



## New approaches to extraction techniques in determination of 4,4'-methylenebis(2-chloroaniline) in air and water solutions

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### ABSTRACT

Extraction techniques for 4,4'-methylenebis(2-chloroaniline) (MOCA) in air samples and water solutions were developed and compared. Classic techniques for air sampling of MOCA were enhanced by incorporating a derivatization step (3,5-dinitrobenzoyl chloride solution in toluene), thus increasing the limit of detection and limit of quantification. Sampling of MOCA from water solution was performed using novel nanoporous polymeric (polypyrrole and polythiophene) fiber coatings and solid phase microextraction. Samples were analyzed by high-performance liquid chromatography coupled with a UV detector. Using the modified method for air sampling of MOCA, we found that the limit of detection was  $7.90 \text{ ng m}^{-3}$  and the limit of quantification was  $23.8 \text{ ng m}^{-3}$ . In contrast, the limit of detection for MOCA in water samples was  $11.26 \text{ ng mL}^{-1}$  (polypyrrole) and  $84.62 \text{ ng mL}^{-1}$  (polythiophene) and the limit of quantification for MOCA was from  $33.78$  (polypyrrole) and  $253.86 \text{ ng mL}^{-1}$  (polythiophene). Correlation coefficients were 0.9997 for air and 0.8790–0.9852 for water samples, respectively. The techniques presented provide alternative methods for the determination of MOCA in air samples and in water solutions that are more sensitive, quicker and less expensive than previously established procedures.

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### 1. Introduction

Beginning in the 1980s, use of DAPSONE has been substituted with MOCA in the polymer industry as a curing agent for urethane and epoxy resins [1,2]. Despite similarities in the structures of DAPSONE and MOCA (Fig. 1), substitution of a sulphone group in DAPSONE to a methylene group in MOCA as well as the addition of two chloride atoms in MOCA have altered the toxicological and carcinogenic properties of MOCA as compared to DAPSONE [3].

DAPSONE is a non-toxic compound with a variety of medicinal uses, including antibacterial [4], antimalarial [5] and antiprotozoan [6] applications. In contrast, MOCA has been placed on the Special Health Hazard List [7] because of its toxic and carcinogenic effects observed in mammals [8,9]. Despite these health hazards, MOCA continues to be used in polymer industry, which has spurred investigations to determine the concentration of MOCA in the air of the work environment as well as the industrial wastewater. Currently, recommendations exist only for air exposure limits to MOCA.

The National Institute for Occupational Safety and Health (NIOSH) and American Conference of Industrial Hygienists (ACGIH) recommend that the airborne exposure limits for MOCA are  $0.003 \text{ mg m}^{-3}$  and  $0.11 \text{ mg m}^{-3}$  averaged over a 10- and 8-h work

shift, respectively. In case of direct skin contact, however, an over-exposure of MOCA may occur even if air level concentrations are below the limits provided by NIOSH and ACGIH [7]. Because of the extensive use of MOCA in polymer industry, as mentioned above, parallel water analysis should be also taken under consideration. Rice et al. [13] analyzed MOCA in combination with other aromatic amines obtained from river water, however a sample preparation method was not employed.

In the last two decades, there has been extensive development of direct sampling methods. One such method is solid phase microextraction (SPME), which has been deemed one of the most accurate techniques for direct sampling [20]. An advantage of using SPME is that it allows the use polar and non-polar sorbents, which allows for the selection an appropriate coating for the determination of analytes [19].

Sample preparation and analysis of air samples containing MOCA typically consists of sampling on a fiberglass filter and tube with silica gel. After desorption with a predetermined (organic or aqueous) solution, the sample may be analyzed using liquid chromatography coupled with spectrophotometer [10–12], electrochemical detector [3,12–14] or a mass spectrometer [15]. Likewise, MOCA may be also analyzed following appropriate derivatization using gas chromatography, as described in Vaughan et al. [14–16]. Despite the monetary and ease of use benefits of gas chromatography in the detection of MOCA, liquid chromatography coupled to spectrophotometric detection is advantageous because

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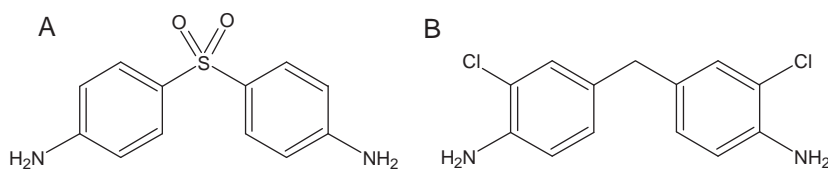


Fig. 1. Chemical structures of DAPSONE (A) and MOCA (B).

of its lower limit of detection ( $0.3 \mu\text{g mL}^{-1}$  [17]) as compared to gas chromatography ( $4 \mu\text{g mL}^{-1}$  [18]).

The main objective of this work was to evaluate the effectiveness of two synthesized polymeric coatings, polypyrrole and polythiophene, as SPME sorbents for the determination of MOCA concentrations in water samples under optimized chromatographic conditions. In addition, we determined MOCA concentrations in air samples using extraction on filters followed by derivatization. The scientific novelty of the present work is the use of a miniaturized extraction technique using two synthesized polymers as sorption phases. In comparison with the traditional extraction method using a glass filter followed by chemical derivatization, this proposed SPME technique has two major advantages: simplified assembly and use as well as decreased solvent use.

## 2. Experimental

### 2.1. Materials and reagents

The following reagents were used in the study: 2,2'-dichloro-4,4'-methylenedianiline from Riedel-de Haën (Seelze, Germany). Acetonitrile, methanol and toluene were supplied from J.T. Baker (Deventer, The Netherlands) and high-purity water produced by the Milli-Q apparatus (Millipore Intertech, Bedford, USA). HPLC grade reagents were used throughout the study. Sulfuric acid and sodium hydroxide were supplied from POCh (Gliwice, Poland) and fiberglass filters with 37 mm diameter from Whatman GF/A (Maidstone, UK).

### 2.2. Instrumentations

#### 2.2.1. Air samples

The HPLC 1200 system (Agilent Technologies, Waldbronn, Germany) with quaternary pump, automatic sample injector and UV detector was used. Chromatographic separations were performed by using the analytical HPLC  $C_{18}$  column ( $250 \text{ mm} \times 4.6 \text{ mm}$ ,  $d_p = 5 \mu\text{m}$ ) from Restek (Bellefonte, USA). Agilent Technologies ChemStation software was used for data acquisition. Gilair 5 (Sensidyne, Clearwater, FL, USA) aspirator was used for air sampling. Promax 1020 (Heidolph, Schwabach, Germany) mechanic shaker was applied for MOCA recovery from the filter. A water bath (Laboplay, Bytom, Poland) or a microwave oven (Panasonic, Kusatsu, Japan) for heating the reaction mixture.

#### 2.2.2. Water samples

The HPLC 1100 system (Agilent Technologies, Waldbronn, Germany) with quaternary pump, automatic sample injector, and UV detector was used. Chromatographic separations were performed by using the analytical HPLC  $C_{18}$  column ( $150 \text{ mm} \times 4.6 \text{ mm}$ ,  $d_p = 5 \mu\text{m}$ ) from Supelco (Bellefonte, USA). Agilent Technologies ChemStation software was used for data acquisition. For sample evaporation a Labconco CentriVap DNA concentrator (Kansas City, USA) was used.

### 2.3. Air samples preparation and analytical procedure

Air samples (100 L) were transferred through 2 filters contains sulfuric acid solution and line connected. Subsequently, each filter was placed in a separate conical flask. 2 mL of distilled water were used for the recovery of MOCA disulfate deposited on the filter and then filters were left for 1 h. Subsequently, 2 mL of sodium hydroxide solution ( $135 \text{ mmol dm}^{-3}$ ) and 1 mL of toluene were added. The flasks were shaken for 30 min. The solutions from both filters were transferred into test tubes and left there to stand until equilibrium between the aqueous and toluene phase was reached. Subsequently, 0.5 mL of the toluene layer was taken and transferred into 2 mL vessels to which 20  $\mu\text{L}$  of 3,5-dinitrobenzoyl chloride solution in toluene ( $1 \text{ mg mL}^{-1}$ ) was added. The derivatization reaction was carried out using two procedures: on a water bath ( $80^\circ\text{C}$ ; 30 min) or in a microwave oven (600 W;  $2 \times 2 \text{ min}$ ). The toluene was evaporated in nitrogen stream and the dry residue was dissolved in 500  $\mu\text{L}$  of acetonitrile. The resulting solutions were subjected to chromatographic analysis. The procedure of air sample preparation is shown in Fig. 2a.

The determination of MOCA was carried out using a HPLC type Ultra  $C_{18}$  column. Measurement temperature was  $23^\circ\text{C}$ . The mobile phase flow rate was  $1 \text{ mL min}^{-1}$ . Acetonitrile/water was used as the mobile phase (70/30, v/v). The volume of injected samples was 20  $\mu\text{L}$ . An UV diode array detector (DAD) detector with an analytical wavelength of  $\lambda = 232 \text{ nm}$  was used. Owing to such conditions, MOCA could be assayed in the presence of other aromatic amines, such as: aniline, benzidine, 4,4'-methylenedianiline and 2-chloroaniline.

### 2.4. Water samples preparation and analytical procedure

The polypyrrole and polythiophene fibers prepared via electrochemical polymerization and were used as an adsorbent for solid phase microextraction. Preparation of fibers was done using a homemade system connected with a new generation potentiostat–galvanostat, as described previously [20]. SPME fibers had a thickness in range 200–220 and 265–285  $\mu\text{m}$  for polypyrrole and polythiophene, respectively. Scanning electron microscopy (SEM) micrographs were used to visualize applied fibers (Fig. 3).

Standard solutions were prepared by dilution of stock solution in using a concentration range from 0.2 to  $20 \mu\text{g mL}^{-1}$ . Extraction time profiles using polypyrrole and polythiophene fibers were established previously (citation) and include 10 min for adsorption and 5 min for desorption experiments. Selection of desorption solutions of polypyrrole and polythiophene fibers were performed using pure methanol, methanol/water (80/20, v/v), methanol/water (50/50, v/v), methanol/ammonia, methanol/acetic acid and pure acetonitrile. Before each experiments polymeric fibers were conditioned in methanol/water (90/10, v/v) mixture. Adsorption and desorption were performed in 1.5 mL of appropriate solution. Samples after adsorption and desorption were dried under low pressure and then resolubilized in 100  $\mu\text{L}$  of mobile phase (MeOH/ $\text{H}_2\text{O}$ , 30/70, v/v). The procedure of water sample preparation is shown in Fig. 2b.

Samples were analyzed using HPLC system with UV–vis. Isocratic elution while MOCA analysis was applied. The flow rate was  $1 \text{ mL min}^{-1}$  and sample volume injection was 15  $\mu\text{L}$ . Wavelength

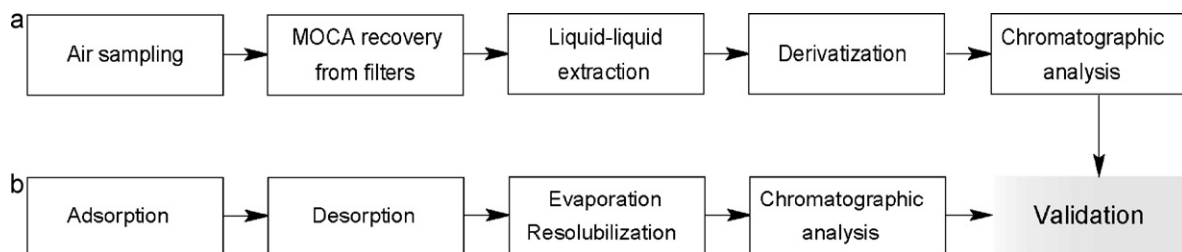


Fig. 2. The procedure of air (A) and water (B) sample preparation.

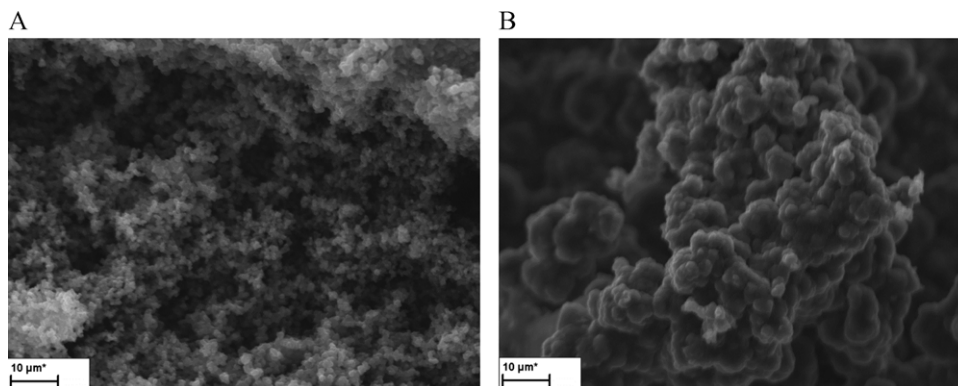


Fig. 3. SEM micrographs of polypyrrole (A) and polythiophene (B) SPME fiber coatings.

was adjust at  $\lambda = 232$  nm. Supelcosil LC-18DB column (250 mm  $\times$  4.6 mm) with particle diameter 5  $\mu$ m was applied during the analysis.

### 3. Results and discussion

#### 3.1. Analysis of MOCA from air samples

The method of sampling MOCA from air using two consecutively placed glass filters filled with sulfuric acid is a very well-known approach. We found that derivatization using 3,5-dinitrobenzoyl chloride (DNB) promoted a more rapid sample preparation step and also allowed for a lower limit of detection (LOD) and quantification (LOQ).

Derivatization and extraction of MOCA from filters at room temperature could not be accomplished in less than 1 h, which was based on the continued observation of MOCA in the chromatogram during this time period. In contrast, derivatization at higher temperature (80 °C) allowed for the extraction of MOCA within 30 min as observed with the disappearance of MOCA from the chromatogram during this time period. A similar observation was made after derivatization in microwave (600 W – 2  $\times$  2 min), however this approach allowed for a shortened time of derivatization to a total of 4 min.

Additionally, lower LOD and LOQ values allowed us to measure MOCA in the air at a concentration range from 2 to 40  $\mu$ g m<sup>-3</sup>, which was impossible using the OSHA method.

#### 3.2. Analysis of MOCA from water samples

Determination of the most appropriate desorption solution with use of pure polypyrrole and polythiophene coatings were performed using six different solutions: methanol, methanol/water (80/20, v/v), methanol/water (50/50, v/v), methanol/ammonia, methanol/acetic acid and pure acetonitrile (Fig. 4). We found that the most appropriate desorption solution for the polypyrrole coating was methanol with acetic acid, whereas the most

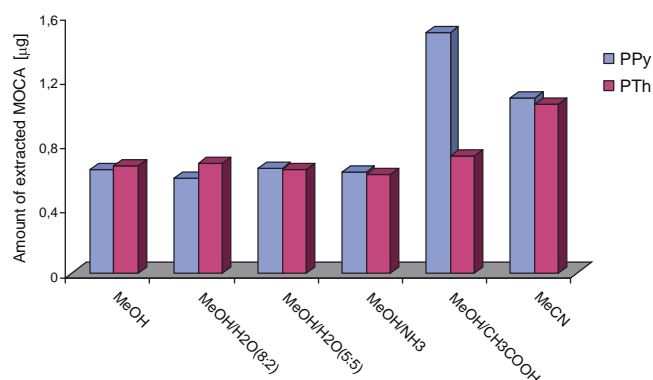


Fig. 4. Selection of desorption solution using polypyrrole and polythiophene coatings.

appropriate desorption solution for the polythiophene coating was pure acetonitrile. These desorption solutions were used in all further experiments. Methanol, methanol/water mixtures and methanol with small addition of ammonia did not allow for optimal extraction and thus were not used in the further investigations. Standard deviations calculated for all fibers and solvent mixtures were in range from 43.5 to 105.9 ng mL<sup>-1</sup>.

Prepared fibers were then used for the analysis of MOCA within an aqueous solution. These experiments were performed using six concentrations of MOCA ranging from 1 to 20  $\mu$ g mL<sup>-1</sup>. A linear dependence was observed between the quantity of MOCA extracted and the known concentration of MOCA in aqueous solution using

Table 1

Extraction of MOCA from aqueous solution and using PPy and PTh SPME coatings.

SPME coating	Slope	Intercept	RSD [%]
PPy	0.1218	0.4458	1.30
PTh	0.0338	0.7192	1.96

**Table 2**  
Validation parameters calculated for developed method for sampling of MOCA.

Parameter	Method		
	Air samples	Water samples	
	Filter/H <sub>2</sub> SO <sub>4</sub>	PPy/SPME	PTh/SPME
Concentration range	2–40 μg m <sup>-3</sup>	1–20 μg mL <sup>-1</sup>	1–20 μg mL <sup>-1</sup>
Correlation coefficient	0.9997	0.9852	0.8790
Limit of detection (LOD)	7.90 ng m <sup>-3</sup>	11.26 ng mL <sup>-1</sup>	84.62 ng mL <sup>-1</sup>
Limit of quantification (LOQ)	23.8 ng m <sup>-3</sup>	33.78 ng mL <sup>-1</sup>	253.86 ng mL <sup>-1</sup>

both the polypyrrole ( $R=0.9852$ ) and polythiophene ( $R=0.8790$ ) fibers.

Sensitivity for MOCA is four times greater for polypyrrole fibers as compared to polythiophene fibers. Differences observed in slope values (Table 1) suggest that polythiophene fibers exhibit minimal sensitivity toward MOCA and that changes in amount of extracted MOCA were minimal even as concentrations of MOCA in aqueous solution was increased.

This lower extraction efficiency for polythiophene fibers may be explained by worse adsorption properties of this material toward applied analyte as compared to polypyrrole. Likewise, decreased extraction efficiency also be a result of irreversible binding of MOCA to the surface of polythiophene via strong interactions between MOCA and the sulfur atom in thiophene (memory effect).

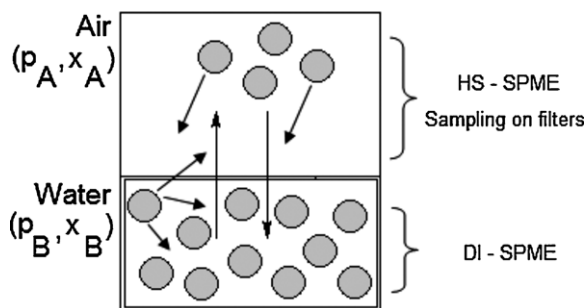
### 3.3. Comparison of developed procedure to analysis of MOCA from air and water samples

Sampling methods for MOCA from air samples (2–40 μg m<sup>-3</sup>) and water solutions (1–20 μg mL<sup>-1</sup>) were compared. Calculated validation parameters are presented in Table 2. As compared to OSHA methods [21] for detection of MOCA from air samples, our method for the detection of MOCA from air was much more sensitive allowing for a LOD nearly 20 times lower than OSHA methods.

Solid phase microextraction of MOCA from water using a polypyrrole coating ( $R=0.9852$ ) allowed for a better sorption efficiency as compared to a polythiophene coating ( $R=0.8790$ ). Similarly, LOD and LOQ for MOCA using a polypyrrole (LOD: 11.26 ng mL<sup>-1</sup>; LOQ: 33.78 ng mL<sup>-1</sup>) coating was eight times lower than that of a polythiophene coating (LOD: 84.62 ng mL<sup>-1</sup>; LOQ: 253.9 ng mL<sup>-1</sup>).

### 3.4. Relation between content of MOCA in water solution and air under the water surface

We have proposed an environmental model system (Fig. 5) for MOCA and have incorporated the presented approaches for detection of MOCA in air and in water. Based on this model system, it could be considered that if MOCA molecules are in liquid phase, MOCA may escape from the surface of the liquid and remain in



**Fig. 5.** Model of environmental system together with method using in analysis of MOCA molecules in water (liquid phase) and air (vapor phase) states.

vapor phase. Additionally, as more molecules enter to the vapor phase, the possibility of re-entering liquid phase increases as well and vice versa. Such interactions could be described by Raoult's law, which states that the vapor pressure ( $p$ ) of a solvent in a solution equals the vapor pressure ( $p_A, p_B$ ) of the pure solvent multiplied by its mole fraction ( $x_A, x_B$ ):  $p = p_A x_A + p_B x_B$ .

Using Raoult's law, it is possible to calculate the concentration of the specific compound in liquid or in vapor phases. Hence, methodologies of sample preparation of MOCA in water solutions as well as in air samples are necessary and should be taken under consideration for all analytical approaches.

## 4. Conclusions

We have presented an analytical method for the detection of MOCA from air and water samples using an HPLC system with spectrophotometric detection. In addition, a solid phase microextraction procedure has been optimized and developed for the determination of MOCA in water samples. This work is novel in that we have performed simultaneous isolation and enrichment of MOCA using homemade SPME polypyrrole and polythiophene fibers. These polypyrrole- and polythiophene-coated SPME fibers are a useful alternative for MOCA determination. In comparison with previous method based on the use of HPLC-DAD [10], our approach is more sensitive and requires a small aliquot of water sample for a single sample preparation step (1.5 mL of water in comparison to 100 dm<sup>3</sup> air). Furthermore, we have developed a novel method for clean-up of water samples using SPME techniques. This method resulted in better extraction efficiencies of target compound and was faster, easier and cheaper than previously reported procedures [10].

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