Tosylation of Endocrine-Disruptive Alkylphenolic Compounds in a Solid-Phase Reaction System Consisting of C_{18}-Modified Silica Gel and Aqueous Buffer Solution

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The tosylation reaction of endocrine-disruptive alkylphenolic compounds in a solid-phase aqueous system was investigated with the aim of developing an environment-friendly and efficient derivatization method for HPLC analyses of environmental samples. The phenols were rapidly and efficiently converted to the tosyl derivatives on a commercially available ODS solid-phase cartridge by passing an aqueous buffer solution through it. The solid-phase aqueous tosylation system has been incorporated into a preconcentration step performed by solid-phase extraction from environmental water.

Key words tosylation reaction; solid-phase reaction; environmental sample; solid-phase extraction; high-performance liquid chromatography

Recently, much attention has been paid to synthetic organic reactions in aqueous media instead of in organic solvents such as silica gel-dispersal and polymer-dispersal aqueous systems from the point of view of environmental conservation.[1—3] The results suggest that the total synthetic process in aqueous systems is simple and the interface between the solid phase and the aqueous liquid provides specificity and diversity in the synthetic reactions. On the other hand, many attempts at chromatographic determination of analytes coupled with the derivatization reaction have been made to overcome the problems of low detection and insufficient separation.[4] However, the derivatization techniques utilizing the solid phase and solid phase-dispersed reactions have only been discussed in terms of efficiency in the analytical procedure, that is, efficient conversion of the analytes to the derivatives, high recovery, and shortening of the analysis time.[5—9] In particular, trace analyses of biological samples have been developed coupled with on-column derivatization and micro solid-phase extraction techniques.[6,7] However, environmental analyses still essentially require an enrichment step such as preconcentration and a derivatization step to achieve low detection limits prior to chromatographic separation.[10] The solid-phase aqueous reaction is a desirable method for environmental analyses from the point of view of environmental conservation as well as efficiency in the analytical procedure.

The derivatization reagents characterized by an active sulfonyl chloride to the phenolic hydroxyl group such as tosyl chloride (4-methylbenzenesulfonyl chloride), dansyl chloride, and ferrocenesulfonyl chloride are entirely used for precolumn derivatization to overcome the disadvantage of the background response. Precolumn dansylation is applied to the HPLC analysis of endocrine-disruptive alkylphenolic compounds in environmental water.[10] The solid-phase reaction of the sulfonyl chlorides is important in improving the analytical methods. In this study, we have developed a direct tosylation method for alkylphenolic compounds preconcentrated in a C_{18}-modified silica gel (ODS) cartridge used for their enrichment step using a heterogeneous system consisting of an aqueous medium and ODS instead of organic solvents, as a prototype of the solid-phase derivatization method of the phenols with sulfonyl chlorides.

Experimental

Chemicals Tosyl chloride as a derivatization reagent was purchased from Nacalai Tesque and used as received without further purification. The samples used were alkylphenols [4-*tert*-butylphenol, 4-*sec*-butylphenol, 4-(1,1-dimethylpropyl)phenol, 4-(1,1,3,3-tetramethylbutyl)phenol, and 4-nonylphenol] which are recognized as estrogenic compounds.[10] They were of the best available grade from Nacalai Tesque and Tokyo Chemical Industry and were used as received. The ODS powder was purchased from Nacalai Tesque and was used in the solid-phase disperse system as a solid-phase catalyst.

Preparation of the Standard of Tosyl Phenols The standard of the tosyl derivatives of the alkylphenols was synthesized by addition of a phosphate buffer (pH 12) to a mixture of equimolar tosyl chloride and the phenols in CH_3CN. The reaction mixture was kept at 60 °C for 30 min and then extracted with ether. The crystals of the tosyl phenols were obtained by evaporation of the solvent from the extract after washing with 0.1 mol/l of KOH solution. The structure and purification of the tosyl derivatives were determined by mass spectrometry and elemental analyses.

Preparation of the Solid-Phase Extraction Cartridge Used for the Tosylation Reaction Waters Sep-Pak® cartridges packed with ODS were used for the solid-phase tosylation field of the phenols. Tosyl chloride was adsorbed to ODS in the cartridges prior to solid-phase extraction (SPE) of alkylphenols as follows. A 1-ml aliquot of a CH_3CN solution containing 5 mg of tosyl chloride was run in the inlet of the cartridges, and then the cartridges were washed with 20 ml of water.

HPLC Apparatus and Conditions The chromatographic separation was performed with a Hewlett-Packard (HP) 1050 series HPLC unit equipped with an UV-Vis detector set at 250 nm. The tosyl phenols were separated on a Cosmosil $5C_8$-MS packed column (4.6×150 mm) with a mobile phase consisting of a water–CH_3CN mixture (1 : 1) at a flow rate of 1.0 ml/min. A 10-μl aliquot of the sample solutions was injected into the HPLC column. Data collection and manipulation were performed using HP Chem Station software for HPLC analyses.

Results and Discussion

Tosylation Reaction in Solution Derivatization of phenols with sulfonyl chlorides such as tosyl chloride and dansyl chloride is usually carried out in aqueous acetone saturated with sodium carbonate with a long reaction time at a relatively high temperature.[4] The optimal conditions are set with regard to pH in the reaction mixture, which significantly affects the competitive rates of the labeling reaction and the hydrolysis of sulfonyl chlorides. The reaction mixture was prepared by the addition of 1.0 ml of an acetone solution of tosyl chloride to a mixture of 1.0 ml of the aqueous sample solutions and 1.0 ml of a buffer solution. The dependence of

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pH of the buffer solutions and reaction time on the reaction yields is shown in Figs. 1 and 2, respectively. The buffer solutions with pH less than 7 did not allow the reaction to proceed smoothly. On the other hand, hydrolysis of tosyl chloride prevented the derivatization reaction from proceeding with high yield at pH higher than 12. The reaction at 60 °C quickly gave high yields of the labeling product compared with that at room temperature. The reaction yields of 81.6% and 78.5% were obtained at 60 °C for 15 min and at room temperature for 20 min, respectively, using the carbonate buffer (pH 10) for 10^{-5} mol/l of 4-*tert*-butylphenol. The other phenols were also converted to the tosyl derivatives under the same conditions in yields of 72.0—82.2%. The results indicate that the mild conditions in the usual tosylation system in solution do not give the excellent yields of the tosylated products.

Tosylation Reaction on ODS Solid Phase The solid phase-dispersed aqueous reaction has been preliminarily investigated in a heterogeneous system consisting of tosyl chloride adsorbed on ODS and a aqueous buffer solution of the phenols. The results suggested that ODS is suitable for solid-phase catalysis for the tosylation reaction of phenols, probably owing to good retention of both the phenols and the tosylated product.

The tosylation reaction in the ODS solid-phase aqueous system was examined in the following procedure in consideration of coupling with SPE of phenols. An aqueous sample solution (10 ml) of alkylphenols was flowed into the ODS cartridge containing tosyl chloride, followed by a run of a buffer solution as a pH control and a reaction initiator. The reaction mixture on ODS was eluted with 10 ml of CH_3CN. Figures 1 and 2 show the dependence of the yield of the solid-phase tosylation against 4-*tert*-butylphenol on pH of the buffer solutions and on the reaction time, respectively. The phenols were tosylated quickly and easily under aqueous solid-phase conditions compared with the reaction in solution. The results indicate that the derivatization of the solid phase is completed within a few minutes at room temperature using the buffer solution of pH 12. We realized that the tosylation reaction proceeded more effectively and selectively on ODS under mild conditions than in solution. The optimal conditions for the ODS aqueous reaction system are set at the reaction time of 5 min using the buffer solution of pH 12 at room temperature. The other phenols were also derivatized into the tosylated products under the same conditions in high yields of 89.3—98.2%.

The calibration curves were linear over a range of 1.0×10^{-2} to 3.0×10^{-6} mol/l for the phenols in water sample solutions with correlation coefficients between 0.9981 and 0.9997. The wide dynamic range indicates that the derivatization is carried out with high yields regardless of the concentrations of the sample solutions. The detection limit (6.0×10^{-7} mol/l) is the same as that of the standards of the tosyl phenols.

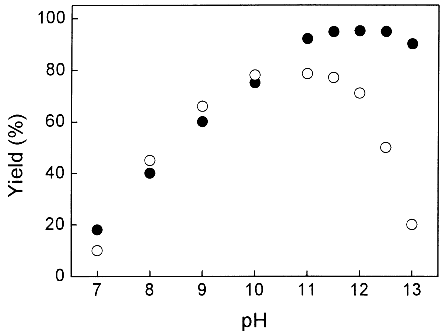

Fig. 1. Dependence of Yields of the Tosylation Reaction of 4-*tert*-Butylphenol on pH of the Buffer Solutions in the Usual Solution (○) and ODS Solid-Phase Systems (●)

The reaction yields were estimated for the reaction time of 20 min at room temperature in the solution system and for 5 min at room temperature in the solid-phase system.

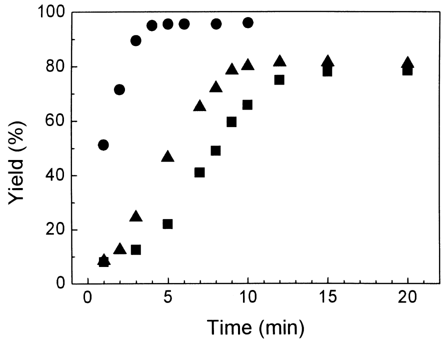

Fig. 2. Dependence of the Reaction Yields of the Tosylation Reaction of 4-*tert*-Butylphenol on the Reaction Time in an Aqueous Acetone Solution of pH 10 (■ at Room Temperature, ▲ at 60 °C), and in the Solid-Phase System at Room Temperature Using a Buffer Solution of pH 12 (●)

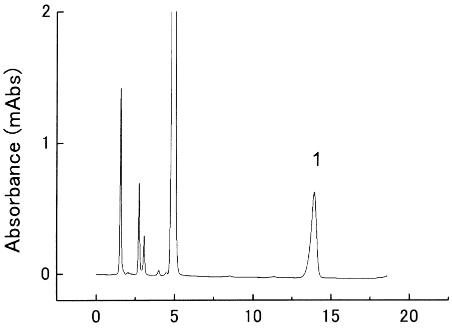

Fig. 3. Typical Chromatogram Obtained for 4-*tert*-Butylphenol 10^{-8} mol/l Spiked in Tap Water

The phenol was preconcentrated 100-fold on the ODS cartridge used for the tosylation reaction. Peak 1 corresponds to tosylated 4-*tert*-butylphenol.

SPE Followed by Direct Derivatization It is well known that commercially available ODS cartridges for SPE are suitable for the extraction and concentration of endocrine-disruptive alkylphenolic compounds from environmental water.[10] An additional advantage of the solid-phase derivatization is to simplify the analytical procedure, combined with an enrichment step by SPE of the samples. The alkylphenolic compounds in environmental water were extracted using the cartridge and were simultaneously and easily converted into the tosyl derivatives in the same cartridge with aqueous basic buffer solution. Figure 3 shows a typical chromatogram obtained for 10^{-8} mol/l of 4-*tert*-butylphenol in tap water using the present method coupled with 100-fold enrichment. The phenols at concentrations less than the detection limit give clear chromatograms without interference from a large amount of 4-methylbenzenesulfonic acid (an aqueous decomposition product of tosyl chloride)

Table 1. Recovery of 4-*tert*-Butylphenol Spiked in Tap Water Measured with the Present Solid-Phase Tosylation Method

4-*tert*-Butylphenol (mol/l)	Sample volume (ml)	Preconcentration ratio	Recovery (%)	RSD (%)
5.0×10^{-5}	100	10	97.5	2.1 ($n=10$)
5.0×10^{-6}	100	10	96.5	3.0 ($n=5$)
3.6×10^{-6}	1000	100	87.2	4.5 ($n=5$)
5.0×10^{-7}	1000	100	83.0	6.7 ($n=5$)
2.5×10^{-8}	10000	1000	72.3	18.5 ($n=3$)

RSD, relative standard deviation.

and are easily quantified by the present derivatization method. Table 1 lists the recovery of 4-*tert*-butylphenol (10^{-5} to 10^{-8} mol/l) spiked in tap water. The enrichment in the ratio of less than 100 gave high recovery with the relative standard derivation (RSD) of 2.1—3.0%. However, an increase in the ratio lowered the recovery, probably due to insufficient retainment of the phenols resulting from the prolonged run of a large volume of the sample water into the SPE cartridge. The recovery of the other phenols spiked in tap water (10^{-5} to 10^{-6} mol/l) was also examined with the present solid-phase tosylation method involving an enrichment step at a ratio of less than 100. High recovery rates (90.2—96.6%) were obtained for all sample solutions with a RSD of 3.1—5.2% ($n=5$).

In conclusion, we have developed a new approach to derivatize alkylphenolic compounds into detectable products linked by the sulfonyl group on ODS. The aqueous solid-phase derivatization is easily coupled with SPE of the samples, and it is expected that trace analyses of endocrine-disruptive alkylphenolic compounds in environmental samples can easily be recognized in an environment-friendly way, coupled with the precolumn derivatization using dansyl chloride and ferrocenesulfonyl chloride as derivatization reagents for highly sensitive fluorometric and electrochemical detection, respectively.

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