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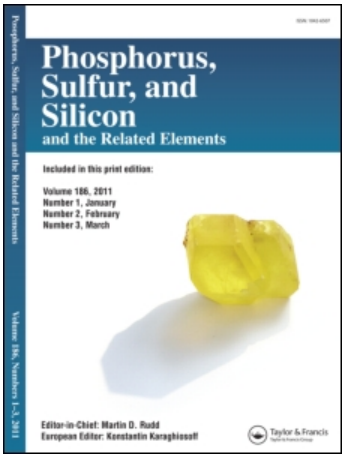

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2,4,6-Triphenylpyrylium Cations as Derivatization Reagents for Sulfide Ions Detection in TLC

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To cite this Article Zakrzewski, Robert , Ciesielski, Witold , Ulanowska, Agnieszka and Martínez-Máñez, Ramón(2009) '2,4,6-Triphenylpyrylium Cations as Derivatization Reagents for Sulfide Ions Detection in TLC', Phosphorus, Sulfur, and Silicon and the Related Elements, 184: 5, 1139 — 1148
To link to this Article: DOI: 10.1080/10426500902855158
URL: http://dx.doi.org/10.1080/10426500902855158

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Phosphorus, Sulfur, and Silicon, 184:1139–1148, 2009
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ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500902855158

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2,4,6-Triphenylpyrylium Cations as Derivatization Reagents for Sulfide Ions Detection in TLC

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We report a study of the use of 2,4,6-triphenylpyrylium salts as derivatization reagents for the simple detection of sulfide ions in thin-layer chromatography (TLC). The principle of the presented method is based on the transformation of 2,4,6-triphenylpyrylium compounds into the parent thiopyrylium derivatives upon reaction with sulfide ions. The derivatization reaction took place in a tube or directly on the TLC plate before the developing step. As a consequence of the reaction of sulfide with the 2,4,6-triphenylpyrylium derivatives, the spots became visible as yellow or blue spots on a colorless background. Spots were stable for several hours. The detection limit is at pmol per spot level and depends on the triphenylpyrylium salt used and on the detection method.

Keywords Sulfide ions detection; TLC; 2,4,6-triphenylpyrylium salts

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INTRODUCTION

Sulfide is an inorganic anion used in a large number of applications by humans, and in fact, sulfide anions can be found in water due to industrial processes, but also due to the microbial reduction of sulfate by anaerobic bacterium. In addition to the disagreeable "rotten eggs" smell of sulfide, there are important reasons to develop simple

Received 17 December 2007; accepted 20 January 2008.

Dedicated to Professor Marian Mikołajczyk, CBMiM PAN in Łódź, Poland, on the occasion of his 70th birthday.

Financial support from the University of Łódź, Łódź, Poland, is gratefully acknowledged.

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methods to detect this anion, which are related with its high toxicity. For instance, it has been reported that sulfide can irritate mucous membranes and, in serious intoxication, can even cause respiratory paralysis. Therefore, the design of new and improved methods for detection and sensing of the sulfide anion can be of interest, and in fact, the detection of sulfide anions has played a significant role within the analytical society.[1,2] Most of the determination procedures, including the widely used methylene blue method,[3–5] face problems, most of which are related with the necessity of reaching a high sensitivity free of interferences. Ion chromatography using special separation resins and suppressor column coupled to a number of detection systems (e.g., conductometric,[6] electrochemical,[7,8] ion-selective electrode,[9] photometric,[10] ICP-MS[11]) has been a commonly used tool for separation and detection of sulfide ions from complex matrices. Sometimes also derivatization[12,13] or post-column[14] reactions have been applied. However, most of these procedures are expensive and not suitable for in situ or at site determinations. As an alternative, there is recent interest in the development of a new generation of chromogenic sensing molecules that have the advantage of showing easily observable color changes in the presence of target guests. In this respect, anion detection by using chromogenic reagents is an area of emerging importance within the field of anion chemistry. There are not too many examples of chromogenic reagents for anion detection, and in this sense, the search for new, easy-to-handle methods for the colorimetric detection of target guests such as sulfide in aqueous environments is still a challenge.

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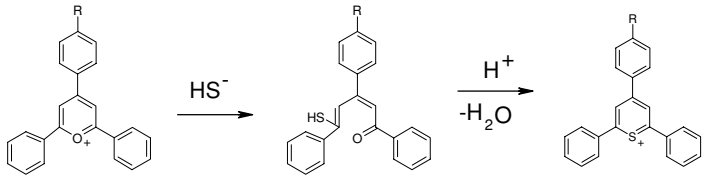
Thin-layer chromatography (TLC) is still one of the simplest separation methods and can be used for identification (as a retention factor R_f and/or color of spots), as well as quantification (as a visual inspection or applying of TLC scanner) of analytes.[15] In chromatography, detection is an important stage of successful analysis and is constantly progressing, because the analyses are getting highly demanding and the quantity of analyte that must be detected is usually very low. In many analyses, it is necessary to apply a selective and sensitive reagent for pre- and/or post-chromatographic detection. A TLC procedure of separation of sulfides from inorganic ions has been proposed.[16]

We report herein a study related with the possibility of using 2,4,6-triphenylpyrylium cations as a pre-chromatographic detection reagent to improve sulfide ion detection selectivity and sensitivity. It is know that the pyrylium cation can be easily transformed into the corresponding thiopyrylium derivative according to Scheme 1.[17]

Such a transformation has never been applied for the development of derivatization reagents, since most of the triarylpyrylium and



Where:

R = H	L1	L2	L3
R = $N(CH_3)_2$	LN1	LN2	LN3

SCHEME 1 Pyrylium to thiopyrylium transformation.

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triarylthiopyrylium cations have a similar color (usually yellow). However, when this transformation is carried out with a triarylpyrylium derivative containing a *N*,*N*-dimethylamino group, there is a remarkable color modulation from magenta (LN1) to blue (LN3).[18]

RESULTS AND DISCUSSION

The transformation of the 2,4,6-triphenylpyrylium compounds to the corresponding 2,4,6-triphenylthiopyrylium derivatives takes place in two steps that involve (i) the nucleophilic attack of the sulfide anion to the pyrylium group followed by (ii) cyclization in acidic conditions to give a thiopyrylium ring (see Scheme 1). The studies carried out (see Detection section) were directed to determine the detection limit for sulfide when this anion reacts in a chromatographic support with 2,4,6-triphenylthiopyrylium derivatives and to study the effect when using different modes of manipulation of different derivatization procedures.

Separation

Several solvent mixtures were examined in order to resolve in TLC triphenylpyrylium and triphenylthiopyrylium derivatives. From the different mixtures tested, Table I shows the combination of solvents that displayed an acceptable capability of resolving the analytes and that additionally do not show interfering effects on the derivative spots.

TABLE I Chromatographic System for Detection of 2,4,6-Triphenylthiopyrylium Salts

			R_f	
	Stationary phase	Solvent	Mean	R.S.D. [%]
L1	Silica gel	Methanol/dichlorometane 1 : 5 v/v	0.95	1.5
L2			0.70	2.1
L3			0.72	2.3
LN1	Cellulose	Phosphoric buffer (pH 6.0) : acetonitrile : 1,4-dioxane 4 : 2 : 1 v/v/v	0.97	1.4
LN3			0.71	2.1

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Derivatization Procedure

As explained above, the reaction of L1 (2,4,6-triphenylpyrylium cation) or LN1 (4-[*p*-(*N*,*N*-dimethylamino)phenyl]-2,6-diphenylpyrylium cation) with sulfide ions results in the formation of the corresponding compounds L2 or LN2, which could further be transformed into the derivatives L3 or LN3 upon treatment with acidic solutions. The optimum reaction conditions for different derivatization procedures for the formation of the corresponding thiopyrylium derivatives were determined by varying the composition of the reagent solution, the concentration of each ingredient, the pH of the derivatization mixture, and the duration of the derivatization reaction. For the derivatization of sulfide ions such as L2 and L3 products (derivatization procedure in a tube), the 2,4,6-triphenylpyrylium salt was used.[18] In order to find the most efficient composition of the derivatization solution, a number of combinations were prepared and tested. From them, the most suitable mixture was the following: TRISMA (0.1 M; pH 9.0):L1 solution (10 mM):acetonitrile (10:10:4 v/v/v). Additionally, when 30 μL 36% hydrochloric acid was added to this reagent solution, cyclization of compound L2 occurs and L3 is obtained. In further studies, we also determined the general conditions for the derivatization of sulfide ions using the 4-[*p*-(*N*,*N*-dimethylamino)phenyl]-2,6-diphenylpyrylium reagent (LN1).[18] In this case, it was found that sulfide ions react rapidly and adequately with the LN1 derivative to yield LN2 in a solution that consists of sodium hydroxide (1 M):LN1 (10 mM): acetonitrile:water (2:7.5:60:40 v/v/v/v). After 10 min, the addition of 75

μL 36% hydrochloric acid resulted in the rapid transformation of LN2 to LN3.

From part of our studies we also found that by using the in situ derivatization, the number of the stages in the entire process was reduced, resulting in a less time-consuming and straightforward method than when the derivatization in the tube was used. The interfering effect of this particular stationary phase was also studied, and it was also found that the sequence in which the spots were placed on the plates played an important role. Thus, for instance, spotting the sulfide ions solution on a cellulose plate followed by the application of derivatization reagent resulted in lower detection limit for LN3. However, the observed detection limits for L3 with NP plates were lower in the case of applying the opposite order of spotting.

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Detection

Table II shows the detection limits in pmol/spot observed for the detection of sulfide with the derivatization reagents L1 or LN1 using diverse detection procedures (i.e., color of the spot, UV, iodide chamber, etc.). Table II also shows the detection limit using both derivatization methods, i.e., in tube and in situ. As explained above, the reaction of L1 or LN1 with sulfide ions results in the formation of the corresponding L2 or LN2, which can be transformed to L3 or LN3 with acidic solutions. When using L1 as derivatization reagent, sulfide ions form pale yellow derivatives (L2 and L3) that are similar in color to L1. In clear contrast, when the magenta compound LN1 is employed as derivatization reagent, sulfide ions form the blue derivative LN3. According to the results summarized in Table II, the detection limits established for L2, L3, and LN3 using simple visual observation of the spots are similar for all three compounds when using derivatization in a tube. On the contrary, by using derivatization in situ, the detection limit obtained for LN3 is significantly lower than that found for L3. Also it is significant that the detection limit (spot color detection) for L3 is much higher using the procedure of derivatization in situ than in a tube. It was presumed that different interactions between the silica gel stationary phase and the strong polar molecules taking part in derivatization somehow inhibit the reaction so strongly that the detection limit is dependent on where the derivatization is carried out. In fact the detection limit for L3 in the derivatization in situ procedure shows quite high detection limits of ca. 1600 pmol/spot independent of the detection system used (spot color, UV, iodine chamber, iodine-azide, or iodine chamber + starch). In contrast, the detection limit when LN3 is formed from

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TABLE II Detection Limits (pmol/spot) for the Determination of Sulfide Ions[a]

	Derivatization in a tube				Derivatization in situ				
	Spot color	UV	Iodine chamber	Iodine-azide detection system	Spot color	UV	Iodine chamber	Iodine-azide detection system	Iodine chamber + starch
L2	240	240	—[b]	650	—	—	—	—	—
L3	200	200	300	800	1600	1600	1600	1600	1600
LN3	240	1200	—	—	60	160	—	—	120

[a]For the chromatographic system, see Table I.
[b]Not tested.

LN1 in derivatization in situ via spot color changes was as low as 60 pmol/spot. When LN3 was used for detection in derivatization in a tube, the detection limit was higher than the one obtained in derivatization in situ using simple spot color detection.

The detection limit for LN3 obtained with UV procedure in derivatization in a tube is considerably higher than the one obtained for L3 (detection limits are 1200 and 200 pmol/spot for LN3 and L3, respectively; see Table II). The blue LN3 spot is weakly visible on fluorescent background of the plate leading to a poor detection limit. Using also derivatization in a tube, the detection limit for L3 when spot color is observed is slightly lower (detection limit 200 pmol/spot) than the one obtained with iodine procedure (detection limit 300 pmol/spot). This was because the yellow spot of L3 became pale brown on the yellow background when using the iodine procedure, making the distinction a bit more difficult.

We also applied an iodine-azide detection system for L2. It has been reported that azide reacts with iodine only in the presence of a sulfur(II) compound (e.g., a thiol) and this offers a means of selective and sensitive TLC detection of thiols as inductors of the iodine-azide reaction.[19] Unfortunately, the detection limit obtained with this procedure is much higher among the tested procedures. This is due to the fact that when the plate was treated with iodine-azide, the spot remained yellow and did not become white,[19] making the visual observation on a violet-gray background quite difficult. When the plate was treated with a buffer at pH 6.0, further shift of the equilibrium L2/L3 to the right took place and the increase of the detection limit is caused by the fact that L3 is not an inductor of iodine-azide reaction.

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Interferences

Table I shows the solvent systems, which were selected in order to have a clear separation of the spots for L1 and LN1 and the corresponding spots after reaction with the sulfide anion. Additionally we also observed that there was not any interference when other anions are present for the formation of the blue color when LN1 is transformed to LN3. This result is in agreement with those shown previously.[18] Some additional studies were conducted, and it was concluded that neither MESNA, nor cysteine, nor octanethiol form 2,4,6-triphenylthiopyrylium derivatives, and therefore these compounds are not interferents in sulfide detection procedures.

CONCLUSIONS

A new TLC system for sulfide anions detection has been developed. The principle of the method is based on the use of 2,4,6-triphenlpyrylium salts as pre-chromatographic derivatization reagents. The cations L1 (2,4,6-triphenylpyrylium) or LN1 (4-[*p*-(*N*,*N*-dimethylamino)phenyl]-2,6-diphenylpyrylium) were used in this study. These reagents react with sulfide ions to yield the parent 2,4,6-triphenlpyrylium salts. The derivatization reactions were assayed in a tube or directly on the TLC plate before the developing step. The proposed detection procedure allows selective and sensitive detection for sulfide anions at several dozen pmol/spot. For instance, a detection limit as low as 60 pmol/spot was observed when the spot color of product LN3 was observed in derivatization in situ. We believe that the presented system of sulfide anion detection may provide an efficient, easy, and undemanding tool for sulfide determination in environmental analysis using simple TLC procedures.

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EXPERIMENTAL

Solution and Reagents

Chemicals: sodium sulfide, sodium azide, iodine, starch, 2,4,6-triphenylpyrylium tetrafluoroborate, and all organic solvents were obtained from Sigma-Aldrich (Steinheim, Germany), LABSCAN Analytical Science (Dublin, Ireland), or POCH (Gliwice, Poland). 4-[*p*-(*N*,*N*-dimethylamino)phenyl]-2,6-diphenylpyrylium perchlorate was synthesized according to the previous report.[18]

Sulfide ions stock solutions: 0.4 Mmol of sodium sulfide was dissolved in 0.2 mL of 1 M sodium hydroxide and diluted to 10 mL with water to obtain 0.04 M concentration of sulfide ion solution. Sulfide ions standard solution: specific volumes of stock sulfide ions solution were added to 0.2 mL 0.1 M TRISMA (pH 9.0) and diluted to 10 mL with water.

Derivatization solution: 1 Mmol of L1 or LN2 was dissolved in acetonitrile and diluted to 10 mL with acetonitrile.

Solvents systems: Specified volumes of organic solvents were mixed (for details see Table I).

Spraying solution for the iodine-azide procedure: 25 mL aqueous starch solution containing 2.5 g starch was added to 20 mL aqueous sodium azide solution containing 2 g sodium azide. The mixture was adjusted to pH = 6.0 with 0.1 M hydrochloric acid solution and diluted

to 50 mL with water to obtain 4% and 1% solution for sodium azide and starch, respectively. All solutions were prepared fresh daily.

Derivatization in a Tube

Sulfide ions detection as L2 or L3: The appropriate amount of standard sulfide ions solution, 200 μL 0.1 M TRISMA, 100 μL of 10 mM L1 solution, and 400 μL of acetonitrile were placed in a stoppered tube; afterwards the reaction solution was diluted with water to 1 mL. When L2 was detected 30 μL 36% hydrochloric solution was added. The plates were spotted with 0.5 μL L2 or L3 solution and developed with a mixture of suitable solvents.

Sulfide ions detection as LN3: The appropriate amount of standard sulfide ions solution, 20 μL of 0.1 M sodium hydroxide solution, 75 μL of 10 mM LN1 solution, and 600 μL of acetonitrile were placed in a stoppered tube, and the reaction solution was diluted with water to 1 mL. The reaction time was 5 min. After the reaction was completed, 75 μL of 36% hydrochloric solution was added. The plates were spotted with 0.3 μL of LN3 solution and developed with a mixture of suitable solvents.

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Derivatization in Situ

Derivatization solutions (7 mM L1 or 5 mM LN1) were spotted on the plate with micropipette of the 0.5 μL range (Brand, Wertheim, Germany). Subsequently, derivatization dots were spotted with sulfide ions solution by means of a 0.1–0.5 μL range micropipette (Brand, Wertheim, Germany). Afterwards, the dots where spotted with 1 μL 0.5 M hydrochloric solution when L3 and LN3 were detected. The plate was placed in a chromatographic chamber for 10 min in order to reach completion of the prechromatographic derivatization and the development with a mixture of suitable solvents was initiated.

Planar Chromatography

Chromatography was performed at room temperature on HPTLC silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany; 10 $\times$ 5 cm, 0.2 mm thick layer) or cellulose F aluminum sheets (Merck, Darmstadt, Germany; 5 $\times$ 5 cm, 0.2 mm thick layer). The horizontal DS-Chamber (Chromdes, Poland) equilibrated for 10 min and was applied to the process for L3 and LN3, respectively. The developing distance was 4 cm

with a starting line positioned at 0.5 cm from the edge of the plate by the use of the solvent systems indicated in Table I.

Detection

The spot color procedure: The air-dried developed plates were examined under daylight. The compounds were detected as pale yellow (L2, L3) or blue (LN3) spots.

The UV_{254} procedure: The air-dried developed plates with a fluorescent indicator were examined under UV light (254 nm). The compounds extinguished the fluorescence of the plate background.

The iodine procedure: The air-dried developed plates were exposed to iodine vapor for 3 min. The spots became visible as brown spots on a yellow background.

The iodine-azide procedure: The TLC-Sprayer (Merck) was utilized to spread the mist of homogeneous sodium azide and starch mixture on the air-dried plate. Subsequently the plate was exposed to iodine vapor for 5 sec. Yellow spots on a violet-gray background appeared.

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