STUDIES ON THE DECONTAMINATION OF SURFACES EXPOSED TO CYTOTOXIC DRUGS

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Introduction

It is known a broad spectrum of decontamination techniques for biological and chemical contaminants from different surfaces and new physical and chemical processes continue to be developed[1]. The main reasons for development of new methods for decontamination are both dangerous properties of some decontamination agents, and possible hazardous residues formed after their application. An optimal chemical decontamination agent should demonstrate high-level disinfection properties, ability to remove and/or breakdown of biochemical and chemical contamination, high activity, material compatibility, lack of toxicity to humans and the environment, odorless, non-staining, unrestricted disposal, prolonged reuse life and shelf life, easy to use, ability to be monitored for concentration, cost-effective, and may be validated[2]. **V**apor **P**hase **H**ydrogen **P**eroxide (VPHP) seems to be one of the favorable methods that fit the above-mentioned remarks.[3]

VPHP represents a new technology, which was widely proved as an appropriate method for decontamination of rooms. The attacks on many cities revealed the difficulty of decontaminating buildings that had been exposed to biological weapons. The well-known effects of mold on allergies and asthma are also creating demand for improved building air quality. VPHP has become the method of choice for many bio-decontamination requirements in the pharmaceutical, biomedical and healthcare sectors. VPHP is used to treat pharmaceutical manufacturing clean rooms and laboratory toxicology rooms.

There is rising interest in application of VPHP for decontamination of chemical warfare and biologically active compounds. But there is only limited information about influence of VPHP on chemical substances.[4] The aim of this study was to examine the removal and deactivation of chemical substances from surfaces. Because VPHP is still „frontier“ area of research, one of the goal was to explain basics of this decontamination process and study parameters that play important role in this process (temperature, relative humidity, concentration of gaseous hydrogen peroxide, synergistic effect of UV radiation[5]).

Using mass spectrometry (GC/MS, LC/MS) and NMR were studied decomposition products of chemical substances. Furthermore, using HPLC with ESI-MS/MS or GC/MS were monitoring kinetics of decontamination.

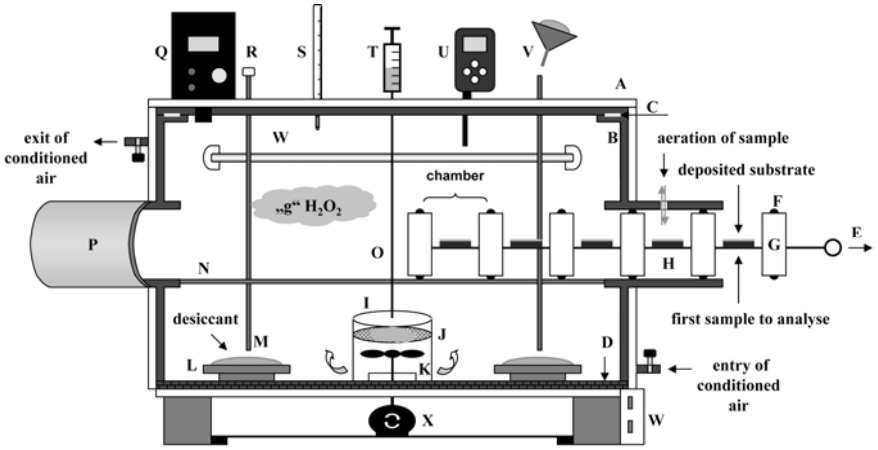
Experimental

Experimental equipment

Appropriate unique, sophisticated equipment, called “peroxybox”, was developed for laboratory tests of VPHP for chemical substances decontamination (Fig. 1). It allows to do controlled decontamination of chemical substances by VPHP under various parameters (temperature, hydrogen peroxide concentration, relative humidity) and monitor the kinetics of decontamination of organic substances. Furthermore, it is possible to study the synergistic effect of VPHP with UV (wavelenght 254 nm) radiation too.

Decontamination procedure

The typical VPHP decontamination cycle consists of four phases in this peroxybox, namely: dehumidification, conditioning, decontamination and aeration. The tested compound is deposited on a microscopic slide and placed in the holder. The holder is inserted into the peroxybox, where is left for a defined period in the atmosphere of VPHP. The holder can be pushed out segment-by-segment without disrupting the atmosphere in the peroxybox to follow the kinetics of decomposition. The microscopic slide is afterwards washed with appropriate solvent and studied by GC/MS, and LC/MS or is evaporated to dryness and reconstituted in deuterated solvent for NMR analysis.



A thermal isolation	**J evaporation surface**	**R plug** (polypropylen)
B body of peroxybox (glass)	(stainless steel net with cotton or glass fibres)	**S thermometer**
C seal of cover (silicon)	**K fan** (PVC)	**T injektor of aqueouse H_2O_2**
D heating surface	**L grid at dessicant** (stainless steel)	(polypropylen syringe or membrane pump)
E holder (stainless steel)	**M transporter of desiccant** (glass)	**U hygrometer**
F o-ring (viton)	**N rail guide** (glass)	**V loader of desiccant**
G seal of chamber (teflon)	**O cannula** (teflon)	**W UV lamp**
H microscopic slide (glass)	**P compensatory bag** (PVC)	**X motor of fan**
I evaporation stand (glass)	**Q sonda of VPHP concentration**	**Y electric source**

Figure 1: The model of the peroxybox.

LC/MS

HPLC system consisted of dual pump Varian ProStar 210, degasser and autosampler Varian 410 (Varian Inc, USA) connected to a triple quadrupole mass spectrometer Varian 1200L (Varian Inc., USA).

GC/MS

It was used Varian Saturn 2000 (Varian Inc, USA) with ion trap allowing tandem mass spectrometry.

NMR

NMR spectra were measured on a Varian ^{UNITY}*Inova*-400 spectrometer (399.89 MHz and 100.55 MHz, respectively) and Varian Mercury 300 (300.07 MHz, and 75.47 MHz, respectively). Residual signal of solvent was used as an internal standard. ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, ROESY, gHSQC, and gHMBC spectra were measured using standard manufacturers' software (Varian Inc., Palo Alto, U.S.A.). 1D NMR spectra were zero filled to fourfold data points and multiplied by window function (for ^{1}H NMR with two-parameter double-exponential Lorentz-Gauss function, for ^{13}C NMR was applied line broadening 1 Hz) before Fourier transformation to improve resolution.

Results and discussion

The initial tests of the peroxybox proceeded on a series of substituted benzaldehydes to get basic information about decontamination process. The aldehydic group is very sensitive to VPHP but decontamination process is sensitive to substitutents of the benzene ring. The introduction of hydroxyl group led to change in the degradation pathway. The aldehydic group was not only oxidized to a carboxylic acid but also replaced by hydroxyl group. Substitution by halogens like chlorine and bromine prevented any transformation. Vanillin (4-hydroxy-3-methoxybenzaldehyde), which was chosen as the model substrate for next experiments, for it's stability on air, was decomposed in relatively short time and could be easily monitored by gas chromatography. The major primary degradation products of degradation of vanillin by VPHP are shown in Fig. 2.

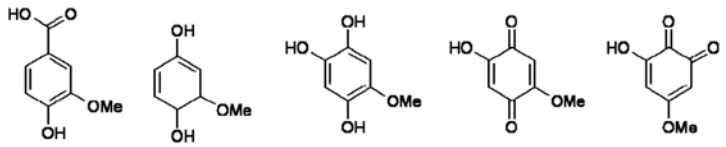


Figure 2: The major primary products of degradation of vanillin by VPHP.

The Fig. 3 demostrates the degradation of vanillin under different conditions. The curves (a)-(c) represent the attempts that were provided in the same operating mode (constant - temperature, and initial relative humidity). It was approved the synergistic effect UV with VPHP, which is related to radical formation which are stronger oxidation agents than hydrogen peroxide. Application of desiccant during the degradation process has negative influence on the degradation rate. It seems that the microcondensation has a positive influence on the course of degradation process. The „dry" VPHP degradation process (Fig. 3c - with desiccant) had a very long inductive period (2 hours) after which relatively fast degradation followed. This behavior can be associated with the formation of an optimal relative humidity or definite value of microcondensation.

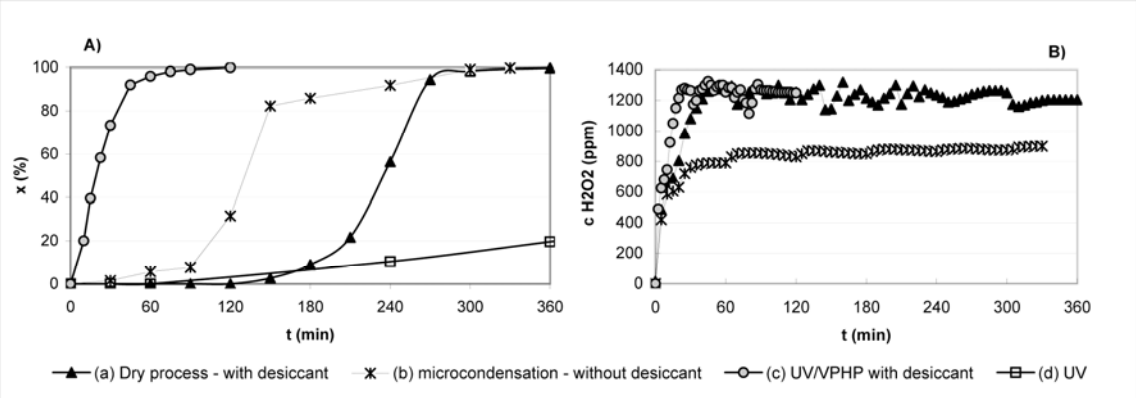


Figure 3: Degradation of vanillin by VPHP under the different conditions: A) the dependence of conversion of vanillin on time, B) time path of VPHP concentration.

Fig. 4 demonstrates degradations of vanillin (dry process – with desiccant) that were provided by different values of initial relative humidity. The higher the initial humidity is the higher is the initial reaction rate.

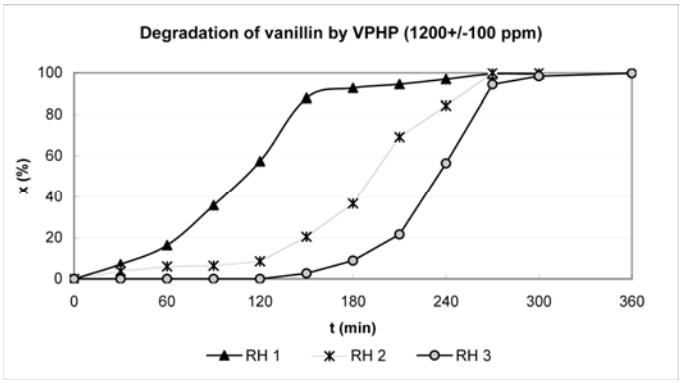


Figure 4: Degradation of vanillin by VPHP under different initial relative humidity

One of the pharmacologically most important groups of indole alkaloids is the ergoline, or ergot alkaloids. These alkaloids are isolated from the dried sclerotium of the

fungus Claviceps purpurea (Hypocreaceae) (ergot). VPHP degradation was tested on lisuride, pergolide, nicergoline, ergotamine and bromocryptine. Ergot alkaloids or their derivatives were possible to decompose by VPHP. Degradation of ergopeptines leads to a cyclol moiety (peptide part of ergopeptines) like only decontamination product. It can be explained by initial N-oxide formation at nitrogen N-6 of ergoline or ergolene moiety. An N-oxide can not be formed in cyclol part of ergopeptines, which further remains unchanged.

It is in good agreement with our experiments on tertiary amines. All compounds contain tertiary aminy were sensitive on VPHP. It was established Cope elimination[6] as an initial mechanism of their decontamination (creation an amine oxide to form an alkene and a hydroxyl amine).

Conclusion

The work was focused on the decontamination by VPHP. It was build the appropriate laboratory equipment for tests of VPHP method for organic compounds. It was tested on a series of benzaldehydes derivatives and ergot alkaloids. The decontamination process was followed by GC/MS, LC/MS, and NMR to get deeper insight into the process. It was possible to determine sensitive functional groups and study the kinetic of degradation under the different conditions. It was revealed substantial structure sensitivity on the course of the decontamination. N-Oxide formation is the most probably the initial step in the ergot-alkaloid decontamination. Ergopeptine decomposition product is the intact cyclol moiety. VPHP seems to be a method of choice for degradation of organic molecule like pharmaceutical substances.

Acknowledgements

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