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PREPARATION AND CHARACTERIZATION OF *MENTHA X VILLOSA* HUDSON OIL–β-CYCLODEXTRIN COMPLEX

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Inclusion complex between the essential oil of *Mentha x villosa* Hudson and β-cyclodextrin, with a 1:9 mass/mass oil–β-cyclodextrin ratio was prepared by co-precipitation and kneading methods in a hydroethanolic medium. The GC/MS analysis showed a total volatile content of 99.5% in the *Mentha x villosa* oil. The characterization of the complex involved the analysis of the original essential oil, the surface and the total extracted oils. Among 28 detected compounds in the original essential oil, 13 are monoterpenes and 10 sesquiterpenes, furthermore, piperitenone-oxide is the major component (35.4%). 12 compounds were totally and 11 partially complexed, 3 have been adsorbed only on the surface of the β-CD and 2 have not been detected neither in the surface oil nor in the complexed oil. A 13.6% encapsulation efficiency was observed, while the total oil and volatiles retention was 15 and 77%, respectively. Non-parametric statistic analysis of the data showed that the profile of the volatiles were not significantly different comparing the original oil and the complexed oil (p>0.04). The results of thermogravimetry-mass spectrometry and XRD analysis have proven the inclusion complex formation between the essential oil and cyclodextrin.

Keywords: *β-cyclodextrin, essential oils, evolved gas analysis, inclusion complex, Mentha x villosa Hudson, thermogravimetry-mass spectrometry*

Introduction

The *Mentha x villosa* Hudson is an aromatic plant from the Lamiaceae family, known in Brazil as 'small leaved mint', 'creeping mint' or 'regular mint' [1, 2]. It is a hybrid from the crossing between the *Mentha spicata* L. and *Mentha suaveolens* Ehrh. Their leaves are used for cooking because of their flavouring properties. The plant itself has different pharmacological activities: anti-parasitic and central nervous system depressant, used as tranquilizer, anti-hypertensive and bradicardizant. It also causes contraction of skeletal musculature, relaxation of smooth musculature and has an analgesic effect, explaining its popular use in stomach disorders and menstrual pain [2–8]. The essential oil of *Mentha x villosa* is relatively well known and the major constituent is rotundifolone, an oxygenated monoterpene (cetone), also known as piperitenone oxide or 1,2-epoxipulegone. Some other constituents including monoterpenoids, sesquiterpenoids, triterpenoids and steroids were also identified [9–14].

Cyclodextrins have been widely used to prepare inclusion complexes to improve the stability and solubility, modify the release of drugs [15] and turn liquid substances into stable and free flowing powders [16]. Inclusion complexation of volatile oils with β-CD has been applied to protect essential oils against oxidation, heat and light degradation, evaporation and moisture. This protection is due to the fact that the flavor molecules are tightly held within the β-CD molecule [17–27].

It was shown that molecular encapsulation of the essential oil with β-cyclodextrin improves physical and chemical stability mainly of terpenoid and phenylpropane derivatives [22]. Essential oils are one of the most important raw materials in food, perfumery and also pharmaceutical industries. In this way it is useful to improve thermal and chemical stability and facilitate handling which could increase the potential uses in new dosage forms [28]. Stabilisation of essential oils using maltodextrin and β-cyclodextrin, furthermore the analysis of the formed products was reported in [29, 30].

The complexes can be formed in different ways. During the selection of a preparation method several factors, including good yield, simplicity, rapidity,

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simplicity of scaling up, low cost and characteristics of the end product should be taken into account [31].

The most common methods used to evaluate and characterize the complex formation between essential oils and cyclodextrins are the extraction with organic solvents and simultaneous steam distillation/solvent extraction, followed by gas chromatography analysis, because it involves the identification and determination of the compounds of the original oil as well as the total amount of the complexed oil [21, 27, 32–35].

The aim of the present study was the inclusion complex preparation of the *Mentha x villosa* oil with β-CD, using a co-precipitation and kneading method, characterization and evaluation of the complex by GC/MS and evolved gas analysis using TG-MS combined technique. The amount as well as the composition of the surface-adsorbed and entrapped guest was measured and compared to the composition of the initial oil (Standard oil). Besides, the efficiency of the complexation was also investigated.

Thermoanalytical techniques – providing rapid and cost-sparing analysis – are frequently applied for the investigation of cyclodextrins and their inclusion complexes by the comparison of the thermoanalytical curves of the guest, host, their mechanical mixtures and the putative complexes. The thermal properties of cyclodextrins have been summarized in [36], while the application of thermal analysis to prove the existence of the inclusion complex formation between cyclodextrins and different guest molecules is the topic of several research papers [37–40]. In most cases, when the guest is crystalline itself, DSC is a powerful tool to study the complexation. However, when liquid state guests are complexed, the inclusion complex formation can be proven by evolved gas analysis. During the analysis of these compounds both qualitative and quantitative information is required (e.g. melting temperature, mass loss, etc.). Over the conventional measurements, TG-MS combined technique can be an effective tool showing the thermal fragmentation of the parent cyclodextrin (host) and the liberation of the entrapped guest especially when it is volatile.

Experimental

Standard oil extraction

Mentha x villosa fresh leaves (12 kg) collected in the medicinal plant garden of Cidade da Esperança Park in Natal – Brazil were submitted to hydrodistillation using a modified Clevenger apparatus, followed by extraction with hexane. The oil was dried over anhydrous sodium sulphate and stored at 4°C. Then it was analyzed by GC/MS using a Hewlet-Packard 5890 gas chromatograph coupled to a Hewlet-Packard 5971 mass spectrometer.

The standard oil was used to prepare the standard oil solution as well as the complexes.

Standard oil solution: 20 mg of the standard oil were dissolved in 1 mL of hexane containing 2 mg of menthone, as internal standard.

Capillary GC-MS analysis

The standard oil and the oils extracted from the complexes (total oil and surface oil) were analyzed by GC/MS procedure, using a Hewlet-Packard 5890 GC Series II interfaced to a HP 5971 mass selective detector operating in the scan mode (*m*/*e* 30–300) under the following conditions: column DB-1-Dimethylpolysiloxane fused silica capillary (30 m×0.25 mm; film thickness 0.1 μm); carrier gas: helium (1 mL min^{-1}), injector temperature 250°C, detector temperature and type: 300°C, total ion chromatogram (TIC), programmed column temperature: from 35–180°C at 4°C min^{-1}, then 180–250°C at 20°C min^{-1}, electron impact: 70 eV. Menthone (Fluka) was used as internal standard.

The identification of the compounds was made by computer library search based on matchings of MS fragments and Kovats Indices followed by visual comparison of the mass spectra [41–43].

For all injections 4 μL of the concentrated extracts were used in a splitless injection mode [27].

Preparation of mechanical mixture

A mechanical mixture was prepared in a 1:9 mass/mass oil:β-CD ratio. 10 mL of 10% ethanol solution of *Mentha x villosa* oil in ethanol (10 mL) was added to 10 mL 2:1 *v*/*v* water:ethanol solution containing 9 g of β-CD and was gently mixed. This substance was then vacuum-filtered and dried at room temperature (to a desiccator) to obtain constant mass. The recovered powder was stored in airtight glass containers in refrigerator prior to analysis.

Complexation by co-precipitation method (in organic-aqueous solution)

The complex was prepared maintaining 1:9 mass/mass% oil:β-CD ratio using a co-precipitation method in hydroethanolic medium, described in [27]. A 10 mL of 10 *v*/*v*% *Mentha x villosa* oil–ethanol solution was slowly added to 10 mL of hydroethanolic solution (2:1) containing 9 g of β-CD (Kleptose® – ROQUETTE – France) at 55°C. The mixture was stirred continuously for 5 h while its tem-

perature decreased spontaneously to 25°C. Then it was kept at about 4±2°C for 16 h.

The cold precipitated material was recovered by vacuum filtration. The precipitate was dried at room temperature (in a desiccator) until constant mass. The recovered powder was stored in airtight glass containers in the refrigerator prior to analysis.

Complexation by kneading

The complex was also prepared keeping a 1:9 mass/mass oil:β-CD ratio by the kneading method. 10% ethanol solution of *Mentha x villosa* oil (10 mL) was added to 9 g of β-CD powder into a mortar and kneaded for 45 min. This material was dried at room temperature (into a desiccator) until constant mass. The recovered powder was stored in airtight glass containers in the refrigerator prior to analysis [34].

Determination of the total oil content

Distilled water (8 mL) plus 4 mL of hexane and 0.2 g of the sample were put in a glass tube which was kept in a water-bath at 85°C for 20 min with intermittent shaking. The organic phase containing the volatile compounds was decanted. This procedure was repeated 3 times. Then, hexane (1 mL) and the internal standard (2 mg) were added to the decanted sample, then the extract was concentrated to approximately 1 mL using rotary evaporator and stored around 4°C in a vial till the GC/MS analysis [35].

To calculate the total oil content of the complex powder, the following equation was used [21]:

$$\text{total oil (mass/mass\%)} = \frac{SdVA_e}{WA_s} \qquad (1)$$

where S is the oil content (v/v%) in the standard oil solution (see above), V is the volume of solvent used in the total oil extraction, A_e and A_s are the total area of the peaks in the GC chromatograms of the extracted oil (total oil) and standard oil respectively, W is the mass of the complex submitted to extraction, d is the density of the standard oil which was determined by gravimetric method as described in the Brazilian Pharmacopea.

The total oil corresponds to the amount of complexed guest in the β-cyclodextrin cavity plus the surface-adsorbed oil.

Determination of surface-adsorbed oil content

The volatile compounds adsorbed on the surface of the cyclodextrin were determined by washing 3 g of powder with 20 mL hexane which was shaken for 20 min. The suspension was then filtered and the residue was further washed with hexane (10 mL). Then, hexane (1 mL) and the internal standard (2 mg) were added to the extract which was concentrated and stored as it was described above [27, 35].

The amount of the surface oil (mass:mass%) was calculated according to Eq. (1), where now V is the amount of the solvent used for the surface-adsorbed oil extraction and A_e is the total peaks area of the surface-adsorbed oil.

The difference between the total oil and the surface-adsorbed oil is the amount complexed in the β-cyclodextrin cavity.

Statistical analysis

Statistical analysis of the volatile components from the original oil, total oil and surface-adsorbed oil was done using Statistica 6.0 software.

Mann–Whitney U test was used to compare 2 groups. The investigated parameter was the mean concentration of volatiles in each oils (%). The significance level was established as α=4%.

Total volatile substances (TVS%)

The concentration of volatiles in the *Mentha x villosa* oil was determined by calculating the response factor (RF) of piperitenone oxide using Eqs (2) and (3) [27].

RF of piperitenone oxide was determined and used for the quantification of all volatile compounds. During the calculation it was assumed that RF values for the components are nearly the same.

$$\text{RF} = \frac{M_p A_{is}}{A_p M_{is}} \qquad (2)$$

$$\text{TVS} = \frac{A_s M_{is} \text{RF} \cdot 100}{A_{is} M_s} \qquad (3)$$

where M_p, M_{is} and M_s are the amount of piperitenone oxide, menthone (internal standard) and standard oil (in milligrams), respectively, and A_p, A_{is} and A_s are the corresponding peak areas.

Encapsulation efficiency

The encapsulation efficiency (E, %) which determines the quantity of oil entrapped into the β-CD cavity, was calculated by Eq. (4) [27].

$$E = \frac{(M_t - M_a) \cdot 100}{M_c - M_t} \qquad (4)$$

where M_t, M_a and M_c are the masses of total oil, surface-adsorbed oil and of the dry complex powder (which was kept in desiccator), respectively.

Thermal analysis

Thermoanalytical measurements were carried out using TA Instruments (Newcastle, Delaware, USA) STD 2960 simultaneous TG-DTA unit under helium purging connected to a Balzers Thermostar GSD 300T quadrupole mass spectrometer through a heated silica capillary (t=200°C) transfer tube. Considerations concerning the operation modes of the mass spectrometer were discussed in [28, 44]. The experimental conditions are summarized in Table 1.

Table 1 Experimental parameters of thermogravimetric–mass spectrometric measurements

Methods	Parameter	
TA	flow rate/L h^{-1}	15
	heating rate/K min^{-1}	10
	temperature interval/°C	35–400
	sample mass/mg	8–8.5
MS/MID mode	observation time/s $channel^{-1}$	0.5

X-ray diffraction

X-ray investigations were carried out using a Jena-Zeiss HZG4 Freiberger Prazisions Mechanik Diffractometer with CuK_α (λ=1.5405 Å and 2θ=2 to 44°), operating at room temperature under settings of 30 kV and 25 mA.

EGD analysis

The evolved gas detection (EGD) experiments have been done applying DuPont 916 (Carle 3000) Thermal Evolution Analyzer equipped with a hydrogen–air flame ionisation detector which provides signal for the organic compounds but not for the inorganic ones e.g. water, CO, CO_2, NH_3, etc.

Results and discussion

Table 2 shows the recovered powder after co-precipitation of the *Mentha x villosa* oil with β-CD at a 1:9 mass/mass% ratio. The co-precipitation method seems to be good to prepare inclusion complex since the recovery of the complexed powder was 95.9%. The relatively low material loss can be attributed to the selected way of preparation (i.e. a part of the oil remained in the solution and/or some evaporation loss took place during the long complexation process. The yield is close to that of [35].

GC-MS analysis

The standard oil composition was similar to that reported in the literature [5, 8, 9]. The amount of piperitenone oxide was 35.4 mass/mass%, probably due to the high water solubility compared to other essential oil constituents [6, 10].

The GC/MS profiles of the standard oil as well as the surface-adsorbed and the total oils are presented in Figs 1–3, respectively.

Among the twenty-eight compounds detected in the original oil, 13 were monoterpenoids, 10 were sesquiterpenoids, one phenylpropanoid, one ester and three others were not identified (Table 3). The qualitative and quantitative composition of the volatiles of *Mentha x villosa* surface-adsorbed oil (Fig. 2) was different from the standard oil (Fig. 1). On the other hand, the chromatographic profile of the total oil was similar to the standard oil.

Table 3 shows the compounds related to their chromatographic peaks and their amount in the standard, surface-adsorbed and total oils. The concentration in the complexed oil was obtained by subtraction of the amount of the surface-adsorbed oil from the amount of the total oil.

The results showed that 12 compounds were completely complexed: α-pinene, sabinene, *p*-cimenene, linalool, anethole, β-elemene, β-cubebene, γ-cadinene, β-germacrene, δ-cadinene, trans-muurolol and an unidentified component. In addition, 11 were partially complexed (included both in the cyclodextrin cavity and adsorbed on its surface), distributed with different ratios: β-myrcene, limonene, 1,8-cineole, *trans*-ocimene, thymol acetate, piperitenone oxide, piperitone, *trans*-caryophyllene, γ-muurolene, α-humulene, 1,10-di-*epi*-cubenol.

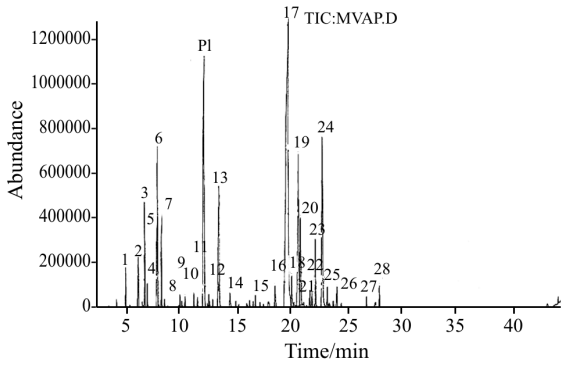

Fig. 1 GC/MS chromatogram of the standard *Mentha x villosa* Hudson oil

Table 2 Recovery of the co-precipitated *Mentha x villosa* β-cyclodextrin powder from 2:1 *v*/*v* water:ethanol solution

Mentha x villosa oil:β-CD ratio	Theoretical amount of co-crystallized complex/g	Starting material/g	Recovered powder/g	Recovery/%	Loss/%
1:9	9.1	10	8.7	95.9	4.1

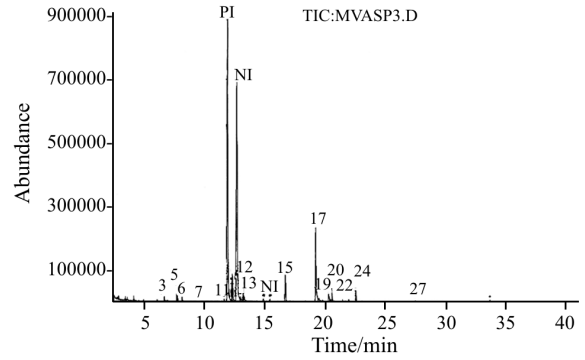

Fig. 2 CG/MS chromatogram of the surface oil extracted from the complex powder

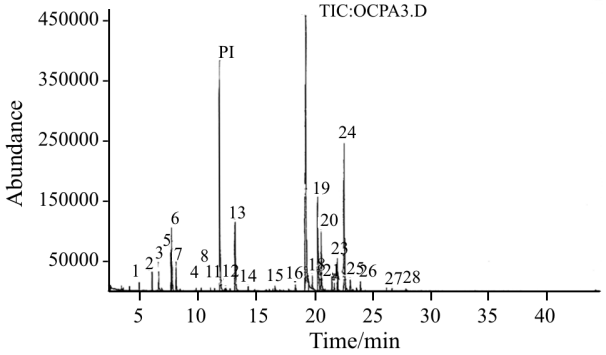

Fig. 3 CG/MS chromatogram of the total oil extracted from the complex powder

Five other compounds were not complexed, 3 had only been adsorbed on the surface of the β-CD and 2 had not been detected neither in the surface-adsorbed oil nor in the complexed oil. One possibility that has to be considered in the case of the compounds not found in the surface oil (those that were not detected anywhere as well as those that were assumed totally included in the β-CD) is the evaporation of these compounds during the drying process [35].

The Mann Whitney test indicated no significant difference (p>0.04) between the volatile compounds of the standard and the complexed oil.

A higher inclusion efficiency was found (13.6 mass/mass%) compared to other results [21, 26, 35] where 8–12% maximum theoretical flavour load for β-CD was reported. This result suggests that the molecular masses of the complexed components of *Mentha x villosa* are higher, than that has been reported by Harangi and Bhadhari.

The molecular mass of the monoterpenes and sesquiterpenes found in the *Mentha x villosa* oil is between 136 and 222 g mol^{-1}. The complexation ratio between them was similar despite the molecular size of sesquiterpenes is more appropriate to form an inclusion complex. In addition, the polarity of the compounds seems to play an important role in the process, since the less polar sesquiterpenes were complexed into a higher ratio (Table 2).

The estimated retention of the predominant flavor compound (piperitenone oxide) is 72% [35], which reached 87% as a maximum retention starting from a 9:91 initial mass/mass ratio composition between lemon oil and β-CD.

Since there is no difference between the chromatographic profile of the standard oil and the total oil extracted from the complexed powder, one can suppose that after complexation the *Mentha x villosa* oil maintains its organoleptic property as well as its pharmacological activity. This justifies the use of the complexed oil in the food and pharmaceutical industries.

Thermogravimetric results

Mentha x villosa oil extract, its two mechanical mixtures (freshly prepared and stored ones), and two complexes, kneaded and co-precipitated with β-cyclodextrin were subjected to thermal analysis.

The TG curves of the *Mentha x villosa* oil–β-CD system are summarized in Fig. 4. By their data analysis it can be seen that the major fraction (90%) of the essential oil evaporates up to 160°C (Fig. 4, curve A). In this temperature interval β-CD looses its water content causing about 14% of mass loss. Until 250°C no further mass change for the host molecule was recorded. The decomposition of the β-CD started above 270°C (curve here is not presented). The TG curve of the freshly prepared mechanical mixture can be considered as the superposition of the pure compounds. Two overlapping steps were exhibited causing 49.8% of mass loss, related to the evaporation of the essential oil and the water release from the CD up to 160°C (Fig. 4, curve B). (The Authors wish to remark that due to the in situ preparation the amount of guest was

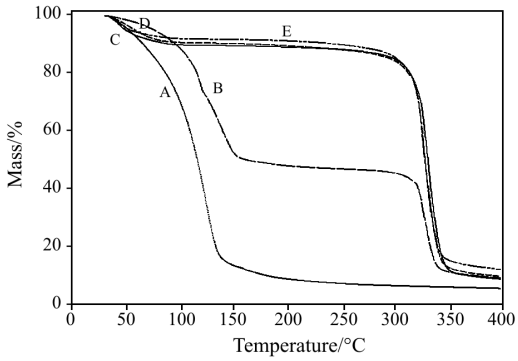

Fig. 4 TG curves of *Mentha x villosa*–β-cyclodextrin samples; A – pure oil, B – freshly prepared mechanical mixture, C – one-month stored mechanical mixture, D – kneaded complex, E – co-precipitated complex

Table 3 Characterization of *Mentha x villosa* Hudson oil and its β-cyclodextrin complex

Original oil				Extracts		Concn./% difference of extracts from original *M. villosa* oil	
Peak	volatile	T_R/min	conc./%	surface oil	total oil	compl. oil/%	complexation ratio
1	α-pinene	5.00	0.67	0	0.62	0.62	total
2	sabinene	6.13	1.38	0	1.25	1.25	total
3	β-myrcene	6.70	2.05	0.41	1.70	1.29	1:3
4	heptanol-2,6-dimethyl	6.92	0.57	0	0	–	–
5	limonene	7.77	2.60	0.51	2.14	1.63	1:3
6	cineole-1,8	7.84	3.48	0.37	3.43	3.06	1:8
7	*trans*-ocimene	8.17	2.10	0.35	1.72	1.37	1:4
8	*para*-cimenene	9.83	0.40	0	0.20	0.20	total
9	linalool	10.29	0.40	0	0.25	0.25	total
10	octanol acetate-2	11.16	0.42	0	0	–	–
11	not identified	11.38	0.40	0.20	0.18	–0.02	none
	menthone (I.S)	12.03	12.42	38.71	17.19	–21.52	–
12	borneol-endo	12.43	0.37	0.30	0.19	–0.11	none
13	thymol acetate	13.36	6.38	1.21	5.28	4.07	1:3.4
14	anethole	14.29	0.84	0	0.40	0.40	total
15	not identified	16.5	0.52	2.45	0.44	–2.01	none
16	not identified	18.44	1.28	0	0.60	0.6	total
17	piperitenone oxide	19.67	35.35	9.91	34.92	25.01	1:2.5
18	β-elemene	19.95	1.21	0	0.93	0.93	total
19	piperitone	20.61	9.64	1.39	7.49	6.10	1:4.4
20	*trans*-caryophyllene	20.76	3.36	0.89	4.09	3.20	1:3.6
21	β-cubebene	21.58	0.52	0	1.00	1.00	total
22	α-humulene	21.77	0.51	0.26	0.53	0.27	distributed
23	γ-cadinene	22.08	2.20	0	2.38	2.38	total
24	γ-muurolene	22.75	8.47	1.01	11.35	10.34	1:10
25	germacrene B	23.16	0.64	0	0.76	0.76	total
26	δ-cadinene	24.02	1.01	0	0.99	0.99	total
27	*epi*-cubenol-1,10-di	26.70	0.42	0.28	0.62	0.34	distributed
28	*trans*-muurolol	27.90	0.79	0	0.70	0.70	total

remarkably higher in the freshly prepared mechanical mixture.) The measured mass losses for the freshly prepared mechanical mixture confirm that the thermal process does not result in complex formation between the pure components.

Table 4 Mass losses for the *Mentha x villosa* oil–β-CD system in two different temperature intervals

Sample	Mass loss/%	
	30–160°C	160–250°C
β-CD	14.3	–
Essential oil	87.8	4.9
Freshly prepared mechanical mixture	49.8	3.3
1 month stored mechanical mixture	10.5	1.2
Co-precipitated complex	8.3	1.8
Kneaded complex	9.8	2.0

The co-precipitated inclusion complex exhibits 8.3%, the kneaded 9.8%, and the stored mixture 10.5% of mass loss up to 160°C (Fig. 4, curves E, D and C), respectively. It can be attributed mainly to the water loss and to the release of a small amount of guest from the samples.

Between 160 and 250°C 1–5% further mass change was recorded which may due to both to the evaporation of a *Mentha x villosa* oil fraction possessing lower volatility and/or to the partial release of the guest components from their inclusion complexes. (Few representative mass loss values are summarized in Table 4).

Evolved gas detection/evolved gas analysis

The EGD curve of β-CD has been interpreted in [44]. The evaporation of the majority of the pure

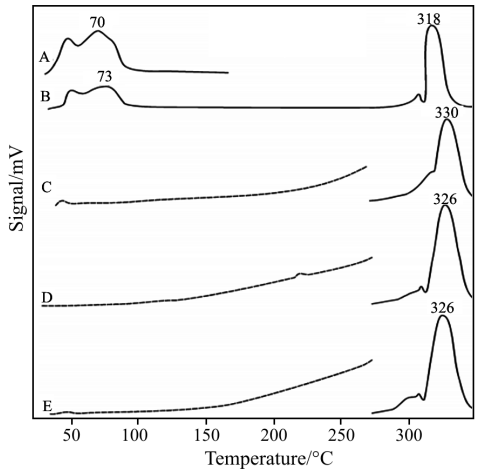

Fig. 5 EGD curves of *Mentha x villosa*–β-cyclodextrin samples; A – pure oil, B – freshly prepared mechanical mixture, C – one-month stored mechanical mixture, D – co-precipitated complex, E – kneaded complex

Mentha x villosa oil occurs up to 150°C. The shape of the evaporation peak indicates the presence of fractions with different volatility and tension (Fig. 5, curve A). The EGD curve of the freshly prepared mechanical mixture bears the thermoanalytical characteristics of the individual components: the broad overlapping peaks below 100°C represent the evaporation of the oil extract, whereas the peak over 300°C indicates the decomposition of the β-CD ring excluding the possible interaction between the components during the thermal run (Fig. 5, curve B).

On the contrary, the thermal features of the one-month stored mechanical mixture are similar to that of the complexes proving that complexation eventuates upon storage (Fig. 5, curves C–E) indicating the formation of the inclusion complex between *Mentha x villosa* and β-cyclodextrin. The slight deviation from the baseline refers to the evaporation of the oil components from their inclusion complex.

Mass spectrometric results

The systematic comparison of the three-dimensional figures of the *Mentha x villosa*–β-CD systems provides convincing evidence for inclusion complex formation. (Figures are drawn in the *m*/*e*=45–120 range.) The interpretation of the mass spectral curves of β-CD and few inclusion complexes has been written elsewhere [28, 44, 45]. The characteristic SAC curves of the *Mentha x villosa* oil extract can be seen in Fig. 6a. The distance between the characteristic groups of peaks is usually 14 specific mass/charge unit, which is equal to the mass of the CH_2^+ fragment. The evaporation of the individual components starts at room temperature. The majority of the oil extract leaves up to 160°C but the process becomes complete well above this temperature (see TG curves of the pure guest, Fig. 4 curve A). According to the SAC curves of the freshly prepared mechanical mixture (Fig. 6b), the *Mentha x villosa* oil evaporates in the same way from the mixture as with standard oil is alone. The thermal fragmentation of the β-CD takes place between 270–330°C. From the one-month stored mechanical mixture the evaporation of the oil takes place between 250–350°C. Since there was no any special mechanical treatment and/or agitation (disregard of a gentle blending of the extract and β-CD) applied on the preparation of this sample it means, that the blending followed by storage at room temperature was sufficient to obtain a *Mentha x villosa*–β-cyclodextrin inclusion complex (Fig. 6c).

The above finding is appropriately supported by the SAC curves of the co-precipitated and kneaded complexes (Figs 6d and e). The thermal behaviour of these two samples – as well as their fragmentation patterns – are concordant and correspond to the thermal behaviour (and fragmentation) of the one-month stored mechanical mixture.

In case of the co-precipitated complex some fragmentation starts at about 110–130°C, while in case of the kneaded product the same occurs around 150–170°C. It can be explained in two different ways. In one hand the retardation of the β-CD when kneading as preparation method was applied is better. On the other hand it might happen that upon kneading a part of the *Mentha x villosa* oil is released, which itself would have formed a lower thermal stability complex with the β-CD (if this fraction would have remained in the system). Since, due to the applied preparation method the amount of the guest has decreased and did not form inclusion complex. Consequently its thermal decomposition/fragmentation cannot be expected.

X-ray diffraction

The X-ray diffraction patterns of solid samples are summarized in Fig. 7. The upper one is belonging to the crystalline β-CD, itself. The most representative lines are marked with an asterisk. The X-ray diffraction curve of the stored *Mentha x villosa*–β-CD mechanical mixture indicates the occurrence of a slightly crystalline substance. The most representative diffraction lines of the β-CD are also evident with less intensity. Besides, a new band (marked with #) has appeared indicating a presence of a new phase which should be the *Mentha x villosa*–β-CD complex which formed upon storage (Fig. 7, curve B). The diffraction curves of the two *Mentha x villosa* oil–β-CD complexes (Fig. 7, curves C and D) are very similar to that of the stored mechanical mixture, confirming the inclusion complex formation in case of the co-precipitated as well as in the kneaded complex.

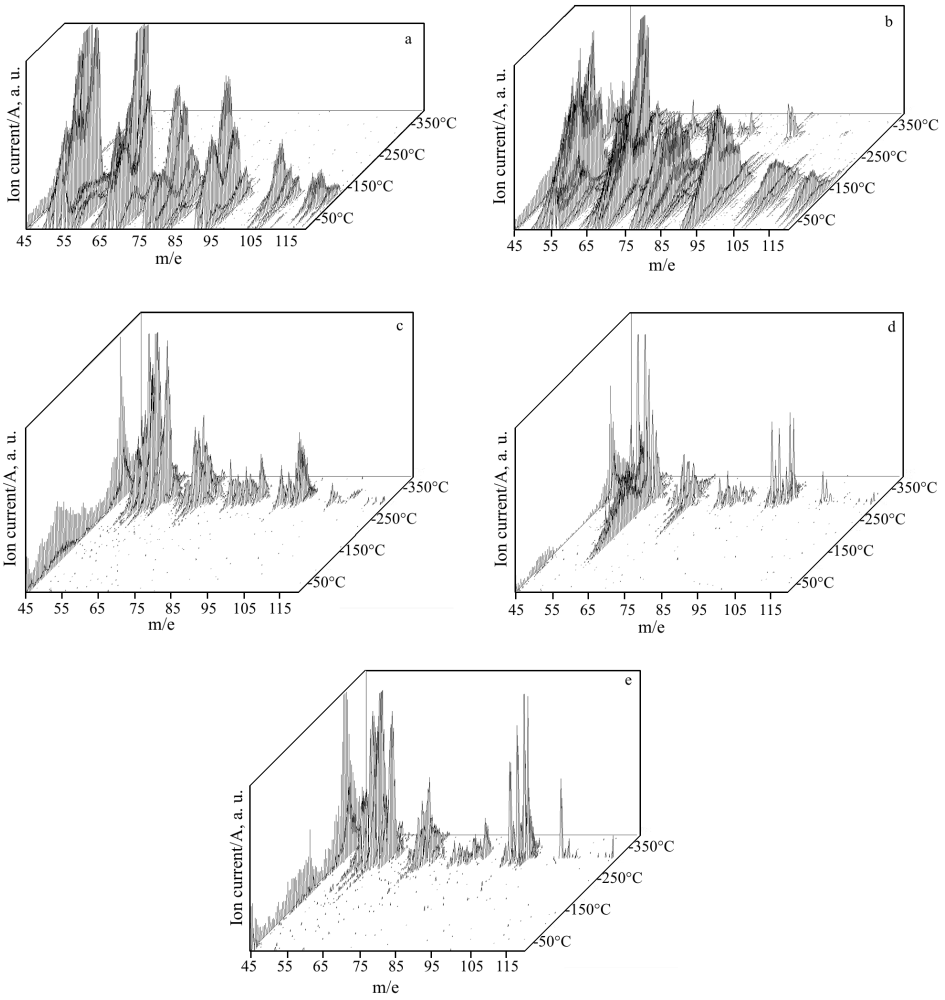

Fig. 6 SAC curves of a – *Mentha x villosa* oil extract, b – freshly prepared mechanical mixture, c – one-month stored mechanical mixture, d – co-precipitated complex, e – kneaded complex

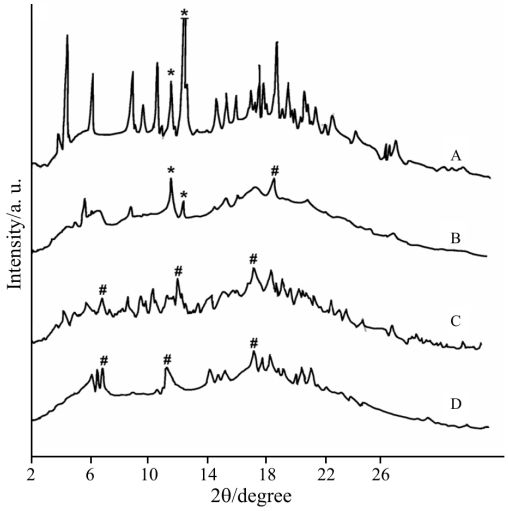

Fig. 7 X-ray diffraction patterns of *Mentha x villosa*–β-cyclodextrin samples; A – β-cyclodextrin, B – one-month stored mechanical mixture, C – co-precipitated complex, D – kneaded complex

Conclusions

It was found that *Mentha x villosa* oil can be successfully complexed with β-CD by the co-precipitation method in hydroethanolic medium with a 96% powder recovery. The product was similar to the original *Mentha x villosa* oil in respect of the major volatile components. The volatile flavour composition of the surface adsorbed *Mentha x villosa* powder was different from the original *Mentha x villosa* oil.

The retention of volatiles was approximately 78%, while the total volatile compounds in the original oil was 99%.

From the detected compounds in the original oil, 13 are monoterpenes, 10 are sesquiterpenes, 1 ester, 1 phenylpropanoid and 3 not identified. The major component of the oil is piperitenone oxide. Its degree of complexation was 72%.

12 compounds were totally complexed, 11 compounds were partially complexed and 5 compounds were not complexed. It is supposed that 3 were only adsorbed on the β-CD surface

and 2 were absent. The calculated complexation efficiency was 13.6%.

The statistical analysis (Mann Whitney U test) did not show significant differences between the original oil composition and the complexed oil.

Among thermoanalytical techniques TG method is not sensitive enough to prove the inclusion complex formation.

EGD technique itself is effective however the flame ionization detector does not provide a sample specific signal to monitor and to follow the release of the individual components of the extracted oil.

The method developed for the TG-MS combined technique is powerful since it indicates the evaporation of the different oil components.

Complex formation was found between *Mentha x villosa* oil and β-CD in case of the one-month stored mechanical mixture. It indicates the high complex forming ability of the oil extract which does not require the selection and application of any of the known inclusion complex preparation techniques.

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