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# Preparation and Biological Thermal Properties of Sp- Bound Composites

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**Abstract:** Sporopollenins (Sp) are modified, using a two-step process with amine and benzoyl- isothiocyanate groups. This article describes, the synthesis and characterization of Sp modifi- ed with a series of new benzoylthiourea compounds. The Sp-bound benzoylthiourea derivati- ves of ethylenediamine; Sp-N,N'–(ethane-1-methylbenzamide-2-benzoylthiourea) **1**, diethylen triamine; Sp-N,N'–(N',N'-diethylamine-1,3-dibenzoylthiourea) **2**, N',N''-bis(3aminopropyl)-a- mine; Sp-N,N'–(N',N'-dıpropylamine-1,3-dibenzoylthiourea) **3,** N',N''-bis(3aminopropyl)-eth ylenediamine; Sp-N,N'''-(N',N''-dipropyl-ethanediamide-dibenzoylthiouurea) **4** are synthesi- zed. The compounds are characterized by elemental analysis, FT-IR, UV–VIS, TGA, SEM, GPC and conductance measurement. Sp bound compounds are discussed in terms of biologi- cal, electrical and thermal properties. The antimicrobial activities of Sp bound compounds are also evaluated against seven species of bacteria and mold. The present work shows that ben- zoylthiourea could be utlized to increase the conductivity of insulating Sp.

**Key words:** Sporopollenin · Modification · Benzoylthiourea · TGA · Antimicrobial

## INTRODUCTION

Sporopollenin (Sp) is a natural biopolymer derived from plants (moss, fern, pollen). It has aliphatic, aromatic, hydroxyl, carbonyl/carboxyl groups and ether functions in various portions in its polymeric structure, but its chemical structure is not fully known. Sp is remarkable because of its resolute chemical and molecular structure, high holding capacity and low cost [1-3].

The biological effects of the biopolymers are studied. For example; chitosan is a natural nontoxic biopolymer. Chitosan and its derivatives have attracted considerable interest owing to their antimicrobial and antifungal activities [3] but until now, there has been no study on the biological activity of Sp. Over the past two decades, the modified forms of Sp have been utilized as highly selective reagents [4-6]. It is a well-known polymer as because of its numerous environmental and analytical applications. Additionally, some polymer- bound benzoylthiourea derivatives are used in separation processes. [7-9]. For example, these reagents are very important in the removal and recovery of metal ions. They are generally used in the process of coordination of transition metal ions with N,O and S donor atoms.

Here, we propose to synthesize of Sp-benzoylthiourea composites and examine their biological activity. Sp-benzoylthiourea compounds have been prepared by Sp reaction with amine groups and benzoyl isothiocyanate. The process of Sp modification has been studied using FTIR and elemental analysis. The results show that Sp can be modified with these groups. The modification reaction of different compounds with Sp is depicted in Schemes 1 and 2. Reaction conditions are described in the experimental section.

**Experimental:** Sp, amine derivatives and process assistants (commercial grades) were obtained from the market. Melting points were determined on an Electrothermal 9100**.** UV spectrum was obtained on a Shimadzu UV 2200 spectrophotometer (1 mg/100 ml in ethanol). IR spectra were recorded with a potassium bromide disk on JASCO 300 E FTIR. Elemental analysis of

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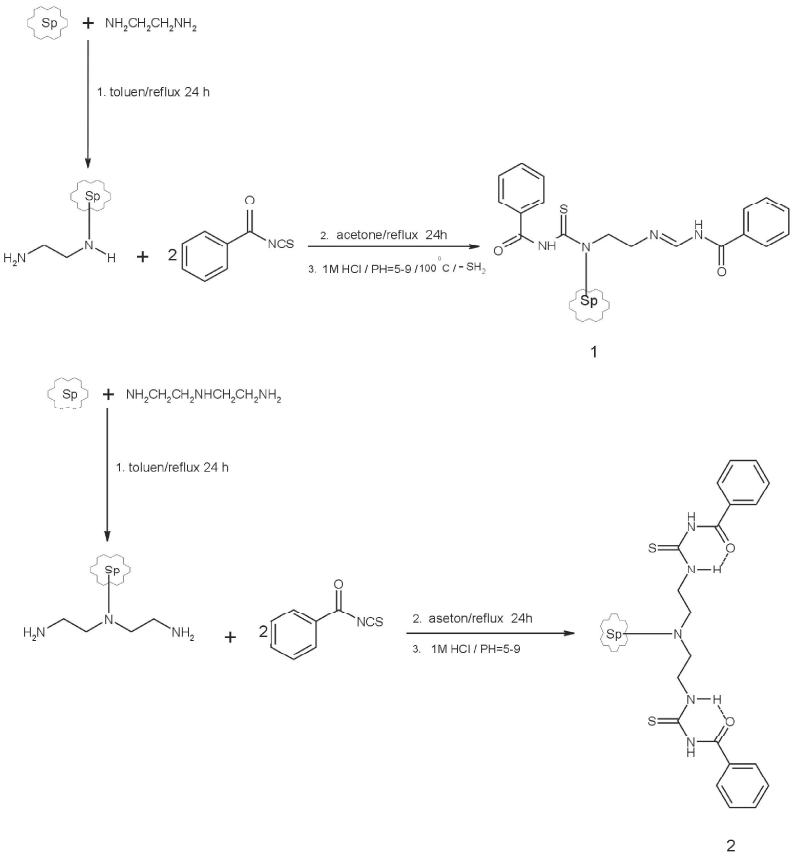
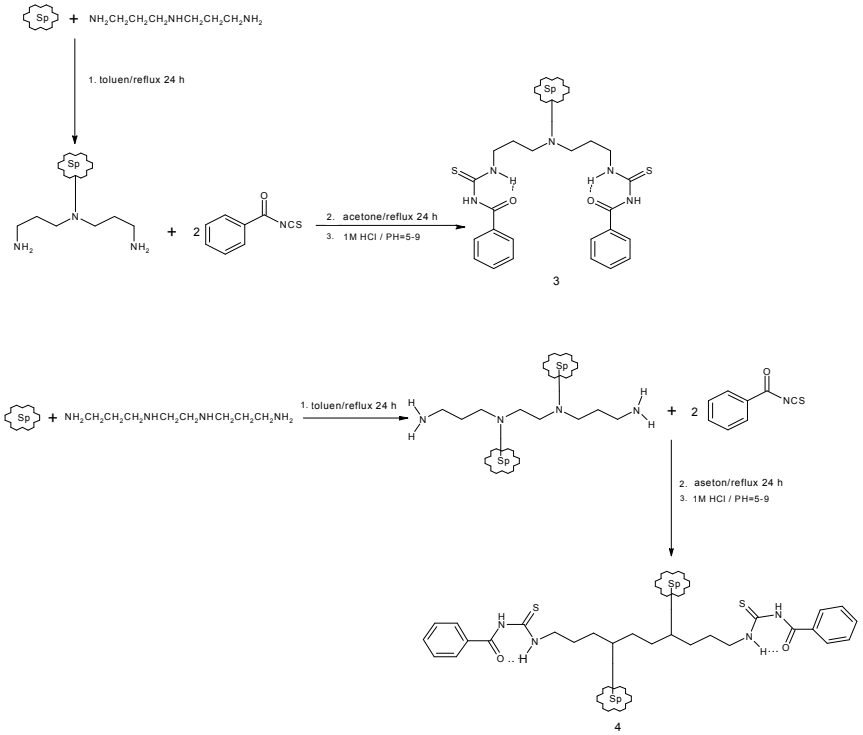



Scheme 1: Synthesis of 1 and 2




Scheme 2: Synthesis of 3 and 4

the samples was performed on a CHNS-932 of Leco. The average molecular weights were determined by GPC with a Shimadzu LC prominence. (Detector: refractive index, fluid phase: N,N-dimethyl formamide). Scanning electron microscopy (SEM) (JEOL, Japan JSM-6360LV) was employed to study the surface of all tested samples. Thermal stabilities were measured on with a Shimadzu DT-40 thermal analyzer.

**Chemical Modification**

**Synthesis of Sp-polydentate Amine Ligands:** Sp (10 g) was dissolved in 100 ml toluene. A 10g-solution of Sp was added to 30 ml ethylenediamine, 40 ml diethylenetriamine, 35 ml N',N''-bis(3aminopropyl)-amine and 48 ml N',N''-bis (3aminopropyl)-ethylenediamine. After addition, the mixture was heated under reflux for 24 h. The isolated solids were filtrated, washed with diethylether and dried to yield.

**Synthesis of Sp-Benzoylthiourea Ligands:** Benzoyl isothiocyanate was synthesized by condensation of benzoyl chloride with KSCN. Benzoyl isothiocyanate (21 ml) was added to a solution of Sp-polydentate amine ligands in anhydrous acetone. The mixture was refluxed for 24 h. Finally, the mixture was cooled in an ice bath and 1M HCl (250 ml) was added. The precipitate was collected by filtration and was washed with diethyl ether.

**Sp-N,N'–(ethane-1-methylbenzamide-2-benzoylthiourea) 1:** Sp-$C_{18}H_{17}N_4O_2S$; Color: yellow. m.p: 210°C-230°C. Elemental analysis: Found: C:53,04; H:6,3; N:8,0; S:4,2, Calc.for: N:8,1; S:4,7. IR (KBr) $\nu_{max}/cm^{-1}$: 3511-3423 (Sp), 3291 (N-H), 3065 aromatic (C-H), 2928-2852 aliphatic (C-H), 1972-1822 (C=C), 1677 (C=O), 1643 (C=O), 1554-1258 (C-N), 1169 (C=S). UV vis($CH_2Cl_2$,abs ): 240; 420; 480.

**Sp-N,N'–(N',N'-diethylamine-1,3-dibenzoylthiourea) 2:** Sp-$C_{20}H_{22}N_5O_2S_2$; Color: orange. m.p: 200-230°C. Elemental analysis: Found: C:58,0; H:6,0; N:9,4; S:8,0, Calc.for: N:9,3; S:8,4. FT-IR (KBr) $\nu_{max}/cm^{-1}$: 3374 (Sp), 2928-2859 aliphatic (C-H), 2062-1980 (C=C), 1677 (C=O), 1684 (C=O), 1547-1269 (C-N), 1162 (C=S). UV-vis ($CH_2Cl_2$,abs ): 220; 260; 280; 420.

**Sp-N,N'–(N',N'-dıpropylamine-1,3-dibenzoylthiourea)3:** Sp-$C_{22}H_{26}N_5O_2S_2$; Color: green. m.p: 290-300°C. Elemental analysis: Found: C:54,4; H:7,4; N:8,5; S:8,1, Calc.for: N:8,5; S:8,1. IR (KBr) $\nu_{max}/cm^{-1}$: 3400 (Sp), 3120 aromatic (C-H), 2720-2820 aliphatic (C-H), 2040 (-SH), 1840-2000 (C=C), 1660 (C=O), 1574-1272 (C-N), 1162 (C-S). UV-vis($CH_2Cl_2$,abs ): 220; 240; 260; 420.

**Sp-N,N'''-(N',N''-dipropyl-ethanediamide-dibenzoylthiourea) 4:** Sp-$C_{24}H_{30}N_6O_2S_2$. Color: orange. m.p: 232°C-245°C. Elemental analysis: Found C: 54,9; H:6,8; N:9,2; S:7.9, Calc.for: N:9,2; S:7,0. IR (KBr) $\nu_{max}/cm^{-1}$: 3401 (Sp), 3058 aromatic (C-H), 2935-2859 aliphatic (C-H), 1719 (C=C), 1664 (C=O), 1574-1266 (C-N), 1179 (C=S). UV-vis($CH_2Cl_2$,abs): 220; 260; 440.

**Biological Studies:** Biological activities the compounds were tested against fungi and bacteria such as Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus mutans, Salmonella enteritidis, Staphylococcus aureus and Candida albicans that are standard. Antimicrobial activities were determined by "disk diffusion" method. The compounds have been prepared in two concentrations, 15mg/ml (300µg/perdiscs) and 5mg/ml (100µg/perdiscs) in DMSO. The sterilized blank antibiotic disks (Oxoid) having a diameter of 6mm are impregnated with 20µL of these two solutions. The disks have been placed in the plates and incubated at 37°C for 24 h; fungi were incubated for 48 hours. The diameter of the zone of inhibition around each disk has been measured and results have been recorded. Ceftri- axone (CRO-30mcg) and gentamycin (GM-10mcg) have been used for positive checking.

## RESULTS AND DISCUSSION

**FT-IR Analysis:** FT-IR spectra of compounds are examined and the FT-IR spectra for compounds (nanocomposites) are shown in Figures 1 and 2. Sp peaks are observed in the range of 3400-3512 $cm^{-1}$. N-H bands are not observed because of the broad band of Sp. Characteristic Car–H stretching vibrations are found in the 3058–3120 $cm^{-1}$ range. The overtone of C=C bands is calculated in 1800-2000 $cm^{-1}$ frequency ranges. These Car–H and C=C bands prove of the presence of benzene rings. The strong C=O bands of compounds are seen at approximately 1664, 1660, 1674 and 1680 $cm^{-1}$. These bands are found in low wavenumbers due to the conjugated resonance formed as a result of the binding of the phenyl ring to carbonyl and the double-band character of the intramolecular hydrogen bond with N–H. N–C–N, hence, both symmetric and asymmetric N–C–N vibration shifts to higher values. The C=S stretching vibrations are discovered in 1179, 1169 and 1162 $cm^{-1}$. Furthermore, our ligands can exhibit thione–thiol tautomerism because of the thioamide –NH–C=S functional group. The bands in the 2400-2500 $cm^{-1}$ range indicate the existence of S–H group.

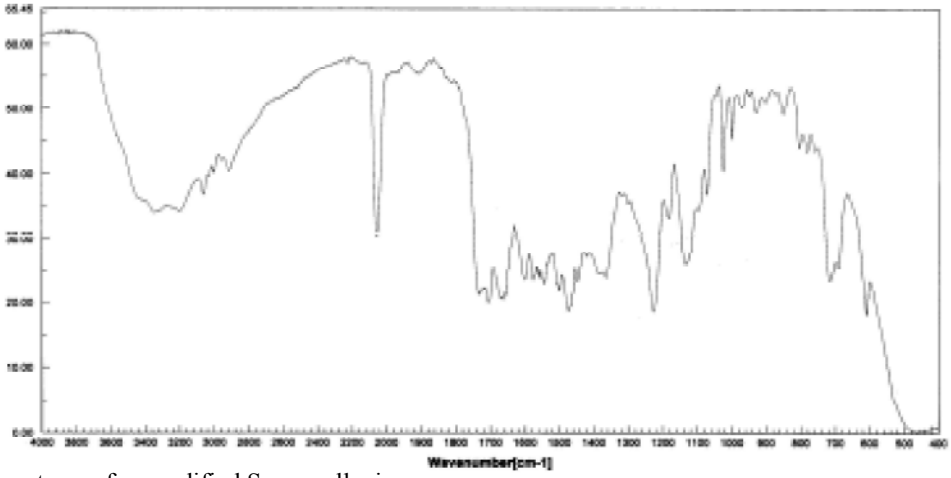



Fig. 1: FTIR spectrum of unmodified Sporopollenin

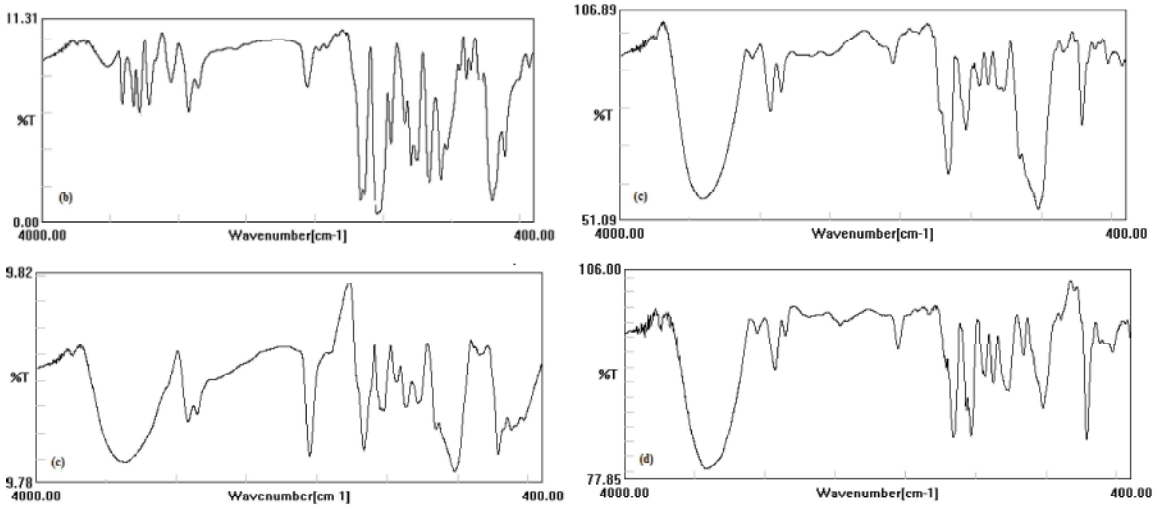



Fig. 2: FTIR spectrum of 1 (b), 2 (c), 3 (d) and 4 (e)

**UV-VIS Analysis:** Compounds have been analyzed with UV–VIS spectra and the electronic spectra of the compounds have been recorded. Absorption bands around 220, 260, 280, 420, 440 and 480 nm are discovered. According to results the compounds show n→□*, n→Π* and Π→Π* transitions while the presence of phenyl rings is confirmed from bands about 240–280 nm. These results are shown in Figure 3.

**Electrical Properties, Average Molecular Weight and Pore Dimension Studies of the Modified Sporopollenin:** These properties of nanocomposites are summarized in Table 1. The average molecular weight has been measured with GPC. These results are shown in Figures 4 and 5. The average five benzoylthiourea groups have been bound by an Sp bipolymer. PDI (Mw/Mn) can demonstrate that compounds are formed from a single-type polymer. The compounds' conductance was measured by a conductometric apparatus. The compounds were dissolved in a dielectric solvent such as DMSO. Conductivity was subsequently measured using an electrode. The results show that the Sp-benzoylthiourea composites have higher electrical conductivity than pure Sp. Benzoylthiourea is known to be more conductive owing to the high degree of conjugation. We perhaps can utilize these conditions to increase the electrical conductivity for insulating Sp. The dimension of the pores was determined through scanning electron microscopy (SEM) analysis (Figures 6,7 and 8). The analysis shows the dimensions of pores to be 10, 30 and 50 $\mu m^2$; these pore dimensions are different from pure Sp.

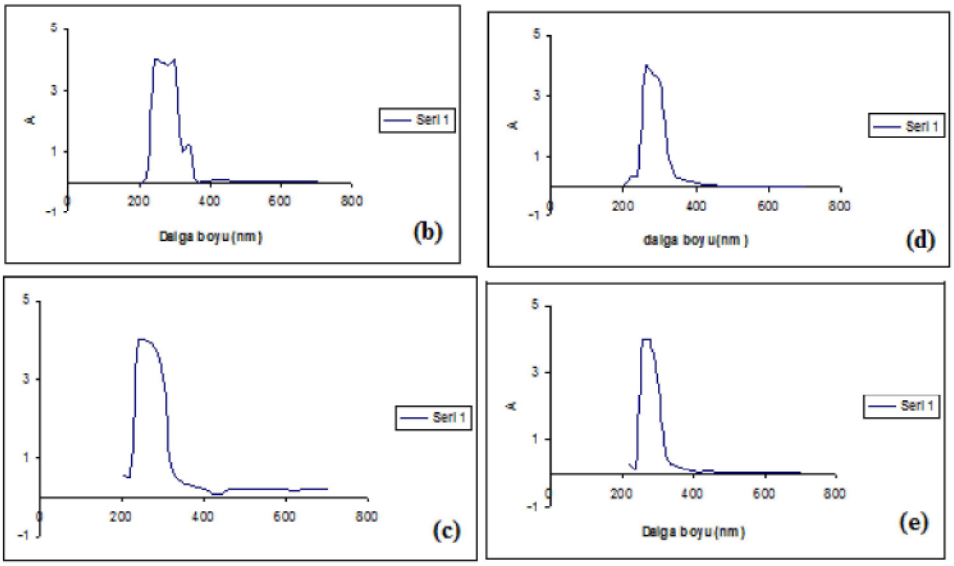



Fig. 3: UV spectrum of 1 (b), 2 (c), 3 (d) and 4 (e)

Table 1:

| Composites | Conductance (µs) 25°C | Density (g/ml) | Pore dimension (µm$^2$) | Tg (°C) |
|---|---|---|---|---|
| 1 | 21,5 | 0,48 | 50 | 60,00 |
| 2 | 8,8 | 0,42 | 30 | 53,36 |
| 3 | 12,3 | 0,4 | 10 | 59,82 |
| 4 | - | 0,17 | 10 | 67,78 |

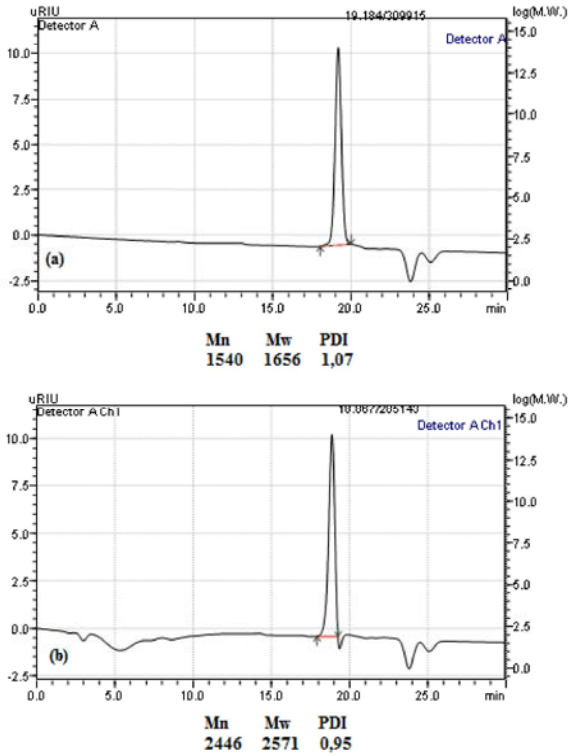



Fig. 4: Molecular weight of Sporopollenin (a) and 1 (b)

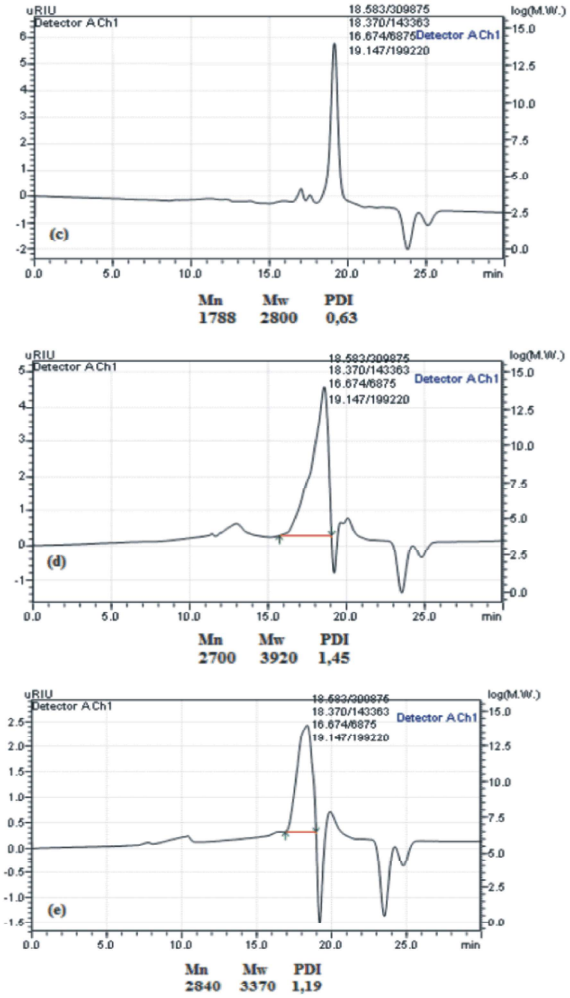



Fig. 5: Molecular weight of 2 (c), 3 (d) and 4(e)

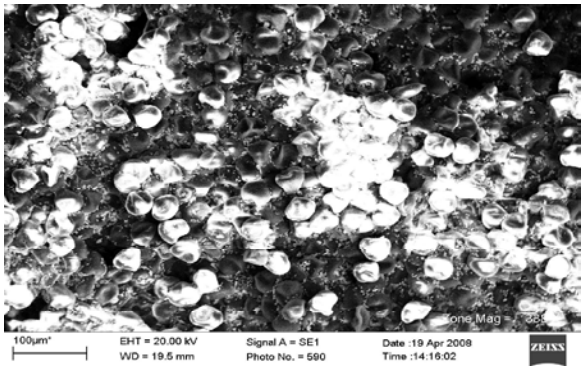



Fig. 6: SEM photograph of Sporopollenin

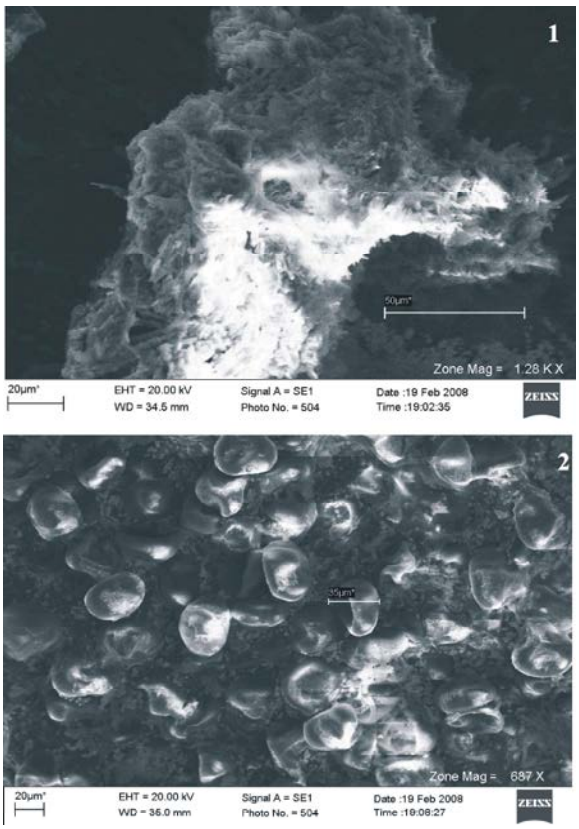



Fig. 7: SEM photograph of 1 and 2

**Thermogravimetric Studies of the Modified Sporopollenin:** The thermal behavior of the compounds is investigated with TGA/DTA analysis. These results are shown in Figures 9 and 10. Total mass loss of compound (1) is% 84,891 in the range of 80-600 °C. The first mass loss was observed in the 180-240 °C range with%22,225 while the second decomposition is in the range 240-420°C with%47,509 mass loss. Two endothermic peaks, 202°C and 228°C have been observed in DTA curves. Total mass loss of compound (2) is%82,211. TGA curve at the first decomposition with%4,557 mass loss in the 20-140°C

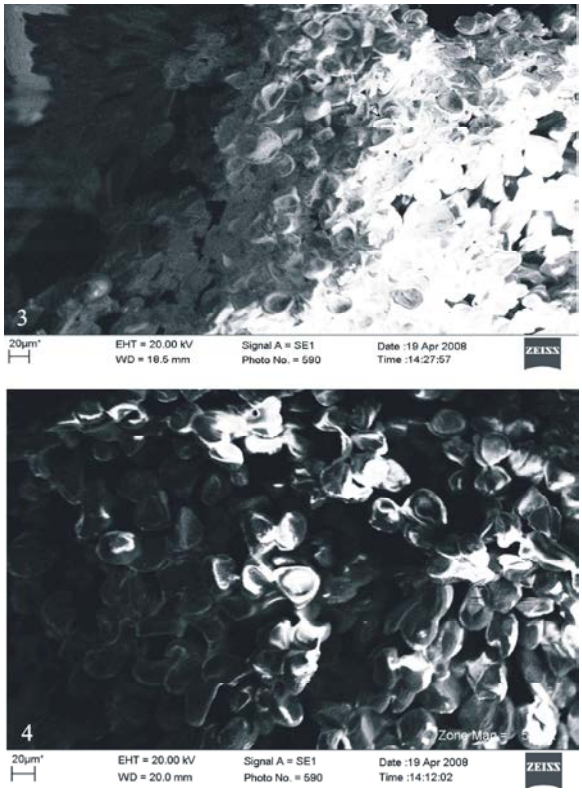



Fig. 8: SEM photograph of 3 and 4

range, second decomposition with%55,008 mass loss in the 140-380°C range and third decomposition with%14,78 mass loss in the 380-510°C range have been observed. Four endothermic peaks from DTA analysis were observed in the 53,36, 255,96, 316,67 and 417,46°C ranges. Total mass decomposition of compound (3) is 85,236% in the range of 20 -600 °C. The first mass loss has been observed in 20-120°C with 6,045%, the second mass loss that is 19,39% has been observed in 180-280°C range. The last decomposition was found in the 280-420°C range with 45,987% a rapid mass loss. The remaining masses are approximately 15% and these can be explained with the resolute structure of Sp. Five endothermic peaks have been observed in the DTA analysis. The maximas of these peaks are found in 59,82, 260,71, 342,44, 362,76 and 423,76°C, respectively. Endothermic peaks are generally explained in connection with reduction, oxidation and decomposition. The glass transition temperatures (Tg) of polymer materials are defined from TGA/DTA curves. The first peaks in DTA curves are explained in connection with Tg. Total mass loss of compound (4) is 83,99% in the range 20-600 °C. The first mass loss has been observed in the 20-120 °C range with 4,08%. The second decomposition that is 21,97% was observed in the range

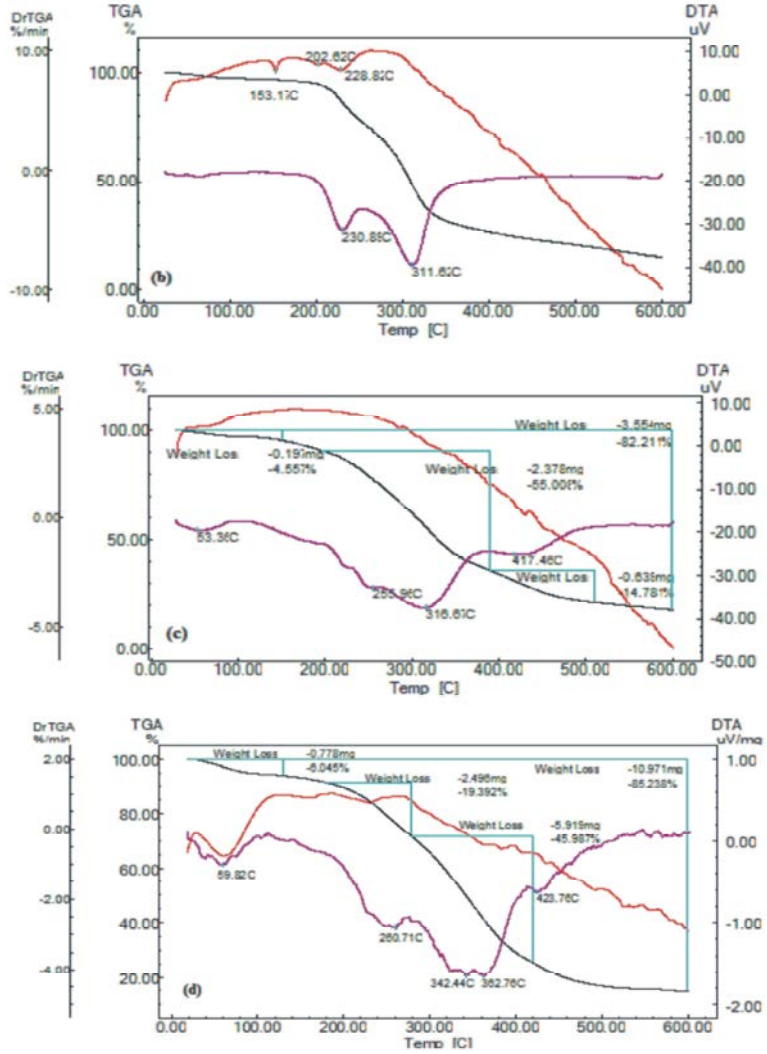



Fig. 9: TGA/DTA spectrum of 1 (b), 2 (c) and 3 (d)

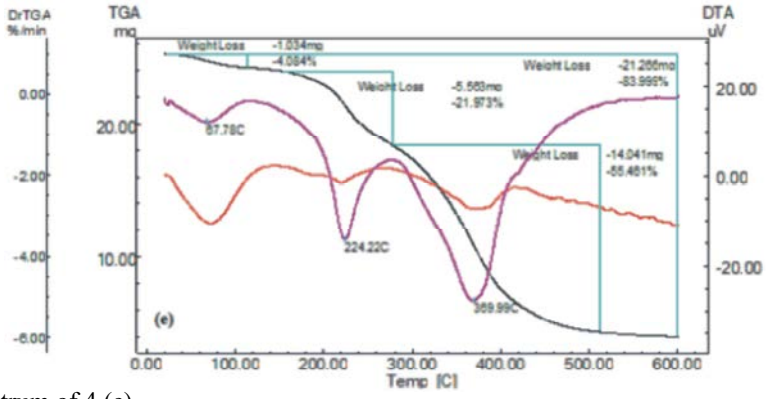



Fig. 10: TGA/DTA spectrum of 4 (e)

120 -280°C range. The last decomposition was observed in the 280-520°C range with 55,46% rapid mass loss. Five endothermic peaks have been observed in 67,78, 224,22, 369,91, 202 and 228°C in DTA analysis. Tg of the new compounds are compared. Results are summarized in Table 2.

Table 2:

| Compounds | E.C. 300µg | L.M. 300µg | P.A. 300µg | B.S. 300µg | C.A. 300µg | S.M. 300µg | S.E. 300µg | S.A. 300µg |
|---|---|---|---|---|---|---|---|---|
| Sporopollenin | - | - | - | - | - | - | - | - |
| 1 | - | - | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - | - | - |
| 3 | 9mm | - | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - | - | - |
| Gentamycin | 25 | 24 | 22 | 30 | 30 | 20 | 32 | 24 |
| Ceftriaxone | 25 | 24 | 22 | 30 | 30 | 20 | 32 | 24 |

**Antibacterial and Antifungal Activity of the Modified Sporopollenin:** Biological sensitivity to compounds is tested and the inhibition values of compounds are summarized in Table 2. Sp-N,N'–(ethane-1-methylbenzamide-2-benzoylthiourea), Sp-N,N'–(N',N'-diethylamine-1,3-dibenzoylthiourea), Sp-N,N'''-(N',N''-dipropyl-ethanediamide-dibenzo ylthiourea) are not active against fungi and bacteria, but, at the same time, the growth of *E.coli* has been inhibited by Sp-N,N'–(N',N'-dipropylamine-1,3-dibenzoylthiourea), which is a compound shown to have weak activity. This consequence can be the result of modified groups. L. monocytogenes, P. aeruginosa, B. subtilis, S. mutans, S. enteritidis, S. aureus and C.albicans have not been inhibited by compounds.

## CONCLUSION

The present work deals with the modification of sporopollenins (Sp). Sp-bound benzoylthiourea compounds have been prepared from the reaction of benzoyl isothiocyanate with Sp-bound amine derivatives. The structural characterization of polymeric compounds is not usually easy. In this study, all of functional groups have been confirmed with elemental analysis and FTIR. Some images of compounds were compared with pure Sp. As a consequence, bound groups have changed the structure of pure Sp. Another consequence is that compound (3) has shown antibacterial activity against *E. coli*.

## ACKNOWLEDGMENTS

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