INCLUSION COMPOUNDS OF MONOCHLOROTRIAZINYL-β-CYCLODEXTRIN GRAFTED ON PAPER SUPPORTS

ANA-MARIA GRIGORIU, CONSTANTIN LUCA, NARCISA VRÎNCEANU[*]
and FLORIN CIOLACU

Faculty of Chemical Engineering and Environmental Protection,
"Gheorghe Asachi" Technical University, 71A, D. Mangeron Blvd., 700050, Iasi, Romania
[*]Faculty of Chemistry, "Alexandru Ioan Cuza" University, 22, Carol Blvd., 700090, Iasi, Romania

Received December 20, 2010

Invaluable heritage documents are largely affected by the influence of the environmental conditions, namely light, temperature, moisture and microorganisms. To prevent the damaging activity of these factors, a simple and non-invasive conservation method is the treatment of paper supports with different protective substances.
The present study investigates the efficiency of a grafting treatment using the reactive derivative of a cyclic oligosaccharide, β-cyclodextrin (*i.e.*, monochlorotriazinyl-β-cyclodextrin). Due to their characteristic molecular structure, cyclodextrin and its derivatives may form inclusion compounds with a wide range of guest compounds (for their antimicrobial protective properties, ferulic acid, Michler's ketone and allantoin were selected). The cellulosic support used was Japanese veil.
Grafting of the reactive compound on the cellulosic paper support was realized by a relatively simple pad-dry-cure treatment, under mild conditions, while inclusion of the protective substances – by wet treatment with a guest solution. The treated supports were analysed by Fourier Transform-Infrared Attenuated Total Reflexion Spectroscopy (FT-IR ATR), Scanning Electronic Microscopy (SEM), tensile testing and by antimicrobial tests.

Keywords: inclusion compound, Japanese veil, antimicrobial activity, ferulic acid, Michler's ketone, allantoin

INTRODUCTION

After more than a century since their discovery (1891), cyclodextrins (CDs) are the guest molecules most frequently used, due to their large applicability[1,2] in the pharmaceutical, chemical and food industries, in cosmetics, agriculture, etc. Their main advantages are: availability and capacity of natural renewal of the raw material (starch); a relatively simple green procedure to obtain CDs, at an acceptable price; multiple possibilities of synthesis of CD-based inclusion compounds and derivatives, with minimum toxicity.

Cyclodextrins (CDs) present nanocavities (0.57-0.97 nm in diameter) in which molecules of the same size order may be included. The most advanced studies in this extremely dynamic field refer to nanosciences and nanotechnologies. At present, CDs are used as nanoreactors, nanoprotectors, nanocapsules, drug releasing agents at a nanometric level.[3,4]

Paper treatment with CDs has been scarcely reported in literature.[5-7] The paper is devoted to new aspects of CDs properties – of forming chemical derivatives and inclusion compounds, namely grafting of a reactive CD derivative on paper support and the subsequent inclusion, in the grafted material, of guest compounds with different properties (antimicrobial activity, UV protection, etc.).

EXPERIMENTAL

Materials

Chemically-modified cyclodextrin monochlorotriazinyl-β-cyclodextrin – MCT-β-CD – with a degree of substitution of 0.46 per anhydrous glucose unit, as host) was purchased from Wacker Chemie Gmbh, Germany.

Sodium carbonate (as analytical reagent grade) was obtained from Sigma Chemical Co, Bucharest, Romania. As a *grafting substrate*, Japanese veil (25 microns thick and 15 g/m^2 grammage) from CTS Europe Co., was used.

As *guests*, a few substances (of analytical reagent grade, from Sigma Chemical Co, Bucharest, Romania) with different chemical structures and protection properties were used: cinammic derivatives (ferulic acid – FA – (trans) [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid]), urea derivatives (allantoin – Al – 5-ureidohydantoin or glyoxyldiureide) or ketonic derivatives (4,4'-bis(dimethylamino) benzophenone – Michler's ketone – MK).

Methods

Grafting of MCT-β-CD to a paper substrate was performed according to a modified procedure reported by Grigoriu and Luca,[1,2] to treat linen fibers.

The paper samples were dipped for 20 min or brushed with an aqueous alkaline solution (pH = 10, 60 g/L sodium carbonate) of MCT-β-CD (120 g/L), at room temperature, without stirring. After drying in an oven, the samples were cured at 150 °C, for 10 min. Finally, the paper samples were washed under running water for 5 min and then with distilled water, to remove the non-reacted substances, and again dried in vacuum at 80 °C for 24 h. The quantity of MCT-β-CD bonded to the papers was estimated gravimetrically, as a weight difference before and after the above-mentioned curing process.

The conditions for guest inclusion were adapted from research works on cellulosic fibers,[1,2] namely:

- calculating the quantities of guest compound for a grafted sample, for a grafting degree of 6% (at a 1:1 inclusion ratio);
- weighing of guest compounds and preparation of 10 g/L solutions in an ethylic alcohol/water mixture (7/3) (ferulic acid and allantoin solving was incomplete);
- immersion of samples in the previously prepared solutions (at room temperature) for 4 h, and their magnetic stirring every 10 min for the formation of the inclusion compound;
- 4 successive warm (40 °C) and cold washings with distilled water (15 min each) for the removal of reagents in excess;
- cold extraction (20 min) of the products in excess in an ethylic alcohol/water mixture (7/1), at a bath ratio of 1:40;
- drying of samples in the air at room temperature, for 12 h;
- conditioning.

RESULTS AND DISCUSSION

FT-IR ATR analysis

FT-IR ATR analysis was carried out on a Multiple Internal Reflectance Accessory (SPECAC, USA) with KRS-5 (thallium bromide – iodide) ATR crystal, with 25 reflections, 250 scans and an investigation angle of 45 degrees, in the 1800-600 cm^{-1} range. This accessory was attached to the FTIR-IR Affinity-1 (Shimadzu, Japan) Spectrophotometer.

The FT-IR absorption spectrum (Figs. 1, 2) of the paper modified with MCT-β-CD has the same characteristic bands for the triazinyl nucleus, compared to the spectrum of MCT-β-CD (*i.e*. stretching vibrations υ(C=N)) at 1572 and 1455 cm^{-1} for functionalized papers, respectively at 1471, 1570 and 1608 cm^{-1} for MCT-β-CD). This means that the Japanese veil has been indeed functionalized with the reactive product monochlorotriazinyl-β-CD.

The small shifts of the characteristic bands for the triazinyl nucleus are due to the substituent modification in the reaction between MCT-β-CD and cellulose (the chloride atom has been replaced by O-Cel). After the grafting reaction, two new absorption bands, characteristic of the triazinyl nucleus too, will appear as small shoulders, at 820 and 807 cm^{-1}.

At the same time, the absorption band from 785 cm^{-1}, assigned to the stretching vibration υ(C-Cl) from the reactive compound (which disappears after grafting), does not appear, anymore, in the grafted products, which indicates that the reaction between MCT-β-CD and paper takes place.

The FT-IR-ATR absorption spectra for the Japanese veil functionalized with MCT-β-CD and treated with different guests (ferulic acid – FA, allantoin – Al, and Michler's ketone – MK) are presented in Figures 3-5.

The characteristic IR absorption bands for 4,4'-bis(dimethylamino) benzophenone (Michler's ketone) (MK) (Fig. 3) are: 1593 and 1479 cm^{-1} – υ(C=C) stretching vibrations of the p-substituted aromatic ring; 1479 cm^{-1} – $\delta_{as}(CH_3)$ asymmetrical deformation of the CH_3 groups; 1232 cm^{-1} – δ(C-H) in-plane of ring H bending; 826 and 815 cm^{-1} – δ(C-H) *p*-disubstituted aromatic ring out-of-plane deformation of 2 adjacent H.

These bands are also present in the functionalized Japanese veil treated with MK (1595; 1479; 1233; 828, 815 cm^{-1}).

The characteristic IR absorption bands for ferulic acid (FA) (Fig. 4) are: 1691 and 1663 cm^{-1} – υ (C=O) stretching vibrations of the acid group; 1621 cm^{-1} – υ (C=C) stretching vibrations of side chain non-saturation; 1621 and 1595 cm^{-1} – υ(C=C) stretching vibrations from the 1,2,4 trisubstituted aromatic ring;

1467 cm^{-1} – $\delta_{as}(CH_3)$ asymmetrical deformation of the CH_3 groups; 1265 cm^{-1} – υ(C–O) stretching vibrations of the acid groups; 851 and 800 cm^{-1} – δ(C-H) 1,2,4-trisubstituted aromatic ring out-of-plane deformation of 2 adjacent H; 851 cm^{-1} – δ(C-H) deformation vibrations of the C-H groups of the non-saturated side chain (-HC=CH-). These bands are also present in the grafted paper treated with FA (1691, 1621, 1514, 1265, 851 and 800 cm^{-1}).

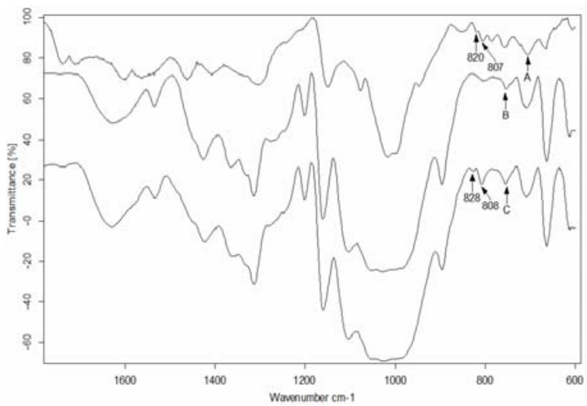

Figure 1: FT-IR-ATR spectra for: A – MCT-β-CD, B – Japanese veil, C – Japanese veil functionalized with MCT-β-CD (impregnation method)

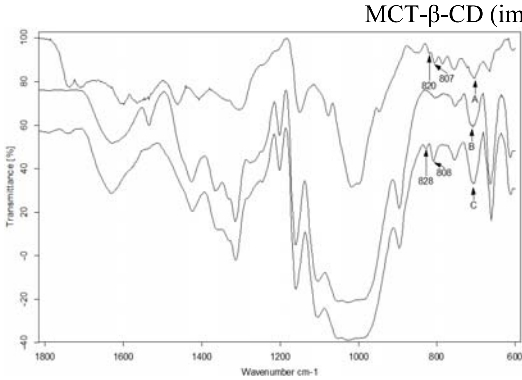

Figure 2: FT-IR-ATR spectra for: A – MCT-β-CD, B – Japanese veil, C – Japanese veil functionalized with MCT-β-CD (brushing method)

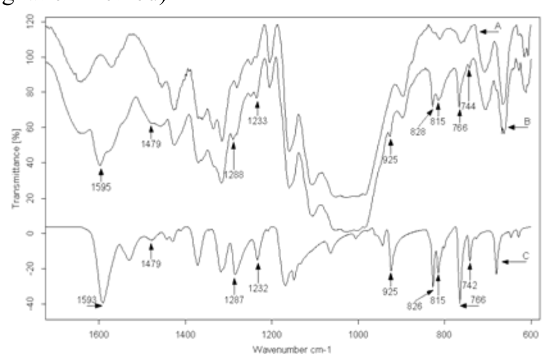

Figure 3: FT-IR-ATR spectra for: A – Japanese veil functionalized with MCT-β-CD (brushing method), B – Japanese veil functionalized with MCT-β-CD and treated with MK, C – MK

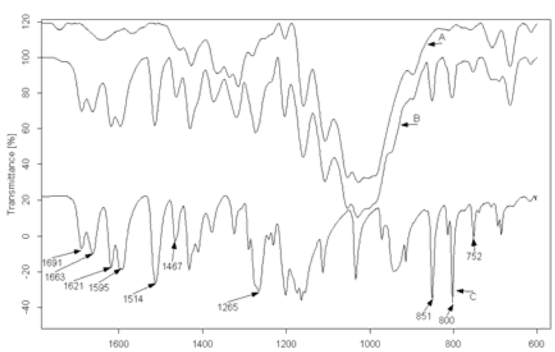

Figure 4: FT-IR-ATR spectra for: A – Japanese veil functionalized with MCT-β-CD (brushing method), B – Japanese veil functionalized with MCT-β-CD and treated with FA, C – FA

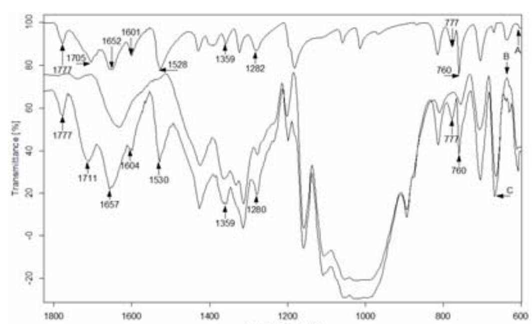

Figure 5: FT-IR-ATR spectra for: A – Allantoin (Al), B – Japanese veil functionalized with MCT-β-CD (brushing method), C – Japanese veil functionalized with MCT-β-CD and treated with Al

The characteristic IR absorption bands for allantoin (Al) (Fig. 5, A spectrum) are: 1705 cm^{-1} – υ (C=O) stretching vibrations from the >C=O conjugated group; 1652 cm^{-1} – υ

(C=O) stretching vibrations from H_2N-(C=O)- and -NH-(C=O)-C (AMIDE I) and υ (C=O) stretching vibrations from -NH-(C=O)-HN- as urea cyclic structure; 1528 cm^{-1} – C-(N-H)- from -NH-(C=O)-C, a combined band from υ (C-N) with δ (N-H) (AMIDE II), δ (N-H) from -NH-(C=O)-HN-, as an urea cyclic structure; 1282 cm^{-1} – -C-(N-H)- from -NH-(C=O)-C as a combined band υ (C-N) cu δ (N-H) (AMIDE III).

The above-mentioned characteristic IR absorption bands for allantoin are also present in the C spectrum (Fig. 5) of the Japanese veil functionalized with MCT-β-CD and treated with allantoin (1717, 1652, 1527 and 1280 cm^{-1}), proving allantoin inclusion in the grafted support.

Scanning Electron Microscopy (SEM)

The SEM investigation was performed on a VEGA II LSH scanning electronic microscope (TESCAN, Czech Republic), entirely computer-operated, containing an electron gun with tungsten filament with a potential between 200 V and 30 kV, and a 3 nm resolution at 30KV, with a magnifying power between 13 and 1000000X in the resolution mode, a scanning speed between 200 ns and 10 ms per pixel. The working pressure is lower than $1x10^{-2}$ Pa.

The comparative SEM analysis of the non-functionalized (Figs. 6, 7) and functionalized (Figs. 8-10) Japanese veil samples allows the visualization of rough micro deposits of reactive product on the surface of the modified cellulosic support. At the same time, one may observe that, after a mild curing treatment (150 ºC) during grafting, the fibrils were not thermally degraded, maintaining their forms and dimensions. Under such conditions, the only heating effect of grafting was thermal activation of the polymer surface.

Tensile strength properties

The treated and untreated paper samples were conditioned for 24 h in a standard atmosphere (at 23 °C and 50% relative humidity), prior to testing for weight, thickness, tensile strength. Tensile testing was carried out on 15 mm wide strips between jaws set 100 mm apart, using a Zwick universal testing machine, according to the international standards for paper.[8]

The effects of grafting and inclusion treatments on the mechanical properties of the Japanese veil are summarized in Table 1. As can be seen, the immediate effect of the chemical treatments is of increasing tensile strength (expressed as tensile index) up to 32% for simple grafting, and up to 46% for grafting and inclusion treatment.

The stress-strain curves show altered conformations, proving the existence of more and more resistant linked networks (Fig 11). The values recorded for breaking strain show that the grafting treatment has no effect on the development of a rigid fiber network (which would result in decreased tensile strain). On the contrary, an increase of tensile strain has been noticed. Furthermore, grafting resulted in a substantial increase of tensile energy absorption (TEA), proving the ability of the new structure to absorb a higher amount of energy, up to the loss of its integrity. Anisotropy decreased after paper grafting, as demonstrated by the ratio of tensile strength values in the two directions (R). This has also proved that the grafting treatment resulted in an increased resistance of fiber links (constituent elements of the network) rather than in an increased strength of fibers (which would have raised network anisotropy).

Table 1
Tensile strength properties of Japanese veil

Sample	Tensile index, Nm/g		Breaking strain, %		Tensile energy absorption TEA, J/m^2		Anisotropy ratio R
	MD	CD	MD	CD	MD	CD	MD/CD
Unmodified	63.84±7.74	14.54±0.66	1.56±0.09	1.83±0.15	9.75±1.65	2.93±0.30	4.39
Grafted	84.72±7.63	24.23±1.49	2.18±0.25	3.11±0.36	19.04±3.55	8.79±1.52	3.50
Grafted & included	93.41±8.22	24.82±1.76	2.13±0.11	3.32±0.42	21.42±2.57	9.65±1.77	3.76

X = x±σ: MD – machine direction; CD – cross direction

Figure 6: SEM image (x720) of non-functionalized Japanese veil

Figure 7: SEM image (x2500) of non-functionalized Japanese veil

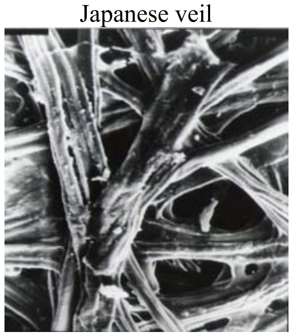

Figure 8: SEM image (x890) of functionalized Japanese veil

Figure 9: SEM image (x4600) of functionalized Japanese veil

Figure 10: SEM image (x5100) of functionalized Japanese veil

Microbiological testing

The antimicrobial activity of non-grafted and grafted samples was tested on ATCC strains, according to the standard method.[9] Four different strains of microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were selected for the study. The first two are of human origin (cutaneous or intestinal), *Pseudomonas aeruginosa* is an environmental bacterium (from air, water, plants and land), while *Candida albicans* is a yeast of human origin. All are pathogenic and/or opportunistic microorganisms.

The antimicrobial effects of untreated and treated paper supports are presented in Figures 12, 13.

The results obtained show that microbial growth was important in the medium pressed with nongrafted and, respectively, grafted paper samples. For all four microorganisms, microbial proliferation in the growth medium was totally inhibited underneath the modified papers and the inhibition zone (around the paper samples grafted with MCT and treated with hosts), varying from 2.3 to 7.4 mm. This demonstrates the satisfactory antimicrobial effect of the three derivatives even after inclusion in CDs. Antimicrobial efficiency decreases in the order: ferulic acid > allantoin > Michler's ketone.

The treated paper samples present an enhanced efficiency against Gram-positive bacteria (*Staphylococcus aureus*), compared to the Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) ones and fungi (*Candida albicans*).

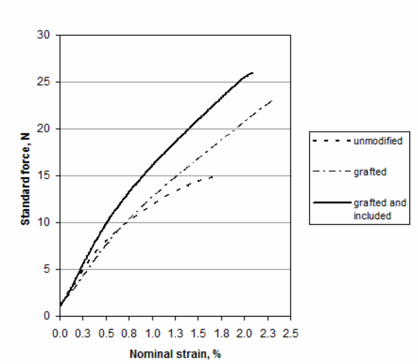

Figure 11: Force-strain curve for different kinds of Japanese veil

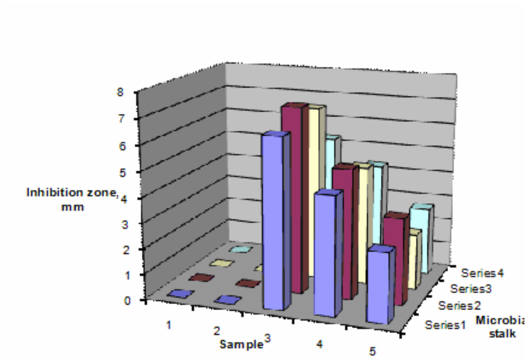

Figure 12: Antimicrobial activity for: non-functionalized Japanese veil (sample 1), impregnation grafted Japanese veil (sample 2), functionalized Japanese veil with MCT-β-CD and treated with FA (sample 3), Al (sample 4) and MK (sample 5); Series 1 – *Escherichia coli* (-); Series 2 – *Staphylococcus aureus* (+); Series 3 – *Pseudomonas aeruginosa* (-); Series 4 – *Candida albicans*

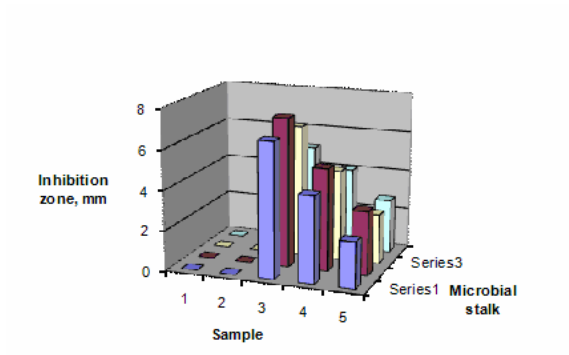

Figure 13: The antimicrobial activity for: non-functionalized Japanese veil (sample 1), brushing grafted Japanese veil (sample 2), functionalized Japanese veil with MCT-β-CD and treated with FA (sample 3), Al (sample 4) and MK (sample 5); Series 1 – *Escherichia coli* (-); Series 2 – *Staphylococcus aureus* (+); Series 3 – *Pseudomonas aeruginosa* (-); Series 4 – *Candida albicans*

A comparison between the grafting methods shows that the impregnation variant is as efficient as the brushing one, demonstrating the superficial localization of coating in both cases.

CONCLUSIONS

- FT-IR-ATR and SEM studies have proved that the paper samples were successfully grafted with the reactive product monochlorotriazinyl-β-CD. The inclusion of the three substances (ferulic acid – FA, allantoin – Al and Michler's ketone – MK) on the grafted material was also confirmed by FT-IR analysis.
- The chemical treatments increase the tensile index up to 32% for simple grafting, and up to 46% for grafting and inclusion treatment. Stress-strain curves have altered conformations, which proves the formation of more resistant linked non-rigid networks. Grafting results in a substantial increase of tensile energy absorption (TEA), demonstrating the ability of the new structure to absorb a higher amount of energy, up to fiber integrity loss. The decrease of anisotropy has demonstrated that the grafting treatment resulted in an increased resistance of links rather than in an increased strength of fibers (which would have raised network anisotropy).
- For further application in the field of heritage documents conservation, superficial grafting of MCT-β-CD on papers has provided hosting nanocavities that can include a wide variety of chemicals, as follows: cinammic derivatives (ferulic acid-(*trans*) [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid]), urea derivatives (allantoin-5-ureidohydantoin or glyoxyldiureide) or ketonic derivatives (4,4'-bis(dimethylamino) benzophenone–Michler's ketone) with antibacterial protective effects.

- The papers finished with these derivatives possess satisfactory antimicrobial activity against four microbial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*), especially against *Staphylococcus aureus*. The potential of MCT-β-CD grafted paper supports, including the selected guests to provide sterile surfaces, has been demonstrated. The experiments have clearly shown that the decreased number of vital microorganisms was caused by the included guest compounds. Therefore, the guest compounds maintain their antibacterial and antifungal activities after inclusion and the newly obtained inclusion complexes appear as promising alternatives in the field of heritage paper materials.

ACKNOWLEDGEMENTS: This paper was supported by the PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649) and POSDRU/89/1.5/S/49944 projects financed by the European Social Fund and the Romanian Government.

REFERENCES

[1] A.-M. Grigoriu and C. Luca, in "Noi aplicatii ale ciclodextrinelor in industria textila" (in Romanian), Performantica Publishing House, Iasi, 2010, pp. 17-18, 53-60.
[2] A.-M. Grigoriu, PhD thesis, Polytechnic University of Iasi, Romania, 2009, 196 pp.
[3] A. Popa, C. Luca and G. Mihailă, in "Compusi de incluziune" (in Romanian), Scientific Publishing House, Bucuresti, 1992, p. 116.
[4] C. Luca and A.-M. Grigoriu, *Rev. Chim.*, **57**, 248 (2006).
[5] N. Vrinceanu, in "Metode neconvenționale pentru stabilizarea suporturilor celulozice" (in Romanian), Performantica Publishing House, Iasi, 2009, p. 22.
[6] T. Furuta, Y. Kusuya, T. L. Neoh, L. Rehmann, S. H. Beak and H. Yoshii, *J. Incl. Phenom. Macrocycl. Chem.*, **56**, 107 (2006).
[7] R. C. Bergamasco, G. M. Zanin and F. F. De Morales, *J. Incl. Phenom. Macrocycl. Chem.*, **57**, 157 (2007).
[8] ISO 1924-2:1994, Paper and board – Determination of tensile properties, Part 2: Constant rate of elongation method, 1995.
[9] SR EN ISO 20645, Materiale textile. Controlul activitatii antibacteriene. Încercare de difuzie pe placa gelozata, 2005.