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Short communication

High-performance liquid chromatography–atmospheric pressure negative chemical ionisation mass spectrometry of 2,4-dinitrophenyl derivatives of amines
Applications in epoxy chemistry

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Abstract

Characterisation of unknown amines in complex matrices — as commonly encountered in the analysis of polyamide-amine- or polyamine–epoxy adducts — requires the use of universally applicable, interference free and robust analytical methods. A reversed-phase high-performance liquid chromatography (RP-HPLC) method coupled to mass spectrometry (MS) is used to detect 2,4-dinitrophenyl (DNP) derivatives of amines by means of negative chemical ionisation at atmospheric pressure (APCI). The high selectivity and good comparability of UV and MS detection of DNP derivatives of amines is exemplified by an amine mixture resulting from hydrolytic degradation of a polyamide-amine–epoxy adduct. The origin of hydrolytic degradation products of a commercially available epoxy–amine adduct is discussed. © 1997 Elsevier Science B.V.

Keywords: Amines; Epoxy resins; Epoxy hardeners

1. Introduction

Amine based hardeners are one of the most important commercially available hardeners for epoxy resins. A multitude of different amines, amine formulations and reaction products allow the creation of hardeners for almost any application — from carbon fibre prepregs to coating systems and from heat curable one-component systems to ambient curing two-component systems. New polyamine- or polyamide-amine–epoxy adduct based water emulsifiable hardeners [1,2], as used in the coatings industry, represent just one type of increasingly complex commercially available epoxy hardeners (Fig. 1).

Due to their structural complexity and the diversity of adducts used, analysis of these hardeners requires a multitude of different analytical methods (see Fig. 2). Although direct spectroscopic investigations yield some useful information [3], the characterisation of amines can only be performed after hydrolytic degradation of polyamine–epoxy adducts. As described in [4], derivatisation is necessary to make these amines chromatographically amenable. As shown below, derivatisation with 2,4-dinitrofluorobenzene (DNFB) — first described by Sanger [5] — has substantial advantages, compared with other derivatisation methods for amines in aqueous phases, especially for the identification of unknown amines.

High-performance liquid chromatography–mass

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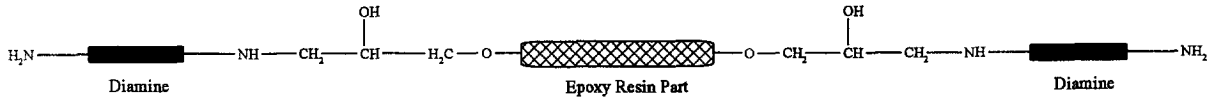


Fig. 1. Idealised structure of an amine adduct of (1 mol) epoxy resin with (2 mol) of diamine.

spectrometry (HPLC–MS) with atmospheric pressure chemical ionisation (APCI) is a well established technique for a multitude of different substance classes. Negative chemical ionisation as an ionisation method [6] proved to be a versatile technique, especially for the dinitrophenyl (DNP) derivatives of amines or amino acids [7]. The possibility of using negative chemical ionisation at atmospheric pressure MS for the detection of DNP derivatives of amines, combined with the separation power of RP-HPLC makes this method an excellent tool for the identification of amines in a wide variety of different matrices.

2. Experimental

2.1. Chromatography

A Thermo Separation Products (TSP, San Jose, CA, USA) HPLC system consisting of a quarternary pump (P 4000) an autosampler (AS 1000) a UV detector (UV 1000) and a PC 1000 data acquisition unit was used. Separation of the derivatised amines was performed on an Ultremex 5 C_{18} column (125× 4.6 mm I.D., 5 μm particle size) from Phenomenex (Torrance, CA, USA). The gradient profile used is shown in Table 1. Chromatography was performed at ambient temperature (ca. 21°C) at a flow-rate of 1 ml/min. The injection volume was 10 μl and the equilibration time before injection 20 min. UV detection was carried out at 355 nm. HPLC grade acetonitrile (Fluka, Buchs, Switzerland) and water purified with a Milli-Q reagent water system from Millipore–Waters (Milford, MA, USA) was used.

2.2. Atmospheric pressure chemical ionisation mass spectrometry

A Perkin-Elmer Sciex API III+ triple quadrupole mass spectrometer with a heated nebulizer as APCI device (Perkin-Elmer Sciex, Toronto, Canada) was used for the detection and identification of the derivatised amines. The HPLC system was connected via a steel capillary (0.1 mm I.D.) to the heated nebulizer, which was held at 490°C. Nitrogen (>99.999%) was used for both, nebulisation and auxiliary gas at a flow-rate of 0.6 resp. 2.0 l/min to nebulise the complete HPLC-flow of 1 ml/min. For negative chemical ionisation the corona discharge needle supplied a discharge current of ~3 μA at −4500 V. The interface plate was held at −650 V and the orifice at −45 V. The curtain gas flow (nitrogen >99.999%) was maintained at 0.8 l/min.

If not mentioned otherwise, the first quadrupole of the mass spectrometer was scanned with a step rate of 0.3 between 250 and 1200 mass-to-charge ratio (m/z), a dwell time of 0.61 ms and a pause time of 0.02 ms resulting in 0.48 scans per second.

2.3. Reagents, solvents and preparation of the samples

Commercial hardener samples are dried in an air-circumventing oven at 120°C for 2–4 h to evaporate water and solvents. 2–3 g of the resulting residue are then sealed with approx. 25 ml hydrochloric acid (30%) in a glass tube and treated as shown in Fig. 2.

Aqueous phases after hydrolytic degradation were adjusted to a pH of approximately 7 to 9 with sodium hydrogen carbonate. To assure complete derivatisation 1 ml of a solution of 25 mg 2,4-dinitrofluorobenzene (Fluka, Buchs, Switzerland) in 1 ml acetone was added under thorough vortexing. After 30 min at room temperature 2 ml tetrahydro-

Table 1
Gradient programme for the elution of DNFB derivatives of amines

Time (min)	Acetonitrile (%)	Water (%)
0.0	0	100
40.0	100	0
50.0	100	0

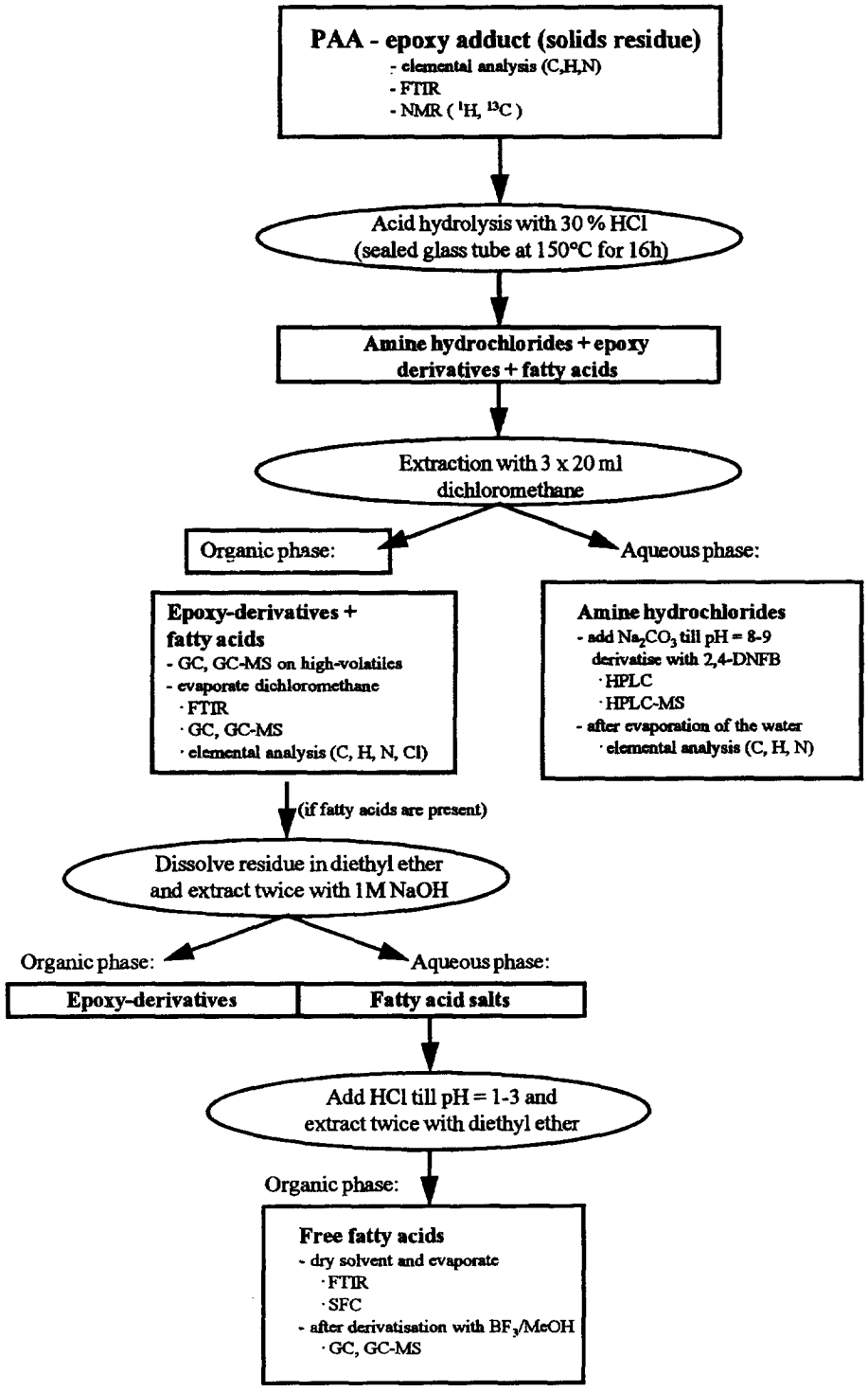


Fig. 2. Scheme of the hydrolysis of an epoxy–amine adduct (an epoxy–polyamidoamine adduct, respectively) with hydrochloric acid and subsequent treatment of the reaction mixture.

furan was added. The sample prepared in this manner can directly be used for subsequent HPLC analysis (see also Ref. [4]).

3. Results and discussion

As mentioned in Section 1, most of the constituents of polyamine- or polyamide-amine–epoxy adducts can only be characterised after hydrolytic degradation. The hydrolysis method described above releases free amines and other amine based degradation products into a highly acidic aqueous phase that still contains polar degradation products of the epoxy adduct arising from the epoxy moiety. Due to the fact that complete extraction of highly polar amines into an organic phase can only be achieved with difficulty by using ion-pairing reagents, it is necessary to focus on a derivatisation method that allows reaction of amines in an aqueous phase with high selectivity while yielding easily detectable derivatisation products. As described in [4], DNFB allows selective and fast derivatisation of both, primary and secondary amines with high conversion in aqueous phases even at high ionic strength. The DNP derivatives can be separated in a HPLC eluent lacking any buffer salts due to the complete loss of basicity attributable to the electron-withdrawal of the 2,4-dinitro substituents. Underivatised or only partly reacted amines are not eluted under these conditions (for an exception see [4]). The highly characteristic absorption of DNP derivatives of amines at 355 nm is very selective and allows interference-free UV detection. Excellent ionisation by negative APCI — resulting in low detection limits [4] — and good comparability between UV and MS detection facilitate the identification of unknown amines.

Fig. 3 shows the chromatograms of the aqueous phase of a typical hydrolytic degradation mixture of a commercially available waterbased epoxy–amine adduct hardener after derivatisation with DNFB followed by UV and MS detection without any further purification. As can be seen, UV and MS detection yield almost the same pattern. Missing peaks in the MS chromatogram — due to masses out of the scanned range (peaks 1 to 5) — and slight response variabilities are the only differences. Table 2 shows the corresponding amine derivatives as far as identified.

2,4-Dinitrophenol (peak 1) is a derivatisation by-

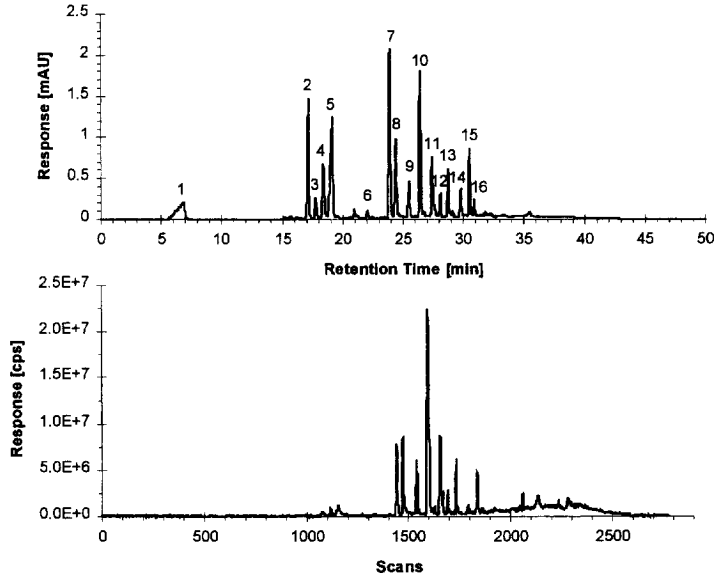
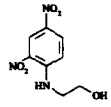
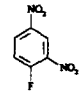
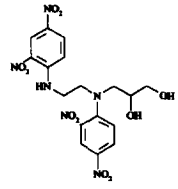
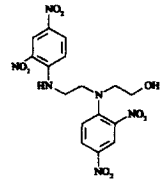
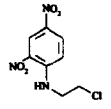
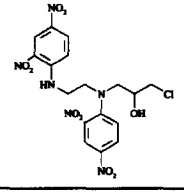
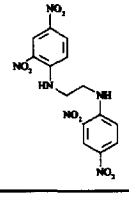
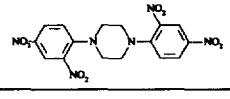


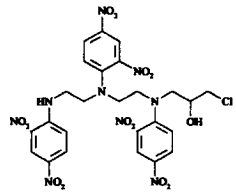
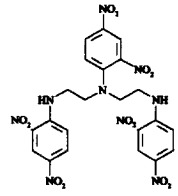
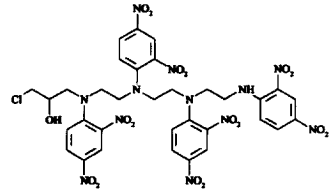
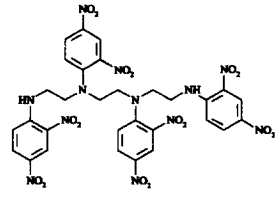
Fig. 3. Chromatogram of the aqueous phase of a typical hydrolytic degradation mixture of a commercially available waterbased epoxy–amine adduct hardener after derivatisation with DNFB with UV (355 nm) and MS detection (scan: 250–1200, resolution: 0.3 u), respectively. For details see Section 3.

Table 2
Structures corresponding to the chromatogram in Fig. 3

Peak Number	Molecular Weight [g/mol]	Structure
1	184	
2	227	
3	284	unknown
4	296	unknown
5	186	
6	466	
7	436	
8	245	
9	484	
10	392	
11	418	

(continued on p. 198)

Table 2 (*continued*)

12	693	
13	601	
14	902	
15	810	
16	670	unknown

Masses of peaks 1 to 5 were assigned using a scan from 180 to 500 *m/z*.

product that forms easily under extremely strong basic conditions. An increase of it can sometimes be observed when derivatised sample solutions are stored for several days, indicating hydrolysis of excess DNFB or of DNP-amine derivatives. With the exception of peak 5 (excess derivatisation agent), all identified amine derivatives contain at least one ethylene amine moiety. Thereby it may be assumed, that they originate from a poly(ethyleneamine) or a mixture of poly(ethyleneamines) like triethylene tetramine (112-24-3) and tetraethylene pentamine (112-57-2) being cleaved during the harsh conditions of hydrolysis. Piperazine, although present in some poly(ethyleneamines) as by-product [8], is another typical degradation product of poly(ethyleneamine) based adducts. The existence of 1,2-propylene glycol and 1-chloro-2-propanol substituted amines in the hydrolysis mixture besides the typical amine signals (peak 6, 9, 12 and 14) provides satisfactory evidence that in fact amines were reacted with an epoxy resin. These degradation products of the poly(ethyleneamine)–epoxy adducts show, that the cleavage of the epoxy–amine link occurs unspecifically at several different positions (see Fig. 4), and that the (C–N) bonds of the poly(ethyleneamines) are easily susceptible to hydrolysis.

The degradation of poly(ethyleneamines) proceeds

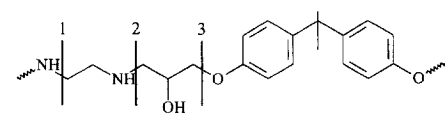


Fig. 4. Cleavgae points of the epoxy–amine link by hydrolytic degradation as observed throughout this work.

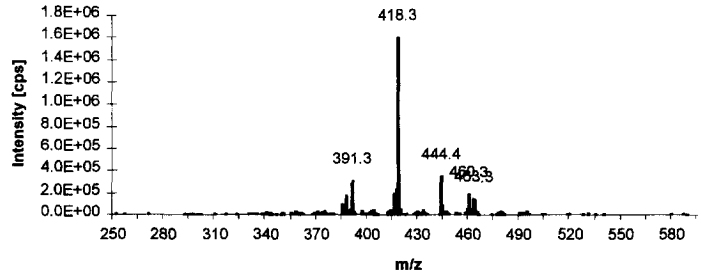


Fig. 5. Spectrum as obtained by negative APCI of the DNP derivative of piperazine (418 g/mol).

quickly and thus it is impossible to distinguish between homologues higher than triethylene tetramine. One possible explanation is cleavage of one or more (C–N) bonds and possible formation of an olefine structure, which further decomposes under the drastic hydrolysis conditions (150°C). This seems comparable to the situation observed with ethers subjected to temperatures of ≥150°C and leading to formation of the corresponding alcohols and olefines[1]. Although different approaches — with different acid concentrations, temperatures and reaction times — have been applied, none lead to significantly better results. Even the use of sulphuric acid for hydrolysis does not reduce elimination and therefore does not allow distinction between higher homologues of poly(ethyleneamines). Polyethylene and polypropylene glycol di- and triamines (Jeffamines, Texaco Chem.) that are sometimes used in such hardeners get cleaved, too. In general, only the terminal amine moiety and the fragment with a directly adjacent glycol unit can be detected after hydrolytic degradation, confirming the easy cleavage of the (C–N) and (C–O) bond. In contrast, this is not the case for a multitude of different other amines like 1,2-cyclohexanediamine (694-83-7), isophorone diamine (80-52-4) or *m*-xylylenediamine (1477-55-0) that show only the substituted and the free amine after hydrolytic degradation. A further advantage of this method is, that the amine based degradation products are not influenced by the type of epoxy resin used for adduct formation. This means, that the degradation products are identical for adducts made of either aliphatic epoxy resins or aromatic ones, which makes the interpretation of the results much easier, especially when adducts are built up of different epoxy resins.

Interpretation of the mass spectra obtained by negative APCI is very straightforward. In general, only $[M-H]^-$ ions are detected. Thermally induced fragmentation or elimination occurs only with those substituted amine derivatives, that easily eliminate hydrochloric acid or water [4]. The only exception encountered throughout the extensive study of degradation products of different adducts is the DNP derivative of piperazine (structure 11) with a molecular mass of 418 g/mol.

The spectrum (Fig. 5) clearly shows a very intense signal at 418 *m/z*, thus providing evidence that the DNP derivative of piperazine does not form a $[M-H]^-$ ion under negative APCI conditions. The formation of the M^- ion has not been investigated any further as it was not in the scope of these investigations.

4. Conclusions

The amines and amine derivatives released after hydrolytic degradation of polyamine– or polyamidoamine–epoxy adducts are reacted at high yields to the corresponding 2,4-dinitrophenyl derivatives. Derivatisation was carried out on the crude hydrolysis mixture after prior neutralisation of the acid aqueous phase and pH adjustment to about 8 to 9. No further sample clean-up was necessary before subsequent separation by HPLC. Due to the loss of any basic character as a consequence of the electron withdrawal of the 2,4-dinitro moiety, HPLC separation could be performed without addition of buffers

[1]Polyamines of e.g., the triethylene triamine or tetraethylene pentamine type can be considered as "polyaza ethers".

or ion-pairing reagents and thus yields sharp peaks and excellent separation. A further advantage of this chromatographic technique, is the marked reduction of background noise normally observed during MS detection in the presence of high loadings of buffer and ion-pairing reagents. For this reason, the highly efficient negative APCI method could be easily applied for structural elucidation of degradation products providing both, high sensitivity and high specificity of detection.

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