Synthesis and Characterization of Polyurea Microcapsules Containing Essential Oils With Antigerminative Activity

Paola Scarfato,[1] Elvira Avallone,[1] Pio Iannelli,[2] Vincenzo De Feo,[2] Domenico Acierno[3]

[1]*Department of Chemical and Food Engineering, University of Salerno, 84084 Fisciano (SA), Italy*
[2]*Department of Pharmaceutical Sciences, University of Salerno, 84084 Fisciano (SA), Italy*
[3]*Department of Materials and Production Engineering, University of Naples ''Federico II'', 80125 Naples, Italy*

Received 24 November 2006; accepted 13 March 2007
DOI 10.1002/app.26420
Published online 5 June 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this paper we report on the preparation and characterization of polyurea-based microencapsulated systems, containing essential oils as core materials, for potential applications in controlled-release formulations of agrochemicals. Microcapsules were synthesized by interfacial polymerization in o/w emulsion between polyfunctional isocyanates and diamines, to investigate the effect of the monomer kind on the morphology and properties of the produced samples. The synthetic conditions that gave the best results were used to microencapsulate four essential oils, able to interfere with the seed germination and radicle elongation of some test plants. The produced samples were characterized, with the aim to analyze their morphology and to verify the effectiveness of essential oil microencapsulation. Moreover, preliminary bioassay based on seed germination and subsequent radical growth were carried out to study the effects of the microencapsulated essential oils. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 3568–3577, 2007

Key words: microcapsules; polyurea; interfacial polymerization; essential oils; antigerminative effects

INTRODUCTION

Microencapsulated systems for controlled-release of bioactive molecules or for protection of functional materials have attracted much interest in the last years.[1–6] Many industrial sectors are interested in microencapsulation processes: health, cosmetic, food, agricultural, chemical, etc. Among them, the agricultural one especially demands coating techniques at the lowest possible cost and at highest efficacy, for applications in controlled-release formulations of agrochemicals e.g., insecticides, pesticides, or herbicides. Although the obtained microparticulate systems may have a higher cost than the conventional formulations, they meet numerous environmental advantages: smaller quantities of active substances need to be applied with increased duration of their effectiveness; evaporative losses of volatile agents are reduced; the handling of liquid substances becomes more easy and safe, changing them in solid systems; environmental degradation is slackened; toxicity against plants and mammalian is lowered.

Several methods have been proposed to realize microencapsulated systems for controlled-release of active substances.[1,6–8] One of the most interesting agricultural application is the microencapsulation by interfacial polymerization in emulsion.[8–11] This technique ensures high structural and performance versatility, in terms of morphology, hardness, porosity, thermal properties, and permeability of the microcapsule shell. Moreover, depending on microcapsule composition, it can also be fairly biostable and environmentally compatible.[12–17] Finally, opportunistically selecting the emulsion composition, it allows to microencapsulate both hydrophilic and lipophilic molecules.

Nowadays, the interfacial polymerization is used to microencapsulate a wide range of active agents within various polymeric shells, mostly polyureas.[1] In fact, the isocyanate group easily reacts with nucleophilic agents and its reaction with amines is particularly fast compared with that one occurring with alcohols and water. For this reason, isocyanates and amines are suitable monomers to be used in microencapsulation by interfacial polymerization in emulsion, where a fast reaction is required to avoid emulsion destabilization and shell deformation and/or breakage. Moreover, polyureas are cheap and safe for the environment.

In this paper we report on preparation, by interfacial polymerization in oil in water (o/w) emulsion, and characterization of polyurea microcapsules containing active essential oils in the core, for agricultural applications. It is well known, in fact, that many essential oils are able to inhibit the seed ger-

Correspondence to: P. Scarfato (pscarfato@unisa.it).
Contract grant sponsors: Università degli Studi di Salerno.

mination of other plants than the ones producing them[18–24] and thus may be useful as ''natural product herbicides'' for organic farming systems. Nevertheless, because of their high volatility, high level of application is generally required, to obtain long duration of their effectiveness. In the first part of the work the effects of the kind of reacting monomers on the morphology and the properties of synthesized microcapsules were investigated. Later, the synthetic conditions that gave the best results were used to microencapsulate four essential oils (lemon balm, lavender, sage, and thyme) with antigerminative activity. The synthesized microcapsules were submitted to chemical–physical characterization to evaluate their morphology and to verify the effectiveness of essential oil microencapsulation. Moreover, they were assayed in preliminary tests against the germination and the successive radical growth of seeds of three different species (radish, lettuce, and garden cress) with the aim to verify if the inhibitory activity of the encapsulated oils was preserved.

EXPERIMENTAL

Materials

Poly[(phenyl isocyanate)-co-formaldehyde] (PPI, oligomer, average Mn 400 ca., activity $\sim$ 3.2 isocyanate groups/molecule), tolylene 2,4-diisocyanate 95% (TDI), hydrazine monohydrate 98% (HH), ethylenediamine (EDA), and butanediamine (BDA), pentyl acetate 99% (PA) were purchased from Aldrich and used without further purification. Polyoxyethylenesorbitan monooleate (Tween 80) was obtained from Fluka. All the chemicals purchased were reagent grade.

Essential oils used for microencapsulation experiments were extracted from lemon balm (*Melissa officinalis* L.), lavender (*Lavandula angustifolia* Miller), sage (*Salvia officinalis* L.), and thyme (*Thymus vulgaris* L.). Aromatic plants were grown at the Garden of Medicinal Plants in the Campus of the University of Salerno. Aerial parts were collected at full flowering stage. A voucher specimen of each plant is deposited in the Herbarium of the Medical Botany Chair, Faculty of Pharmacy, University of Salerno.

Seeds of radish (*Raphanus sativus* L. cv. ''Saxa''), garden cress (*Lepidium sativum* L. cv. ''Inglese''), and lettuce (*Lactuca sativa* L. cv. ''Grand Rapid''), used for antigerminative tests, were purchased from Imperatore Co. (Naples, Italy).

Oil extraction

Fresh picked aerial parts of aromatic plants were cut into small pieces, air dried, and then submitted to hydrodistillation for 3 h, according to the standard procedure reported in the European Pharmacopoeia (1975).[25] Pale yellow essential oils were usually obtained in $\sim$ 0.3% yield on dry weight basis (lavender, 0.49%; lemon balm, 0.25%; sage, 0.46%; thyme, 0.26%).

Microencapsulation

Polyurea microcapsules were synthesized by interfacial polymerization in o/w emulsion between various polyisocyanates and diamines, using several oily core materials. The preparation conditions and the corresponding adopted nomenclature are summarized in Tables I and II. The general preparation procedure was the following. In o/w emulsion the aqueous phase was composed by 170 mL of deionized water containing 0.5 wt % of Tween 80 as emulsifier, whereas the organic phase was a mixture of essential oil and isocyanate monomer (TDI and/or PPI in the appropriate ratio), dissolved in the minimum volume of PA. The two phases were emulsified at room temperature at 3000 rpm. Then, the second monomer (HH, EDA, or BDA), previously diluted in 30 mL of deionized water, was added to the emulsion and the system was left to react for 3 h under agitation at 150 rpm. The resultant microcapsules slurry was filtered, washed two times with water, and left to dry at room temperature. All syntheses were made in triplicate, to check reproducibility.

TABLE I
Sample Nomenclature and Synthetic Condition Used for Preparation of Polyurea Microcapsules from Different Polyisocyanate and Diamine Monomers

	Oily phase		Reacting monomers				
Sample	PA (mL)	PPI (g)	TDI (g)	HH (g)	EDA (g)	BDA (g)	NH_2/NCO molar ratio
S1	12	–	3.00	1.00	–	–	1.16
S2	12	0.75	2.25	1.00	–	–	1.25
S3	12	1.50	1.50	1.00	–	–	1.37
S4	12	2.25	0.75	1.00	–	–	1.50
S5	12	3.00	–	1.00	–	–	1.66
S6	12	3.00	–	–	1.50	–	2.00
S7	12	3.00	–	–	–	1.98	1.87

TABLE II
Sample Nomenclature and Synthetic Condition Used for Preparation of Polyurea Microcapsules Containing Essential Oils

	Oily phase			Reacting monomers	
Sample	PA (mL)	Essential oil	(mL)	PPI (g)	HH (g)
S8	1	Lemon balm	3	3.00	1.00
S9	1	Lavender	3	3.00	1.00
S10	1	Sage	3	3.00	1.00
S11	1	Thyme	3	3.00	1.00

The same preparation procedure was also used to synthesize blank microcapsules without essential oil in the core, used for comparison.

The powder products obtained were stored at −20 °C in sealed glass bottle to protect them against light and oxygen.

Bioassay

A bioassay based on seed germination and subsequent radical growth was used to study the effects of the microencapsulated essential oils on seeds of three different species usually used as targets for antigerminative studies: *Raphanus sativus* L. (radish), *Lepidium sativum* L. (garden cress), and *Lactuca sativa* L. (lettuce). The seeds were surface-sterilized in 95% ethanol for 15 s before testing. The bioassays were made using germination chambers, prepared following a procedure suggested by Oleszek.[26] According to this procedure, 0.5 g of microcapsules were placed in a small petri dish (ϕ, 30 mm) in turn placed in an other petri dish (ϕ, 90 mm) containing five layers of Whatman filter papers, impregnated with 7 mL of distilled water and sown with 10 seeds. Then, the germination chambers were incubated at (22 ± 1)°C and exposed to white light (light photoperiod, 12 h; light intensity, 25 μmol m^{-2} s^{-1}). Seed germination process was observed with a stereomicroscope at steps of 12 h (a seed was considered germinated when the protrusion of the radicle became evident[27]). The radicle elongation was determined by measuring radicle length (in mm) after 120 h (on the fifth day). Each determination was repeated on 10 petri dishes and the results were expressed as the mean ± standard deviation.

The results were compared with those obtained from similar bioassay performed using the essential oils alone instead of microcapsules. With this aim, the tests were replicated, charging 1.5 mL of neat essential oil in the small petri dishes of the germination chamber, and so assuring the saturation of larger petri dish by the oil evaporation.

Analytical techniques

Emulsions were prepared by a Silverson L4RT high-shear mixer (Silverson Machines, Inc., East Longmeadow, MA), operated at 3000 rpm for 60 s at room temperature.

FTIR measurements were carried out on neat microcapsule samples in the range of 4000–650 cm^{-1}, using a Nexus ThermoNicolet spectrometer equipped with a SmartPerformer accessory for ATR analyses.

Scanning electron micrographs were obtained using a LEO 420 apparatus (LEO Electron Microscopy Ltd.). The samples, sprinkled onto a double-sided carbon adhesive tape that had previously been secured on aluminum stubs, were coated with an AuPd alloy using a high vacuum sputter coater before analysis.

Microcapsules were analyzed for their size distribution. Dried particles were dispersed in distilled water containing 1% by wt. of Tween 20 surfactant and sonicated in water bath for 5 min before sam-

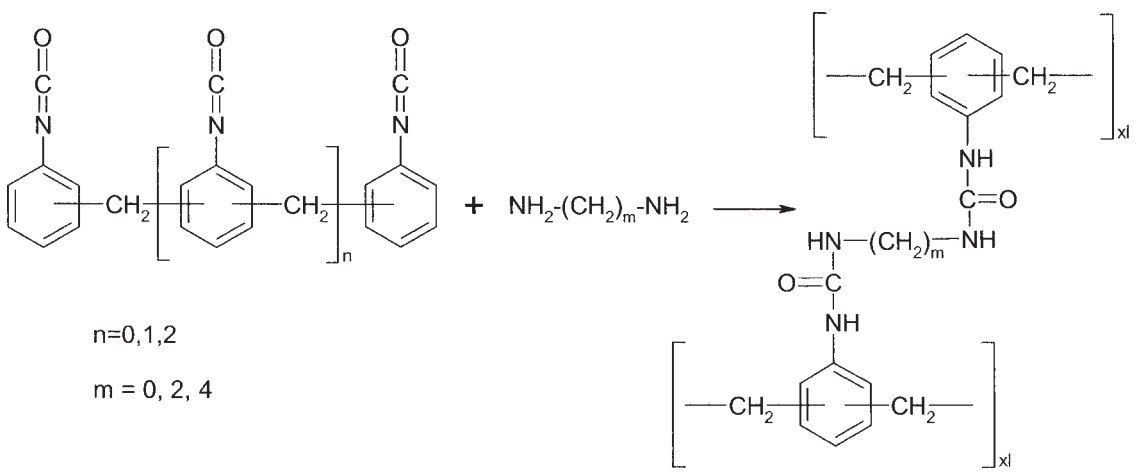

Figure 1 Scheme of the reaction between the PPI and the diamines.

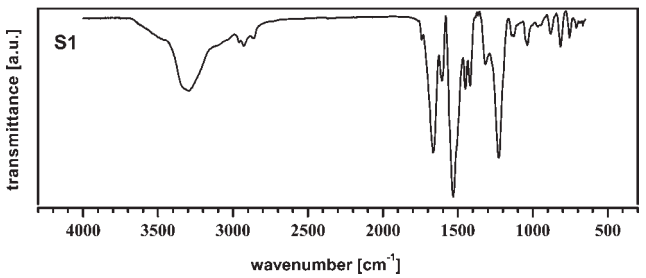

Figure 2 FTIR spectrum of S1 polyurea microcapsules, prepared from TDI and HH monomers.

pling. Particle size analysis was obtained using a laser Malvern Mod. Mastersizer S instrument.

Thermogravimetric analyses (TGAs) were performed with a TA Instruments Q500 thermogravimetric analyzer at a heating rate of 20°C/min. The samples were tested in the temperature range of 30–550°C in nitrogen atmosphere.

The amount of microencapsulated oil was determined by quantitative HPLC using a Waters Breeze HPLC System with an UV-Vis Dual λ Absorbance Detector mod. 2487 and a Differential Refractive Index (dRI) Detector mod. 2414. Column: Symmetry C18 5μm (i.d. 4.6 × 150 mm); eluent: methanol; flow rate, 1.00 mL/min; injection, 10 μL of methanol solution; extractive solution, 0.300 g per 20 mL. The UV detection parameters were the following: (1) lemon balm, λ = 274 nm; (2) lavender, λ = 232 nm; (3) sage, λ = 320 nm; (4) thyme, λ = 274 nm. The correlation coefficients were always above 0.99 (5 points, 3 assays).

RESULTS AND DISCUSSION

To investigate the effects of the kind of reactive monomers on the structure, properties, and release behavior of microencapsulated systems, blank polyurea microcapsules, containing pure PA as core substance, were first synthesized by interfacial polymerization in o/w emulsion.

In this study, PPI and TDI monomers, bearing about three and two isocyanate groups, respectively, were selected with the aim to obtain shell membranes with different crosslinking degrees, by varying PPI/TDI ratio. HH, EDA, and BDA monomers, having two terminal amine groups but different length, were chosen with the aim to modulate membrane stiffness and permeability. As an example, the reaction between the PPI and the diamines was sketched in Figure 1. The synthetic conditions for each sample were detailed in Table I. The amino/isocyanate feed ratio was arbitrarily fixed to a value higher than the stoichiometric one ($1.16 < n_{NH2}/n_{NCO} < 2.00$), in the attempt to let completely react the isocyanate units. In fact, isocyanate must be absent in the final product to avoid chemical hazard when microcapsules are used as biocompatible system.

Figures 2 and 3 show the FTIR spectra of pure PA solvent and microcapsule samples from S1 to S7. The spectrum corresponding to S1 sample (Fig. 2) shows that the resultant wall membrane of all microcapsules is polyurea, as it results by the disappearance of the N=C=O characteristic absorbance peak at around 2275 cm^{-1} and by the outbreak of the N—H and C=O absorbance peaks, in comparison to the FTIR traces of the pure PPI and TDI monomers (not shown).[28] Accordingly, the spectrum exhibits a strong broad band at around 3300 cm^{-1}, due to the N—H stretching vibrations, and an urea carbonyl

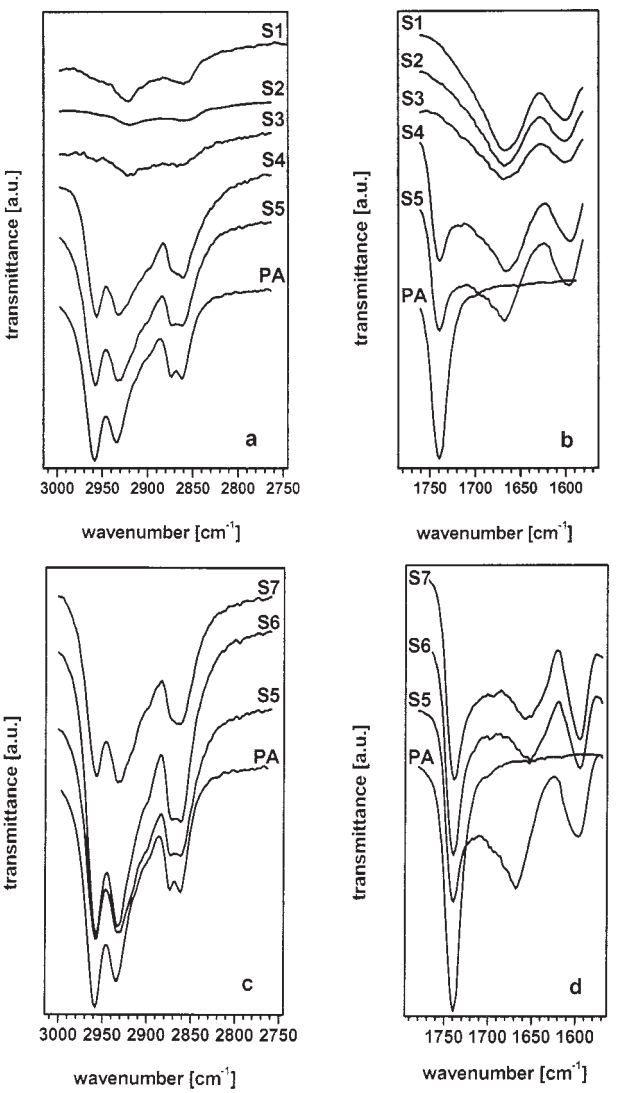

Figure 3 FTIR spectra of S1–S7 polyurea microcapsules having different shell composition: Effect of the PPI/TDI ratios (a, b) and effect of the kind of diamine monomer (c, d). The FT-IR response of pure pentylacetate used as core material is also shown for comparison.

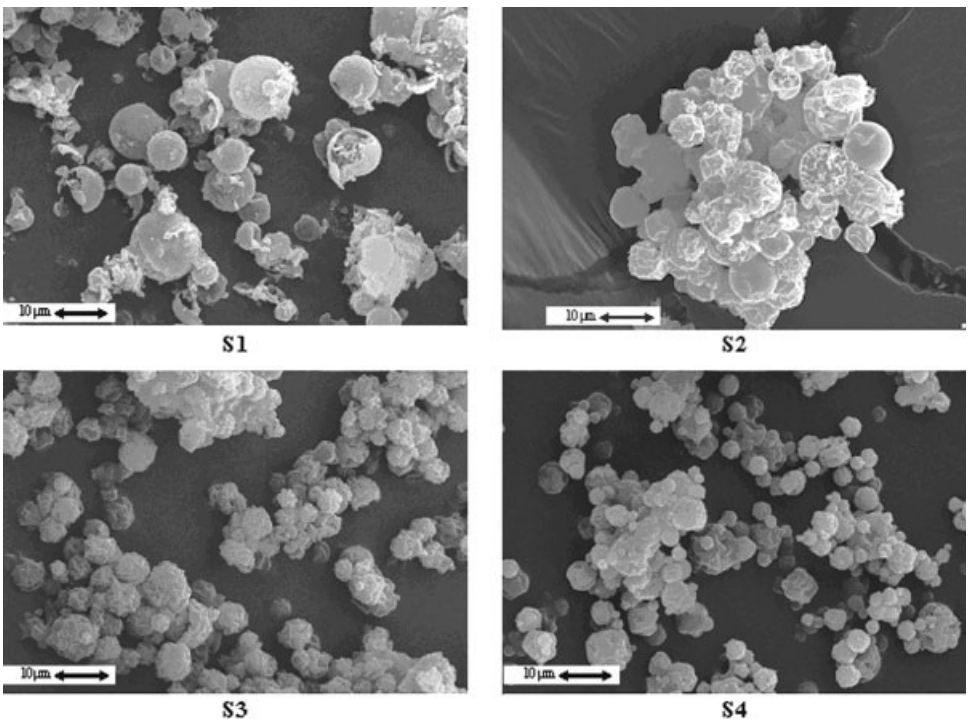

Figure 4 SEM images of S1–S4 polyurea microcapsules prepared with increasing PPI/TDI ratios.

absorption band at 1670 cm^{-1}. The FTIR response also demonstrates that no significant competitive reaction between the isocyanate monomers and the Tween 80 surfactant (having three OH groups) occurred in our experimental conditions, since no peak is present at the absorption frequency ($\sim$ 1709 cm^{-1}) of the carbonyl group of the urethane linkage.

Similar FTIR traces are also obtained analyzing all the other samples, but when the PPI percentage in the shell composition becomes higher than the TDI one, as in the case from S4 to S7, new peaks corresponding to PA solvent used as inner phase appear (Fig. 3). In particular, Figures 3(a,c) show the outbreak of the asymmetrical and symmetrical C—H stretching vibrations of methyl groups and methylene units at 2960–2930 and 2875–2860 cm^{-1}, whereas Figures 3(b,d) show the appearance of the strong acetate C=O absorption band at 1740 cm^{-1}. The intensity of all these bands decreases passing from S5 to S7 samples, suggesting that the amount of entrapped PA solvent is lower in the case of microcapsules having flexible diamine monomers in the shell. This conclusion is also supported by sample morphology and thermogravimetric data, discussed below.

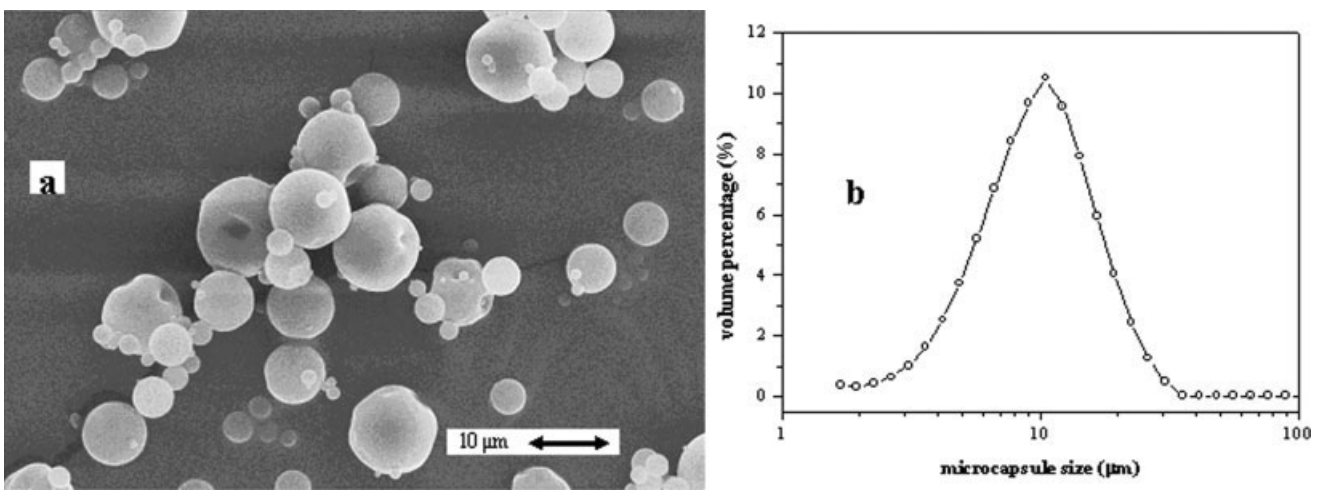

Figure 5 SEM image (5a) and particle size distribution (5b) of S5 polyurea microcapsules prepared from PPI and HH monomers.

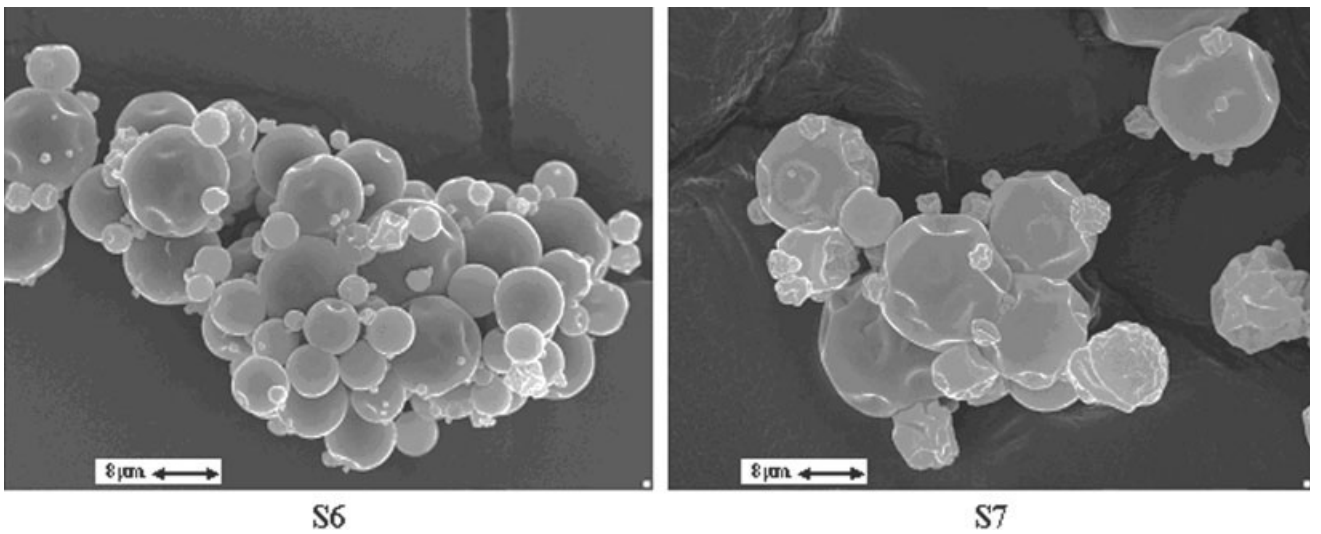

Figure 6 SEM images of S6–S7 polyurea microcapsules prepared by reaction of PPI with EDA and BDA monomers, respectively.

To investigate the effect of the shell composition on microcapsule morphology, the scanning electron micrographs of samples are compared. Figure 4 reports the SEM images of S1–S4 samples, prepared using HH as bifunctional amine monomer and increasing PPI/TDI ratios. The micrographs show that S1 microcapsules have spherical shape and quite smooth surface; however, their membrane is very brittle, as revealed by the numerous broken microcapsules and shell fragments. A globe-shaped morphology without coalescence can also be observed for the S2–S4 samples, prepared with increasing amounts of the polyfunctional PPI monomer; nevertheless, thanks to the crosslinkage due to PPI, these microcapsules appear intact. In the case of S5 sample, based on 100% PPI, the SEM micrograph [Fig. 5(a)] shows microcapsules having a well developed spherical shape and a very smooth surface; no coalescence phenomena and no fragments are evident. The mean diameter of the particles was 9.1 $\pm$ 1.9 μm, as determined by particle size distribution analysis [Fig. 5(b)]. These SEM observations suggest that the dispersed phase was successfully microencapsulated in the S5 sample and evidence that a high degree of crosslinking is necessary to ensure microcapsule formation and solvent incorporation in our experimental conditions.

SEM micrographs of S6 and S7 samples, prepared from PPI but using EDA and DBA as diamine monomers, are compared in Figure 6. The images show that such microcapsules, though globe-shaped, appear deflated. This result can be related to the flexible nature of the EDA and BDA, due to the presence of 2 and 4 methylene spacers in the chains, respectively. As a consequence, the resulting shell membrane has a reduced mechanical consistency, allowing the entrapped solvent evaporation (see discussion below). This effect, of course, becomes more pronounced increasing the length of the methylene chain and it can be of interest when modulation of the release rate is needed.

Qualitative information about the relationships between composition, thermal stability, and permeability of the shell were obtained from thermogravimetric analysis. TGA traces of S1–S7 samples are reported in Figure 7. Comparing the curves, two different types of TGA traces can be distinguished. In the case of S1, S2, and S3 samples, having PPI weight percent $\leq$ 50, the weight loss signal exhibits the typical trend of polyureas:[29] It remains about constant up to the initial decomposition temperature of $\sim$ 230–240°C, after decreases gradually by three

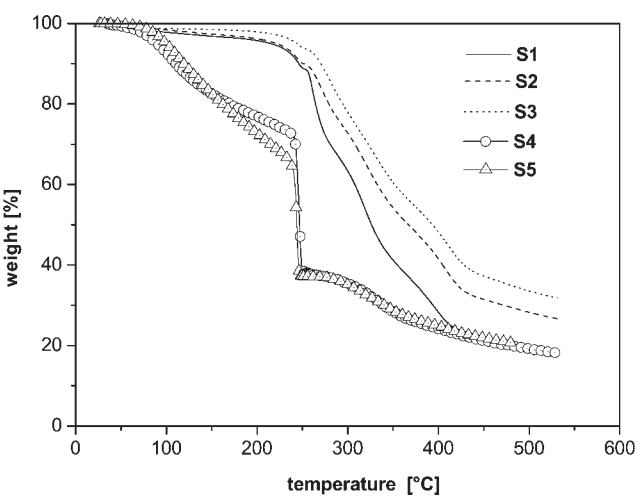

Figure 7 Comparison between the TGA traces of S1–S5 microcapsules, prepared with increasing PPI/TDI ratios.

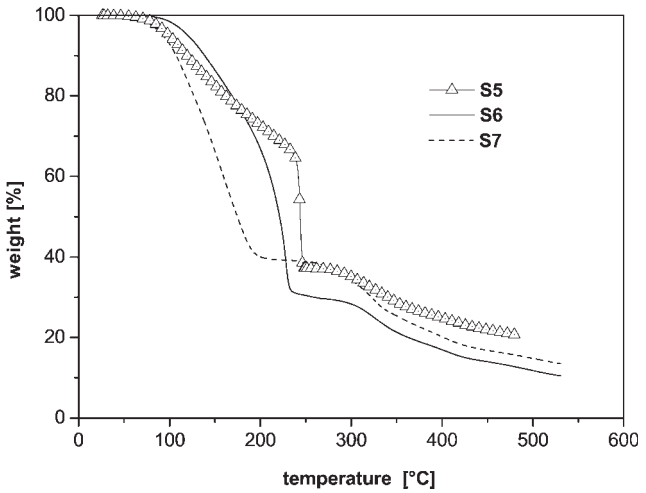

Figure 8 Comparison between the TGA traces of S5, S6, and S7 polyurea microcapsules, prepared by reaction of PPI with HH, EDA, and BDA monomers, respectively.

steps in a wide temperature range (240–430°C), reaching a residual weight of ~ 20–30% at 550°C. This TGA response corresponds mainly to the degradation of the shell materials alone, since the solvent was not encapsulated in these samples, as suggested by SEM images and FTIR traces previously reported. On the other hand, in the case of samples S4 and S5, having higher PPI content (≥50%), the thermogravimetric curve has to be explained by relating the weight loss to the evaporation of the encapsulated PA solvent. The graph shows that, after an initial constancy up to 100°C, the S4 and S5 weight loss signals decrease quite linearly on temperature up to ~ 240°C, where drop suddenly: in fact, until the shell remains intact, the PA is slowly released across the membrane pores, but as soon as the shell starts to degrade, the solvent is discharged at once. Afterwards, the weight loss curves go on decreasing more gradually reaching a residual weight of ~ 20% at 550°C. Similar results were obtained for microcapsule samples synthesized from EDA and BDA as diamine monomers, as shown in Figure 8. However, because of the lower consistency of these membranes, the encapsulated PA solvent is completely released before that the shell begins to degrade; the release is shifted towards the more low temperatures, the longer methylene spacer chain has the diamine.

All the results reported above revealed the critical role of the kind of monomers in determining the morphology and the release properties of the microcapsule shells. On the basis of such preliminary investigation, the membrane composition corresponding to the S5 sample seemed the most interesting choice for the essential oils microencapsulation performed in the continuation of the research. Four essential oils extracted from aromatic plants (lemon balm, lavender, sage, and thyme) were employed for the experiments, performed using the synthetic conditions detailed in Table II. The obtained microcap-

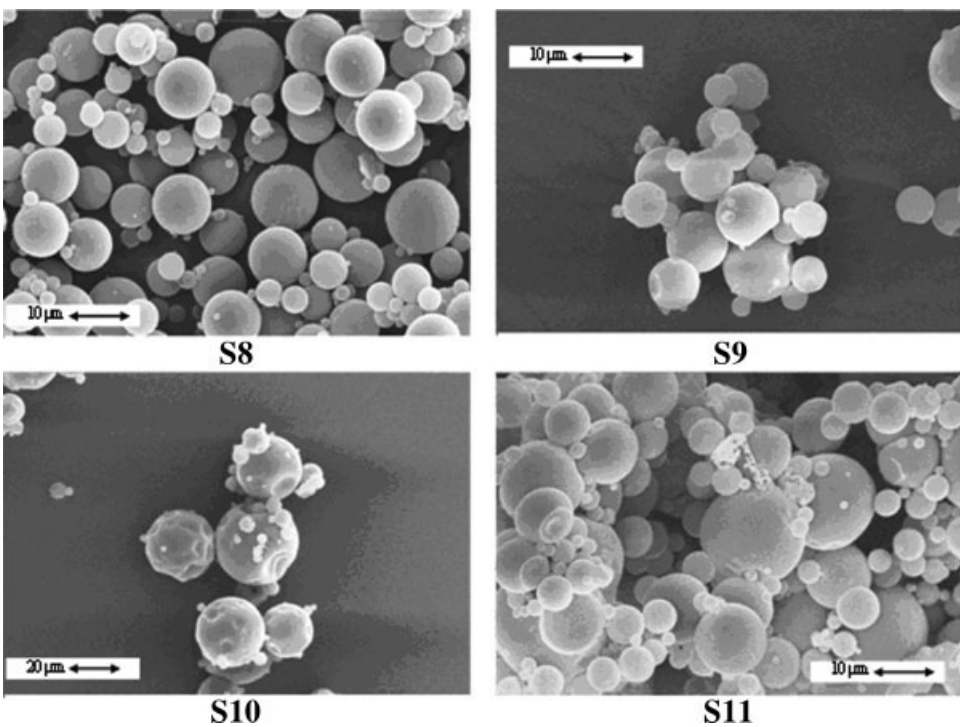

Figure 9 SEM images of S8–S11 microcapsules containing essential oils in the core (S8, lemon balm; S9, lavender; S10, sage; S11, thyme).

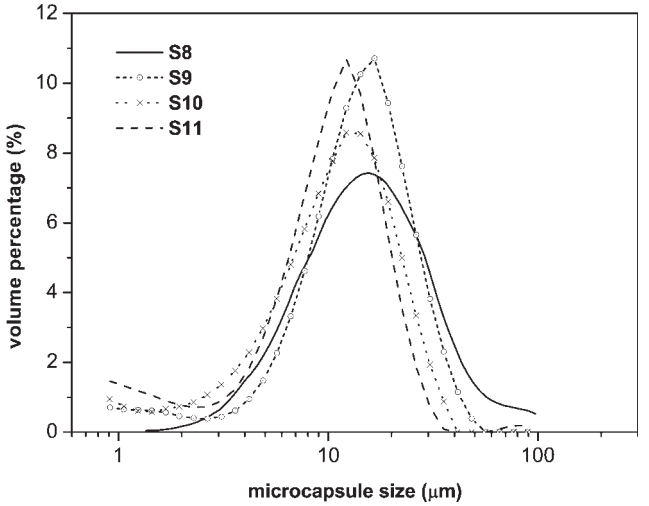

Figure 10 Particle size distributions of S8–S11 microcapsules containing essential oils in the core (S8, lemon balm; S9, lavender; S10, sage; S11, thyme).

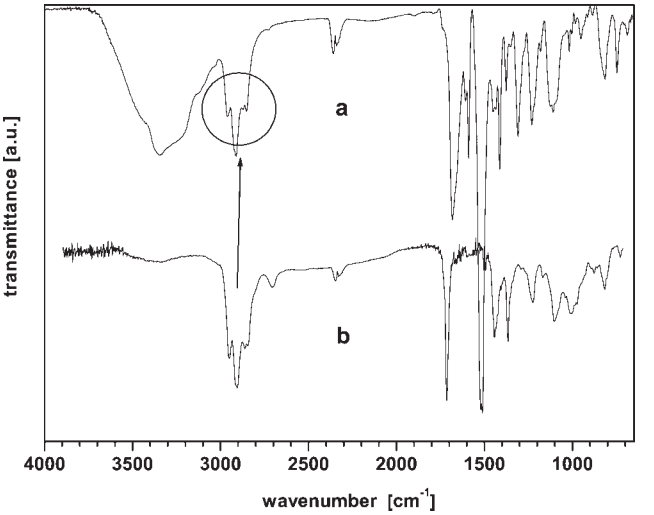

Figure 11 Comparison between the FTIR spectra of S8 sample and neat lemon balm essential oil.

sules were characterized in terms of morphology and properties and compared with S5 unloaded microcapsules, used as a reference.

The SEM images and the size distributions of the S8–S10 samples are given in Figures 9 and 10, respectively. The micrographs show that all samples exhibit similar morphology to the unloaded S5 one, having a quite perfect spherical shape with smooth surfaces. Particles seem perfectly dry, dusty, with no flocculation, and they can be spread easily with spatula. The mean diameter of the samples was in the range of $\sim$ 10–15 μm and the size distributions wideness was dependent on the kind of essential oil used in the synthesis.

To verify the actual incorporation of the essential oil into the shell and to put in evidence eventual interference phenomena of the oil with the shell formation reaction, FTIR measurements of S8–S11 microcapsules and neat oils were performed. As an example, FTIR spectra of S8 sample and lemon balm essential oil were compared in Figure 11. The graph clearly shows that the oil was successfully microencapsulated, as it results from the outbreak of the strong absorption band centered at around 2900 cm^{-1} in the infrared response of the S8 sample. As for the rest, all the other signals are typical of a polyurea and no significant differences can be observed respect to the trace of S5 blank microcapsules. Similar results were obtained also analyzing all the other samples.

The amount of essential oils microencapsulated was determined by HPLC and the results are reported in Table III. The essential oil loadings are included in the range of 25–50 wt % and appear markedly dependent upon the oil nature. This is an unsurprising result, to which several factors contribute. In fact, because of the different viscosity of the oils, the droplets of the dispersed phase produced in the emulsifying step have dissimilar sizes and then unequal oil content in the core; moreover, because of the complex composition of these oils, different losses of oil mass may occur during the synthesis as a consequence of the differences in the volatility, aqueous solubility, or reactivity of some oil constituents in the reaction medium. Then, the synthetic conditions (emulsion composition, reaction temperature, stirring rate, etc.) should be tuned case by case to maximize the essential oil loadings.

To investigate the potential antigerminative activity of microencapsulated essential oils, preliminary bioassays were carried out *in vitro* on seed germination (SG) and radical elongation (RE) of three different species of seeds (radish, garden cress, and lettuce), according to the procedure described in the experimental section. The tests were conducted both on neat and microencapsulated essential oils, for comparison. All the results were reported in Tables IV and V and an example of a germination chamber, used for testing the effects of microencapsulated lemon balm essential oil on SG and RE of radish, is shown in Figure 12 (unexposed (a) and exposed (b) radish seeds 120 h after application). The data clearly show that exposure of seeds to neat essential

TABLE III
Essential Oil Loadings in Polyurea Microcapsules Determined by Hplc Method

	Essential oil			
	Lemon balm	Lavender	Sage	Thyme
Essential oil loading (wt%)	40.0 ± 0.5	24.6 ± 0.3	50.5 ± 0.8	31.0 ± 0.5

TABLE IV
Comparison Between the Activity of the Microencapsulated and Neat Essential Oils on Germination (%) of Radish, Cress, and Lettuce, 120 h After Sowing

	Seeds					
	Radish		Garden cress		Lettuce	
Essential oil	SG (%)[a]	% Inhibition[b]	SG (%)[a]	% Inhibition[b]	SG (%)[a]	% Inhibition[b]
None (control)	92 ± 3	–	93 ± 1	–	62 ± 2	–
Lemon balm						
Encapsulated (S8)	53 ± 2	42	53 ± 2	42	6 ± 1	90
Neat	0	100	10 ± 1	89	0	100
Lavender						
Encapsulated (S9)	87 ± 2	5.4	96 ± 3	−3.2[c]	64 ± 3	−3.2[c]
Neat	0	100	10 ± 1	89	0	100
Sage						
Encapsulated (S10)	87 ± 3	5.4	77 ± 3	17	60 ± 2	3.2
Neat	10 ± 1	89	0	100	0	100
Thyme						
Encapsulated (S11)	72 ± 2	22	5 ± 1	95	0	100
Neat	15 ± 1	84	0	100	0	100

[a] SG data are expressed as mean percentage of germinated seeds ± standard deviation. Each petridish contained 10 seeds; each determination was repeated 10 times.
[b] Inhibition percentages are calculated according to the formula: [(SG control − SG oil treated)/SG control]100.
[c] Negative values indicate that seed germination is positively affected [Ref. 30].

oils to vapor drastically influences the germination and radical elongation of all three seeds, resulting in a complete inhibition at 120 h in most cases. SG and RE are affected by the essential oil release from microcapsules too. However, the microencapsulation process sensibly modifies the inhibitory activity of all tested oils, inducing a general decrement of their efficacy whose entity depends on the species of treated seed. Only the S11 microcapsules, containing thyme oil in the core, exhibit similar biological activity to the neat oil, totally inhibiting lettuce and garden cress and inhibiting radish over 70% in radical growth. Instead, in the case of S9 microcapsules, containing lavender oil in the core, an activity promoting both germination and radical elongation of lettuce and garden cress seeds was even observed. This occurrence can be found in nature, where a lot of allelochemicals show, in a dose-dependent way, an activity promoting the plant growth.[30]

All the results obtained from bioassays may be justified hypothesizing a change in the composition of essential oils during the microencapsulation process.

TABLE V
Comparison Between the Activity of the Microencapsulated and Neat Essential Oils on Radicle Elongation (mm) of Radish, Cress, and Lettuce, 120 h After Sowing

	Seeds					
	Radish		Garden cress		Lettuce	
Essential oil	RE[a] (mm)	% Inhibition[b]	RE[a] (mm)	% Inhibition[b]	RE[a] (mm)	% Inhibition[b]
None (control)	32 ± 3	–	60 ± 2	–	12 ± 1	–
Lemon balm						
Encapsulated (S8)	11 ± 1	66	20 ± 2	67	4 ± 1	67
Neat	0	100	2 ± 1	97	0	100
Lavender						
Encapsulated (S9)	23 ± 2	28	60 ± 3	0	12 ± 2	0
Neat	0	100	2 ± 1	97	0	100
Sage						
Encapsulated (S10)	27 ± 3	16	58 ± 2	3.3	11 ± 1	8.3
Neat	8 ± 1	75	0	100	0	100
Thyme						
Encapsulated (S11)	9 ± 2	72	1 ± 1	98	0	100
Neat	9 ± 1	72	60 ± 4	0	0	100

[a] RE data are expressed as mean radical length ± standard deviation. Each petridish contained 10 seeds; each determination was repeated 10 times.
[b] Inhibition percentages are calculated according to the formula: [(SG control − SG oil treated)/SG control]100.

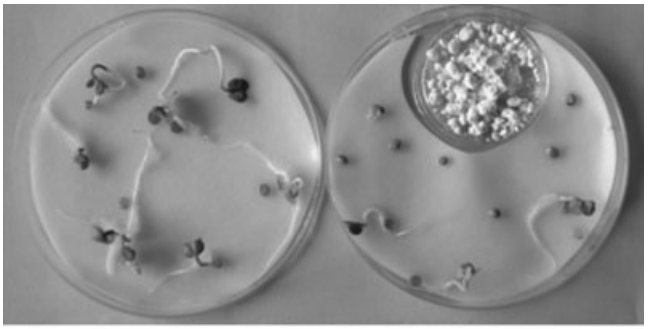

Figure 12 Effects of microencapsulated lemon balm essential oil on germination and radical elongation of radish, 120 h after application: unexposed (a) and exposed (b) radish seeds.

Since the antigerminative activity is a result of the synergetic interaction of some oil constituents, each compositional variation occurred might modify it. Other investigations are in progress to better investigate these issues.

CONCLUSIONS

In this study polyurea microcapsules with different shell compositions were synthesized by interfacial polymerization in emulsion using different polyfunctional isocyanates and amines. The obtained samples were then characterized to investigate the effects of the reacting monomers on their morphology and properties. Microscopy, FTIR, and TGA analyses have shown that, increasing crosslinking degree of polyureas, microcapsules with quite perfect spherical shape and good mechanical resistance can be obtained. The consistency of the shell membrane can be tuned changing the diamine monomer from a hard (as HH) to a more flexible one (as BDA). On this basis, the synthesized microcapsules appeared suitable for realizing sustained release systems. Therefore, using the same procedure polyurea microcapsules containing several essential oils with herbicidal activity were also prepared and characterized. The morphology of the systems was similar in all cases but the encapsulation yield appeared dependent on the kind of essential oil used. Also the antigerminative activity of microencapsulated oils, tested by *in vitro* bioassay on germination and radical elongation of three species of seeds, was sensibly modified with respect to the neat oil, showing a general decrement whose entity depends both on the kind of oil and treated seed. One cause of these results may be due to compositional changes occurring during the microencapsulation process, now under scrutiny.

References

1. Benita S., Ed. 1996. Microencapsulation: Methods and Industrial Applications; Marcel Dekker: New York.
2. Conti, B.; Giunchedi, P.; Conte, U. Cellulose microparticles in drug delivery. S.T.P. Pharma Sci 1997, 7, 331.
3. Ravi Kumar, M. N. V. Nano and microparticles as controlled drug delivery devices. J Pharm Pharmaceut Sci 2000, 3, 234.
4. Chang, T. M. S. Eur J Pharmaceut Biopharmaceut 1998, 45, 3.
5. Obeidat, W. M.; Price, J. C. J Microencapsul 2006, 23, 195.
6. Kydonieus A. F., Ed. Controlled Release Technologies: Methods, Theory and Applications. CRC Press: Boca Ratón, Florida, 1980.
7. Giunchedi, P.; Conte, U. S.T.P. Pharma Sci 1995, 5, 276.
8. Yeom, C. K.; Oh, S. B.; Rhim, J. W.; Lee, J. M. J Appl Polym Sci 2000, 78, 1645.
9. Yeom, C. K.; Kim, Y. H.; Lee, J. M. J Appl Polym Sci 2002, 84, 1025.
10. Tarcha P. J. Polymers for Controlled Drug Delivery. CRC Press: Boca Ratón, Florida, 1991.
11. Hirech, K.; Payan, S.; Carnelle, G.; Brujes, L.; Legrand, J. Microencapsulation of an insecticide by interfacial polymerization. Powder Technol 2003, 130, 324.
12. Matsunami, Y.; Ichikawa, K. Int J Pharmaceut 2002, 242, 147.
13. Hong, K.; Park, S. Mater Chem Phys 2000, 64, 20.
14. Hong, K.; Park, S. J Appl Polym Sci 2000, 78, 894.
15. Jabbari, E.; Khakpour, M. Biomaterials 2000, 21, 2073.
16. Hong, K.; Park, S. React Funct Polym 1999, 42, 193.
17. Łukaszczyk, J.; Urbaś P. React Funct Polym 1997, 33, 233.
18. Vokou D. Essential oils as allelochemicals. Fourth World Congress in Allelopathy held at Charles Sturt University (CSU); 21–-26 Aug 2005; Wagga Wagga, NSW, Australia. Available: http://www.regional.org.au/au/allelopathy/2005/1/3/2685_vokoud.htm via the INTERNET. Accessed 1 August 2006.
19. Paranagama, P. A.; Abeysekera, K. H. T.; Abeywickrama, K.; Nugaliyadde, L. Lett Appl Microbiol 2003, 37, 86.
20. De Feo, V.; De Martino, L.; Quaranta, E.; Pizza, C. J Agric Food Chem 2003, 51, 1177.
21. Tworkoski, T. Weed Sci 2002, 50, 425.
22. De Feo, V.; De Simone, F.; Senatore, F. Phytochemistry 2002, 61, 573.
23. Abrahim, D.; Braguini, W. L.; Kelmer-Bracht, A. M.; Ishii-Iwamoto, E. L. J Chem Ecol 2000, 26, 611.
24. Harborne J. B. Introduction to Ecological Biochemistry, 3rd ed., Academic Press: New York, 1989.
25. EDQM-Council of Europe, European Pharmacopoeia, Vol. 3. Maisonneuve, S. A.: Sainte-Ruffine (France); 1975, pp 68–71.
26. Oleszek, W. Plant Soil 1987, 102, 271.
27. Bewley, D., Black, M. Seeds. Physiology of Development and Germination. Plenum Press: New York, 1985.
28. Silverstein, R. M., Webster, F. X.; Kiemle, D. Spectrometric Identification of Organic Compounds, 7th ed.; Wiley: New York, 2005.
29. Chantarasiri, N.; Chulamanee, C.; Mananunsap, T.; Muangsin, N. Polym Degrad Stabil 2004, 86, 505.
30. Stebbing, A. R. D.; Hormesis—the stimulation of growth by low levels of inhibitors. Sci Tot Environ 1982, 22, 213.