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ORIGINAL ARTICLE

Bioactive microparticles (10): thermal and oxidative stability of nicotine and its complex with β-cyclodextrin

Daniel Ioan Hădărugă · Nicoleta Gabriela Hădărugă · Gallia Butnaru · Călin Tatu · Alexandra Gruia

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Abstract The paper presents a comparative thermal and oxidative stability study between nicotine/β-cyclodextrin microparticles and commercial nicotine. It is well known that the nicotine is the bioactive compound in formulations used for smoking cessation and no studies among the stability of nicotine in cyclodextrin-containing formulations were reported. The non-enzymatic and enzymatic oxidation of nicotine can lead to cotinine (an alkaloid/metabolite with a lower toxicity), but another way is the obtaining of the cancerigene *N*-nitroso-nicotine derivatives by nornicotine derivative intermediates (like nornicotine and myosmine). The present study demonstrates the protecting capacity of β-cyclodextrin for commercial nicotine against thermal and oxidative factors: for the non-complexed nicotine the thermal and oxidative degradation led to a decrease of the relative concentration of nicotine from 96 to 92% for an increasing temperature from 30 to 90 °C (in the presence of air at normal pressure), with an increase of the relative concentration of the corresponding oxidized compounds (like cotinine and furthermore myosmine up to 0.7%, and up to 4.7%, respectively). For the nicotine/β-cyclodextrin complex the interaction selectivity was higher for nicotine and the stability of this bioactive compound against oxidation was also higher in comparison with the non-complexed nicotine (around 98% in all cases).

Keywords Nicotine · Cotinine · *Nicotiana tabacum* L · Microparticles · Cyclodextrins · Thermal and oxidative stability

Abbreviations

bCD	β-cyclodextrin
KI	Kovats Index
TG	Thermogravimetry
GC-MS	Gas chromatography-mass spectrometry
SEM	Scanning electron microscopy
KFT	Karl Fischer titration

Introduction

The main alkaloid found in various *Nicotiana* species is nicotine ((*S*)-(-)-3-(1-methyl-2-pyrrolidinyl)pyridine); it was confirmed in about 34 from 65 species, while

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D. I. Hădărugă (✉)
Faculty of Industrial Chemistry and Environmental Engineering, Organic Chemistry and Technology Department, Politehnica University of Timişoara, P-ţa Victoriei 2, Timişoara 300006, Romania
e-mail: daniel.hadaruga@chim.upt.ro
URL: www.chim.upt.ro

N. G. Hădărugă
Faculty of Food Processing Technology, Food Quality Department, Banat's University of Agricultural Sciences and Veterinary Medicine, C. Aradului 119, Timişoara 300645, Romania

G. Butnaru
Faculty of Horticulture and Forestry, Genetic Engineering in Agriculture Department, Banat's University of Agricultural Sciences and Veterinary Medicine, C. Aradului 119, Timişoara 300645, Romania

C. Tatu · A. Gruia
Regional Centre of Immunology and Transplant, County Hospital Timişoara, Bv. Iosif Bulbuca 10, Timişoara 300736, Romania

nornicotine (3-(2-pyrrolidinyl)pyridine) was the principal alkaloid in 19 from 65 species studied. The third most important alkaloid from these plants is anabasine (3-(2-piperidinyl)pyridine). Tobacco leaves contain the highest concentration of nicotine, roots have less, and stalks have the least. The maximum concentration of nicotine has been found in *Nicotiana rustica* (grown under favorable conditions) in range up to 14% in leaves, while for the commercial tobacco (*Nicotiana tabacum* L.) the range of alkaloid content between 0.1 and 5% has been found (nicotine and other alkaloids like nornicotine, anabasine, cotinine, myosmine, nicotyrine, anatabine, nicotelline) [1, 2]. Analysis of the nicotine level in different species of plants or in different food products like milk can be evaluated by using either liquid chromatography, gas chromatography or thin layer chromatography [3, 4].

The nicotine metabolism in humans is P450 2A6-catalyzed 5′-oxidation with the formation of cotinine (by the nicotine 1′(5′)-iminium ion intermediate), as the main metabolite. Nicotine may also be oxidized to *N*′-(hydroxymethyl)nornicotine, which can be transformed in nornicotine by non-enzymatic decomposition [5]. Smoking determines some alkaloids from tobacco to be transformed in other substances, among these the *N*-nitrosamines being the most dangerous due to the carcinogenic properties. Previous reports indicate that the nicotine and cotinine reduce the mutagenicity of these nitrosamines, but the more potent inhibitor of CYP 2E1 activity (the enzyme which enhance the formation of the main carcinogenic nitrosamines—*N*-nitrosodimethylamine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) is nicotine, as well as the aqueous cigarette tar extract [6].

Nicotine in small doses can act also as a respiratory stimulant, though in larger doses it causes respiratory depression. Other therapeutic uses were ectoparasiticide and anthelmintic. Despite the vast array of evidence linking tobacco smoking and cancer, the smoking habit continues throughout the world, and tobacco remains a major crop plant [1]. In order to evaluate the smoking cessation even by using medication like bupropion or placebo, the cotinine concentration can be measured. The urinary cotinine levels in young children can estimate the exposure to the environmental tobacco smoke [7]. For measuring the total smoke volume (which is correlated with the exposure to the toxins from the tobacco smoke) the salivary cotinine can be evaluated by using the digital image analysis [8]. Cigarette smoking is a major source of mortality and increase of medical costs in all over the world. In order to increase the smoking cessation and/or to reduce the toxicity of cigarette smoke, more graphic and salient warning labels on cigarette packs and mild, light, and ultra-low-tar cigarettes had to be used [9, 10]. Smoking is causing about 5 million premature deaths worldwide each year, being the major risk factor for lung cancer and one of the main risk factor for peripheral arterial disease [11].

In order to enhance the bioactive compound properties (like water-solubility), to protect them against action of environmental and biological factors (protect against oxidation, humidity, light) and to obtain biosystems with controlled release properties micro- (by means of polymer coating process) and/or nanoencapsulation (by means of liposome techniques or molecular inclusion methods) are widely used. A great number of natural, semisynthetic or synthetic oligomers and polymers (or even copolymers) are used as matrices for obtaining the corresponding particles. Among these, cyclodextrins represent an important class of such matrices, which can allow obtaining particles with above mentioned properties. Cyclodextrins are cyclic oligosaccharides, formed by α-D-glucopyranose moieties, which have shapes like truncated cones with the primary and secondary hydroxyl groups (primary-A and secondary-B faces) oriented to the exterior and furnish the water solubility and with a hydrophobic inner cavity which can get a geometrically compatible hydrophobic compounds by molecular encapsulation (host–guest interaction, molecular inclusion compounds) [12–14]. In the pharmaceutical field, cyclodextrins are widely used in order to enhance the apparent water solubility, but also as drug carriers. The most used cyclodextrins as drug carries and solubilizing agents are natural α-, β-, and γ-cyclodextrin, and also the semisynthetic oligosaccharide 2-hydroxypropyl-β-cyclodextrin, and the most used drugs for encapsulation are cephalosporins, prostaglandins, steroids (testosteron, estradiol), antibiotics, anti-inflammatory, anti-ulcerative, and antihistaminic drugs, anticholinergics and antispasmotics (scopolamine and the butylated form, respectively), antidiabetics (tolbutamid) and antihypertensive or vasodilators drugs (clonidin, nitroglycerin) [12, 13]. For other drugs the main goal is to eliminate the bitter/irritating taste or unpleasant odor (i.e., orally administrating drugs like anti-inflammatory drugs or amino-acid containing formulations) [13]. Cyclodextrins alone or in combination with other polymers (like polysaccharides) are used in order to reduce the volatility of some compounds or extracts and to obtain products with controlled release properties, with applications in perfumery, cosmetics, and toiletry. Thus, encapsulation of pure volatile compounds such as pinenes and limonene (monoterpenes), carvone, eugenol, vanillin, allyl isothiocyanate or flavoring aldehydes and ketones, as well as volatile biosystems like essential oils (e.g., *Carum carvi* or *Salvia sclarea*) were reported [15–17]. Polyphenols are among other natural bioactive compounds reported to be encapsulated in cyclodextrins in order to improve their properties [18].

As above mentioned bioactive compounds, some alkaloids were complexed with cyclodextrins in order to

enhance their properties. For example, alkaloid-containing ophthalmic drugs were investigated for increasing the aqueous solubility, aqueous stability and bioavailability, and also to decrease drug irritation (like the antiglaucoma agent pilocarpine). Other alkaloids like harman or harmine were encapsulated in natural or chemically modified cyclodextrins in order to modify some properties, like fluorescence intensity [19]. Nicotine is often used in smoking cessation in different formulations like chewing gum, sublingual tablet, nasal spray, transdermal delivery systems or even as food additives. These formulations usually contain nicotine-cyclodextrin complexes [20–22]. Cyclodextrins can be used in mobile phase of liquid chromatography in order to enhance the enantiomeric separations of racemic nicotine and related compounds, and analysis of them in formulations used for smoking cessation (like chewing gum) [23–25].

In this paper we continue the studies on the obtaining, analysis, and stability of the bioactive natural compounds or systems/cyclodextrin micro- or nanoparticles [26–29] and try to evaluate the protection capacity of β-cyclodextrin of nicotine against thermal and oxidative factors which can occur in the processing and/or using formulations containing nicotine/β-cyclodextrin complex. In this study the term "microparticle" (as is revealed by SEM analysis) for the nicotine/cyclodextrin complex aggregate is used.

Materials and methods

Materials

Nicotine (reagent grade), methanol (>99%), ethanol 96% (v/v), and propanol (>99%) used for the synthesis of microparticles were purchased from SC Chimopar SA, Bucharest, Romania. β-Cyclodextrin (bCD) used for the obtaining of microparticles was purchased from Merck&Co., Inc., New Jersey, USA, and was a reagent grade product (>99%). Hexane used for the recovering of the compounds from microparticles has GC purity and was purchased from Merck&Co, Inc., New Jersey. The alkane standard solution C_8–C_{20} used for the determination of Kovats indices (KIs) of the bioactive compounds from the GC-MS analysis was purchased from Fluka Chemie AG, Switzerland. Titrant 5 apura®, Solvent apura®, and Water standard 1% apura®, used for two-component Karl Fischer water titration, were purchased from Merck&Co., Inc.

Obtaining the nicotine/β-cyclodextrin microparticles

bCD was dissolved (or suspended) in 2-4 mL distilled water at 30–70 °C, and then 2-4 mL alcoholic solution (ethanol, methanol or propanol), containing 0.5–1.5 mmoles nicotine, was added in 0.5 h to the bCD solution, under continuous stirring; the suspension was stirred for another 15 min at the same temperature. The suspension was then cooled at the environment temperature in 0.5-4 h, in a water bath, and stored at 4 °C for 1 or 18 h in a refrigerator, in order to complete the crystallization. The suspension was then filtered, washed with 1.5 mL alcohol and dried in desiccator.

Degradation of nicotine and nicotine/β-cyclodextrin microparticles

$\sim$100 µl Nicotine or $\sim$100 mg cyclodextrin microparticles were uniformly distributed at the bottom of the degradation flasks (100–300 mm^2 surface area), which are then purged with air (oxidative atmosphere), sealed, and put in a bath with controlled temperature. The samples were maintained under degrading conditions for 2 or 6 h. After cooling, degraded nicotine samples were dissolved in 5 × 1 mL hexane and solutions have been analyzed by GC–MS; in the case of degraded microparticles, these were suspended in water and the bioactive compounds have been recovered by hexane extraction.

Recovering of the bioactive compounds from microparticles

After cooling the degraded samples of microparticles these were dissolved/suspended in 4 × 1 ml distilled water and the bioactive compounds have been extracted in a liquid–liquid extractor with 2 mL hexane at 60 °C for 20 min three times (the fourth extract do not contain any kind of compound, revealed by GC–MS analysis). The combined extracts were dried over anhydrous $CaCl_2$ and analyzed by GC–MS.

GC–MS analysis

The analysis of bioactive compounds, those degraded in various conditions and those recovered from non-degraded or degraded microparticles has been performed by a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass selective detector (GC–MS) system (calibration factor 1.0). A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used for the GC system. The temperature program was set up from 50 to 250 °C with 6 °C/min, both the injector and detector temperatures were 280 °C and He was used as carrier gas. 1 µl sample was injected in all cases. Ionization energy EI of 70 eV was used for mass detector, with a source temperature of 150 °C, scan range 30–600 amu, scan rate 1 s^{-1}. The compounds were identified by comparing the mass spectra with those from the

NIST/EPA/NIH Mass Spectral Library 2.0 or by using the Kovats indices computed from the GC–MS analysis of the C_8–C_{20} alkane standard mixture.

TG analysis

A TG 209 Netzsch thermogravimetric apparatus was used for the thermal analysis of the nicotine/bCD microparticles. The temperature program was from 20 to 500 °C with an increasing rate of 10 °C/min. For commercial nicotine the rate was 4°C/min. All determinations were conducted under nitrogen atmosphere. Data acquisition was performed with the TG Netzsch 209-Acquisition Soft/2000 and the data analysis was realized with the Netzsch Proteus-Thermal Analysis ver. 4.0/2000 soft.

Karl Fischer titration

Volumetric Karl Fischer water determination in cyclodextrin microparticles was carried out by using a Karl Fischer 701 Titrando apparatus from Metrohm; a Metrohm 10 dosing system and 703 Ti Stand mixing system were also used. The two-component technique was used for water determination (Component 1: Titrant 5 apura® and Component 2: Solvent apura®). The titer of component 1 was performed by using Water standard 1% apura®, standard for volumetric Karl Fisher titration. The sample amount was in the range of 0.01–0.084 g. The method parameters were: I(pol) of 50μA, end point and dynamics at 250 mV, maximum rate of 5 mL/min, drift was used as stop criterion, with a stop drift of 25 μL/min. The extraction time was 360 s. Almost all determinations were done in triplicate (Table 2).

SEM analysis

For morphological and dimensional evaluation of the nicotine/bCD particles scanning electron microscopy (SEM) technique was used. An INSPECT S SEM apparatus, with a voltage of 12.5 kV, 3 000 × magnitude level, and focusing of 13.9 mm was used.

Molecular modeling and docking experiments

Molecular modelings of nicotine and of β-cyclodextrin, as well as the docking experiments have been realized with the MM+ molecular mechanics program from the HyperChem 5.1 package, using a RMS of 0.005 kcal/mole and conjugated gradient Polak-Ribiere algorithm.

Molecular modeling was performed in the following way: the starting molecular structure of β-cyclodextrin was builded according to the chiral structure known from X-ray analysis of the crystals. For nicotine, the natural enantiomer was used in modeling and docking experiments. All torsion angles corresponding to flexible bonds and rings were used in conformational analysis (Conformational Search program from HyperChem package) and only the conformation of molecules with minimal internal energy was used for docking experiments.

For docking experiments, the nicotine and β-cyclodextrin structures in minimal energy conformations were set up at distances of $\sim$8 Å between the gravity centres of these two molecules, and the nicotine structure was oriented with the pyridine or pyrrolidine moieties in front of the primary (A) or secondary (B) faces of cyclodextrin (the axis corresponding to the pyridine–pyrrolidine bond was perpendicular to the A or B plan of cyclodextrin). The complex was modeled in absence of water molecules or in water periodic box by using the same MM+ program and the interaction was stopped when the RMS gradient was lower than 0.01 kcal/mole. The nicotine–cyclodextrin interaction energy was evaluated as the difference between the overall energies of these two molecules and the complex energy.

Results and discussion

The GC–MS analysis of the commercial nicotine (Fig. 1) used for thermal degradation and complexation revealed that nicotine is the major component, with a concentration of 96% (Table 1). Other compounds identified are myosmine (3.1%), nicotyrine, isonicoteline (2,3′-bipyridyl), and cotinine. The experimental MS spectra for nicotine and of the corresponding oxidized analogue (cotinine) are presented in Fig. 1.

The study of thermal stability of commercial nicotine in the presence of air-oxygen revealed that the relative concentration of nicotine (evaluated from the GC–MS analysis by using the percentage of the corresponding peak area from the sum of overall peak areas) was lower in the case of degradation temperature of 60 and 90 °C, especially for a long degradation time. Thus, the relative concentration of nicotine in the case of degradation at temperatures of 30, 60, and 90 °C for 2 h in the presence of air at normal pressure was 95.9, 94.3, and 93.7%, respectively. By increasing of the degradation time to 6 h, these concentrations become lower: 95.7, 94.7, and 92.6%, respectively (Table 1). In both cases, the decrease of the nicotine concentration vs. the degradation temperature is approximately linear.

Other minor alkaloids identified in commercial nicotine become more concentrated, especially in the case of cotinine, which probably results by oxidation of nicotine (the relative concentration increase up to 0.7% both for 2 and 6 h degradation time); the same variation can be observed

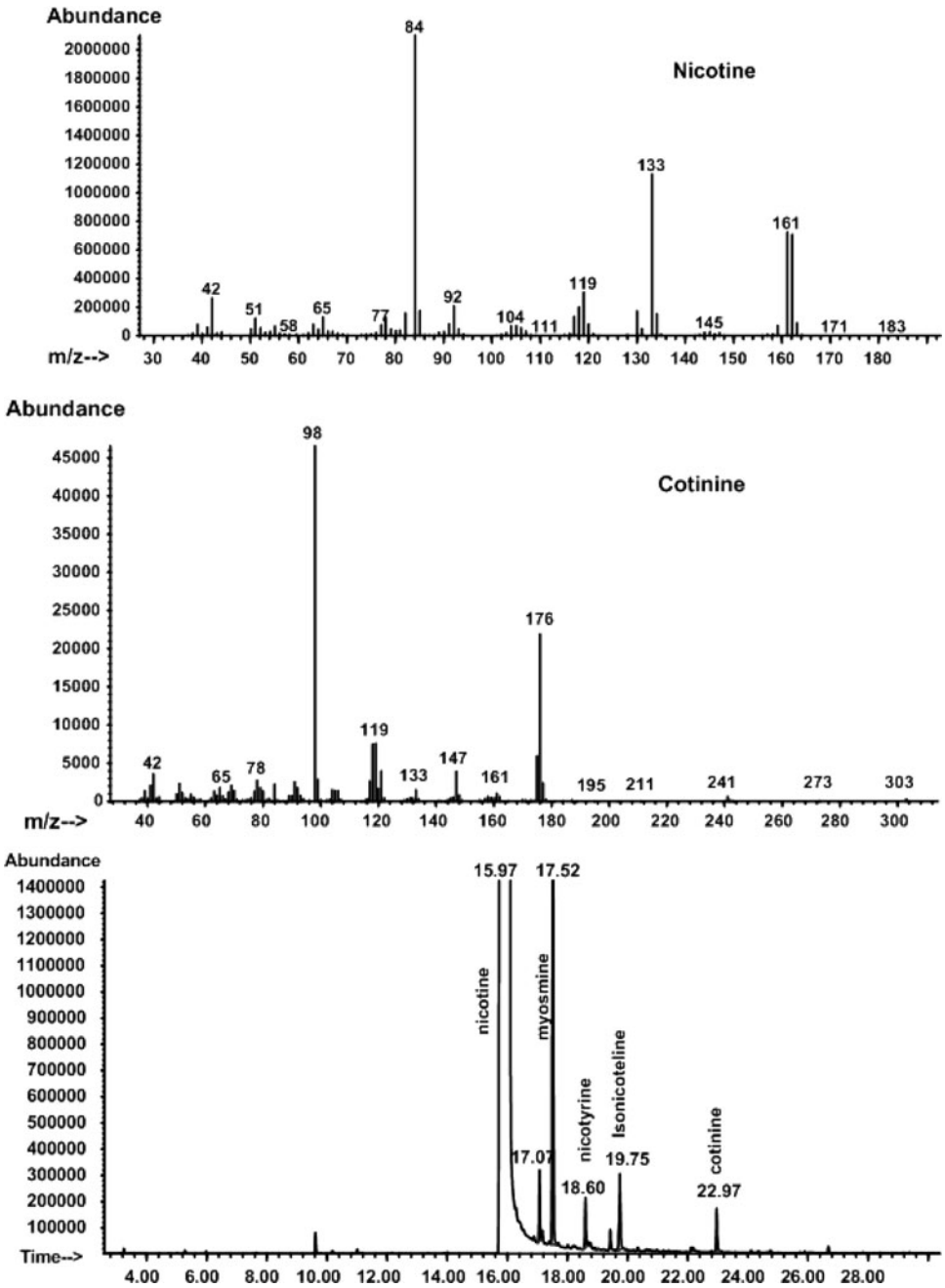

Fig. 1 Chromatogram from the GC–MS analysis of the raw nicotine sample (*down*) and the corresponding MS spectrum for nicotine (*up*) and for cotinine (*middle*)

for another oxidation product of nicotine, nicotyrine, with an increase of relative concentration up to 0.6% for the case of 6 h degradation time at 90 °C, and for some possible oxidation products of nornicotine, like myosmine (up to 3.7 and 4.7% for 2 and 6 h degradation time, respectively). No significant variation of the relative concentration of isonicoteline (∼0.4%) can be observed; this compound cannot be easily oxidized, due to the presence of two aromatic moieties in structure (2,3′-bipyridyl). Additional data are given in supplementary material.

The protection of nicotine against oxidation in the presence of air/oxygen could be achieved by complexation in matrices such as cyclodextrins. In order to evaluate the possibility of complexation, theoretical molecular modeling and docking experiments were conducted [30]. Thus, the interactions between natural nicotine ((*S*)-(-)-nicotine) and *β*-cyclodextrin (in minimal energy conformations) were evaluated for the both sides of cyclodextrin (side A—primary OH-groups, side B—secondary OH-groups) and with the nicotine structure oriented with pyridine as well as with pyrrolidine moieties to the cyclodextrin. The best interaction was obtained with the nicotine oriented with the pyridine moiety to the secondary side of cyclodextrin (Fig. 2), both in the absence of water molecules

Table 1 Relative concentrations for the main alkaloids from commercial nicotine (N), nicotine samples thermally (30, 60, 90 °C) degraded for 2 h (N–O-t1,2,3) and for 6 h (N–O-t4,5,6), and for the corresponding nicotine/β-cyclodextrin microparticles, non-degraded and degraded in the same conditions for 6 h (NbCD and NbCD-O-t4,5,6)

No	Code	Main alkaloid content (%)				
		Nicotine	Myosmine	Nicotyrine	Isonicoteline	Cotinine
	KI[a]	1363	1437	1489	1546	1714
1	N[b]	96.0	3.1	0.2	0.5	0.2
2	N–O-t1	95.9	2.8	0.2	0.4	0.1
3	N–O-t2	94.3	3.3	0.2	0.4	0.2
4	N–O-t3	93.7	3.7	0.3	0.4	0.7
5	N–O-t4	95.7	2.3	0.1	0.3	0.6
6	N–O-t5	94.7	3.3	0.3	0.4	0.2
7	N–O-t6	92.6	4.7	0.6	0.4	0.7
8	NbCD[b]	98.5	0.10			
9	NbCD-O-t4	98.4	0.08			
10	NbCD-O-t5	98.8	0.09			
11	NbCD-O-t6	98.0	0.05			

[a] Kovats index computed by using a C_8–C_{20} alkane standard mixture; [b] RSD (relative standard deviation) of the nicotine content was <0.06% (for three determinations); almost all other determinations were done in duplicate and the difference between nicotine concentrations was lower than 0.05%

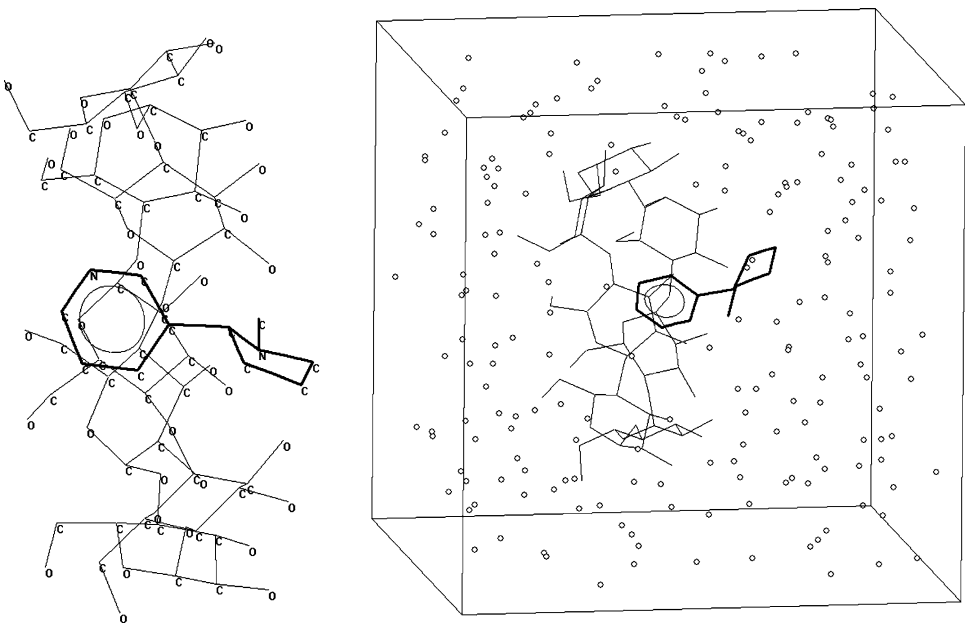

Fig. 2 The nicotine/β-cyclodextrin complex modeled in the absence of water molecules (*left*) or in the water periodic box (*right*)

(interaction energy of 21 kcal/mole) and in the water periodic box (interaction energy of 129 kcal/mole). The hydrophobicity of nicotine (log $P = 1.98$, calculated with the QSAR Properties program from the HyperChem package), the compatible geometry, and the interaction energy denote that the nicotine/β-cyclodextrin complex can be achieved.

For the complexation of commercial nicotine with β-cyclodextrin the alcohol-water solution method has been used. The main parameters which have been considered were the nicotine:β-cyclodextrin ratio (1:1, 2:1, and 3:1), hydrophobicity of alcohol-water system (ethanol, methanol, and 1-propanol were used), alcohol:water ratio (2:1 and 1:2), complexation temperature (30, 60, and 90 °C), crystallization and holding time (0.5 or 4 h for cooling time, and 1 or 18 h for holding time). Increasing the nicotine:bCD ratio from 1:1 to 3:1 (codes NbCDE_r1,2,3) in the case of ethanol–water system conduct to a decrease of the overall yield from 80 to 68%. The TG analysis of these microparticles revealed that the mass loss in the range of 20–150 °C decreased from 10.5 to 8.3% with the increase of the nicotine:bCD ratio (Table 2), but the mass loss exists

even up to 250 °C, especially for the case of 2:1 ratio. These facts demonstrate the formation of the nicotine (or other alkaloids)/bCD complex. On the other hand, nicotine (a volatile alkaloid) is loss almost entirely in the range of 20–160 °C (even if the boiling point is higher: 247 °C), but in the case of complexes it is released only at higher temperatures, as is revealed by TG analysis (Fig. 3). At longer crystallization time, the yield increases with approximately 5%. The ethanol:water ratio seems to have no significant importance to the overall yield, as well as the temperature of complexation; by increase of the temperature complexation from 30 to 70 °C (codes NbCDE_t1,2), the yield does not varies (83–85%), but the TG analyses of the obtained complexes are very different (Table 2). The method used for obtaining the nicotine/bCD complex allows to achieve the equilibrium between non-complexed and complexed nicotine; therefore the nicotine concentration in the complex is relatively low, but the complex is well formed and easily to characterize (i.e., comparatively with the kneading method). Thus, in the case of complexation temperature of 30 and 50 °C, a mass loss higher than 1.5% from 100 to 250 °C exists (when the release of the complexed alkaloids can appear; most probably only nicotine and relatives are released from the complex in the range of 150–200 °C, where the mass loss is up to 0.8% for NbCDE_r1,2 complexes and 0.8% for NbCDE_t1 complex, Fig. 3 and Table 2), but in the case of 70 °C complexation temperature, the mass loss is very close to the bCD (~13% up to 100 °C), which corresponds to the water/alcohol release, and a significant mass loss appear only beyond 200 °C (we can presumes that the nicotine concentration is ~0.46% as the mass loss between 150 and 200 °C). The higher mass loss in the range of 200–250 °C can be due to the uncomplexed, only surface-bound nicotine. According to Table 1, the nicotine content in the recovered alkaloid mixture is ~98%; therefore the nicotine concentration (by mass) in the bCD complexes can be expressed as the mass loss in the range of 150–200 °C from the TG analysis. By reducing the cooling/crystallization time from 4 to 0.5 h the complexation is reduced, as is revealed by TG analysis (mass loss of 13% up to 100 °C and no mass loss beyond this temperature, with the exception of the degradation of bCD at ~300 °C). The complexation in methanol–water system is lower (a mass loss of 12.4% up to 150 °C, and up to 0.7% in the range of 150–250 °C, code NbCDM_r1). The complexation is better with 1-propanol (code NbCDP_r1), as is revealed by TG analysis (a nicotine concentration of ~1.1%, evaluated by mass loss in the range of 150–200 °C), but it is possible that this alcohol to be complexed in higher concentration than ethanol. As a conclusion to the complexation process, the best results seems to be obtained in ethanol–water system, for a starting nicotine:bCD ratio of 1:1 or 2:1, a complexation temperature of 50 °C and longer cooling and holding times.

The water content of nicotine/cyclodextrin microparticles has been determined by using the classical Karl Fischer water titration (KFT; Table 2, Fig. 4). In all cases the KFT revealed a water content little bit higher than the mass loss corresponding to the water release from the complex, evaluated by using the TG analysis up to 150 °C (the major quantity of water is released up to 100 °C), where it is possible that some volatile compounds (like nicotine) to be also released. The difference is due to the water loss (approximately 1–1.5%) from the TG analysis at 20 °C under nitrogen flow, until the balance is stabilized and the recording starts. Thus, the water content of NbCDE_r1 complex is 11.3% (10.5% by TG) and this value increases for the NbCDE_r2,3 due to the higher hygroscopicity of these complexes (for NbCDE_r3 complex the KFT value

Table 2 Thermogravimetric analysis (TG, up to 150 °C, where the most of water content are released, and for ranges of 150–250 and 150–200 °C, where the volatile bioactive compounds—such as nicotine and relatives—can be released) and classical Karl Fischer water titration (KFT) for the nicotine (N)/β-cyclodextrin (bCD) complexes obtained in ethanol–water system at different N:bCD ratios (1:1, 2:1, and 3:1, at 50 °C, codes NbCDE_r1,2,3), at various temperatures (50, 30, and 70 °C, codes NbCDE_r1, and NbCDE_t1,2, respectively), and in methanol- or propanol-water systems at 50 °C and N:bCD ratio of 1:1 (codes NbCDM_r1 and NbCDP_r1, respectively)

No	Code	TG mass loss (<150 °C) (%)	TG mass loss (150–250 °C) (%)	TG mass loss (150–200 °C) (%)	Water (%) (by KFT)
1	NbCDE_r1	10.5	1.3	0.4	11.3 ± 0.7 ($n = 3$)
2	NbCDE_r2	9.3	2.0	0.8	11.4 ± 0.1 ($n = 3$)
3	NbCDE_r3	8.3	1.6	0.3	–
4	NbCDE_t1	10.5	1.8	0.8	12.0 ± 0.2 ($n = 3$)
5	NbCDE_t2	13.3	1.5	0.5	13.6 ($n = 2$)
6	NbCDM_r1	12.4	0.7	0.4	12.0 ± 0.4 ($n = 3$)
7	NbCDP_r1	9.8	2.2	1.1	–
8	bCD	13.0	–	–	13.9 ± 0.4 ($n = 5$)

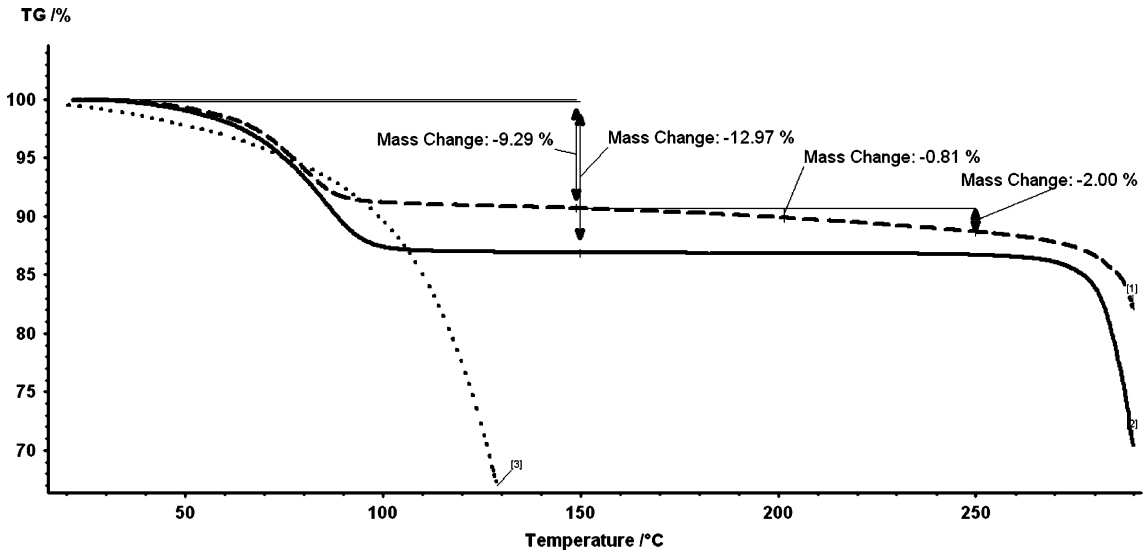

Fig. 3 Superimposed TG diagrams for β-cyclodextrin (*continuous line*), commercial nicotine (*dotted line*), and nicotine/β-cyclodextrin microparticles (2:1 for nicotine:cyclodextrin ratio, temperature of 50 °C) obtained by using the ethanol–water system (*dashed line*)

cannot be achieved). For the complexes obtained at lower or higher temperatures (NbCDE_t1,2) the KF water content was close to the TG mass loss up to 150 °C (12 and 13.6% by KFT, 10.5 and 13.3% by TG, respectively). For the commercial β-cyclodextrin (bCD), the water content is 13.9% (by KFT), very close to the value indicated by the manufacturer ($\sim$14%), but in the TG analysis, the mass loss up to 150 °C was lower, as well as in the case of nicotine complexes. This means that the mass loss in the range of 150–250 °C is especially due to the release of nicotine from the complex. All representative TG and KFT analyses are given in the supplementary material.

The recovered alkaloids from this complex revealed that the relative concentration of nicotine is higher (98.5%) than in the commercial nicotine, probably due to the better interaction of this alkaloid with the inner cavity of bCD comparatively with others alkaloids and shifting of the association–dissociation equilibrium to the formation of the nicotine/bCD complex (nicotine is in much higher concentration than other alkaloids).

The nicotine/bCD microparticles (revealed by SEM analysis, Fig. 5), obtained with nicotine:bCD ratio of 2:1, were used for the degradation studies. In all cases the relative concentration of the recovered compounds from the degraded complex samples was higher than 98% (Table 1), even at 90 °C temperature degradation for 6 h (NbCD-O-t6 sample). No significant concentration of degradation compounds could be identified. As a

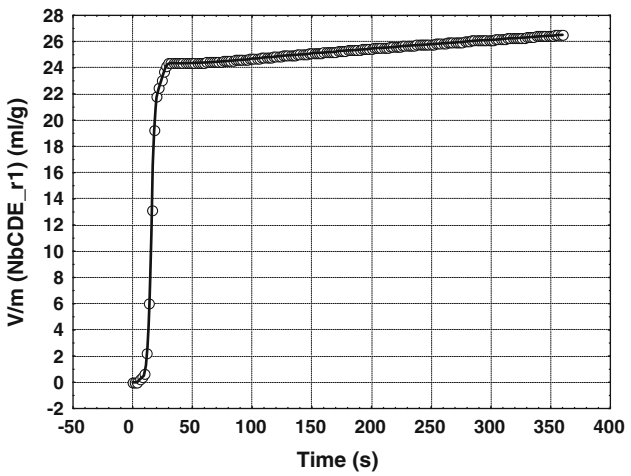

Fig. 4 Karl Fischer water titration curve (V/m vs. Time) for nicotine/β-cyclodextrin complex (code NbCDE_r1)

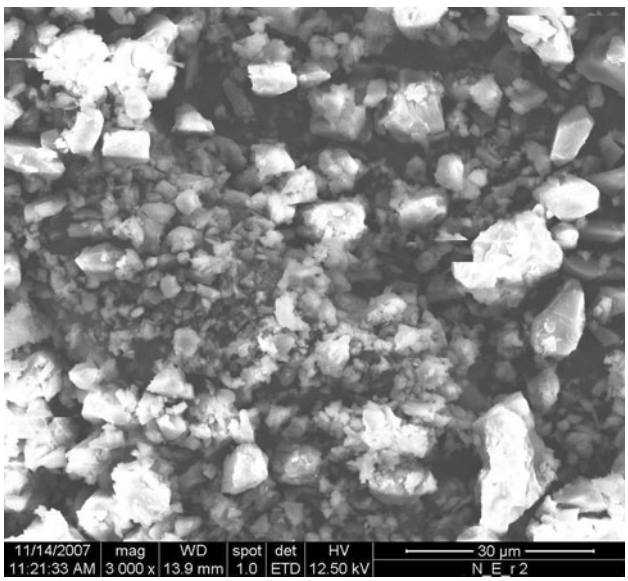

Fig. 5 SEM analysis of nicotine/β-cyclodextrin microparticles

conclusion, the protective capacity of bCD against thermally and/or oxidative degradation of nicotine is very good even at higher temperatures, comparatively with the case of non-complexed nicotine.

Conclusion

In comparison with the non-complexed nicotine, the cyclodextrin complexed product pointed out a higher stability, being protected against thermal and/or oxidative degradation even if the temperature is high. Thus, the β-cyclodextrin can act as a protecting host-molecule for nicotine-containing formulations used in smoking cessation and improve the quality and/or the stability of this bioactive compound against thermal and oxidative degradation.

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