CHAPTER 15
ANALYSIS OF WATER DISINFECTION BY-PRODUCTS.

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ABSTRACT
This paper outlines the causes of the formation of water disinfection by-products. It discusses the impact of these by-products on the quality of water and describes the main methods, both organic and inorganic, of analysis of the by-products that occur when using chlorine, chlorine dioxide and ozone. Special attention has been drawn to the occurrence and analysis of biodegradable organic matter. The role of biodegradable organic matter, from the point of view of water biostability, is also discussed in some detail.
1 WATER TREATMENT – AIMS AND METHODS.
Water is one of the basic human needs. The role of water treatment companies is to acquire water from its natural sources, which may be found either underground or on the surface, and prepare it so as it does not endanger the health or life of the consumer. The quality of drinking water is strictly enforced both on the international level, by the European Directive, and on a national level by the Health Ministry. Under the law, water quality is measured by taking into account chemical and microbiological factors. There are three parameters within which water quality is tested:

- microbiological parameters, which provide the most essential requirements for microbiological purity of water,
- chemical parameters, which provide the purity of water in terms of harmful or dangerous chemical compound (or ion) content,
- organoleptic parameters, such as taste, smell, turbidity and color of water which make it either fit or unfit for drinking, especially in the opinion of the consumer.
Despite the rather ‘descriptive’ character of these parameters, they are very important from the point of view of the consumer, e.g. water having a bad smell does not necessarily have to be bad for health. On the other hand, water which is turbid may indicate some sort of risk connected with the presence of bacteria or viruses adsorbed on the colloids or suspended matter.

The task of water manufacturers is to prepare the water so it fulfils the current regulations. The process of preparing the water before passing it on into the distribution network is called water treatment or water conditioning. Water conditioning depends mainly on the quality of untreated water – there are major differences between the conditioning of underground and surface water.

- Physical processes- these are mainly to do with separating the solid phase from the water, which means eliminating the suspension, through straining, filtration, sedimentation and flotation. Adsorption is also a physical process used in the water conditioning process, and through it, organic micropollutants are eliminated. There are also membrane processes, which not only eliminate colloids but also high and low molecular chemical compounds. Water disinfection by means of UV rays is also classed as a physical process.

- Chemical processes – these are widely used in water conditioning; they employ a wide range of oxidizers, such as oxygen from the air, ozone, chlorine, chlorine dioxide, chloramines, and potassium permanganate. These processes are used when oxidizing some inorganic water admixtures (Fe$^{2+}$, Mn$^{2+}$, NO$_2^-$, H$_2$S etc.), they assist in oxidizing organic components and water contaminants, and they are used as disinfectants.

- Biological processes- these are used both knowingly and unconsciously in water conditioning. For example, it is accepted that the process of eliminating Fe and Mn during filtration through rapid filters is aided by ferric and manganese bacteria. Nitrification, denitrification and aerobic elimination of organic matter from water are conscious and intentional processes in water treatment. Biological processes are also used when purifying water in slow filters, and are used intensively when infiltrating surface water to water-bearing deposits. This phenomenon is used on a large scale during artificial infiltration. Biological processes such as the biochemical oxidation of sulphides or autotrophic denitrification of water-bearing deposits, can lead to significant changes in water quality, for example by increasing water mineralization.
The most important aim of water conditioning is microbiological purity and even though the disinfection process may lead to many chemical by-products, the microbiological quality of water takes priority; there cannot be a compromise between the microbiological quality of water and the quantity of disinfection by-products. After going through the above unit processes, the water must fulfil the requirements set by the Health Minister’s decree from 19 November 2002 [69]. This decree regulates the allowed concentration of about 70 parameters of water, which means that water is the best-controlled consumer product on the market. The decree also regulates the allowed concentration of disinfection by-products. These are shown in Table 1.

TABLE 1. Permissible concentration of disinfection by-products according to the Health Ministry’s decree [69].

<table>
<thead>
<tr>
<th>D. Disinfection by-products in µg/l</th>
<th></th>
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<tbody>
<tr>
<td>46</td>
<td>Bromates</td>
</tr>
<tr>
<td>46a</td>
<td>Bromates</td>
</tr>
<tr>
<td>47</td>
<td>Bromodichloromethane</td>
</tr>
<tr>
<td>48</td>
<td>Chloramines</td>
</tr>
<tr>
<td>49</td>
<td>Chlorates</td>
</tr>
<tr>
<td>50</td>
<td>Chlorites</td>
</tr>
<tr>
<td>51</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td>52</td>
<td>Tetrachloromethane (carbon tetrachloride)</td>
</tr>
<tr>
<td>53</td>
<td>Trichloroacetic aldehyde</td>
</tr>
<tr>
<td>54</td>
<td>Trichloromethane (chloroform)</td>
</tr>
<tr>
<td>55</td>
<td>2,4,6-trichlorophenol</td>
</tr>
<tr>
<td>56</td>
<td>Σ THM</td>
</tr>
<tr>
<td>56a</td>
<td>Σ THM</td>
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</tbody>
</table>

\(^5\) The range of values are given in § 12 of the decree. According to Appendix No 2 the values for bromates shown under 46a and those for THM shown under 56a are in use since 1 January 2008.

It is worth noting here that only about 1-1.5% of water produced by water treatment companies is used for consumption purposes, the rest being used outside the ‘food industry’.
2 DISINFECTION- THE MOST IMPORTANT STAGE OF WATER CONDITIONING.

Water disinfection can be carried out using physical and chemical methods. UV radiation, ultrasound and thermal methods all come under the category of physical processes. UV rays are widely used in disinfection of water in the food industry, e.g. bottled water is usually disinfected using UV radiation. Water disinfection by means of UV rays is used sporadically in distribution networks because the water must be of a very high quality. Using UV radiation for disinfection of water does not give any by-products but it does not guard the distribution network against secondary bacterial contamination. Using ultrasound in disinfection is very effective but from the economic point of view, it is quite unprofitable because of the high-energy input needed. Thermal methods- boiling, in other words, is obviously impossible to put into practice because of the amount of water needed for public requirements. Chemical methods implement strong oxidizers such as chlorine, chlorine dioxide, chloramines, ozone and potassium permanganate.

Chlorine is the most widely used method in disinfection because of its efficacy, and because it is economical. It has been used as a disinfectant since the 19th century. Its use has largely contributed to the disappearance of many infectious diseases carried in water, which were in the past the cause of many an epidemic. For example, in Poznań in the 18th century, scores of cholera epidemics caused the town’s population to decrease by a third! If that were to happen today, the figure would reach the 200 000 mark. With the introduction of chlorine into the water conditioning process, the number of epidemics caused by diseases carried in the water drastically declined. Fig. 1 illustrates the influence of introducing chlorine into the water disinfection process on deaths caused by typhoid fever.

![Graph](image)

Figure 1. Typhoid fever mortality vs. disinfection of water using chlorine.
- typhoid fever mortality rate, ■ population of treated water users
Using chlorine results in by-products, which are mainly chloroorganic compounds. Bromo- and chlorobromoorganic compounds can also occur. These compounds occur as a result of a reaction between hypochlorous acid or hypochlorite with the so called by-product ‘precursors’, which is the natural organic matter found in the water. Organic matter is mainly made up of humus substance which is soluble in water. It is therefore no wonder that process engineers have turned their attention to organic compounds found in the water that were previously thought entirely harmless.

These by-product precursors also react with chlorine dioxide, ozone, and chloramines. There are fewer by-products when using chloramines rather than chlorine, and they are also more durable in the water but they are evidently less germicidal. Chlorine dioxide is a disinfectant that was at first thought not to react with organic matter and its main by-products to be chlorate and chlorite. However, chlorine dioxide does not produce chloroorganic by-products and in this way, it is a much safer disinfectant.

Ozone reacts intensively with organic matter, causing bigger particles of humus to decay into smaller ones, and more than that, it generates many low molecular organic compounds that belong to aldehydes, short chain carboxylic acids and ketoacids. These compounds are easily biodegradable and thanks to this characteristic, they can be removed in the filtration process by biologically active filters.

3 BY-PRODUCTS OF WATER DISINFECTION USING CHLORINE, CHLORINE DIOXIDE AND OZONE.

Interest in water disinfection by-products was sparked in 1974 with the almost simultaneous publication of two papers [1,2] proving the formation of chloroform as a result of disinfecting water with chlorine. This information was then associated with published reports that claimed that this compound was carcinogenic. The two papers triggered off studies that led to the discovery of many other compounds, by-products of using chlorine as well as new ways of conditioning water. These discoveries also led to a much better understanding of processes occurring during disinfection and of using strong oxidizers in treating water. They were made possible by the development in trace analysis, since by-products of chlorination usually occur at ppb or ppt concentration level. Studies into by-products of ozonization have been recognised as very important since they have brought to attention the crucial role that biodegradable organic matter plays in water.

3.A By-products of water chlorination (THM, haloacetic acids, MX).

Chlorine, depending on its pH, is introduced into water as the soluble gas Cl₂, hypochlorous acid HOCl, and hypochlorite ion ClO⁻. In the case of sodium hypochlorite being introduced into water, the result is hypochlorite ion, as shown below:

At pH>2 :  
\[ \text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HOCl} + \text{Cl}^- \]

At pH>7.5 :  
\[ \text{HOCl} \rightarrow \text{H}^+ + \text{ClO}^- \]

As results from the above equations, chlorine, when it is introduced into water, undergoes disproportionation after which it may appear in the water as undissociated hypochlorous acid or hypochlorite ions. The existence of Cl₂ as a dissolved molecule is limited to a very narrow range of very low values of pH<2. The ratio of undissociated HOCl
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molecules and ClO\(^-\) ions depends on the solution pH. Because pK\(_a\) \(\text{HOCl} = 7.5\), one can say that in case of pH = 5.5 almost 100% of HOCl will occur in the undissociated form, but in case of pH = 9.5, hypochlorite ions alone will almost entirely be present in the water. It is possible to estimate the HOCl fraction of dissociation at a given pH level, as fig. 2 shows. The dissociation curves of HOBr, of which pK\(_a\) = 9.0, are also shown. The figure shows that HOBr, as a product of oxididation of bromides by hypochlorous acid, will occur in the water mainly in the undissociated form, which explains why bromoorganic compounds are formed so easily in the process of chlorination.

![Dissociation curves of HOCl and HOBr](image)

Figure 2. Dissociation of HOCl and HOBr as a function of solution pH.

This analysis is significant when taking into consideration the main aims of chlorination. HOCl and ClO\(^-\) differ decidedly with respect to their bactericidal properties. On the other hand, undissociated HOCl reacts more easily with organic matter, giving chlorination by-products.

There are perhaps hundreds (even thousands) of compounds which occur when organic substances present in raw water react with chlorine, and which all come under the name of by-products. These compounds occur in small amounts, but because of their nature (toxic, mutable or carcinogenic) they must all be taken into account in the final assessment of the quality of the conditioned water. The source of organic carbon in reactions yielding chlorination by-products, may be naturally present organic substances, such as humus, as well as compounds that are anthropogenic, such as phenol. The most common chlorination by-product formation reaction can be presented in the following way:

\[
\text{THM precursors} + \text{chlorine} \rightarrow \text{THM} + \text{other by-products}
\]

Most chlorination by-products are chloroorganic compounds, however there are some substances, which, under the influence of this oxidizer, do not contain chlorine in the molecule, e.g. aldehydes or carboxylic acids. Bromo- and chlorobromoorganic compounds constitute a separate group of by-products, which occur when bromides present in
untreated water are oxidized. (Iodoorganic compounds can even occur in water containing iodides)

The measure that gives the total amount of haloorganic compounds after chlorination is called total organic chlorine (TOX). It is a good indicator of chlorination by-products. Another parameter well correlated in most cases with TOX, or with a potential of THM formation, is water absorbance when $\lambda = 254$ nm [3].

Chlorination by-products can be divided into two groups: non-volatile haloorganic compounds (NVOX) and volatile haloorganic compounds (VOX). Usually, the amount of NVOX equals around 70-80% of TOX whilst the amount of VOX equals around 20-30% TOX. It is worth remembering that it is the volatile group of by-products which is well known and researched, and so it is this group that is usually determined quantitatively. NVOX compounds are mainly haloorganic acids and a whole range of other products that do not yet have any fixed chemical structures.

As has been already said, using chlorine in the process of conditioning water entails the danger of potentially dangerous by-products occurring. Listed below are the most important chlorination by-products:

**Trihalomethanes (THM):** chloroform, bromodichloromethane, chlorodibromomethane, bromoform

**Haloacetonitriles:** bromochloroacetonitrile, dibromoacetonitrile, dichloroacetonitrile, trichloroacetonitrile

**Haloorganic acids:** chloroacetic, dichloroacetic acid, trichloroacetic acid

**Haloketones:** dichloroacetic aldehyde, trichloroacetic aldehyde

**Halogenates:** 1,1-dichloropropanone, 1,1,1-trichloropropanone, 1,1-dichloro-2-butanone, 1,1,1-trichloro-2-butanone

**Chlorophenol:** 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol

**Others:** chloropicrine, cyanogen chloride, MX.

Additionally, like in all oxidation processes, some by-products are formed which do not contain chlorine, such as formaldehyde and acetic aldehyde.

The presence of trihalomethanes in chlorinated water was first observed in 1974 [1,2]. Because chloroform, the main representative of this group, is classed by the World Health Organisation as a potentially carcinogenic substance and because the presence of trihalomethanes in chlorinated water is common, water which is to be used for consumption purposes has a permissible concentration of THM. Since that time, a great deal of research has been performed on the by-products of oxidizers used on water. The use of chlorine has been gradually cut down (especially where it was used as a technological oxidizer) and other oxidizers and disinfectants have been implemented. Furthermore, all methods of removing organic matter from water have been given a higher status.

It is also necessary to emphasize the other properties of hypochlorous acid: when it reacts with bromide ion, depending on pH, HClO oxidizes it quantitatively to hypobromous acid HOBr or to hypobromite ion BrO$^-$$^-$, in accordance with the reaction:

\[
\text{HOCl} + \text{Br}^- \rightarrow \text{HOBr} + \text{Cl}^- \\
\text{OCl}^- + \text{Br}^- \rightarrow \text{OBr}^- + \text{Cl}^- 
\]

The resulting hypobromous acid can also react with the organic matter present in water. These reactions lead to the occurrence of bromorganic or chlorobromorganic compounds during water chlorination. Examples of such compounds are dichlorobromomethane, chlorodibromomethane and bromoform. The reader may find
exhaustive discussion of the problems resulting from the presence of bromides in conditioned water in the extensive review articles [69,70].

The second most important group of compounds, by-products of water treatment are haloacetic acids; these can be chloro-, bromo- and mixed chlorobromoacetic acids. It has been proven that they always occur with trihalomethanes.

Mutagenicity of water may well characterize surface as well as conditioned water. This activity is the result of mutagenic compounds present in the water. Mutagenicity is defined as the capacity of a compound to induce mutations, and it is recognized as one of the indications of genotoxicity. Proving that a chemical compound is mutagenic, may also mean that it is carcinogenic [6]. Studies on carcinogenicity have to be carried out on animals. They are expensive and long lasting (a few years). Mutagenicity is studied on bacteria and the universally used Ames test takes only a few days [6]. This test is performed on a bacterial strain called *Salmonella Typhimurium*. The test organism contains the (his-) mutation that makes it incapable of producing one of the enzymes taking part in synthesis of histidine, an essential ingredient of proteins. As a result of such a mutation the bacteria is unable to grow on a substrate not enriched with histidine. This phenomenon is called food mutation. Back mutation reinstates the ability of histidine synthesis and at the same time makes it possible for the bacteria to grow on a mineral substrate. The level of back mutation is considerably increased if the bacteria are exposed to some mutagenic factors. The Ames test permits a quantitative assessment of this type of activity. More information about the Ames test can be found in „Zarys ekotoksykologii” [6]. The significance of the mutagenic activity for humans is that there is a high correlation between the mutagenicity in the Ames test and the incidence of tumours (cancer) in long-term tests done on animals. Epidemiological studies reveal a correlation between the amount of organic compounds in drinking water and the incidence of tumours. However, giving extracts of water to experimental animals does not confirm the danger. A rise in mutagenicity of water after chlorination has repeatedly been demonstrated. Chloramination and disinfection with chlorine dioxide have a lesser effect on mutagenicity. Results with ozone, however, are inconclusive. A number of aldehydes, the by-products of water ozonization (formaldehyde, acetaldehyde, glyoxal, methylglyoxal), are mutagenic. The range of mutagenic compounds formed during chlorination is much bigger. Among the many already identified mutagenic chlorination by-products, are [11]: bromomethane, dibromomethane, bromochloroacetonitrile, dichloroacetonitrile, bromobutane, bromochloromethane, bromodichloromethane, 1,2-dichloroethane, bromoform, dichloropropene, bromopropane, iodoethane, trichloroacetic aldehyde, chlorodibromomethane. This diversity of mutagenic compounds in chlorinated water is responsible for but 10% of total mutagenic activity of the water.

An especially mutagenic substance is a compound at first isolated from chlorinated wood pulp and afterwards found in drinking water, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, also known as MX [14-16]. Figure 3 presents the structure of MX.
3.B By-products when using ClO₂.

When using ClO₂ in conditioning water, the main by-products are chlorates and chlorites. When chlorine dioxide reacts with inorganic, reductive components of natural water, 60-70% of it is a single electron reaction:

\[
\text{ClO}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{ClO}_2^-
\]

\[
2\text{ClO}_2 + \text{Mn}^{2+} + 2\text{H}_2\text{O} \rightarrow 2\text{ClO}_2^- + \text{MnO}_2 + 4\text{H}^+
\]

\[
2\text{ClO}_2 + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow 2\text{ClO}_2^- + 2\text{H}^+ + \text{NO}_3^-
\]

Minor amounts of chlorates (ClO₃⁻) can usually be detected as a result of the decay of ClO₂ in water. The reaction between ClO₂ and organic matter is also a single electron one. However, this type of reaction is much slower than that with inorganic matter. The products of the reaction with organic matter are aldehydes and low molecular organic acids. As opposed to chlorine, chlorine dioxide does not produce any chloroorganic by-products. This is one very clear advantage of ClO₂ over chlorine.

Therefore, the analytics of the by-products of usage of chlorine dioxide, should include both inorganic products (chlorates and chlorites) as well as organic by-products like aldehydes and carboxylic acids. Chlorine dioxide is used chiefly as a disinfectant and as such, the disinfection by-products occur mainly in the distribution networks. This may have a significant effect on the quality of water.

3.C By-products of ozonization

In contrast to the other, already mentioned disinfectants, ozone cannot be used in the final stages of disinfection, due to its instability in water. An ozone molecule lives for ca. 20-30 minutes in water at neutral pH (the time depends heavily on the pH) which does not safeguard the distribution network against secondary bacterial infection. When ozone reacts with organic matter, it also leads to the formation of many by-products. Ozone attacks primarily all C=C double bonds. The results of such reactions are carbonyl bonds, chiefly aldehydes. Ozone reacts relatively easily with aromatic rings, results in a decrease in water coloration. Reacting with humus, ozone causes macromolecules to break down into smaller ones. Ozonizing humus present in the water leads to the formation of aldehydes. These are chiefly formaldehyde, glyoxal, acetaldehyde
and methylglyoxal. The dominating product is formaldehyde, the concentration of which varies from a couple to a couple of dozen µg/l \([58,59,60-62]\). Aldehydes occur as a result of the degradation of humus, and this process comes about through the cycloaddition reaction of \(\text{C} = \text{C}\) double bonds or through an attack on the aromatic rings present in the complex molecules constituting humus.

Formation of aldehydes during ozonization depends on a number of factors; inter alia, the amount of organic matter (TOC), pH of water, its temperature, and the presence of other substances (carbonates, hydrogencarbonates) to name but a few. \([58,63,59,64-66]\).

Another effect of the reaction is formation of low molecular carboxylic acids. Both aldehydes and carboxylic acids belong to a group of easily biodegradable compounds. Formation of these compounds, easily available for bacteria is another important reason why ozone is not used in the final stages of disinfection, just before the water is passed into the distribution network. Analysis of ozonization by-products should consequently include these two groups of compounds. The results of research done in the institute has shown that all oxidizers used in disinfection generate aldehydes and carboxylic acids, in other words products which biodegrade easily. These products are formed relatively quickly during ozonization (the ozone is usually in contact with the water for only 10-20 minutes) and they are quickly removed in the filtration stage on the beds of biologically active carbon filters. As concerns chlorine and chlorine dioxide, the reactions in which these biodegradable products are formed, occur at a much slower rate and, what is more, they occur in the distribution networks. The process of formation of these compounds always occurs at the expense of the disinfectants (chlorine or ClO\(_2\)) – in other words, the concentration of the disinfectant in the distribution network decreases while the concentration of carboxylic acids and aldehydes increases. This process puts the quality of water at risk especially when the disinfecting reagent is chlorine dioxide.

4 METHODS OF ANALYSING THE BY-PRODUCTS OF WATER CHLORINATION.

4. A. Methods of analysing trihalomethanes.

There are four compounds which usually fall under the name of trihalomethanes: chloroform, dichlorobromomethane, chlorodibromomethane and bromoform. The quantitative ratio between them depends on the amount of bromide in the water and on the given dose of chlorine. The higher the concentration of bromide, the bigger the bromoorganic THM content. The natural concentration of Br- in water is very low and does not exceed a couple to a couple of dozen micrograms per litre. A higher concentration of bromides is observed when the acquired water is contaminated with sea water or simply contains salt (e.g., mine water). Trihalomethanes are always analysed using the gas chromatograph with an electron capture detector as the chromatograph is very compatible with the type of compounds being analysed in addition to high sensitivity of the detector. In this way, individual methods of analysis differ from each other in the procedure of the sample preparation. The same course of analysis can also be used to determine other chloro and bromoorganic compounds which appear after chlorination. The most frequent method of sample preparation used for analysis of trihalomethanes is as follows:

- extraction of THM into an organic solvent: n-pentane, n-hexane, n-heptane, petroleum ether, methylcyclohexane, 2,2,4-trimethylpentane \([4]\),
- head space analysis, in both static and dynamic versions, as well as with the solid phase microextraction method,
- extraction into the solid phase - both the classic phase in small columns and solid phase microextraction,
- dosing water samples directly onto the chromatographic column.

Before analysing water for the presence of THM, the analyst should be aware of the concentration of THM to be expected in the samples. Chlorinated water, which fulfils the current standards, should not contain more than 30 µg of chloroform per litre. Therefore, the standard solutions prepared should correspond to this concentration level.

4.A.1. Liquid-liquid extraction

Because liquid-liquid extraction requires high purity solvents, all the solvents should be tested for the presence of trihalomethanes, prior to use. The respective analytical procedures should include determination of the calibration curve obtained by extraction of model aqueous solutions. Subsequently, the differences in extraction recoveries between the individual trihalomethanes become negligible. However, if the calibration curve is based directly on standard solutions of individual THMs in an organic solvent, the differences in recovery should be taken into account. The differences in the recovery result from the differences in the distribution ratio. These differences can be neglected when the extraction is complete, i.e., when even the substance most difficult to extract is almost entirely transferred to the organic phase. This condition is usually met when using a fairly large amount of organic solvent. Nonetheless, it should be remembered that such an approach yields relatively diluted extracts.

4.B. Methods of analysing haloacetic acids.

The haloacetic acids – by-products of water chlorination – include: monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA). If bromides are present in the disinfected water, bromo- and chlorobromoacetic acids can also occur: monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), dibromochloroacetic acid (DBCAA) as well as dichlorobromoacetic acid (DCBAA).

Determining the haloacetic acids means acidifying the water sample to pH<2. This reverses the acid dissociation, making it possible to extract them into the organic solvent immiscible with water. Methyl tertiary-butyl ether (MTBE) is usually used for this purpose. The extraction of these polar compounds is aided by salting out. The extracted acids are methylated with diazomethane into respective methyl esters, these esters being separated using gas chromatography with an electron capture detector. Diazomethane is generated from diazole. The detection limit of this method is ca. 0.5 µg/l, except for chloroacetic acid that has a limit of about 1 µg/l [45].

4.C Methods of analysing MX.

As has been mentioned before, MX is a compound which is especially mutagenic and comparable in this respect only to aflatoxins. Many fears were raised when MX was first discovered in chlorination by-products. The role of analytics was particularly important, as this compound is present in water at concentrations varying from several to several dozen ng/l. Development of effective methods of isolation, preconcentration and analysis of MX was particularly difficult as there are many other compounds in the water at this level of concentration. The first commonly used method of MX determination in tap water was proposed by Kronberg [46]. It was based on preconcentration of analyte from water samples by adsorption on XAD Amberlites and
extracting it using ethyl acetate. After evaporating the solvent in the stream of nitrogen, the dried analyte was methylated with a 2% solution of sulphuric acid in methanol. Subsequently, the reaction mixture was extracted with n-hexane, and the hexane extract was analysed using the GC-MS system, usually with a high-resolution mass spectrometer.

As mentioned above, before determining the MX in tap water extracts, the analyte undergoes derivatization with methanol, so that the hydroxyl group changes to a methoxyl group. The process of derivatization with methanol has many undeniable advantages, such as simplicity, low temperature of the process, as well as simple separation of the derivatization product from the excess of reagents used, especially the aggressive sulphuric acid.

The methyl pseudo-ester is identified with the aid of a mass spectrum. The two isotope ions having the highest intensity, have an m/z ratio of 147 (primary peak) and 149 (see fig. 1.2), formed by splitting the dichloromethyl group from the molecular ion. In spite of their high relative intensity, these ions are not used either in MX identification or in quantitative determination in the low-resolution mass spectrometry system, due to their small specificity in the complex drinking water extract. A triplet of isotope ions of m/z ratio 199, 201, and 203, obtained by the way of fragmentation [M – OCH3], is used for this purpose. (The triplet is the result of the presence of three chlorine atoms participating in this fragmentation.) A disadvantage of this triplet is its comparatively low relative intensity: 12.6% (199), 21.5% (201) and 13.2% (203) defined vs. the primary peak, i.e. the isotope ion of the dominating fragmentation [M –CHCl2] with an m/z of 147 (the isotope ion [M –CHCl2] including the nuclide $^{35}$Cl). Theoretically, the mutual ratio of intensity of an ion isotope having 3 chlorine atoms is equal to 100/97/32 [47]. In the case of an MX methyl pseudo-ester, the mutual ratio of intensity of the triplet is 58/100/61 because of the superposition of the fragmentation ion of m/z 201 formed in the [M– (H+CO)] fragmentation. It is possible to distinguish two different ions of m/z 201 only using a high-resolution mass spectrometer [48]. Because of the low relative intensity of the triplet of m/z 199/201/203 and interference of the ion of m/z 201 derived from a different fragmentation process, detecting low concentrations of MX in water is difficult. The second drawback of the method is the occurrence of methylation by-products, known as acetals, formed as a result of the furanone ring opening. Such compounds were found in many chlorinated hydroxyfuranones, including MX [49].
It was necessary to find a derivatization method which would give a characteristic triplet of greater intensity in the GC-MS technique by changing the alcohol used. Such research was carried out in the Department of Water Treatment Technology at the Adam Mickiewicz University in Poznań.

Of all the many alcohols tested, the ones giving the best results were isopropyl and sec-butyl (both enantiomers of the latter were also tested). Isopropyl alcohol turned out to be the best derivatization reagent for MX [50,51], as well as other similar hydroxyfuranones [52]. Figure 1.3 presents the mass spectrum of MX after derivatization with isopropanol. The domination of the \([\text{M-((CH}_3)_2\text{CHO})]\) fragmentation with the triplet of isotope ions of m/z 199,201,203 is visible.

Figure 4. Mass spectrum of MX after derivatization with methanol

Figure 5. The mass spectrum of MX after derivatization with isopropanol.
MX derivative obtained with isopropyl alcohol had the highest signal coefficient of all the derivatives when using the MSD detector. This means it has the lowest threshold of detection. Figure 6 shows the results obtained for several alcohols generating MX derivative that had the highest detector signal coefficient.

![Figure 6](image-url)

Figure 6. Intensity of isotope ions of m/z 199,201,203 in relation to the type of derivatization. (Me - MX + methanol, iP - MX + isopropanol, iB - MX + isobutanol, sB1 – first MX peak + sec-butanol, sB2 – second MX peak + sec-butanol, nB - MX + n-butanol).

The relatively high coefficient of the MSD detector signal, as compared with a methanol MX derivative, made the use of the low-resolution mass detector in MX analysis possible. At the same time, it reduced but did not eliminate the problem of qualitative identification of a compound in a complex matrix, such as tap water at this concentration level. As in the case of a methanol derivative, it was still one peak of one derivative, which made it impossible to both identify and get a quantitative result in the case of interference from a pollutant.

However, in the case of sec-butyl alcohol, two chromatographic peaks were obtained which clearly differed in retention time and intensity and were comparable to those of the respective isotope ions of the isopropyl derivative [53]. The existence of two triplets results from the fact that the C5 carbon atom in the MX molecule and other chlorinated hydroxyfuranes is an asymmetric carbon. Sec-butyl alcohol has in its skeleton a chiral carbon atom (carbon C2). The results of this derivatization are four diastereoisomers (RR, RS, SR, SS) of which two pairs are separated. RR is separated from RS and SR from SS, meaning those diastereoisomers which are not enantiomers. Thus, the intensity of each of those two peaks is the sum of the intensity of two diastereoisomers (RR and SS) for one peak, and SR and RS for the second peak. Using one of the sec-butanol enantiomers for derivatization resulted in the formation of two diastereoisomers which are not enantiomers, i.e., may be separated by typical chromatographic methods. Using the S(+) sec-butanol enantiomer, two peaks were obtained corresponding to butyl derivatives SS and SR MX, while using the R(-) sec-butanol enantiomer led to the formation of butyl derivatives RS and RR. Application of sec-butanol enantiomers instead of a mixture of enantiomers decreased the probability of getting derivatives of another compound of a similar isotope ion mass and identical retention time. [54].
The chromatogram of selected ions (EIC) of the [M-((CH₃)₂CHCH₂O)] fragmentation being the result of MX derivatization with sec-butyl alcohol along with the mass spectrum of both separated pairs of diastereoisomers is shown in fig. 1.5.

Figure 7. The chromatogram of selected ions (EIC) and the mass spectrum of MX after derivatization with sec-butanol enantiomer (both diastereoisomers of shorter and longer retention time).

A schematic diagram of the respective analytical procedure is shown in figure 8.
**A 1-2l sample of water**

- pH correction to 2 using HCl
- Vacuum concentration of water sample
- Extraction with ethyl acetic (3 x 4 ml)
- Extract vaporisation
- Derivatization:
  - 2-propanol (85 °C 1h) or
  - S(-) or R(-) 2-butanol (90 °C 1h)
- Extraction with hexane(3 x 0.3 ml)
- Chromatographic analysis GC-LRMS SIM m/z= 199, 201, 203

---

Figure 8. Analytical procedure for determination of MX using vacuum concentration of the sample and derivatization with isopropanol or enantiomeric sec-butanol.

## 5 BY-PRODUCTS OF USING CHLORINE DIOXIDE: CHLORATE (III) AND (V)

### 5.A Colorimetric titration

Method of simultaneous determination of all forms of active chlorine, ClO₂ and ClO₂⁻ ions with N,N-diethyl-p-phenylenediamine (DPD)

The method of determining chlorine dioxide and ClO₂ ions with N,N-diethyl-p-phenylenediamine is an extended DPD method of determining free chlorine and chloramines in water and is based on the reaction of various forms of chlorine with DPD at pH=6.5, with formation of colored meroquinone, also known as Wurster’s red. In the colorimetric method, the extinction of the solution is measured at 515nm. This method is described in Standards Methods [55].

### 5.B Amperometric determination of Cl₂, ClO₂, ClO₂⁻, ClO₃⁻.

Determination of free chlorine in presence of chlorine dioxide, chlorates (V) and chlorates (III) is also possible by amperometric titration based on a sequence of reactions taking place at various pH:

at pH = 7 the following reaction occurs:

\[
2 \text{ClO}_2 + 2 \Gamma \rightarrow \text{I}_2 + 2 \text{ClO}_2^-
\]

at 2 < pH <7 the following reaction occurs:

\[
\text{Cl}_2 + 2 \Gamma \rightarrow \text{I}_2 + 2 \text{Cl}^-
\]
at 0.1 < pH < 2:

\[ 2 \text{Cl}_2 + 10 \text{I}^- + 8 \text{H}^+ \rightarrow 5 \text{I}_2 + 2 \text{Cl}^- + 4 \text{H}_2\text{O} \]

\[ \text{ClO}_2^- + 4 \text{I}^- + 8 \text{H}^+ \rightarrow 2 \text{I}_2 + \text{Cl}^- + 2 \text{H}_2\text{O} \]

at pH < 0.1:

\[ \text{ClO}_3^- + 6 \text{I}^- + 6 \text{H}^+ \rightarrow 3 \text{I}_2 + \text{Cl}^- + 3 \text{H}_2\text{O} \]

The analytic procedure takes into consideration reactions taking place at different pH and so is made up of the following stages:
- Cl\(_2\) is determined and so is one fifth of ClO\(_2\) at pH = 7 (reactions 1+2)
- pH is lowered to 2 and the remaining four fifths of ClO\(_2\) are determined as well as all chlorates (III) (reactions 3 +4).
- Another sample of water is prepared, which is bubbled with nitrogen to remove ClO\(_2\), and the remaining Cl\(_2\) is determined at pH = 7
- A third sample of water is prepared, its pH lowered to around 0.1 (about 5M HCl) and all forms of chlorine are determined (reactions 1+3+4+5).

A drawback of this method of determining chlorate ions in water is a relatively high detection limit for chlorates (V) equal to 0.25 mg/l. The actual concentrations of chlorates may be lower than this value. This method is described in Standards Methods [55].

5.C Determination of ClO\(_2^-\) and ClO\(_3^-\) ions using the ion chromatography method

The ion chromatography method is the only truly selective and reliable method of determining chlorates (III) and chlorates (V).

The EPA recommends the ion chromatography method - the 300.0 [56] method – for determination of chlorates (III) and chlorates (V) in drinking water. The chromatographic separation is performed using the IonPac AS9-HC column, which permits the determination of trace amounts of disinfection by-products in water in the presence of common anions. Determining low concentrations of chlorates (III) and chlorates (V) in water is carried out by direct injection of the sample into the ion chromatograph.
Figure 9. A chromatogram showing the separation of chlorates (III) and chlorates (V) in the presence of typical ions occurring in water.

The limits of detection for chlorates (V) and chlorates (III) [57] were found using simulated water which contained 50 mg/l chlorides, 150 mg/l carbonates and 50 mg/l sulphates. The results of 7 samples are given below:

TABLE 2. Detection limits for chlorates (V) and (III) using the ion chromatography.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Amount [µg/l]</th>
<th>Odch. STD [µg/l]</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorates(III)</td>
<td>10</td>
<td>0,76</td>
<td>7.99</td>
</tr>
<tr>
<td>Chlorates(V)</td>
<td>10</td>
<td>0,34</td>
<td>4,38</td>
</tr>
</tbody>
</table>

It is important to maintain linearity when determining small amounts of chlorate (V) and (III) ions in the presence of a large quantity of other ions in the water. The measurements of the range of linearity [57] has shown that linearity is maintained within a concentration range of 20 – 500 µg/l. The chosen range reflects that met in analyses of real drinking water samples.
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6  BY-PRODUCTS OF OZONIZATION: ALDEHYDES AND CARBOXYLIC ACIDS.

6.A. Aldehydes
The methods of determining aldehydes can be divided into two groups:
- spectrophotometric methods, non-specific, usually leading to the determination of the sum of aldehydes
- chromatographic methods, specific, using derivatization reactions

6.A.1. Determination of formaldehydes in water based on procedure according to Polish Standards (PN-71/C-04593) [17].
This method allows the determination of formaldehyde content in water and in wastewater within the concentration range from 50 to 1000 µg/l. The procedure is based on a reaction of formaldehyde with chromotropic acid at elevated temperature, and subsequently measuring the intensity of coloration proportional to the concentration of formaldehyde in the sample tested. The formaldehyde content in the sample is determined photometrically or visually by comparing sample coloration with the color of a standard scale. The drawback of this method is that determining other aldehydes and low concentrations of formaldehyde (below 50 µg/l) is impossible. This method is not recommended when acetaldehyde is present in the sample [17].
Phenol, some metals and oxidizing agents may hinder the analysis. These substances can be removed but this complicates the analysis. Phenol is eliminated by distilling the sample in high pH, however in the case of metals and oxidizing agents, the distillation should take place at low pH [17]. This method is inadequate for determination of aldehydes - by-products of ozonization.

6.A.2. Colorimetric method recommended by the Hach company.
This method serves to find the concentration of formaldehyde in water (at ppb level) [56, 57]. The method is based on photometric measurements of the intensity of coloration of the complex compound formed as a result of a reaction between formaldehyde and 3-methyl-2-benzotiazolinohydrazine (MBTH). The course of this reaction is shown below:
Figure 10. The reaction of formaldehyde derivatization using MBTH.

The colorimetric reagent, which is added in excess, reacts with formaldehyde forming an azine derivative (compound (a) in the figure). The excess of the reagent is oxidized in the presence of FeCl$_3$ and sulphamic acid. The oxidized form of MBTH (compound (b) in the figure) reacts with the azine derivative of formaldehyde, which leads to the formation of a colored product (compound (c) in the figure). The intensity of the product’s (c) coloration is proportional to the amount of formaldehyde in the sample tested. The weakness of this method is that there are about 20 interfering substances, e.g. ammonia, calcium, chlorides, iron (III), manganese, mercury, nitrates, nitrites, phenols, phosphates, zinc and others [18]. Other aldehydes also interfere during the analysis, as there is no way of separating these substances. In reality, the concentration of the sum of aldehydes is determined, not only the amount of formaldehyde in the sample. As in the previous method, this one is unsuitable for determining aldehydes - by-products of disinfection.


DNPH is used in determining aldehydes and ketones both in aqueous and gaseous samples [19-33]. This method is based on the reaction between analysed compounds and a derivatization reagent, and chromatographic analysis of the resulting hydrazones:
In practice, the SPE (solid phase extraction) technique is implemented as it allows simultaneous derivatization and extraction of the analysed substances. The derivatization reagent (DNPH) is applied to a previously purified bed (such as a C\textsubscript{18} type), after which the tested sample is passed through the sorbent prepared in this way. Products of the derivatization are eluted with an organic solvent (the most common being acetonitrile) and are chromatographically analysed in an HPLC/UV configuration [21, 23, 26, 27]. The derivatization method can be used in conjunction with extraction in the liquid-liquid system. A solution of DNPH in HCl is added to the water sample. After the reaction is complete, the resulting hydrazone is extracted to dichloromethane and analysed by the HPLC/UV [19].

The derivatization method using DNPH is selective and very sensitive, which allows carbonyl compound determination at the ppb level. Authors of papers describing this method ascribe it two major imperfections. The first has to do with a high background originating from the sorbents used in the SPE technique (containing mainly formaldehyde, acetaldehyde and acetone). The second is interference from ozone - present in both aqueous and gaseous samples. A thorough preliminary purification of sorbents used in analysis and elimination of the interference from ozone by applying sodium thiosulphate or potassium iodide are therefore required.

6.A.4. Determining aldehydes by derivatization with the aid of O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBOA).

H. Yamada and I. Somiya proposed the use of PFBOA as a reagent in the derivatization process [34]. This method has been later modified by authors of various studies [35, 36]. The reaction is shown in fig. 8. The products of the reaction are oximes, which are characterised by a high volatility (higher than derivatives of carbonyl compounds and DNPH), which makes it possible to analyse them chromatographically (GC/ECD), and with good aldehyde detectability (ppb). This method is very simple and does not require any preliminary treatment of the sample. The derivatization reaction occurs directly in the water sample and, subsequently, the obtained oximes are extracted with hexane. Neither does this method require a large volume of the sample (a couple to a dozen or so ml) nor the solvent (1 – 2 ml).

In studies, which have used this method, different conditions of the derivatization process were employed: temperature (room temperature or elevated to 45\textdegree C), reaction time (1 – 24 hours), pH (neutral, acidic), and amount of PFBOA (1 – 10 mg) [20, 35, 36, 37]. By using the method based on derivatization with PFBOA, it is possible to determine aliphatic aldehydes, plus mono and diketones as well as ketoacids (when using additional derivatization, e.g. methylation of COOH groups) [34].
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Figure 12. Scheme of the derivatization reaction of carbonyl compounds with PFBOA.

This is the method we use in the study of ozonation by-products, as well as in the analysis of the formation of aldehydes as a result of chlorination and the use of ClO₂ for water disinfection. We also use this method to analyse the aldehydes in bottled water. A typical chromatogram of aldehyde standards is shown in fig. 13.

Figure 13. Separation of oximes on a Rtx-5MS column, (30m x 0.25mm x 0.25 µm), Sample: 0.5µl.

The ability of determining aldehydes on the microgram per litre level, allowed us to observe the dependence of the formation of aldehydes in the process of water ozonization on the dose of ozone and contact time. This dependence for formaldehyde is shown in the figure below.
We also use aldehyde analysis in evaluating bottled water. Aldehydes (especially acetaldehyde) are undesirable for organoleptic reasons. Their main source is the package material from which they migrate into the water. Temperature and sunlight are very favourable to such migrations. This method is also excellent in determining ketones, as can be seen in the chart (fig. 15.).

Figure 14. Effect of dose and contact time of ozone on the formation of aldehydes in the water feeding the water supply system in Poznań.

![Figure 14](image)

Figure 15. Influence of storage conditions of bottled water on the aldehyde content.

![Figure 15](image)
In methods of determining aldehydes, not only is the derivatization method important, but also the way the analysed substance is isolated and preconcentrated. Up until now, derivatization in the sample and liquid-liquid extraction of the obtained oximes were the most widely used methods. Nonetheless, derivatization in an SPE bed was also used [71], and recently intensive work has been carried out on the application of solid phase microextraction (SPME) for this purpose. SPME can be used in several ways:
- derivatization of aldehydes occurs in the sample and the sorption of the oximes formed takes place during the immersion of fibre in the solution,
- derivatization takes place in the tested sample of water but the sorption is carried out from the head space,
- the liquid stationary phase of the SPME fibre is saturated with a derivatization reagent, the aldehydes from the head space are sorbed and derivatization occurs on the fibre,

The versions of the SPME technique used in determining aldehydes

![Image of SPME technique versions]

Derivatization in water, oxime extraction to fibre

Derivatization in water, oxime extraction from the head space

Extraction of aldehydes to fibre, derivatization on fibre

Oxime desorption, qualitative and quantitative analysis

Figure 16. Ways of utilizing the SPME technique for the determination of aldehydes.
The result of optimisation of the above mentioned methods, conducted in our laboratory, show that the best effects are achieved when derivatization takes place in the sample and sorption of the oximes occurs to the fibre from the head space. Cancho et al presented similar results [72]. The following figures present the most significant differences between determining aldehydes by derivatization in the sample and the sorption from the head space, and determining these same aldehydes by derivatization on the fibre. In this latter method, chromatographic peaks from PFBOA appear which interfere with formaldehyde and acetone bands, while glyoxal and methylglyoxal peaks are significantly smaller than in the method using derivatization in the sample.

Figure 17. Chromatogram of aldehydes (GC – ECD) after derivatization in the water sample and oxime sorption from the head space.

Figure 18. Chromatogram of aldehydes (GC – ECD) after sorption from the head space and derivatization on the fibre. 1 formaldehyde, 2 acetaldehyde, 3 acetone, 4 propane, 5 butanal, 6 pentanal, 7 heksanal, 8 heptanal, 9 octanal, 10 benzaldehyde, 11 nonanal, 12 trans-2-nonenal, dekanal, 14 glyoxal, 15 methylglyoxal.
6.A.5 Determining aldehydes by 2,4,6-trichlorophenylohydrazines (TCPH).

This method is based on a derivatization reaction between carbonyl compounds and TCPH. Using TCPH as the derivatization reagent makes GC/ECD chromatographic analysis possible because of the presence of the chlorine atom in the TCPH molecule and because of the volatility of the derivatives. The method is rapid, simple and has good detectability (ppb). In the case of gas samples, the sample is passed through the C$_{18}$ type bed (impregnated earlier with TCPH solution), and then a small column with the sorbent and adsorbed substances is kept at temperature of 100°C for a few minutes. After cooling, the products of the reaction are extracted with the aid of an organic solvent (acetonitrile), and the extract analysed chromatographically. A longer reaction time (over 24 hours) is required in the case of water samples.

Both aldehydes and ketones can be analysed using this method, however, the recoveries may vary between the analytes. Compounds with the molecular mass bigger than that of propanal reacted rapidly, while acetaldehyde and propanal were characterized by poor sorption on the bed [38]. When using this method, the recovery for the majority of aldehydes was over 99%, and only 80 and 94% in the case of acetylaldehyde and propanal respectively.

The reaction can be schematically presented in the following way [38]:

![Figure 19. Scheme of derivatization reaction of carbonyl compounds with TCPH.](image)

When using this method, possible interference from ozone present in the tested samples should be taken into consideration. In case of such interference, sodium thiosulphate should be used to avoid oxidizing the derivatizational products in the presence of ozone [38]. Authors of studies on this method do not recommend the use of potassium iodide because of the possibility of the formation of iodoorganic compounds.
This method is based on the derivative reaction shown below:

![Derivatization reaction of carbonyl compounds with DNSH](image)

Carbonyl compounds (aldehydes, ketones) react with DNSH giving hydrazines, which are analysed using the HPLC technique with a fluorescence detector [20, 39, 40]. In practice the on-line system is often used, in which the gas sample is passed through sorbent beds (e.g. C18 type), impregnated with a derivatization reagent, placed in a special SPE column connected to the HPLC system. The on-line system allows higher sensitivity and facilitates the analysis due to automation.

The derivatization reaction requires temperatures between 50 and 70°C (10 to 30 minutes). Adsorption of the analysed substances on the bed, impregnated with a derivatization reagent, is employed in the case of water samples, and, after the completion of the derivatization on the bed, the obtained hydrazines are eluted with dichloromethane. The solvent should be evaporated, and the hydrazines dissolved in acetonitrile and analysed using the HPLC technique. The detection limit of this method is low (pg) as a sensitive fluorescence detector is used.

6.A.7. Method of determining formaldehyde using the derivatization technique with fluoral P.
This method is used for determining low concentrations of formaldehyde (at ppb level) in liquid samples (water, alcoholic beverages). This analysis is based on determining the concentration of the derivative (DDL, in other words 3,5-diacetyl-1,4-dihydrolutidine) which is the result of the reaction between formaldehyde and the derivatization reagent [41, 42]. The reaction is shown below:
Figure 21. Reaction between formaldehyde and fluoral P.

This reaction is used in the flow-injection analysis. The sample is fed into the flow-reaction column containing a derivatization reagent. The column is connected to a fluorescence detector. The method is quite simple as the analysis can be automated which facilitates routine determination of formaldehydes in environmental samples.

The efficiency of the derivatization process depends on: pH, reaction temperature, and the concentration of the derivatization reagent. The method cannot be used to determine other aldehydes but their presence does not interfere in the analysis because the reaction is highly specific. The presence of other aldehydes in the sample does not trigger the fluorescent detector even when the concentration of these substances is very high (200 times greater than that of formaldehyde). Among the compounds that were tested for possible interference are the following groups: aldehydes (e.g. acetaldehyde, propanal, heptanal, butanal, benzaldehyde), ketones (e.g. acetone, ethylmethyl ketone), solvents (e.g. methanol, ethanol, benzyl alcohol, ethyl acetate, n-hexane, chloroform, benzene, water) and sugars (glucose, fructose, lactose).
This method is based on the reaction between aldehydes, derivatization agents and ammonium ions, as shown on the scheme below:

$$\text{CHD} + R_1\text{C}=O + \text{NH}_4^+ \rightarrow \text{R}_1\text{C}=\text{N} + \text{H}_2\text{O}$$

Figure 22. Scheme showing the derivatization reaction of carbonyl compounds with CHD.

This method is simple and highly sensitive (ppb). The derivatization reagent and ammonium acetic is added to the tested sample as a liquid solution, and then heated for a given time. The cooled solution is analysed using HPLC chromatography with a fluorescence detector [43, 44]. Research done on optimising the method included factors such as: pH, reaction time, the concentration of ammonium ions and the derivatization reagent, repeatability of determination, stability of derivatization process products [43]. The optimal reaction time of the derivatization was ca. 1 hour (at 60°C), pH of ca. 5, and the amount of ammonium ions (in the form of ammonium acetic) equalled ca. 12.5-g/100 ml. When the concentration of ammonium ions was higher, the efficiency of the reaction decreased significantly, which was caused by an increase in viscosity of the solution. In the case of formaldehyde, the derivatization efficiency was good, even at low levels of concentration of the derivatization reagent, but in the case of other aldehydes, an excess of the derivatization reagent was needed [43].


Ozonization causes the occurrence of such acids as formic acid, oxalic acid, acetic acid, ketomalonic acid, and pyruvic acid, where formic and oxalic acids strongly dominate. The acids were determined with ion chromatography using the Dionex DX-500 (USA) system. The chromatographic separation was done in an IonPac AS-9-HC (4x250mm) analytical column working in conjunction with a conductometric detector CD-20 Dionex. The analytical column was preceded by an IonPac AG-9-HC (4x50mm) protective column. As a mobile phase, 9 mM sodium carbonate solution was used. The
determination was done by introducing a 100-µl sample directly into the ion chromatograph. As mentioned before, control of the formation of organic acids plays a vital role in the control of the correct course of ozonization reaction. It is important to control not only the formation of acids and ketoacids during the derivatization, but also to control the process of their removal during filtration through biologically active beds. Carboxylic acids are easily assimilated by bacteria and in consequence, the acids should not be allowed to penetrate into the water distribution networks. In the figures below, the dependence of organic acids formation on the ozone dose and contact time is shown. It should be noted that carboxylic acids are formed in much larger amounts than aldehydes – hundreds of µg/l vs. tens of µg/l. Thus, it is clear that carboxylic acids are primarily responsible for the increase in biodegradable organic carbon after ozonization. The figures illustrate how to use carboxylic acids analysis for the optimisation of the ozonization process- choosing an appropriate ozone dose and contact time. It has been demonstrated that the formation of carboxylic acids proceeds with considerably higher efficiency than the formation of aldehydes, and the process does not, in principle, depend on the contact time. This in turn points to the rapidity of the reaction of ozone with organic matter present in the water.

The next figure shows the kinetics of the removal of carboxylic acids along the biologically active carbon filter bed. A 2 metre high bed eliminates carboxylic acids already in the upper 50cm layer. This proves that carboxylic acids are easily absorbed by bacteria residing in the bed, and also that the most intense processes of biodegradation occur mainly in the upper layer of the biologically active bed.

![Figure 23 The influence of ozonization conditions on the amount of the sum of formic and oxalic acids produced][67]
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**Amount of formic acid at different levels of F 400 carbon**

![Graph showing amount of formic acid at different levels of F 400 carbon](image)

Figure 24. The removal of formic acid along the Chemviron F-400 biologically active carbon filter bed.

### 6.B.2 Determination of carboxylic acids using ion-exclusion chromatography

Another method of determining short chain carboxylic acids we use in our laboratory is ion-exclusion chromatography. In this type of chromatography, the stationary phase is a strong cation exchanger, while the mobile phase is a sulphuric acid solution of pH 1.5-2. Under these conditions, active groups of the strong cation exchanger are dissociated and the fraction of dissociation of each of the analysed carboxylic acids depends on the pK\textsubscript{a} value (in other words, dissociation constant). The weaker the acid (the higher the pK\textsubscript{a}) the more acid molecules will exist in an undissociated state. Undissociated molecules (chargeless) are able to penetrate the cation exchanger pores (stationary phase) and undergo retention due to the hydrophobic interactions, for example. Stronger acids will be dissociated to a larger extent, so in the mobile phase they will be present as ions - anions with a negative charge. Because the surface of the cation exchanger, serving as the stationary phase, has also a negative charge, the acid anions will be repelled by this surface and they will not be able to penetrate the stationary phase pores. In other words, the stronger the acid, the weaker the retention in this type of chromatography. One could say that the separation is achieved approximately according to the increase in the pK\textsubscript{a} value of the analysed acids. Formic and acetic acids, as well as ketoacids, can be determined relatively well by this method. By contrast, determining oxalic acid is more difficult because its pK\textsubscript{a} = 1.4. However, the determination of oxalic acid is made possible by lowering the pH in the mobile phase to a level of 1.5. This method is less sensitive than ion chromatography with conductimetric detection [73,74].

### 7 BIODEGRADABLE ORGANIC MATTER- ITS IMPORTANCE AND METHODS OF DETERMINATION.

The terms biodegradable organic matter and biodegradable carbon are used interchangeably, and describe the sum of organic compounds in water that are susceptible to biochemical decomposition. Earlier, the capacity of the organic matter dissolved in water for biochemical decomposition was characterised by substitute
parameters, e.g., biological oxygen demand (BOD5) or chemical oxygen demand (COD). These days, we know more and have a better understanding of the processes that occur in water, which is why we try to determine directly that part of organic matter which is susceptible to biochemical decomposition. We also have a much better understanding of the role that biodegradable organic carbon may play in water distribution networks. What is more, we are trying to gain more knowledge about which specific compounds constitute the biodegradable organic matter. But why is biodegradable organic matter being discussed in the context of disinfection by-products? Because the compounds which are formed reactions between the disinfectants (strong oxidizers) and natural organic matter found in water are, to a large extent, biodegradable. The biodegradability of organic matter increases significantly in the presence of ozone. At the same time, BDOC is an important qualitative indicator of water quality, determining the biological stability of water in distribution systems.

Organic matter found in water can be divided into two groups:
- biodegradable,
- refractory – not undergoing biodegradation in a reasonable period of time.

A part of BDOC is available organic carbon (AOC) – it is that part of BDOC, which is quickly metabolised and incorporated into the cell’s matter. It is accepted that the rest of BDOC is used only as a source of energy. Modern methods of determining BDOC include determining the whole of biodegradable organic carbon as well as determining available organic carbon. These methods are as follows:

- **van der Kooij's method of determining AOC** – a method utilizing growth of P17 strain of the bacteria *Pseudomonas fluorescens* or NOX Spirillum;
- **Werner’s method of determining AOC** – a method utilizing the growth rate of water bacteria bred under laboratory conditions;
- **Servais’s method of determining BDOC** – measurement of organic carbon susceptible to biodegradation based on measuring the growth of the biomass;
- **Joret’s method of determining BDOC** – this method is based on observing the loss in the dissolved organic carbon (DOC) in the tested water, inoculated with bacterial flora characteristic for the given water.

In the Department of Water Treatment Technology, we use Joret’s method, which in our opinion best characterizes BDOC in the given water because, for biodegradation, it utilizes bacterial flora characteristic for this water. Moreover, the BDOC determined in this manner indicates the amount of organic matter which can potentially be used as a source of energy, therefore facilitating the growth of bacteria in the water supply system. The measurement of BDOC is done within a couple of days (usually 5 days) which is why this parameter, in our opinion, indicates well the potential dangers posed to the biostability of water by the presence of BDOC.

The BDOC content in water is not legally regulated but many authors suggest that such regulation is necessary. Different sources suggest different allowed levels of BDOC and AOC, e.g.,
- 10 µg AOC/l and 200 µg BDOC/l or
- 10 µg AOC/l and 160 µg BDOC/l in non chlorinated water
- 50-100 µg AOC/l and 150 µg BDOC/l for water chlorinated at 20°C and 300 µg BDOC/l at 15°C.

Serious discrepancies are clearly visible. The variability of this parameter is another complication: in the process of water distribution through the network, reactions take
place between the disinfecting agents and the organic matter, resulting the increase in BDOC.

Ozonization and disinfection using chlorine dioxide increase the amount of BDOC in the water. This is shown in the figure below - water from the Mosina source (for the needs of Poznań) after the first stage of its treatment contains only 0,26 mg BDOC/l. Ozonization with a dose of 1,96 mg/l increases the amount of BDOC threefold to 0,96 mg/l. Moreover, it is demonstrate that chlorine dioxide disinfection (24 hour contact) substantially increases the amount of BDOC as well. This increase depends on the dose of the oxidizer.

![Figure 25. Increase in the amount of biodegradable organic carbon after ozonization or chlorine dioxide disinfection for water taken from the Mosina source after the first stage of its treatment.](image)

Figure 25. Increase in the amount of biodegradable organic carbon after ozonization or chlorine dioxide disinfection for water taken from the Mosina source after the first stage of its treatment.
It is easily proven that the amount of BDOC formed in the process of ozonization depends on the ozone dose. This is shown in the following figure.

Figure 26. Dependence of biodegradable organic carbon concentration on the ozone dose.

At the same time, it is possible to show that BDOC correlates very well with the total amount of organic acids formed during ozonization, which is shown in the figure below:

Figure 27. Correlation between the total amount of carboxylic acids (by-products of ozonization) and biodegradable organic carbon.
This excellent correlation between the total amount of carboxylic acids and BDOC only means that carboxylic acids constitute an essential part of BDOC. However, the molecular composition of BDOC is rather poorly known, and as regards the chemistry, only 25-30% of organic substances contributing to the growth of BDOC in the process of ozonization, have been identified, as illustrated by the figure below.

![Pie chart showing BDOC and organic substances](image)

Figure 28. Carboxylic acids and aldehydes share in biodegradable organic carbon after the ozonization of water from the Mosina source with a dose of 2.8 mgO3/l

As can be seen from the above illustration, the composition of biodegradable organic carbon is still, to a large degree, a mystery to be solved.

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