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CONTROLLED RELEASE PROFILES OF *EUGENIA CARYOPHYLLATA*, *ARTEMISIA ANNUA* AND *CARUM CARVI* VOLATILE OILS FROM ORGANIC FUNCTIONALIZED MCM-41 SUPPORT

Dan Eduard MIHAIESCU, Dragos GUDOVAN* and Anca MARTON

Organic Chemistry Department, Politehnica University of Bucharest, Faculty of Applied Chemistry and Materials Science
313 Splaiul Independentei, 060042 Bucharest, Roumania

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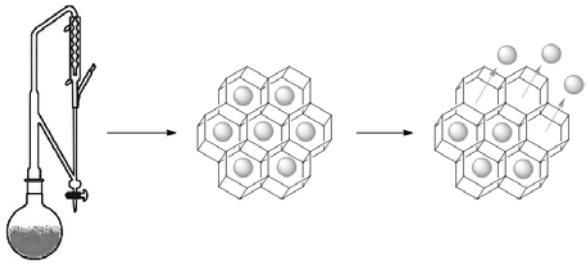
Dimethyldichlorosilane (DMDCS), a tetrahedral organosilicon compound, was used for the organic functionalization of the MCM-41 material. For all the release experiments, 3 volatile oils were used, extracted from *Eugenia caryophyllata* (clove), *Artemisia annua* (sweet wormwood) and *Carum carvi* (Caraway). The volatile oils were extracted by hydrodistillation technique, using a Neo-Clevenger apparatus, and analysed by GC-MS technique and FT-IR. For the study of the release profiles a continuous monitoring system was used, using a modified HPLC system. For all the volatile oils used, the release profile shows a slow curve, at a medium loading (30 mg/100 mg substrate), making the DMDCS functionalised MCM-41 a very good candidate for the controlled release of volatile oils. The DMDCS functionalised MCM-41 loaded with volatile oils can be a good antiseptic material in bandages and other types of media which have a significant water content.



INTRODUCTION

Volatile oils have been widely investigated as an alternative to the use of antibiotics[1] which usually have many side-effects apart from the main function, due to the better biocompatibility and lower allergenic response. Given the active presence of the volatile compounds in the environment (food products, air refreshers, toilet hygiene products), the human body rejection of the volatile oils is lesser than that of antibiotics. Also, given the constant adaptation of bacteria to antibiotics, alternate means of treatment are of great importance. However, some volatile oils have a lesser antimicrobial activity than antibiotics, which yields an increase in the dosage required in order to get the desired antimicrobial effect. This can be an issue when dealing with oral ingestion, but usually it is not an issue in the case of skin applied bandages.

Volatile oils have also been used as an alternate preservative, for the use in bio-products that degrade rapidly, like tomatoes, cucumber, bell peppers, green peppers and such. Polymer coatings containing volatile oils are a good counter-candidate for old-fashioned polypropylene coatings currently used, which are a constant source of pollution due to their resilient nature and lack of bio-degradability.

* Corresponding author: dragosgudovan@gmail.com

MCM-41 materials have known one the largest interests in the market since Mobil Corporation first discovered the material, interest proven in the scientific environment by the thousands of articles published since the first mention. Articles concerning MCM-41 materials are still published due to the many advantages they present. The high surface area, the possibility of tuning the pore size through modification of the reaction conditions,[2] the narrow distribution of pore size, are among the characteristics which are highly desirable in a porous material, difficult to obtain in a natural porous material, yet easy to obtain and to control in the MCM-41 material.[3] One of the many advantages of the MCM-41 material is its excellent biocompatibility.[4] Organic functionalized MCM-41 materials [5] have grown in interest due to the fact that they allowed further upgrade of the applications range.[6] In this study we present the way the organic functionalized MCM-41 silica perform after being loaded with the selected volatile oils, and to perform the release of the oils. Controlled release of the oils – if managed – would be a goal for the use of MCM-41 in various applications, including therapeutic.

Controlled release studies have grown in importance, the application of controlled release experiments being various, from the most simple ones, like longer lasting air refreshers, toilet disinfectants, longer lasting air or water purifiers, to more complex applications, concerning controlled release drug delivery or even targeted release drug delivery.[7]

EXPERIMENTAL

The vegetal material used for the extraction of the volatile oils used in the experiments was a commercially available dry material, which was finely grinded right before the extraction. The extraction was done by hydro-distillation using a Neo-Clevenger apparatus. The resulting products were recovered and separated from the water in a separatory funnel. The volatile oils were dried using anhydrous sodium sulphate (Sigma Aldrich) and diluted in dichloromethane (GC purity, Sigma Aldrich) for GC-MS analysis.

The chromatographic analysis was done on a HP-5 MS column, 30 m long, 0.320 mm internal diameter and 0.320 µm film thickness. The injection volume used was 1 µL, the column flow 1 mL/min H_2, oven program was 40°C initial, 5°C/min to 250°C, 10°C/min to 300°C, and hold for 10 minutes. The temperatures on the MS transfer line and inlet were kept at 280°C. All major components were identified using the NIST spectral library.

The MCM-41 material was synthesized using a method proposed by Melendez-Ortiz and his colaborators[7] with minor modifications. Cetyltrimethylammonium bromide (CTAB), ethyl alcohol, tetraethylorthosilicate (TEOS) and ammonium hydroxide (25% NH_3 basis) were all purchased from Sigma Aldrich and used without further purification. The water used came from a Millipore Elix 5 system, 15 MΩ resistance. In a typical experiment, 0.5 g of CTAB were mixed with 96 mL of pure water, then 34 mL of ethyl alcohol were added, and 10 mL of ammonium hydroxide. After 5 minutes stirring, 2 mL of TEOS were added and the mixture was left to stir for 3 hours at room temperature. The resulting precipitate was recovered by filtration and dried overnight at room temperature. The dry powder was thermally treated in an oven at 540°C in order to completely remove the CTAB and purify the MCM-41 material.

For the organic functionalization reaction, 400 mg of MCM-41 material were suspended in 2 mL acetonitrile in a 6 mL reaction vessel with Luer-lock valve. Dimethyldichlorosilane (0,5 mL) was added with a syringe in acetonitrile through the septum of the reaction vessel. The reaction mixture was kept at 100°C for 4 hours. The resulting material was washed five times with 5 mL of acetonitrile and dried at room temperature. The solid obtained was used for loading with volatile oils. All materials were loaded with 40 mg of volatile oil. 100 mg of MCM-41 material functionalized with DMDCS were put in a covered 10 mL Berzelius beaker and 40 mg of volatile oil, carefully weighed, were added on top. The container was immediately covered after the weighing was over. The beakers were left to stand overnight. Controlled release experiments were made the next day.

FT-IR spectra were recorded using a Thermo Nicolet 6700 series spectrometer, with µATR accessory, using a ZnSe window. The spectra were recorded in the 650-4000 cm^{-1} wavenumber range.

Controlled release experiments were made using a modified HPLC system in order to allow the continuous monitoring of the experiment. The experiment uses the pump and the detector of the HPLC system. The complete method is detailed in one of our previous articles.[8] For all the studied volatile oils, a calibration curve was recorded to help trace the amount released. The good calibration criteria used was $r^2>0.990$. A total of five calibration points were used. The wavelengths used for spectra are *Eugenia caryophyllata* – 300 nm, *Artemisia annua* – 210 nm and *Carum carvi* – 210 nm. The choice of the wavelenghts was based on the calibration curve and on the UV-VIS absorbtion of the major compounds present in each volatile oil. The absorbtion maximum was not always a criterion because of the saturation phenomena in the detector.

RESULTS AND DISSCUSION

The FT-IR technique provided a quick check for the loading. FT-IR spectra – presented below – were recorded for each volatile oil, for MCM-41 reference material, and for MCM loaded material. FT-IR spectra of the MCM loaded material prove the successful loading by the present peaks corresponding to functional groups existing in the loaded volatile oils.

The MCM-41 mesoporous material was analyzed using transmission electron microscopy (TEM) and BET surface area measurement. BET

technique showed a type 4 curve, corresponding to a mesoporous material. Average pore size found from BET analysis was 2.45 nm, and BET surface area found was 1224 m^2/g. This result is in accordance with the IUPAC mesoporous definition,[9] which establishes the range for mesoporous materials between 2 and 50 nm. TEM analysis complies with the definition, showing pores with outer diameter between 2.8 and 3.2 nm (Fig. 2). Also from the high resolution TEM analysis the typical hexagonal pore configuration of MCM-41 material can be noticed.

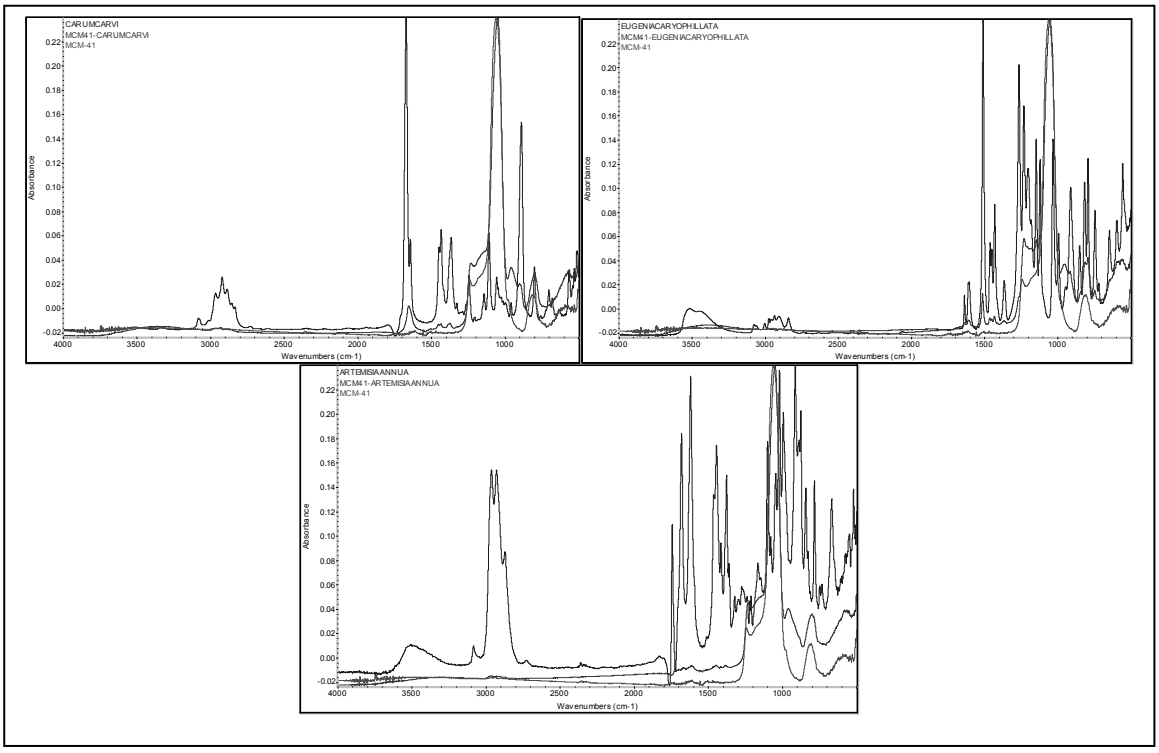


Fig. 1 – FT-IR spectra of MCM-41 material loaded with *Carum carvi* (upper left), *Eugenia caryophillata* (upper right) and *Artemsia annua* (lower middle).

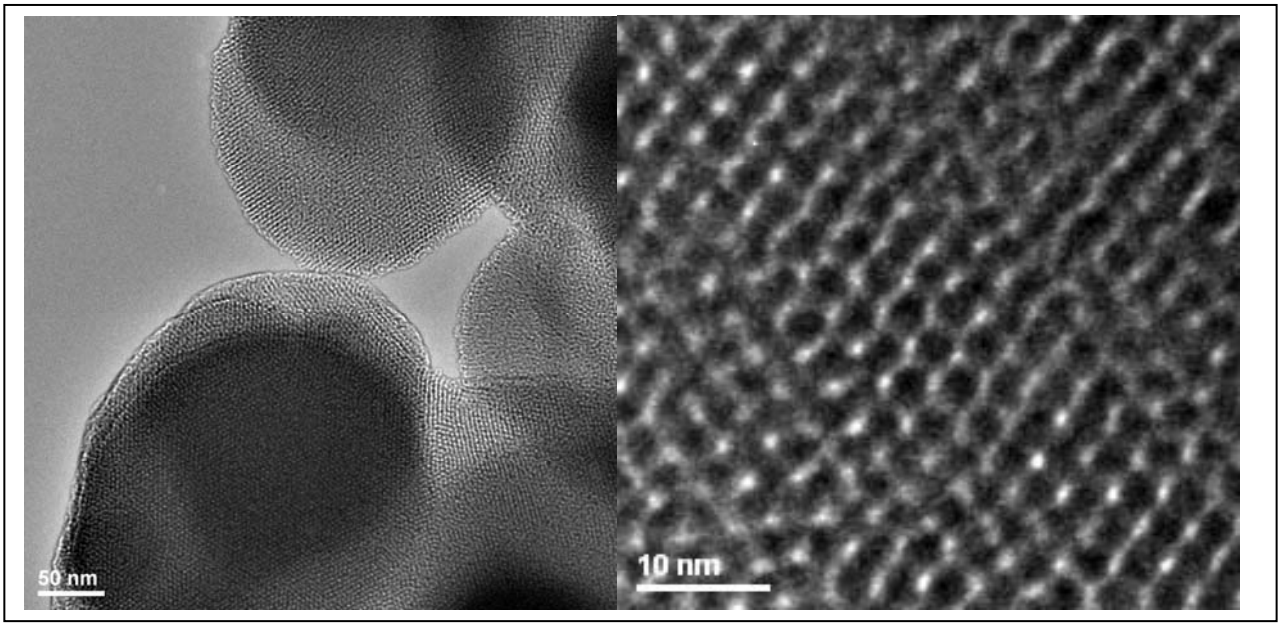


Fig. 2 – HR-TEM analysis of the MCM-41 sample (overall image left-hand side, and detailed image right-hand side).

Based on the GC-MS analysis, in Tables 1-3 the identification of all the compounds found in the sample is presented. An estimation of the amount present in each volatile oil is based on area percentage. Our research proves that in each volatile oil there are some major compounds: in the case of *Eugenia caryophylatta* it is the eugenol (more than 90%), in the case of *Artemisia annua* there are the artemisia ketone (37%), camphor (18%) and eucalyptol (8%), and in the case of Carum carvi there are carvone (72%) and limonene (25%).

Table 1

Identified compounds for *Eugenia caryophyllata* volatile oil

Nr.	Retention time (min)	Area percentage	Identified compound
1	6.381	0.005	ethyl caproate
2	6.994	0.006	p-cymene
3	7.117	0.006	m-mentha-1,8-diene
4	7.203	0.010	eucalyptol
5	7.779	0.032	2-heptenoic acetate
6	7.889	0.006	o-cymene
7	9.422	0.01	2-nonanone
8	11.862	0.032	benzyl acetate
9	12.021	0.012	ethyl benzoate
10	12.193	0.011	terpinen-4-ol
11	12.757	0.138	methyl salicylate
12	15.038	0.063	chavicol
13	18.716	92.843	eugenol
14	18.862	0.030	α-copaene
15	20.200	3.999	β-caryophyllene
16	21.218	0.379	α-caryophyllene
17	22.002	0.010	β-guaiene
18	23.130	0.028	himachalene
19	23.437	0.040	δ-cadinene
20	23.756	1.927	eugenol acetate
21	25.043	0.273	caryophyllene oxide

Table 2

Identified compounds for *Artemisia annua* volatile oil

Nr. crt.	Retention time (min)	Area percentage	Identified compound
1	6.737	0.227	carene
2	6.994	0.136	p-cymene
3	7.178	8.506	eucalyptol
4	8.147	0.363	terpinolen
5	8.416	37.404	artemisyl ketone
6	9.091	2.600	artemisyl alcohol
7	9.790	2.228	farnesene epoxide
8	10.832	3.672	pinocarveol
9	11.016	18.854	camphor
10	11.665	2.409	pinocarvone
11	11.764	0.292	borneol
12	11.874	0.136	α-terpine-ol
13	12.021	0.505	isocamphone
14	12.181	1.074	δ-terpineol
15	12.684	0.362	β-terpineol
16	12.868	0.638	myrtenol
17	18.814	2.160	copaene
18	20.151	5.794	β-caryophyllene
19	21.193	0.294	bisabolene
20	21.573	0.607	α-caryophyllene
21	22.088	3.512	cadinene
22	22.211	0.554	selinene
23	23.437	0.519	cadinene

Table 3

Identified compounds for *Carum carvi* volatile oil

Nr. crt.	Retention time (min)	Area percentage	Identified compound
1	7.154	25.235	limonene
2	7.865	0.027	o-cymene
3	9.631	0.049	geraniol
4	10.231	0.065	carveol
5	10.746	0.028	pinocarveol
6	12.193	0.020	δ-terpineol
7	12.684	0.071	α-terpineol
8	12.868	0.624	dihydrocarvone
9	13.113	0.334	dihydrocarvone
10	13.640	0.118	dihydrocarveol acetate
11	13.836	0.111	dihydrocarveol acetate
12	14.731	72.293	carvone
13	20.114	0.070	β-caryophyllene

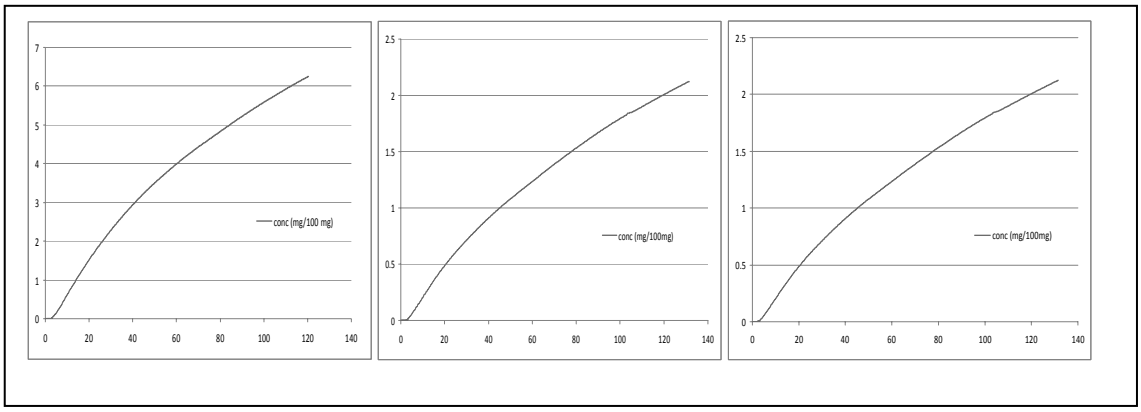


Fig. 3 – Release profiles for Eugenia caryophyllata (left), Artemisia annua (middle) and Carum carvi (right) volatile oils.

The release experiments show a slow release for *Carum carvi* and *Artemisia annua* volatile oils and a slightly higher release for *Eugenia caryophyllata* volatile oil. Still, the release is around 10% in the case of *Artemisia annua* and *Carum carvi* and about 18% in the case of *Eugenia caryophyllata*, according to the calibration data, for a time period of 140 minutes. The release experiments show a significant change related to the profile of non-functionalized MCM-41. We consider that this change in behavior is related to the stronger connection between DMDCS functionalized MCM-41 and the volatile oil, than in the normal case of the MCM-41. This is also strengthened by the high linearity of the release profile, and the linear dependence of the released concentration on the measured absorbance.

CONCLUSIONS

The slow release profile of volatile oils make the DMDCS functionalized MCM-41 material a good candidate for applications involving slower releases. The change in release rate induced by the stronger binding of the volatile oil components to the slightly more reactive chlorine atom in the DMDCS compared to the siloxanic groups of the MCM-41 material can be exploited in various applications, such as the development of polymer protective films for the food industry. MCM-41 is a highly versatile material, easy to synthesize and relatively cheap, since the development of the silicon compounds made those types of materials a great deal more inexpensive that they were. In future studies, we aim to integrate this type of material in skin applied bandages containing a thin layer of MCM-41 material. The thin layered MCM-41 material could be loaded with active compounds, for the beginning such volatile oils, to be then released at a controlled rate into the skin. Thus, future studies could integrate the present one in respect to the controlled release of volatile oils. Volatile oils could be integrated in non-toxic bio-degradable polymers which could be a very good alternative to non-degradable plastic coatings presently used. This alternative could prove a cost effective, green and marketable solution for the plastic films used today.

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