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EFFECT OF VANADIUM ON NEW SILSESQUIOXANE-NANO SILICA ENCAPSULATED BIO-ACTIVE COMPOUNDS WITH POTENTIAL FOR BIO-MEDICAL APPLICATION

Rodica-Mirela NIȚĂ[1], Ioana LACATUŞU[2], Nicoleta BADEA[3], Cornelia NICHITA[4], Aurelia MEGHEA[5]

În lucrarea de față sunt prezentate efectele induse de vanadiu în procesul de încapsulare asupra fluorescentei extractului vegetal de flori de begonia într-o matrice nano-silicică. Efectele structurale şi electronice induse de compuşii cu vanadiu au fost determinate cu ajutorul metodelor spectroscopice. S-au observat efecte speciale de conjugare şi delocalizare elecronică induse de vanadiu matricei nano-silicice datorate interacției specifice stabilite, efecte care pot fi corelate cu potentiale aplicatii medicale.

Into the present article are presented the vanadium induced effects on the fluorescence effect on begonia vegetal extract encapsulated into nano-silica. Structural and electronic effects induced by vanadium compounds have been revealed by spectral measurements. Special electronic conjugated effects of vanadium promoted by nano-silica hosts on the specific interaction between vegetal extract and vanadium compound have been observed that could be correlated with their potential for bio-medical application.

Keywords: nano-silica, vanadium compounds, electronic effects

1. Introduction

Free oxygen radicals seem to be the key factors acting in a still unclear certain pathologic mechanisms. They are apparently involved into different cancer pathology development stages [1], have an important role in launching and subsequent development of athero-sclerosis [2, 3], similar destructive mechanisms in inflammatory damages associated with asthma disease and by oxidation of lens proteins being suggested as promoters of cataract [5, 6].

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[1] PhD Student, Dept. of Applied Physical Chemistry and Electrochemistry, University POLITEHNICA of Bucharest, Romania, e-mail: Rodica.Pop@parexel.com

[2] PhD, Dept. of Applied Physical Chemistry and Electrochemistry, University POLITEHNICA of Bucharest, Romania

[3] PhD, Dept. of Applied Physical Chemistry and Electrochemistry, University POLITEHNICA of Bucharest, Romania

[4] National Institute for Chemical-Pharmaceutical Research & Development, Bucharest, Romania

[5] Prof., Dr., Department of Applied Physical Chemistry and Electrochemistry, University POLITEHNICA of Bucharest, Romania, e-mail: a.meghea@gmail.com

There are some important researches focused on developing products with a high level of antioxidant effect, but only few prospective clinical studies in humans have predicted therapeutical efficiency *versus* placebo or related numbers of adverse effects. Most of them are considering high antioxidant potential of flavonoids – natural active compounds class, isolated from plant metabolism and having different phenolic structures [7-9]. Free oxygen radicals may induce some important autoimmune destructive mechanisms at the level of β cells [10].

Vanadium compounds are relatively well known both as free oxygen radicals generators in some inorganic and bio-reaction mechanisms [11, 12] and as complex activators or inhibitors of many enzymes involved in carbohydrate or lipid metabolic pathways. Vanadium seems to increase insulin receptor substrat -1 phosphorylatio, acting as an insulin-sensitizing agent. The specific action mechanism for vanadium species involved in insulin receptors modification is still unknown and a lot of efforts are invested by clinical and biological research in solving this problem [13-15].

The aim of present research was synthesis of a new class of nano-sized colloidal encapsulated compounds with pharmacologic potential, using active principles from flowers of begonia plant. Special fluorescent properties of some encapsulated vegetal active substances, induced by vanadium sub-oxide species effect, used as co-activators have been considered as first step in evaluation of biocompatibility and biomimetic degree of obtained nano-material.

Nano-silica based carrier is not a food source for micro-organisms, is not toxic and it is biologically inert material. A positive role of vanadium, widely used in animal models and human diabetes, acting as an insulin-mimetic drug was expected. Vanadium compounds are relatively well known as complex activators or inhibitors of many enzymes involved in carbohydrate or lipid metabolic pathways [16-18].

The key point for the design of new hybrids encapsulated materials is to extend the accessibility of the inner interfaces for including a new pre-designed vanadium complex able to remain bonded at the surface in the active bio-mimetic conditions and to transfer its electronic effect to the active reaction center. The nature of the interface or the nature of the links and interactions that the organic and inorganic components exchange has been used to design these hybrids.

The success of this strategy is also clearly related to the ability to control and tune hybrid interfaces.

2. Experimental

Biological active principles were obtained from a vegetal extract sampled from flowers of begonia plant. An amount of 85 g of petals were subject of a continuous extraction process in 100 mL of ethanol. The extraction was completed after 24 hours, extracts was filtered and concentrated under vacuum.

A classical polymeric sol-gel method using tetraethylorthosilicate (TEOS), water and ethanol in a molar ratio of 1: 2: 3.8 and nitric acid as catalyst (pH ~ 3) has been used. A silsesquioxane silica precursor – octaisobutiltetraciclo [7.3.3.15,11] octasiloxan-*endo*-3,7-diol was selected as primary ordered silica precursor for begonia active compounds encapsulation, – in a molar ratio 0.053 : 1 with TEOS.

$VOSO_4$ was selected as a co-activator of begonia active compounds, in a standard ratio 0.15 mg per 1 g silica compound (identical with standard treatment dose in patients with diabetes).

UV-VIS-NIR spectra were recorded between 200 and 1850 nm using a Jasco double – beam V570 Spectrophotometer. The samples were illuminated with a 260 nm excitation light and the fluorescence emission was collected at 305 nm. Fluorescence assays were performed on solid samples of native extract and encapsulated extract in the inorganic silica and hybrid silica-silsesquioxane networks. Vanadium species evolution during sol-gel preparative steps has been monitored by ESR spectroscopy, JES-FA series, using 9445.023 MHz frequency for a 350.401 mT field centre.

An optimization step of the synthetic process was necessary in order to obtain reproducible results and especially to preserve the specific properties and activities of encapsulated compounds [24]:

- hydrolysis initiated under acid catalysis,
- to avoid possible flavonoid extract degradation the hydrolysis was performed separately: at acid pH the alkoxide can be hydrolyzed in the absence of the biomolecule and without causing a rapid condensation of silicic acid.

3. Results and discussion

UV-VIS-NIR spectra (table 1) registered for humid and dry gel hybrid materials show two main bands associated with V^{4+} Td (tetrahedral coordination) and Sq (square pyramidal coordination) species and both are result of a charge transfer between metal ion and ligands. The existence of two different vanadium species V^{5+} and V^{4+} in tetrahedral or square pyramidal coordination is a very well literature documented fact for vanadium containing sol-gel materials, together with a possible oxidation of V4+ ions during gel formation steps. After a short exposure of samples to ambient atmosphere, the colour changes from light yellow to dark yellow and an additional peak emerges at 380 nm in the visible spectra. This band increases and at the same time a decrease of specific band at 245 nm is observed.

Table 1

UV-VIS-NIR spectral characteristics

Sample	Charge transfer V^{5+} (Td) → Ls	Charge transfer V^{5+} (Sq) → Ls	Charge transfer V^{4+} (Sq) → Ls	d → d	Flavonoid specific n → π^* transitions	Hydrogen bonds
Encapsulated V- nanosilica	362 s	380 s 431 s	245s 936 s,br	942 sh		
Rutin					304 sw, 394 sh	1520sh
Encapsulated begonia extract – nanosilica					308 shw, 358sh	1602sh
V-encapsulated begonia extract-nanosilica	374 s	463 w	925 s,br		328 sh, 372 sh	1614shb

Abbreviations: s = strong; br=broad; w = weak; m=medium; sh=shoulder

According to literature, band at 380 nm is assigned to V^{5+} species in interaction with water molecules [19, 20] but these are different from possible new formed species which appear in hydrated meso-porous (MCM) samples.

As compared to native rutin, in encapsulated rutin samples a hipsocrom effect of 36 nm was observed, as a proof of encapsulating process with participation of *n* electrons. A similar effect is observed for V-encapsulated begonia extract-nanosilica samples, but the amplitude of hipsocrom effect is higher possibly due to an electronic effect induced by vanadium on extension of conjugation of electronic participating orbital. The aspects related to the structural conjunctions of hydroxyl groups of flavonoidic structure pointed out by NIR bands can be related to possible weak interactions (e.g. hydrogen bonding) with OH groups from surface of silica polymeric network, indicating the appearance of coupling phenomena and a conformational structural rearrangement favourable for transmission and amplification of fluorescence properties.

The fluorescence activity of vanadium, begonia extract and vanadium-begonia samples encapsulated in nano-silica matrices were determined comparatively to that of native begonia vegetal extract, by measurements of fluorescence emission intensity (Fig. 1). All the samples were excited at a wavelength specific for flavonoidic compounds (λ = 260 nm), obtaining two characteristic emissions for native begonia extract and immobilized extract samples, at 306 and ~450 nm.

Fig. 1 shows an intensive response from encapsulated nanosilica samples against natural begonia extract. Even if vanadium has no fluorescent signal at the characteristic emission wavelength, a low fluorescent activity is detected for encapsulated vanadium nanosilica samples. A possible resonance energy transfer phenomenon of fluorescence could be supposed, which involve a nonradiative transfer from a fluorescent donor molecule (nanosilica ordered polymeric chains) to an acceptor molecule in close proximity, which is not fluorescent (vanadium metallic centre), via dipole-dipole interaction.

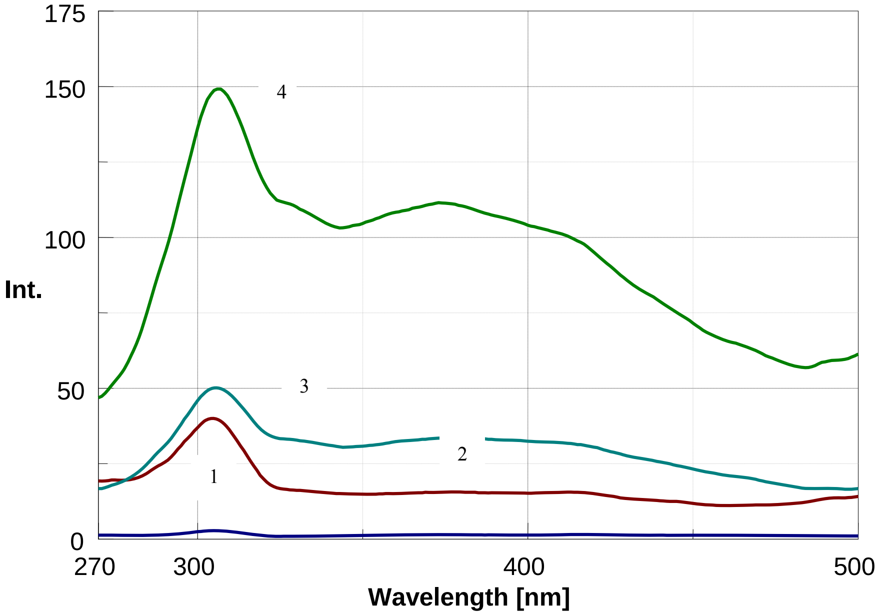

Fig 1. Fluorescence spectra of begonia vegetal extract (1), encapsulated V- nanosilica (2), encapsulated begonia –nanosilica (3) and vanadium-begonia-encapsulated nanosilica (4)

A very important observation is related to nano-encapsulation effect on fluorescence properties of both species vanadium and begonia vegetal extract, the structural confinement induced by encapsulation refines the structural properties of encapsulated species and is favourable to supplementary electronic transfers and conjunctions. A cumulative effect on fluorescence properties of vanadium-begonia nano-silica samples is observed, fluorescence response being by 3 times higher than for begonia encapsulated nano-silica.

IR characterization of vanadium-begonia encapsulated nano-silica (Fig. 2) shows three major peacks associated with vanadium oxide and sub-oxide species, at 1047 and 600-660 cm^{-1}, identified, respectively as a V=O stretching vibration and a V-O-V stretching vibration. A very interesting observation could be associated with the stretching frequencies of the monomeric “V=O species”. Similar to a previous study on IR frequencies of different V_2O_5 alkali promoted species compared to different vanadyl sulfate species [21], the hypothesis of two different monomeric “V=O species” co-existence at nano-silica surface seems to be the most plausible interpretation of the important shift in V=O frequency: the major signal is located at 1047 cm^{-1} (shifted from 1020 cm^{-1} in V_2O_5) with an associated broad shoulder at 966 cm^{-1}.

Considering the fluorescence results, the existence of this shift of monomeric V=O frequencies, could be a consequence of d_π - p_π metal-oxygen interactions which acts to produce a stronger bond, hence a higher ν (V-O), normaly located to 825 cm^{-1}. These influences are considered in literature [21] as a lattice induced modification which enhances the d_π - p_π interaction and especially affects the electron delocalization of the surface.

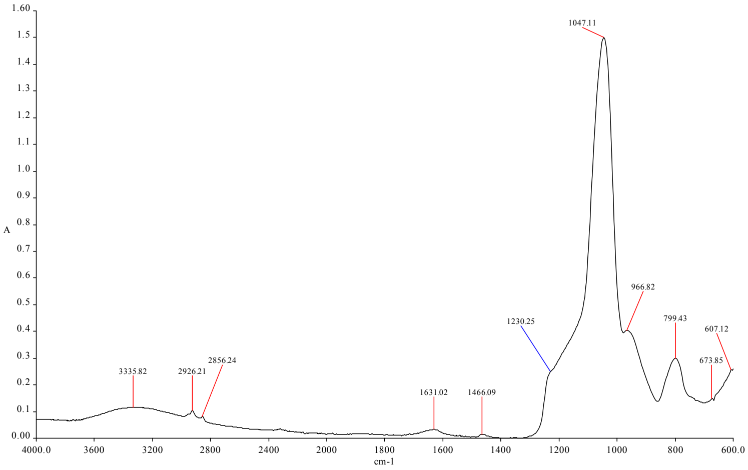

Fig. 2. IR spectrum of vanadium-begonia-encapsulated nano-silica

Thus any lattice modification which enhances the d_π - p_π interactions would be expected to increase bond strength with the corollary that a diminution in d_π - p_π interaction would result in a weaker bond and lower ν (V-O).

However, such electron delocalization could be an explanation for the enhanced fluorescence of vanadium begonia encapsulated nano-silica compounds.

ESR characterization of specific vanadium signal evolution during sol-gel preparation was recorded for different temperature following the preparation process steps (Fig. 3). Since the products were synthesized with a V^{4+} precursor, most of the V^{4+} species were oxidized during synthesis process [22, 23].

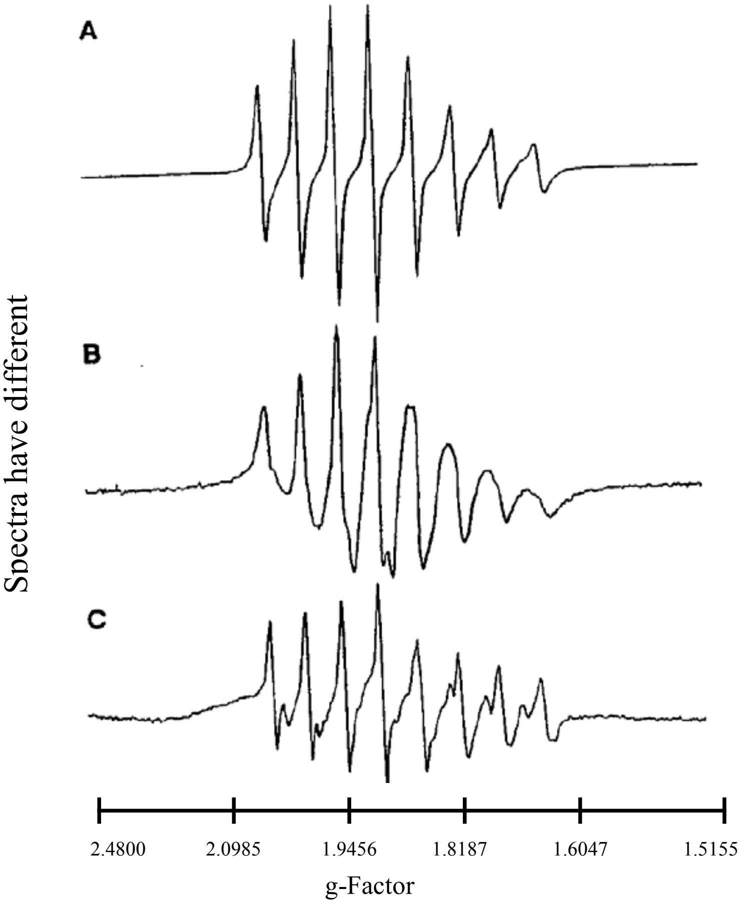

Fig 3. *In-situ* gel formation steps measured by ESR spectroscopy

However, two different V^{4+} sites were observed, as for V-MCM-41 and V-silicalite-1 [19, 20], with g values and hyperfine coupling constants typical for VO^{2+} ions in a square pyramidal coordination.

The spectra presented in Fig. 3 at ambient temperature exhibits an eight-line spectrum with a $g_{\|} = 1.916$ (± 0.001 cm^{-1}) and $A_{\|} = (-114.6 \pm 0.5)$ x10^{-4} cm^{-1} characteristic to monomeric V(IV) species. The spectra evolution under gel formation process shows the presence of possible two similar V(IV) species with different hyperfine splitting constants due to the steric effects of polymeric silica chains formation.

Finally, by corroborating all the spectral measurements there are various evidences on the physical interactions of hydroxyl groups of flavonoidic structure with OH groups from surface of silica polymeric network; these interactions are clear indications on the appearance of coupling phenomena resulting in a conformational structural rearrangement favourable for transmission and amplification of fluorescence properties. In this way, participation of vanadium electronic effects in this possible conformational supramolecular arrangement seems to be substantial for recommending such compounds for bio-medical application.

4. Conclusion

Vanadium compounds have important structural and electronic effects on properties of begonia extract encapsulated materials.

Electronic spectra reveal significant band shifts specific to electronic effects induced by vanadium on extension of conjugation of electronic participating orbital.

NIR and IR domains support the existence of hydrogen bonds established between silica or silica-silsesquioxane hybrid matrices with the bio-active flavonoid compounds existent in begonia flower extract.

ESR spectra of VO^{2+} ions illustrate the evolution in stereochemistry of paramagnetic species during sol-gel preparation process step.

Enhancement of fluorescence after nano-encapsulation of begonia extract within silica matrix is the result of a favourable rearrangement of structural conformation, this being equivalent with biocompatibility conferred by such type of compounds.

One can be concluded, our results show that by nano-encapsulation some specific bio-properties of vegetal extracts could be maintained, while some specific activators could enhance these properties to a level of applicability for health and clinical use.

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REFERENCES

[1] *B. Halliwell*, “Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?” in Lancet , **vol. 344**, 1994, pp. 721–724

[2] *D. Dreher, A.F. Junod*, “Role of oxygen free radicals in cancer development” in Eur. J Cancer, **vol. 32A,** 1996, pp. 30–38

[3] *K.J. Davies*, ”Protein oxidation and proteolytic degradation. General aspects and relationship to cataract formation” in Adv. Exp. Med. Biol., **vol. 264,** 1990, pp. 503–511

[4] *A. Ceriello*, “Oxidative stress and glycaemic regulation” in Metabolism, **vol. 49**, 2000, pp. 27-29

[5] *J. Kuhnau*, “The flavonoids - a class of semi-essential food components: their role in human nutrition” in World Rev. Nutr. Diet, **vol. 24**, 1976, pp. 117–191

[6] *D. Steinberg, S. Parthasarathy, T. E. Carew, J. C. Khoo, J. L. Witztum*, “Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity” in N. Engl. J. Med., 1989, **vol. 320**, 1989, pp. 915–924

[7] *C.A. Rice-Evans, N.J. Miller, G. Paganga*, ”Structure - antioxidant activity relationships of flavonoids and phenolic acids” in Free Radic. Biol. Med., **vol. 20,** 1996, pp. 933–956

[8] *E. Jr. Middleton, C. Kandaswami, T.C. Theoharides*, “The effects of plant flavanoids on mammalian cells: Implications for inflammation, heart disease and cancer” in Pharmacol. Rev., **vol. 52**, 2000, pp. 673–751

[9] *M.G. Hertog, E.J. Feskens, D. Kromhout*, ”Antioxidant flavonols and coronary heart disease risk” in Lancet, **vol. 349**, 1997, pp. 699-705

[10] *P. Knekt, R. Järvinen, R. Seppänen, M. Heliövaara, L. Teppo, E. Pukkala*, “Dietary flavonoids and the risk of lung cancer and other malignant neoplasms” in Am J Epidemiol, **vol. 146**, 1997, pp. 223–230

[11] *L.A. Esposito, S. Melov, A. Panov, B.A. Cottrell, D.C. Wallace*, **”** Mitochondrial disease in mouse results in increased oxidative stress” in Proc. Natl. Acad. Sci. USA ,**vol. 96**, 1999, pp. 4820-4825

[12] *T. Ide, H. Tsutsui, S. Kinugawa, H. Utsumi, D. Kang, N. Hattori, K. Uchida, K. Arimura, K. Egashira, A. Takeshita*, **“**Disruption of inducible nitric oxide synthase improves β-adrenergic inotropic responsiveness but not the survival of mice with cytokine-induced cardiomyopathyin” in Circ. Res., **vol. 85**, 1999, pp. 357-363

[13] *C.E. Heyliger, A.G. Tahiliani, J.H McNeill*, ”Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats” in Science, 1985, **vol. 227**, 1985, pp. 1474–1477

[14] *G. Boden, X. Chen, J. Ruiz, G.D. van Rossum, S. Turco*, “Effects of Vanadyl Sulfate on Carbohydrate and Lipid Metabolism in Patients with Noninsulin - Dependent Diabetes Mellitus” in Metabolism, **vol. 45**, 1996, pp. 1130–1135

[15] *S.M. Brichard, A.M. Pottier, J.C. Henquin,*”Long Term Improvement of Glucose Homeostasis by Vanadate in Obese Hyperinsulinemic fa/fa Rats” in Endocrinology, **vol. 125**, 1989, pp. 2510–2516

[16] *Pugazhenthi, F. Tanha, B. Dahl, R.L. Khandelwal*, “Decrease in protein tyrosine phosphatase activities in vanadate-treated obese Zucker (fa/fa) rat liver” in Mol. Cell. Biochem., 1995, **vol. 153**, pp. 125–129

[17] *J. Anrather, V. Csizmadia, M.P. Soares, H. Winkler,* “Regulation of NF-kappa B RelA Phosphorylation and Transcriptional Activity by p21ras and Protein Kinase Czeta in Primary Endothelial Cells” in J. Biol. Chem., **vol. 274**, 1999, pp. 13594–13603

[18] *N. Sizemore, S. Leung, G.R. Stark*, ”Activation of Phosphatidylinositol 3-Kinase in Response to Interleukin-1 Leads to Phosphorylation and Activation of the NF-kB p65/RelA Subunit” in Mol. Cell. Biol.,**vol. 19**, 1999, pp. 4798–4805

[19] *Z. Luan, J. Xu, H. He, J. Klinowski, L. Kevan*, “Synthesis and Spectroscopic Characterization of Vanadosilicate Mesoporous MCM-41 Molecular Sieves” in J. Phys. Chem., **vol. 100**, 1996, pp. 19595-99

[20] *M. Morey, A. Davison, H. Eckert, G. Stucky*, “Pseudotetrahedral $O_{3/2}V{=}O$ Centers Immobilized on the Walls of a Mesoporous, Cubic MCM-48 Support: Preparation, Characterization, and Reactivity toward Water As Investigated by ^{51}V NMR and UV−Vis Spectroscopies**”** in Chem. Mater., **vol. 8**, 1996, pp. 486-492

[21] *D.V. Fikis, W.J. Murphy, R. Ross*, “Active surface centres in vanadium pentoxide/alkali metal sulphate heterogeneous catalysts for 2-propanol decomposition**”** in Can. J. Chem., **vol. 57**, 1979, pp. 2464-2470

[22] *C.G. Barnaclough, L. Ewis, R.S. Nyhol*, “The stretching frequencies of metal–oxygen double bonds” in J.Cherm. Soc., 1959, pp. 3552-3558

[23] *M.Y. Al-Janab, N.J. Ali. Z.*, “Reaction of Vanadyl Ions with Alkyl Dinitriles” in Naturforsch., **vol. 31a**, 1976, pp. 1696-1772

[24] *I. Lacatusu, N. Badea, R. Nita, A. Murariu, F. Miculescu, I. Iosub, A. Meghea*, - “Encapsulation of fluorescence vegetable extracts within a templated sol–gel matrix - Optical Materials, **vol 32**, (2010), pp. 711–718.