



Thiosulfonate functionalized polystyrene resin: Preparation and application in the isolation and identification of electrophilic mutagens

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Abstract

A new approach for isolation and identification of electrophilic mutagens from complex matrix was developed. Thiosulfonic anion was immobilized onto polystyrene beads and used as separation media. Potassium polystyryl-thiosulfonate, prepared from polystyryl-sulfonyl chloride and KHS, was observed to selectively react with model electrophilic mutagens such as alkyl halides, α -chloroketones and α -chloroesters to produce polystyryl-thiosulfonic esters. After separation from other nonreactive organic compounds, the beads then reacted with ethanethiol to produce unsymmetrical ethyl disulfides which are easily detected by GC/MS. For one mutagenic compound, only one unsymmetrical disulfide was found to contain its structure part. Thus, the structure of the parent mutagens could be deduced from that of the unsymmetrical disulfides. The degree of functionalization of the potassium polystyryl-thiosulfonate resin was 1.11 mmol/g. Its reactivity was discussed and its recycling method was reported here.

Key words: functionalized polystyrene; potassium polystyryl-thiosulfonate; unsymmetrical ethyl disulfides; electrophilic mutagens

Introduction

Toxicants, such as direct-acting mutagens, arise through normal metabolic sequences or from anthropogenic processes such as the chemical disinfection of water (Cheh *et al.*, 1980). However, only a small percentage of these compounds have been positively identified in environmental samples due to the absence of appropriate separation methodology. Thus, the identification of reactive components of toxicological importance from complex matrices remains a significant issue in environmental chemistry and toxicology.

An evaluation of the structural characteristics of the various classes of toxic compounds indicates that the vast majority have functionality with inherent electron insufficiency. It has been the approach to develop a series of complementary electron-rich derivative agents to selectively label the reactive mutagenic electrophiles in samples to facilitate the isolation and identification. Among them, polymer supported reactive nucleophilic agents are of interest as they are anticipated to immobilize the toxic electrophiles that can be separated from complex matrices. Since Merrifield (1963) created the solid-phase peptide synthesis, a variety of polymer-supported reagents have been investigated for their different purpose (Sherrington and Hodge, 1988). In our case, the polymer supported nucleophilic agents should be reactive to immobilize the toxic electrophiles which will be then liberated from the polymer as labeled adducts after separation. Some special agents

such as cross-linked polystyrene beads supported thiol (Chiu and Anderson, 1976) or sulfenic anion (Frechet *et al.*, 1982) were reported to be prepared. They are reactive to electrophilic mutagens to generate immobilized sulfides and sulfones, respectively. Unfortunately, the sulfides and sulfones are stable and would not be easily cleaved for subsequent detection. For solving the problem, it is necessary to create a new polymeric nucleophilic agent. This report discussed the design and preparation of a new cross-linked polystyrene beads supported thiosulfonate and its application as medium in isolation and identification of electrophilic mutagens.

1 Experimental

1.1 Instrument and chemicals

The GC/MS instrument was a Hewlett Packard 5790-5970. NMR Spectra were taken on an Ibm/Bruker NRA 200 MHz instrument. The GC was a Perkin Elmer F-42 with FID detector (Germany) and a three foot by one-eighth inch column packed with 20% SP 2100/0.1% CW-1500 on Supelcoport[®] or as noted otherwise. IR spectra were taken with Nicolet 5ZDX instrument. Bio-Beads S-X2[®] obtained from Bio-Rad Laboratories served as the starting polymer for synthesis: benzyl ethyl disulfide as a standard sample was prepared according to the literature (Jayasuriya and Regen, 1992) from ethyl 2-pyridyl disulfide which is synthesized from 2,2-dithiobispyridine. The intermediate and product were purified by silica-gel

column chromatography and checked by NMR and MS. Ethyl 2-pyridyl disulfide: $^1\text{H-NMR}$ (CDCl_3): δ 8.43–8.37 (m, ^1H , aromatic), 7.72–7.59 (m, ^2H , aromatic), 7.02–7.00 (m, ^1H , aromatic), 2.77 (q, ^2H , SCH_2Me), 1.29 (t, ^3H , CH_3); MS (EI): m/z 171 (M^+), 111 (base peak), 78. Benzyl ethyl disulfide: $^1\text{H-NMR}$ (CDCl_3): δ 7.34–7.25 (m, ^5H , aromatic), 3.89 (s, ^2H , $\text{PhCH}_2\text{S-}$), 2.42 (q, ^2H , $\text{MeCH}_2\text{S-}$), 1.20 (t, ^3H , CH_3). MS(EI): m/z 184(M^+), 91 (base peak), 77, 65.

Other organic chemicals used in this work were obtained from Aldrich Chemical Company.

1.2 Procedure

1.2.1 Reaction of potassium thiosylate with electrophiles

Potassium thiosylate (1.0 mmol) and the electrophiles (1.0 mmol) were combined in DMF (2.0 ml) and stirred for 6 h at 60°C . The reaction mixture was poured into water and extracted with chloroform. The extract was washed with water, dried over sodium sulfate and concentrated. The residue was dissolved in methylene chloride (2.0 ml) and 1.0 mmol each of thiol and triethyl amine (in 1.0 ml of methylene chloride) were added at 0°C . After 30 min, the reaction mixture was washed with water, dried over anhydrous sodium sulfate and analyzed with GC/MS. Alternatively, the residue from the first step was dissolved in 7:1 DMF- H_2O (2.0 ml) with dropwise addition of the thiol (1.0 mmol), followed by stirring at room temperature for 2 h. The resulted mixture was taken up in methylene chloride, washed with water, dried and concentrated.

1.2.2 Preparation of polystyryl-sulfonyl chloride (beads 1)

S-X2[®] polymer beads (2.60 g) were stirred in chloroform (100 ml) and chlorosulfonic acid (20 ml) was added dropwise. The mixture was subsequently stirred over night under reflux and was then filtered. The beads were stirred for 30 min successively in chloroform and acetonitrile, followed by thoroughly washing with water, acetonitrile and ether, then vacuum dried to a constant weight (5.02 g). The IR spectra indicated absorption at 1170 and 1370 cm^{-1} characteristic of the sulfonyl chloride group (Fig. 1a).

1.2.3 Preparation of potassium polystyryl-thiosulfonate (beads 2)

Five grams of beads 1 were suspended in 25 ml of 2 mol/L KHS solution. The reaction mixture was kept at 55°C for 3 h with stirring. Then the beads were collected on a filter, washed with water, ethanol and ether, then vacuum dried to constant weight as 5.97 g. The elemental analysis showed the contents are: C 38.71%; H 4.67%; S 19.31%. The IR spectra are shown in Fig. 1b. The degree of functionalization (DF, the determination method were mentioned later) of this beads 2 was 1.11 mmol/g.

1.2.4 Preparation of sodium polystyryl-thiosulfonate

Sodium sulfide nonahydrate (8.0 g) and sodium hydroxide (0.25 g) were dissolved in water (50 ml) in which beads 1 (5.0 g) were suspended by stirring. The mixture was maintained at 95°C for 72 h. Then the beads were

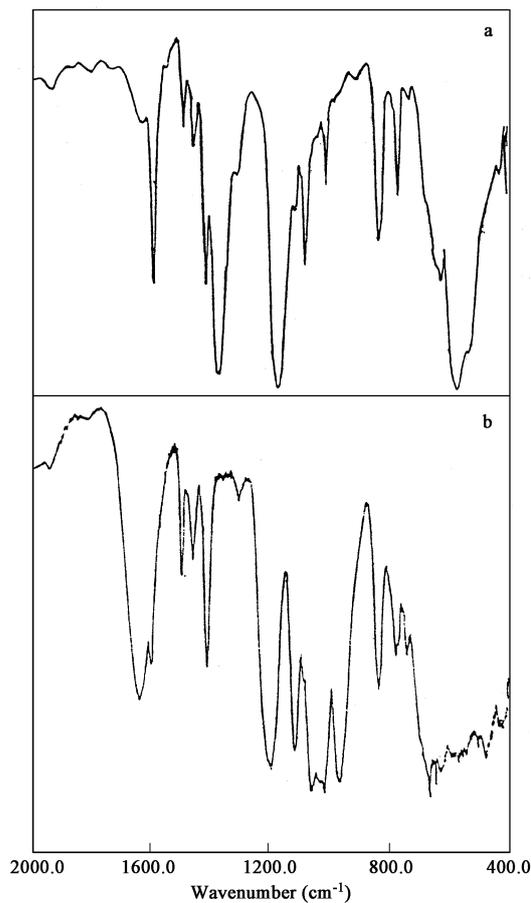


Fig. 1 IR spectra of polystyryl-sulfonyl chloride (a) and potassium polystyryl-sulfonate (b).

collected on a filter and thoroughly washed with water, ethanol and ether, dried in vacuum to a constant weight as 5.58 g. The elemental analysis showed: C 39.23%; H 5.71%; S 16.09%; Cl 0.13%. Its DF is 0.32 mmol/g.

1.2.5 Reaction of beads 2 with electrophiles and cleavage reaction

Single or mixed electrophiles (0.3 mmol) were added to a slurry of beads 2 (200 mg in 1.0 ml of 95% ethanol) and stirred at 60°C . After 6 h, the ethanol was removed by filtration. The beads were washed with ethanol and dried to get polystyryl-thiosulfonic esters (beads 3). Then the beads 3 were placed in a vial containing 2 drops of ethanethiol in 1.0 ml of acetonitrile and allowed to react in room temperature for 12 h. Disulfide produced were observed by direct injection of the reaction solution into the GC-MS.

1.2.6 Detection of electrophiles in water samples via a thermostatic column packed with beads 2

The beads 2 (0.5 g) were put inside of a small glass column which was warmed up to 60°C outside. Three liter of water samples containing 10–20 mg/L of single or mixed electrophiles passed through the column at a flow rate of 3 ml/min (to each 3 L of water sample added 5 ml of methanol to increase the solubility of electrophiles). Then the beads were taken out to a vial and treated with 100 mg of iodomethane for 8 h at room temperature. The cleavage and detection procedure is just as above mentioned.

1.2.7 Determination of degree of functionalization (DF)

First, using standard benzyl ethyl disulfide sample to prepared the above to exactly determine the response curve of the peak area integration to the injection volume of GC. The curve is quite linear. Then the polystyryl-thiosulfonate polymer beads (200 mg) were stirred in 2.0 ml of 95% ethanol and 50 mg of benzyl chloride was added to this slurry at 60°C. After 6 h, the ethanol was removed by filtration and the beads were washed by water and ethanol. The beads after dried were placed in a vial and 1.0 ml of ethanol containing 50 mg of ethanethiol was introduced. The mixture was stirred at room temperature overnight, and the solution was quantitated by peak area integration on GC. After calculation through the slope of response curve, the amount of benzyl ethyl disulfide obtained via one gram of the polystyryl-thiosulfonate beads were thought to be the degree of functionalization.

1.2.8 Recycling of the used polymer

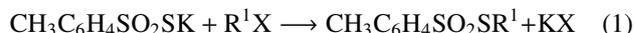
After cleavage reaction, the polystyryl-thiosulfonic esters (beads 3) transferred to polystyryl-sulfinic acid (beads 4). Two grams of the used beads 4 was suspended in 50 ml of ethanol-water (1:1) to which 200 mg of sulfur and 50 mg of hexadecyltrimethyl ammonium chloride were added. The reaction mixture was kept at 90°C with stirring for 16 h. After filtration, the beads were washed with water, ethanol and ether, and then dried to get 2.02 g of hexadecyltrimethyl ammonium polystyryl-thiosulfonate (beads 2'). The DF of the recovered beads 2' is 1.04 mmol/g.

2 Results and discussion

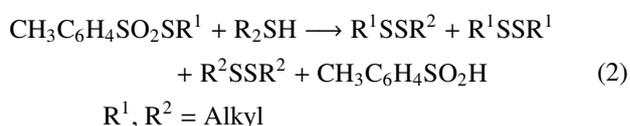
2.1 Design and preparation of potassium polystyryl-thiosulfonate resin (beads 2)

The need to develop effective isolation and detection methods for electrophilic mutagens directed our attention to the use of polymer to which a nucleophilic group would be covalently attached. One choice of the electro-rich derivative agents is thought to be the thiosulfonate anion because it is known that in versatile synthetic purposes the metal salts of thiosulfonic acid could react with alkyl halides to produce thiosulfonic esters in which sulfur-sulfur bond is able to be cleaved (Chandra and Field, 1986; Boehme and Lenz, 1984; Singh and Field, 1988; Scholz, 1984). But if the group is immobilized and these reactions are used for analysis in solid phase, it is still necessary to

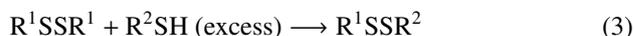
check whether there is any by-reaction and to see if the by-products present interfere in the analysis or not. For this purpose, before preparing polystyryl-thiosulfonate resin, potassium thiosulfate was used as a matrix to react with electrophiles to produce toluene thiosulfonic esters:



where, R^1X represents electrophilic model compounds such as alkyl halides. The sulfur-sulfur bond in toluene thiosulfonic esters is able to be splitted by reducing agent to produce thiols (Kushko, 1971) or by thiols to produce disulfides (Parsons *et al.*, 1965; Field *et al.*, 1961, 1965). In the former case, the thiols produced readily oxidized in the air to form mixed disulfides which would cause considerable complication in the subsequent analysis. In the later case, thiols react with the thiosulfonic esters and produce unsymmetrical disulfide as main product with two symmetrical disulfides as by-products:



Obviously, the reaction mixture was not able to determine the structure of electrophilic analytes R^1 because one of them would be involved in two products or even more in complicated system. For solving this problem excess thiol (R^2SH) was used to transfer the symmetrical disulfides which contain electrophilic analytes R^1 to the unsymmetrical disulfide:



The experiment proved that in this way the only measurable product containing electrophilic analytes R^1 became the expected unsymmetrical disulfide (R^1SSR^2). The other symmetrical disulfide (R^2SSR^2) is from chosen thiol and would not interfere with the detection. Thus, the structure determination of the parent electrophiles (R^1X) could be carried out through identification of the obtained unsymmetrical disulfide (R^1SSR^2) by GC-MS or other instrument. Table 1 represents the MS data of the unsymmetrical disulfides (R^1SSR^2) from alkyl halides (R^1X) via toluene thiosulfonic esters. Fortunately, for one alkyl halide only one unsymmetrical disulfide was found in GC-MS. The results are rather encouraging.

However, as mentioned above, it is not convenient to isolate and identify mutagenic electrophiles from complex

Table 1 MS data of disulfides produced via the reaction of organic electrophiles and potassium thiosulfate

Electrophiles R^1X	Cleavage reagent R^2SH	Disulfides produced	EI MS m/z (% relative abundance)
$\text{C}_2\text{H}_5\text{I}$	$\text{HSCH}_2\text{COOCH}_3$	$\text{C}_2\text{H}_5\text{SSCH}_2\text{COOCH}_3$	166 (M^+ , 60), 106 (86), 79 (58), 60 (66), 45 (100)
$(\text{CH}_3)_2\text{CHCH}_2\text{Br}$	$\text{CH}_3\text{CH}_2\text{SH}$	$(\text{CH}_3)_2\text{CHCH}_2\text{SSC}_2\text{H}_5$	150 (M^+ , 75), 94 (71), 57 (100), 41 (90)
$\text{C}_6\text{H}_5\text{CH}_2\text{Cl}$	$\text{HSCH}_2\text{COOCH}_3$	$\text{C}_6\text{H}_5\text{CH}_2\text{SSCH}_2\text{COOCH}_3$	288 (M^+), 197, 169, 137, 105 (each < 10%), 91 (100)
$\text{CH}_3\text{C}(\text{O})\text{CH}_2\text{Cl}$	$\text{C}_5\text{H}_{11}\text{SH}$	$\text{CH}_3\text{C}(\text{O})\text{CH}_2\text{SSC}_5\text{H}_{11}$	192 (M^+ , 14), 122 (15), 58 (14), 43 (100)
$\text{BrCH}_2\text{CH}:\text{CHCOOCH}_3$	$\text{HSCH}_2\text{COOCH}_3$	$(\text{C}_5\text{H}_7\text{O}_2)\text{SSCH}_2\text{COOCH}_3$	236 (M^+ , 15), 99 (95), 71 (100), 59 (80), 45 (75)
ClCH_2CN	$\text{C}_5\text{H}_{11}\text{SH}$	$(\text{CN})\text{CH}_2\text{SSC}_5\text{H}_{11}$	175 (M^+ , 8), 71 (17), 43 (100), 41 (36)
$\text{CH}_3\text{CH}(\text{Cl})\text{COOCH}_3$	$\text{HSCH}_2\text{COOCH}_3$	$\text{C}_4\text{H}_7\text{O}_2\text{SSCH}_2\text{COOCH}_3$	224 (M^+ , 13), 192 (13), 105 (20), 59 (100), 45 (47)
$\text{ClCH}_2\text{COOCH}_3$	$\text{C}_5\text{H}_{11}\text{SH}$	$\text{CH}_3\text{OC}(\text{O})\text{CH}_2\text{SSC}_5\text{H}_{11}$	208 (M^+ , 9), 138 (9), 106 (16), 71 (27), 43 (100)
$\text{BrCH}_2\text{CH}:\text{CH}_2$	$(\text{CH}_3)_2\text{NCH}_2\text{CH}_2\text{SH}$	$\text{C}_3\text{H}_5\text{SSC}_4\text{H}_{10}\text{N}$	177 (M^+ , 2), 105 (5), 71 (3), 58 (100), 42 (38)

were found in this experiment.

It is found that more active electrophiles such as allyl halides or benzyl chloride reacted more readily with beads 2 than alkyl halides. After beads 2 reacted with excess of equimolar mixture of methyl 4-bromocrotonate, 1-bromopentane, 2-bromopentane, 2-bromopropane, 1-bromo-1-methyl-propane, benzyl chloride, methyl 2-chloropropionate and then cleaved with ethanethiol, only the disulfides derived from the more reactive benzyl chloride and methyl 4-bromocrotonate could be detected. Obviously, because the amount of beads 2 is relatively insufficient to the mixture of electrophiles, and more reactive benzyl chloride and methyl 4-bromocrotonate reacted with beads 2 much faster than other compounds, and firstly occupied all active sites on the beads 2. In other words, only if the amount of beads 2 is sufficient, it could be possible to detect the other compounds in the electrophiles mixture. This is significant and interesting because more reactive electrophiles are more toxic to human beings, and need to be isolated and detected firstly.

The experiments result also represented that the beads 2 had apparent steric effect. When equimolar mixture of linear and branched halogenated compounds were subjected to this procedure, the peaks of the disulfides from linear compounds were observed higher than that from branched analogues. Fig.4 shows the peak area of ethyl *n*-pentyl disulfide from 1-bromopentane through the polymer media was twelve times that of the peak from 2-bromopentane. The same phenomenon was observed in the case for methyl chloroacetate and methyl 2-chloropropionate.

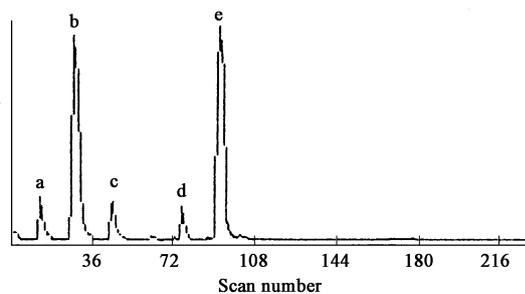


Fig. 4 TIC of the disulfides produced via the reaction between the polymer beads 2 and mixture of alkyl bromides then cleaved with ethanethiol. (a) EtSSCH(CH₃)₂; (b) EtSSCH₂CH₂CH₃; (c) EtSSCH₂CH(CH₃)₂; (d) EtSSCH(CH₃)C₃H₇; (e) EtSS(CH₂)₄CH₃.

As a further test, the beads 2 was packed in a column and subjected to water samples. About 0.5 g of the beads 2 was put inside of a small glass column which was warmed up to 60°C outside. 3 L of water samples containing 10–20 mg/L of single or mixed electrophilic model compounds passed through the column at a flow rate of 3 ml/min. Then the beads were taken out to a small bottle and reacted with excess iodomethane at room temperature for 8 h so as to transfer all the thiosulfonate anion left in the beads to polystyryl-thiosulfonic ester group. After filtration and washing, the beads were treated with ethanethiol in acetonitrile. The reaction solution was injected to GC-MS directly. Besides methyl ethyl disulfide and diethyl disulfide, the unsymmetrical disulfides found are listed in Table 3. All samples have been detected out at this concentration level. Samples 1, 2 and 3 are single component system, just as expected, for every sample, only one peak of unsymmetrical disulfide was found, besides methyl ethyl disulfide which was leak out together with diethyl disulfide. It is interesting that for disubstituted 1,2-dichloroethane in sample 3, only bis-disulfide EtSSCH₂CH₂SSEt was found. The explanation maybe is that in the dilute sample solution, when one side of 1,2-dichloroethane had been bound to the beads 2, the other side of the molecule would be easier to react because it is nearer to the active site on beads 2 and odds of impact would be larger than that of other molecules of 1,2-dichloroethane in water solution. Sample 4 and Sample 5 are tri-component system. The effect of reactivity of the model electrophiles was also observed here. The concentrations of 2-chloroethanol in sample 4 and in sample 5 are the same, but the peak area of ethyl hydroxyethyl disulfide derived from 2-chloroethanol in sample 4 was smaller than that in sample 5. The reason is that the other two bromo-compounds BrCH₂CH₂CH₃ and Br(CH₂)₃CH₃ in the sample 4 are more active than the two other chloro-compounds ClCH₂COOC₂H₅ and ClCH₂CH₂Cl present in the sample 5. The ability of competition of 2-chloroethanol in sample 4 would be lower than that in sample 5. The more reactive, the faster the electrophiles reacted with the thiosulfonate group in the polymer. The chance of reaction for compounds which are not so active must be decreased and the yield of the disulfide would be lower. Of course, the more reactive, the electrophilic mutagens are more important in toxicology, this procedure is able to selectively detect electrophilic toxicants from complex

Table 3 Detection of water samples via potassium polystyryl-thiosulfonate by GC-MS

Sample number	Component conc. of electrophile in water sample (mg/L)	Disulfides detected	Retention time (min)	EI MS (<i>m/z</i>) (% relative abundance)
1	ClCH ₂ COOC ₂ H ₅ (10)	EtSSCH ₂ COOC ₂ H ₅	18.12 ^b	180 (M ⁺ , 100), 120 (28.5), 107 (36), 106 (71), 93 (25), 79 (60), 60 (50)
2	ClCH ₂ C ₆ H ₅ (10)	EtSSCH ₂ C ₆ H ₅	11.01 ^a	184 (M ⁺ , 10), 91 (100), 65 (17), 45 (14)
3	ClCH ₂ CH ₂ Cl (20)	EtSSCH ₂ CH ₂ SSEt	25.36 ^b	214 (M ⁺ , 2.5), 154 (12), 121 (58), 93 (100), 61 (17), 59 (23)
4	ClCH ₂ CH ₂ OH (20)	EtSSCH ₂ CH ₂ OH	14.31 ^b	138 (M ⁺ , 100), 107 (7), 94 (82), 79 (35), 66 (41), 45 (48)
	BrCH ₂ CH ₂ CH ₃ (20)	EtSSCH ₂ CH ₂ CH ₃	6.10 ^a	136 (M ⁺ , 47), 94 (64), 79 (8), 66 (46), 43 (100)
	Br(CH ₂) ₃ CH ₃ (20)	EtSS(CH ₂) ₃ CH ₃	13.85 ^b	150 (M ⁺ , 75), 94 (100), 79 (7), 66 (25), 57 (33)
5	ClCH ₂ COOC ₂ H ₅ (20)	EtSSCH ₂ COOC ₂ H ₅	18.12 ^b	180 (M ⁺ , 100), 120 (28.5), 107 (36), 106 (71), 93 (25), 79 (60), 60 (50)
	ClCH ₂ CH ₂ OH (20)	EtSSCH ₂ CH ₂ OH	14.31 ^a	138 (M ⁺ , 100), 107 (7), 94 (82), 79 (35), 66 (41), 45 (48)
	ClCH ₂ CH ₂ Cl (20)	EtSSCH ₂ CH ₂ SSEt	25.36 ^b	214 (M ⁺ , 2.5), 154 (12), 121 (58), 93 (100), 61 (17), 59 (23)

^a and ^b are the same as Table 2.

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