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Determination of formaldehyde and cyanide ion in human nasal discharge by using simple spectrophotometric methods

Research Article

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Abstract: Environmental tobacco smoke (ETS) contains many toxic compounds which include substances classified as aldehydes (e.g. formaldehyde) and inorganic substances such as cyanide ions. The information on the determination of these compounds in water is available, but the monitoring data on the level of these substances in human body fluids are still lacking. In this work the procedure for determining cyanide ions and formaldehyde in samples of human nasal discharge by simple spectrophotometric technique is presented.

Keywords: Cyanide ion • Formaldehyde • Environmental tobacco smoke • Nasal discharge • Spectrophotometry © Versita Sp. z o.o.

1. Introduction

Tobacco smoking has a negative impact on health and is considered a chronic illness. During cigarette smoking a complex mixture is inhaled into respiratory system [1]. Tobacco smoke can be divided into mainstream smoke (MS) which is inhaled by a smoker directly from the burning cigarette, and sidestream smoke which is emitted into the air from the smoldering cigarette [2].

A number of aldehydes have been identified in mainstream smoke, *e.g.* acetaldehyde, formaldehyde, acrolein, propionaldehyde and others. A certain percentage of aldehydes are forming from such precursors as polysacharides, pectins, proteins, tobacco triglycerides and also from natural tobacco sugars as well as sugars added to tobacco in the production process. Besides tobacco smoke, aldehydes are found in many environmental compartments such as foods (fruits and vegetables, alcohol, wine, milk and milk products), beverages and car exhaust fumes. Mainstream smoke from one cigarette produces from 70 to 100 μ g of formaldehyde. A smoker can inhale from 0.4 to 2.0 mg of formaldehyde from one cigarettes formaldehyde emission

value is 30-57 µg [4]. Formaldehyde is a very reactive compound which can cause the irritation of eyes, skin and mucous membranes [3]. The first reported adverse health effects from exposure to formaldehyde like irritation of the eyes and upper airways took place in the mid 1960s. The International Agency for Research on Cancer (IARC) in 2004 has classified formaldehyde as carcinogenic for humans (Group 1). This classification is based on cohort mortality studies of people who are exposed to formaldehyde in work with an increased incidence of nasopharyngeal cancer [4]. Discussion about formaldehyde as a possible carcinogen started in 1980 when the research indicated that the exposure to formaldehyde is associated with the occurrence of cancer of the nose and throat in humans, and squamous cell nasal cancer in rats and mice [3,4]. Formaldehyde is rapidly absorbed in the respiratory system because of its high solubility in water. As an electrophile, it can react with biogenic compounds in the body with nucleophilic property. Scientific Committee on Health and Environmental Risks (SCHER) has classified the formaldehyde as a compound of concern in indoor air. Today, formaldehyde is the best known indoor air pollutant [4].

Another contaminant present in tobacco smoke is cyanide ion which originates from hydrogen cyanide. Cyanide ions are mainly present in glycosides from which they are released under the influence of gastric juice. They also occur in various food products such as, plants belonging to the Brassicaceae family (cabbage, broccoli and cauliflower), almonds, nuts, leguminous plants, bamboo, beans, flax seeds and the pits of cherries, peaches, apricots, prunes and apples as well as mustard, cow milk and cheeses [5,6]. Moreover, cyanide ions are formed endogenously by bacteria in the large intestine [5]. The amount of cyanide ions originating from food products is however rather small. Larger concentrations of this ion result from the tobacco smoke metabolism. Cyanide ion is metabolized to thiocyanate ion. The pertinent reaction is catalyzed by the enzyme rodanase and mainly takes place in liver mitochondria. The half-life of SCNion is 6 days which is significantly longer than the half-life values of other substances, for example, nicotine [7].

Cyanide ions enter the organism via alveolar sacs, mucous membranes of oral cavity, stomach and skin [5]. During the inhalation of hydrogen cyanide the symptoms such as dryness of the mouth and the irritation of ocular conjunctiva, mucous membranes, nose and larynx appear within a couple of seconds. Low level poisoning manifests itself by anxiety, fear, headache and dizziness as well as substernal chest pain. In the case of acute cyanide poisoning, convulsions accompanied by the loss of consciousness are observed within minutes followed by the cessation of blood circulation and

breathing. In poisoning caused by large doses of cvanide the symptoms begin very fast, *i.e.*, within a couple of seconds or minutes from swallowing the substance. In people who are chronically exposed to low doses of HCN the accumulation effect has not been observed because cyanide ions are transformed into thiocyanate ions and excreted from the body. Due to its small size the cyanide ion is easily absorbed by mucous membranes and alveolar sacs. Absorption also takes place in the stomach which is facilitated by the presence of non-ionic cyanide forms in an acidic environment. After absorption in the digestive tract cyanide ions enter biochemical pathways in the liver. Small amounts of cyanides are excreted via lungs, sweat, saliva and urine [8]. The cyanide ion toxicity is caused by its affinity to iron in the mitochondrial cytochrome C. Cyanide blocks the final stage of electron transport chain which results in cellular hypoxia and changes leading to ATP depletion, metabolic acidosis and consequently to cellular death [<mark>8</mark>].

Information on many analytical techniques for determining cyanide ion and formaldehyde, particularly in water samples [9-14], food and other samples [15-20] and biological samples [23-28] can be found in literature. The techniques differ with regard to sensitivity as well as time consumption and the costs of analysis. The compilation of advantages and disadvantages of various analytical techniques used for determining formaldehyde and cyanide ion in water and other samples are presented in Table 1. Based on the data contained in the table, it is apparent that the spectrophotometric technique is simple, inexpensive

Table 1. Advantages and disadvantages of various analytical techniques used for determining HCHO and CN in water and other samples.

| Analyte | Sample | Analytical technique | LOD, μg L ⁻¹ | Sample preparation | Extraction | Detection | High cost of equipment/ analysis | Short time of analysis | Difficulty level of analysis | Labor intensity | Ref. |
|-----------------|-------------------|---|-----------------------------------|---|--------------------------------|-------------------------------------|---|------------------------------|------------------------------------|--------------------|------|
| нсно | Wastewater | Gas chromatography | 10 | on-fiber derivatization | solid-phase microextraction | mass spectrometry | +/+ | - | + | + | [9] |
| CN | Drinking water | lon chromatography | 0.68 | stabilized by addition of 2.0 g of 50% (w/w) sodium hydroxide solution | - | pulsed amperometric | +/+ | - | + | + | [10] |
| нсно | Dried food | Fluorometry | 0.02 | Ultrasonic and filtration with micropore membrane | - | - | -/- | + | - | + | [12] |
| нсно | Water, air | Spectrophotometry | 15 | pre-column derivatization | - | UV | -/- | + | - | - | [13] |
| нсно | Frozen fish | Flow injection analysis with an incorporated gel-filtration chromatography column | 2500 | homogenisation | Liquid extaction | - | +/+ | - | - | + | [14] |
| нсно | beers | air-driving flow injection | 3 | - | - | - | +/+ | - | - | + | [15] |
| нсно | water | Flow injection spectrophotomeptry | 1.5 | derivatization | - | UV | +/- | + | - | -/+ | [16] |
| CN | water | Flow injection spectrophotometry | 0.5 | - | - | UV | +/- | + | - | -/+ | [21] |
| CN [.] | water | luminescence spectrometry | 3 | - | Liquid extraction | room temperature phosphorescence | -/- | + | - | -/+ | [22] |
| + yes, - n | 10 | | | | | | | | | | |

and fast. Formaldehyde and cyanide ion are most frequently determined in water samples however there is no information indicating that the level of contamination with these compounds is being monitored in human body fluids. Spectrophotometric methods can be very sensitive and have other advantages therefore they are often applied to analytical studies.

The data presented in Table 1 shows that the most analytical techniques used for the determination of HCHO and CN in biological samples requires a very time-consuming and costly step of sample preparation including the extraction steps. The most sensitive method is ion chromatography, flow injection fluorometry and spectrophotometry. The lowest sensitivity was obtained in the case of flow injection analysis with an incorporated gel-filtration chromatography column. Spectrophotometric methods are characterized by relatively high sensitivity (LOD of the horizontal from a few to several).

Not all analytical procedures which are used to determinate HCHO in water samples can be used for analyzing the samples of human body fluids. Limited data on HCHO and cyanide ion determination in body fluids of humans exposed in different ways to the components of ETS can be found in literature. Information regarding determination of these contaminants in human body fluids such as nasal discharge is lacking.

The aim of the performed study was to develop procedures that make possible the use of technology for the spectrophotometric determination of selected pollutants from tobacco smoke in samples of nasal discharge. Until now no information has been published on biomarkers of ETS exposure in the nasal discharge samples determined by spectrophotometric techniques. Spectrometric technique was chosen as a technique because it is relatively cheap and fast. In addition, samples of nasal discharge using other techniques (chromatographic) may lead to soil columns. In spectrophotometry sample has no contact with sensitive elements of instrument which are susceptible to contamination. Determination of cyanide and formaldehyde is very important because of the need for biological monitoring of toxic substances. Therefore, the procedure described can be used for rapid determination of pollutants in samples of nasal discharge.

2. Experimental procedure

2.1. Methods and reagents

Cyanide ions react with a chlorinating agent to form cyanogen chloride, which in turn reacts with 1,3dimethylbarbituric acid in the presence of pyridine to form a violet dye (König reaction) (Reaction 1). This complex is measured at 570 nm.

$$cn^{-} + clo^{-} + 2H^{+} \longrightarrow clcn + H_{2}O$$

$$clcn + \bigcap_{N} + 2H_{2}O \longrightarrow O = CHCH = CHCH_{2}CH = 0 + H_{2}NCN + HCI$$
(1)

Formaldehyde was determined spectrophotometrically based on the reaction with chromotropic acid. In a solution acidified with sulphuric acid, formaldehyde reacted with chromotropic acid to form a violet dye that was measured at 585 nm (Reaction 2).



Data on parameters of calibration curves, limits of detection and quantification, as well as precision of the obtained analytical results are presented in Table 2. The limit of detection was calculated using the method of blind samples (solutions were prepared for the CN-ion and formaldehyde). From the mean and standard deviation of each measurement, the LOD was calculated from the formula:

LOD = $x_{average}$ +3S, where S is the standard deviation. The precision was calculated using the relative standard deviation expressed as a percentage obtained in the analysis of samples of standard solutions. Deionized water was obtained from a Millipore Gradient A10 (resistivity : 18.2 M Ω cm at 25°C) water purification system (Millipore, Bedford, MA, USA). A calibration curve obtained by dissolving various concentrations of standards in distilled water showed a linear relationship between concentration and absorbance (Table 2).

2.2. Sample preparation and the characteristic of sample donors

Samples were collected from healthy individuals exposed in different ways to harmful ETS components, and placed into sterile plastic vials. A schematic representation of the analytical procedure is shown in Fig. 1.

Samples collected from smokers, passive smokers and the control group, the latter consisting of individuals not exposed to tobacco smoke neither at work nor at home, were analyzed. The values of percent share of the specific sample donor groups are presented in Fig. 2.

| Technique | | Equation of the calibration curve | Correlation coefficient (r) | LOD, µg L ⁻¹ | Precision [%] | Expanded uncertainty | |
|-----------------------------|-------------------------|-----------------------------------|-----------------------------|-------------------------|------------------|-------------------------|--|
| Spectrophotometric | HCHO CN ⁻ | y=1.23x+0.010 y=52.079x-0.4775 | 0.996 0.995 | 50 2 | 5 5 | 10 10 | |
| HPLC HPLC/photometric (s | standards) | y=151x+90.7 y=0.0070x-0.41 | 0.992 0.996 | 0.40 | 4 | 10 - | |

Table 2. Concentration range values of spectrophotometric tests used for analyzing the samples of nasal discharge.

Fifty-nine samples of nasal discharge were taken. In all of them cyanide ion was determinated, in 29 of them the formaldehyde was determinated. Over 90% of all donors were between 21 and 30 years old.

3. Results and discussion

The analyzed compounds were detected in all samples of nasal discharge. The obtained results, calculated per one gram of sample, are presented in Table 3.

The aim of this research was to determine formaldehyde and cyanide ion concentrations in 93 samples of nasal discharge that had been collected from individuals with different exposure levels to ETS components. The highest concentrations of both compounds were measured in samples collected from smokers. High HCHO concentrations were observed in the non-smoker group which may indicate exposure to this compound from sources other than ETS. In the case of cyanide ion, its mean concentration in the smoker group was 7 times higher as compared to that in the non-smoker group. The obtained results indicate that cyanide ion concentrations in the nasal discharge samples of passive smokers are two times higher than those measured in the samples collected from nonsmokers. This confirms the hypothesis that passive exposure to ETS components also leads to substantial accumulation of toxic compounds in the human body. The concentrations of cyanide ion and formaldehyde measured in the nasal discharge samples from different donor groups are presented graphically in Fig. 3.

In order to compare the standard deviation (variance) for concentration of CN⁻ ions and HCHO identified in samples of nasal discharges for a series of results obtained from three of person groups: nonsmoking and passive smoking person, non-smoking and active smoking person, and passive smoking and active smoking person the F-Snedecor test has been applied. As a null hypothesis (H₀ : $\mu_1 = \mu_2$) assumed that the calculated values of standard deviations (variances) for comparing a series of results (nonsmoking and passive smoking person) are not statistically significantly different ($F < F_{crit}$). While the alternative hypothesis (H₁ : $\mu_1 \neq \mu_2$) assumed that the calculated values for the



Figure 1. Schematic representation of the analytical procedure for determining cyanide ion and formaldehyde in the nasal discharge samples.

compared series of results are statistically significantly different ($F > F_{crit}$).

Additionally, in order to compare the mean values for the two series of results indicated ions in samples of nasal discharges for two types of groups: nonsmoking person and passive smoking person, the t-Student test has been applied. As a null hypothesis $(H_0 : \mu_1 = \mu_2)$



Figure 2. Percent share of sample donors exposed to ETS components at varying levels.

assumed that the calculated mean values for the compared series of results (nonsmoking and passive smoking person) are not statistically significantly different ($t < t_{crit}$). While the alternative hypothesis ($H_1 : \mu_1 \neq \mu_2$) assumed the calculated mean values for the compared series of results are statistically significantly different ($t > t_{crit}$).

Analogous statistical operations were performed for a series of results obtained for ions identified in the samples assayed from nasal discharges for two groups: active smoking and nonsmoking person. In order to compare the standard deviation (variance) for concentration of selected ions identified in samples of nasal discharges for sets of results from these



Figure 3. Concentrations of HCHO (A) and ion CN⁻ (B) in the nasal discharge samples collected from non-smokers, passive smokers and smokers.

| Table | З. | Concentration range values for CN and HCHO content in | | | | | |
|-------|----|---|--|--|--|--|--|
| | | the processed samples of nasal discharge in groups of | | | | | |
| | | different exposure level to ETS components. | | | | | |

| | HCHO × 10 ⁻³ [mg g ⁻¹] | CN ⁻ × 10 ⁻³ [mg g ⁻¹] |
|-----------------------|--|---|
| NON-SMOKERS | | |
| Average concentration | 7.09 | 1.21 |
| Maximum concentration | 14.9 | 5.01 |
| SD | 6.4 | 1.5 |
| PASSIVE SMOKERS | | |
| Average concentration | 20.0 | 1.22 |
| Maximum concentration | 102 | 3.86 |
| SD | 31 | 1.0 |
| ACTIVE SMOKERS | | |
| Average concentration | 20.3 | 8.70 |
| Maximum concentration | 57.2 | 74.4 |
| SD | 1.4 | 1.5 |

SD-standard deviation

types of groups the F-Snedecor test has been used. As a null hypothesis (H_0 : $\mu_1 = \mu_2$) assumed that the calculated values of standard deviations (variances) for the compared series of results (active smoking

and nonsmoking person) are not statistically significantly different ($F < F_{crit}$). While the alternative hypothesis ($H_1 : \mu_1 \neq \mu_2$) assumed that the calculated values of variance for comparing the results of the series are statistically significantly different ($F > F_{crit}$).

In addition, the mean values for the two series of results indicated ions in samples of nasal discharges for two types of groups: active smoking and nonsmoking person, has been examined using the t-Student test. As the null hypothesis ($H_0 : \mu_1 = \mu_2$) was adopted, that calculated mean values for the compared series of results (active smoking and nonsmoking person) are not statistically significantly different ($t < t_{crit}$). While the alternative hypothesis ($H_1 : \mu_1 \neq \mu_2$) assumed that the calculated mean values for the compared series results are statistically significantly different ($t < t_{crit}$).

Taking into consideration the values of appropriate statistical parameters calculated on the basis of F-Snedecor and *t*-Student test, it was observed that concentration of CN^- ions and HCHO were statistically significantly different in one investigated group of smokers - active smoking person and nonsmoking person. The obtained results confirmed the influence of the toxic constituents of environmental tobacco smoke on the content of ions in nasal discharge samples.

| | | нсно | | CN ⁻ | | | | |
|---|--|---------------------------------------|--|---|---------------------------------------|--|--|--|
| | Calculated Calculated F _{sample} value F _{crit} value | | Verification of the null hypothesis against the alternative one | Calculated F _{sample} value | Calculated F _{crit} value | Verification of the null hypothesis against the alternative one | | |
| Non-smokers and smokers person | 0.22314 | 2.95824 | μ=μ | 0.01095 | 2.09206 | μ=μ | | |
| Non-smokers and passive smokers person | 0.04585 | 3.32583 | $\mu = \mu$ | 2.05914 | 2.53424 | $\mu = \mu$ | | |
| Passive smokers and smokers person | 0.48673 | 0.48673 2.60215 μ=μ | | 0.00532 | 2.63712 | $\mu = \mu$ | | |
| | | нсно | | | CN ⁻ | | | |
| | Calculated t _{sample} value | Calculated t _{crit} value | Verification of the null hypothesis against the alternative one | Calculated t _{sample} value | Calculated t _{crit} value | Verification of the null hypothesis against the alternative one | | |
| Non-smokers and smokers person | 1.93000 | 1.73960 | μ≠μ | 1.71000 | 1.68385 | μ≠μ | | |

| Tabel | 4. | Calculated F | and tampia | values fo | or analy | tes determinate | ed in | nasal | discharge | samples |
|-------|----|--------------|------------|-----------|----------|-----------------|-------|-------|-----------|---------|
|-------|----|--------------|------------|-----------|----------|-----------------|-------|-------|-----------|---------|

Table 5. Effect of interfering ions on the determination of formaldehyde and cyanide ion with the optimal reactant concentrations.

| Species | Tolerance limit | | | | |
|---|-----------------|-----------------|--|--|--|
| | нсно | CN ⁻ | | | |
| Ca ²⁺ [mg L ⁻¹] | 1000 | 1000 | | | |
| F [.] [mg L ^{.1}] | 100 | 1000 | | | |
| NH₄⁺ [mg L⁻¹] | 1000 | 1000 | | | |
| Cu ²⁺ [mg L ⁻¹] | 100 | 10 | | | |
| Cd ²⁺ [mg L ⁻¹] | 1000 | 100 | | | |
| NaCI [%] | 5 | No data | | | |
| NO ₂ - [mg L ⁻¹] | No data | 10 | | | |

The calculated $\rm F_{sample}$ and $\rm t_{sample}$ values for analytes determinedin nasal discharge samples are presented in Table 4.

3.1. Interference study

Nasal discharge samples are very specific materials to analyze. Therefore, it is necessary to investigate the effect of matrix on the analysis results. Performed investigation of the effect the content of selected substances on the outcome of the determination of cyanide and formaldehyde in nasal discharge samples. The effect of various potential interferences concurrently present in the sample on the determination of formaldehyde and cyanide ion was investigated. The effect of the conditions employed in fluoride, calcium, cadmium, ammonium, copper ions do not interfere in this study even at 100 or 1000 times higher concentration than formaldehyde and cyanide ion concentration. The results showed that the developed method had a good selectivity, and was not subject to the interferences. Summary data are presented in Table 5.

3.2. A comparison between already applied procedures of HCHO and CN⁻ determinations in human samples

In the literature can be found data about the determination of cyanide and formaldehyde in biological samples. The procedure involved the extraction step and derivatization of the sample. The literature data are available for the determination of cyanide and formaldehyde in biological samples. In 1998 Chinaka et al. developed a procedure for the determination of cyanide in blood samples using ion chromatography with fluorescence and ultraviolet detection. The procedure involved the extraction step and derivatization of the sample. It was used for cyanide determination in blood samples of smokers and fire victims. It is a method for mean cyanide ions at a low level (3.8 pmol mL). The selected sample preparation is time consuming, but necessary in the case of samples such as blood, which contain significant amounts of proteins and other substances that may affect the outcome of the analysis [23]. In 2012 Desharnais et al. developed procedure for the determination of cyanide in blood samples and gastric contents collected from the

victims of the fires and suicides. They used the technique of headspace gas chromatography coupled to mass spectrometry. The detection limit was 0.02 mg mL⁻¹ [24]. Free cyanide ion was determined by Meng *et al.* in 2009 using a home-made hollow fiber-protected liquid-phase headspace microextraction to capillary electrophoresis followed with UV detection in urine and saliva samples. The limit was 0.01 detection μ mol L⁻¹ [25].

In 2001 Luo *et al.* developed methods to determination of formaldehyde in blood plasma by high performance liquid chromatography with fluorescence detection. The limit of detection was 0.46 μ g mL⁻¹. They determined the fluorescent product of the chemical reaction between formaldehyde and ampicillin which was extracted from the matrix with diethyl ether [26]. Formaldehyde was also measured in urine samples using HPLC [27]. Another method for the determination of formaldehyde in samples of blood plasma resonance is fluorimetry technique. The procedure was developed in 2011 by Wang *et al.* Samples were determined after extraction, and they obtained LOD = 3.80 ng mL⁻¹ [28].

Each of these methods requires time-consuming step of sample preparation for analysis, including various types of extraction techniques. We have developed a

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procedure that allows the determination of cyanide ions without extraction and sample preparation is limited to dilution with deionized water and centrifugation). Using spectrophotometric techniques without extraction stage for nasal discharge samples is possible due to the lack of color, which is not possible with blood samples.

4. Conclusions

The proposed analytical procedure allows fast and inexpensive determination of CN⁻ ion and HCHO contents in the nasal discharge samples by means of spectrophotometric technique. The research indicates that nasal discharge is a biological material which - when analyzed for the presence of chosen contaminants originating from ETS - can be used to assess the exposure level to mainstream or sidestream ETS.

The obtained results show that the highest concentrations of CN^{-} ion and HCHO were measured in the samples collected from smokers. Moreover, it is noteworthy that the mean concentration of these contaminants measured in passive smokers is two times higher as compared to the control group.

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