

## WATER ACTIVITY RESTRICTION BY APPLICATION OF ESSENTIALS OILS

MILOSLAV MILICHOVSKÝ,\* BŘETISLAV ČEŠEK,\* ONDŘEJ MIKALA\*\* and  
JAN GOJNÝ\*

\*University of Pardubice, Faculty of Chemical Technology, Institute of Chemistry and Technology of  
Macromolecular Materials, 95, Studentská, Pardubice 532 10, Czech Republic

\*\*Mondi Štětí a.s., 272, Litoměřická, Štětí 411 08, Czech Republic

✉ Corresponding author: Břetislav Češek, [bretislav.cesek@upce.cz](mailto:bretislav.cesek@upce.cz)

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The antimicrobial activity of essential oils (EO) is mainly connected to the restriction of water distribution in reticular porous systems of bioactive matters, but the whole process is more complex. The reversibility of the EO desorption process in the water vapour environment of porous lignocellulosic materials, such as paper, is mainly controlled by the relative humidity (RH) of the atmosphere. At low RH, the reversibility is low in comparison with that at high RH. The reason of this behaviour is due to different diffusivity of penetrating water molecules at low RH and high RH, thus water diffusivity at low RH is higher and that at high RH is lower than EO diffusivity. During EO desorption process at low RH, e.g. of 49%, non-condensed water molecules spread rapidly through a porous material and the EO molecules do not have the time to escape from the non-uniform pores of lignocellulosic material. In contrast, the desorption process at high RH is more reversible.

**Keywords:** cellulose, humidification, essential oils, absorption reversibility

### INTRODUCTION

The epimolecular structure of biomatter is formed from molecules through micro-bricks, micro-segments, micro-prefabricates (parts of cell wall) *etc.*, supramolecular structures, of oriented (crystalline) and non-oriented (amphoteric) character, organized into a macroscopic hypermolecular porous system, constructed by the “bottom up” mechanism. Meanwhile, usually the activities of other surrounding molecules have an opposite “top down” character, *i.e.* environment molecules enter biomatter through its hypermolecular system. Therefore, knowledge about the influence of essential oils upon the hypermolecular structure of biomatter plays a key role in better understanding the antimicrobial activity of such oils. A typical material with relatively well-defined hypermolecular structure is paper and, respectively, paperboard.

Essential oils (EO) are complex mixtures of natural substances extracted from plants, consisting most often of terpenes and terpenoid derivatives, hydrocarbons, alcohols, aldehydes, ketones and carboxylic acids, which have an important antimicrobial effect. Some EO are used

as a preservative in the food industry, *e.g.* *Thymus vulgaris* (thyme) and *Ocimum basilicum* (basil).<sup>1</sup> In medicine, significant inhibitive effects of oregano essential oil were demonstrated, when applied to *Listeria innocua*, *Staphylococcus aureus* and *Salmonella enteritidis*.<sup>2</sup> The bactericidal effects of EO components isolated from eucalyptus (eucalyptol,  $\alpha$ -pinene, p-cymene and limonene) were detected against *Staphylococcus aureus*.<sup>3</sup> Two types of sage (*Salvia officinalis* and *Salvia triloba*), growing in southern Brazil, have found important applications in natural pharmacy and food industry, due to the bacteriostatic and bactericidal activity of their main components, *e.g.* eucalyptol, camphor, borneol and  $\beta$ -pinene.<sup>4</sup> The use of essential oils has been also reported to protect archival documents (paper, leather *etc.*) and appears to be an environmentally friendly preservation method for books that are stored under unsuitable conditions.<sup>5-16</sup> To better understand the aging principle of cellulose materials, it is necessary to examine the mechanism of their degradation, such as acid

hydrolysis, oxidation, photo-oxidation or microbiological attack.<sup>11-17</sup> On the other hand, new antibacterial cellulosic materials are developed as a means to prevent the spread of disease-causing microorganisms.<sup>18</sup>

Microbial attack on cellulose and lignocellulose materials is mainly dependent on the humidity of the environment and the amount of condensed, *i.e.* absorbed water, in the cellulosic matter – in paper. Experiments have confirmed<sup>19</sup> that the evolution of microbial growth occurs at the relative humidity of the air of about 70-75%. Our accumulated experience has revealed a noticeable antimicrobial effect of EO vapours, only if the air relative humidity is at least 70-75%. However, a noticeable sporicidal effect of EO vapours has been observed in environments with  $RH \leq 15\%$ . All the EOs have approximately the same influence, but with different efficiency, depending upon their composition. It seems that, generally, the substances or groups of substances such as linalyl-acetate, limonene, eucalyptol, citral and ocimene have the highest antimicrobial effect. For this reason, it is beneficial to use a special chromatographic device to separate the EO vapours before their application.<sup>20</sup>

The evolution of the concentration of water and EO vapours condensed in lignocellulosic materials depends on the relative air humidity

(RH) of the environment in which these are placed. The competitive absorption mechanisms for the condensation of water and essential oils in the porous structure of paper have been described in detail by Češek *et al.*<sup>8</sup> As indicated in Figure 1,<sup>8</sup> the absorption capacity of water vapour for different pulp types increases exponentially with the increase of relative air humidity, being influenced to a small extent by pulp quality, and to a high extent by the presence of EO molecules.<sup>8</sup> For example, the presence of EO vapours caused a decrease in the hygroscopic capacity of lignocellulosic materials at  $RH = 75\%$  to the level of that at  $RH = 50\%$ . This is probably the main explanation of the microbicidal effect of all EO vapours.

Additionally, with decreasing environment humidity, living microorganisms have the tendency to turn to their non-active spore state, as minimum water content is constantly present in micropore systems at low relative air humidity ( $RH \leq 20\%$ ). However, as documented in Figure 1 (see curve for pure water), the application of EO vapours suppresses the water content in the micropores practically to zero, which is probably the main reason for the high sporicidal effect of EO vapours applied at low RH ( $RH \leq 15\%$ ) – compare the absorption curves of pure water *vs.* EO in water environment.

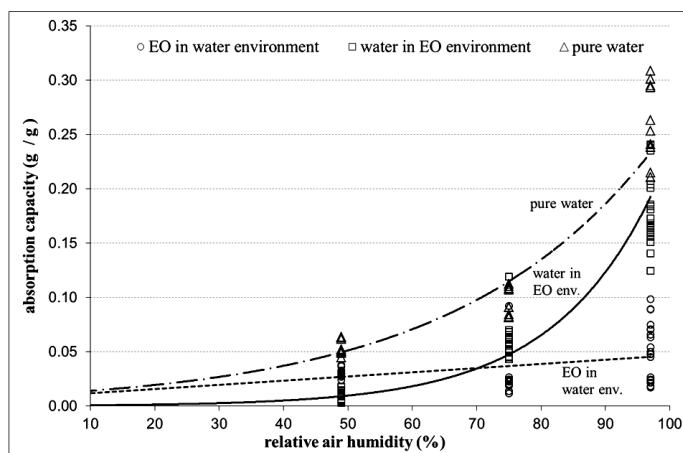


Figure 1: Absorption capacity (at 49%, 75% and 97% RH) of EO and water,  $Y_e$  (g/g of dried pulp substance) *vs.* relative air humidity (%); the data are presented for 4 types of lignocellulosic materials and 6 types of EO vapours (Češek *et al.*<sup>8</sup>)

It is well known that paper, as a typical cellulosic or lignocellulosic material, is a substrate prone to microbial attack, but bacterial growth requires a moist environment to grow well. Otherwise, a “false”, or preventive,

antibacterial effect will be obtained. The present study aims to better understand all the processes of mutual interaction and competition between water and a few chosen essential oils with high antibacterial activities in the hypermolecular

matrix of cellulosic matter. Obtaining valuable information about these processes would enable the evaluation of the kinetics of the absorption-desorption processes and their reversibility.

## THEORETICAL BACKGROUND

### Water vapour influence upon reversibility of EO absorption in porous lignocellulosic materials

It was demonstrated that the kinetics of paper products moistening,<sup>21,22</sup> or one-component vapour absorption of organic liquids,<sup>23</sup> is satisfactory described by the following, originally empirical, later theoretically derived and experimentally verified Equation (1):<sup>24</sup>

$$\Delta Y = \frac{at^d}{b + t^d} \quad (1)$$

where  $\Delta Y (= Y - Y_i)$  is the increment of moisture content (mass of liquid per unit mass of dry solid, w/w), given as the difference between current moisture content,  $Y$ , and initial moisture content at the start of the experiment,  $Y_i$ ; by the use of sample moisture content  $y$  (w/w) according to the relation  $Y = y/(1-y)$ ;  $t$  is the duration (days) of sample storage at a given relative air humidity;  $d$  characterises the rate of steady-state adjustment; ( $a = \Delta Y_e$ ) is the hygroscopicity or absorption capacity of the porous material and  $b$  ( $\text{day}^d$ ) is a parameter related to the mobility of diffusing molecules by the following equation:

$$b = x_{ra}^2 \varphi / (d D_{ap} \Delta Y_e) \quad (1a)$$

The steady-state moisture or steady-state absorption capacity of a porous flat sample,  $Y_e$ , is given as  $Y_e = a + Yi$ . If  $d > 1$  or  $d < 1$ , the porous web material with the thickness,  $2x_{ra}$ , has a stratified structure with poor uniformity, where vapour diffusion takes place more slowly or rapidly than in an ideal web porous material, with increased sample uniformity. Additionally, it is possible to remark that, if  $d \leq 1$ , the humidification kinetic curve has a monotonic ascending character and if  $d > 1$ , the humidification kinetic curve has a typical S character, with the inflection point being moved to higher time values with increased  $d$  values.

The apparent or pseudo-diffusion coefficient calculated as:

$$D_{ap} = D_{ap}^1 / d \quad (1b)$$

where:

$$D_{ap}^1 = \varphi x_{ra}^2 / b \Delta Y_e \quad (1c)$$

is hence proportional to a given relative air humidity,  $\varphi$ . For better comparison of diffusivity results, we have relativized these coefficients with the apparent diffusion coefficient of pure water under comparable conditions according to Equation (2) defined as  $D_{rel} = D_{ap}/D_{ap(H_2O \text{ at } 49\%)}$ , *i.e.* the ratio of  $D_{ap}$  of water or EO vapours during a common absorption process at given relative air humidity (RH) to the apparent diffusion coefficient of pure water at RH of 49%,  $D_{ap(H_2O \text{ at } 49\%)}$ .

$$D_{rel} = (b \cdot a \cdot d)_{\text{pure water vapour at RH } 49\%} / (b \cdot a \cdot d)_{\text{water or EO vapours at given RH}} \quad (2)$$

The process of vapour condensation, *i.e.* vapour absorption, in a porous web material takes place by diffusion of the vapour from the surrounding atmosphere into the pores of the porous flat medium, followed by quick condensation in suitable pores. Suitable pores are all the pores whose size is characterized by the so-called equivalent cylindrical pore radius  $r \leq r(\varphi)$ . The value of maximal equivalent cylindrical pore radius,  $r(\varphi)$ , occupied by the condensed liquid, *e.g.*, water in the pore material at constant temperature and relative humidity, is expressed by the well-known Kelvin equation.

If vapour concentration of absorbed molecules in the surrounding atmosphere, *i.e.* the partial tension of the sorbing component, decreases under equilibrium, an inverse desorption process is evoked. Supposing the reverse process, *i.e.* desorption, is taking place according to a similar mechanism to that of sorption, but only in the reverse mode, a weight increment monitored gravimetrically during sorption experiments is then written generally in the form of Equation (3). Supposing the behaviour of multicomponent EO vapour to be similar to that of one-component vapour of water, the EO absorption-desorption kinetics can be hereby expressed by Equation (3):

$$\Delta Y = \frac{a_{(H_2O)} t^d}{b_{(H_2O)} + t^d} - \frac{a_{(EO)} t^d}{b_{(EO)} + t^d} \quad (3)$$

where  $a_{(H_2O)}$ ,  $a_{(EO)}$  and  $b_{(H_2O)}$ ,  $b_{(EO)}$  are the absorption capacities and mobility coefficients of water or EO component absorbed or desorbed in the porous materials, respectively.

The typical absorption kinetics of water and EO vapours is described similarly, but with the plus sign before the second summand of Equation (3). The reversibility, respectively irreversibility, of sorption processes is then calculated as the difference between the absorption capacity

$a_{(EO)absorption}$  achieved during the typical absorption process and  $a_{(EO)desorption}$  of the typical desorption process under comparable conditions. If the difference is zero, the sorption process is fully reversible, otherwise it is irreversible.

## EXPERIMENTAL

An environment was modelled with different RH values and with the presence of a selection of essential oil vapours, with the best demonstrated microbicidal (bactericidal, fungicidal and sporicidal) effects;<sup>25,26</sup> as well as microporous materials, *i.e.* paperboard sheets with different hydrophilicity. During the experiments, the weight increment of all the condensed components was monitored gravimetrically. The experimental conditions were limited by safety requirements regarding the vapour diffusion, so the tests were performed in a glass weighing bottle, without air circulation.

### Lignocellulosic materials and essential oils

Paperboard sheets (grammage  $400 \pm 15 \text{ g m}^{-2}$ ) were prepared with the following composition:<sup>8</sup> DM (virgin groundwood – high-yield lignocellulosic pulp) consisting of 90% groundwood (beating degree according to Schopper-Riegler (SR) equal to 60) and 10% of MgBi-sulphite spruce bleached pulp (SR 25); Sa J (bleached sulphate softwood pulp – low-yield lignocellulosic pulp), SR 25; Sa L (bleached sulphate hardwood pulp – low-yield lignocellulosic pulp), SR 25; Si (MgBi-sulphite spruce bleached pulp – low-yield lignocellulosic pulp), SR 25. After defibration and beating in a standardized Valley laboratory beater, the sheets were formed according to the ISO 5269-2 standard.

A method of molecular groping of pores was used to characterize the hypermolecular structure and surface-molecular properties of the pore interface of the paperboard samples. The method is based on controlled humidification and vapour absorption of selected well-defined organic substances (toluene and methyl-ethyl ketone) in chosen paperboards.<sup>22,23</sup> Before the start of the experiments, all the samples were stored for 10 days in a desiccator filled with dry silica gel, at room temperature ( $23 \pm 1 \text{ }^\circ\text{C}$ ). At least three values measured were averaged to give a mean value, which was reported in this paper.

According to previous results,<sup>8</sup> papers prepared from bleached pulps are more hydrophilic in comparison with those containing predominantly groundwood, *i.e.* typical lignocellulosic fibre. The porosities of paperboard, composed of groundwood, bleached sulphate softwood pulp, sulphate hardwood pulp and sulphite spruce pulp were 71.6, 59.29, 63.2 and 55.2%, but the effective mean pore sizes were 2.3, 6.8, 4.9 and 4.7  $\mu\text{m}$ , with pore size distribution of 3.4, 26, 10 and 12  $\mu\text{m}$ , respectively. Compared with paperboards from bleached pulps, the one containing

groundwood is distinguished by its higher porosity, smaller pore size and pore size distribution. The differences between the bleached sulphate and sulphite pulps, as well as between long-fibre and short-fibre pulps, are small.<sup>8</sup>

The surface-molecular pore interface parameters characterised by adhesion tension of pore interface with water, surface tension of pore interface with air (including non-polar and polar parts of this tension) and other surface-molecular parameters of the used lignocellulosic materials are presented in Češek *et al.*<sup>8</sup>

The following essential oils were selected: lavender (*Lavandula angustifolia*), lime (*Citrus aurantifolia*), myrtle (*Myrtus communis*), cinnamon (*Cinnamomum zeylanicum*), juniper (*Juniperus communis*) and citronella (*Cymbopogon nardus*). The essential oils were purchased from Essentials Company (Mladá Boleslav, Czech Republic). The chemical composition of these essential oils was determined at the Institute of Analytical Chemistry of the Czech Academy of Science (Brno, Czech Republic).

All the samples of these oils were diluted in n-heptane and analysed by the GC-MS method.<sup>27</sup> All six essential oils contained 1-3 main components that presented 67-91 wt% of the total mass of the respective essential oil. The amount of other components was much lower (less than 5 wt%). Eucalyptol and  $\alpha$ -pinene were found as the main components of *Myrtus communis* (35.7 wt% and 35.3 wt%, respectively). The compound  $\alpha$ -pinene was identified as the primary component of *Juniperus communis* (54.1 wt%). Limonene (51.4 wt%) and citronellal (40.4 wt%) were the main components of *Citrus aurantifolia* and *Cymbopogon nardus*, respectively. Cinnamaldehyde was the principal component of *Cinnamomum zeylanicum* (62.0 wt%) and linalool of *Lavandula angustifolia* (51.4 wt%). The antimicrobial activity analysis of these EO is described in detail in Křůmal *et al.*<sup>27</sup> The purchased concentrated oils were not added into the medium, but dripped on open Petri dishes in amounts of 2 mL of EO per desiccator.

### Methods

The time dependence of mutually competitive absorption and desorption of water and essential oil vapours by porous lignocellulosic materials was measured by the gravimetric method.<sup>8</sup> All the measurements were carried out in a constant climate room at a temperature of  $23 \pm 1 \text{ }^\circ\text{C}$  and relative air humidity of  $50 \pm 5\%$ , with paper strip samples previously stored for 10 days in a desiccator filled with dry silica gel. The strips with a constant width of 15 mm were cut from the paperboard and were placed in a weighing bottle cell, with a lid, which was closed before weighing. The samples were then exposed to the influence of water and EO vapours. At the bottom of the glass desiccator, an evaporating dish with 2 mL of EO was inserted, as well as a container with a solution of salt corresponding to the desired relative

humidity of 49%, 75% and 97%, achieved by means of a saturated solution of  $\text{KNO}_2$ ,  $\text{NaCl}$  and  $\text{K}_2\text{SO}_4$ , respectively. As a reference, an experiment was realised only with pure water atmosphere at the same relative humidity (RH) of 49%, 75% and 97%, respectively. Triplicate samples were assayed.

### Irreversibility of sorption processes

Paper strips with the width of 15 mm were put in weighing bottle cells with open lids, which were closed before weighing, and were deposited in a desiccator to stabilize the humidity of the paper strips at a relative air humidity of 49%. This curing of the paper strips before the measurements lasted for at least 10 days. After this, the paper strips were inserted into the desiccator in the presence of the selected EO on a Petri dish in an amount of 2 mL for twelve days. Last, the proper absorption-desorption gravimetric measurements were realised as usual in the desiccator, with pure water atmosphere at 49%, 75% and 97% relative humidity. The characteristic kinetic measurements performed with lavender vapours are presented in Figures 2 and 3. The other essential oils had similar profiles.

At least two measured values were averaged to give a mean value, which was reported in this paper. All the data were evaluated using the theoretical relations (1) and (3) (e.g. in Figs. 2 and 3). Then, the absorption capacities were evaluated by Equation (3) for both water and essential oil (EO) components, specifically  $a_{49(\text{EO})}$ ,  $a_{75(\text{EO})}$  and  $a_{97(\text{EO})}$ , i.e. absorption capacities of EO vapours at 49%, 75% and 97% RH, respectively, and both diffusion parameters (Eq. (2)):  $D_{2\text{rel}(\text{H}_2\text{O})}$  – relative diffusion coefficient of water (compared with the apparent diffusion coefficient of pure water under comparable conditions, i.e. RH and the type of EO and paperboard); and  $D_{1\text{rel}(\text{EO})}$  – relative diffusion coefficient of EO (compared with the apparent diffusion coefficient of pure water under comparable

conditions, i.e. RH and the type of EO and paperboard).

## RESULTS AND DISCUSSION

Considering the obtained results for the paperboards, it is possible to formulate a simple model for a non-structuralised fragment of lignocellulosic material, composed of effective cylindrical and conical pores, to describe its behaviour in environments with different RH (Fig. 4). The simplified theoretical model simulating the experimental findings (Fig. 4) shows that, in contrast to the penetration of liquid water, it essentially never occurs for water vapours to fulfil the pores completely, and a considerable portion of the pores (~75%), in particular the largest ones remain unfilled. The reason for this is not only the different size, character and shape of the pores, but also their different adhesiveness to the vapours and other components that make up the air molecules, characterized by the appropriate adhesive tension,  $\sigma_T$ . The walls of the pore may be qualitatively homogeneous, but consist of micro- and nanosites with positive (adhesion to vapours and cause condensation to inclination) and negative (the molecules are from the pores, cracks, etc., pushed) adhesion. This shows that a positive value of the adhesion tension to the water bonding lignocellulosic materials is not too high (maximum 40 mN/m for highly refined cellulose materials, but usually of 10 mN/m), and is dependent on the content of lignin (lignin as a hydrophobic component –the adhesion tension decreases), the size of the pores (with a decrease of size, the adhesion tension increases) and the shape of the pores.<sup>8,9</sup>

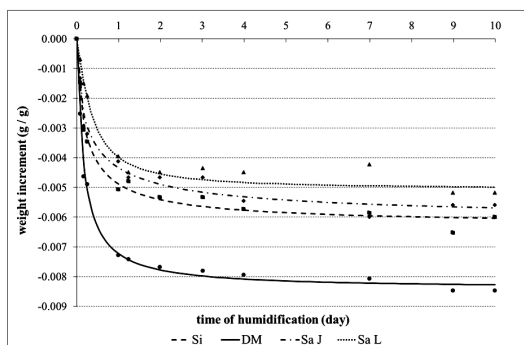


Figure 2: Weight increment during a typical humidification and lavender vapour desorption process in paper webs of different composition (relative air humidity = 49%)

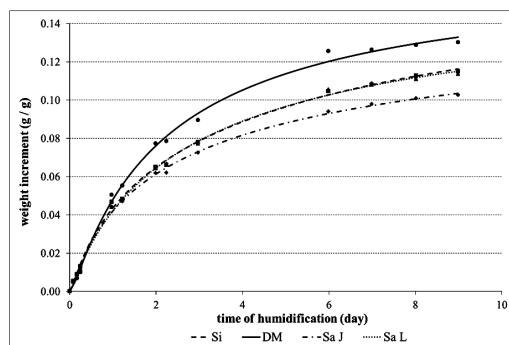


Figure 3: Weight increment during a typical humidification and lavender vapour desorption process in paper webs of different composition (relative air humidity = 97%)

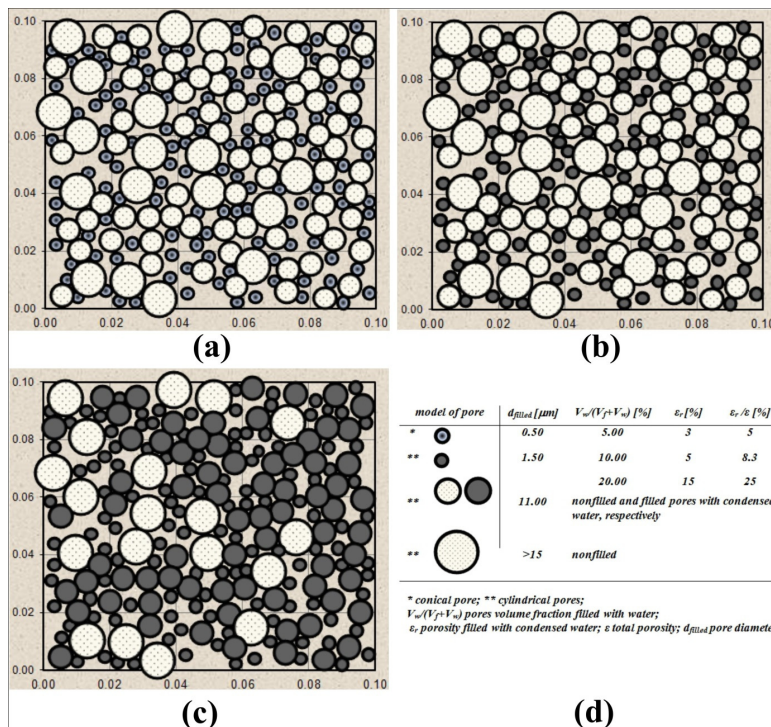


Figure 4: Theoretical model of a porous material; outline of a horizontal cut through porous matter of plane A = 0.1x0.1 mm and total porosity  $\epsilon = 60\%$ , filled with condensed water ( $\epsilon_r$ ) at (a) RH = 50% – only separated micropores with condensed water, (b) RH = 75% – discontinuous clusters of condensed water, and (c) RH = 97% – continuous cluster of condensed water; (d) explanatory note to the picture

It is logical and has been confirmed by experiments that the evolution of microbial growth occurs only if there is a blending of the individual islands with condensed water in the whole of microcellular matter. In addition, unlike non-polar, hydrophobic molecules, EO's affinity and respectively adhesion to water is dependent on the hydrophilicity of the walls of the pore. The pore walls with increasing hydrophilicity raise the adhesion tension to water. In theory (Fig. 4), it can be concluded, and experiments have confirmed it,<sup>20</sup> that the first contact of the pores filled with water up to the blending of individual islets with condensed water occurs only after exceeding the relative humidity of the air environment of about 70-75%.

As experimental results have confirmed, the absorption-desorption process itself is given by the condensation of water vapour and the evaporation of EO molecules in the appropriate pores and its kinetics is very well described by the theoretical equation (3) (Figs. 2 and 3). As indicated in Figure 2, at low RH, a weight increment decrease is typical during common humidification of paper strips with EO, because

the evaporation of EO from the paper pores predominates, in contrast with no or small water vapour condensation. However, at high RH (see Fig. 3), a weight increment increase is typical during common humidification of paper strips with EO, because water vapour condensation predominates, prior to EO vapour evaporation. Due to this, using Equation 3, it is possible to calculate the absorption capacities of both water and EO vapours, and so to determine the reversibility of the absorption-desorption processes. Moreover, it is not a problem to obtain these parameters for a typical absorption of water and EO vapours. Compared with pure water, the absorption capacity of EO in a porous non-structuralised material, such as paper, is not constant at different RH, and with increasing RH, it increases as well, but at a relatively smaller extent. Further, the absorption capacity of water vapour is markedly higher for the absorption from pure humid atmosphere, compared with a polluted environment, e.g. by EO. Moreover, it was observed as well that water absorption in a pure water vapour environment exceeds the sum of absorbed components in a more complex

atmosphere including water vapour (Fig. 1). Logically, the absorption capacity of water vapour for different pulp types increases exponentially with the increase of relative humidity, being influenced to a small extent by pulp quality and the presence of EO molecules. It is worth noting

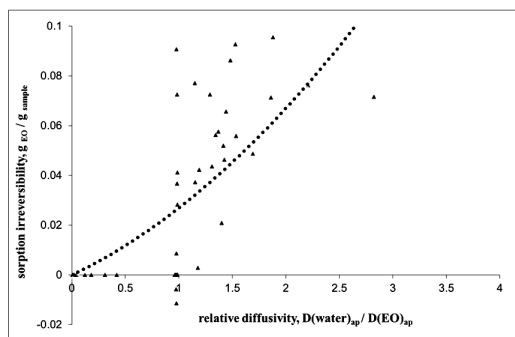


Figure 5: Relationship between the relative diffusivity of water vapour (the ratio of apparent diffusion coefficients of water and EO vapours at comparable RH) and sorption irreversibility of EO vapours

Obviously, as has been also described in Češek *et al.*,<sup>8</sup> with increasing water absorption accompanied by a dramatic decrease in its diffusivity, with and without the presence of EO vapours, a preferred diffusion of EO vapour molecules is evoked, followed by their diffusion in appropriate, not yet occupied pores. Additionally, due to following massive stepwise water condensation at RH 97% in the pores occupied by the EO vapour molecules, we have to expect a possible partial capture of these molecules in the hollow non-permeable cavities of the pores. Though the total absorbed amount of all the components is lower in comparison with that of clean water, the process is then continued not only by a decrease of the water absorption capacity, but controversially, even by a small increase of EO absorption capacity. As a result of this behaviour, the relative absorption increase of EO vapour is accompanied by a relative decrease of its diffusivity (see Fig. 5).

By the application of EO vapours, water is reversibly removed from the paper and cardboard, when the relative air humidity is below RH = 50%. At higher RH, an increase of the total absorbed mass occurs because of common condensation of EO and water vapours. This means that EO occupies additional suitable places

this behaviour at low RH (<20%), where in common water and EO atmosphere, the EO molecules completely excluded the water molecules from the micro-porous matter.

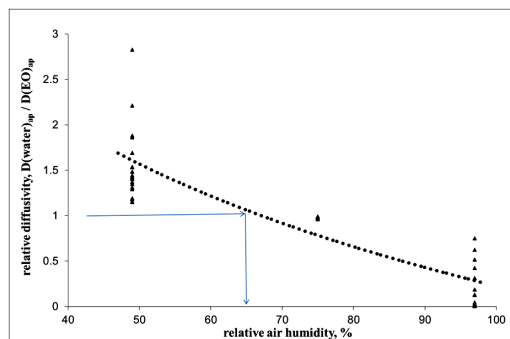


Figure 6: Dependence of relative diffusivity of water vapour (the ratio of apparent diffusion coefficients of water and EO vapours under comparable conditions, *i.e.* RH) upon the relative humidity of the environment

(pores) in the porous matter, which are normally unoccupied and water displacement does not take place. The paper moisture reduction due to EO vapour influence is not, therefore, such as when the common mode of current moistening in the EO vapour environment is generated, where the sum of the relative weight of condensed water and EO even no exceeds the absorption of clean water (see Fig. 1), and in particular, when the displacement of EO by water vapour exists at high RH. Upon EO vapours failure, the EO are expelled with water vapour by the reversible process if RH is higher than 65% (see Fig. 6). As a rule, the molecules of a substance slowly permeating porous mass crowd out those which permeate quickly. This behaviour is then related to the reversibility of absorption-desorption processes. Due to water vapour, with the growth of the permeation velocity of EO vapours through the porous environment of lignocellulosic materials relative to water vapour, the reversibility of their release increases. *Vice versa*, with the growth of the relative permeation velocity of water vapour, the displacement of water vapour from the porous environment is increased due to EO vapours, *i.e.* the reversibility of water displacement increases. This reality is evidenced also by the irreversibility of these

processes (see Fig. 5). It means that if the ratio of the apparent diffusion coefficient of water vapour and essential oils increases (*i.e.* the relative diffusivity of water vapour), the sorption irreversibility of EO vapours also increases.

However, because the relative diffusivity of water vapour is dependent on the relative humidity (RH) of the atmosphere (see Fig. 6), the irreversibility of EO release decreases with an increase of RH, and *vice versa* for water, in this case, it is increased with the growth of RH. Additionally, as follows from Figures 5 and 6, the permeation of water vapour, in comparison with the vapour of EO, is faster when the RH is lower of about 65% and *vice versa*. The rate of displacement of EO from lignocellulosic porous materials (the reversibility of the sorption-desorption process) rises with the growth of air RH and becomes complete when the air relative humidity reaches 65%.

In contrast to EO, the rate of water expulsion from the porous lignocellulosic materials rises with decreasing air RH, but it is usually not complete. This is due to the increasing affinity of the smallest pores to water, characterised by adhesion tension, which increases with a decrease in pore size and further it is dependent on surface hydrophilicity of the pore walls and exceeding the adhesion tension of more hydrophobic liquids, represented by EO.

From a practical point of view, in order to achieve maximal microbicidal and sporocidal effects, it is reasonable to dose EO vapours at RH of 65-75% and lower to about 20%, respectively. There are a lot of devices dosing EO vapours, with different design and control units, but based on a common principle – dosing only mixtures of vapours evolved from EO liquids. Recently, a static EO vapour releaser has been developed, which is provided with a “chromatographic” unit, enabling to separate the EO vapours.<sup>19</sup> For example, this EO releaser can separate linalool and linalyl-acetate from vapours of lavender EO.

## CONCLUSION

Experimental observations have confirmed that the antimicrobial activity of EO vapours is mainly due to their ability to expel condensed water from nano- and micropores of biomatter. The reversibility or irreversibility of the EO desorption process in the water vapour environment of porous lignocellulosic materials is mainly controlled by the relative humidity (RH) of the atmosphere. The reversibility of the EO

absorption-desorption process is increased with an increase of RH and is completely fulfilled at 97% RH. The absorption, *i.e.* condensation, process of water and EO substances is predominantly controlled by the extent of condensation of diffusing water and EO molecules, with marginal influence of the porous lignocellulosic matter and type of EO. As a rule, with an increase of absorption, the diffusion is depressed, and *vice versa*. Due to this, the diffusion of water molecules through a porous system is usually slower at higher RH, in comparison with that of other molecules. It was shown that at low RH, *e.g.* at 49% RH, due to the rapid spread of non-condensed water molecules through a porous material, the EO molecules do not have enough time to escape from the pores of the lignocellulosic material, and in contrast, the desorption process at high RH is more reversible. An explanation of this behaviour has been proposed, based on the formation of a stratified structure of fulfilled non-uniform pores, composed of water and EO condensed liquids and their vapours. During the following desorption process, *i.e.* a cleaning of this porous material with quick removal of the pure water vapour atmosphere of 49% RH, the absorbed EO molecules hidden in big pores are not released, because the relatively highly mobile water vapours do not allow both the condensed or vapour forms of EO to escape, in contrast with the cleaning process at 97% of RH.

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