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4-Nitro-7-piperazino-2,1,3-benzoxadiazole as a Reagent for Monitoring of Airborne Isocyanates by Liquid Chromatography

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4-Nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ) is presented as a new reagent for the determination of mono- and diisocyanates in air samples. NBDPZ readily reacts with the airborne analytes, thus yielding the corresponding urea derivatives, which are subsequently separated by means of reversed-phase liquid chromatography. On a phenyl-modified stationary phase, excellent baseline separation for numerous mono- and diisocyanate derivatives is obtained. Both diode array and fluorescence detection are performed with limits of detection of 11−35 and 5−9 nmol/L for the individual derivatives, respectively. In contrast to established derivatizing agents for the analysis of isocyanates, NBDPZ provides for increased selectivity due to the favorable detection wavelengths in the visible range (UV/visible, absorption maximums ∼480 nm; fluorescence, excitation maximums ∼470 nm, emission maximums ∼535 nm). In addition, the high molar absorptivities of the reagent and the derivatives provide excellent sensitivity that is superior to most literature-known methods. Finally, air sampling methods comprising both the use of impingers and test tubes are developed and successfully applied to the determination of isocyanates in gaseous samples. Excellent recovery reaching values of >90% is observed for each of the two techniques investigated.

Today, both mono- and diisocyanates find widespread application in the industrial production of pharmaceuticals, pesticides, and polyurethanes (PUR), representing one of the most important classes of polymers, the annual global consumption of which has reached over 6 million tons.[1] PUR-based foam materials are mainly used for insulation or furniture while solid PUR can even be found in medical implants. Additionally, prepolymers with terminal isocyanate groups are applied as glues or sealants.

Due to their high reactivity, isocyanates are significantly toxic and exposure to these compounds during production or treatment of PUR materials may cause severe irritations to the mucous membranes of the respiratory system, thus finally leading to either acute pulmonary edema[2] or even chronic asthma.[2,3] Therefore, identification and sensitive quantification of mono- and diisocyanates in air is of crucial importance to workplace analysis.

As the analytes readily react with a wide range of interfering compounds, e.g., water, direct spectroscopic methods are not applicable to the analysis of individual isocyanates and derivatization has turned out to be inevitable. While first methods were mainly based on colorimetric techniques possessing only restricted selectivity and sensitivity,[4−8] modern analytical approaches predominantly comprise the derivatization with nucleophilic compounds such as alcohols or amines, which is followed by chromatographic separation and, in most cases, photometric or fluorometric detection. Over the past decades, especially the use of amine-based reagents has proven to show best results with respect to the analysis of isocyanates.[9−22] Although a number of different derivatizing agents is currently available, only few of them offer sufficient sensitivity, e.g., 9-(*N*-methylaminomethyl)-an-

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thracene (MAMA)[13,14] or dibutylamine (DBA).[17−22] Applying MAMA, detection can be performed by means of both UV/visible and fluorescence spectroscopy, but owing to absorption maximums in the short-wavelength range, this may be prone to matrix interferences. In contrast, DBA, which lacks a chromophoric or fluorophoric moiety, is mostly restricted to mass spectrometric detection which—although quite sensitive—requires a more expensive instrumental setup than photometric or fluorometric detectors, which are found in almost any of today's analytical laboratories.

Derivatizing agents based on the 2,1,3-benzoxadiazole backbone have found widespread application especially for bioanalytical and environmental purposes.[23−32] They are mainly used as tagging reagents for amino acids,[29] carboxylic acids,[30] amines,[29] alcohols,[31] and thiols[32] but also for the quantification of aldehydes and ketones in air samples.[25] Although all benzoxadiazoles are likely to be excellent chromophores that possess UV/visible absorption maximums in a range >350 nm in conjunction with high molar absorptivities, fluorescence is restricted to a limited number of compounds. The influence of substituents on spectroscopic properties is thoroughly discussed in the literature.[33]

This paper describes the quantification of airborne isocyanates making use of 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ), which has formerly been applied only to the determination of carboxylic acids.[30] The analyte derivatives are separated by means of reversed-phase (RP) high-performance liquid chromatography (HPLC). Owing to the versatile spectroscopic characteristics that are introduced with the NBDPZ reagent, detection can be performed by both UV/visible and fluorescence spectroscopy. Regarding selectivity, derivatization of isocyanates applying NBDPZ thus allows photometric detection in the visible range of the electromagnetic spectrum where spectral interferences resulting from matrix constituents are not quite likely to occur. Additionally, the possibility of the NBDPZ method to perform fluorescence spectroscopy leads to a further improvement of sensitivity when compared to photometry.

EXPERIMENTAL SECTION

Chemicals. Methyl isocyanate was obtained from ChemService (West Chester, PA). 4,4′-Methylene bis(phenyl isocyanate) was purchased from Lancaster (Eastgate, White Lund, Morecamb, U.K.). All other isocyanates used, trifluoroacetic acid (TFA), piperazine, and ammonium formate were from Aldrich (Steinheim, Germany). Solvents used for synthesis and spectroscopy were obtained from Grüssing (Filsum, Germany) in the highest quality available. Acetonitrile (Elution grade) was from Merck Eurolab (Fontenau S/Bois, France), and water for chromatography was purchased from Merck Eurolab (Briare le Canal, France). 4-Chloro-7-nitro-2,1,3-benzooxadiazole and formic acid were delivered by Fluka (Neu-Ulm, Germany) in the highest quality available.

Safety Considerations. Dealing with volatile isocyanates—especially methyl, ethyl, propyl, butyl, pentyl, hexyl, and phenyl isocyanate—inevitably recommends handling in a hood with appropriate safety precautions, e.g., gloves, safety goggles, and, optionally, inhalation precautions.

Instrumentation for Product Identification. ^{1}H NMR measurements were performed with an AM 300 spectrometer from Bruker (Bremen, Germany). The solvent for all NMR measurements was hexadeuterated dimethyl sulfoxide (DMSO-d_6). All peaks are given as δ in ppm and are related to the signal of remaining nondeuterated DMSO content (2.49 ppm vs TMS). FT-IR spectral information was obtained for the products in potassium bromide pellets using an IFS 48 from Bruker. Mass spectra were recorded on a MAT 212 from Varian (Darmstadt, Germany). Elemental analyses were performed with a vario EL III CHNOS analyzer version H from Elementar Analysensysteme GmbH (Hanau, Germany). Melting points were determined with a Mel-Temp II from Holliston. The analytical data for the reagent derivatives are provided as Supporting Information.

Photometer. UV/visible spectra were recorded with a HP 8453 diode array spectrophotometer (Hewlett-Packard, Waldbronn, Germany) equipped with HP Chem Station 845x-biochemical UV/vis system.

Spectrofluorophotometer. A RF 5301-PC spectrofluorophotometer from Shimadzu (Duisburg, Germany) with software version 1.10 was used to record fluorescence spectra.

Syntheses. (1) 4-Nitro-7-piperazino-2,1,3-benzoxadiazole. A 5.00-g amount of 4-chloro-7-nitro-2,1,3-benzoxadiazole (25 mmol) in 300 mL of dichloromethane was added dropwise to a solution of 8.74 g (100 mmol) of piperazine in 400 mL of methanol. During this procedure, the product precipitated as red crystalline material, which was filtered off and washed with water and cold methanol. Subsequently, the product was allowed to dry for 12 h in a vacuum. The yield was 66%. The purity of the product was examined by means of HPLC. The product was fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

(2) Methyl Isocyanate NBDPZ Derivative (MI−NBDPZ). For the synthesis of MI−NBDPZ, 555 mg (2.23 mmol) of NBDPZ was dissolved in 140 mL of dichloromethane. Afterward, 500 mg (8.77 mmol) of methyl isocyanate was rapidly added to the stirred solution and the mixture was allowed to react for 1 h under stirring. After 1 min, the MI−NBDPZ precipitated as reddish crystalline material. To remove excess amounts of methyl isocyanate, 2 mL of methanol was added to the mixture. Finally, the product was filtered off, washed with water and 20 mL of cold methanol, and dried in a vacuum for at least 12 h. The yield was 77%. The urea derivative was fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

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(3) NBDPZ Derivatives of Ethyl, Propyl, Butyl, Pentyl, Hexyl, and Benzyl Isocyanate (EtI−NBDPZ, PI−NBDPZ, BI−NBDPZ, PeI−NBDPZ, HI−NBDPZ, Bz-NBDPZ, Respectively). These derivatives were prepared by adding a solution of 4.00 mmol of the respective isocyanate in 2 mL of dichloromethane to a stirred solution of 250 mg (1.00 mmol) of 4-nitro-7-piperazino-2,1,3-benzoxadiazole in 70 mL of the same solvent. The mixture was allowed to react for 2 h. To remove the excess amount of isocyanate, 2 mL of methanol was added. For the precipitation of the formed urea derivatives, 70 mL of hexane was subsequently added in portions. The orange-reddish product was filtered off, washed with water and cold methanol, and dried in a vacuum for at least 12 h. If necessary, the product was recrystallized from acetonitrile. Yields ranged from 54% to 90%. All urea derivatives were fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

(4) NBDPZ Derivatives of Phenyl and Naphthyl Isocyanate (PhI−NBDPZ and NI−NBDPZ, Respectively). A 4.00-mmol sample of the isocyanate in 2 mL of dichloromethane was added to a solution of 250 mmol of 4-nitro-7-piperazino-2,1,3-benzoxadiazole in 70 mL of dichloromethane. The reaction mixture was stirred for 2 h. To remove the excess amount of isocyanate, 2 mL of methanol was added. The urea derivatives precipitated as orange-reddish crystalline material, which was subsequently filtered off, washed with water and cold methanol, and finally dried under vacuum for 12 h. The yield was 61% for PhI−NBDPZ and 87% for NI−NBDPZ, respectively. The products were fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

(5) NBDPZ Derivatives of 2,6- and 2,4-Toluene Diisocyanate (2,6-TDI−NBDPZ and 2,4-TDI−NBDPZ, Respectively). An amount of 1.00 mmol of 4-nitro-7-piperazino-2,1,3-benzoxadiazole was dissolved in 60 mL of dichloromethane. Afterward, a solution of 0.26 mmol of the respective diisocyanate in 2 mL of the same solvent was added to the stirred reagent solution. After a few seconds, the product precipitated as orange-reddish crystalline material. The latter was filtered off, washed with cold methanol, and dried under vacuum for 12 h. The yield was 87% for the 2,6-TDI derivative and 81% for the 2,4-TDI compound, respectively. The ureas were fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

(6) 1,6-Hexamethylene Diisocyanate NBDPZ Derivative (HDI−NBDPZ) and Isophorone Diisocyanate NBDPZ Derivative (IPDI−NBDPZ). First, 1.00 mmol of 4-nitro-7-piperazino-2,1,3-benzoxadiazole was dissolved in 60 mL of dichloromethane. Subsequently, 0.25 mmol of the respective diisocyanate in 2 mL of dichloromethane was added to the stirred solution. The mixture was allowed to react for 2 h. To precipitate the urea derivatives, 40 mL of *n*-hexane was added in 10-mL portions. The solids were then filtered off, washed with water and cold methanol, and dried under vacuum overnight. If necessary, the products were recrystallized from acetonitrile. The yield was 74% for HDI−NBDPZ and 45% for IPDI−NBDPZ, respectively. The urea compounds were fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis. These data are provided as Supporting Information.

(7) 4,4′-Methylene Bis(phenyl isocyanate) NBDPZ Derivative (MDI−NBDPZ). Initially, 1.31 mmol of 4-nitro-7-piperazino-2,1,3-benzoxadiazole was dissolved in 90 mL of dichloromethane. To the stirred solution, 0.27 mmol of 4,4′-methylene bis(phenyl isocyanate) in 10 mL of dichloromethane was added. The corresponding urea derivative precipitated within a few seconds and was filtered off after a reaction time of 30 min. The product was washed with 20 mL of cold methanol and 20 mL of ethyl acetate. Finally, the crystalline material was additionally suspended in 20 mL of hot acetonitrile. After the MDI derivative had been filtered off again, it was washed with cold methanol and allowed to dry under vacuum for 12 h. The yield was 72%. The product was fully characterized by means of ^{1}H NMR, IR, UV/visible, MS, melting point, and elemental analysis.

HPLC Instrumentation and Analysis. All quantitative liquid chromatographic measurements and reactivity investigations were performed on a Shimadzu (Duisburg, Germany) HPLC system comprising the following parts: two LC-10AS pumps, GT-104 degasser unit, SIL-10A autosampler, sample loop with variable injection volume of up to 50 μL, SUS mixing chamber (0.5 mL), CTO-10ACvp column oven, SPD-M10Avp diode array detector, RF-10AXL fluorescence detector, CBM-10A controller unit, and Class LC-10 software version 1.6. Stability tests and chromatographic method development were carried out on a Shimadzu HPLC system consisting of the following: two LC-10AS pumps, GT-104 degasser unit, Rheodyne six-port valve (series 7725i) with 5-μL sample loop, SUS mixing chamber (0.5 mL), CTO-10ASvp column oven, SPD-10AVvp UV/visible detector, CBM-10A controller unit, and Class LC-10 software version 1.6.

For liquid chromatographic analysis, the following columns were used:

(Column 1) ProntoSIL 120-5-Phenyl (Bischoff Chromatography, Leonberg, Germany); particle size 5 μm; pore size 120 Å; column dimensions 250 mm × 3 mm; guard column 10 mm × 3 mm. (Column 2) Discovery RP18 (Supelco, Deisenhofen, Germany); particle size 5 μm; pore size 200 Å; column dimensions 150 mm × 3 mm; guard column 20 mm × 3 mm. (Column 3) ProntoSIL 120−5-C18-ace-EPS (Bischoff Chromatography); particle size 5 μm; pore size 120 Å; column dimensions 150 mm × 3 mm; guard column 20 mm × 3 mm. (Column 4) LiChrospher RP-18ec in ChromCart cartridges (Macherey-Nagel, Düren, Germany); particle size 5 μm; pore size 100 Å; column dimensions 250 mm × 3 mm; guard column 8 mm × 3 mm. (Column 5) Discovery RP 18 (Supelco, Deisenhofen, Germany); particle size 5 μm; pore size 200 Å; column dimensions 20 mm × 3 mm.

For separation, binary gradients with the profiles shown in Table 1 were chosen.

Reactivity Measurements. (1) Reactivity of NBDPZ toward 2,6-TDI. For the investigation of the reactivity of NBDPZ toward 2,6-toluene diisocyanate, 46.4 μL of a 5.0×10^{-2} mol/L solution of 2,6-TDI in acetonitrile was added to 100 mL of a 2.0×10^{-4} mol/L solution of NBDPZ in acetonitrile. After thorough mixing, the reaction solution was immediately analyzed by liquid chromatography using column 5 and applying gradient C. Detection was performed by means of fluorescence spectroscopy (excitation wavelength $\lambda = 470$ nm; emission wavelength $\lambda = 538$ nm).

Table 1. Profiles of Binary Gradients

Gradient A: flow, 0.65 mL/min; *T*, 45 °C; (A) acetonitrile (0.05% (v/v) TFA), (B) water (0.05% (v/v) TFA)

time (min)	0	27.5	29.0	30.0	32.0	35.0	(stop)
c_A (%)	20	60	100	100	20	20	

Gradient B: flow, 0.60 mL/min; *T*, ambient; (A) acetonitrile, (B) buffer pH 3.5 (1000 mL of water, 60 μL of formic acid, 265 mg of ammonium formate)

time (min)	0	1.0	15.0	16.0	18.0	19.0	21.0 (stop)
c_A (%)	35	35	60	100	100	35	35

Gradient C: flow, 2.00 mL/min; *T*, ambient; (A) acetonitrile, (B) water (0.05% (v/v) TFA)

time (min)	0	0.6	1.0	1.5	1.8	2.5 (stop)
c_A (%)	15	15	100	100	15	15

Gradient D: flow, 2.00 mL/min; *T*, ambient; (A) acetonitrile, (B) water (0.05% (v/v) TFA)

time (min)	0	1.0	1.5	2.0	2.3	3.0 (stop)
c_A (%)	50	50	100	100	50	50

Gradient E: flow, 2.00 mL/min; *T*, ambient; (A) acetonitrile, (B) water (0.05% (v/v) TFA)

time (min)	0	1.0	15	17	18	20	23 (stop)
c_A (%)	30	30	60	100	100	30	30

(2) Reactivity of MAMA toward 2,6-TDI. For the investigation of the reactivity of 9-(*N*-methylaminomethyl)anthracene toward 2,6-toluene diisocyanate, 61.7 μL of a 5.1 $\times$ 10^{-2} mol/L solution of 2,6-TDI in acetonitrile was added to 100 mL of a 3.0 $\times$ 10^{-4} mol/L solution of MAMA in acetonitrile. After thorough mixing, the reaction solution was rapidly analyzed by means of liquid chromatography using column 5 and applying gradient D. Detection was performed by means of fluorescence spectroscopy (excitation wavelength $\lambda = 254$ nm; emission wavelength $\lambda = 412$ nm).

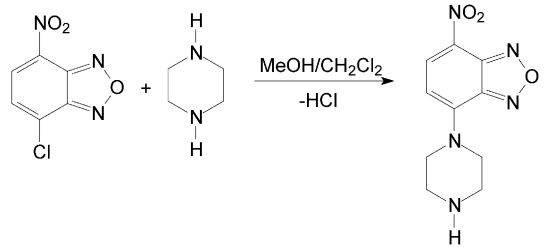
Preparation of NBDPZ Sampling Solution and NBDPZ Sampling Tubes. Preparation of a NBDPZ reagent solution for impinger sampling: In a 250-mL volumetric flask, 31.2 mg of 4-nitro-7-piperazino-2,1,3-benzoxadiazole was suspended in 100 mL of acetonitrile and the resultant mixture was treated in an ultrasonic bath for 1 min. Afterward, the flask was filled to the calibration mark.

Preparation of NBDPZ sampling tubes: 6-mL glass cartridges with Teflon frit (Supelco) were packed with 500 mg of Supelclean LC-18 material (particle size 45 μm, pore size 60 Å), also from Supelco. The packed sorbent was topped with a glass wool layer. These test tubes were conditioned first with 1 mL of acetonitrile, and afterward, the packing material was coated with NBDPZ by allowing 10 mL of a 2.3 $\times$ 10^{-3} mol/L reagent solution to seep through the tubes. Subsequently, the cartridges were dried in a gentle stream of nitrogen.

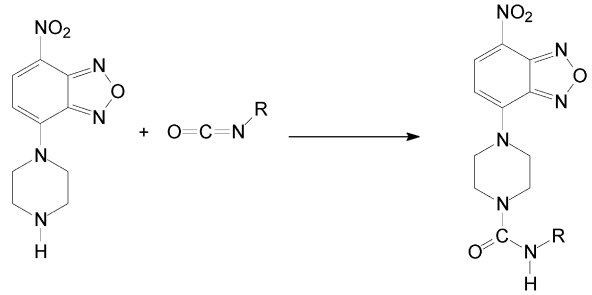
Air Sampling Procedure and Analysis. Air sampling was performed using a two-channel air sampler pump (model 1067) from Supelco. Flow rates were determined applying a 1000-mL bubble flowmeter from Supelco. The generation of simulated isocyanate atmospheres was performed in thoroughly dried glass apparatus to avoid hydrolysis of the reactive analytes. The instrumental setup used in this work is described in the following: Initially, air is pumped through a 100-mL impinger filled with 50 mL of concentrated sulfuric acid, which is connected with a second impinger serving to avoid contamination of the rest of the setup. The latter was followed by a heatable flask in which the temperature-supported evaporation of defined analyte amounts was performed. In series, either two reagent solution-filled impingers (sampling and backup) or two reagent-coated cartridges (sampling and backup) were placed. All connections were Teflon tubing and were kept as short as possible to avoid analyte loss owing to adsorption phenomena. The simulated air sampling with a defined amount of the analyte(s) was performed by pipetting the respective volume of an acetonitrile solution containing a known concentration of isocyanate(s) into the heatable flask. Afterward, a constant air stream was pumped through the setup for a defined period of time. After sampling, the test tubes were eluted with 10 mL of acetonitrile. A 10-μL portion of this solution was injected into the HPLC system. After the impinger sampling procedure was finished, the volume was made up to the calibration mark and subsequently, 10 μL of the solution was injected into the HPLC system. For separation, column 2 and gradient E were used. Detection was performed photometrically at 478 nm.

RESULTS AND DISCUSSION

The aim of the present study was to develop a reagent for the derivatization of mono- and diisocyanates in air samples serving for both UV/visible and fluorescence spectroscopic detection. Furthermore, the new method should additionally provide for good chromatographic separation as well as for sufficient reactivity toward the analytes. Although a number of derivatizing agents have already been described in the literature, each one having its individual advantage, none of them fulfills all needs, which are directed to a modern derivatization method. Within this work, NBDPZ is first described for the derivatization of mono- and diisocyanates. The reagent has been applied earlier to the determination of carboxylic acids.[30] NBDPZ can easily be synthesized in a nucleophilic substitution reaction of piperazine with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBDCl).[30]



Isocyanates readily react with the NBDPZ reagent, thus forming the corresponding urea derivatives:



All NBDPZ ureas show attractive chromophoric properties, e.g., absorption maximums in the range of 480 nm and high molar

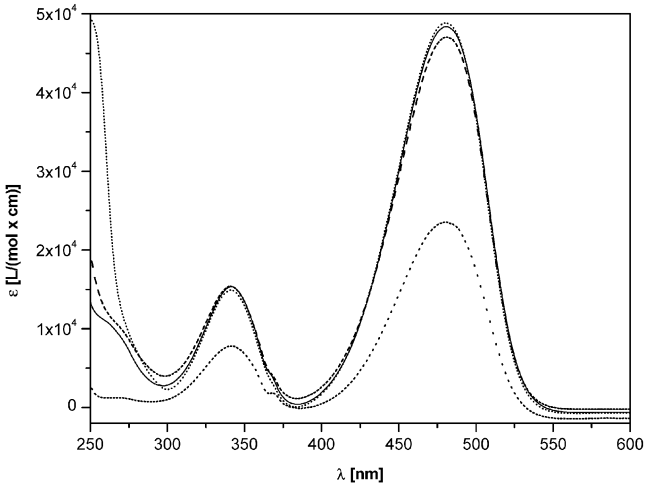


Figure 1. UV/visible spectra of selected NBDPZ isocyanate derivatives dissolved in acetonitrile/dimethyl sulfoxide 20:1 (v/v): methyl isocyanate (dotted), 2,6-toluene diisocyanate (dashed), methylene bis-(phenyl isocyanate) (short dotted), and isophorone diisocyanate (solid). Concentrations range from 1.2 to 1.6 $\times$ 10^{-5} mol/L.

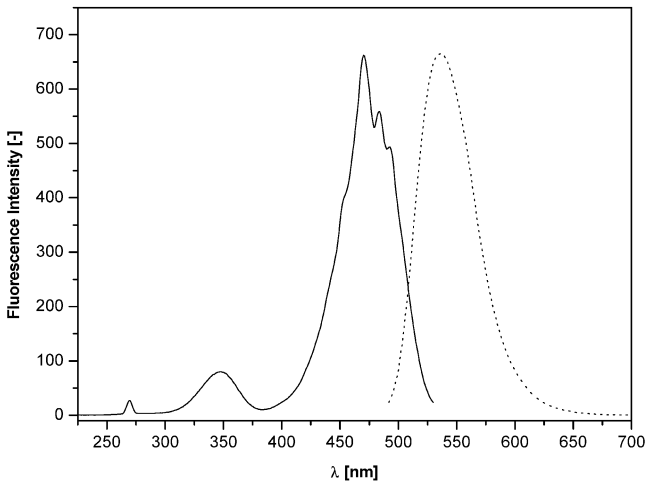


Figure 2. Fluorescence spectrum of methyl isocyanate NBDPZ (MI−NBDPZ) in acetonitrile/dimethyl sulfoxide 20:1 (v/v). $c_{\mathrm{MI-NBDPZ}}$ = 1.3 $\times$ 10^{-5} mol/L; λ_{exc} = 470 nm; λ_{em} = 537 nm.

absorptivities. Figure 1 presents UV/visible spectra of selected isocyanate derivatives. Obviously, the spectra are mainly characterized by the NBD backbone independent of the derivatized analyte. In accordance with a derivatization at both functionalities, molar absorptivities of diisocyanates are ∼2 times higher than those of monoisocyanate derivatives. Additionally, all NBDPZ compounds show good fluorescence characteristics with excitation wavelengths at ∼470 nm and emission wavelengths at ∼535 nm. Figure 2 presents the excitation and emission spectra of MI−NBDPZ, which is characteristic for the other isocyanate derivatives discussed within this paper. Compared to all established amine reagents as MAMA,[13,14] DBA,[17−22] or MPP,[10,11] NBDPZ provides for the most red-shifted absorption maximums (Figure 3, Figure 4), thus guaranteeing spectroscopic detection with limited matrix interferences and going along with highest selectivity. With respect to molar absorptivities, NBDPZ is by far superior to most established amine reagents apart from MAMA (Figure 3). This results in excellent limits of detection (LOD). Table 2 summarizes the instrumental detection limits of several NBDPZ ureas obtained

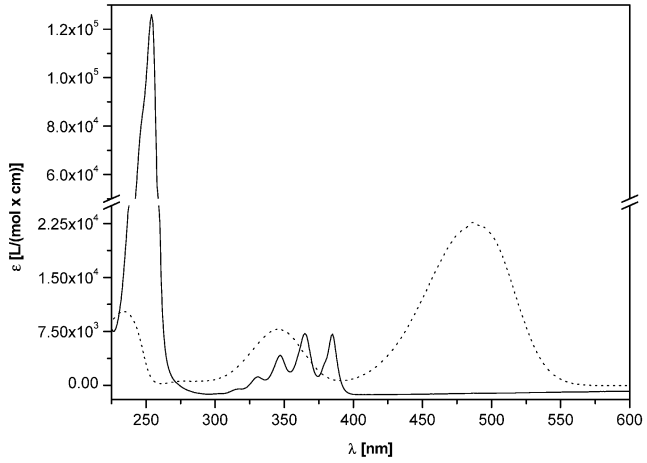


Figure 3. UV/visible spectrum of 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ, dotted) compared to that of 9-(*N*-methylaminomethyl)anthracene (MAMA, solid). Solvent, acetonitrile; c_{NBDPZ} = 1.1 $\times$ 10^{-4} mol/L; c_{MAMA} = 1.9 $\times$ 10^{-5} mol/L.

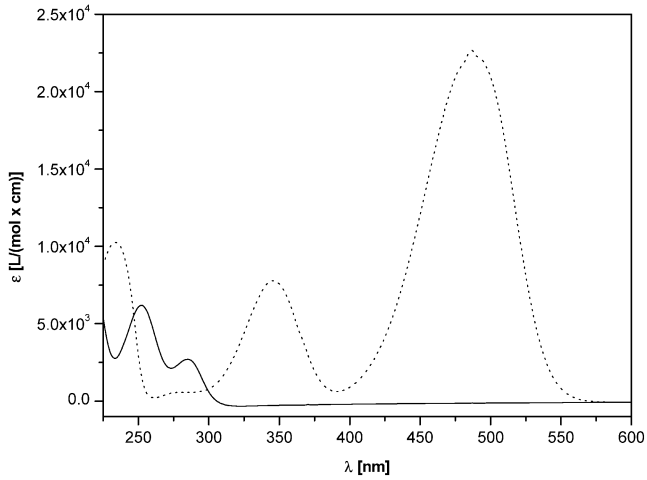


Figure 4. UV/visible spectrum of NBDPZ (dotted) compared to that of 1-(2-methoxyphenyl)piperazine (MPP, solid). Solvent, acetonitrile; c_{NBDPZ} = 1.1 $\times$ 10^{-4} mol/L; c_{MPP} = 1.1 $\times$ 10^{-4} mol/L.

by means of liquid chromatographic separation followed by photometry and fluorometry, respectively. As for fluorescence detection, LOD range from 5.1 $\times$ 10^{-9} mol/L for benzyl isocyanate (BzI) to 8.3 $\times$ 10^{-9} mol/L for isophorone diisocyanate (IPDI). LOD for photometry range from 1.1 $\times$ 10^{-8} mol/L for 2,4-toluene diisocyanate (2,4-TDI) to 3.5 $\times$ 10^{-8} mol/L for 1-naphthyl isocyanate (NI). Photometric LOD are in good accordance with the molar absorptivities stated above because diisocyanate derivatives can be detected more sensitively due to the presence of two NBD moieties. Although fluorescence spectroscopy yields significantly lower LOD than UV/visible detection—up to 6.2 times lower as observed for BzI—there is no decreasing effect on LOD for bifunctional analytes in fluorescence spectroscopy. Unexpectedly, detection limits are even slightly worse than for monofunctional analytes. However, the applicability of fluorescence spectroscopy especially provides higher selectivity when isocyanates in complex matrixes are determined. Linear dynamic ranges for all NBDPZ derivatives were determined and comprise 3 orders of magnitude for fluorescence and four decades for UV/visible detection.

As a selective analysis of isocyanates by means of derivatization requires an excellent separation of reagent and derivatives, a

Table 2. Instrumental Limits of Detection (LOD) for the HPLC Determination of NBDPZ Derivatives Performing UV/Visible and Fluorescence Spectroscopy[a]

	fluorescence detection $\lambda_{ex} = 470$ nm; $\lambda_{em} = 538$ nm		UV/visible detection $\lambda = 480$ nm	
NBDPZ	LOD (10^{-9} mol/L)	LOD (pg of analyte)	LOD (10^{-8} mol/L)	LOD (pg of analyte)
MI	7.8	4	2.5	14
EtI	7.2	5	3.0	21
PI	6.8	6	3.1	26
BI	6.0	6	3.1	31
PhI	5.6	7	3.2	38
BzI	5.1	7	3.2	43
HDI	6.4	11	1.5	25
2,4-TDI	7.2	13	1.1	19
NI	5.7	10	3.5	59
IPDI	8.3	19	1.3	29

[a] These data were obtained using column 3 and gradient B.

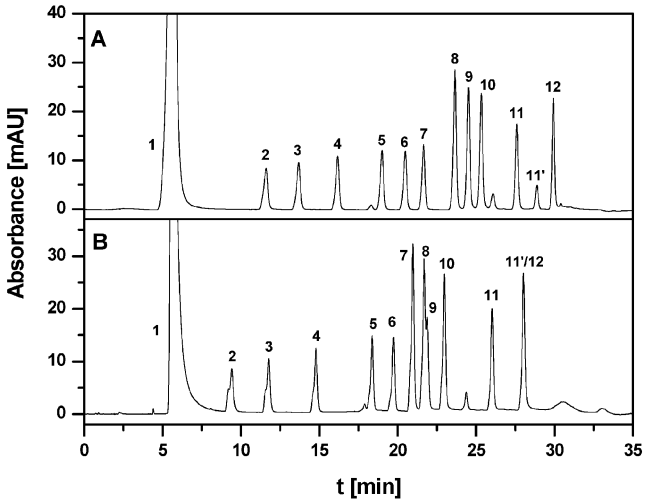


Figure 5. (A) LC separation of a series of mono- and diisocyanate NBDPZ derivatives on a phenyl-modified stationary phase (column 1, gradient A). (B) LC separation of the same compounds on a RP-18 column (column 4, gradient A). Peaks: NBDPZ (1), methyl isocyanate (2), ethyl isocyanate (3), propyl isocyanate (4), butyl isocyanate (5), pentyl isocyanate (6), phenyl isocyanate (7), 2,6-toluene diisocyanate (8), 1,6-hexamethylene diisocyanate (9), 2,4-toluene diisocyanate (10), *cis*-/*trans*-isophorone diisocyanate (11/11′), and methylene bis(phenyl isocyanate) (12).

number of reversed-phase columns for liquid chromatography were tested with respect to their applicability to the NBDPZ method. Performing a binary gradient that consisted of an aqueous buffer and acetonitrile, most commercially available RP-18 columns revealed good results for the separation of the reagent and its corresponding analyte derivatives. Long-wavelength detection at ~480 nm is advantageous as the UV chromatographic baseline is quite flat even when running strong gradients. The observed fronting of peaks 2−4 in chromatogram B (Figure 5) was because the sample injected was dissolved in 100% of acetonitrile while the gradient applied started with an amount of only 20% of the same solvent. Although a baseline separation for the 2,4-TDI and the 2,6-TDI urea was obtained on all octadecyl-modified silica (ODS) columns, coelution of 2,6-TDI and HDI (Figure 5, chromatogram B) could not be avoided even by means of gradient or

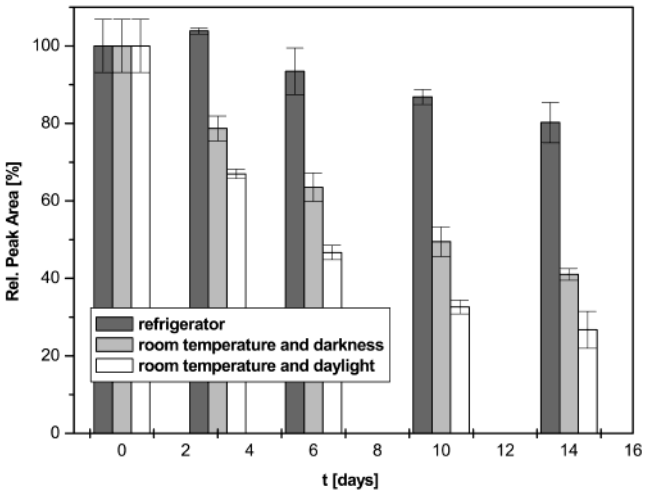
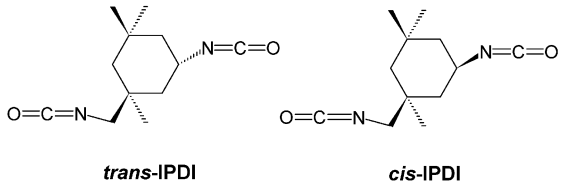


Figure 6. Stability of NBDPZ dissolved in acetonitrile applying three different storage conditions. $c_{NBDPZ} = 5.5 \times 10^{-6}$ mol/L; HPLC analysis was performed using column 1 and gradient A; detection wavelength was 478 nm.

eluent modification. To overcome the coelution problem, a phenyl-modified reversed-phase column turned out to show the best selectivity for the given separation problem (Figure 5, chromatogram A). Here, the crucial pair of peaks (8/9) is well separated, and analogous to the separation on ODS columns, two peaks for the IPDI derivative are observed again. This is due to the fact that the commercially available IPDI contains both the cis and the trans isomer whose derivatives chromatographically behave differently.



Compared to the MAMA method, NBDPZ provides for a better liquid chromatographic separation, which is due to the smaller NBD backbone. Therefore, structural differences of the derivatized analytes are more likely to influence the separation process. For MAMA derivatives, steric demands of the anthracene moiety dominate, thus showing worse separation of structurally related compounds.

The performance of analytical derivatization methods is strongly dependent on the stability of the reagent used as well as on the stability of the formed analyte derivatives. Therefore, storage stability of NBDPZ and its derivatives dissolved in acetonitrile was investigated by applying different conditions: An amount of the sample was stored at room temperature and under the influence of daylight, another part was also stored at room temperature but kept in the darkness, and a last sample was stored in the refrigerator at 5 °C. All solutions were regularly monitored by means of HPLC with subsequent UV/visible detection at 478 nm. Figure 6 summarizes the results that were obtained for NBDPZ. When stored in a cooled environment, the reagent solution turned out to be stable for at least 3 days. Over a period of two weeks in

the refrigerator, the peak area decreased down to ~80% of its initial value. In contrast, both storage experiments performed at ambient temperature revealed a significantly larger decrease in peak area, which went down to 41% (room temperature, darkness) and 27% (room temperature, daylight), respectively. As for HPLC analysis, this was accompanied by the occurrence of a new peak that eluted significantly later in the chromatogram, thus indicating the formation of a more nonpolar compound. First assumptions that this might result from an noncovalent adduct formation of two NBDPZ molecules could be falsified by adding an excess of isocyanate to the sample. This showed that the peak area of the unknown product was not influenced by this experiment. In contrast, all NBDPZ derivatives turned out to be stable under the given storage conditions. This leads to the conclusion that the NBD backbone does not undergo any kind of degradation reaction but that the secondary amine function of the piperazine group seems to be responsible for the formation of a new compound, the identity of which has not been elucidated yet. Stability problems of amine-based agents are not restricted to NBDPZ and have already been described in the literature. For example, MAMA was reported to be not very stable and was recommended to be freshly prepared from its hydrochloride prior to use.[3] Although the NBDPZ reagent revealed only limited stability when stored in organic solution, storage as a solid in the refrigerator yielded no degradation over a period of at least six months. With respect to the application of NBDPZ in sampling solutions, the results observed are not significantly crucial as the reagent can freshly be dissolved in organic media prior to use. Furthermore, the good stability of the reagent when stored as a solid offers the opportunity to develop sampling methods based on NBDPZ-coated test tubes.

To study the reactivity toward the analytes, the reaction of NBDPZ with isocyanates was investigated by means of fast HPLC. For these measurements, an amount of an isocyanate was added to an excess of the reagent dissolved in acetonitrile. Immediately after additon, samples were injected into the HPLC system at constant intervals. For separation, a short gradient program was applied, and the peak area of the derivative was observed with time. Conditions for reactivity measurements are described in detail in the Experimental Section. The reaction of 4-nitro-7-piperazino-2,1,3-benzoxadiazole with 2,6-toluene diisocyanate was complete within less than 10 min, and the recovery was ~95%. Compared to NBDPZ, the reaction of the MAMA reagent with 2,6-TDI was obviously slower with the reaction being complete after ~25 min. Although the reaction monitoring carried out within this work is much too slow to effectively monitor relative reaction rates as described in the literature,[34] the experiments performed indicate that the rate enhancement of NBDPZ relative to MAMA is consistent with that reported by Streicher et al. for the two piperazine reagents 1-(9-anthracenylmethyl)piperazine (MAP) and MPP versus MAMA.[15] Therefore, NBDPZ can also be considered as a good alternative to MAMA with regard to reactivity.

In the following, NBDPZ was used to develop active (pumped) sampling methods for the determination of isocyanates in the gas phase. The generation of analyte atmospheres was performed by introducing a robust setup that is described in the Experimental Section. Initially, air sampling by means of impingers for a single compound was tested. As summarized in Table 3, recovery rates for 2,4-toluene diisocyanate range from 82% to 95%. For all measurements, no breakthrough into the backup impinger was observed. The large standard deviations from 5% to 15% may result from technical circumstances that occurred during sampling procedure. Based on these promising results, impinger sampling was subsequently performed with respect to the simultaneous determination of PI and PhI, the results of which are presented in Table 4. On one hand, good results were obtained for the aromatic analyte showing recovery from 79% up to 91% without any breakthrough into the backup impinger. On the other hand, recovery for the aliphatic propyl isocyanate was significantly lower, and additionally, breakthroughs occurred. This can be explained on the basis of the lower reactivity of aliphatic isocyanates toward nucleophiles compared to the aromatic representatives. If the recoveries from the sampling and the backup impinger are summed up, total recovery of propyl isocyanate of >80% is obtained.

In addition to air sampling by means of impingers, a method for the determination of airborne isocyanates using test tubes was developed. These were filled with a supporting material that was coated with the reagent prior to use. In contrast to impinger sampling, the application of test tubes is less laborous and can be performed by nonexperienced personal. As for method development, a number of commercially available solid-phase extraction (SPE) tubes filled with ODS material were tested. Although the material could be easily coated with NBDPZ, thus yielding a sufficient reagent excess, recovery turned out to be far below 50% of the calculated value. This was due to adsorption of the airborne analytes on the tube walls, which were made out of polypropylene. Further method development was performed using

Table 3. Recovery Rates for the Investigation of Simulated 2,4-TDI Atmospheres Obtained by Means of Impinger Sampling[a]

sample	$n_{2,4-TDI-NBDPZ}$ (mol)	$c_{2,4-TDI}$ ($\mu g/m^3$)[b]	recovery (%)	no. of samples
1	2.3×10^{-7}	1830	82 ± 15	3
2	1.2×10^{-7}	916	95 ± 10	3
3	5.8×10^{-8}	458	86 ± 5	3
4	2.9×10^{-8}	229	86 ± 5	3

[a] Flow rate was determined to be 369 mL/min; sampling time was 60 min. The evaporation flask was heated to 130 °C. [b] c is calculated on the idealized assumption that the analyte is continuously evaporating.

Table 4. Recovery Rates for the Simultaneous Determination of Propyl (PI) and Phenyl Isocyanate (PhI) Obtained by Impinger Sampling[a]

sample/ analyte	$n_{analyte}$ (mol)	$c_{analyte}$ ($\mu g/m^3$)[b]	recovery (%)	breakthrough (%)	no. of samples
1 (PI)	4.0×10^{-7}	1520	71 ± 2	10 ± 1	3
1 (PhI)	3.4×10^{-7}	1820	88 ± 1		3
2 (PI)	2.0×10^{-7}	761	77 ± 4	9 ± 4	3
2 (PhI)	1.7×10^{-7}	909	91 ± 2		3
3 (PI)	5.0×10^{-8}	190	71 ± 2	11 ± 1	3
3 (PhI)	4.2×10^{-8}	227	79 ± 3		3

[a] Flow rate was 369 mL/min; sampling time was 60 min. The evaporation flask was heated to 130 °C. [b] c is calculated on the idealized assumption that the analyte is continuously evaporating.

(34) Kuck, M.; Ballé, G.; Slawyk, W. *Analyst* **1999**, *124*, 933−939.

Table 5. Recovery Rates for the Investigation of Simulated 2,4-TDI Atmospheres Obtained by Means of Glass Cartridges Filled with NBDPZ-Coated ODS Material[a]

sample	$n_{2,4-TDI-NBDPZ}$ (mol)	$c_{2,4-TDI}$ ($\mu g/m^3$)[b]	recovery (%)	no. of samples
1	1.0×10^{-7}	878	89 ± 4	3
2	5.0×10^{-8}	439	99 ± 7	3
3	2.5×10^{-8}	220	86 ± 14	3

[a] Flow rate was 330 mL/min; sampling time was 60 min. The evaporation flask was heated to 150 °C. [b] c is calculated on the idealized assumption that the analyte is continuously evaporating.

Table 6. MDI Concentrations during the Thermal Treatment of a Commercially Available Polyurethane (PUR) Foam Determined on the Basis of the NBDPZ Method Performing Test Tube Sampling[a]

		UV/vis detection $\lambda = 478$ nm	
sample	amount of foam (g)	n (10^{-7}mol)	c (mg/m^3)[b]
1 (RT)	10		
2 (130 °C)	13	2.5 ± 0.1	3.2 ± 0.1
3 (150 °C)	17	8.0 ± 0.3	10.1 ± 0.3

[a] Flow rate was set to 330 mL/min; sampling time was 60 min. The PUR sample was investigated under three different temperatures. [b] c is calculated on the idealized assumption that the analyte is continuously evaporating.

glass cartridges that were manually packed with the respective filling material. Table 5 summarizes recovery experiments for 2,4-toluene diisocyanate using ODS-filled glass tubes for sampling experiments. Recoveries ranged from 86% up to 99% and breakthrough of the analyte into the backup tube was not observed. Good recovery within the lower concentration range thus suggests that workplace concentrations can be effectively monitored, e.g., the German maximum workplace concentration (MAK) of 70 $\mu g/m^3$.[35] To sum up, with the introduction of glass tubes filled with NBDPZ-coated ODS material, a new sampling setup for individual workplace monitoring of isocyanates that is easy to use has been established.

To investigate the applicability of the NBDPZ test tube method for the determination of real samples, emissions resulting from a PUR foam based on 4,4′-methylene bis(phenyl isocyanate) (MDI) were investigated. The foam, which is mainly used for insulation purposes, was freshly sprayed into a flask. Immediately afterward, emissions from three PUR samples were analyzed: The first sample was kept at room temperature, and the second and the third samples were heated to 130 and 150 °C, respectively. The amounts of MDI that were determined on the basis of the NBDPZ test tube method are presented in Table 6. For the nonheated PUR foam, no MDI emission was detected, while the thermally treated samples revealed significant release of residual monomeric MDI that was higher than the German MAK value for MDI of 50 $\mu g/m^3$.[2] With respect to MDI, the thermal treatment of freshly sprayed PUR foam is prone to yield an aerosol. Collection of an aerosol is more difficult for reagent-coated test tubes than TDI vapor because diffusion is much slower for the aerosol. This is often likely to result in breakthroughs. However, those could not be observed during the analyses described. Although the calculation of the concentration determined was based on the assumption of a continuous evaporation of the analytes and despite the fact that a rather concentrated MDI atmosphere was generated within the 250-mL evaporation flask, the results obtained clearly demonstrate the risk resulting from heated PUR materials containing residual amounts of monomeric isocyanates.

(35) *Deutsche Forschungsgemeinschaft MAK− und BAT-Werte-Liste der Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe*; VCH: Weinheim, 1996.

CONCLUSIONS

NBDPZ has been applied as a new reagent for the determination of mono- and diisocyanates in air samples, thus revealing numerous advantages compared to established derivatization methods. A series of NBDPZ isocyanate derivatives, all of which are new compounds, has been synthesized and fully characterized by means of melting point, infrared spectroscopy, ^{1}H NMR, mass spectrometry, and UV/visible and fluorescence spectroscopy. Both the reagent and its urea derivatives, which are formed with the isocyanates, are characterized by the most red-shifted absorption maximums of all established amine reagents as well as by high molar absorptivities (ϵ). Compared to the MPP reagent, ϵ for NBDPZ compounds are ~4 times higher. In addition, all ureas show good fluorescence, which is characterized by emission wavelengths in the visible range of the electromagnetic spectrum. Owing to the excellent spectroscopic properties, selectivity of this method is higher than for any other reagent described, and the limits of detection are superior to most known derivatizing agents. Stability in organic solution and as a solid has been thoroughly tested and has turned out to be very good for the ureas dissolved in acetonitrile as well as for the solid compounds, while reagent solutions were stable for at least 3 days only when stored in a refrigerator. NBDPZ and the corresponding derivatives can be separated on a number of octadecyl-modified silica HPLC columns while highest chromatographic selectivity is obtained on a phenyl-modified phase. Thus, chromatographic resolution of the new method is quite advantageous, and it is superior to that of the MAMA method, where coelution of isocyanate derivatives is more likely to occur. The reactivity of NBDPZ toward the analytes has been investigated in organic media and has turned out to be faster than that of MAMA. Regarding sampling procedures, active (pumped) methods based on impingers and reagent-coated test tubes were developed and successfully applied to the analysis of isocyanate-containing air samples.

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SUPPORTING INFORMATION AVAILABLE

Text presenting melting point, IR, ^{1}H NMR, MS and elemental analysis data for methyl isocyanate NBDPZ urea derivative (MI-NBDPZ), ethyl isocyanate NBDPZ urea derivative (EtI-NBDPZ), propyl isocyanate NBDPZ urea derivative (PI-NBDPZ), butyl isocyanate NBDPZ urea derivative (BI-NBDPZ), pentyl isocyanate NBDPZ urea derivative (PI-NBDPZ), hexyl isocyanate NBDPZ urea derivative (HI-NBDPZ), phenyl isocyanate NBDPZ urea

derivative (PhI-NBDPZ), benzyl isocyanate NBDPZ urea derivative (BzI-NBDPZ), 1-naphthyl isocyanate NBDPZ urea derivative (NI-NBDPZ), 1,6-hexamethylene diisocyanate NBDPZ urea derivative (HDI-NBDPZ), 2,6-toluene diisocyanate NBDPZ urea derivative (2,6-TDI-NBDPZ), 2,4-toluene diisocyanate NBDPZ urea derivative (2,4-TDI-NBDPZ), 4,4′-methylene bis(phenylisocyanate) NBDPZ urea derivative (MDI-NBDPZ), isophorone diisocyanate NBDPZ urea derivative (IPDI-NBDPZ), and 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ). This material is available free of charge via the Internet at http://pubs.acs.org

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