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3

Homogeneous and Heterogeneous Free-Based Porphyrins Incorporated to Silica Gel as Fluorescent Materials and Visible Light Catalysts Mimic Monooxygenases

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1. Introduction

The use of enzymes and whole cells as biocatalysts in biotechnology and organic chemistry is recognized as being of great economic potential in the increasing production of unique and valuable compounds present in nature in an insufficient amount. Bioconversion with living microbial cells, which have the ability to regenerate their own respective cofactors and exhibit a spectrum of enzymatic activity, is a very useful tool in bioorganic synthesis. There is no doubt that the advantage of microbial biotransformations is the possibility to induce enzymes of defined, desired activity, simply by the suitable preincubation methods (Griffin et al., 2001). However, this kind of biocatalysts has several limitations in application to oxidative biotransformations, including stability and activities, especially to those substrates and products that are toxic and require being performed in reaction media other than water. A disadvantage might be the membrane barrier and diffusion problems. Recent advances in addressing these problems include molecular and reaction engineering approaches (Burton, 2003).

A broad field of activity of biological heme-containing oxygenases like cytochromes P450 has provoked the development of the invention of artificial catalytic systems based on porphyrins mimicking the controlled and selective oxidation reactions of these hemoproteins. The concept of artificial enzymes and biomimetic chemistry was introduced by Breslow in 1972 (Breslow, 1972), that started the field of *bioinspired* or *biomimetic catalysis*, wherein researchers try to copy Nature in designing the catalyst with the structure and properties similar to those of the active sites of enzymes. Such biomimetic systems are expected to work in organic media and have high stability toward toxic organic compounds in comparison with traditional catalysis employing enzymes or microorganisms.

The porphyrins, due to their synthetic versatility and reactivity, are especially attractive in the construction of biomimetic analogues of monooxygenases through elaboration of the

superstructure of the macrocycle or chelate complexes. Firstly, the brief description of the physicochemical properties of porphyrins is given in this chapter together with the main contributions of metalloporphyrin model studies to the cytochromes P450 chemistry and oxidation catalysis. The reactions of terpenes oxidation are singled out. Secondly, we elaborated a matter of porphyrins working as photoactive catalysts in the oxidative transformation of limonene and α-pinene into the products attracting great interest by the fragrance and flavour industry. The effects of substrate concentration, wavelength of visible light during the photochemical excitation, kind of solvent and oxidant on the efficiency of the porphyrin-based homogeneous system was discussed. Finally, two types of porphyrins (water-soluble and water-insoluble) incorporated into the silica gel are described pointing out to the advantages and limitations of the silica matrices prepared by the sol-gel method. Here, the immobilization of porphyrin catalyst in an insoluble organic or inorganic support is also briefly outlined. Among the topics included in this part of the chapter are: the effect of porphyrins immobilization on the changes of their photophysical properties, photodegradation or aggregation, as well as correlation between photoactivity of free-base porphyrins in various organic solutions and those entrapped in silica matrices. The correlation between the biocatalytic efficiency of porphyrins entrapped in the sol-gel matrix and their intensity of fluorescent emission and absorbance was also examined. The reasons for much better photooxidation of α-pinene by OEP/SiO_2 than by HmP/SiO_2 are discussed, and the mechanism of pinene photooxidation based on proton transfer and then on two possible routes namely, electron abstraction by the light-excited OEP/SiO_2 matrix and the photosensitized singlet oxygen generation is proposed. It is worth emphasizing that the systems investigated are new and have not been reported before.

2. The biological monooxygenation. New opportunities and innovations for the improvement biocatalysis efficiency

The use of enzymes, either derived from natural sources or generated through directed evolution methods, has increased significantly over the past decades (Tao & Cornisch, 2002; Wackett, 2004). There are essentially two approaches to choosing a biocatalyst: the screening for novel biocatalysts, and the screening for new activities among the existing biocatalysts. The latter approach also includes the improvement of known biocatalysts through protein engineering, either through molecular biology or direct modification of the protein, and the modification of biocatalyst properties by »media engineering« (Adamczak & Krishna, 2004; Turner, 2009).

Replacing the aqueous milieu with organic solvents, ionic liquids, supercritical fluids, fluoro-hydrocarbons etc. enables biocatalysis to increase the chemo-, regio- and enantio-specificity as well as catalytic efficiency of many enzymes (Castro & Knubovets, 2003). It should be kept in mind that supremely good selectivity of enzymes is weighted against their lack of stability (Thomas & Raja, 2005). As the application of enzymes continues to grow within both academia and industry, it is evident that, in order to satisfy the stringent requirement of industrial processes with respect to catalyst turnover and productivity, protein engineering techniques will be essential to the growing incorporation of this technology into synthetic chemical processes. This requires a profound understanding of protein structure and molecular biology techniques for manipulation of proteins, as well as the computational methods. The rational mutagenesis of amino acids based on a knowledge of structure or mechanism is targeted at specific residues and is hence popularly known as

side-directed mutagenesis. This technique is a good tool for investigating enzyme mechanism or altering catalytic attributes. It may be possible to change the electrostatic environment of an active side, perhaps by changing a positively charged lysine to negatively charged glutamic acid. Of course, some amino acid side chains are more susceptible to damage by oxidation than others, notably those containing sulfur, such as cysteine and methionine. It was postulated that mutating the methionine residue in the active side of subtilisin at position 222 to other residues like alanine, serine and cysteine may decrease susceptibility of the enzyme to inactivation through oxidation (Estell et al., 1985).

As the number of structures of enzymes increases and more is learned about the molecular and structural determination of substrate recognition and transformation by enzymes, there is increasing move towards the computational design for enzyme activities that as yet have no equivalent in natural enzyme chemistry (Arnold, 2001; Rothlisberger et al., 2008). The powerful combination of *in silico* design and directed evolution suggests that protein evolution strategies in future may be credibly targeted towards entirely new enzymes for which no natural enzymes currently exist. Directed evolution involves repeated rounds of (i) random gene library generation, (ii) expression of genes in a suitable host and (iii) screening of libraries of variant enzymes for the property of interest. Both *in vitro* screening–based methods and *in vivo* selection–based methods have been applied to the evolution of enzyme function and properties (Turner, 2009).

Another aspect of diversity that attracts an increasing level of attention within the biocatalysis community is that of catalytic promiscuity. This can loosely be defined as the ability of an enzyme to catalyze a new chemical reaction additional to that to which it is usually attributed. Such reactions of new substrates have been recognized as a valuable research and synthesis tool (Hult & Berglund, 2007). Many examples of catalytic promiscuity exist within the literature (Bornshcheuer & Kaslauskas, 2004). One theory suggests that a low level of promiscuous activity in an enzyme can assist the evolution of that enzyme into one that has optimum levels of activity for new reaction. For example, a phosphotriesterase (PTE) enzyme that catalyses the degradation of the herbicide paraoxon is thought only to have acquired this ability relatively recently, as previously, the pesticide substrate would not have occurred in the environment.

Among enzymes, heme monooxygenases (such as cytochrome P450 and peroxidases) are probably the most versatile biocatalysts because of their capability of catalyzing the regio- and stereoselective transfer of an oxygen atom from O_2 into a vast range of substrates, including monoterpenes. It is one of the most widespread enzymatic activities occurring in all forms of life including both prokaryotes and eukaryotes (Li et al., 2002). Cytochrome P450 was found in a wide range of organisms from bacteria to mammals where it exists in both soluble and membrane-bound states playing at least two key roles. Firstly, it catalyzes many oxidation steps involved in the biosynthesis or biodegradation of endogenous substrates such as steroid hormones, fatty acids or prostaglandins. Secondly, they participate in the crucial step in the oxidative metabolism of exogenous compounds such as drugs and other xenobiotic products, allowing their elimination out from living organism (Mansuy, 1990). The family of these enzymes is involved in the reactions as diverse as e.g. hydroxylation, N-, O- and S-dealkylation, sulphoxidation, epoxidation, deamination, desulphuration, dehalogenation, peroxidation, and N-oxide reduction.

Most monooxygenases are metal-enzymes where metallic complex active center is located in the prosthetic site of the molecule. The active centre of P450 contains the heme prosthetic group (protoporphyrin IX), which is bound to the apoprotein by the coordination to the iron

of the tiolate group of cysteine at the proximal side of porphyrin ring, (and also through salt bridges and hydrogen bonds) (Poulos at al., 1987) **(Fig. 1)**. Unlike P450, a heme in peroxidases (except for chloroperoxidase) is ligated with a proximal histidine residue and uses hydrogen peroxide as an oxidant instead of O_2.

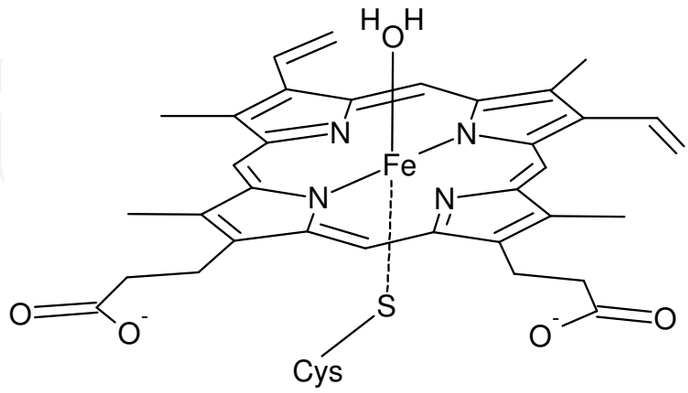


Fig. 1. Protoporphyrin IX. The prosthetic group of cytochrome P450.

One should bear in mind that despite impressive biotechnological impact, the potential of monooxygenases has not been fully exploited. Isolation and purification of the relevant enzymes is difficult and expensive. Moreover, instability and other difficulties associated with using monooxygenases under industrial conditions limit their implementation in applied biocatalysis.

Notwithstanding progress of enzymology and biochemistry which permits to work directly with the proteins themselves, it is still usually difficult to purify the cytochrome P450 dependent enzymes and isolate the corresponding gene using amino acid sequence information for the purified protein. Application of isolated P450-monooxygenases as oxidation biocatalysts is also limited due to the requirement for the costly cofactor NAD(P)H.

Site-directed mutagenesis, directed evolution and chimeragenesis, design of substrate binding cavities, chemical modification of prosthetic groups and covalent attachment of metal cofactors were applied in improving the activities and changing of the substrate specificity of various P450s or in inducing a new reactivity in a non-metal protein (Bell, 2001; Kato et al., 2002; van de Velde et al., 2001; Branco et al., 2008). Despite the fact that some efforts meet with spectacular success, it seems to be still too early for these improved P450 systems to be applied in the industry (Bühler & Schmid, 2004).

3. Porphyrins as monooxygenase mimics in hydrocarbon oxidative biocatalysis under homogeneous conditions

A wide variety of activity of biological heme-containing monoxygenases has inspired the preparation, characterization, and application of artificial catalytic systems resembling the structure as well as the activity of these enzymes. There are two main, strictly connected, objectives in the investigation of biomimetic catalysis: i) insight into the nature of the enzymatic processes and ii) the necessity in both biotechnology and industrial chemistry to obtain more efficient biocatalysts working with high regio- and stereo- selectivity, under mild temperature and pressure (Bartocci et al., 1996). There has been observed growing

interest in the use of metalloporphyrins as biomimetic catalysts in recent years (Que & Tolman, 2008).

Porphyrins belong to the naturally occurring compounds participating in many important biological processes such as: oxygen transport and different catalytical conversions. Porphyrins can be treated as derivatives of porphine, which consists of four pyrrole subunits joined via -CH= bridges. This aromatic macrocycle has 22 π electrons, although according to Hűckel rule, only 18 of them undergo delocalization (4n+2, n=4). The size of the cavity in this macrocycle is suitable for the adaptation of different metal ions. The exchange of two protons in H_2-porphyrin by a metal ion leads to the origination of metalloporphyrin, a complex compound with a binegative macrocyclic anion. The compounds of Fe, Co, Zn and Ni are the best known metaloporphyrins. Hemes (iron complexes) and chlorophylles (magnesium complexes) are the examples of porphyrins having special importance in biological systems, the latter in photosynthesis. In turn, vitamin B_{12} has a cobalt complex in its structure with coryne ligand structurally similar to porphyrin. The well known is the role of B_{12} in catalysis of isomerization reactions of methyl group transfer in biological systems. Hemoproteins (complexes of Fe) serve as oxygen carriers (hemoglobin) or oxygen stores (mioglobin) and participate in: electron transfer processes (cytochrome b and c), activation of O_2 (cytochrome P450). The porphyrins have become an indispensable component in the evolution of living organisms, due to many types of chemical reactions, characteristic of this group of compounds, such as coordination, polymerization, aggregation, oxidation and reduction, catalysis, sorption and photochemical changes.

Several synthetic models based on porphyrins for heme oxidases have been developed in order to understand principles of the complex mechanism of dioxygen activation and oxygen atom transfer processes, and to obtain valuable information on the nature and reactivity of intermediates produced in the catalytic cycles of metalloenzymes in living organisms (Hessenauer-Ilicheva at al., 2007; Dallacosta et al, 2007; Nam, 2007; Suzuki et al., 1999). The extensive investigation of metalloporphyrins catalyzed oxidation reaction has concentrated on using iron (III) and manganese (III) and the role of pyridine and imidazole ligands. The proposed mechanism for the reaction proceeds through the formation of a high valent metal-oxo species, the first model of which was reported by Groves *et al.* in 1981 using the system based on the Fe(III) complex of the *meso*-tetramethylphenylporphyrin Fe(TMP)Cl with peroxyacids (Groves et al., 1981) and later confirmed by iron tetraphenylporphyrinate, Fe(TPP)Cl in the presence of iodosobenzene (PhI=O) as an oxidant or acetic anhydride as a donor of acyl cations (Mandon et al., 1989; Oligario et al., 2002). The mechanistic developments in biomimetic research have been supported by cross-linked investigation (directed mutagenesis, computational methods, physical biochemistry) using a broad array of the spectroscopic techniques. The detailed mechanistic aspects and structural characterization of intermediates are very well described elsewhere (Shteinman, 2001; Watanabe, 2001; Sligar et al., 2005).

Monooxygenases require a metal center (transition metal ions, predominantly iron and copper) and two electrons to reductively cleave atmospheric dioxygen, producing only a single water molecule in the process while saving the second atom for substrate functionalization and formal oxidation:

$$RH + O_2 + 2\bar{e} + H^+ \rightarrow ROH + H_2O$$

In cytochrome P450, the electrons necessary for the reduction of diooxygen are transferred from a cellular reductant NADH. They are delivered directly one by one by the cytochrome P450-reductase, a huge protein which contains two flavin cofactors, FAD and FMN. The role of metal is reduced to the activation of molecular oxygen and decrease in the kinetic barrier to its reaction with hydrocarbons due to the formation of reactive metal-oxygen intermediates.

The commonly accepted catalytic mechanism operating in the P450 monooxygenases involves six steps (Shteinman, 2001; Sligar et al., 2005; Bernhardt, 2006). Catalytic turnover starts with the binding of substrate to the active site with displacement of water coordinated to the open axial ligand position thereby moving the iron (Fe^{III}) to a high-spin ferric manifold, which increases the redox potential of the complex and facilitates the transfer of the first electron forming the ferrous heme (Fe^{II}) that could bind oxygen (Sligar et al., 2005). In the next steps, further reduction and protonation of the resulted oxy-ferrous intermediate leads to the generation of the so called "active oxygen species", ferric peroxoanion, ferric hydroperoxo, high-valent iron-oxo complex (compound I-ferryl porphyrin cation radical), responsible for the hydroxylation of the substrates. The last species was also found in peroxidases and well characterized (Penner-Hahn et al., 1986). It was postulated (by the heme model systems according to the similar assumption for P450) that two different electrophilic oxidants can hydroxylate alkanes in the catalytic cycle of P450. One of them, hydroperoxide, is a predominantly epoxidizing agent, and another oxidant, ferryl, is a predominantly hydroxylating agent (Vaz et al., 1998; Newcomb & Toy, 2000; Watanabe, 2001; Jin et al., 2003), although the density functional theory (DFT) calculations excluded the former intermediate as a reactive, electrophilic oxidant (Groves, 2003).

Despite the increasing knowledge of structure-function relationship and physico-chemistry of monooxygenase function, the question how the formation and ultimate chemical reactivity of the intermediate states of metal, oxygen and substrate is controlled to have an effect on efficient catalytic processing still remains open.

The second reason for the considerable interest in monooxygenases mimetic is connected with the design of the perfect chemical model system for a growing number of synthetic transformations (*e.g.* for oxidation of aliphatic and olefinic hydrocarbons). Such catalyst could be applied in the conversion of petroleum products to valuable commodity chemicals (Groves, 2000), preparation of hydroxylated metabolites of drugs, oxidation of agrochemicals, pollutants, and other xenobiotics (Meunier et al., 2004), as well in the catalytic production of unique and valuable compounds present in nature in an insufficient amount. Thus, one of the most extensively studied groups of compounds in both the enzymatic and biomimetic transformations are terpenes, hydrocarbons derived from the isoprene units, which are the largest class of plant secondary metabolites. Together with terpenoids (oxidized terpenes), terpenes are the main flavour and fragrance impact molecules in the essential oils of higher plants and flowers, however, their natural resources are limited to a few plant growing regions. In the perfumery, as well as in the flavour industry at present, a lot of effort is devoted to the replacement of expensive naturally occurring raw materials or the reproduction of original organoleptic effects by the use of new chemicals. Terpenes have also drawn increasing commercial attention because of their beneficial healthcare effects. Numerous terpenoids have been found to be effective in chemoprevention and chemotherapy of several diseases and to exhibit antimicrobial, antiviral, antihyperglycemic, anti-inflammatory and antiparasitic activities (Paduch et al., 2007). Therefore, a special attention should be paid to introduction of new terpenoid

products in modern therapies. Monoterpenes are known to play an important role in chemical ecology, where they act as pollinator attractants, repellents, sex pheromones, alerting pheromones, antifungal defences, or as part of defense secretion systems against predators (Banthorpe, 1994).

Most of the microbial hydroxylation of terpenes and other exogenous substrates described so far are initiated by cytochrome P450 dependent monooxygenases (Duetz et al., 2003; Bicas at al., 2009). A number of microorganisms have been reported to convert limonene and pinene to notable monoterpenoid compounds (Chatterjee and Bhattacharyya, 2001; Yoo & Day, 2002; Duetz et al., 2003; Adams et al., 2003; Trytek & Fiedurek, 2005; Bicas at al., 2009), yet the amounts obtained (a few milligrams per litre) were insufficient for industrial applications. The drawbacks of this type of biotransformation systems are excess volatility of terpenes, insolubility in aqueous solutions, and their toxicity towards microorganisms (Krings & Berger, 1998).

The development of synthetic metallic complexes that reproduce the activity of P450 enzymes and enable to carry out the reaction (in the case of water insoluble precursors) in organic solvents has been the subject of intense research (Meunier, 1992; Collman et al., 1993; Suslick, 1999; Bernadou & Meunier, 2004; Simões et al., 2006). Metalloporphyrins in homogeneous systems, with the structures based on the well known *meso*-tetraphenylporphyrin (called the first-generation catalyst), are amongst the catalysts commonly studied (Maraval et al., 2002; Guo et al., 2005; Milaeva et al., 2007). The largest bulk of examples has been published for the manganese (III) and iron (III) porphyrins (Traylor & Tsuchiya, 1987; Gonsalves et al. 1991; Mansuy& Fontecave, 1984; Martins et al., 2001; Maraval et al., 2002; Rebelo et al., 2005), but the complexes of porphyrins with Cr (Groves & Krupper, 1979; Schmidt et al., 2005), and Ru(II) (Groves et al., 1997; Murahashi & Komiya, 1998; Simonneaux & Maux, 2002) have also been demonstrated as useful catalysts for the oxygen atom transfer reactions to olefin or alkane-type substrates. A wide variety of oxidative reactions (mainly epoxidation and hydroxylation of the olefins) catalyzed by metalloporphyrins has been studied (Montanari & Casella, 1994; Kadish at al., 2000, Meunier, 2000; Simões et al., 2006), and numerous classes of substrates have been given in transformation including pre- and post-emergence herbicides (Chauhan & Kumari, 2007), amino acids (Mukherjee & Ray, 2007), monoterpenes such as: geraniol, nerol, limonene, α-pinene, α-terpinene, 1,8-cineole, carvacrol, thymol, and cymene (Martins et al., 2001; Maraval et al., 2002, Guo et al., 2005; Simões et al., 2006). In the specific case of terpenes, Skrobot et al. (2003) showed the production of epoxides from these compounds. In the similar work, the oxidation of monoterpenes by hydrogen peroxide catalyzed by porphyrins was also described (Martins et al., 2001).

Almost in all investigations, strong oxidants were used as oxygen atom transfer reagents to metalloporphyrins, such as PhIO, alkylhydroperoxides, peroxyacids, hypochlorites, C_6F_5IO, potassium monopersulfate. Lately, the use of more environmentally benign, H_2O_2 has been preferred. This concept is favoured by the fact that cytochromes P450 (in the absence of NAD(P)H) and peroxidases[1] *in vitro* can also use hydrogen peroxide and other exogenous peroxides to catalyze the hydroxylation of substrates under mild conditions (Yuchun et al., 2001; Trytek & Fiedurek, 2002). The monooxygenation by metalloporphyrins with these oxidants requires the presence of pyridine or 1-methylimidazol, 4-butylpyridine, imidazole,

[1] Though peroxidase forms the ferryl intermediate, it cannot hydroxylate alkanes because of steric hindrance for the attack to the C-H bond by O atom in this enzyme.

or ammonium acetate, which were shown to act both as axial ligands and cocatalyst used to promote the desired heterolytic cleavage of peroxide and also to stabilize the active species Mn(V)=O formed in the oxidation cycle (Meunier, 1992; Gonsalves et al., 1996; Maraval et al., 2002; Susana et al., 2003; Cantonetti et al., 2004). These agents substantially increased the rate of the regio- and stereoselectivity reaction, and their role resembles somewhat the action of the strongly nucleophilic thiolate axial ligand in cyt-P450, which is crucial for heterolytic cleavage of the O-O bond of hydroperoxo intermediate by donating the electron density to this bond (Suzuki at al., 1999; Watanabe, 2000). Cytochromes P450 mimics using O_2 itself as an oxygen atom donor has also been developed. In this case a reducing agent and/or proton source must be used (especially Zn powder, CH_3COOH, acetaldehyde or borohydrides) (Mansuy, 1990, Groves, 2000) and higher degree of organization of the catalyst is required due to competition between the substrate and reducing agent for the active oxidant (Shteinman, 2001).

Models with a tight coordination of the axial ligand are in general not good catalysts as thiolate or porphyrin is rapidly oxidized in the presence of oxidants (Meyer et al., 2005). However, for some porphyrins-based systems, the oxidation reactions usually provide very high conversions of the substrates (often exceeding 90% after a few hours), and even some systems are able to convert olefin to epoxides in a stereospecific manner, and alkanes to the corresponding alcohols and ketones in a good yield. Nevertheless, application of conventional homogeneous systems in the porphyrin catalysis is frequently complicated by the oxidative degradation instability of the metalloporphyrin rings, difficulty of recovering this rather expensive catalyst, and poor degree of regioselectivity. The systems also lead to generation of chemically contaminated effluents that have a great environmental impact.

The stability of porphyrin catalysts was substantially improved by the introduction of electron-withdrawing substituents into the phenyl group of the porphyrins and then by the complete halogenation of the porphyrin ring (second and third generation of porphyrin) **(Fig 2)**. The decreased oxidative degradation of the porphyrin ligand and the increased reactivity of the intermediate were also achieved to some extent when the bulky or chiral groups were attached to the porphyrin periphery (*ortho*-position of the phenyl ring of H_2TPP or *meso*-position of the porphyrin), which provides some steric protection for the active oxygen species preventing from a too fast destruction of the porphyrin ring (Suslick, 1999). In practice, both electronic and steric properties of the substituents are manipulated to improve oxidative robustness of the porphyrins and must also be considered along with the regio- and stereochemical factors (Suslick, 1999; Maraval et al., 2002; Guo et al., 2005).

The two main strategies towards a new generation of more regioselective and efficient artificial hemoproteins have been envisioned. The first exploits the systems with simple sterically hindered porphyrins and shaped-selective catalysts that have a superstructure designed to recognize and bind incoming substrates. As an extension of the basic structure of the porphyrin macrocycle, there has been a multitude of approaches to synthesize porphyrins with a variety of peripheral substituents or even with more than four pyrrole rings, leading to the modification of the macrocyclic ring size, planarity, number of π electrons and aromaticity, and ultimately, the biocatalytic activity. Diverse structures have been created in this fashion (**Fig. 3.)** and more elegant systems including stereoselective substituents are described and shown in greater details in the literature (Kadish et al., 2000).

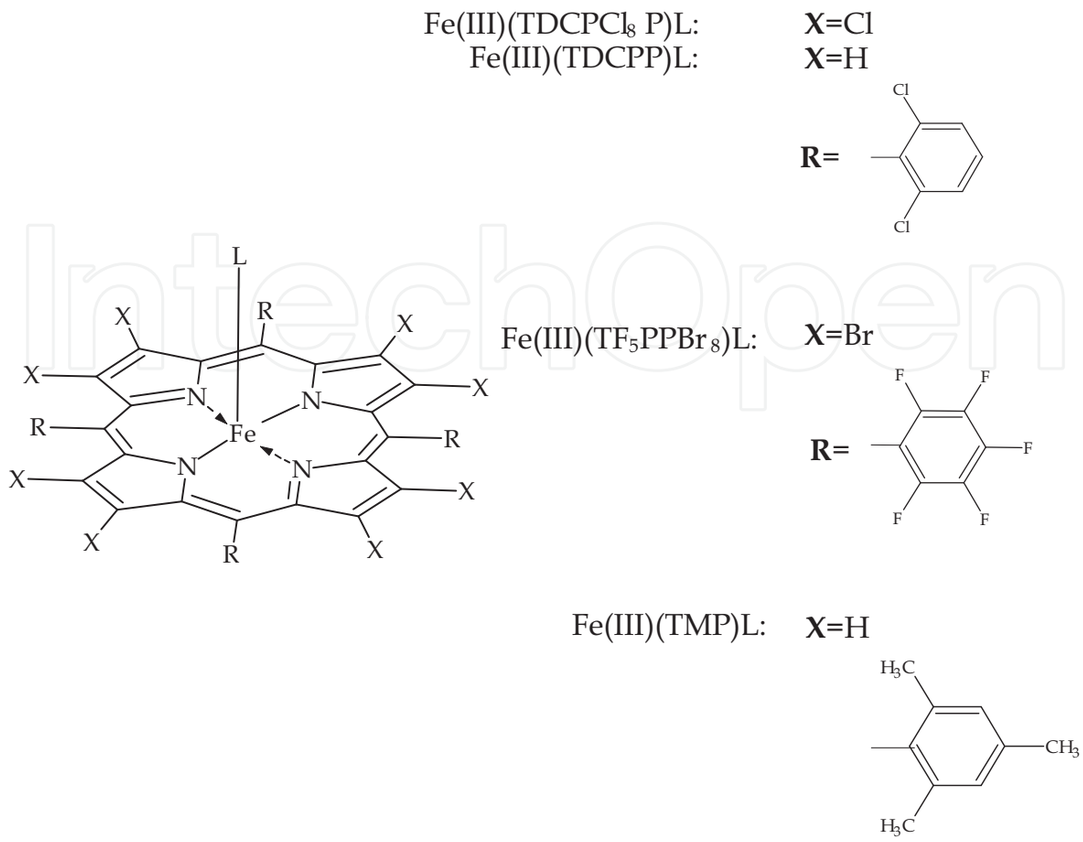


Fig. 2. The second and third generations of porphyrin. Halogenated porphyrins exhibit the increased stability.

The examples of extraordinary compounds which can be used as efficient catalysts in the future are: amino acid functionalized porphyrins (Robertson et al., 1994; Geier. & Sasaki, 1997), porphyrins with the substituents at a pyrrolenic nitrogen atom, the so-called "*N*-substituted porphyrins" (Ito et al., 1996), the porphyrin complexes composed of more than one porphyrin ring, for example "sandwich" or "shish kebab" porphyrins, where several macrocyclic rings are joined by metal ions. The porphyrins are also suitable elements of more complicated systems, which can find application in biomimetic catalysis like that of the larger macrocyles prepared by covalent bonding of porphyrins with hydrophobic cavities (cyclodextrins, cyclophanes) (Hartmman et al., 1999; Feiters et al., 2000; Woggon, 2010), cobaltacarborane-porphyrin and carbohydrate-porphyrin conjugates, carboranylporphyrins, multiporphyrin arrays (e.g. cofacial bisporphyrins) (Chang et al., 2004; Hao, 2007). The porphyrin complexes with other compounds, such as dendrimer metalloporphyrins (Suslick, 1999) as fullerenes (Nierengarten et al., 1998), as well as "expanded" porphyrins, for example texaphyrins (Sessler et al., 1994; Lisowski et al., 1995), "porphyrin wheels" (Schenning et al., 1996), saphyrins, platyrins and others (Jasat & Dolphin, 1997; Lim et al., 2009) seem to be also very promising.

Secondly, supramolecular mimetic approaches with modulation of heterogeneous environment to restrict substrate orientation or access have been used. Many interesting examples of activity of porphyrins and metalloporphyrins in such systems have been reported, for example: amphiphilized metalloporphyrins in the micellar media for

epoxidation of limonene (Mancini et al., 2001; Cantonetti et al., 2004), complex imbedded in a self-assembled lipid or surfactant bilayer (Groves & Neumann, 1989; Feiters et al., 2000), porphyrins appended with β-cyclodextrines (Breslow, 1997; Woggon, 2010), polyethylene glycol-modified hemin (Takahashi et al., 1986), surfactant-heme complex (Kamiya et al., 1997), association of polyoxometalates with porphyrins (Santos et al., 2005), association of monoclonal anti-porphyrin antibodies (so-cold hemoabzymes) with metalloporphyrin cofactor (Nimri & Keinan, 1999; Ricoux et al., 2007). The most promising solution to improve the stability of porphyrin catalysts is to immobilize or encapsulate in a solid, usually inorganic, matrix.

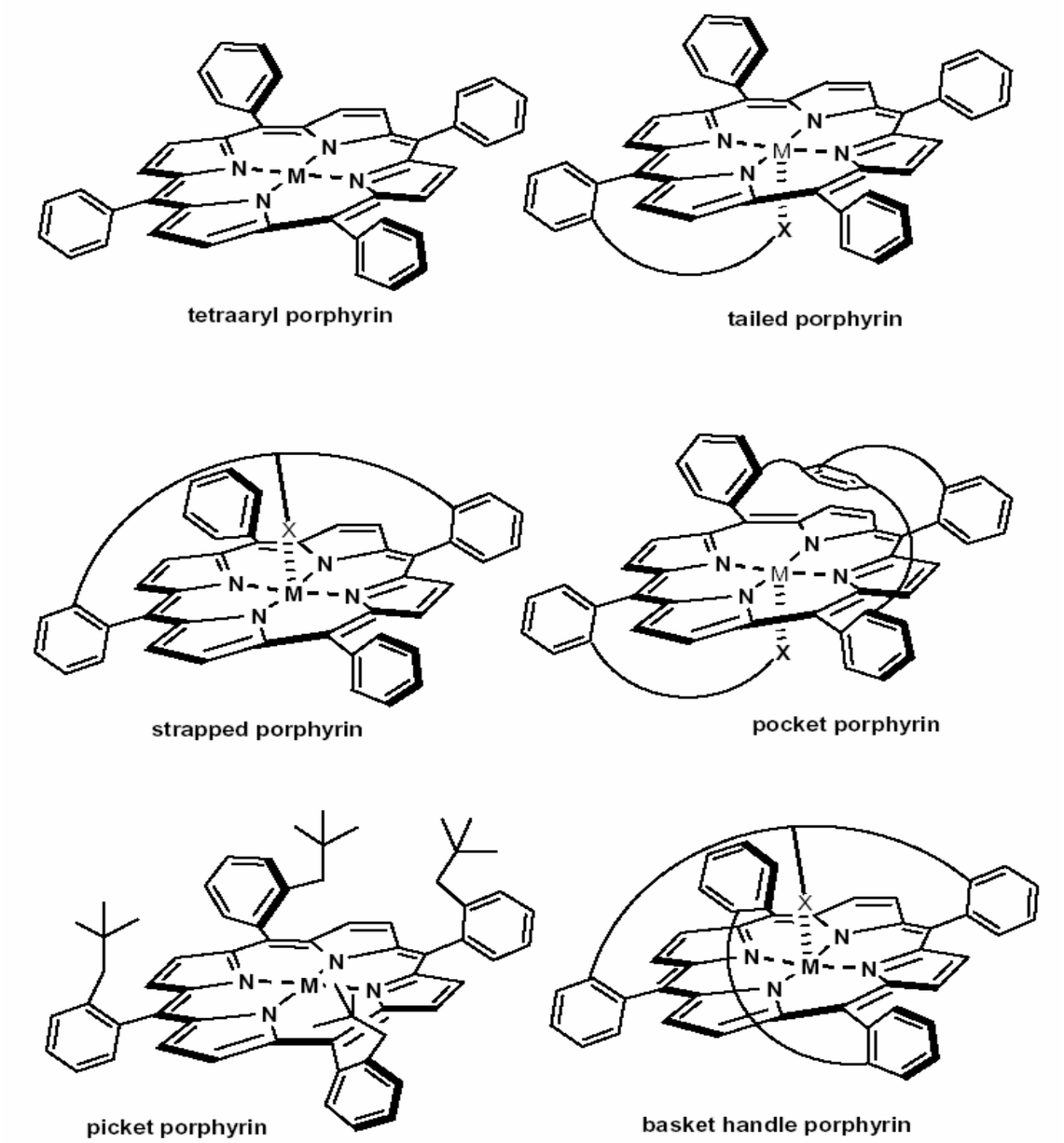


Fig. 3. Examples of the most frequently used porphyrin classes as monooxygenase mimics.

3.1. Porphyrins as photocatalysts for oxidation of organic compounds

The subsequent synthetic method searching for biomimetic monooxygenase analogues is the use of photoactive porphyrins as the sensitizer catalysts for the aerobic oxidation. The conceptually attractive entry for the employment of porphyrins as photocatalysts and photons as the energy source in biomimetic catalysis consists in the fact that in plants and living organisms four crucial prerequisites are found, which favour the photoprocess on bioactive molecules. These criteria are: (I) presence of natural sunlight providing visible spectrum irradiation; (II) good light-absorbing properties and proliferation of photosensitizers (e.g., tannins, porphyrins, and chlorophyll) in the environment; (III) pervasive molecular dioxygen; and, finally, (IV) abundance of oxidizable substrates, such as terpenes, in the immediate vicinity (Margaros et al., 2006).

The role of porphyrins in photocatalysis and photosynthesis processes or in the function of solar cells is related to the intensive absorption of UV-visible light by these compounds. It is known that the colour of many simple complex compounds is the result of electronic transitions with the participation of metal d-electrons. The transitions between d-orbitals are forbidden in respect to symmetry and therefore their intensity is low. However, the porphyrins case is somewhat different, i.e. the absorption spectra of porphyrins are dominated by the strong bands corresponding with the π-π* transitions in the porphyrin ligand. The two kinds of bands are recognized in the absorption spectra of porphyrins: B (Soret band) and Q. According to Gouterman (1961), the essential features of these spectra can be explained based on the model of four molecular orbitals.

The absorption bands in the porphyrin systems result from the electronic transitions between two HOMOs and two LUMOs energy levels. The metal center and the substituents on the ring affect the relative energies of these transitions. Mixing splits these two states of energy, forming a higher energy 1 eu state with larger oscillator strength (Soret band) and a lower energy 1 eu state with smaller oscillator strength (Q-bands). The shape and position of B and Q are closely related to the aggregation of porphyrins, their protonation with the formation of dications or axial ligation, as well as their metallation. The Soret band in the blue and the Q-band in the red are major porphyrin bands, which represent an important component of sunlight.

Peculiar spectral properties and ability of the porphyrins to undergo photoinduced multielectron transfer without changing their structure has opened new fields of their application not only for light-driven biocatalysis but also for application in solar energy conversion, photodegradation of a wide range of organic and inorganic chemicals in air and water, elimination of bacteria, viruses, cancer cells and splitting of water as well as construction of sensing materials to mimic the human nose *(electronic nose*) and photosynthetic systems (Paolesse et al., 2002).

There are a lot of contributions of porphyrin model studies to the photooxidation catalysis in homogeneous systems (Weber et al., 1994; Quici et al., 1993; Maldotti et al., 1996; Funyu et al., 2003). Simple photoexcited Fe^{III}porphyrins were used to induce hydrocarbon oxygenation to the corresponding ketones under aerobic conditions. For example, in pure cyclohexane, high yields of cyclohexanone with small amounts of cyclohexanol as a by-product were obtained (Bartocci at al., 1996). The photo-oxidation of phenol and monochlorophenols by irradiation with visible light in aqueous alkaline solution in the presence of 5,10,15,20-tetrakis(4-carboxy-phenyl)porphyrin and oxygen has been described by Wöchrle and co-workers (Gerdes, et al., 1997). For the visible light-photocatalytic oxidation of hydrocarbons, a bis-iron(III)-l-oxo pacman porphyrin was used (Rosenthal et al., 2006).

Trytek et al. (2007) described a comparative study of photoexcited system involving metal free and metal-containing porphyrin catalysts in the photooxidation of monoterpene, *R*-(+)-limonene using dioxygen from the air dissolved in organic solvents. The largest photocatalytic activity was registered by using metal free porphyrins such as 5,10,15,20-tetraphenylporphyrin (H_2TPP) and hematoporphyrin IX (HmP-IX). Later investigations of light-promoted biooxidation of α-pinene have revealed that octaethylporphine (H_2OEP) and porphyrins chelating a metal ion with a closed-shell electron configuration, especially zinc 5,10,15,20-Tetra (4-pyridyl)-21H,23H-porphine are also very efficient photocatalysts. Pyridyl porphyrin (II) and aryl porphyrin (III) with relatively longer substituents had twice as low catalytic efficiency. Also Zn(II) and Co(II) tetraphenyl porphyrins had lower catalytical properties. A mixture of carvone and another unknown product **(1)** (with a mass spectrum similar to that of verbenone) in the concentrations of up to 3.4 g/L and 6.0 g/L, respectively was obtained from the chloroform solution consisting of catalytic amount of H_2TPP and 90% (v/v) limonene, within the period of 70-hour exposition to the sunlight. The authors have checked the effects of substrate concentration and kind of oxidant on the efficiency of the porphyrin-based homogeneous system, as well as determined some photoexcitation conditions and mechanism for monoterpene transformation. At lower substrate concentration of about 1-5%, and high photoirradiation intensity (>1600 μmol/m²s, where mol is the number of photons), the yield of by-products, especially (1S,4R)-*p*-mentha-2,8-diene 1-hydroperoxide and 1,2-limonene oxide, increases significantly. Substantial amounts of *trans*- and *cis*-carveol, perilla aldehyde, perilla alcohol, and other hydroperoxides were also identified. The productivity of terpenoinds has been improved (about 1.6-fold), when 0.5% (v/v) of H_2O_2 or *tert*-BuOOH was added to the solution. An increase in oxidant concentration caused oxidative degradation of the porphyrin ring under the strong oxidizing conditions.

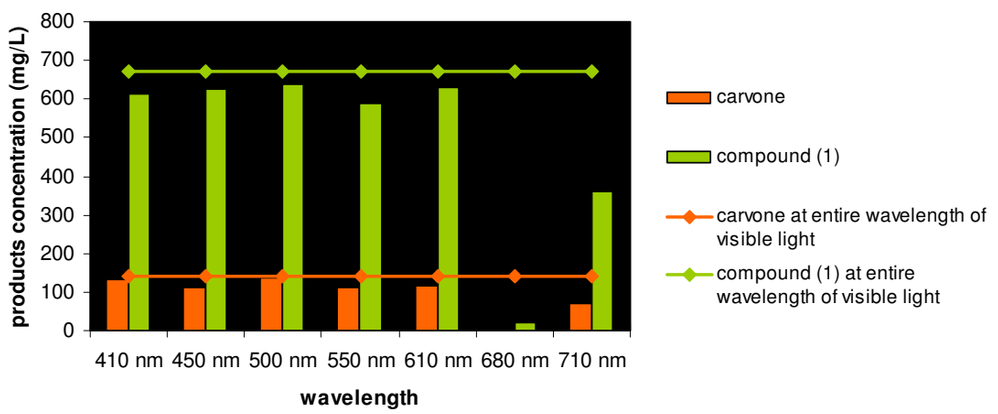


Fig. 4. Effect of wavelengths of visible light on limonene photooxidation with H_2TPP.

It has been shown that the photooxidation of limonene does not depend significantly on the particular wavelengths of visible light. The best yields of products were achieved within the entire wavelength range of the visible light which is profitable from an economic point of view, since the costs of the kind of light in photocatalytic reactions are taken into consideration. However, at 670 nm remarkable decrease in terpenoids accumulation was detected in the course of the photooxidation process **(Fig 4)**. This phenomenon was explained by low absorption coefficient of H_2TPP in this spectral region and the formation

of the protonated porphyrin in the system, which is known to have the enhanced deactivation of the excited state (Chirvony et al., 2000). In fact, it was established based on the absorption spectra of porphyrins recorded before and after photocatalysis **(Fig 5)** that porphyrin in the system with a low concentration of limonene exists in a protonated form of

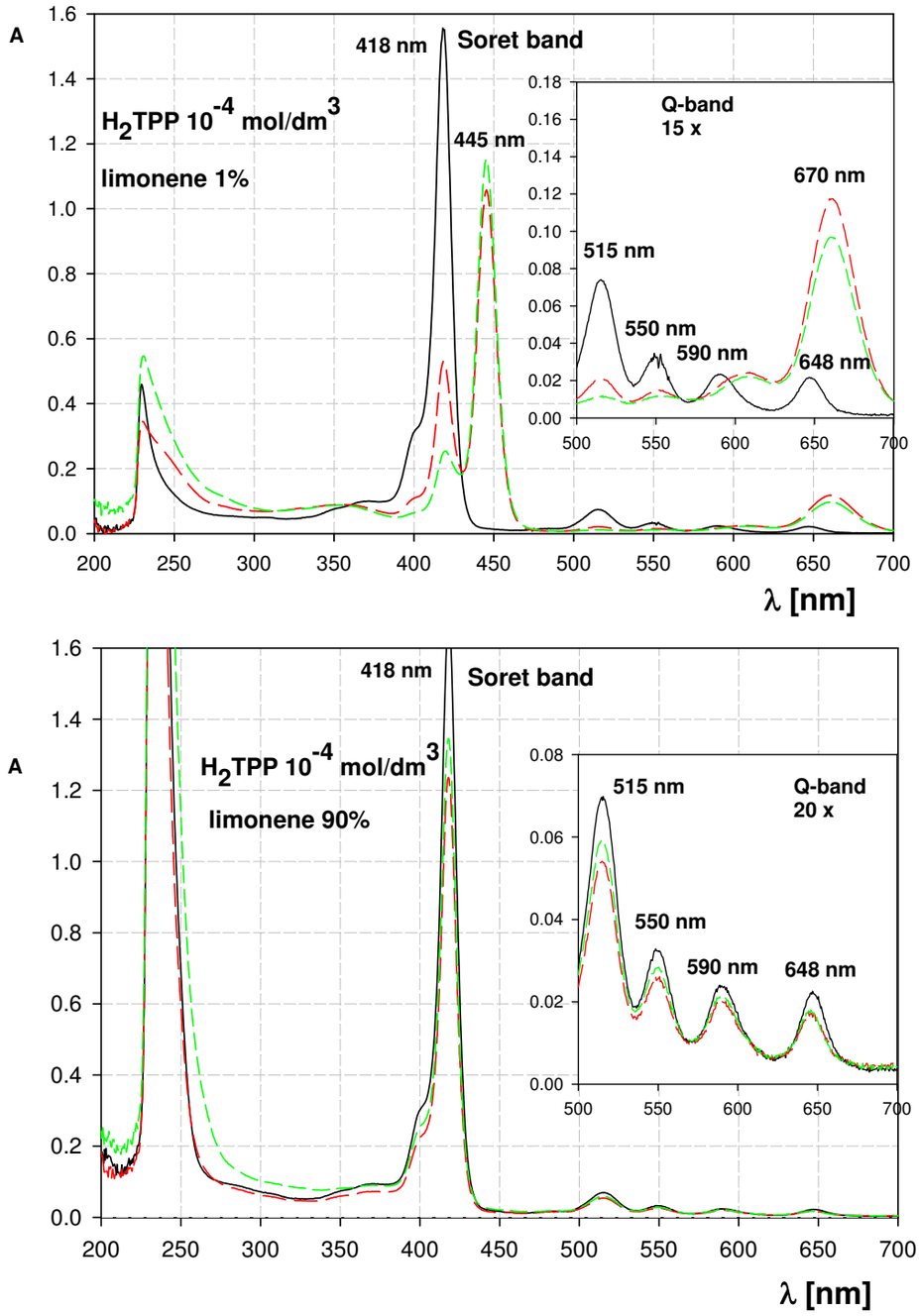


Fig. 5. Absorption spectra of the H_2TPP-limonene system (at two different substrate concentrations) in chloroform; before photoreaction (black solid line), after 36 h (red dash line) and 126 h (green dash line) exposition to the sunlight (Trytek at al., 2007).

dication H_4TPP^{2+}. As a characteristic Q-band was only observed for free-base H_2TPP (500-650 nm) and dication H_4TPP^{2+} (670 nm), this means that the catalytic system is based just on photoexcited porphyrine and the metal contamination is excluded. The porphyrin spectra with a high 90% concentration of limonene during photoexcitation showed that, neither Soret nor Q bands were shifted, and only their intensity was slightly lower after irradiation than before it, which does not indicate that the catalyst is readily decomposed under the applied oxidizing conditions. In this context, it is worth taking into account that the presence of a large excess of readily oxidized substrates will provide kinetic protection against oxidative destruction of the biomimetic catalysts (Suslick, 1999).

The authors concluded that such spectral changes are the evidence for the existence of two different mechanisms of monoterpenes photooxidation by H_2TPP, to the extent extremely dependent on the substrate concentration. For the systems with a lower substrate concentration, a proton accepting mechanism is more important, whereas in the systems with a larger amount of monoterpene, H_2TPP rather plays a role of a photosensitizer and oxidative transformation is most probably based on a free radical chain pathway initiated by photoexcited porphyrin producing singlet oxygen. Singlet oxygen is a powerful electrophile, so the reaction may proceed just as well by electron transfer from the electron rich monoterpenes to it. The common mechanism of action of the sensitizer is rather complex. The absorbed light promotes the excited state of the porphyrin catalyst. The singlet-excited state (S_1) undergoes intersystem crossing to the triplet state (T_1), which, in turn, reacts with oxygen (or first with the substrate) producing singlet oxygen ($^1O_2$), superoxide radical anions ($O^{2\bullet-}$), and hydroxyl radicals (**Fig. 6**). In most of the cases, the concurrent involvement of these reactive oxygen species determines the mechanism of the photooxidation (Chacon et al., 1988; Krishna et al., 1991; Ahmad et al., 2004).

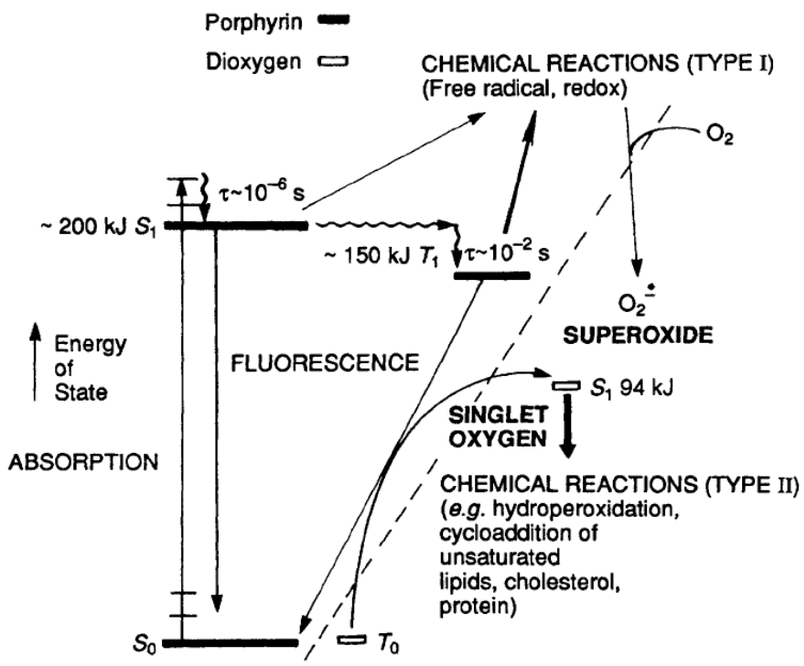


Fig. 6. Generation of excited porphyrin states and reactive diooxygen species (Bonnett, 1995) - *Reproduced by permission of The Royal Society of Chemistry, copyright* 1995.

We applied the photoexcited porphyrin using diooxygen under atmospheric pressure to oxidize α- and β-pinene. The system enables the production of pinocarvone, *trans*-pinocarveol and myrtenol from α-pinene, whereas from β-pinene, myrtenol as the most abundant and pinocarvone being by-product, were obtained. Some porphyrins have been found to be characterized by unusually high reactivity toward pinene hydroxylation. For instance, in the α-pinene oxidation studies, up to 17 mg/L of pinocarvone was achieved after 24-hour photoirradiation of 50% (v/v) of the substrate solution in the presence of H_2OEP (Trytek et al., in preparation).

Homogeneous catalysts in general are inclined towards an easy deactivation and are difficult to be recovered from reaction mixtures, leading to many restrictions in large scale-processes. Furthermore, the porphyrins typically used as photosensitizers are poorly soluble and together with their decomposition derivatives, can contaminate the reaction products and make the purification step really troublesome. For example, the sensitizer hematoporphyrin is only moderately soluble in most organic solvents (e.g., ether, chloroform), and nearly insoluble in water. In this case, one possible solution might be the use of heterogeneous catalysts through the immobilization of the porphyrins on organic and inorganic solid supports.

4. Heterogeneous catalysis using biomimetic porphyrins

Much work has been devoted to investigation of solid supports for the immobilization of porphyrins with respect to easier recovery, recycling and increased stability of this expensive catalyst. Numerous proposals have appeared: the use of cross-linked polymers (Griesbeck & El-Idreesy, 2005; Ribeiro et al., 2008), clays and layered materials (Bedioui, 1995; Evans & Lindsay Smith, 2001), biopolymers (Huanga et al., 2005), cationic ion-exchange resins (Campestrini, S. & Meunier, 1992); silanized kaolinite (Nakagaki et al., 2004); different types of zeolites (Skrobot et al., 2003; Haber et al., 2004), modified silica (Milaeva et al., 2007), and also microporous porphyrin framework solids (Suslick et al., 2005). Noteworthy results for the immobilized porphyrins have been obtained with ionic porphyrins immobilized on cationically functionalized polystyrene (Inbaraj et al., 2003), porphyrins copolymerized with polystyrene (Griesbeck et al., 2004), a soluble polyethylene glycol-supported tetrahydroxyphenyl porphyrin (Benaglia et al., 2002), photosensitizers ionically bound at polymeric ion-exchanging resins (Gerdes et al., 2001), and a cross-linked polyacrylamide hydrogel serve as a scaffold for the photosensitizer hematoporphyrin (Rogers et al., 2005) and as well with chitosan-supported metallotetraphenylporphyrin complexes (Huanga et al., 2005).

Manganese(III) 5-(pentafluorophenyl)-10,15,20-tri(2,6 dichlorophenyl)porphyrin, and manganese(II) 2,3,7,8,12,13,17,18-octachloro-5-(pentafluorophenyl)-10,15,20-tri(2,6-dichlorophenyl)porphyrin, have been covalently attached to aminopropylated silica and used in hydrocarbon oxidation by iodosylbenzene and hydrogen peroxide (Doro et al., 2000).

A few supported porphyrins have been tested in the oxidation of several monoterpenes. A tetracationic porphyrin, *meso*-tetrakis(4-*N*benzylpyridyl) porphyrinato manganese(III) being supported in a zeolite, NaY, have proved to be an active catalyst for the epoxidation of (*R*)-(+)-limonene and α-pinene, as well as in the hydroxylation of carvacrol and thymol, using H_2O_2 and ammonium acetate in CH_3CN at room temperature and under atmospheric pressure. This catalyst has enabled the 40-50% conversion of α-pinene after 6 hours of reaction, not exceeding 25% of conversion for the other substrates, even after 24 hours of

reaction. The 24-hour oxidation of limonene (>21% of conversion) gave rise to the mixture of 1,2- and 8,9-epoxide products. The oxidation of α-pinene afforded mainly the formation of corresponding epoxide and campholenic aldehyde, whereas carvacrol (>25% of conversion) and thymol (>18% of conversion) produced thymoquinone with 100% selectivity. However, in the presence of H_2O_2, the irreversible deactivation of catalyst took place, by leaching of the porphyrin complex from zeolite, followed by a loss of activity when the catalyst was reused (Skrobot et al., 2003).

Tangestaninejad *et al.* have described the tetra(4-pyridyl)porphyrinato manganese(III) anchored in chloromethylated styrene–divinylbenzene copolymer as an efficient and reusable catalyst for the (*R*)-(+)-limonene epoxidation by sodium periodate, among several other alkenes (Moghadam et al., 2004).

The Chinese team has utilized manganese(III) 5,10,15-tris(tolyl)-20-(4-hydroxyphenyl) porphyrin covalently attached to the Merrifield's peptide resin for the highly diastereoselective epoxidation of cholest-5-ene derivatives (Du, et al., 2004). The similar sort of material has been used by Gonsalves et al. (2008) in photooxidation of monoterpenes. They have covalently linked the chlorosulfonation activated porphyrins to the Merrifield-modified polymers. These immobilised porphyrins were used then as effective photosensitizers in monoterpenes oxidation using chloroform as the solvent. Photooxygenation of α-terpinene originated ascaridole as the main product, citronellol gave isomeric hydroperoxides. The latter oxidative transformation represents an important industrial application of a singlet-oxygen ene reaction as the first step of the route to the fragrant chemical specialty *rose oxide* (Pickenhagen & Schatkowski, 1998). From α-pinene and β-pinene, followed by reduction of the corresponding hydroperoxide products with triphenylphosphine, *trans*-pinocarveol and myrtenol have been obtained. These products are expected as a result of the singlet oxygen ene addition to terpenoic olefins, although in the case of pinenes, non-ene products were also formed probably through a pathway involving a mechanism of electron transfer between the excited photosensitizers and the substrates (Ribeiro et al., 2008).

Presently, the sol–gel processes offer new and promising possibilities of physical trapping or covalent bonding of porphyrin catalysts inside the silica network. The process is based on the evolution of inorganic networks through the formation of a sol and gelation with formation of a network in a continuous liquid phase. The precursors of the synthesis of these sols consist of a metal or a metalloid element bonded with various reactive ligands. The most popular are metal alkoxides since they react readily with water. The most known alkoxides are the alkoxysilanes, such as teramethoxysilane (TMOS) and tetraethoxysilane (TEOS). Aluminates, titanates or borates are also used in the sol-gel process.

Besides simplicity of preparation, low-temperature encapsulation, silica sol–gels are also characterized by optical transparency, chemical and photochemical inertness, as well as photochemical, thermal, and mechanical stability. Moreover, those materials have a negligible swelling in organic solvents and aqueous solution compared to most organic polymers. The matrices appeared to have additional two roles: in protection of porphyrin from oxidative degradations and in induction, by tunable porosity, of the selective positioning of the substrate with respect to the porphyrin cofactor (Kandimalla et al., 2006). The sol-gel process allowing the preparation of optically transparent porous silica gel seems to be excellent technology for development of the photoactive porphyrin catalyst.

The classical work by Avnir et al. (1984) concerning the spectra of rhodamine 6G entrapped in silica prepared by the sol-gel method, started successful research on direct

bioencapsulation in silica, organosiloxanes and hybrid sol-gel polymers, also active biological substances with heat-sensitive and fragile molecules, such as enzymes, proteins, DNA, RNA, catalytic antibodies and even the whole cells of microbes (Kandimalla et al., 2006). Such materials have many potential applications due to their catalytic, biosensing, optical, photo-electronic, photonic, and nanobiophotonic properties (Lan et al., 1999; Gill & Ballesteros, 2000; Ariga et al., 2007; Escribano et al., 2008).

Porphyrins have been encapsulated in sol-gel materials (either in monolithic blocks or as thin layers on various supports) because of their ability of photoconduction and photoemission, in the context of their application as fluorescent materials (Papkovsky et al., 2000; García-Sánchez et al., 2006; De la Luz et al., 2007) optical sensors (Delmarre & Bied-Charreton, 2000; Im et al., 2005), dye sensitive solar cells (Grätzel, 2001; Ray et al., 2001), and non-linear absorption (Sun et al., 1997), mesoporous materials (Ariga et al., 2007), as well as sensitizers in photodynamic therapy (PDT) (Reisfeld, 2001; Podbielska et al., 2006).

5. Spectral and biocatalytical properties of porphyrins immobilized in the silica gels

The physicochemical characterization of the active element is considered of primary importance at the initial stages of the design of a new optical and photocatalytic device. The most commonly employed techniques in the investigation of porphyrin systems are: nuclear magnetic resonance (NMR), absorption, excitation and emission spectroscopy (Oulmi et al., 1989) or circular magnetic dichroism (Purrello et al., 2000). The influence of different factors on the absorption, emission and luminescent spectral properties of the porphyrins can be shown in the best way when the cases of porphyrins intercalated in different matrices are considered. The most spectacular examples of spectroscopic properties of porphyrin intercalates are reported below.

Dargiewicz et al. (2002) studied the UV-Vis absorption spectra of water soluble porphyrins: tetrakis[4-(trimethylammonio)phenyl] (H_2TTMePP) and tetrakis(1-methyl-4-pirydyl)(H_2TmePyP) in the monolith silica aerogel and solid thin films obtained by the sol-gel method (**Figs. 7-8**).The authors found the bands intensity decrease, their broadening and shift in both porphyrins toward longer wavelengths when the sol solution was compared with the aerogel and monolithic sol-gel material. The changes in Q bands were more complex, i.e. the bands $Q_{y(1,0)}$ and $Q_{y(0,1)}$ were blue shifted while the bands $Q_{x(1,0)}$ and $Q_{x(0,1)}$ were red shifted for aerogel, which was interpreted as a result of silica matrix polarity decrease in comparison with sol. In turn, the decrease of components in Q bands from four to two when the spectra of porphyrins in sol were compared with these for the monolithic sol-gel material was explained as a consequence of free base porphyrins protonation in acidic soda-lime glass with formation of dications H_4P^{2+}. The dimerization constants K_d of both porphyrins in the monolithic sol-gel glass, calculated based on the Soret bands intensity changes, were found to be in the range: $1\text{-}6.8\times10^5$ $M.^{-1}$. Their decrease with time of gel drying was the result of steric obstacle in dimers formation when the SiO_2 network was formed and monomer porphyrins were preferable.

Pereira et al. (2005) studied the spectra of mesoporphyrin IX in the TiO_2 and SiO_2 matrices **(Fig. 9)**. The authors found that in the initially formed titania matrices the porphyrin exists in a similar environment and a significant proportion relates to its neutral monomer, especially within the matrices with DMF. Without DMF more of the ionised porphyrine is present, both cation and dication. The quantity of the neutral form decreases with time and

without DMF the spectrum of porphyrine within the titania monolith broadens and shifts to red accompanied by a decrease of fluorescence time. In the presence of DMF this effect is not observed, although the amount of monomer decreases and the presence of aggregates, photoproducts as well as ionised porphyrine is possible. The final forms of the silica matrix also show the effect of DMF addition. In the absence of DMF the main form of porphyrine is the cation.

Sanchez et al. (2004; 2006; 2009) reported about the free bases of the substituted tetraphenylporphyrins $H_2T(o\text{-}NH_2)PP$, $H_2T(p\text{-}COOH)PP$ and $H_2T(p\text{-}OH)PP$, which were bound to the silica matrix by the use of the functionalized alkoxides 3-isocyanatopropyltriethoxysilane or 3-amminopropyltriethoxysilane, by the sol-gel method. It was shown, based on the UV-Vis spectra of all xerogels, that porphyrins are in stable forms inside the xerogel pores. The red fluorescence observed in the solutions of the substituted porphyrins was preserved even after these species were covalently bound to the

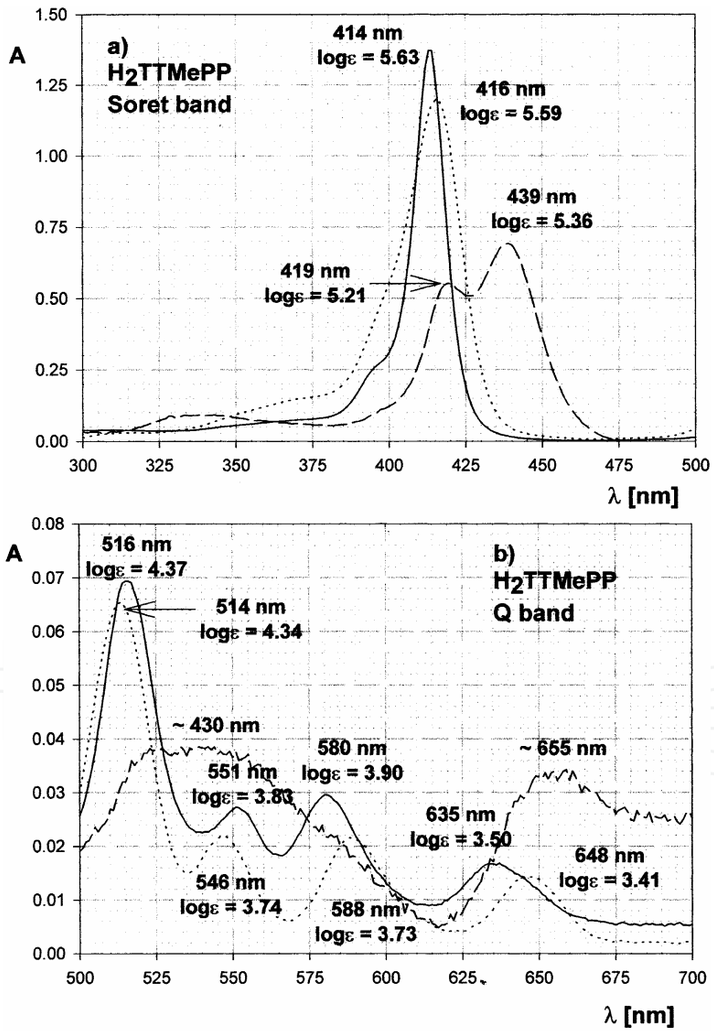


Fig. 7. Absorption spectra of the $H_2TTMePP$ in: aqueous solution (solid line), monolithic sol–gel material (dotted line) and thin film (dash line); ε =molecular extinction coefficient; *reproduction with permission from Colloids and Surfaces A, copyright 2002.*

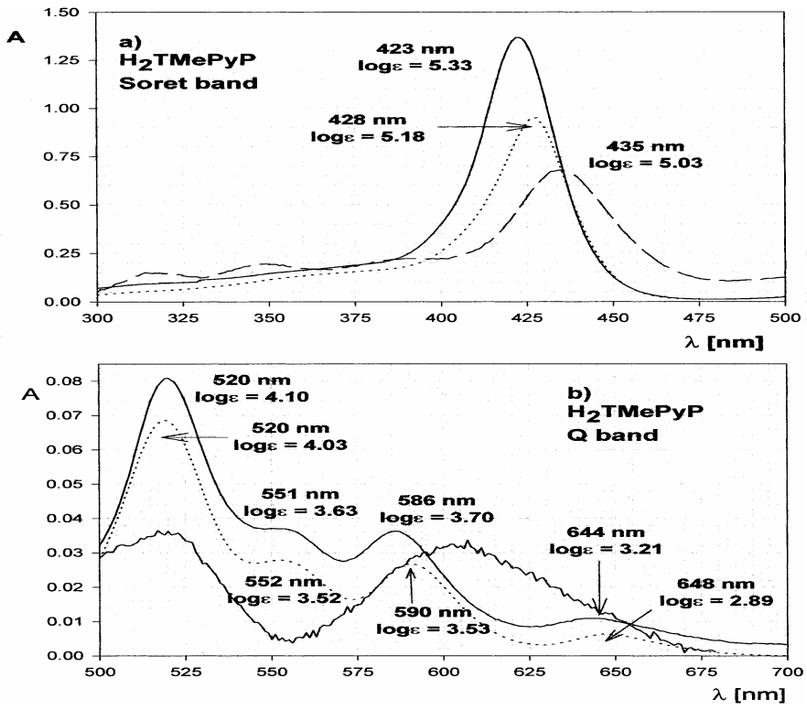


Fig. 8. Absorption spectra of the H_2TMePyP in: aqueous solution (solid line), monolithic sol–gel material (dotted line) and thin film (dash line); ε =molecular extinction coefficient; *reproduction with permission from Colloids and Surfaces A, copyright 2002.*

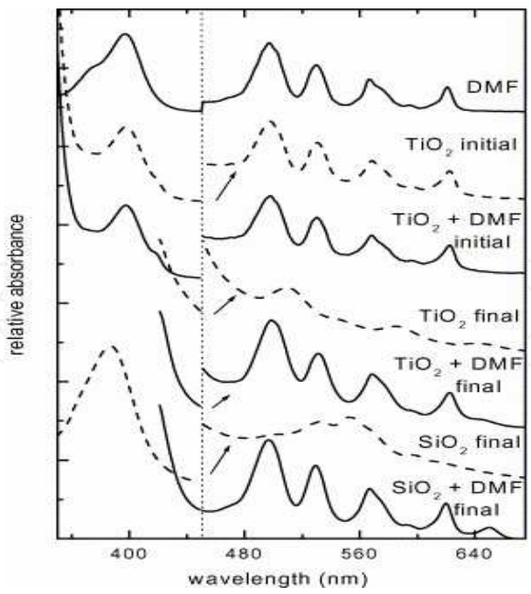


Fig. 9. Absorption spectra for MP in sol–gel-derived matrices, both with and without addition DMF. The absorption spectrum in DMF is given for comparison and the *Q* band region has been amplified for clarity; *reproduction with permission from Journal of Photochemistry and Photobiology A: Chemistry, copyright 2005.*

silica matrix. The best results, in terms of fluorescence preservation, were obtained by using a TPP substituted with -NH_2 groups in the *ortho* position of the phenyl rings, i.e $H_2T(o\text{-}NH_2)PP$. The UV-Vis spectrum of these species showed no signs of protonation. The authors found that cobalt tetraphenylporphyrins substituted with hydroxyl groups: (a) in *ortho* and *para* positions, and (b) with amino groups in *para* positions can be trapped in SiO_2 by the sol-gel method. The addition of DMF, methanol or pyridine is necessary for successful immobilization of macrocycle in the silica network. The addition of pyridine to the gelling mixture results in the inhibition of demetallation and protonation of the macrocycles.

Dargiewicz et al. (2004) reported the strong luminescence of Eu complex with methyl-pyridylporphyrin ($H_2TmePyP$) intercalated in the silica gel. The authors observed strong bands in the emision spectrum of Eu complex located at 604, 560 and 554 nm when the excitations: 423, 443 and 530 nm were applied. Pure $EuCl_3$ intercalated in gel did not show the luminescence, whereas the free base porphyrin spectra had the pairs of peaks placed at 653, 716; 658, 719 and 655, 715 nm respectively (at the excitation wavelengths: 423, 443, 530 nm), red shifted when compared with the Eu complex. The authors suggested that the red luminescence of Eu complex results partially from the π-π electron interaction of porphyrin rings and from the interaction of methyl pyridyl porphyrin peripherals with Si-O-Si or Si-O groups located at the inner surface of gel pores.

Delmaire et al. (1999) studied the UV-Vis spectra of two porphyrins: 5,10,15,20-tetra(4-N-methylpyridinium)porphyrin (H_2TMPyP^{4+}) and 5,10,15,20-tetra(4-sulfonato)porphyrin (H_2TPPS^{4-}) in the silica sol-gel matrix. The Soret band underwent a remarkable shift upon the pH change of buffer solutions, which were equilibrated with gel samples. In the case of H_2TMPyP^{4+}, a shift of 12 nm was observed from 422 nm in the solution to 434 nm in the sol-gel materials. This was probably the result of the presence of positive charges on the porphyrin ring favouring strong interactions with the deprotonated silanols of the surface. In turn when a solution of H_2TPPS^{4-} at pH 6 was incorporated in the sol-gel material a green colour was observed probably due to the protonation of the central nitrogen. It was possible to determine the dissociation constant pK of protonated H_4TPPS^{2-} species inside a sol-gel matrix and from this determination to find the local pH in the sol-gel. It was concluded that pH sensor is a possible application.

Polska and Radzki (2008) found very strong fluorescence enhancement of tetrakis[4-(trimethylammonio)phenyl]porphyrin ($H_2TTMePP$) by the addition of concavaline A both in the solution and in the silica sol-gel matrix. Based on the absorption spectra in the solution authors concluded about the 1:1 associate formation between the porphyrine and concavaline A with the binding constant $K=4.35\times10^5$ mol^{-1}. During inspection of the emission spectra of transparent monolithic silica gels doped with concavaline A and porphyrin associate **(Fig. 10)** authors found that emission of concavalin is excited at the absorption near 260 nm, while excitation at 430 nm is not observed. The excitation at the Soret band results in the remarkable enhancement of porphyrin fluorescence in the associate, and this phenomenon probably results from energy transfer from protein to porphyrin. Fluorescence of the associate was 10-fold more intense than luminescence of the gel doped by porphyrin alone. The intensification of fluorescence was observed only for the low concentration of the associate (1.25×10^{-5} mol/dm^3). For the higher concentrations of porphyrin and porphyrin-protein associate the luminescence quenching was observed. The authors suggested practical application of the studied system in luminescent materials.

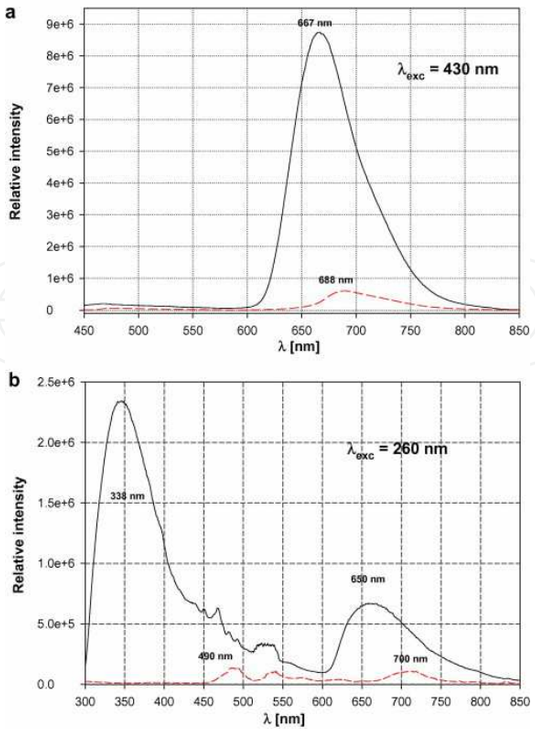
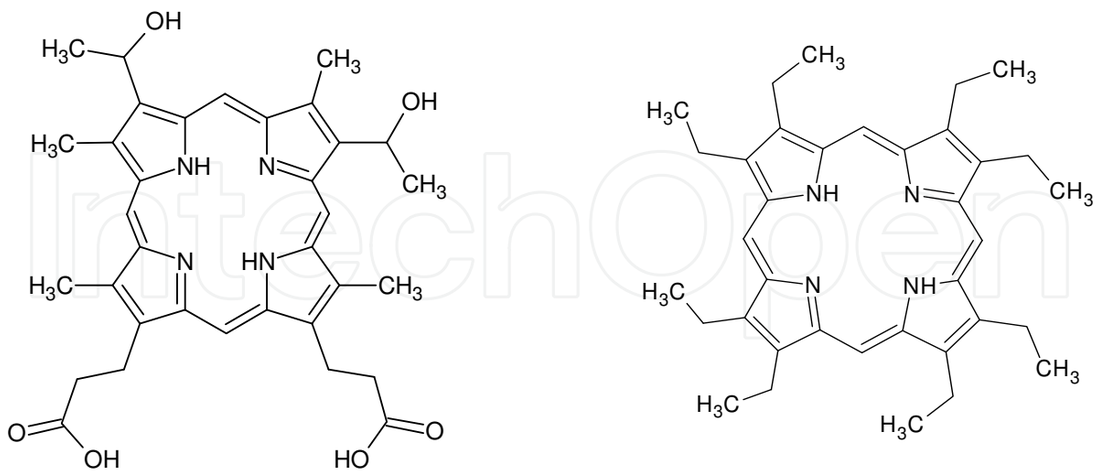


Fig. 10. Emission spectra of the monolithic silica gels: doped with 1.25×10^{-5} mol dm^{-1} H_2 TTMePP (dashed line) and 1.25×10^{-5} mol dm^{-1} H_2 TTMePP/Con A 1:1 associate (solid line) excited at 430 nm (a) and doped with 5×10^{-5} mol dm^{-1} Con A (solid line) and 5×10^{-5} mol dm^{-1} H_2 TTMePP/Con A 1:1 associate (dashed line) excited at 260 nm (b); *reproduction with permission from Optical Materials, copyright 2008.*



HmP-IX H_2OEP

Fig. 11. Chemical structures of porphyrins encapsulated in silica gel showing biomimetic activity in photooxygenation of monoterpenes.

Trytek et al. (2009) studied the fluorescent and biocatalytical properties of water soluble and insoluble prophyrins intercalated in the silica sol-gel matrix. The inspection of UV-Vis absorption spectra of tetraphenylporphyrin H_2TPP showed that the intensity of the Soret band is lower in sol than in a solution. After gelation and 33 days of drying further lowering and broadening of the Soret band was observed, which was explained on the one hand by the changing polarity of the gel environment (the transition from hydro to alcogel) and by the dimerization and agglomeration of the porphyrin. The spectrum of a dried sample differed from sol as well as from the samples before drying. The Soret band was shifted from 414 to 437 nm, whereas in the Q band region the new, very intensive band at 655 nm was observed and its existence was probably the result of porphyrin protonation with consequent dication formation. In the emission spectrum of H_2TPP the fluorescence of sol and the dried gel was evidently less intensive than luminescence in the DMSO solution as a result of porphyrin agglomeration. The shift of the luminescence maximum from 650 to 667 nm, when sol was compared with the dried sample, confirmed the dication existence in the dried gel.

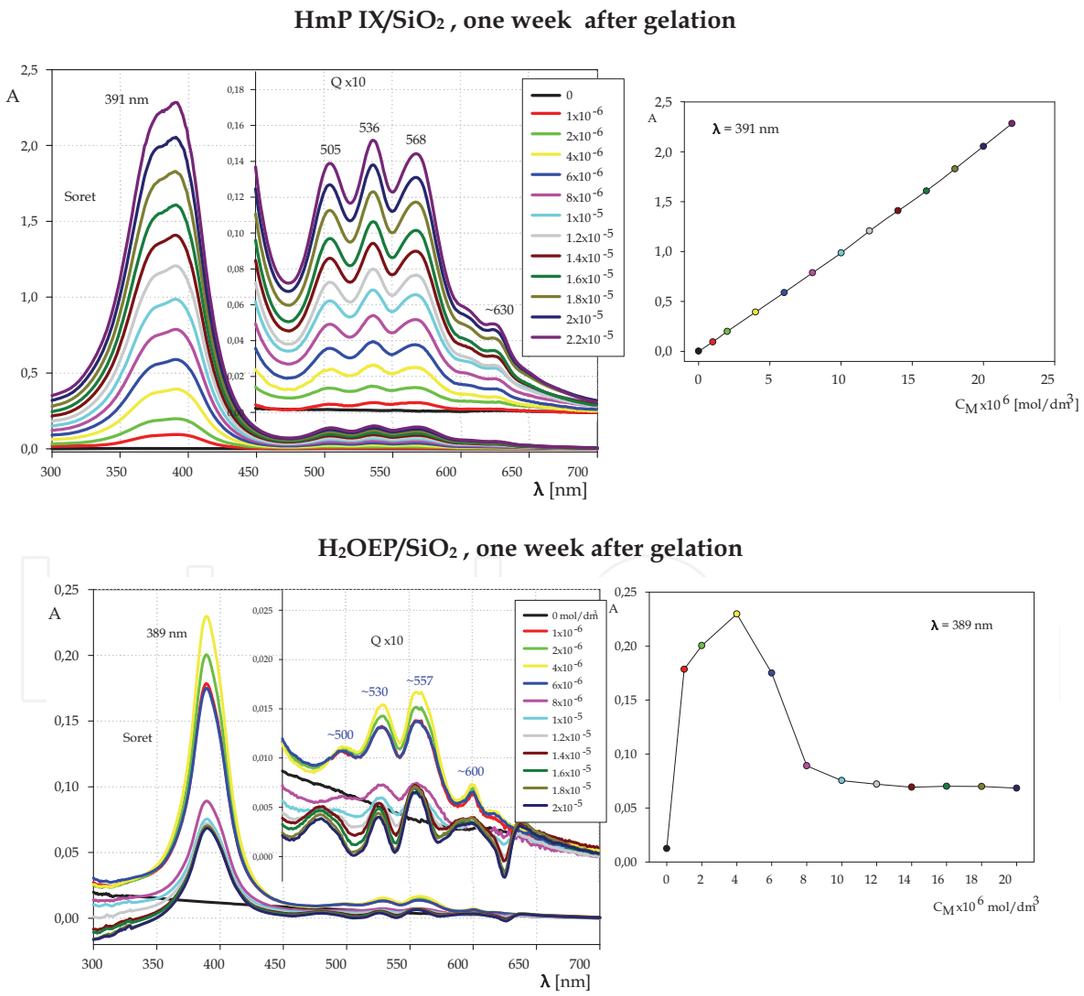


Fig. 12. UV-Vis absorption spectra of HmP IX and OEP intercalated in SiO_2 alcogel (the numbers denote the initial concentrations of porphyrins in silica sol).

The authors compared the behaviour of Hemato-IX porphyrin (HmP-IX) and octaethylporphyrin (H_2OEP) (**Fig. 11**) in silica alco-gel (**Fig. 12)** (Trytek et al., manuscript in preparation). There was an evident straightline increase of absorbance at 391 nm with the concentration of Hemato-IX porphyrine, which probably was the result of its solvation by ethanol molecules and therefore its lack of aggregation in the alcogel matrix. It has been reported that the porphyrin molecules tend to aggregate by hydrophobic and π-π interactions, leading to loss of linearity in the Beer's plot (Takagi et al., 2002).
The essential difference in the spectral behaviour of hematoporphyrine in comparison with H_2OEP resulted probably from the presence of polar groups in its structure, i.e. peripheral hydroxyl and carboxyl groups, attracting strongly solvent molecules in alcogel. In turn, for octaethyl porphyrin the drastic decrease of the Soret band intensity with the porphyrin concentration was the basis for the evaluation of the molar fractions of monomers, dimers and trimers of porphyrin **(Fig. 13)**. It was found that trimers are a predominant form of free base porphyrin in sol, whereas dimers molar fraction in alcogel was much higher than monomers and trimers, and this fact is probably a consequence of steric hindrance in trimers formation in the silica pores.

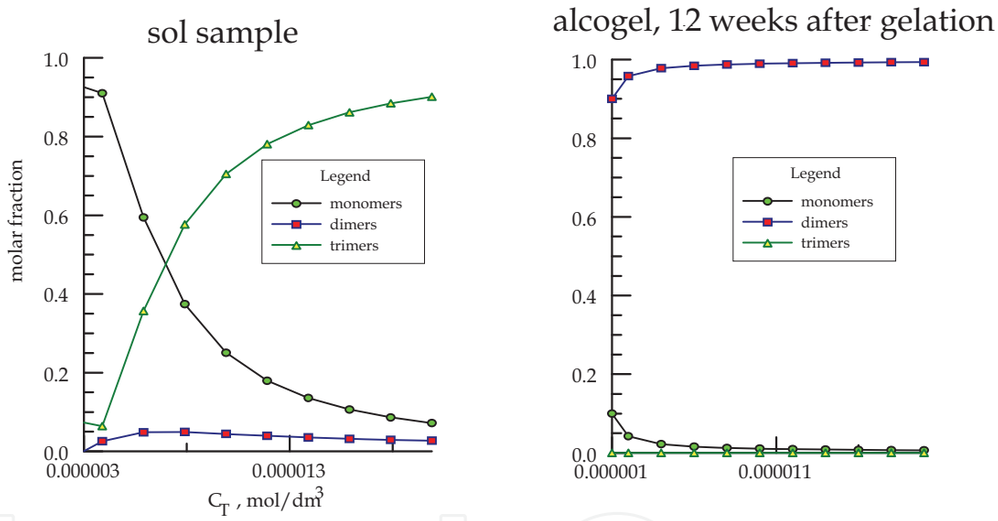


Fig. 13. The molar fractions of monomers, dimers and trimers of octaethylporphyrin in sol and alcogel.

In xerogel both porphyrines: H_2OEP and HmP-IX behave similarly relating to the character of their UV-Vis absorption spectra. There is observable red shift of the Soret bands with their characteristic broadening at the higher concentration of porphyrins **(Fig. 14),** when the xerogel is compared with alcogel (**Fig. 12**), i.e. from 391 to 400 nm for HmP-IX and from 389 to 400 nm for H_2OEP. This fact is explained by many investigators by the agglomeration of tetrapyrrole macrocycles. On the other hand it can not be precluded that the existence of the above mentioned Soret bands red shift may be also referred to the interaction of porphyrin molecules with the silica walls. Based on the FT-IR and Raman spectra formation of hydrogen bonds between the porphyrine and silica walls has been found. The band 1083 cm^{-1} in FT-IR spectrum of pure SiO_2 was shifted toward 1078 cm^{-1} in silica gel doped with porphyrine as a result of interaction of porphyrin with siloxane oxygens via hydrogen bond. In Raman spectrum the band responsible for the O-Si-O bending mode was shifted from 810

cm^{-1} in SiO_2 to 816 cm^{-1} in O-SiO...H_2OEP species. What is more interesting the band 599 cm^{-1}, characteristic of the $(SiO)_3$-ring breathing mode, was observed merely in the case of SiO_2/H_2OEP case as a result of partial rearrangement of tetrahedral silica structure after incorporation of porphyrin and formation of three-membered rings.

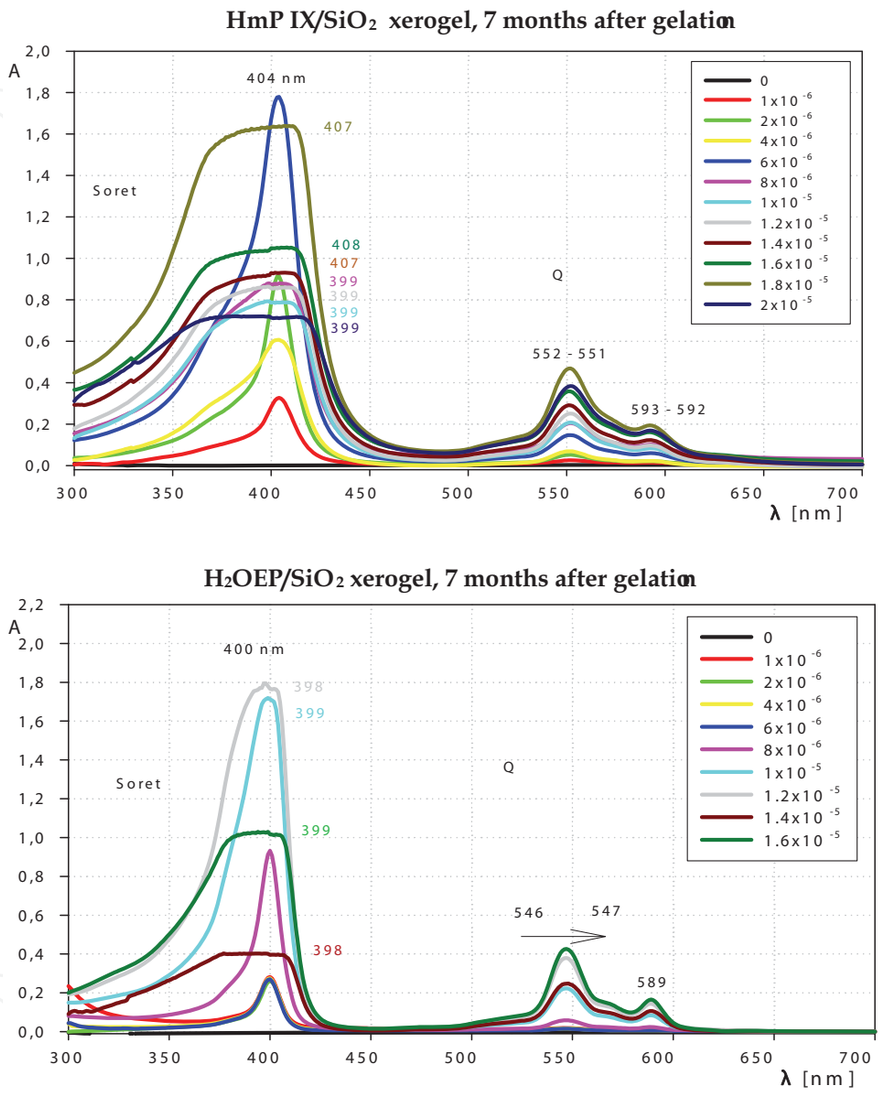


Fig. 14. UV-Vis absorption spectra of HmP IX and H_2OEP intercalated in SiO_2 xerogel (the numbers denote the initial concentrations of porphyrins in silica sol).

Formation of H- (*face to face*) and J-type (*face to tail*) agglomerates of free base porphyrin and its dications is very probable, since splitting of the Soret band in the excitation spectrum into two peaks (**Fig. 15**), both for octaethyl- and hematoporphyrin IX was observed. This is in agreement with the exciton theory, in which the excited-state energy level of a monomeric dye splits into two during aggregation. Separation of the Soret band into two components is not the same for both porphyrins. For HmP-IX porphyrine the intensity of the bands 345 and 425 nm is similar, which means that the concentrations of H- and J-aggregates are

approximately equal. In the case of H_2OEP porphyrin the 355 band intensity is significantly higher than that for 412 nm. Formation of H-aggregate is therefore more advanced in comparison with the J-aggregate.

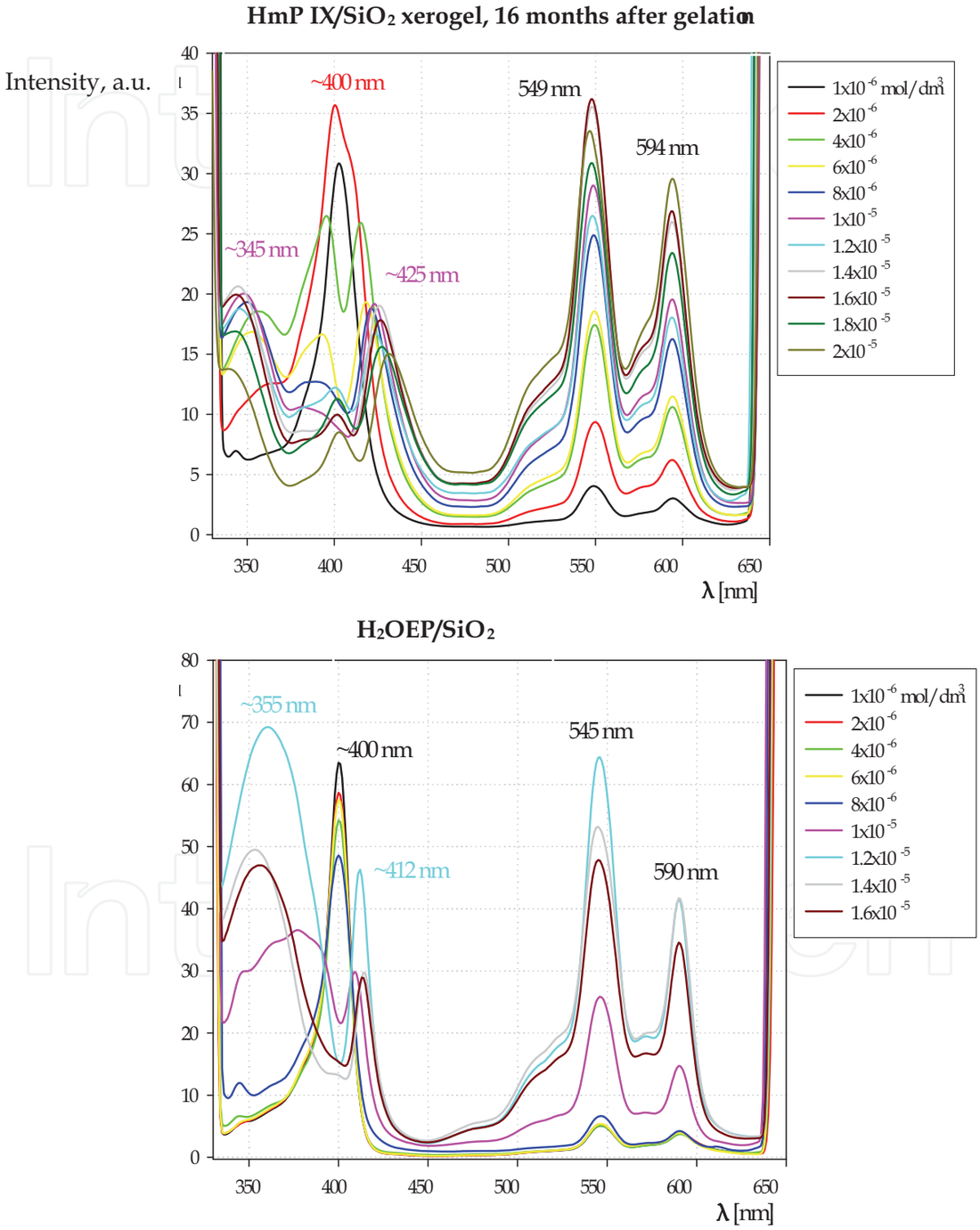


Fig. 15. Excitation spectra of porphyrins intercalated in silica xerogel (16 months after gelation, emission at 650 nm).

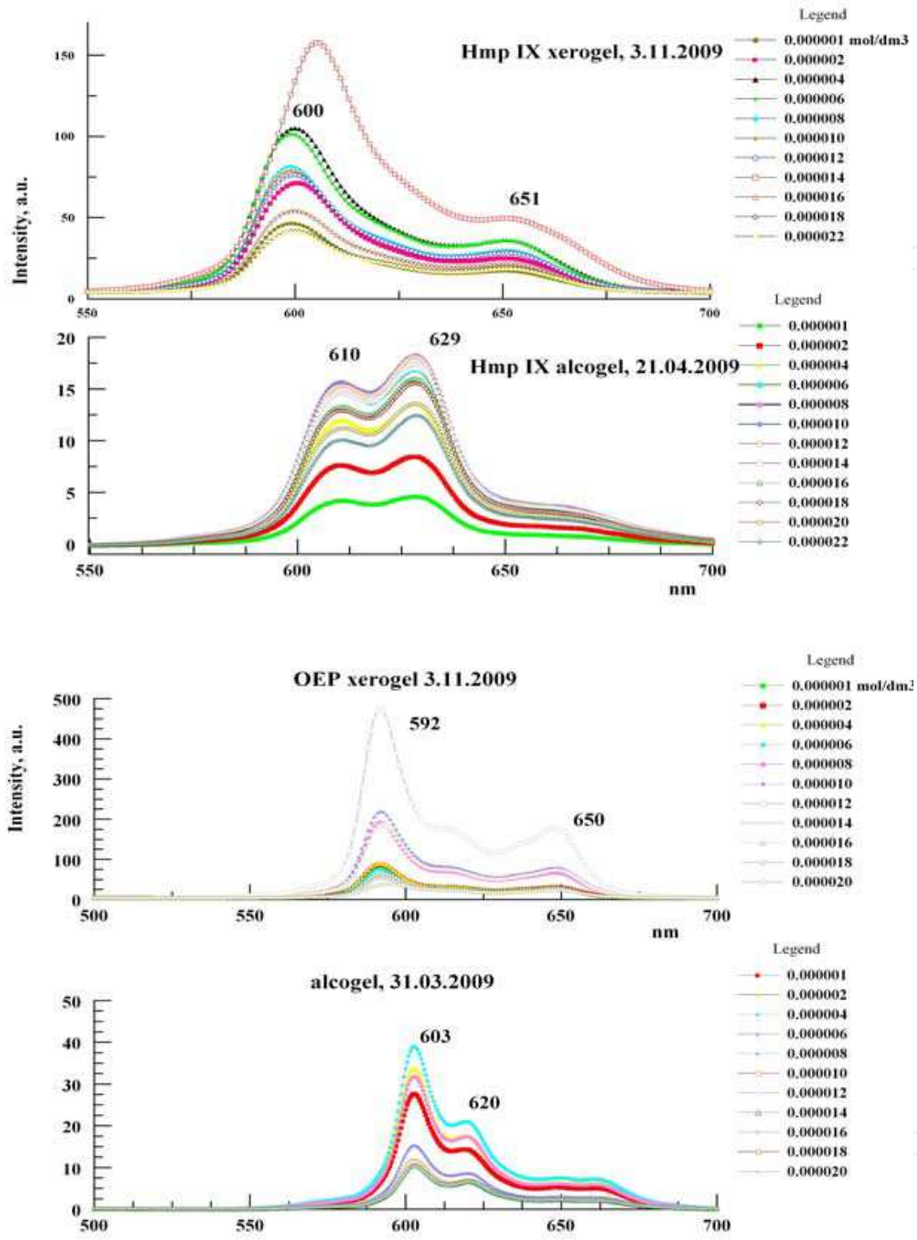


Fig. 16. The emission spectra comparison of HmP IX, H_2OEP in alco- and xerogel (excitation wavelength 390 nm).

Textural characteristics of silica xerogel based on N_2 adsorption revealed the microporous character of adsorbent, however, apart from the micro-, the meso- and macropores were present in the silica structure with the pore size diameter: 17-3000 Å; therefore the accommodation of J- and H-dimers of porphyrines inside the gel structure is possible without steric obstacles. It must be emphasized that besides optical applications, the silica materials should have very small pore sizes and if the functionalized porphyrin systems are used as biocatalysts or chemical sensors, the passage of reagents and products in and out of

the pores becomes really important (Garcia-Sanchez et al., 2009). Control of many operational factors involved in the hydrolysis and condensation during the sol-gel process is essential for achieving a proper balance between non-leaching of the entrapped bioactive porphyrins and their accessibility to the reagents.

Emission spectra **(Fig. 16)** contain two bands at 610, 629 nm for Hemato IX porphyrin and 603, 620 nm for H_2OEP. These bands correspond to the presence of dications and free base porphyrins. When passing from alco- to xerogel there can be seen the blue shift of bands to 600 and 592 nm and the emergence of a new band at 650 nm, which probably is related to the agglomeration of free base porphyrins and their protonated forms. On the other hand, it is well known that the agglomerated and protonated forms of porphyrins have less intensive fluorescence compared to free-based porphyrin monomers. In our case if they really exist in the dried gel (xerogel), protonated porphyrin dimers possess characteristic of very strong red fluorescence with greater intensity in compared to that porphyrins dissolved in sol and to those with the aryl substituents immobilized in gel such as H_2TPP/SiO_2.

Lee and Okura (1997) reported the sensor oxygen prepared from immobilized platinum octaethylporphyrin. The luminescence spectra of porphyrin in the silica matrix were very sensitive to the oxygen presence. The strong luminescence quenching was found with the oxygen concentration rise, i.e. intensity of the 645 nm band decreased up to 97.5% changing from the totally deoxyganated to oxygenated conditions. This result seems to be dependent on the porous structure of the inorganic matrix prepared by the sol-gel process, having channels for oxygen. According to us, it is most probable that in the presence of oxygen there occur non-radiative processes concurrent to fluorescence, for example quenching of the porphyrin excited state by $^3O_2$ leading to energy transfer and $^1O_2$ formation. The UV-Vis absorpion spectra of PtOEP-doped silica glass and initial PtOEP/dimethylformamide solutions differed markedly, i.e. the bands 400, 550, 586 nm in the sol-gel glass were red shifted in comparison with those of the initial PtOEP/DMF solution, which appeared at 375, 500, 534 nm. This fact was explained as arising from the less polar silica matrix.

The investigations of tetrakis-(N-methyl-4-pyridinium)porphyrin encapsulated in mesoporus silica by Yoshida and co-workers (2003) showed the evident changes in the UV-Vis absorbance and fluorescence spectrum when free base porphyrin in solution is compared with that in gel. Shift of the Soret band in direction of shorter wavelengths was observed and explained by the dimerization of porphyrin and interaction of porhyrine with the solid surface. Shift of the flurescence band from 650 to 665 nm was also found with the increase in the adsorbed TMPyP amounts. The luminescence intensity varied depending on the adsorbed amounts and the variation was not simple. Occurence of luminescence quenching including self-quenching was suggested.

Xu et al. (2001) reported about the formation of monomeric and aggregated terakis(p-sulphonatophenyl)porphyrin encapsulated within an aluminosilicate mesostructure, i.e in MCM-41, under different pH conditions. Stabilization of MCM-41 was necessary through the use of a silylation reagent, (aminopropyl)triethoxysilane, which cross-linked oxygen on the surface, rigidified the mesoporous material walls and functionalized the interface for proper guest-host interaction. The authors found that the encapsulated free-base monomer exhibited the blue shift for its Soret band (396 nm) when compared with the Soret band (413 nm) of the solution monomeric species. The blue shift for the absorption was also established for the encapsulated aggregate when compared with the solution aggregate, i.e. from 490 to 487 nm respectively. Such shifts were explained in terms of charge transfer

caused by the host-guest interaction, connected with the steric effects associated with the MCM pore structure. The broad structure for the Soret band for TSPP-M/(MCM-41) was interpreted as site-specific absorptions for encapsulated TSPP, suggesting that the monomeric species is distributed in various positions within the cage and experiences a range of perturbations. Based on the analysis of absorbance and fluorescence spectra the authors concluded about the formation of J-aggregates by porphyrin in gel, which had the zigzag or spread deck of cards structure **(Fig. 17).**

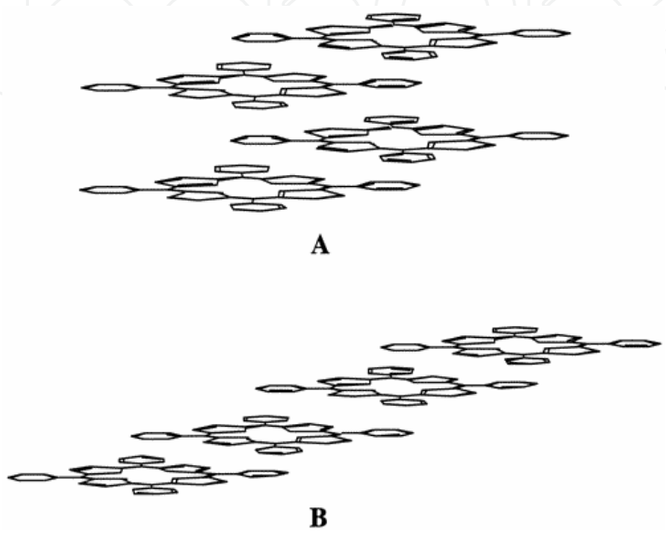


Fig. 17. Two possible linear J-aggregates (A and B, relating to the zigzag and spread deck of cards, respectively) resulting upon protonation of the macrocycle; *reproduction with permission from Journal of Physical Chemistry B, copyright 2001.*

Holland et al. (1998) investigated the encapsulation of *meso*-Tetrakis(5-trimethylammoniopentyl)porphyrin (TMAP) in the molecular sieve MCM-41 via the hydrothermal synthesis or by the surfactant/porphyrin ion-exchange reaction with MCM-41. Both methods allowed for the incorporation of porphyrin into the mesoporous channel system. The inspection of the UV-Vis spectra showed that porphyrin dimers or aggregates were formed during ion-exchange, whereas TMAP molecules remained isolated during hydrothermal synthesis. In the latter the UV-Vis spectra were similar to those of TMAP in dilute solution and no significant broadening of Soret and Q bands was noticed.

Murata et al. (2000) modified the surface silanol groups of FSM-type mesoporous silica (folded sheet mesoporous material) with 1,4-butanediol and used this material as the adsorbent to accommodate chlorophyll *a* molecules. Pheophytinization (removal of magnesium atom from the porphyrin ligand) was suppressed by the presence of the surface organic groups intercalated into the mesoporous silica. Pheophytinization occurred in the unmodified silica. The UV-Vis spectra showed that the Q_y transition occurred at 665 nm, suggesting that the encapsulated Chl *a* molecules are well dispersed in the mesopores of 1,4BD-C_{18}FSMs. The UV-vis spectrum of 1,4BD-C_{18}FSM containing Chl *a* stored under dark and cold (0^0) conditions for 6 months, was registered in order to estimate the stability of incorporated Chl *a* and the spectrum showed no change. The authors concluded that the obtained results may lead to the construction of *in vitro* biomimetic solar energy conversion and storage systems.

Itoh et al. (2002) investigated the adsorption of chlorophyll *a* to the mesoporous silica FSM. The chlorophyll-FSM conjugate was formed with a nanometer-scale interaction between chlorophyll molecules. There was found the enhancement of photostability for the chlorophyll *a* adsorbed to the pores FSM, along with decrease in the pore volume and specific surface area, as well as a shift in the adsorption maximum to a longer wavelength, i.e from 671 to 675 nm. Such a shift was explained as a consequence of an interaction between two chlorophyll molecules. The evolution of hydrogen gas was observed when the aqueous solution containing chlorophyll-FSM, methyl viologen, 2-mercaptoethanol, and platinum was illuminated with visible light.

As follows from the presented results the spetroscopic behaviour of the porphyrins in the solutions and solid matrices is evidently different but the interpretation of changes in the position and intensity of Soret and Q bands of porphyrins spectra after their intercalation in various matrices is very complexed. The same applies to the formation of UV-Vis absorption bands referred to the porphyrins agglomeration. There are too many effects, such as formation of dications, agglomeration and interactions of porphyrins with the matrix, which prevent the identification of the electronic transitions. Despite the lack of theoretical explanation for complexed porphyrins spectroscopic behaviour, the fact of their high affinity for the solid matrices and the preservation of their luminescent properties during insertion into the solid matrices structure has been used in biocatalytic applications and this is the issue of the next topic.

As most of the silica-gels are transparent to visible light, the mechanisms governing the photoprocesses are strongly dependent on the characteristics of the photosensitizer. Studies on the change of structure conformation and physicochemical properties of porphyrin macrocycles entrapped in silica matrices can be especially helpful in elucidation of mechanism of their photocatalytic oxidation in heterogeneous systems, and in attempts at achievement of more powerful biocatalysts as well as in rationalizing the reaction manifolds accessible to the porphyrin-silica catalysts.

6. Photocatalytic oxygenation using porphyrins intercalated in the sol-gel matrix

Only a few works have been reported on porphyrins immobilized in the sol-gel matrix as the new functional catalytic devices for the production of commercially important compounds.

Iron porphyrin catalysts were anchored on the spherical silica gel beads obtained by the sol-gel route (Biazzotto et al., 2002; Moreira et al., 2005; Papacídero et al., 2006) for the oxidation of (*Z*)-cyclooctene and cyclohexane with PhIO or H_2O_2. Better results were obtained with the electronegatively substituted porphyrin on the SiO_2 supports rendering 86% epoxide yields (Moreira et al., 2005). A similar carrier covalently modified with the metal-free monopyridyltriphenylporphyrin, introduced into a polymer microchannel and the microchip were applied for photodecomposition of phenol under solution-flow conditions (Kitamura et al., 2006). Very recently a visible light photocatalytical system based on water-insoluble tin porphyrin $Sn(OH)_2(TPP)$ immobilized on SiO_2 has been successfully used for the degradation of 4-chlorophenol and acid orange 7 (Kim et al., 2008). Silica microspheres functionalized with 5-(4-allyloxy)phenyl-10,15,20-tri(2,6-dichlorophenyl)porphyrin have been prepared by Huang et al. for the photooxidation of 1,5-dihydroxynaphthalene under visible light irradiation in the aerated aqueous solution (Cai et al., 2009).

Trytek *et al.* (2009) have presented the results concerning two types of porphyrins (water-soluble and water-insoluble) incorporated into the silica gel which act on the one hand as fluorophores, and on the other hand as efficient catalysts in biomimetic photocatalysis of monoterpene, α-pinene, in organic solvent under molecular oxygen. In the presented experiments the authors confirmed that the photochemical excitation of sol-gel immobilized porphyrins is crucial for catalyzed oxidation of monoterpenes, and it can proceed with sunlight or by using an artificial light source. The catalysts based on the porphyrin copper (II) complexes proved to be ineffective in the photochemical system. The largest level of *light promoted biotransformation* was achieved with water insoluble H_2TPP and water soluble, cationic tetrakis(1-methyl-4-pyridinio)porphyrin ($H_2TMePyP$).

We decided to develop the biomimetic system using $H_2TMePyP/SiO_2$ because H_2TPP in silica gel was rapidly protonated in the initial course of the photooxygenation, and without regeneration could not be reused. Among four solvents tested for this reaction, chloroform and dichloromethane proved to be the best with respect to the enhancement of the system productivity. Although the molar yields of products were rather low (about 15%), $H_2TMePyP/SiO_2$ in the powdered form could be repeatedly used for 11 successive biotransformations of each 24-hour cycle without a significant loss of activity, rendering up to 10.5 g/L yield of pinocarvone, 2 g/L of *trans*-pinocarveol and about 1 g/L of myrtenol. It was proved by the invariance of the UV-Vis spectra of the filtered reaction mixture and also on the basis of luminescent intensity for the powder both before and after the reaction that the porphyrin catalyst remains inside the gel pores for thirteen cycles.

The effect of porphyrins immobilization in the silica matrices on the changes and correlation between their photophysical properties and biomimetic activities can be shown from the comparative study on water insoluble SiO_2-encapsulated HmP-IX and H_2OEP (**Fig. 11**), bearing substituents of different polarity (Trytek et al., in preparation). The photoxidation of α-pinene in the organic solvent by molecular oxygen was demonstrated successfully for only octaethylporphine/SiO_2. Significant differences in photobiocatalytic performance of these both intensive luminescent materials may result from strong interaction of the electron-accepting carboxyl groups of HmP-IX with silica, and much stronger electron donating ability of H_2OEP making the central cavity of the porphyrin ring less electrophilic in contrast to HmP (Kane et al., 1998). Such results may be also interpreted in terms of the earlier described spectral investigations, which revealed the contrasting capabilities of HmP-IX and H_2OEP aggregation in both the sol solution and sol-gel matrix, as well as differences in the formation of H- and J-type agglomerates in xerogel.

Since the HmP in silica preserves the fluorescence, we can not preclude the formation of -CO-O-Si ester bridges with the siloxane network by HmP, because according to the other authors the covalent bond between the porphyrin molecules and the silica walls in the interior pores of the gel diminish the interaction of porphyrin molecules with silanol groups (Si-OH) and then the possibility of non-radiating decay of the fluorescent process of porphyrins (Garcia-Sanchez et al., 2009). Thus, the significant drop of the photocatalytic activity of the HmP-IX in silica may be attributed to its chemical binding with SiO_2, probably hindering the undergoing intersystem passing from the singlet excited state of porphyrin to its triplet state, which is an important criterion for a photosensitizer and photochemical activation of dioxygen. As a result, concurrent radiative transition from the excited HmP/SiO_2 state to its singlet ground state may occur in the photochemical system.

Maximal yields of all photooxidation products occurred in more non-polar solvents *e.g.* methyl chloride and chloroform. Hydrophilic solvents such as, for example, MeOH are substantially less efficient in terms of their ability to maintain active porphyrins in the sol-gel. A possible explanation is based on the generally accepted rule that singlet oxygen ($^1O_2$) is the primary oxygen agent responsible for photooxidation by light-sensitive catalysts (DeRosa and Crutchley, 2002). In chloroform, singlet oxygen has a relatively long lifetime and thus photooxygenation resulting faster in comparison with other solvents (Hurst et al., 1982).

However, the GC-MS analysis revealed that the products (pinocarvone, *trans*-pinocarveol and myrtenol were rather not directly obtained in the ene type reaction characteristic of light promoted singlet oxygen reactions where $^1O_2$ reacts with olefins causing a double bond shift and hydroperoxides or alcohols formation (Kenney & Fisher, 1973; Ribeiro et al., 2008). In this context, the behaviour of the photocatalysts encapsulated in silica gel toward α-pinene is unusual and can not be compared to that of several groups of porphyrin photocatalysts and hem iron biocatalyst, which afford the mixtures of hydroperoxides with pinene oxide and verbenol or the pinocarveol and myrtenol as main products after the reduction of the corresponding hydroperoxides (Ribeiro et al., 2008).

In the photooxidation reactions a free radical chain mechanism is also suggested and in this case, competition between the abstraction of the allylic hydrogen to give allylic oxidation products and the addition of the alkylperoxy radical to the double bond resulting in epoxide products is expected. A broad product mixture usually arises from typical radical-initiated autooxidation (Henning et al., 1990; Nakagaki et al., 2004). Since we obtained merely the ketone and alcohol with no hydroperoxides and neither epoxide nor corresponding glycol derivatives being detected in the reaction solutions, we suggest that the cooperative mechanism of α-pinene photooxidation based on two possible routes participates in phototransformation of pinene by porphyrins in silica gel. The first pathway involving electron abstraction by the light-excited OEP/SiO_2 matrix can lead to the formation of minor components during a radical autoxidation mechanism, while the most abundant compounds - pinocarveol and pinocarvon are formed through a photosensitized reaction with singlet oxygen (either by electron transfer from pinene to $^1O_2$ or via a radical mechanism).

Photophysical properties, photodegradation and aggregation changes may have a possible effect on the profile and yields of photooxidation products of substrate (Karapire et al., 2005). The biocatalytic efficiency of porphyrins entrapped in the sol-gel matrix was found to be positively correlated with their intensity of fluorescent emission and absorbance over the range of H_2OEP concentration studied in sol. The maximum turnover number (defined as a mole of product to a mole of porphyrin per hour) was observed to be reached at 10^{-6} for OEP and 10^{-5} for the HmP-IX (**Tab. 1**). Close to these H_2OEP concentrations, the largest luminescence intensity and sharpness of the relative Soret band was noticed, which ruled out the formation of porphyrin aggregated species, in contrary to the larger amount of catalysts in the silica medium. This is in an agreement with the more general rule that the formation of a dication and dimers or higher order aggregates decreases photoactivity of the photosensitizers (Gerdes et al., 1997; Iliev et al., 2000; Tanielian et al., 2001) as the presence of porphyrin stacking does not allow an optimal interaction of the pinene with macrocycles (Paolesse et al., 2002).

Concentration of H_2OEP in sol (M)	Concentration of products (g/L)			Turnover number		
	pinocarvone	*trans*-pinocarveol	myrtenol	pinocarvone	*trans*-pinocarveol	myrtenol
1×10^{-6}	0.087	0.024	-	2 285.7	615.3	-
2×10^{-6}	0.206	0.045	-	2 717.1	579.2	-
4×10^{-6}	0.546	0.093	0.06	3 537.4	588.0	383.9
6×10^{-6}	0.96	0.156	0.111	4225.7	670.1	478.0
8×10^{-6}	0.769	0.128	0.086	2 514.2	408.4	277.7
1×10^{-5}	0.856	0.137	0.102	2 212.8	345.6	260.5
1.2×10^{-5}	0.618	0.107	0.058	1 343.0	226.9	124.5
1.4×10^{-5}	0.686	0.116	0.068	1 279.8	211.2	125.3
1.6×10^{-5}	0.974	0.180	0.125	1 575.6	284.2	199.7
1.8×10^{-5}	0.797	0.123	0.068	1 132.1	170.5	110.8
2×10^{-5}	1.3	0.219	0.198	1 666.2	273.9	250.6

Table 1. Effect of initial concentration of octaethylporphyrin in silica on its biocatalytic efficiency in the α-pinene photooxidation.

However, the results show that the yield of all products increased with an increase in H_2OEP porphyrin concentration from 0.087 up to 1.3 mg/L (**Tab. 1**). We suggested that the dimeric complex of H_2OEP in SiO_2 participates in photooxidation of monoterpenes; if not, the catalytic performance should result in much lower yield of products compared with the homogeneous system in which the same moles of the porphyrin were taken into the reaction (**Fig. 18**). Intercalation of silica with H_2OEP may be expected to originate a system in which the catalytic abilities of the two species (dimers and dications) reinforce each other. As a conclusion, we propose a daring hypothetical primary mechanism step of α-pinene photooxidation by H_2OEP/SiO_2. The essential, photoinduced process, would involve transfer of protons from the dimeric form of porphyrin dication to the lone electron pairs of siloxane oxygen (in ≡Si-O-Si≡) or passing protons to the not-bound silanol groups of silica network. The resulting excited porphyrin in the triplet state can then react in one of

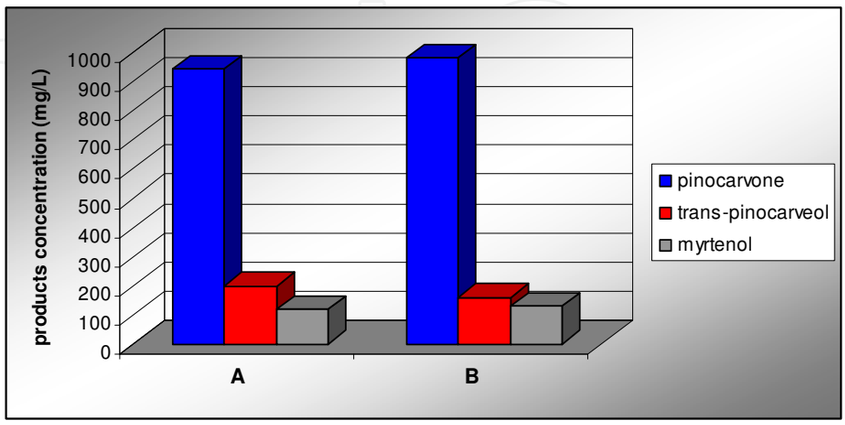


Fig. 18. Comparison of H_2OEP activity in the homogeneous system (A) to the heterogeneous based on the silica immobilized catalyst (B) in the process of α-pinene photooxidation.

two ways, via type I photooxygenation (electron transfer between the excited photosensitizer and the substrate) or II mechanism (energy transfer process during the collision of the excited photosensitizer molecule with triplet oxygen) leading to singlet oxygen generation. These two alternative pathways are well documented in literature (DeRosa and Crutchley, 2002; Griesbeck et al., 2005).

Undoubtedly, the real mechanism of the photooxidation by porphyrin in silica xerogel is more complex, and for example, in the charge transfer inside the silica, the contribution of the holes transport should also be taken into account (Mele et al., 2003).

The kinetics of this process is of the first order in respect to pinene concentration. The straight line dependence of ln (c/co) on time t (where co is the initial concentrations of pinene) proves the first order character of biotransformation reaction. The rate constant K=0.013/h. An independent reactivity experiment corroborated this suggestion, since no oxidative products were detected under typical reaction conditions using *trans*-pinocarveol or myrtenol as the substrates. Therefore one can conclude that all three products: *trans*-pinocarveol, myrtenol, pinocarvone are the result of independent biotransformation reactions of pinene at different oxidation rates. Based on the concentrations of the products of biotransformation the following interesting straight line dependences were found: log[*trans*-pinocarveol] vs log [myrtenol], log[pinocarvon] vs log[myrtenol], log[*trans*-pinocarveol] vs log[pinocarvon]. Hence, it is possible to evaluate the concentration of one product based on the other one. Of course, this will not be an accurate evaluation, since it is difficult to maintain identical reaction conditions, i.e. the same gel, its porosity, and the mixing rate of phases.

The correlation between the photoactivity of free-base porphyrins in organic solutions and those entrapped in the silica matrices indicates that the strategy of incorporation of different porphyrins into the transparent matrix was successful, as the photoactivity of water insoluble H_2OEP in the heterogeneous system was exactly the same as in the homogeneous one (**Fig. 18**). The supplementary characterization of silica pores dimension by the N_2 adsorption method enabled the appreciation of porphyrins encapsulation inside the sol-gel matrix structure and access of the reagents to the active site of catalyst.

Similarly, the high biocatalytic efficiency was achieved for water-soluble H_2TMePyP/SiO_2 with no leaching of the porphyrin complex, followed by retaining catalytic activity in the sol-gel-matrix, when the catalyst was reused. This occurred despite the fact that silica matrix polarity and structure with part of the closed pores might hinder the access of oxygen and pinene to the catalytic site, and diffusion of the oxidation products into the reaction medium. This stability was not only due to physical entrapment but also to additional multiple interactions with SiO_2 through hydrogen, ionic or hydrophobic interactions. The question remains whether the improved photostability of these catalysts is a factor of their immobilization, or simply a result of lower singlet oxygen yields.

Porphyrins in the silica gel work as photoactive catalysts in the oxidative transformation of monoterpenes into the products attracting great interest by the fragrance and flavour industry. Pinocarvone, pinocarveol and myrtenol, the compounds of large commercial value, were the products obtained in the biomimetic oxidation of α-pinene by all porphyrins photoactive in the silica gel, as confirmed by the GC-MS analysis. These structures were previously formed in the α-pinene biotransformation with the use of genera of *Basidiomycetes* and with the intermediates of enzyme degradation pathways of α-pinene occurring in some bacteria strains such as *Pseudomonas putida*, *P. fluorescens* or in the case of *Bacillus pallidus* (Busman & Berger, 1994; Savithiry et al., 1998). Therefore, the catalytic

activity of the examined porphyrins in the sol-gel matrix bears resemblance to enzymatic catalysis involving α-pinene monooxygenase and pinocarveol dehydrogenase.

7. Conclusions

The presence of the porphyrins as the active site of many natural enzymes makes them enticing targets for biocatalysis. Many attempts have been made to design the active site analogues of heme monooxygenases. The huge number of studies performed on iron porphyrin models and on hemoproteins themselves had a strong impact on the elucidation of the enzymatic reaction mechanisms in the catalytic pathway of monooxygenases. Besides the important role they have played in understanding these complex enzymes, porphyrins have allowed to develop many efficient bioinspired catalysts for a variety of oxidation reactions that are of importance for many different industrial processes. The biomimetic oxidation of hydrocarbons by porphyrins under condition of homogeneous catalysis compares very favourably in the efficiency with that catalyzed with enzymes. However, the attempts at process implementation and scale-up to pilot or industrial scales have not been successful yet.

A challenging problem in this area is the construction of the well organized biomimetics showing high regio- and stereoselectivity as well as stability. The development of heterogeneous catalysts, with two types of porphyrins (water-soluble and water-insoluble) as the catalytic units incorporated into the silica gel would allow overcoming some of these limitations with the challenge of improving the catalytic activity and stability. As shown the sol-gel process has proved to be a valid methodology to produce a transparent and stable, designed luminescent porphyrin-based hybrid material, which can be shaped not only for the application in the fields of biomimetic catalysis for the enzyme like cytochrome P450 but also for the electroluminescence, light emitting diodes and lasers, as mentioned in the literature. Although the product analysis has only been conducted for pinene, the catalytic activity extends to a large variety of olefins including terpenes.

From the Raman, FTIR and UV-Vis data it was concluded that the porphyrins form a complex with silica, probably by the hydrogen bond formation of protonated porphyrin molecules, e.g. monomers or dimers of H_2OEP with the Si-O-Si or Si-O groups in the pore structure. The dimerization process in SiO_2 alcogel is advanced for the H_2OEP case whereas for HmP IX the lack of dimerization was observed.

As follows from the comparison of biocatalytic activity of H_2OEP and HmP IX the main goal for the preparation of porphyrin-introduced silica gel, which may be efficiently used in biocatalysis, should be based on the selection of porphyrin with the weak molecular interaction with silanol or siloxane groups; therefore the resulting excited state conformation would facilitate the intersystem crossing from a singlet excited state of porphyrin to its triplet state which, in turn, would affect photocatalyst functionality and therefore oxygen activation, responsible for the oxidation reaction.

The aggregation of the porphyrins is unfavourable for the biocatalytic process. Therefore based on the excitation spectrum of the porphyrins in xero gel it may be possible to select the range of porphyrins concentration where the single Soret band is observed instead of two, that are characteristic of H- and J-aggregates and therefore to obtain the acceptable yield of biocatalytic conversion with these concentrations of catalyst. This statement is valid for biotransformation of α-pinene in the H_2OEP/SiO_2 system where the appearance of Soret band splitting at 10^{-5} mol/dm^3 of H_2OEP accompanied the decrease of its biocatalytic

activity. The confirmation of correlation between the character of the excitation spectra of other porphyrins and their catalytic activity is necessary.
While spectral characterizations have provided considerable insight into the porphyrin-host matrix interaction and the relationship between the structures and the spectroscopic behaviour of porphyrins entrapped in silica matrices, more sophisticated experimental and theoretical methods are needed if the effect of free-base porphyrins immobilization on the changes of their photophysical properties, photodegradation or aggregation, as well as a photocatalytic activity in various organic solutions are to be more fully understood. These studies surely will allow take one step closer to the directed oxidation of organic compounds with dioxygen in the kinetically controlled way.

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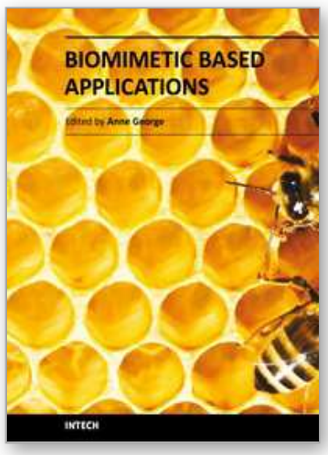

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The interaction between cells, tissues and biomaterial surfaces are the highlights of the book "Biomimetic Based Applications". In this regard the effect of nanostructures and nanotopographies and their effect on the development of a new generation of biomaterials including advanced multifunctional scaffolds for tissue engineering are discussed. The 2 volumes contain articles that cover a wide spectrum of subject matter such as different aspects of the development of scaffolds and coatings with enhanced performance and bioactivity, including investigations of material surface-cell interactions.

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