

# Ship-in-bottle porphyrin – zeolite nanomaterials

V. FARAON<sup>a,b,\*</sup>, R. M. ION<sup>a,b</sup>

<sup>a</sup>Valahia University, Unirii Blvd. 18-20, 130024, Targoviste, Romania

<sup>b</sup>ICECHIM, 202 Splaiul Independentei, 060021, Bucharest, Romania

In this paper, some metallo-porphyrins, like Co(II)-5,10,15,20-tetra-p-phenyl-porphyrin (CoTPP) and Co(II)-tetramethylphenylporphyrin (CoTMPP), were synthesized and encapsulated in different zeolites (X-71, MCM-41 etc.). In the encapsulated form, they exhibit photocatalytic properties and can also be used in the photodynamic therapy of cancer. The synthesized compounds were analyzed by means of some analytical methods, the most important being UV – spectroscopy and X-ray diffraction. Some preliminary photodynamic experiments of these new systems have been achieved (viability and proliferation) on cellular line (EL-4 and K562), by using He-Ne laser.

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## 1. Introduction

Heterogenization is achieved either by encapsulating the metal complex inside the pores of zeolites or by anchoring or tethering them to inert supports (Fig. 1). Grafting and tethering refer to covalent attachment of the metal complex, either directly (grafting) or through a spacer ligand (tethering). The encapsulation (ship-in-a-bottle) approach is convenient and ideal because the complex, once formed inside the cages of the zeolite, is too large to diffuse out and is not lost into the liquid phase during the reaction. As these composite materials mimic biological enzymes, they are also called “zeozymes” (acronym for zeolite mimics of enzymes) [1,2]. On confinement in the zeolite matrix, the metal complex may lose some of its degrees of freedom and adopt unusual geometries that are stabilized by coordination to the zeolite-surface functional groups. In a general sense, the encapsulated complexes mimic enzyme systems in that the porous inorganic mantle (similar to the protein mantle in enzymes) provides (hopefully) the right steric requirement for the metal complex and imposes certain requirements (based on size and shape) to the access of the active site by the substrate molecules (substrate selectivity). Though many porous materials have been used, the most popular ones have been zeolites X and Y possessing large  $\alpha$ -cages ( $\sim 12\text{\AA}$  diameter)<sup>3</sup>.

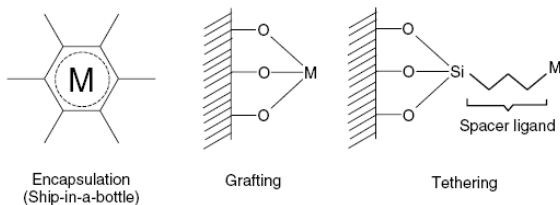


Fig. 1. Methods of heterogenization of homogeneous catalysts (M - metal ion or complex).

One of the most attractive properties of zeolites are their well organized nanopores and nanochannels which serve readily as supporting hosts for various molecules. Encapsulation of catalytically active transition metal complexes inside the nanopores of zeolites, often referred to as ‘ship-in-a-bottle’ systems has been believed to be one of the most promising strategies in the development of viable industrial catalysts [4,5]. Extensive effort has been devoted to the synthesis and catalytic properties of zeolite entrapped metallocomplexes with such ligands as bipyridine, salen, polyamines, porphyrins and phthalocyanines. In most of these syntheses, a so-called ‘assemble-shipinside-bottle’ approach has usually been adopted in which the desired catalytic metallocomplex is synthesized inside the nanopores/channels of the zeolite in the presence of an excess of ligand or its synthetic precursors. As a consequence, what was usually obtained is a mixture of the desired complex with unreacted free ligand as well as side-products. For example, the synthesis of metalloporphyrin (MP) inside zeolites often leads to a significant portion of undesired polymerization product and the unmetallated free base porphyrin ligand [4,6,7].

The preparation of efficient and selective catalysts for the oxidation of organic molecules in mild conditions is one of the biggest challenges for the chemical researcher. A possible strategy is to synthesize a catalyst inspired on enzymatic systems, in many cases formed by macrocyclic structures, which are used by living organisms for selective oxidations [8, 9]. These natural macrocyclic compounds are highly selective and efficient catalytic systems. The use of synthetic metalloporphyrins as bioinspired model compounds of cytochrome P450 to obtain solids with potential homogeneous catalytic activity has attracted a lot of interest over the last decades. However, depending on the structure of the porphyrin ring, some destruction of the catalyst via an autooxidation reaction is observed during the catalytic reactions. One approach to reducing metalloporphyrin self-destruction and promoting catalyst reuse is metalloporphyrin immobilization or encapsulation into solid supports such

as silica, zeolites, cationic exchanger montmorillonite clay, silanized kaolinite, intercalated/delaminated kaolinite, raw halloysite, chrysotile, among other matrices. Moreover, catalyst immobilization and the use of the resulting solid as heterogeneous catalyst offers many advantages: the rigid and highly organized inorganic supports mimic the cavity of the natural enzymes, characterization of the immobilized metalloporphyrin is facilitated, the approach of undesirable activated catalytic species and nonactivated species is prevented, the heterogeneous catalyst can be reused, and a hybrid material that can promote specific selectivity to the catalytic reactions can be obtained [10,11,12]. Selective catalytic materials may result from controlling the formation of either the pore structure of the support or the three-dimensional network of the matrix. In fact, particle morphological control is one of the major challenges regarding the industrial use of silica. Upon immobilization, the homogeneous active species is transformed into a heterogeneous catalyst, while its selectivity and high activity are retained. In this context, amorphous aluminosilicates are a promising support for metalloporphyrin immobilization [13,14].

Amorphous aluminosilicates have been widely employed for a few decades. They have been reported as being good adsorbents, due to their high porosity and large superficial area<sup>8</sup>. A schematic representation of an iron porphyrin immobilization process is presented below (Fig. 2):

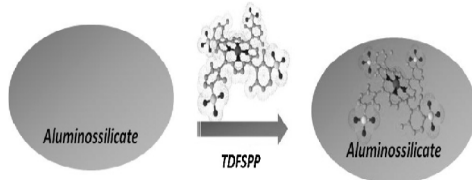


Fig. 2. A schematic representation an immobilization process in the presence of 5,10,15,20-tetrakis (2,6-difluorophenyl-3-sulfonatophenyl) porphyrin (TDFSPP).

Cationic porphyrins have been extensively studied as template in the synthesis of molecular sieves, such as aluminosilicates, aluminophosphates and galophosphates. These porphyrins also have the ideal solubility and are stable under the conditions of both molecular and hydrothermal synthesis [15,16]. One of the methods that attracted a lot of attention consists on the hydrothermal synthesis of faujasite type zeolites in the presence of MeP, referred to as “build-bottle-aroundship” approach. This model is proposed as a complete mimic enzymatic of the inorganic matrix, where the substrate is activated by the porphyrin catalytic centers [15].

## 2. Materials and methods

### 2.1. Materials

For the synthesis of the porphyrins and encapsulated porphyrins discussed in this paper, the following reagents were used:

- pyrrole (Merck), distilled before use;
- benzaldehyde (Merck);
- propionic acid (Aldrich);
- methanol (Chimopar PA);
- acetone, (Chimopar PA);
- Cobalt (II) acetate (Chimopar).
- molecular sieves (MCM-41, AIMCM – 41, X – 71).

### 2.2. Apparatus

1. UV-Vis adsorption spectrophotometer SPECORD M400, Carl Zeiss Jena, with a double beam and microprocessor;
2. IR spectrophotometer, Carl Zeiss Jena, KBr pelettes technique;
3. X-ray DRON CoK $\alpha$  X-ray diffractometer.

### 2.3. Synthesis of 5,10,15,20 – Tetra – p – phenyl – porphyrin (TPP)

Over 300 ml of pure propionic acid were added 5,6 ml freshly distilled pyrrole (0,08 moles) and 8 ml of benzaldehyde (0.08 moles). The mixture was refluxed for 30 minutes and, after that time, it was cooled slowly over night. The TPP crystals were then vacuum – filtered, washed two times with 200ml methanol – acetone portions (10 – 1) and again with two 200 ml methanol portions. The yield was around 54%, with 9% chlorine as impurities. After purification with 2,3-dichloro-5,6-dicyano-benzoquinone, a product with 0.2% chlorine as impurity was obtained. The elemental analysis of the obtained compound shows its purity and uniformity. (Theoretical values: C 85.8%; H 4.97%; N 9.23%; Experimental values: C 85.97%; H 4.92%; N 9.11%).

### 2.4. Synthesis of CoTPP

500 mg TPP were dissolved in 50 ml of pyridine. In the obtained mixture 500 mg of cobalt acetate were added, and the mixture was heated for one hour. After cooling 500 mg of blue – green crystals were obtained. The mixture was recrystallized two times by extraction with 40 ml benzene and 1 mole of pyridine. The preliminary analysis of the dried product was made by elemental analysis (Theoretical values: C 56.41 %; H 35.89% N 5.12%; Co 1.28%, Experimental values: C 56.6 %; H 35.56% N 5.5%; Co 1.134%).

The structures of both porphyrins are shown in Figs. 3a and 3b.

Four purification methods were applied for the above mentioned compounds:

- repeated washes with a methanol – acetone mixture.
- chromatography on a 1 meter column, filled with third degree Brockman alumina.
- treating with dicyano-dichloroquinone (DDQ), which has the capacity to oxidize the existing chlorine.

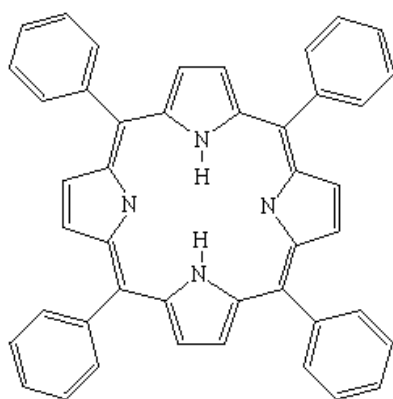
- recrystallization in an acetone – methanol mixture.

A comparison between these methods, regarding the chlorine concentration, is shown in the Table 1.

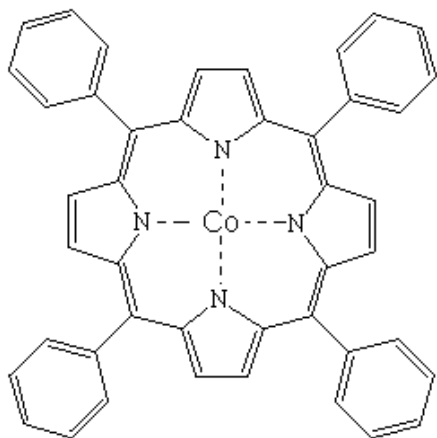
Table 1. Percent of tetraphenylchlorine (TPC) in TPP samples\* after purification.

No.	Purification method	Chlorine content(%)
1.	washing	2,13
2.	chromatography	2,17
3.	DDQ treating	0,44
4.	recrystallization	4,15
5.	unpurified	9,91

\*observation: samples 1 – 4 are from different batches.

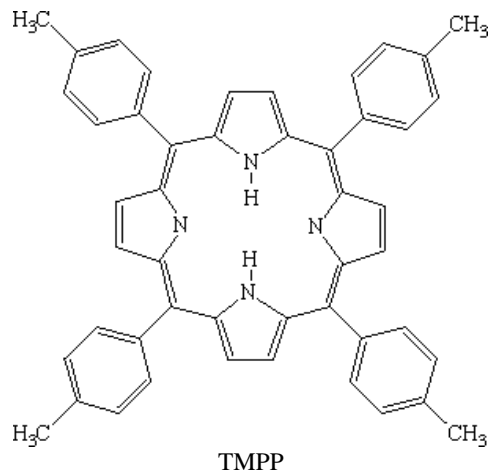


TPP

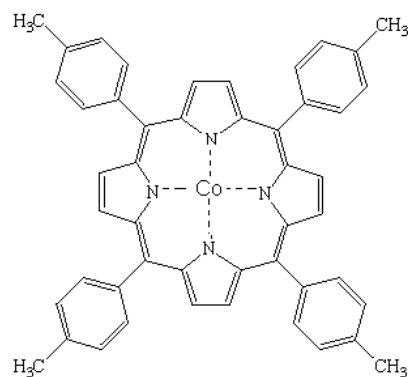


TPP-Co

Fig. 3a. The chemical structures of TPP (up) and TPP-Co (down).



TMPP



TMPP-Co

Fig. 3b. The chemical structures of TMPP (up) and TMPP-Co (down).

## 2.5. Synthesis of Co(II)tetramethylphenylporphyrin (TMPP) encapsulated in a X – 71 zeolite

1 g of X-71zeolite, dried before at 120 °C for 48 hours, was introduced into a 0.025M solution of cobalt acetate, and the mixture was heated at 80 °C for 12 hours, as for the ionic exchange Na – Co to take place. After weighing, the product was introduced into a round – bottomed glass, in which were introduced 5.6 ml of freshly distilled pyrrole, 4.4ml acetaldehyde and 200 ml methanol. The mixture was kept under reflux for 1 hour. A change of colour was observed, from pale pink, in case of the zeolite treated with cobalt acetate, to pale blue, for the dried zeolite, to lavender and brick red for the zeolite with porphyrin. The product was investigated by UV-vis spectroscopy and X-ray diffraction.

## 2.6. Encapsulation of CoTPP in a mesoporous zeolite (MCM-41, ALMCM-41)

1 gram of MCM-41/AlMCM-41 was introduced in a solution of 10<sup>-4</sup>M CoTPP in benzene. The mixture was left about one hour, after which it was filtered, and then the

filtered product dried. Testing of the adsorption of the metalloporphyrin onto the mesoporous zeolites was made by registering the UV-Vis absorption spectrum of the obtained compound. The product was also analyzed by X-ray diffraction.

## 2.7. Biological tests

**Cell cultures:** K562 lymphoblastic human cell line (supplier ATCC Nr.CCL-243), derived from chronic leukemia and EL4 murine lymphoblastic cell line (*ECACC Nr.85023105*) have been used. Cells were maintained in culture or cryopreserved as stated by the supplier. Cryopreservation was performed as stated by the supplier and before utilization cells were maintained in culture at least 3 consecutive passages. In all the PDT experimental systems the cell line was used in RPMI1640 complete medium without phenol red in order to avoid the interference with the detection methods.

**Cell viability:** Cellular viability was assessed as cell membrane integrity with Cytotox96 Non-Radioactive Cytotoxicity Assay kit (Promega), test that quantifies the lactate dehydrogenase (LDH) release from intact cells. Results are expressed as indexes compared to control (LDH index).

**Cell proliferation:** The capacity of tumoral cell line to proliferate was assessed as number of metabolically active cells with CellTiter 96Aqueous One Solution Cell Proliferation kit (Promega). The test measures the activity of intracellular dehydrogenases based on tetrazolium salt reduction to a spectrophotometrically quantifiable formazan compound. Results are expressed as indexes compared to control (MTS index).

**Activation:** The cell suspensions were irradiated with He-Ne laser (30 mW,  $\lambda = 632.8$  nm, 25°C).

## 3. Results and discussion

The spectrophotometrical analysis of the obtained products is presented in Table 2.

Table 2. Spectrophotometrical analysis of the obtained products.

Porphyrin	Wavelength (nm)			Molar absorption coefficient x 105 (M-1cm-1)		
	Soret	$\beta$	$\alpha$	Soret	$\beta$	$\alpha$
TPP	420			420		
TPP-Co	714	845	116	307,1	0043,0	0780,0

The encapsulation of CoTPP in these mesoporous zeolites is deduced firstly by the bathochromic shift of the absorption maximums from 626 nm, 669 nm and 692 nm, and by the band hypsochromicity decrease of intensity from 521 nm. This can be attributed to the change of the tetrahedral geometry by displacing the water or oxygen molecules from the zeolite structure and replacing them with CoTPP. The 675 nm band of the intrazeolitic complex is bathochromically shifted in comparison with the free one (640 nm). The impregnated mesoporous support has an additional band at 795 nm, which is probably associated with the formation of aggregates on the surface. The UV-vis analysis of the encapsulated CoTPP into MCM zeolites gave specific spectra.

Also, to underline the formation of CoTPP inside the X-71 zeolite, an X-ray diffraction was made. From the analysis of these two spectra, the presence in the cavity of the zeolite of a macrocyclic organic compound is observed, but also the presence of Co from the porphyrin structure. The zeolite used was an X-type zeolite, with a FAU topological structure. This zeolite was selected because of its big free volume (0.5cc/cc) determined by a system of large cavities and channels (7.4 Å), this meaning that it belongs to class of zeolites with large pores, having a greater accessibility for the larger molecules.

The X-ray diffractogram initially shows a well crystallized X type zeolite, with a cubical symmetry. The

calculated network constant for the initial sample was  $a_0 = 25,0995 \text{ \AA}$ . From the volume of the elemental network the SI/Al molar ratio can be estimated, having a value of 1.027, which is in the typical range of the X-type zeolite. After the encapsulation of CoTMP, the diffractogram suffers some changes. It is observed, on one side, a slight shift of the diffraction lines towards smaller angles, showing a dilatation of the elemental cell volume by the presence of a big molecule. It was obtained a bigger value of the network constant,  $a_0 = 25,2638 \text{ \AA}$ .

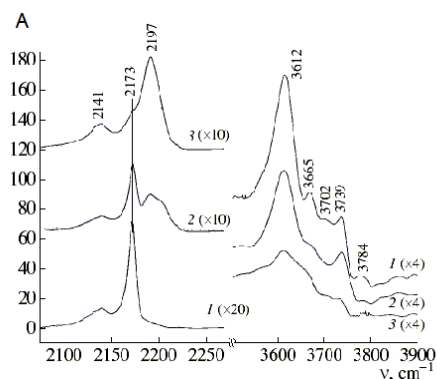


Fig. 4. Vibrational spectra and the diffusion reflectance of AIMCM-41(1), MCM-41 (2) and for CoTPP/AIMCM-41 (3).

The most visible modification is the lowering of the intensities of the diffraction lines, especially to the diffraction lines at smaller angles. This lowering of the intensities shows, in the case of zeolites, the incorporation of a large molecule. Intensity ratios can be used to characterize the filling degree of the zeolite. In this way, the intensities ratio of the diffraction lines corresponding to the planes with Miller indexes 533 and 111,  $I_{533}/I_{111}$  can be such an indicator, the value of this ratio indicating the filling ratio of the pores. In this case, with the encapsulated porphyrin, the ratio was  $I_{533}/I_{111} = 0,943$ , compared with the ratio of the initial sample, of 0.267.

It was concluded that CoTMPP is stabilized inside the zeolite because (1) the size of the metallo-complex formed in the zeolitic cavities is greater than the pore opening in such a way that the complex is closed inside the pore ("ship-in-bottle"), and because of (2) the interaction between the functional groups of the complex with some species from the zeolite wall, which bind the complex to the zeolite.

In the IR spectra, the C= bonds shift from  $1417$  to  $1448\text{ cm}^{-1}$ , additional bands appearing at  $1404$ ,  $1387$ ,  $1306\text{ cm}^{-1}$ . This indicates a change in symmetry of the encapsulated molecule. For a series of encapsulated porphyrins in MCM type zeolites, the encapsulating efficiency rises along the series  $\text{AIMCM-41} > \text{MCM-41}$ . MCM-41 has a mono-dimensional structure, with uniform pores. The pore diameter varies between 15 and  $100\text{ \AA}$ .

After encapsulating CoTPP in mesoporous zeolites of the MCM type, the following X-ray diffractograms were made (Fig. 5, Fig. 6):

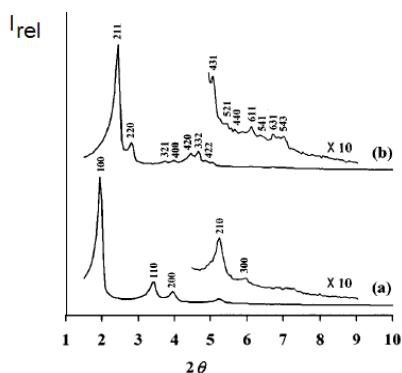


Fig. 5. X-ray diffractograms of AIMCM-41(a) and MCM-41(b).

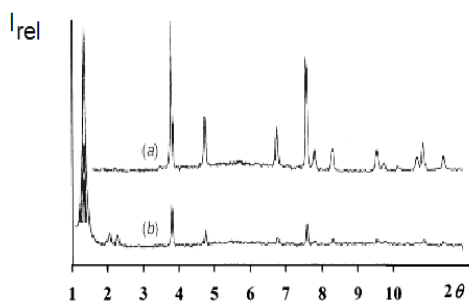


Fig. 6. X-ray diffractograms of CoTPP encapsulated in AIMCM-41(a) and MCM-41(b).

From biological point of view, for EL-4 cells the proliferative capacity has been tested (Table 3), proving that for CoTPP/AIMCM-41, this parameter is continuous decreasing up to  $100\text{ mg/ml}$ . For K562 cells, the membrane integrity (LDH) has been tested, observing that this parameter decreases after 48h after irradiation, and an increased LDH values (Fig. 7).

Table 3. Proliferative capacity of EL-4 cells in the presence of CoTPP/AIMCM-41.

Concentration (mg/ml)	5	15	25	50	100
COTPP/AIMC M 41	0,79	0,051	0,001	0,057	0,002

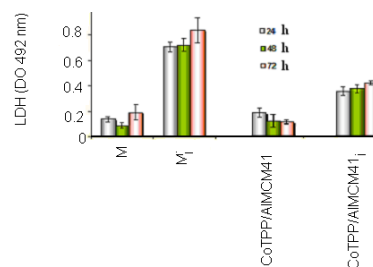


Fig. 7.

#### 4. Conclusions

In this paper some porphyrins (as the organic part) were synthesized by encapsulating them in supports with controlled porosity of the zeolitic type, with large pores (X-71) and mesoporous (MCM-41, AIMCM-41) (as inorganic parts). The results were directed towards the synthesis and physico-chemical characterization of some free-based porphyrins and metalloporphyrins, but also of encapsulated porphyrins into the zeolites. The hybrid materials that were obtained have special properties in the biological domain useful in cancer therapy, the most active being CoTPP/AI-MCM41.

#### Aknowledgemets

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\*Corresponding author: victor.faraon@gmail.com