

Primary oxidation products affect the quantification of formaldehyde in autoxidized fatty alcohol ethoxylates when using DNPH derivatization

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Non-ionic surfactants, e.g. fatty alcohol ethoxylates, are considered to cause less skin irritation than other types of surfactants. However, the autoxidation of alcohol ethoxylates generates products that are both skin irritating and sensitizing, such as formaldehyde. It has been suggested that formaldehyde be used as an indicator of the degree of autoxidation of fatty alcohol ethoxylates, and thus also as an indicator of the sensitizing capacity of the product. Attempts to analyse formaldehyde in autoxidized ethoxylates using 2,4-dinitrophenylhydrazine derivatization resulted in formation of the corresponding hydrazone not only from free formaldehyde but also from autoxidation products during the derivatization reaction. Consequently, this derivatizing agent is inappropriate for the determination of formaldehyde in autoxidized fatty alcohol ethoxylates. Focus on the content of primary oxidation products, i.e. hydroperoxides/peroxides, is suggested, particularly as they also are potent sensitizers.

Key words: 2, 4-dinitrophenylhydrazine; alcohol ethoxylates; autoxidation; formaldehyde; surfactants. © Blackwell Munksgaard, 2003.

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The ethoxylated surfactants have broad applications in industrial and household cleaners, laundry products and pharmaceuticals. Non-ionic surfactants, e.g. fatty alcohol ethoxylates, are often preferred to ionic surfactants, since they are considered to cause less skin irritation than other types of surfactants (1). However, we have shown that the autoxidation of alcohol ethoxylates generates products that are both skin irritating (2) and sensitizing (3–5). The polyoxyethylene chains of fatty alcohol ethoxylates and polyethylene glycols can autoxidize during handling and storage and form various oxidation products (6). We have identified formaldehyde as one of the oxidation products formed, together with formates, aldehydes, and hydroperoxides with various structures (3–5, 7, 8). Formaldehyde is a well-known contact allergen and the ethoxylated aldehydes were found to have a sensitizing capacity of the same magnitude as that of formaldehyde (4).

It has been suggested that formaldehyde be used as an indicator of the degree of autoxidation of fatty alcohol ethoxylates, and thus also as an indi-

cator of the sensitizing capacity of the product. Common methods for formaldehyde determination are based on derivatization with substances such as 2,4-dinitrophenylhydrazine (DNPH), chromotropic acid, 2,4-pentanedione, or dimedone, giving chromophores (9). A frequently used method is derivatization with DNPH and subsequent analysis with HPLC (10). DNPH is used due to its rapid reaction with formaldehyde and the stability of the formaldehyde-2,4-dinitrophenylhydrazone (further designated formaldehyde hydrazone) thus formed. It has been discussed that peroxides and hydroperoxides formed during autoxidation of polymers in edible fats, oils, and natural rubber (11, 12) will interfere with DNPH when used for quantification of carbonyl groups in such products.

It is also known that autoxidation products in ethoxylated surfactants are interfering factors in several biochemical analytical methods (13). Surfactants are used, for example, for isolation of membrane proteins and many such proteins have reactive sulfhydryl groups which will be oxidized

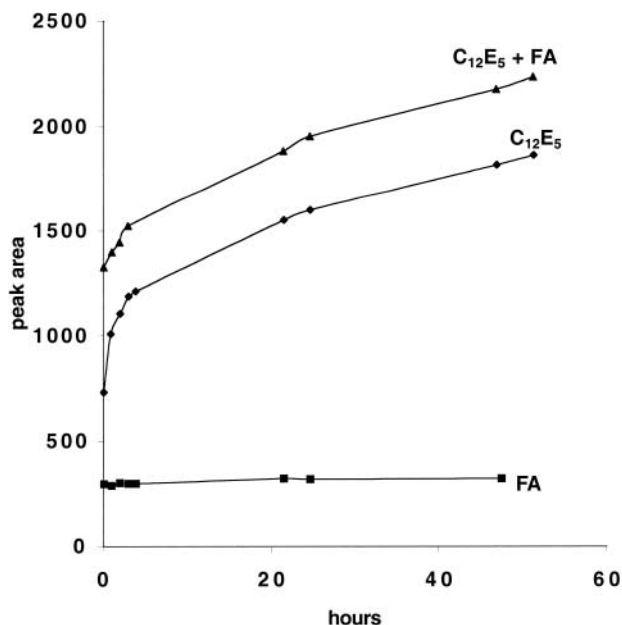


Fig. 1. Formaldehyde hydrazone measured (as peak area in HPLC analysis) at various times after the start at $t=0$ of the DNPH derivatization of formaldehyde in: autoxidized fatty alcohol ethoxylate ($C_{12}E_5$), formaldehyde standard (FA), and the standard added to autoxidized ethoxylate ($C_{12}E_5 + FA$).

by these products. Furthermore, peroxidase assays and other enzymatic assays, e.g. aldehyde reductase, are affected.

The objective of the present study was to investigate the reliability of the DNPH method for the quantification of formaldehyde in autoxidized ethoxylated surfactants.

Materials and Methods

Chemicals

Pentaoxyethylene glycol mono-*n*-dodecyl ether ($C_{12}E_5$) was obtained from Nikkol Chemicals Co. Ltd (Tokyo, Japan). The pure compound and autoxidized samples were studied. (The sample analysed for Fig. 1 was exposed to air for 2 years). 1 nonyl phenol ethoxylate and 2 fatty alcohol ethoxylates (comparable to $C_{12}E_5$) of technical quality from Akzo Nobel (Stenungsund, Sweden) were studied as received and after autoxidation. The autoxidation was achieved by storing the undiluted surfactants in open flasks (covered with aluminium foil) for various times (i.e. months) at room temperature (20–22°C). The samples were stirred gently for 1 h, 4× daily according to previous experience (4) to mimic regular handling.

Acetonitrile and methanol (LiChrosolv grade), formaldehyde (min. 37% solution p.a.), and 2,4-pentanedione (p.a.) were obtained from Merck KGaA, Germany. 2,4-Pentanedione was vacuum distilled before use. The formaldehyde concen-

tration was determined by titration and standards were prepared by dilution with water (e.g. 200 μ L to 250 mL) immediately before use.

2,4-Dinitrophenylhydrazine (p.a.), obtained from Fluka (Buchs, Switzerland), was recrystallized from acetonitrile before use.

Formaldehyde-2,4-dinitrophenylhydrazone was a Supelco Standard from Supelco, Sigma-Aldrich Sweden AB (Stockholm, Sweden). Formaldehyde dehydrogenase (EC 1.2.1.46) 5.7 units/mg and NAD (β -nicotinamide-adenine dinucleotide) were obtained from Sigma-Aldrich Sweden AB.

DNPH method

A 24-mM solution in acetonitrile of 2,4-dinitrophenylhydrazine was prepared and 50 μ L of this solution and 40 μ L of 1 M HCl were added to the different samples (2–40 mg) of surfactants in 310 μ L of acetonitrile. Up to 70 μ L of acetonitrile was substituted for water when compounds in aqueous solutions were added for various tests.

HPLC analyses were performed with a Merck-Hitachi LaChrom D-7000 HPLC System (consisting of pump, solvent degasser, autosampler, column oven, and diode array detector) from Merck KGaA (Darmstadt, Germany). For the hydrazone analysis the column was a Chromolith RP-18e, 100 $\times$ 4.6 mm, kept at 20°C (Merck KGaA). The mobile phase was water:acetonitrile:methanol 60:20:20 (by vol.), and the flow rate 2 mL/min. Fixed wavelength detection was set at 350 nm, and when applicable a spectrum was recorded between 200 and 400 nm. The formaldehyde hydrazone formed was identified using a formaldehyde-2,4-dinitrophenylhydrazone standard by comparing the retention time of the peaks in the chromatograms and by their spectra obtained with the diode array detector. Derivatization was started by adding DNPH at time 0 and samples were taken out for analysis at intervals. The first analysis was performed after 2–3 min due to handling of the sample and processing in the autosampler of the HPLC.

$FeSO_4$ (final concentrations 1 mM and 10 mM) was added to pure and autoxidized $C_{12}E_5$ dissolved in acetonitrile to investigate whether reduction of the peroxides/hydroperoxides would influence the formation of formaldehyde. DNPH was added immediately thereafter and quantification was performed (i.e. after 2–3 min using the HPLC system).

Post column derivatization with 2,4-pentanedione

To estimate the amount of free formaldehyde in the surfactants a method recommended by the Commission of the European Communities for quantification of free formaldehyde in the presence

of formaldehyde releasers was employed (14). The samples of ethoxylated surfactants were dissolved in acetonitrile:water (1:1) and analysed with the HPLC system described above, using 6 mM phosphate buffer pH 2.1 as the mobile phase at 0.5 mL/min. A Discovery RP Amide C16 column, 250 × 4.6 mm; 5 μ m, obtained from Supelco (Sigma-Aldrich Sweden AB) was used. The outlet from the column was mixed in a PEEK Mixing Tee (VICI Jour Research AB, Gothenburg, Sweden) with the 2,4-pentanedione reagent [49 mM 2,4-pentanedione in ammonium acetate (0.81 M) and acetic acid (0.13 M)] delivered from another HPLC pump (LKB Produkter AB, Bromma, Sweden) at 1 mL/min. The mixture was passed through a 5-m (l.) × 0.25 mm (i.d.) Teflon capillary kept in a boiling water bath before entering the detector (set at 420 nm).

Enzymatic method

Autoxidized C₁₂E₅ 10% in acetonitrile (10 μ L) was added to 2.5 mL 0.05 M phosphate buffer pH 7.5, 15 mM NAD (100 μ L) and formaldehyde dehydrogenase (50 μ L, 5 units/mL phosphate buffer). The presence of NADH was recorded after 3 min at room temperature by measuring the absorption at 340 nm.

Results

DNPH method

Figure 1 illustrates the results of quantification of formaldehyde in a sample of autoxidized C₁₂E₅. The formation of formaldehyde hydrazone still proceeds after 50 h in the autoxidized sample, whereas the formaldehyde standard is derivatized rapidly and the amount of formaldehyde hydrazone formed remains at the same level during the entire time period analysed. When the formaldehyde standard is added to the autoxidized C₁₂E₅, the curve obtained is comparable to the sum of the values obtained from the autoxidized sample and the formaldehyde standard. Pure C₁₂E₅ did not give any measurable formaldehyde hydrazone.

Figure 2 illustrates examples of quantification of formaldehyde in different autoxidized ethoxylate surfactants of technical quality using the DNPH method. All samples show the time-related increase of the formaldehyde hydrazone formation.

When FeSO₄ (1 mM) was added to autoxidized C₁₂E₅, the amount of formaldehyde hydrazone increased 9 × compared with the amount measured with no addition of FeSO₄. When FeSO₄ (10 mM) was added the increase was 30 ×. No formaldehyde was detected in samples of pure C₁₂E₅ treated with ferrous ions.

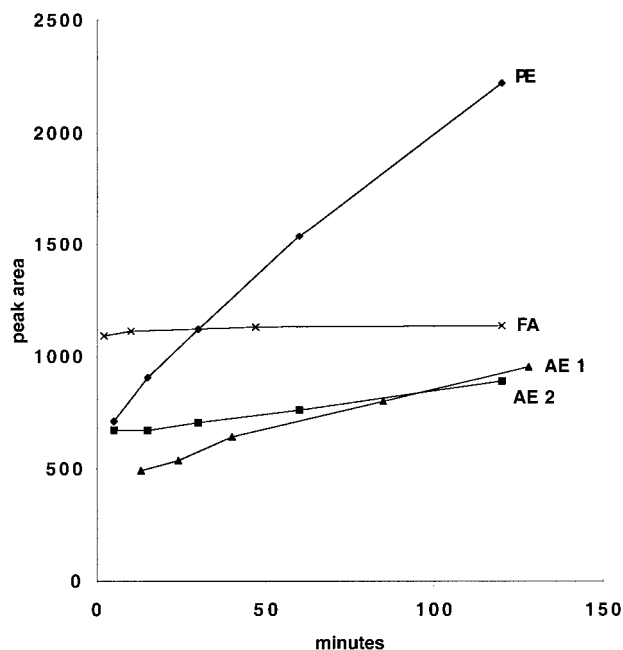


Fig. 2. Formaldehyde hydrazone measured (as peak area in HPLC analysis) in autoxidized technical preparations of ethoxylates after the start at $t=0$ of the derivatization with DNPH. PE = nonyl phenol ethoxylate, AE 1 = technical fatty alcohol ethoxylate, sample 1, AE 2 = technical fatty alcohol ethoxylate, sample 2, FA = formaldehyde standard.

Post column derivatization with 2,4-pentanedione

Autoxidized C₁₂E₅ analysed with this method gave a formaldehyde value 71% of that obtained with the DNPH method for the same sample recorded 2 min after the start of derivatization.

Enzymatic method

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Discussion

It has been suggested that the level of formaldehyde be used as an indicator of the degree of autoxidation of fatty alcohol ethoxylates, and thus also as an indicator of the sensitizing capacity of these products. When the content of formaldehyde in this investigation was determined in autoxidized homologous pure alcohol ethoxylate (C₁₂E₅) using the DNPH method, it was noticed that the formation of formaldehyde hydrazone increased with reaction time and that it did not come to an end within a reasonable time. However, as illustrated in Fig. 1, a formaldehyde standard run in the same system reacted within a few minutes and the

amount of formaldehyde hydrazone formed remains at the same level during the entire time period analysed. When the standard was added to the autoxidized $C_{12}E_5$, the results were comparable to the sum of the 2 individual runs, indicating that free formaldehyde reacts rapidly and that formaldehyde hydrazone formed later probably emanates from precursors. As shown in Fig. 2, autoxidized technical preparations of ethoxylate surfactants analysed with the DNPH method give an extended formaldehyde hydrazone formation similar to autoxidized $C_{12}E_5$.

Autoxidized ethoxylates contain the primary oxidation products hydroperoxides and peroxides and secondary oxidation products like formaldehyde and other aldehydes (4, 8, 15, 16). The primary oxidation products are gradually converted to the secondary products. Our data indicate that DNPH accelerates this reaction, observed as a continuous formation of formaldehyde hydrazone. Our data further show that the formaldehyde formation is not due to acidic hydrolysis of peroxides but merely due to a reaction with DNPH. The extended formaldehyde hydrazone formation started when the DNPH reagent was added, regardless of whether the sample had been kept acidified or not before the addition.

In an attempt to elucidate the amount of free formaldehyde in autoxidized $C_{12}E_5$, 2 other analytical methods were used. The HPLC method included post column derivatization with 2,4-pentanedione for quantification of formaldehyde in the presence of formaldehyde generators as recommended by the EU (14). A drawback is that this method is relatively complicated and requires comprehensive equipment. The other procedure used was an enzymatic method with formaldehyde dehydrogenase. However, the enzymatic analysis must be performed with a UV/VIS photometer in an aqueous system where surfactants cause opalescence that can affect the result. With both these latter methods, formaldehyde content was measured in the autoxidized $C_{12}E_5$ sample to about 70% of that measured after 2 min with DNPH. This indicates that the formaldehyde value obtained with the DNPH method is likely to be overestimated, since it is the result of both free formaldehyde and formaldehyde formed from precursors during the first 2 min of the derivatization reaction with DNPH.

Assuming that peroxides/hydroperoxides in the autoxidized $C_{12}E_5$ were the origin of the formaldehyde formed during the derivatization step, ferrous ions were added to reduce the peroxides. After such treatment, the amount of formaldehyde hydrazone formed after 2 min with the DNPH method was tremendously increased. These experi-

ments indicate that autoxidation products like hydroperoxides are the formaldehyde precursors.

The present investigation shows that DNPH is an inappropriate derivatizing agent for the determination of free formaldehyde directly in autoxidized fatty alcohol ethoxylates, as formaldehyde hydrazone is also formed from precursors during the derivatization reaction. Thus, formaldehyde is not a proper indicator of the degree of autoxidation of ethoxylated alcohols or of the risk of sensitization. Another drawback of using formaldehyde as an indicator of autoxidation of ethoxylates is the volatility of formaldehyde, a factor employed in the semiquantitative chromotropic acid test used for determination of formaldehyde in technical products (17). Focus on the content of primary oxidation products is suggested, particularly since they are also potent skin sensitizers.

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