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Performance of GC-MS and MOSES II, a hybrid modular sensor system, for the quantitative detection of the evaporation of the insect repellent *N,N*-diethyl-*m*-toluamide from two different matrices

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The purpose of this work was to determine the evaporated amount of *N*,*N*-diethyl-*m*-toluamide (DEET), a commonly used insect repellent, from two different matrices medium chain triglycerides (MCT) and Polyethylene glycol 400 (PEG), with GC with mass sensitive detection (GC-MS) and a hybrid modular sensor system (MOSES II). Thus, the binary mixtures are equilibrated at 80 °C for 45 min and then an aliquot of this headspace is analyzed by GC-MS and a sensory system consisting of an array of eight metal oxide sensors and eight quartz crystal microbalances. Both analytical methods allow a sensitive detection of even small amounts of DEET in the headspace from binary mixtures with the MCT or PEG, respectively. The two additives are found to be very different concerning their behavior to the vapor phase; i.e., the lipophilic (MCT) delivers an almost constant headspace of DEET, while the hydrophilic matrix PEG releases smaller amounts of DEET, which continuously decrease when a multiple headspace extraction is performed. Furthermore, the results reveal that both analytical methods lead to comparable results with the MCT/DEET mixtures whereas differences were seen with mixtures containing PEG. This can be attributed to the interaction of volatile portions of PEG with the sensors of the MOSES II.

1. Introduction

The efficiency of an insect repellent strongly depends on its evaporation rate from the skin. To determine this efficiency, laboratory screening tests are usually performed. Examples include the mosquito test either at mans forearm or suitable animals such as guinea-pigs or laboratory rabbits. For further details see the résumé of screening methods compiled by Skinner and Johnson [1]. These tests have the advantages of an *in vivo* method but the disadvantage of great individual differences and of great expense. Hence, there is still a need for an analytical approach to determine the evaporation of an insect repellent from different matrices especially in the area of pharmaceutical pre-formulation. Several analytical approaches were used in the past to determine the degree of evaporated DEET:

- In the early sixties Smith et al. [2] determined the amount of DEET evaporated from a matrix, skin or clothes and also taken along with an air stream. The DEET-containing air stream was then purged through a solvent and the amount of DEET trapped in the solvent was determined by UV-spectroscopy.
- Gabel et al. [3] compared the evaporation rate of five repellents by thermogravimetric analysis. The results showed a linear mass loss over a period of 60 h. However the total mass loss after 100 h was very low (2.26% of the beginning dosage). Evidently, the measurement of a mass-loss was not an adequate method to characterize the appearance of odor.
- Spencer et al. developed an apparatus to evaluate the evaporated amount of insect repellents in 1976 [4]. Like most of the following methods, it was based on the determination of ^{14}C-labeled DEET in a headspace volume by counting enriched samples at certain time intervals (e.g. hourly) in a scintillation counter. This method is expensive because of the demands on working with radioactive materials. The same analytical set up was used to compare some polymer based formulations of DEET and was used for an *in vivo* study [5, 6].
- Reifenrath and Robinson described an evaporation — penetration cell [7], which allows the simultaneous measurement of the penetrated and evaporated amount of DEET. The amount of evaporated DEET is monitored again by counting radioactive signals [8, 9]. Milutinovic et al. determined the evaporated amount of DEET in this cell by GC-MS [9].

Although not complete, this brief survey of examples illustrates that each method has shortcomings. Especially UV-spectroscopy and thermogravimetric analysis do not show an adequate detection threshold. At present, GC-MS is a well accepted analytical approach for the quantification of a variety of chemicals with a very low limit of detection [10]. Alternatively, over the past few years the technology of "electronic noses" has been developed. These chemical sensors are able to detect volatile organic compounds or other gaseous chemicals at great sensitivity. An electronic nose is an instrument, which comprises an array of chemical sensors with partial selectivity and an appropriate pattern-recognition system, capable of recognizing simple or complex odors [11]. Not only chemical sensors but also optical or other transducer signals have been described [12, 13]. The system used in this study is a commercially available instrument; MOSES II [14, 15], which has been used for a variety of applications [16, 17]. It consists of eight quartz crystal microbalances and eight metal oxide sensors. Quartz crystal microbalances (QMB) are mass sensitive, they change their resonance frequency if an analyte is absorbed into the bulk of the sensitive material. The sensor signal is a change in resonance frequency. Metal oxide sensors are semiconductors, which change their resistance after an oxidation or reduction reaction with the analyte at their surface. The sensor signal is a change in electrical conductivity.

The aim of this study was to compare the performance of GC-MS and a modular sensor system for detecting the evaporated amount of DEET from a hydrophilic (polyethylene glycol 400) and a lipophilic (medium chain triglycerides) matrix. Therefore, we assessed wether a corre-

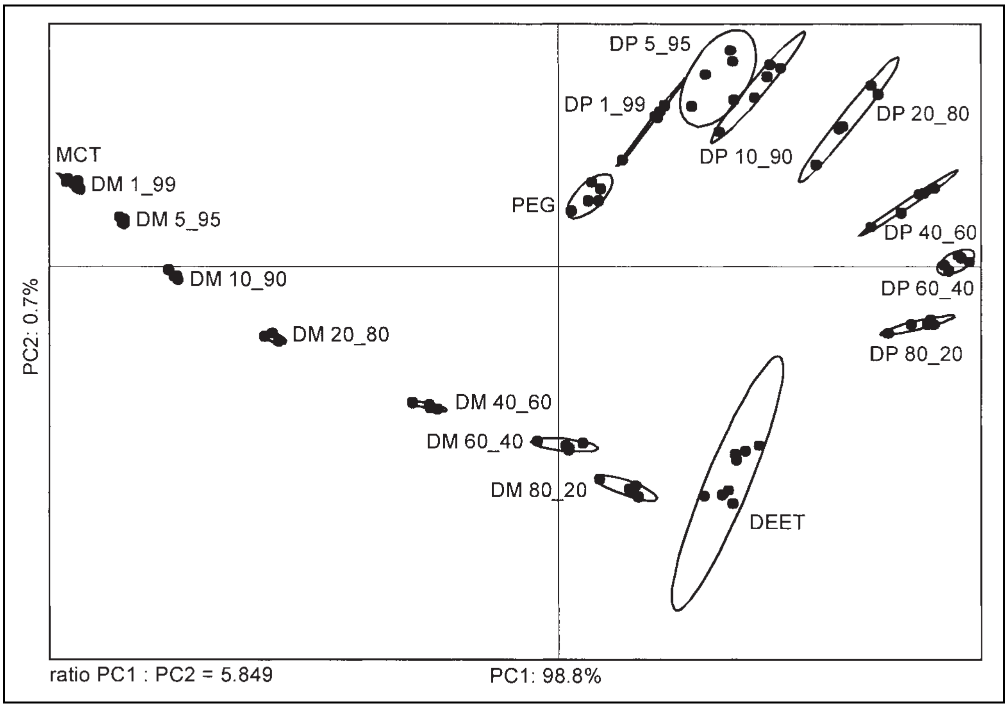

Fig. 1:
Scores plot of the binary mixtures of DEET in MCT and PEG

lation between the concentration of DEET and the sensor signal of the QMBs and the metal oxide sensors exists. In addition, a multiple headspace extraction was carried out. The multiple headspace extraction was developed by Kolb [18] for an analysis of a static headspace irrespectively of matrix effects. We used this approach to establish wether the DEET concentration in the headspace diminishes when DEET is withdrawn repeatedly.

2. Investigations, results and discussion

2.1. Linearity of the sensor signal

As a prerequisite for further evaluations, the linearity of the sensor signal was checked in the concentration range of 0% DEET (= 100% Additive) to 100% DEET (= 0% Additive) with special emphasis on mixtures containing small amounts of DEET (1%). The PCA-Plot in Fig. 1 shows a clear separation for all concentrations (even for the small amounts of DEET). Nevertheless, the two additives behave totally different. For the MCT series the data are highly reproducible over the entire concentration range, while in the PEG series at high PEG concentrations a certain scattering is observed. This scattering increases up to a concentration of 60% PEG and may be attributed to the non-linear response of the individual sensors.

A representative example in Fig. 2 shows the sensor signal of one selected metal oxide sensor for both concentration series. The sensor response is clearly related to the DEET concentration for the MCT series. In this series DEET is the only volatile compound. The affinity of MCT to the sensors is very low and the intensity of the signal is only related to the concentration of DEET. The affinity of PEG to the sensors is totally different. It gives a high sensor signal of its own. In the PEG-series, there is an increasing signal up to 20% DEET, which can be attributed to an increasing DEET-content, and then plateaus up to 80% DEET. An additional DEET-content of more than 20% has no further effect on the sensor signal. This indi-

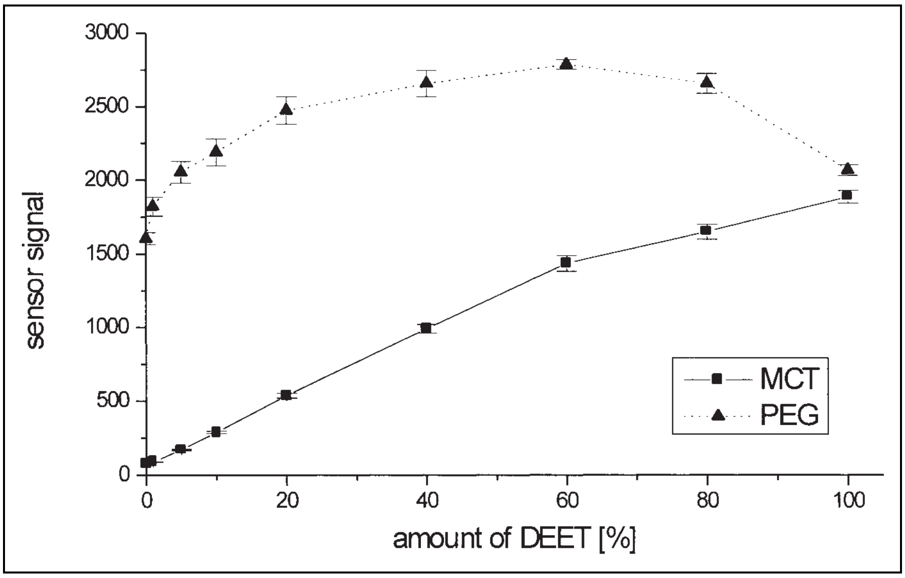

Fig. 2:
Signal of one selected metal oxide sensor for the concentration series of DEET in MCT (solid line) and PEG (dotted line) (mean ± SD, n = 4 for MCT, n = 5 for PEG)

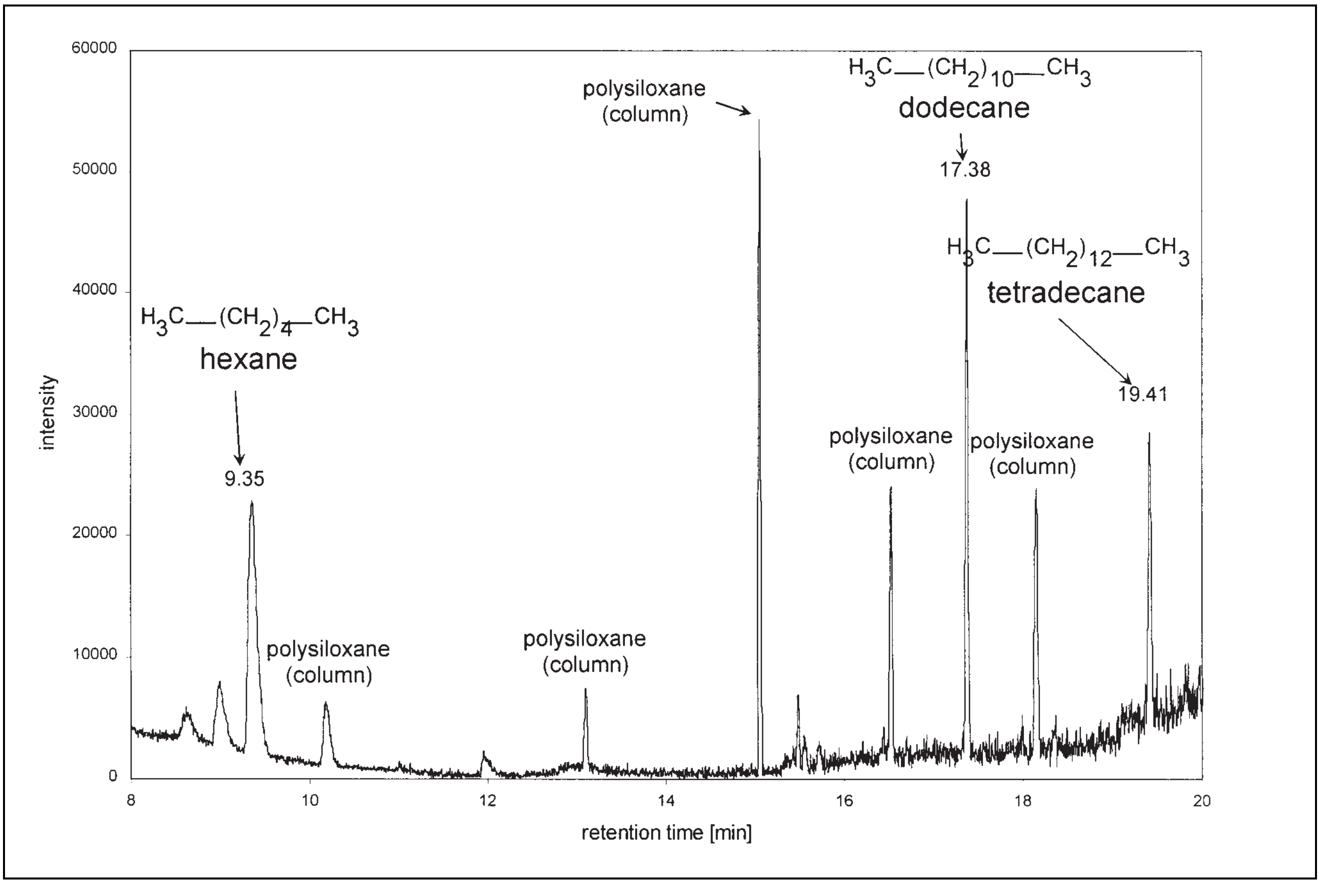

Fig. 3: Gas chromatogram of polyethylene glycol 400. Besides several polysiloxanes three main by-products from the synthesis can be detected and identified

cates that in the concentration range of 20 to 80% DEET (= 80 to 20% PEG) the sensor signal is determined by both substances, DEET and PEG. The use of only one sensor signal provides no further information in this concentration range. In PCA-Analysis, the signal pattern of all eight sensors are used and a separation between the concentration steps becomes possible (Fig. 2).

To obtain more information, PEG is analysed with GC/MS. Fig. 3 shows a typical chromatogram.

The GC-MS chromatogram also shows by-products. Fig. 3 shows hexane (retention time 9.35 min), dodecane (retention time 17.38 min) and tetradecane (retention time 19.41 min). These volatile impurities as well as the volatile low molecular weight PEGs are responsible for the non-linear behavior of the sensor to increasing DEET concentrations. Thus, it is necessary to use more than one sensors in order to discriminate between the various concentrations of DEET with PEG as an diluent. In this particular application, metal oxide sensors appear superior to polymer based sensors. Due to their lower sensitivity, the mass sensitive QMB sensors cannot separate this concentration series as well as the metal oxide sensors [12, 13].

2.2. *Influence of additives on the evaporation of DEET*

To evaluate the influence of the different additives on the continuous evaporation of the repellent from the two matrices, we carried out a multiple headspace extraction ex-

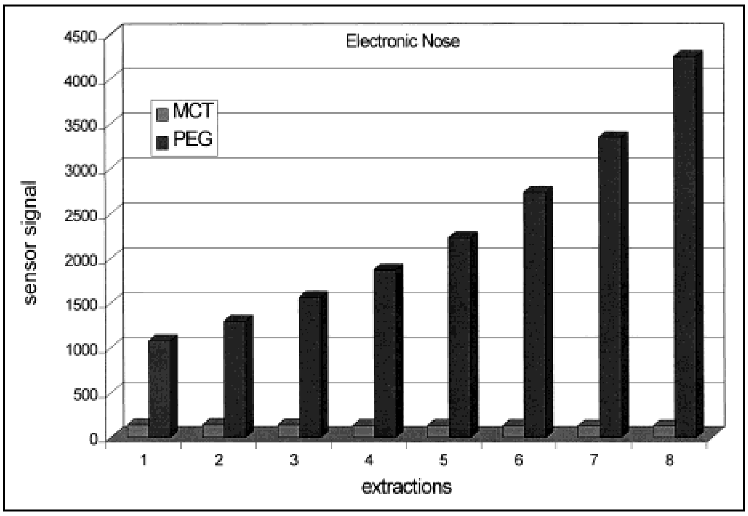

Fig. 4:
Multiple headspace extraction of DEET in MCT and PEG performed by the electronic nose

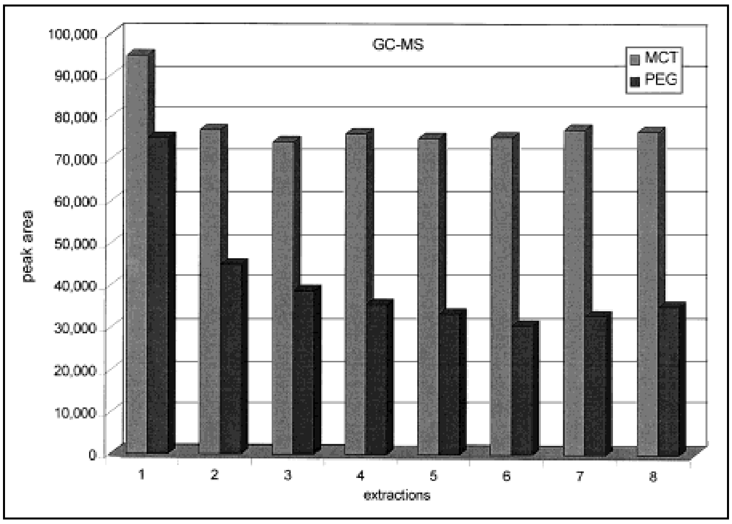

Fig. 5:
Multiple headspace extraction of DEET in MCT and PEG performed by the GC-MS

periment with both electronic nose and GC-MS for headspace analysis. The retention times for DEET were similiar for extraction from both matrices (23.5 min). Figs. 4 and 5 show that both methods indicate that the DEET concentration in the vapor phase above the MCT matrix remains almost constant. As previously shown, the contribution of MCT to the sensor signal is very low and DEET is the only volatile compound in this mixture. GC-MS and MOSES II lead to comparable results. The influence of MCT on the release of DEET is characterized by a constant delivery of the repellent into the vapor phase. The influence of PEG is completely different. For this substance, MOSES shows a massive increase in sensor signal which is not detectable with GC-MS. Obviously PEG has a high affinity for the sensors, thus in this case GC-MS is the more superior analytical method. The initial GC-MS signal is following the first measurement lower than the signal obtained from the MCT-mixture. Moreover, it decreases with every extraction cycle until the 5th extraction. Because there is still a great surplus of the liquid sample, i.e. of the DEET/PEG mixture in the vial at the end of the experiment, the fading of DEET in the headspace cannot be attributed to a total loss in DEET. The effect can be explained by a comparatively slow evaporation of DEET from PEG mixtures. Therefore no equilibrium is reached between to successive extractions and steady state is approached after five cycles. In contrast, MCT delivers higher amounts of DEET and the steady state conditions are obtained already after the first extraction cycle.

In conclusion, in this work the performance of GC-MS and MOSES, a hybrid modular sensor system, for the quantitative detection of the evaporation of the insect repellent DEET from a hydrophilic and a lipophilic matrix is described. The modular sensor system is an innovative tool capable of characterizing the headspace formed from DEET-containing mixtures. Even small amounts of DEET (headspace above 1% DEET) are detectable. The results exhibit differences between the two additives. It is found that the lipophilic matrix MCT is non-volatile. On the other hand, the hydrophilic PEG is volatile and has a high affinity to the sensors itself. Furthermore it contains volatile by-products, which can be identified with GC/MS. The sensor signal from DEET-MCT-mixtures is attributed solely to the DEET content over the entire concentration range, whereas the signal of DEET-PEG-mixtures only increases up to 20% DEET. Multiple headspace extraction experiments show that the hydrophilic matrix PEG substantially reduces the amount of DEET found in the vapor phase in comparison to the lipophilic MCT. This indicates a possible use of PEG as an excipient that can provide a sustained release of DEET.

3. Experimental

3.1. Materials

N,N-Diethyltoluamide was purchased from Merck (D-Darmstadt), medium chain triglycerides (MCT) are received from Hüls (D-Witten) and polyethylene glycol 400 (PEG) was obtained from Clariant (D-Gendorf).

3.2. Methods

3.2.1. GC-MS

For comparative reference studies, a GC-MS system (HP GC 6890/MSD 5973) was used. The following equipment and parameters were used in all measurements: The column was a fused silica column, HP VOC, 60 m, ID 0.32 mm. As carrier gas we used helium 6.0 with a flow rate of 1.0 ml/min. The injector temperature was 250 °C, the injection split 1 : 2. The interface was temperated at 280 °C. The samples are measured with the following temperature ramp: 50 °C isotherm for 6 min, 7 °C/min to 250 °C, 250 °C isotherm for 6 min. The Mass sensitive detector (AutoTUNE) has a EM-voltage of 1400 eV. The scan-range was between 20–350 amu. The samples were temperated in a headspace sampling system at 90 °C oven temperature. The sample loop was temperated at 100 °C, the transfer line at 110 °C. The vial pressure was at 0.4 bar.

3.2.2. MOSES II

As described previously [16, 17], the instrumental set up comprises a hybrid modular sensor system MOSES II connected to a HP 7694 headspace autosampler for sample preparation. The automated sample uptake is maintained by an external carrier gas supply (dry synthetic air) through the thermostated injection needle, sample loop, and transfer line to the sensor system. The sample headspace was carried sequentially through the different modules. The sensor modules include eight polymer coated quartz crystal microbalance oscillators (volume of the measurement chamber: $3\ cm^3$) and an array of eight metal oxide semiconductor gas sensors (volume of the measurement chamber: $12\ cm^3$) in a stainless steel measurement chamber. The coatings of the quartz oscillators are side chain modified polysiloxanes and polyetherurethanes. The flow of the carrier gas was maintained at 25 ml/min. Every 30 min a sample headspace was blown over the sensors. Between the measurements, the sensors are purged with carrier gas to ensure a reset to the initial conditions. This leads to a complete removal of all volatile compounds from the sensor system after each measurement.

3.2.3. Sample preparation

Binary mixtures from DEET and the two additives were prepared in the concentration range of 1 to 80%. For preconditioning the samples, the HP headspace sampler was used for both the GC-MS and the MOSES system. Six 22 ml headspace vials (glass) containing each 2 ml of the binary mixtures or of the pure chemicals were allowed to equilibrate for 45 min at 80 °C.

3.2.4. Multiple headspace extraction (MHE)

MHE experiments were performed with binary mixtures consisting of 10% DEET and 90% additive. From these mixtures, 500 mg were filled into 22 ml headspace vials and allowed to equilibrate. The headspace was withdrawn eight times at an interval of 45 min (equilibration temperature of 80 °C between the extraction cycles) and analyzed by GC-MS or MOSES II, respectively.

3.2.5. Data analysis

The response of n sensors produce multivariate data, which depend on the signals of all n sensors for the same composition of analytes. For data evaluation, methods are required to reduce this n-dimensional chemical sensor feature space. The aim of principal component analysis (PCA) is an optimum description of a given data set in a dimension smaller than n, e.g. in a two- or three-dimensional space. In this case direct visualization of similarities and differences are possible. The different coordinates of this reduced space should be uncorrelated or orthogonal, so that each coordinate represents a property (information), which is completely independent of the other properties. One possibility for determining the coordinate system is the determination of eigenvectors and eigenvalues of the matrix of covariance built from the data matrix. In the terminology of PCA the eigenvectors are named principal components [12]. For typical examples in the application of PCA to quantify odors and gases, see [12, 15–17].

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