}$ | $t_{n-1, p\%}$ |
|-----|-----|-----|-----|-----|
| 0.340 | 0.351 | 0.041 | 0.805 | $t_{8, 90\%} = 1.86$ |
| 0.462 | 0.470 | 0.062 | 0.387 | |
| 0.518 | 0.523 | 0.039 | 0.385 | |
| 0.699 | 0.713 | 0.035 | 1.200 | |

Table 3
Degradation with time of DASS diammonium salt exposed to daylight, in solution and applied to the plate

| Conditions | DASS ($\mu$g per spot) | |
|---|---|---|
| | 0.175 $\mu$g/spot applied to plate | 0.345 $\mu$g/spot applied to plate |
| Obtained from calibration graph immediately after elution | 0.173 | 0.330 |
| Before separation, standard solutions exposed to daylight for: | | |
| 24 h | 0.167 | 0.281 |
| 48 h | 0.135 | 0.232 |
| 96 h | 0.104 | 0.167 |
| Developed plates exposed to daylight for: | | |
| 24 h | 0.173 | 0.322 |
| 48 h | 0.169 | 0.303 |

Analyses carried out with standard solutions of DASS diammonium salt exposed to daylight for 24, 48 and 96 h indicated a systematic increase in the difference between the known and the determined concentrations (Table 3). These differences cannot be attributed to the random errors but to the degradation with time of DASS acid exposed to the light.

The chemical instability of DASS diammonium salt makes it necessary to perform the quantitative analysis on the same day as the preparation of standard solutions, and therefore on the day of neutralization of the DASS acid solution. If the chromatographic plates with spots applied were kept in daylight, the degradation of DASS diammonium salt was not so great, which might be due to the absence of an alkaline aqueous medium.

DASS diammonium salt is very well separated by this TLC method and can be determined by densitometry. The method can also be used for the process control of the electrochemical reduction yield.

## 4. Conclusion

The TLC–densitometric method proposed for the determination of DASS acid is accurate and cheap. Its rapidity is very useful because of the high sensitivity of the compound and its facile degradation.

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