Perfume-Containing Polyurea Microcapsules with Undetectable Levels of Free Isocyanates

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ABSTRACT: Core-shell polyurea microcapsules with a 40% fragrance load were prepared by interfacial polymerization of guanidine and a technical polyisocyanate prepolymer containing mainly the biuret trimer derived from hexamethylene di-isocyanate (HDI). Residual free polyisocyanates were still present at a level slightly above 100 mg NCO functional group per kg as determined by liquid chromatography hyphenated with tandem mass spectrometry of HDI and of its biuret trimer. This level was decreased by a factor of about 10 when the polymerization process was allowed to proceed for a longer time and by a factor of about 500 when guanidine or NaOH were added to the microcapsule suspension to act as scavengers. In these cases, polyisocyanate conversion was observed to proceed for about one month when the microcapsules were stored at room temperature before reaching a plateau at a level below 1 mg NCO/kg. Overall, ammonia was the most efficient polyisocyanate scavenger as no residual HDI biuret trimer and only less than 2 µg NCO/kg as HDI were detected at the end of the process, a level which had dropped below the limit of detection of 0.25 µg NCO/kg after about 40 days of aging at room temperature. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 3074–3080, 2009

Key words: microencapsulation; polyurea; aging; addition polymerization; high performance liquid chromatography (HPLC)

INTRODUCTION

The fragrance compositions commonly used in perfumery applications are mixtures of compounds with a wide variety of chemical structures and different physicochemical properties.[1] Key variable parameters include solubility, evaporation rate, volatility considered in parallel with individual odor threshold, and stability upon exposure to air and light or when formulated in aggressive media such as detergents.[2,3] As a result, maintaining constant hedonic attributes is quite a challenging task. An efficient approach to address this issue consists of encapsulating the fragrance composition within a barrier that will either act as an evaporation retardant and a protective shell or as both. Furthermore, encapsulation may improve the deposition of the fragrance onto different substrates and thereby improve its performance in application. Among numerous encapsulation techniques, an especially promising one for perfumery applications is interfacial polymerization, which is a chemical reaction between an oil-soluble monomer and a water-soluble monomer at the oil and water interface.[4] The fragrance, considered as the oil, can easily be dispersed into an aqueous medium. Also, the versatility of monomers allows the design of microcapsules with well-defined size distribution and release properties.

The selection of monomers and the optimization of reaction processes for interfacial polymerization must take into consideration the toxicity of residual unreacted monomers. This issue is especially important in the case of polyurea-based microcapsules prepared by reacting a polyamine with a polyisocyanate. Although several relatively safe water-soluble polyamines are readily available, there are only limited possibilities for the selection of a polyisocyanate monomer. A first approach toward minimizing the health hazards caused by residual monomers consists of using polymerized species with a much lower volatility. For example, the volatile hexamethylene di-isocyanate (HDI, **1**) can advantageously be replaced by species derived from it such as an uretidone **2**, an isocyanurate **3** or especially a biuret **4** allowing the preparation of microcapsules with a remarkably good performance in application (Fig. 1). Nevertheless, an optimal process for the preparation of polyurea microcapsules would be one ensuring the conversion of all isocyanate groups. We report here on how this goal was achieved in the case of microcapsules prepared by interfacial polymerization of guanidine with a biuret-type prepolymer derived from HDI after various scavenging approaches were assessed. Furthermore, we describe
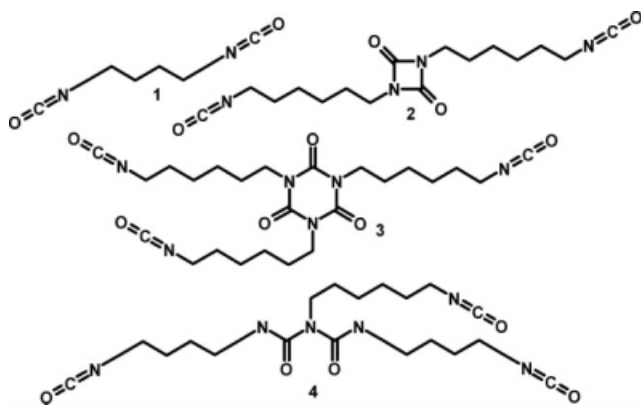
Figure 1 Chemical structures of hexamethylene di-isocyanate (**1**) and of selected prepolymers derived from hexamethylene di-isocyanate: uretidone (**2**), isocyanurate (**3**), and biuret (**4**).

an analytical procedure capable of demonstrating the absence of any residual HDI or HDI-derived biuret trimer, with respective limits-of-quantification of 0.25 and 0.6 μg of NCO functional group per kg of microcapsules suspension.

Quantitative analysis of trace amounts of isocyanates is highly relevant in occupational health monitoring.[5] It is typically achieved by liquid chromatography (LC) after derivatization since the high reactivity of isocyanates precludes their direct chromatographic analysis. The use of almost all available LC detectors has been reported, including ultraviolet detectors, fluorescence detectors, electrochemical detectors, and mass spectrometers, and in each case a number of suitable derivatization reagents have been proposed. Among these methods, LC with mass spectrometric (LC/MS) or preferably tandem mass spectrometric detection (LC/MS-MS) is now recognized as being the most sensitive and selective, and dibutylamine is one of if not the most frequently used derivatization reagent.[6–9] Therefore, this is the approach, we selected. Unfortunately none of the reported sample preparation procedures, most of them developed to determine levels of airborne isocyanates[5] or more rarely to characterize technical polyisocyanates[7] are applicable as such to the perfume-containing microcapsules under investigation. The closest applications were methods for the determination of residual isocyanate monomers in plastics intended for food contact use[10] and for the determination of residual isophorone di-isocyanate trimer in polyester-polyurethane coatings.[11] Therefore, a new sample preparation procedure was developed specifically for the reported study.

MATERIALS AND METHODS

Materials

Polyvinyl alcohol (PVOH, 18–88, dp 2700), tetraethyl ammonium chloride, guanidine carbonate, HDI,

dibutylamine and mass spectrometry grade formic acid were purchased from Fluka (Buchs, Switzerland). Desmodur[TM] N100 (mainly the biuret trimer **4** derived from HDI), Desmodur[TM] N3200 (mainly the biuret trimer **4** derived from HDI but with a different species distribution than Desmodur[TM] N100) and Desmodur[TM] N3300 (mainly the isocyanurate trimer **3** derived from HDI) were purchased from Bayer (Leverkusen, Germany). The model perfume consisting of five fragrance compounds was formulated by mixing equal weights of hexyl salicylate, (±)-methyl 2,2-dimethyl-6-methylene-1-cyclohexanecarboxylate (Romascone®), 3-(4-*tert*-butylphenyl)-2-methylpropanal (Lilial®), cis/trans-4-*tert*-butyl-1-cyclohexyl acetate (Vertenex®), and (±)-2-*tert*-butyl-1-cyclohexyl acetate (Verdox®). Water for LC/MS analysis was prepared using a purification device (Synergy 185, Millipore, Billerca, MA). LC/MS grade acetonitrile was purchased from Merck (Darmstadt, Germany). Analytical grade toluene was from Carlo Erba (Rodano, Italy) and analytical grade pentane was from SDS (Peypin, France). All of these chemicals were used as received.

Microcapsule preparation

Polyurea microcapsules were prepared by an emulsion polymerization process using a procedure well described in the literature after slight modification.[12,12,13] The monomer molar ratio of NCO to NH₂ was fixed at 0.6. The organic phase obtained by dissolving 11.45 g of Desmodur[TM] N100 into 200 g of perfume was dispersed into 274.25 g of 1% PVOH aqueous solution at room temperature. The emulsion was then generated using a rotor/stator system (IKA T25, Staufen, Germany, stator diameter 18 mm, rotor diameter 12.7 mm). The desired size distribution was obtained by adjusting the speed and the time of shearing. The emulsion was then transferred to a 500 mL-reactor and stirred continuously at 600 RPM. Then 2 g of tetraethyl ammonium chloride were added into the reactor. Guanidine carbonate of 4.50 g , dissolved in 9.8 g deionized water were added dropwise over 30 min into the reactor. After being held for 10 min at room temperature, the reaction mixture was heated to 70°C in 45 min. This temperature was kept constant for 2 h. The microcapsule suspension was then divided into five 100 mL samples which were subject to different treatments. Sample A was immediately cooled down to room temperature and used as reference (untreated sample). To sample B, 1.25 g of 10% NaOH solution were added and the temperature was held at 70°C for 2 h before cooling to room temperature. To sample C, 0.6 g of guanidine carbonate were added and the temperature was held at 70°C for 2 h before cooling to room temperature. To sample D, 3.37 g of

a 30% ammonia solution were added and the temperature was held at 70°C for 2 h before cooling to room temperature. Finally, sample E was kept as such but maintained at 70°C for an additional 2 h compared with sample A before being cooled to room temperature. Afterwards, all samples were stored at room temperature and in the dark for a period of 56 days.

Particle size distribution

The particle size and the size distribution of the microcapsules were examined by optical microscopy and laser diffraction measurements (MasterSizer, Malvern Instruments Ltd,., Malvern, England).

Thermogravimetric analysis

Solid content and perfume retention were assessed using a thermogravimetric analyzer (TGA, Mettler-Toledo, Grefensee, Switzerland) equipped with a microbalance having an accuracy of 1 µg and a 35 mL oven. The microcapsule sample (about 14 mg) was introduced into an aluminum oxide crucible and analyzed by TGA under controlled temperature and a constant flow of nitrogen of 20 mL/min. The sample was heated from 25 to 50°C at a rate of 5°C/min and then the temperature was held at 50°C for about 2 hours.

Preparation of authentic standards for LC/MS-MS determination of residual isocyanates

N',N'''-1,6-hexanediylbis(N,N-dibutylurea) (HDI-DBA), the dibutylamine derivative of HDI, was prepared by slowly adding 50 mL of a solution containing 2.93 mmol of HDI in toluene to 50 mL of a solution containing 50.8 mmol of dibutylamine in toluene. The mixture was allowed to stand overnight and the solvent was removed. The white solid was redissolved in dichloromethane, washed with water that had been acidified with formic acid, dried again, and recrystallized in pentane. White crystals of 999 mg were obtained. ^{1}H-NMR (400 MHz, CD$_3$OD): 3.20 (t, J = 7.7, 8H), 3.13 (t, J = 7.1, 4H), 1.56–1.43 (m, 12H), 1.39–1.25 (m, 12H), 0.94 (t, J = 7.4, 12H). ^{13}C-NMR (100 MHz, CD$_3$OD): 159.98 (s), 47.67 (t), 41.50 (t), 31.63 (t), 31.40 (t), 27.61 (t), 21.01 (t), 14.32 (q).

N,N,N'-tris(6-[[(dibutylamino)carbonyl]amino]hexyl)dicarbonimidic diamide, the dibutylamine derivative of the biuret trimer generated from HDI, was prepared by slowly adding 50 mL of a solution containing 0.5 mL of Desmodur[TM] N3200 (mainly the biuret trimer derived from HDI) in toluene to a solution containing 20 mL of dibutylamine in toluene. The mixture was allowed to stand overnight, and the solvent was removed. The oil was redis-

solved in dichloromethane, washed with water that had been acidified with formic acid, and dried again. The target compound was purified from its analog by preparative HPLC on a 250× 8 mm Nucleosil 100-7 C2 column (Macherey-Nagel, Duren, Germany) eluted by a gradient of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. The purity of the 30 s fractions was checked by LC/MS, and pooling or repurification of unsatisfactory fractions was conducted accordingly. A viscous high purity product of 540 mg were obtained after freeze-drying. ^{1}H-NMR (400 MHz, CD$_3$OD): 3.19 (2× t, J = 7.2, 16H), 3.13 and 3.12 (2× t, J = 7.0, 8H), 1.58–1.42 (m, 24H), 1.41–1.22 (m, 24H), 0.92 (t, J = 7.3, 18H). ^{13}C-NMR (100 MHz, CD$_3$OD): 163.43 (s), 160.11 (s), 158.20 (s), 47.77 (t), 43.52 (t), 41.57 (t), 41.52 (t), 41.35 (t), 31.71 (t), 31.41 (t), 30.71 (t), 29.83 (t), 27.71 (t), 27.69 (t), 27.61 (t), 27.28 (t), 21.10 (t), 14.37 (q).

Sample preparation for LC/MS-MS determination of residual HDI and HDI biuret in microcapsule samples

Aliquots of microcapsules of 500 mg were sampled at regular intervals over the 8 weeks of the study. Residual free isocyanates were immediately derivatized by addition of 10 mL volumes of a derivatization solution containing 100 mM dibutylamine in fresh tetrahydrofurane (THF). The suspensions were then exposed for 20 min to a 35 kHz ultrasonic bath (T 460 H, Bioblock, Illkirch, France), and then vortexed for 30 s. The resulting solutions were stored until the end of the study to be analyzed by LC/MS-MS as preliminary investigations had revealed that they were stable enough. They were vortexed before analysis, then allowed to settle for a few minutes, and the supernatants were analyzed without any further sample preparation. All samples were prepared in duplicate.

Sample preparation for LC/MS-MS analysis of reference technical polyisocyanates

Technical polyisocyanates (Desmodur[TM] N100, Desmodur[TM] N3300), 25 mg quantities, were dissolved in 100 mM dibutylamine in fresh THF and allowed to react for 20 min in a 35 kHz ultrasonic bath. The resulting solutions were diluted as required with fresh THF.

LC/MS-MS method

The LC/MS-MS instrument was made up of a Shimadzu (Kyoto, Japan) UFLC XR chromatograph (CBM-20A interface, DGU-20A5 degasser, LC-20AD pumps connected by two SUS-316L semimicro

TABLE I
Parameters of the Mass Spectrometer for the Determination in MRM Mode of the Dibutylamine Derivatives of Two Polyisocyanates Most Relevant for the Microcapsules Under Investigation (HDI 1 and the Biuret Trimer 4), and of the Dibutylamine Derivatives of Six Further Polyisocyanates of Potential Interest for the Study of Other Microcapsule Formulations

Period	Analyte	tR (min)	Precursor (amu)	Product (amu)	Dwell time (ms)	DP (V)	CE (V)	CXP (V)
1	HDI **1**	4.82	427.40	298.25	200	121	25	8
			427.40	130.16	200	121	43	8
2	Uretidone dimmer **2**	6.33	595.49	298.25	200	141	33	8
			595.49	130.16	200	141	35	10
3	Biuret trimer **4**	7.77	866.75	440.36	200	121	47	12
			866.75	130.16	200	121	83	8
	Isocyanurate trimer **3**	8.19	892.73	634.43	50	126	49	20
			892.73	130.16	50	126	65	8
	Biuret/ure-tidone tetramer	8.65	1034.84	440.36	50	126	53	12
			1034.84	130.16	50	126	99	8
	Isocyanu-rate/ureti-done tetramer	9.00	1060.82	931.67	50	41	35	32
			1060.82	130.16	50	41	87	8
4	Biuret pentamer	9.59	1306.11	440.36	200	146	73	12
			1306.11	130.16	200	146	125	8
	Isocyanurate pentamer	10.13	1358.06	970.61	200	151	73	16
			1358.06	130.16	200	151	119	6

tR: Retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential.

mixers placed in series, SIL-20AC HT autosampler held at 20°C, CTO-20AC column oven) and an Applied Biosystems/MDS Sciex (Concord, Canada) hybrid triple quadrupole/ion trap mass spectrometer (4000 Q Trap with Turbo V source and Turbo ion spray probe, ion spray +5000 V, 500°C, gas 1 38 psig, gas 2 48 psig, curtain gas 20, CAD gas 8 psig). Chromatographic separation was achieved on a 250 × 2 mm Nucleosil 100-7 C2 column (Macherey-Nagel, Germany) held at 30°C and eluted at 0.5 mL/min with a gradient of water containing 0.1% formic acid (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). The eluent composition started with 50% B for 1.5 min and was ramped to 80% B in 10 min and then to 95% B in 0.1 min. The column was rinsed for 2 min with 95% B, following which the eluent composition was brought back to the original composition in 0.1 min, and the column reequilibrated for 5 min. Aliquots (5 μL) were injected. The eluent was only allowed to enter the mass spectrometer between 3.5 and 11.5 min by using a diverting valve (C72H-1690ED, VICI, Schenkon, Switzerland). Quantitative MS analysis was performed in multiple reaction monitoring (MRM) mode using the parameters listed in Table I. Only HDI, the most hazardous polyisocyanate due to its volatility which is present at <1% in Desmodur™ N100 according to the specifications, and the biuret trimer which is clearly the most abundant species in the technical polyisocyanate Desmodur™ N100 used to prepare the microcapsules were determined. Nevertheless, the method was developed in a way to render it suitable for the investigation of other capsule formulations that are based on different technical polyisocyanates derived from HDI. Therefore, parameters allowing the determination of a number of analogs are included in the method, which was split into four periods to keep the duty cycle short enough. The capability of the hybrid mass spectrometer to acquire high quality product ion spectra when scanning the third quadrupole as an ion trap was utilized to record confirmation spectra at a scan rate of 4000 amu/s for HDI during period 1 (precursor 427.40 amu, collision energy (CE) 35 V with collision energy spread CES 20 V), the uretidone dimer during period 2 (precursor 595.49 amu, CE 35 V, CES 20 V), and the biuret trimer during period 3 (precursor 866.75 amu, CE 35 V, CES 20 V). The data was reprocessed for quantitative analysis using Analyst 1.4.2 software (11 points smoothing, quadratic fit, 1/x weighting). Limits-of-quantification were determined as being the lowest concentrations at which the data point could still be fitted with an accuracy between 90 and 110%. Sample concentrations were calculated by averaging the four values determined from the duplicate samples using the two MRM transitions programmed for each analyte, after having verified that for any given sample the difference between the two MRM transitions did not exceed 5%.
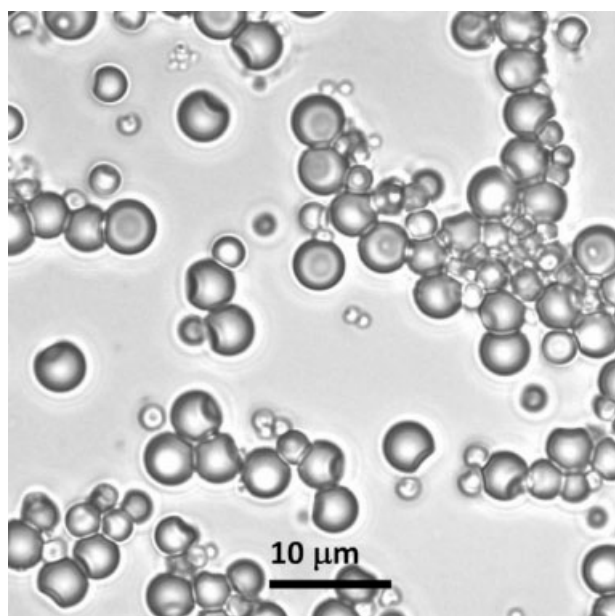
Figure 2 Typical shape of the perfume-containing polyurea microcapsules as observed by optical microscopy (magnification ×100).

RESULTS AND DISCUSSION

Physical characterization of the microcapsules

Observation of the perfume-containing polyurea microcapsules by optical microscopy (Fig. 2) revealed spherical shapes with diameters ranging between 2 and 20 μm. Analysis by laser diffraction (Fig. 3) evidenced a rather broad size distribution with an average diameter D(V,0.5) of 3.4 μm.

The microcapsules were investigated by thermogravimetric analysis to determine their solid content and to gain insights into their perfume retention. This was achieved by monitoring the weight loss of the microcapsules at 50°C under a constant flow of nitrogen, resulting in isotherm plots such as the one displayed in Figure 4 for sample A, the one obtained after 2 h of reaction. These plots showed two regimes. The first one, fast, was dominated by the evaporation of water and nonencapsulated fragrance. The second one, slow, was assigned to the evaporation of encapsulated perfume, which is limited by the diffusion through the polyurea membrane. The perfect plateau observed in Figure 4 is typical of good fragrance retention. A solid content of 44% was determined for the four samples A-D.

After dissolution of guanidine carbonate, the pH of the aqueous phase was around 10.3. The NH_2 groups were then present in both protonated and unprotonated form. On the basis of ability of the unprotonated form to partition with the oil phase, it has been suggested that only this form can take part in the interfacial polyaddition reaction.[12,13] At the end of the polymerization process, the pH of the aqueous phase had decreased to 8.3. The first approach to complete the isocyanates conversion, applied to sample B, consisted of increasing the pH of the reaction medium up to 10 by addition of NaOH solution. In samples C and D, the addition of guanidine carbonate and ammonia solutions increased the pH value up to 9.4 and 10.0, respectively.

LC/MS-MS method for the determination of residual HDI and HDI biuret trimer

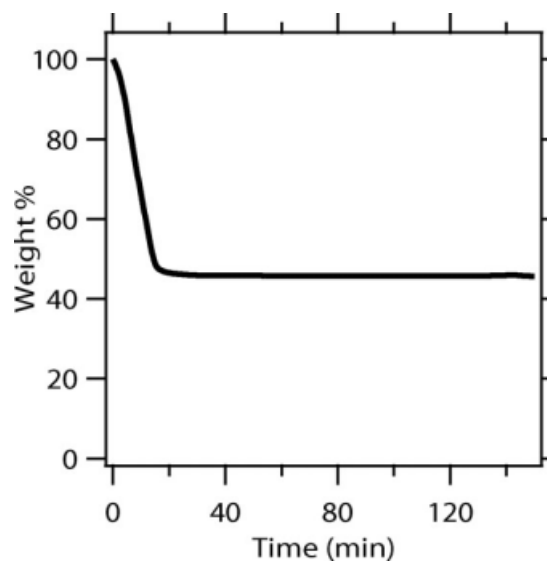Figure 5 displays a reference chromatogram recorded for a solution containing the dibutylamine

Figure 3 Particle size distribution of the perfume-containing polyurea microcapsules.

Figure 4 Thermogravimetric analysis plot of the perfume-containing polyurea microcapsules obtained after 2 h reaction time (sample A).
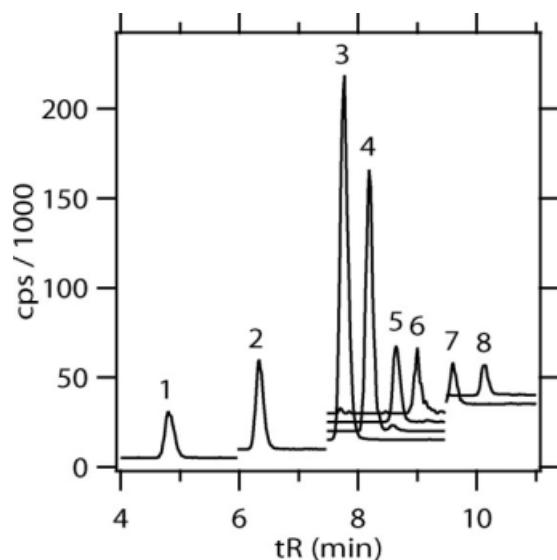
Figure 5 Representative chromatogram of selected mass spectrometric traces recorded from a mixture of dibutylamine derivatives of Desmodur[TM] N100 and Desmodur[TM] N3300. 1: HDI (427.40 → 130.16 MRM transition, zoomed 10x). 2: Uretidone dimer (595.49 → 130.16, zoomed ×10). 3: Biuret trimer (866.75 → 130.16). 4: Isocyanurate trimer (892.73 → 130.16). 5: Biuret/uretidone tetramer (1034.84 → 130.16, zoomed ×10). 6: Isocyanurate/uretidone tetramer (1060.82 → 130.16, zoomed ×100). 7: Biuret pentamer (1306.11 → 130.16, zoomed ×10). 8: Isocyanurate pentamer (1358.06 → 130.16, zoomed ×10).

derivatives of a 1 : 1 mixture of Demodur[TM] N100 (mainly HDI biuret trimer) and Desmodur[TM] N3300 (mainly HDI isocyanurate trimer). Good chromatographic separation of all potentially interesting analytes is obvious. In the specific case of the considered capsules prepared from Desmodur[TM] N100, only the determination of HDI and its biuret trimer (peaks 1 and 3, respectively) was relevant.

Evolution of the free isocyanate levels in samples prepared through different processes

Figures 6 and 7 display the evolution of the levels of HDI and its trimer over the course of 8 weeks. In the reference sample (2 h, no scavenger) a content of about 160 mg NCO/kg as HDI biuret trimer and about 1 mg NCO/kg as HDI monomer were detected immediately after the preparation of the capsules. Allowing the reaction to proceed for an additional 2 h at 70°C resulted in a decrease of these levels by a factor of about 10 and 2, respectively. A much more efficient scavenging approach consisted in adding NaOH or guanidine to let them act as free isocyanate scavengers before allowing the reaction to proceed for an additional 2 h at 70°C. In both these cases, residual free polyisocyanate levels were determined to have dropped by a factor of about 500 in the form of HDI biuret trimer and by a factor of
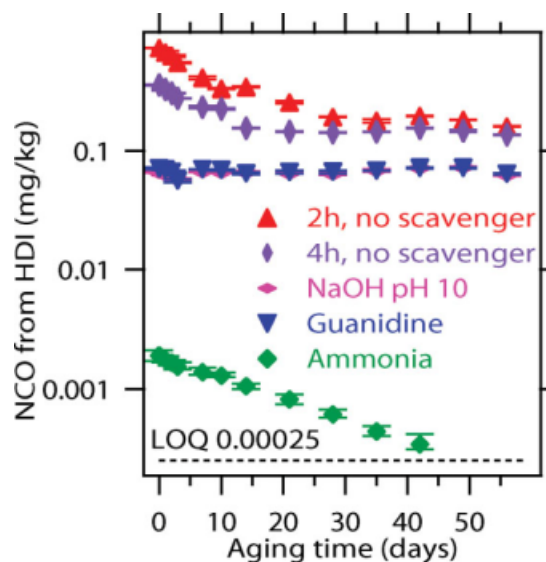

Figure 6 Evolution of the free HDI levels in four perfume-containing polyurea microcapsule formulations over the course of 8 weeks.

about 10 in the form of HDI monomer. In all these cases, residual polyisocyanates were observed to continuously degrade for about 1 month, before reaching a plateau. At that point, total residual polyisocyanate levels were below 0.5 mg NCO/kg in all cases, and lower in the case of the samples that had been treated with NaOH or guanidine carbonate as isocyanate scavengers. Note that, even though these two latter samples exhibited similar free isocyanate levels throughout the study, guanidine is viewed as
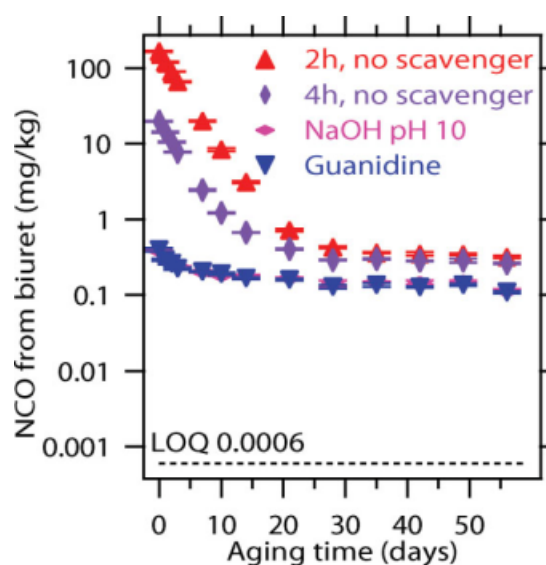

Figure 7 Evolution of the free HDI biuret trimer levels in four perfume-containing polyurea microcapsule formulations over the course of 8 weeks. The samples containing ammonia as a scavenger were also analyzed, but no HDI biuret trimer was detected.

a more valuable approach as addition of NaOH was observed to induce a flocculation of the microcapsules.

An even more efficient free isocyanate scavenger was observed to be ammonia. In this case, no residual HDI biuret trimer at all could be detected even immediately after the preparation of the microcapsules, with a limit of quantification (LOQ) of 0.6 μg NCO/kg. Furthermore, only 2 μg NCO/kg were detected in the form of HDI monomer. This very low level was observed to decrease continuously over time, and had dropped below the LOQ of 0.25 μg NCO/kg after about 6 weeks of aging at room temperature and in the dark. In the case of ammonia used as an polyisocyanate scavenger, no plateau of final residual concentration was observed.

CONCLUSIONS

To the best of our knowledge, this report is the first one to describe bioactives containing polyurea microcapsules with undetectable level of free isocyanates. An analytical procedure is described that allowed the quantitative determination of trace HDI and HDI-derived biuret trimer LOQs of 0.25 and 0.6 μg of NCO groups per kg of microcapsules suspension, respectively. Ammonia was demonstrated to be an especially powerful isocyanate scavenger, resulting in undetectable isocyanate levels within 40 days at room temperature. This duration could be reduced significantly by increasing the concentration of the isocyanates scavenger and/or the temperature of the microcapsules suspensions.

In addition to the reported performance as a controlled-release system,[14] this results reinforce the potential of the polyurea microcapsules in functional perfumery applications. Considering that in consumer products polyurea microcapsules are diluted about 100 times, one could expect to achieve undetectable free isocyanate levels even without sample aging.

References

1. Park, S. J.; Arshady, R. Microcapsules for Fragrances and Cosmetics; Citus Books: London, England, 2003; Vol. 6.
2. Thies, C. A Short History of Microencapsulation Technology. MML Series; Citus Books: London, England,1999; Vol. 1.
3. Quellet, C.; Schudel, M.; Ringgenberg, R. Chimia 2001, 55, 421.
4. Arshady, R. J. Microencapsulation 1989, 6, 13.
5. Henneken, H.; Vogel, M.; Karst, U. Anal Bioanal Chem 2007, 387, 219.
6. Tinnerberg, H.; Spanne, M.; Dalene, M.; Skarping, G. Analyst 1996, 121, 1101.
7. Marand, A.; Dahlin, J.; Karlsson, D.; Skarping, G.; Dalene, M. J Environ Monit 2004, 6, 606.
8. Karlsson, D.; Spanne, M.; Dalene, M.; Skarping, G. Analyst 1998, 123, 117.
9. Karlsson, D.; Dahlin, J.; Marand, A.; Skarping, G.; Dalene, M. Anal Chim Acta 2005, 534, 263.
10. Damant, A. P.; Jickells, S. M.; Castle, L. J AOAC Int 1995, 78, 711.
11. Driffield, M.; Bradley, E. L.; Castle, L. J Chromatogr A 2007, 1141, 61.
12. Kleban, M.; Klug, G.; Weisser, J. U.S. 6,797,670 (2004).
13. Yadav, S. K.; Khilar, K. C.; Suresh, A. K. AIChE J 1996, 42, 2616.
14. Ouali, L.; Benczedi, D. WO 2007004166 (2005).