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REVIEW

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Hydrazine reagents as derivatizing agents in environmental analysis – a critical review

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Abstract Hydrazine reagents are a well-known group of derivatizing agents for the determination of aldehydes and ketones in liquid and gaseous samples. Within this article, the most important hydrazine reagents are critically summarized, and their major applications in different fields, including environmental analysis, food chemistry and industrial analysis are introduced. As 2,4-dinitrophenylhydrazine (DNPH) is the basic reagent for several international standard procedures, its properties are discussed in detail. Particular focus is directed on the chemistry of the hydrazine reagents, and chemical interferences are considered. Recent methods for the determination of various oxidants using hydrazine reagents are presented as well. Due to limited space, this review does not cover the related field of carbohydrate analysis, although many chemical aspects are similar.

1 Introduction

The analysis of reactive substances poses special challenges for the analytical chemist. An analyte which is prone to reaction or decomposition during sampling or analysis has to be determined either by an on-line technique directly in the sample or, in case that this is not available, after chemical derivatization under formation of a more stable product. As short-chain aldehydes are likely to undergo reactions during the analytical process, a large number of dedicated derivatization techniques has evolved in recent years. Some of these are selective for formaldehyde, others allow the simultaneous determination of a large number of aldehydes and ketones in conjunction with a separation method. While a brief general overview on further derivatizing agents for carbonyl compounds is provided in the beginning, the major part of this review will focus on hydrazine reagents in conjunction with chromatographic separations as the currently most widespread group of methods for aldehyde and ketone analysis.

The importance of derivatizing agents for the analysis of aldehydes and ketones becomes apparent when searching in literature for respective analytical developments and applications. Within the Chemical Abstracts database, which covers the literature from 1967 until today, more than 1500 articles are listed which focus on derivatization techniques for the analysis of carbonyl compounds. The assignment of these articles to different groups of derivatizing agents is presented in Fig. 1. It is obvious that almost 50% of all publications are based on the use of 2,4-dinitrophenylhydrazine (DNPH), while the majority of the remaining articles can be traced back on derivatizing agents without hydrazine functionality. Comparably few publications focus on modern developments in the field of hydrazine reagents.

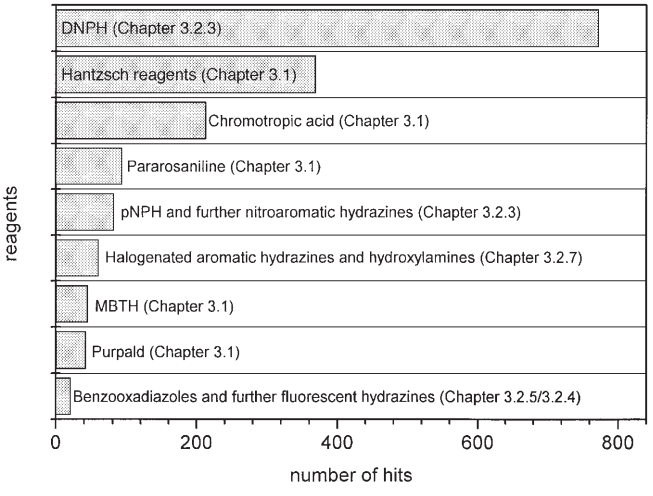

Fig. 1 Survey on the occurrence of different carbonyl reagents in literature

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2 Derivatizing agents for carbonyl compounds – general requirements

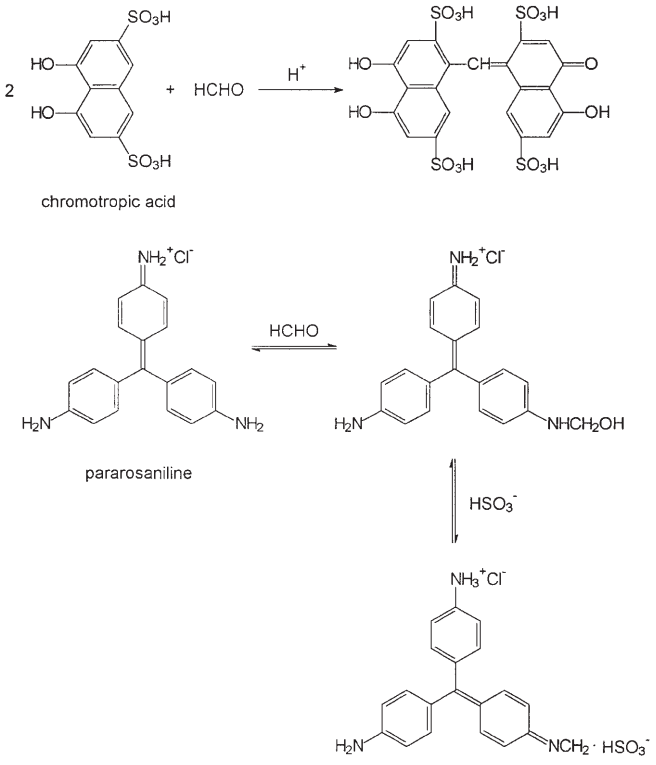
A large number of properties is important when a suitable derivatizing agent for carbonyl compounds has to be selected for a particular application. These include, but are not limited to the following:

- A stable product must be formed in the reaction between reagent and analyte.
- The reaction product must be compatible with the selected separation and detection procedure.
- The velocity of the reaction between reagent and analyte must be high enough to achieve quantitative reaction (exception: for selected automated analyzers, a well reproducible reaction may be sufficient).
- For gas phase sampling, the derivatizing agent should not be too volatile.
- For gas chromatographic analysis, a high volatility of the derivatives is favorable, and their undecomposed evaporation is essential.

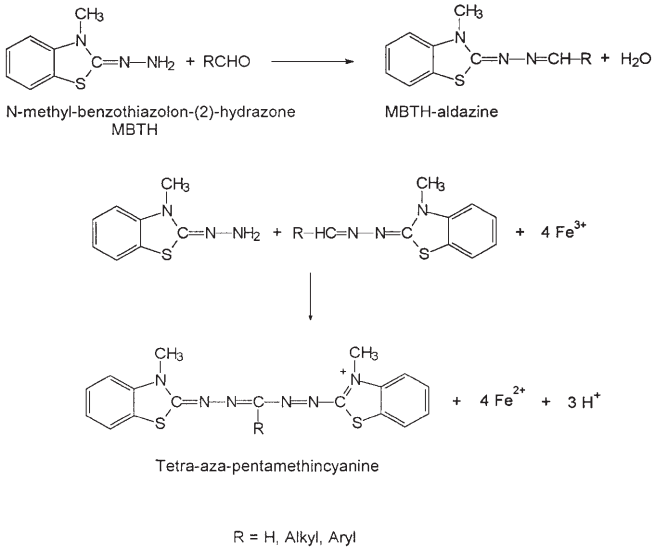
3 Reagents for the determination of carbonyl compounds

3.1 Photometric and fluorimetric methods

Besides hydrazines, a multitude of different groups of derivatizing agents has been established for the analysis of carbonyl compounds. All of these comprise of a condensation reaction of the reagent with the analyte under formation of a colored and/or fluorescent derivative. Therefore, detection may be performed by photometry or fluorescence spectroscopy, but without a chromatographic separation. This is particularly valid if a sum parameter shall be obtained, or if the method provides a sufficient selectivity for formaldehyde. To achieve speciation information, a chromatographic separation is required. Frequently used photometric methods which are selective for formaldehyde, are those based on chromotropic acid [1–5] and pararosaniline [6–8]. The respective reactions are summarized in Scheme 1. Although these methods are well applicable for the determination of formaldehyde in different matrices [1–8], their major drawback is the missing possibility to determine different carbonyls simultaneously within one sample. The N-methyl-benzothiazolon-(2)-hydrazone (MBTH) method is based on an oxidative coupling of formaldehyde and two reagent molecules under formation of an intensively colored product [9–12]. The reaction is depicted in Scheme 2. Although the method has the potential for the simultaneous determination of several carbonyls, its selectivity for different aldehydes and ketones differs considerably depending on the analyte structure and experimental conditions. Another interesting condensation reaction is the base for the 4-amino-3-hydrazino-5-mercapto-4H-1,2,4-triazol (Purpald®) method [13–16] (Scheme 3). The reagent reacts with aldehydes under cyclization and formation of a mercapto-triazolo-tetrazine. Under alkaline conditions, deprotonation leads to the formation of a strongly



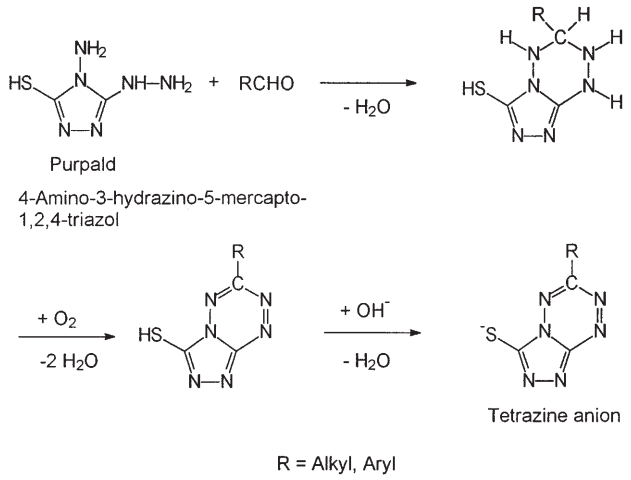
Scheme 1 Reactions of formaldehyde with chromotropic acid and pararosaniline



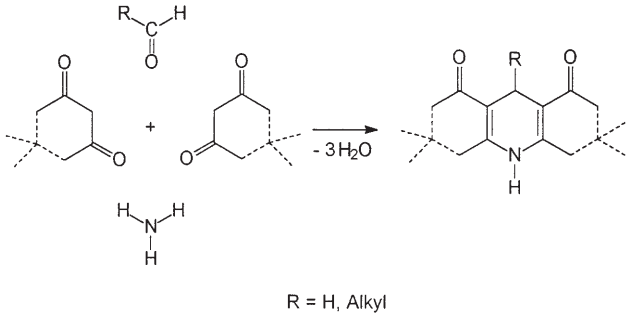
Scheme 2 Derivatization reaction of MBTH with aldehydes

colored product, which may be read UV/vis spectroscopically at a wavelength of 549 nm [14].

A condensation of two molecules of a β-diketone, an aldehyde and ammonia or a primary amine leads to the formation of a dihydrolutidine derivative (Scheme 4). This reaction was introduced by Arthur Hantzsch in 1882 for synthetic purposes [17] and is today named by its inventor. The reaction is frequently used for analytical purposes [18–20], but is has to be applied under very strict time



Scheme 3 Derivatization of Purpald® with carbonyl compounds



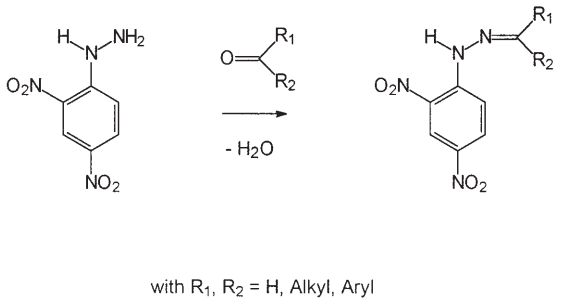
Scheme 4 Condensation reaction of aldehydes with ammonia and acetylacetone (solid line) or dimedone (dotted line)

control due to non-quantitative yields. If acetylacetone is applied as β-diketone, fluorescent dihydrolutidines are the reaction products [18]. The use of 5,5-dimethyl-1,3-cyclohexanedione (dimedone) results in tetrahydroacridines which are excellent fluorophors [20]. The reaction products may be determined by UV/vis spectroscopy and fluorescence spectroscopy, with the latter leading to very low instrumental limits of detection. However, not the signal-to-noise ratio, but the background signal typically limits the applicability of the method for the analysis of real samples [21]. Although simultaneous batch-type analysis for several analytes cannot be recommended due to very high selectivity towards formaldehyde, the Hantzsch reaction is very well suitable for continuous on-line analysis of formaldehyde in liquids and in the gas phase (after stripping into an aqueous solution) [22, 23]. Commercial flow-through analyzers for formaldehyde based on the Hantzsch method are available for atmospheric chemistry and industrial applications [24]. Additionally, a technique using the Hantzsch reaction for post-column derivatization of formaldehyde was presented [25, 26].

3.2 Hydrazine reagents

3.2.1 General aspects

The most popular group of derivatizing agents for carbonyl compounds are hydrazine reagents. These react with aldehydes and ketones under formation of the respective hydrazones as depicted in Scheme 5 for 2,4-dinitrophenylhydrazine (DNPH) as an example of a substituted aromatic hydrazine:

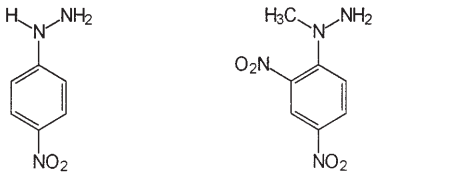


Scheme 5 Derivatization reaction of aldehydes and ketones with DNPH

The hydrazones are typically detected UV/vis or fluorescence spectroscopically after liquid chromatographic separation. As the chromophoric or fluorophoric properties of the reagents are very similar compared to those of the derivatives, the separation step is required to obtain quantitative information on the analyte concentration. Although column liquid chromatography is by far the most important separation technique for the hydrazones, there are developments and applications with almost any modern chromatographic technique, including gas chromatography, capillary electrophoresis and planar chromatography. Established and recent principles and applications of the use of hydrazine reagents for the determination of carbonyl compounds and other analytes will therefore be critically discussed in the following.

3.2.2 History

Most chemists will remember hydrazine reagents already from their basic laboratory classes in organic chemistry, where selected hydrazine reagents, in particular, 2,4-dinitrophenylhydrazine (DNPH), have been used as precipitation reagents for carbonyl compounds. An identification of an aldehyde or ketone is possible using the well defined melting point of the respective hydrazone [27, 28]. This technique has been used for several decades, and the modern developments to be discussed within this work may be considered as refinements and improvements of this original method. First instrumental approaches were based on the identification of carbonyls by thin-layer chromatography [29]. The retention of the derivatives complemented the melting point as a second characteristic parameter for the identification of the analytes. Densitometric evaluation in planar chromatography [30] and the invention of high-performance liquid chromatography and gas chromatography further expanded the possibilities directing to quantitative work. A detailed survey on liquid and gas chromatographic applications of hydrazine reagents is presented below.



4-nitrophenylhydrazine (pNPH) 1-methyl-1-(2,4-dinitrophenyl)-hydrazine (MDNPH)

Scheme 6 Different nitroaromatic hydrazine reagents used in environmental analysis

3.2.3 Reagents based on nitroaromatic hydrazines

3.2.3.1 Structure and spectroscopic properties. Being the oldest group of hydrazine reagents known in analytical chemistry, the nitroaromatic hydrazines have been established as the most important derivatizing agents for the determination of carbonyl compounds. DNPH [31–36], which has been already mentioned above, and 4-nitrophenylhydrazine (pNPH) [37] are the traditional representatives of this class of compounds. Recently, 1-methyl-1-(2,4-dinitrophenyl)hydrazine (MDNPH) has been invented as alternative reagent with interesting properties [38]. The structures of the reagents are presented in Scheme 6.

The UV/vis spectroscopic properties of the nitroaromatic hydrazines are discussed in [39]. Among these, DNPH is the most popular derivatizing agent due to its rapid reaction with the analytes. Besides the excellent crystallization of the derivatives, nitroaromatic hydrazines are characterized by other attractive features. Their synthesis is easily achieved by a nucleophilic substitution of a chloro or fluoro substituted nitroaromatic compound using hydrazine [40] (or methylhydrazine in case of MDNPH) [38]. In a rapid reaction, pure nitroaromatic hydrazines are obtained in high yields [40]. Calibration standards of the derivatives are synthesized by reacting the hydrazine reagent with the carbonyl compound in the presence of an acidic catalyst, typically sulfuric acid [41]. The hydrazones precipitate immediately and are recrystallized from ethanol to yield yellow, orange or red needles [41]. A higher stability of the hydrazones is achieved if excess acid from the reaction mixture is carefully removed by washing the precipitate with bicarbonate [41].

3.2.3.2 Sampling techniques. While first authors described exclusively a reaction between DNPH and the carbonyls in liquid samples [32], a large number of sampling techniques for air monitoring has been used with the DNPH method in recent years [42–50]. For liquid phase analysis, solid phase extraction may serve as a tool for preconcentration of the hydrazones prior to HPLC analysis [43, 51–53]. For gas phase analysis, impingers filled with solutions of DNPH in different solvents have found broad application [54, 55]. Test tubes based on DNPH-coated solid sorbents were also developed [33, 46, 47, 56]. To achieve a fast and quantitative derivatization reaction, both sampling techniques require the use of acidic catalysts, for example hydrochloric [46] or sulfuric acid [57]. As base materials, different silica gel packings [48, 58], e.g., octadecyl modified silica [48] and unmodified material [48], were tested as well as organic polymers [49]. The solid sorbents are eluted with suitable organic solvents, preferably the HPLC eluent, to obtain solutions of the hydrazones [47, 48]. These solutions are injected into the chromatographic system. As high-efficiency sampling techniques for aerosols or for particulate containing gases, which allow the use of very high flow rates of the air sample, denuders of different geometry were applied [50, 59]. Very convenient workplace monitoring is possible by the application of passive sampling devices with DNPH-impregnated filters [60–63].

3.2.3.3 HPLC separation. Today, liquid chromatography with UV/vis detection is the most widespread analytical technique used in conjunction with the nitroaromatic hydrazines and their derivatives. Besides the separation of the hydrazones, it has to be considered that a large excess of the reagent is used for sampling and has to be separated from the DNPH derivatives. A large variety of different columns and mobile phases has been used for HPLC analysis. Separation is easily performed using reversed-phase C_{18} columns and binary [42, 51, 64] or ternary [43] eluents, typically comprising water and one or two organic solvents. Acetonitrile [51, 65], methanol [42, 64] and tetrahydrofuran [66] as organic solvents provide the desired selectivity. In most cases, a binary gradient of acetonitrile and water is used [51, 67], and tetrahydrofuran may be added for the analysis of very complex samples [68]. Non-porous silica [65] instead of porous material [51, 67] as stationary phase provides significantly reduced separation times. It is not possible to generally point out favorable separation conditions, as this strongly depends on the analytical task. For a particular application, the applicant will have to investigate carefully if both stationary and mobile phase meet the specific separation requirements. Due to the limited peak capacity in HPLC, it is only possible to separate all peaks of monocarbonyls up to an alkyl chainlength of three carbon atoms [57]. For the C_4 aldehydes and ketones, up to ten compounds with similar elution properties might already be expected in a sample [57]. It will not be possible to fully resolve all these peaks in HPLC. The occurrence of coeluting peaks of isomeric carbonyls should therefore always be considered.

3.2.3.4 Detection in HPLC. Detection of the DNPH derivatives in HPLC is traditionally performed by UV/vis spectroscopy [43, 69–71] at a wavelength around 360 nm. However, due to significant spectral differences between different groups of analytes [39, 57], the use of a dual wavelength detector [57] or a diode array detector [72] may provide valuable additional information. While the formaldehyde DNPhydrazone is characterized by an absorption maximum around 349 nm, other aliphatic hydrazones exhibit absorption maxima around 360 nm. In contrast, a further red shift to approximately 370 nm and 380 nm is observed for the hydrazones of unsaturated and aromatic aldehydes, respectively [39, 57]. Therefore, dual wavelength detection at 360 nm and 300 nm allows the deci-

sion, whether an unknown peak in a chromatogram may be associated with one of these groups of aldehydes [57]. The peak area ratio at these two wavelengths may be determined precisely and, for routine analysis, automatically by almost any modern HPLC software. The peak area ratio is very characteristic for the different groups of the hydrazones [57]. Coelutions of two peaks, which belong to different groups of hydrazones, may easily be identified using this technique. Only few hydrazones exhibit untypical UV/vis spectroscopic properties: Due to the enlargement of the conjugated system, the DNPhydrazones of glyoxal, methylglyoxal and related α,β-dicarbonyls are characterized by absorption maxima at around 430 nm [73].

An alternative detection of the hydrazones may be used by adding a strongly alkaline solution. The α-hydrogen atom at the hydrazine group is abstracted, and the anion formed is characterized by strong absorbance at 480 nm [74]. However, due to instability of the anions, this detection approach is only suitable for flow injection analysis [75] and it might be used for post-column derivatization.

Mass spectrometry has also been used for the identification and quantification of the hydrazones [66, 73]. While the first publication [76] was based on the moving belt interface, modern approaches use atmospheric pressure chemical ionization (APCI) in the negative mode [77–79]. A deprotonation at the α-hydrogen atom is observed under comparably little fragmentation [77]. The selected ion monitoring (SIM) technique based on the $[M\text{-}H]^-$ ion allows identification and quantification of the hydrazones. Although obviously expanding the selectivity of the method, mass spectrometry has severe limitations as detection technique. Hydrazones of very related analytes, e.g., butanal and isobutanal, are characterized only by small differences even in tandem MS experiments [77]. Compared to UV/vis detection, the MS detection is characterized by significantly larger relative standard deviations for external calibration [79]. Quantification of the carbonyls in HPLC/MS was therefore performed by using deuterated internal standards of the hydrazones [79].

The DNPH method with UV/vis detection has been established in combination with different sampling techniques as standard procedure for the determination of carbonyl compounds in the liquid and gas phases, mostly for workplace air as matrix. Other standardized applications include automobile exhaust monitoring [69] and the analysis of aldehydes in waters [80]. Several standardization bodies, like the Environmental Protection Agency (EPA) [70] and the American Society for Testing and Materials (ASTM) [69] in the United States, the Health and Safety Executive (HSE) [81] in the United Kingdom or the Deutsche Forschungsgemeinschaft (DFG) [82] in Germany have introduced respective official procedures. The results of an interlaboratory comparison for the detection of carbonyls in simulated workplace air are presented in [83].

3.2.3.5 Interferences. As first HPLC methods based on nitroaromatic hydrazines were directed mostly towards workplace monitoring of one single aldehyde, interferences by other components in liquid or gas phase samples were not considered until the late 1980's. Due to increasing interest in aldehyde speciation at low concentrations in atmospheric samples, first investigations regarding potential interferences were carried out at that time. In 1989, two research groups almost simultaneously published their results regarding serious interferences with the DNPH method by ozone [84, 85]. It was found that the reaction of ozone with DNPH on reagent-coated cartridges resulted in the formation of several interfering peaks [84], the identity of which has not been clarified completely until now. However, due to coelution of some of the product peaks with the hydrazones, artifacts may occur [86]. In a recent publication, the identity of some products of the reaction between DNPH and ozone was confirmed as 2,4-dinitrophenol, 2,4-dinitroaniline and m-dinitrochlorobenzene by the use of HPLC/MS and HPLC/NMR [86]. A useful strategy to avoid chemical interferences by airborne ozone is the use of scrubber materials, e.g. potassium iodide [50], mangenese dioxide or copper oxide [87].

The reaction of nitrogen dioxide with DNPH under formation of 2,4-dinitrophenylazide (DNPA) was identified as potential source of chemical interferences in 1993 [88]. DNPA exhibits similar chromatographic properties compared to the formaldehyde hydrazone of DNPH. However, a HPLC separation of these two substances may be achieved under selected conditions [57], and an identification of the interference is easily possible by dual wavelength detection or diode array detection due to the absorption maximum of DNPA at 300 nm (Fig. 2) [57, 88]. Even a determination of nitrogen dioxide in air samples [89, 90] or of nitrite in aqueous samples [91] by using the DNPA peak is possible, as the NO^+ cation is assumed to be the active oxidant in both cases.

In contrast, MDNPH reacts with both ozone and nitrogen dioxide to N-methyl-2,4-dinitroaniline, which may easily be separated from the hydrazones of interest [38]. Therefore, MDNPH might serve as an interesting alternative to DNPH in case of matrices which contain large amounts of oxidants [38]. However, the applicability of MDNPH is

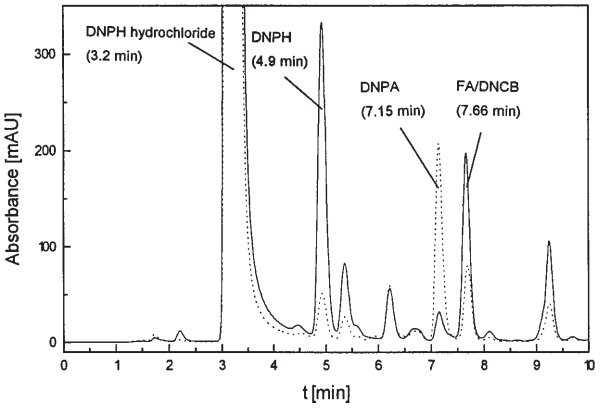


Fig. 2 Chromatogram of an automobile exhaust sample from a diesel-fueled car with dual wavelength detection at λ = 360 nm (—) and λ = 300 nm (·····); DNPA = 2,4-dinitrophenylazide; DNCB = 2,4-dinitrochlorobenzene (reprinted from [57] with permission of the American Chemical Society)

limited by its low reactivity with the carbonyl compounds. This is particularly valid for the reaction of MDNPH with ketones [38].

Although two isomers could be expected for all aldehyde DNPhydrazones starting with acetaldehyde, only a single peak is observed in HPLC [32–34]. However, for asymmetric ketones, two isomers may be separated [92]. As the area ratio of these isomer peaks varies, quantification of asymmetric ketones requires the integration of both peaks to obtain satisfactory results. Up to three peaks, which could be associated with the E,E-, E,Z- and Z,Z-isomers, should be observed for the dihydrazones of dialdehydes. However, in case of glyoxal and glutaraldehyde, only two peaks are separated [62, 93]. Again, the sum of both peaks should be evaluated due to varying peak area ratios. For malonic dialdehyde, not the dihydrazone, but a pyrazol is formed in a reaction of the monohydrazone with the second carbonyl function of the dialdehyde [94–96]. Acrolein is known to yield the acrolein hydrazone plus one additional peak in the chromatogram after sampling the analyte with a DNPH-coated cartridge [46]. Although the identity of the additional peak is not clarified yet, it was suggested to add the peak areas of both peaks at the detection wavelength of 360 nm [46]. This leads to a reasonable approximation for the acrolein concentration [46], but this strategy might result in severe artifacts in case of complex matrices.

3.2.3.6 Selected HPLC applications. Although the major focus of the DNPH/HPLC method was first directed at workplace monitoring [49], several other groups of applications have been added. For gas phase analysis, atmospheric chemistry is one of the driving forces for further developments of the method [47, 50, 58]. Air sampling with different sampling technologies in urban [97] and rural [98] sites has been carried out. Automobile exhaust [67] and industrial exhaust [59] as well as cigarette smoke [44, 68] have been analyzed by several groups. In the liquid phase, drinking water [52, 80, 99], estuary water [100, 101], beer [102] and milk [103] were investigated. Biomedical applications include the analysis of steroidal ketones in samples of human origin [104] and the determination of acetaldehyde in blood [105]. Using the reaction of DNPH with nitrogen oxides to the respective azide, a method for the determination of HNO_2 in the atmosphere has been developed [106]. Additionally, DNPH has turned out to be a valuable tool for workplace monitoring of oximes occurring especially in the polymer industries [107].

3.2.3.7 Gas chromatography (GC). As alternative separation technique for nitroaromatic hydrazones, gas chromatography has been used in combination with flame ionization detector (FID) [108–112] and electron capture detector (ECD) [113]. In contrast to HPLC separation of the DNPhydrazones, the occurrence of syn/anti isomers is characteristic for GC chromatograms. The resulting peak shapes are caused by isomerization reactions during the chromatographic process, thus leading to a plateau comprising syn and anti isomers of the respective hydrazones. Due to the low volatility of the hydrazones, high temperatures and long analysis times are required. Therefore, GC has not been established as a serious alternative to HPLC until now. Recently, new approaches for the gas chromatographic quantification of carbonyls as their DNPH derivatives, especially for the lower aliphatic compounds from C_1 to C_6, were reported [113]. Programmed temperature vaporization (PTV) with solvent split injection has turned out to be a valuable GC injection technique for real sample analysis [113].

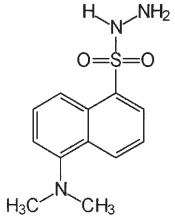
3.2.3.8 Micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC). Only few attempts for the determination of DNPhydrazones using micellar electrokinetic chromatography (MEKC) [114] or capillary electrochromatography (CEC) [115, 116] have been made yet. Stack gas samples were examined, and the hydrazones of formaldehyde, acetaldehyde and acetone were separated by means of MEKC [114]. In contrast to HPLC analysis, where detection limits in the lower ppb range can be achieved, quantification of carbonyls by means of DNPH derivatization with subsequent MEKC [114] or CEC [115] separation is restricted by high detection limits, which are due to short optical pathways.

3.2.4 Dansyl hydrazine

1-Dimethylaminonaphthalene-5-sulfonylhydrazide (Dansyl hydrazine, DNSH, Scheme 7) has been developed as a fluorogenic reagent for the determination of aldehydes and ketones [117–119]. Its naphthalene backbone provides the fluorescent properties, and it can be used for both UV and fluorescence detection.

Dansyl hydrazine is synthesized from dansyl chloride, a well-established derivatizing agent for amino acids and amines, and hydrazine [117]. It has mostly been used for the ultratrace determination of the carbonyls in air samples [120, 121]. Chromatographic separation of DNSH and its carbonyl derivatives is performed by means of HPLC on reversed-phase columns with binary gradients consisting of methanol and water. Additionally, capillary electrophoresis is a useful tool for the quantification of DNSH and the respective hydrazones [119, 122]. This separation technique has successfully been applied to the determination of small carbonyl amounts in raindrops [123, 124]. Microcartridges packed with DNSH coated particles were used to collect atmospheric carbonyls, and the entire sample was injected 'on-line' onto an HPLC column [125]. However, instabil-

Scheme 7 Structure of dansyl hydrazine (DNSH)



ities of the hydrazones due to water interferences have been reported, as hydrolysis of the hydrazide to the sulfonic acid is observed [126].

3.2.5 Reagents based on benzooxadiazole structures

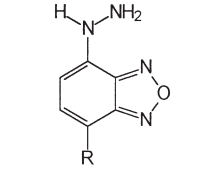
Substituted benzooxadiazoles are another important group of derivatizing agents in analytical chemistry. The benzooxadiazole backbone is substituted in the positions 4 and 7. An electron-withdrawing functionality, e.g., a nitro group (leading to nitrobenzooxadiazole = NBD derivatives), a dimethylaminosulfonyl group (DBD) or an aminosulfonyl (ABD) group is the substituent in position 4, while a reactive group is substituted in position 7. Most applications of the benzooxadiazole reagents focus on the substitution of a fluoro or chloro functionality in position 7 by an amine or an amino acid as analyte, thus leading to strongly fluorescent products, which are subject to chromatographic separation [127, 128]. NBD chloride and NBD fluoride are very important in this concern, and they are also the starting point for the synthesis of hydrazino-functionalized benzooxadiazoles [129]. NBD hydrazine (NBDH) has been introduced several years ago [129, 130] (Scheme 8).

However, despite the promising fluorescence properties and low instrumental limits of detection of the reagent, its application has been limited. This is due to three important factors: First, authors of recent papers have only succeeded in applying the reagent for the determination of aldehydes with alkyl chainlengths of three or more carbon atoms [129]. The most important aldehydes, formaldehyde and acetaldehyde, have not been determined with this reagent yet. Second, the separation of the strongly polar and basic compounds requires the use of base deactivated columns and/or modifiers in the mobile phase in HPLC [129]. Third, NBDH shows light sensitivity thus leading to the formation of degradation products [129].

Based on the experience with MDNPH, the N-methylated reagent MNBDH has also been synthesized and applied for the analysis of aldehydes and ketones [131, 132]. Although the fluorescence properties of the derivatives are only weak, the reagent is characterized by UV/vis spectroscopic limits of detection of its derivatives, which are lower by a factor of two compared to DNPH. The velocity of the reaction between reagent and aldehyde is comparable to that of DNPH, thus being advantageous compared to MDNPH. As for all NBD derivatives with amino- or hydrazino-substituted 4-position, the absorption maximum is located at approximately 480 nm with high molar absorptivities [131]. In the reaction with nitrogen dioxide or ozone, only one product (the strongly fluorescent N-methyl-4-hydrazino-7-nitrobenzofurazan, MNBDA) is formed as in case of MDNPH [38], thus limiting the interferences by oxidants [132].

The fluorescence spectroscopic determination of nitrite traces in waters down to concentrations in the nanomolar range is also possible using HPLC with fluorescence detection or microplate fluorescence spectroscopy without prior separation [133]. This approach is possible due to the extremely weak fluorescence of MNBDH and its hydrazones and the very strong fluorescence of MNBDA [133].

Scheme 8 Hydrazine reagents basing on the benzooxadiazole backbone

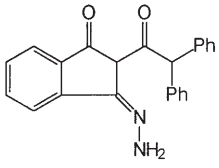


R = SO_2NH_2 (ABDH)
SO_2NMe_2 (DBDH)
NO_2 (NBDH)

3.2.6 Reagents based on indane backbones

For several decades, 2-diphenylacetyl-1,3-indandione-1-hydrazone (DAIH) (Scheme 9) has been known as a derivatizing agent for the quantification of carbonyl compounds [134].

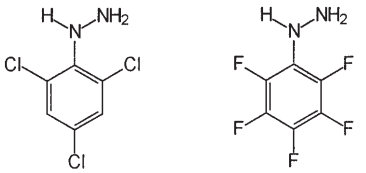
Scheme 9 Structure of 2-diphenylacetyl-1,3-indandione-1-hydrazone (DAIH)



The reagent can easily be synthesized by the reaction of excess hydrazine with 2-diphenylacetyl-1,3-indandione in aqueous methanol [135]. With aldehydes and ketones, DAIH forms azine derivatives which are characterized by good fluorescence properties and UV/vis absorption maxima in a wavelength range between 412 nm for its acetone derivative and 435 nm for the cinnamaldehyde derivative [136]. Because of its fluorescence properties, DAIH was early used for the analysis of selected carbonyl compounds [136]. Thin-layer chromatography has turned out to be a valuable method for the quantification of carbonyls in biochemistry and pharmaceutical chemistry [136]. Sensitive detection of formaldehyde and acetaldehyde in gas phase analysis can be achieved using DAIH-coated silica-gel cartridges. After air sampling, the cartridges are eluted with appropriate organic solvents and the formed hydrazones are separated by means of HPLC and detected by fluorescence spectroscopy [137, 138]. Other research groups have reported on the determination of carbonyls using impingers filled with a DAIH solution [139]. A general disadvantage of the DAIH method is the limited applicability on the quantification of ketones, as the reaction rates are much slower than in the case of aldehyde derivatization.

3.2.7 Reagents based on halogenated aromatic compounds

3.2.7.1 Halogenated aromatic hydrazine reagents. This group of reagents is very well suitable to be applied in combination with gas chromatography followed by electron



Scheme 10 Halogenated phenylhydrazine reagents for GC analysis of aldehydes and ketones

capture detection (ECD) [140, 141] or mass spectrometric detection (MSD) [140]. Established halogenated aromatic compounds for the quantification of aldehydes and ketones include 2,4,6-trichlorophenylhydrazine (TCPH) [141, 142] and pentafluorophenylhydrazine (PFPH) (Scheme 10) [140, 143, 144], which are commercially available.

These reagents are characterized by higher volatility and extremely low limits of detection in GC. TCPH has already been applied to the analysis of airborne carbonyl compounds using small cartridges packed with octadecyl silica and impregnated with TCPH [141]. After the sampling procedure, the cartridges have to be heated to achieve quantitative derivatization [141]. Trichlorophenylhydrazine has also been used for the quantification of carbonyls produced by the decomposition of hydroperoxides [142]. The gas chromatographic separation of TCPH and its formaldehyde derivative is difficult, and no baseline separation could be obtained [141]. In addition to the presented ECD, the FID may also be employed for the detection of the reagent and its derivatives [141]. Due to very slow reaction rates in liquid phases, the TCPH method is not useful for the application in impingers [141].

Pentafluorophenylhydrazine was used in the determination of several carbonyl compounds which are typically produced during the decomposition of lipid hydroperoxides [140] as well as in the analysis of volatile aldehydes from human urine [140]. After derivatization with PFPH followed by solid phase extraction (SPE), GC separation on narrow-bore capillary columns with either ECD or MS detection can be performed [140]. Formation of by-products due to non-controlled nucleophilic substitutions might be expected to occur when using PFPH as a derivatizing agent for carbonyls.

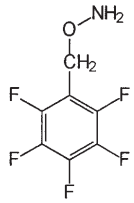
3.2.7.2 O-Alkylated hydroxylamines. Although not being a hydrazine, this reagent is strongly related to the halogenated aromatic hydrazines. With carbonyl compounds, hydroxylamines react to stable derivatives which can easily be separated by means of gas chromatography (GC). For the quantification of aldehydes and ketones, several O-alkylhydroxylamines have been introduced: O-methylhydroxylamine [145], O-benzylhydroxylamine [146–148],

Table 1 Summary of the most important derivatizing agents for aldehyde and ketone determination

Reagents	Analytical methodology	Advantages	Disadvantages	Air sampling techniques[a]
DNPH	HPLC/UV; HPLC/MS (GC/FID; CE; CEC)	international standard procedure; large variety of applications available; stability of reagent and derivatives	interferences with airborne oxidants (NO_X, O_3); limited sensitivity	I, C[b], D, PSD
Hantzsch reagents	UV; fluorescence; HPLC/UV, HPLC/fluorescence	well suitable for on-line analysis; fluorescence detection	slow and non-quantitative reaction; limited to aliphatic carbonyls	I, flow-through analyzers
Chromotropic acid Pararosaniline	UV/vis	only readily available instrumentation required	suitable only for formaldehyde	I
pNPH	HPLC/UV	slightly red-shifted absorption maxima of the derivatives compared to the DNPhydrazones	replaced by DNPH because of its faster derivatization reaction	C, I
Halogenated aromatic hydrazines and hydroxylamines	GC/ECD; GC/FID; GC/MS	improved chromatographic resolution compared to HPLC	slow reaction with carbonyls; E and Z isomers separated	C, I
MBTH / Purpald	UV/vis HPLC/UV (MBTH)	only readily available instrumentation required	selective for formaldehyde but cross-selectivity to other aldehydes	I
DNSH	HPLC/fluorescence	low limits of detection	instability of DNSH and its derivatives towards hydrolysis	C, I
DBDH / ABDH / NBDH	HPLC/fluorescence HPLC/UV	low limits of detection	described only for aldehydes with three or more carbon atoms	only used for liquid phase sampling
MNBDH	HPLC/UV HPLC/MS	strongly red-shifted absorption maxima of MNBDH and its derivatives	not commercially available	C, I, PSD

[a] C = cartridge, I = impinger, D = denuder, PSD = passive sampling devices
[b] reagent coated cartridges are commercially available

Scheme 11 Structure of O-pentafluorobenzylhydroxylamine (PFBOA)



O-(p-nitrobenzyl)hydroxylamine [146] and O-pentafluorobenzylhydroxylamine (PFBOA) (Scheme 11) [144, 149, 150].

Using PFBOA adsorbed on solid-phase microextraction (SPME) fibers, gaseous formaldehyde can be determined in atmospheric samples [150]. The large amount of nitrogen in the reagents allows the use of a selective nitrogen-phosphorus detector instead of the widespread FID. In case of PFBOA, highest sensitivity can be achieved using an ECD [144]. Due to the high resolution of gas chromatography, even E- and Z-isomers of the formed hydroxylamine derivatives are separated [151].

4 Overview

In Table 1, an overview of the most important properties of the discussed derivatizing agents, including the required analytical methodology, advantages and disadvantages is provided to enable the analytical chemist to select a suitable method for his particular application.

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