A POLYMER-DRUG SYSTEM BASED ON REGENERATED CELLULOSE USED IN TEXTILE INDUSTRY

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Biocompatible absorptive materials with porous structure, formed by cavities with different sizes, have many medical applications, such as drug carriers, base for cell regeneration, implants for tissue regeneration.
The immobilization of synthesized indazole derivatives with germicidal, aseptic and anti-inflammatory activity, by diffusion into a regenerated cellulose matrix is studied. The rheological properties of the new composite material are studied, considering the mechanical properties of the materials with potential use in shoe manufacturing for diabetic foot diseases.
The immobilization of indazole derivatives onto the cellulose matrix is influenced by certain parameters, like the derivatives/cellulose ratio and cavity structure of the cellulose matrix.
New polymer-drug blend systems with medical benefits were obtained and the most important physico-mechanical properties of the new composite, such as water retention and tensile strength, were studied.

Keywords: cellulose sponges, immobilization of indazole derivatives, polymeric biocomposite material, water retention, tensile strength measurements, insoles, diabetic foot alternative treatment

INTRODUCTION

The engineering of biodegradable materials compatible with the living tissue is in remarkable progress. Thus, absorbent materials with porous structure formed by cavities have been extensively studied, especially for medical purposes, as drug carriers, along with other bioactive polymers, as bases for cell growth and implants for tissue regeneration.[1-4]

The obtained composite reveals some advantages especially for those suffering from diabetes, which, at the foot level, may induce diabetic neuropathies, caused by damage to the peripheral nervous system. This affection diminishes tactile, pain and thermal sensitivity and may induce, in some parts of the organism, loss of muscular strength or movement capacity. It appears most frequently at the foot level, causing ulcerations and infections, even bone deformation, being the most common type of diabetic neuropathy. Once settled, these ulcerations develop very rapidly to chronic inflammations and, if monitoring is not done daily, they may lead to foot amputation.[5]

Diabetic foot ulcer is the most significant wound care problem in the world. There are now over 60 million diabetics in Europe, with approximately 20-25% foot ulcer incidence over the patient's lifetime. Currently, there are approximately 100000 limb amputations in diabetics each year in Europe. It is difficult to determine the exact cost of care for diabetic foot ulcers, but most likely it represents some billions of dollars. Diabetes and diabetic foot ulcer complications are growing at double-digit rates and have the potential to become more devastating.[6] Therefore, the presence of bioactive compounds in insole structure and at the foot level, which can lead to an aseptic

space, is beneficial. The presence of a bioactive compound inside the shoe creates a clean and aseptic environment, which prevents the appearance of ulcerations, mycoses, infections or fungi. This bio-composite allows local treatment without ingestion of drugs, hepatic transit and local treatment demanding long rest periods.

EXPERIMENTAL

Obtaining of cellulose material

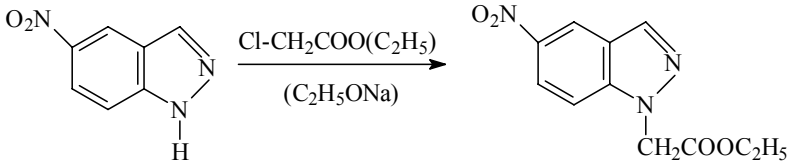
A batch of cellulose sponges, from Beijing Stronger Rich Imp. & Exp. Co. Ltd, was oxidized and compared with the non-oxidized cellulose biomaterial sample. Oxidation involved the treatment[8] of cellulose with sodium metaperiodate in iso-propanol, at a 1:1.5 ratio, whereas the formed dialdehyde cellulose was treated with hydrogen peroxide (2 mol H_2O_2:mol -CH=O).

Synthesis of indazole derivatives

Literature data indicate that heterocyclic hydrazones have bioactive properties: germicidal, aseptic and possible fungicide activities.[9]

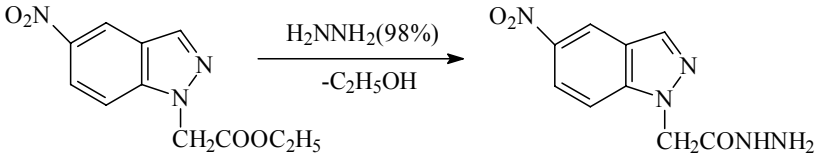
The derivatives prepared from 5-nitro-indazolyl-1-acetic acid are substances with very low toxicity and with germicidal, aseptic and anti-inflammatory potential activity.[10] As intermediate in the synthesis was the ethylic ester of 5-nitro-indazolyl-1-acetic acid (I), obtained by condensation of 5-nitro-indazole with ethyl monochloracetate, in the presence of sodium ethoxide. Derivative (I) was treated with 98% hydrazine hydrate in anhydrous ethanol, the 5-nitro-indazole-1-il-acet hydrazide (II) thus resulting.

Knowing that the –C-NH-N=CN- group gives potentially bioactive materials, compound (II) was condensed with different hydroxy- and nitro-substituted aromatic aldehydes. Thus, by using salicylic- and o- and p-nitro-benzaldehyde, N^1-(5-nitro-indazole-1-il-acetyl)-N^2-o-hydroxy-benzaldehyde-hydrazone (X), N^1-(5-nitro-indazole-1-il-acetyl)-N^2-o-nitro-benzaldehyde-hydrazone (XI) and N^1-(5-nitro-indazole-1-il-acetyl)-N^2-p-nitro-benzaldehyde-hydrazone (XI) were obtained as final products. The structure of these products was established[10] by elemental (C, N, H) and spectral analyses (FT-IR and H-NMR) (Schemes 1-3).



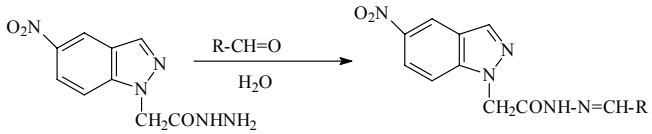
(I)

Scheme 1: Synthesis of ethyl esters of 5-nitro-indazolyl-1-acetic acid (I)



(I) (II)

Scheme 2: Synthesis of 5-nitro-indazole-1-il-acet hydrazide (II)



(X, XI, XII)

X: R = -C_6H_4-OH, XI: R = -C_6H_4-NO_2 (o), XII: R = -C_6H_4-NO_2 (p)

Scheme 3: Synthesis of new products X-XII

Toxic activities of products X, XI, XII

It was made a study regarding hydrazone derivatives X, XI, XII toxicity activity [10]. The activity was evaluated by intraperitoneal administration of the substances in suspension forms in Tween 80 to mice groups of 14 (20±5g)

following the Kärber method.[11] The mice were observed and it was noticed that mortality interfered after 7 days (Table 1).

Toxicological data have confirmed that N-acetyl-hydrazones (X-XII) do not present toxicity, which recommends for germicidal and fungicide tests.

Table 1
DL_{50} values for hydrazone derivatives

Derivatives	DL_{50} mg/kg body weight
X	1520
XI	1050
XII	1025

Inclusion of bioactive compounds

The cellulose samples were immersed in an ethanol solution of hydrazone derivatives (X), (XI) and (XII) of different concentrations (2.5-11%) at 37 °C, for different periods of time (1, 5, 4, 6, 9, 18 h). The new materials were dried at room temperature and weighed to establish the percent of drug included in the cellulose matrix. The bioactive compound recovered from ethanol was weighed again, the experimental results being summarized in Table 2.

These data show that the amount of included drug depends on matrix porosity and size of the drug molecule (Figs. 1-3).

The study of the immobilization process provides information on the parameters influencing the content of bioactive compound in solution (BC%), which may be estimated with the relation:

$$BC\% = [(b-a)/b] \times 100$$

where a – weight of dried cellulose;

b – weight of the immobilized cellulose composite.

The parameter values for the immobilization of bioactive compounds onto cellulose as a function of time are presented in Figure 4.

The size of the bioactive compound molecule, which decreases as the molecule size increases, influences accessibility into the matrix cavities.

Comparative study on physical properties of obtained composites *versus* regular materials used in insole fabrication

Water retention

The aim of this paper is to appreciate water absorption capacity of the composite materials used for medical application insoles. In the experiments, 5 types of regular materials provided by Classico Company (Italy) were used, together with the obtained biocomposite materials. At the same time, samples of cellulose composites reinforced and double-reinforced with carbon fiber network were also prepared, for providing the materials with increased strength in wearing and fabrication processes. Samples with one and two layers of carbon fiber fabric were prepared to wrap the cellulose composite material, for obtaining composites with sandwich structure.

Table 2
Experimental results for immobilization of bioactive derivatives X, XI, XII

Sample №	Bioactive agent	Solution concentration, %	Bioactive agent included in matrix, g/100 g cellulose	Bioactive agent included in matrix, g/100 g oxidized cellulose
1	Derivative X	2.5	68.60	60.87
2		5	73.21	68.00
3		7	74.44	69.77
4		9	75.56	69.39
5		11	74.81	71.54
I	Derivative XI	2.5	44.02	35.54
7		5	52.89	27.08
8		7	37.86	29.41
9		9	46.01	29.41
10		11	50.00	48.35
11	Derivative XII	2.5	60.16	47.57
12		5	74.77	58.25
13		7	58.00	47.89
14		9	45.22	36.21
15		11	33.04	38.78

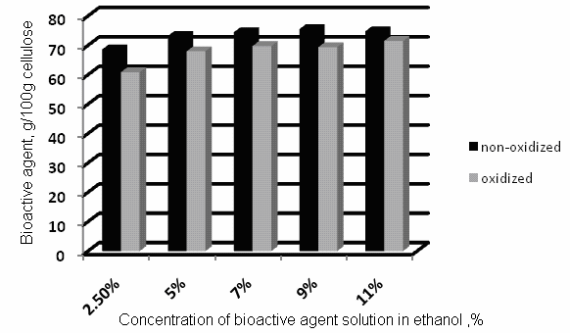


Figure 1: Immobilization of derivative X in cellulose matrix (immersion time: 1.5 h)

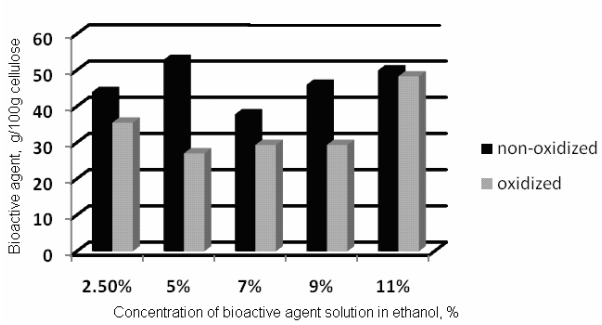


Figure 2: Immobilisation of derivative XI in cellulose matrix (immersion time: 1.5 h)

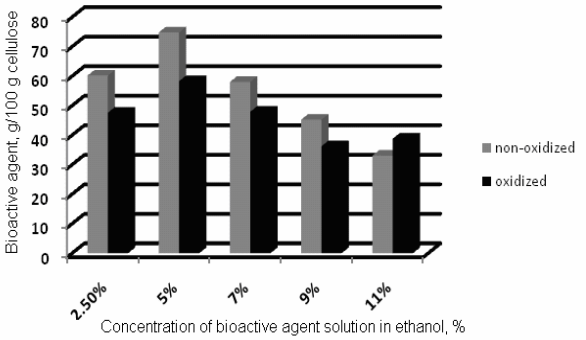


Figure 3: Immobilisation of derivative XII in cellulose matrix (immersion time: 1.5 h)

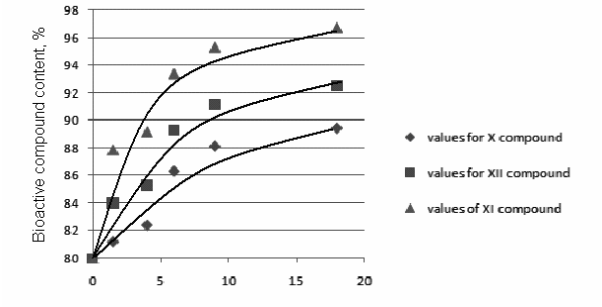


Figure 4: Content of bioactive compounds X, XI and XII (BC%) *vs*. time

The water absorption of insoles was determined according to European standard SR-EN 12770:1999. To this end, a weighing machine with a precision of 0.01 g, a hydraulic cutting device equipped with a square knife (50 ± 1 mm x 50 ± 1 mm), filter paper and distilled water were used. The samples with the above-mentioned dimensions were weighed and immersed in water for 6 h, then the excess water was eliminated using filter paper and the samples were weighed again. The entire process was carried out at standard pressure and 20 ± 2 ºC. According to the standard, water absorption represents the mass increase reported to the surface unit of the tested sample due to water absorption, over one or several time intervals.

To determine water absorption – W_A – the following equation was used:

$$W_A = \frac{M_F - M_0}{A} \text{ [g/m}^2\text{]}$$

where M_0 – initial mass of the dry sample, in grams;

M_F – final mass of the wet sample, in grams;

A – sample surface, in square meters.

To highlight the good matrix properties, the results were compared with the data obtained for regular materials.

The tests were performed using 4 samples from each set, named after the commercial title of the material contained, the results being presented in Table 3. The significantly high values for water sorption are attributed to the regenerated cellulose present in the composite structure, which is a highly porous and hydrophilic material.

The influence of the absorption properties of such materials during wearing was determined by applying a 1.9 kg/cm^2 pressure over the cellulose composite, considered as characteristic of adults weighing 70-80 kg, in stand-up position.

An absorption factor regarding the sample volume was calculated:

$$W_A' = \frac{M_F - M_0}{V} \text{ (g/cm}^3\text{)}$$

where M_0 – initial mass of the dry sample, in grams;

M_F – final mass of the wet sample, in grams;

V – volume of probe, in cm^3.

The results are presented in the chart plotted in Figure 5.

In a worn-out state, when the samples were pressed at maximum pressure levels, the sorption capacity remained within the standard range, assessing the good characteristics of the composite for orthopedic insole production.

Tensile strength of composite materials

The tensile strength of the conditioned samples (SR EN ISO 2419:2003 standard: 48 h at 20 ± 2 °C and 65% ± 5% relative humidity), was measured on a Tensile Testing Centre STM 466 SATRA dynamometer, under the following conditions: maximum load – 2.5-5 kN, speed – 1-1000 mm/min

in 1 mm/min increments, with an accuracy of 0.2% and positioning precision of 0.1 mm. The tensile strength device was equipped with a CAD/CAM software, which allowed to calculate a wide range of values.

Cellulose composite (compound XI included) samples reinforced and double-reinforced with carbon fiber network were also prepared. They contained one and two layers of carbon fiber fabric to wrap the cellulose composite material, sandwich structure composites being thus obtained.

Sample denomination:
Sample 1 – LEATHER INSOLE STRENGTHENED WITH CARBON FIBERS
Sample 2 – PERFUMED POLYURETHANIC FOAM INSOLES WITH COTTON FABRIC
Sample 3 – CELLULOSE COMPOSITE (XI) MATERIAL WITH CARBON FIBER REINFORCEMENT COVERED WITH LEATHER
Sample 4 – CELLULOSE COMPOSITE (XI) INSOLES WITHOUT CARBON FIBER REINFORCEMENT
Sample 5 – LEATHER INSOLE
Sample 6 – CORK INSOLES REINFORCED WITH COTTON FIBER
Sample 7 – CELLULOSE COMPOSITE (XI) INSOLES DOUBLE-REINFORCED WITH CARBON FIBERS
Sample 8 – THERMOINSULATED INSOLES
Sample 9 – REGULAR INSOLES

RESULTS AND DISCUSSION

The behavior of the obtained materials is evidenced by the most important factors characterizing tensile strength (Table 4): 1st peak peel strength (N/mm), load at break (N), elongation (%), tensile strength (N/mm²), Young's modulus (N/mm²). All these values reveal that the best behavior of the cellulose material can be observed on the samples reinforced with carbon fibers, which presented improved strength and higher reliability for insole production. Tensile strength is dependent on the structure of this material (Fig. 6).

For better understanding the reinforcement benefits, a comparison between non-reinforced and reinforced cellulose with one and two layers of carbon fiber was made (Fig. 7). The importance of carbon retrofitting is also shown for leather insoles by comparison of the reinforced with the non-reinforced samples (Fig. 8).

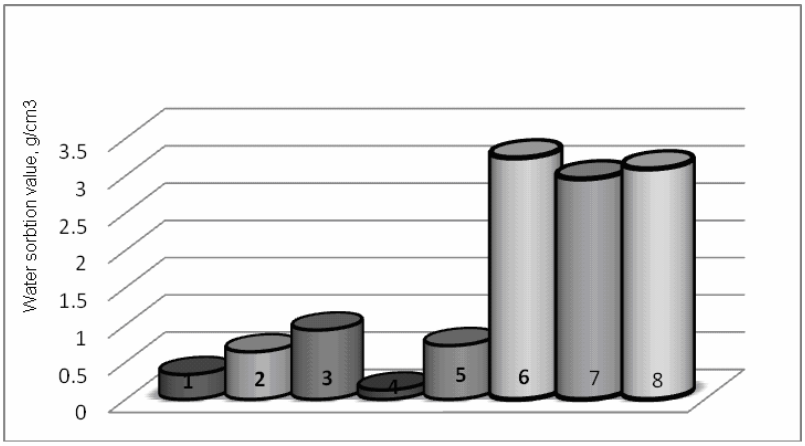


Figure 5: Water retention in sample volume set 1-8

Table 3
Average values of absorbed water W_A [g/m^2] for regular materials in insole fabrication

Set №	Sample set type	Components	W_A (g/m^2)
1	Absorbent cellulose insoles	Cellulose reinforced with cotton fibers	199
2	Perfumed insoles	One layer of polyurethane foam coated with cotton fiber fabric for esthetic purposes	2035
3	Thermal insulation insoles	One layer of polyurethane foam and one layer of wool fibers and polyamide fibers	3456
4	Cork insoles	Cork layer and one covering layer of linen fabric	225
5	Leather insoles	Leather	490
6	Composite insoles (compound X included)	Cellulose with bioactive compound X included into the matrix	8029
7	Composite insoles (compound XI included)	Cellulose with bioactive compound XI included into the matrix	9235
8	Composite insoles (compound XII included)	Cellulose with bioactive compound XII included into the matrix	8345

Table 4
Average values of data recorded for the materials in tensile strength tests

Recorded data	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
1st peak peel strength (N/mm)	608.2	27.0	129.1	0.6	521.8	58.7	190.7	11.5	64.0
Load at break (N)	110.3	15.26	43.13	31.30	288.2	61.17	182.3	27.83	94.43
Elongation (%)	37.44	15.96	22.45	54.67	15.72	31.91	17.31	98.70	34.75
Tensile strength (N/mm²)	30.41	1.36	6.45	0.12	26.09	4.07	9.61	0.58	3.21
Young's Modulus, (N/mm²)	2466	17.66	805.06	2.52	205.95	14.73	751.41	0.50	18.80

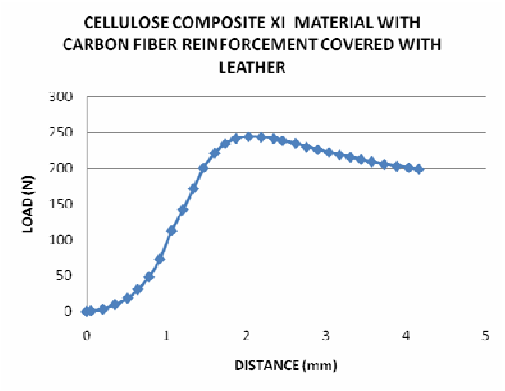
CONCLUSIONS

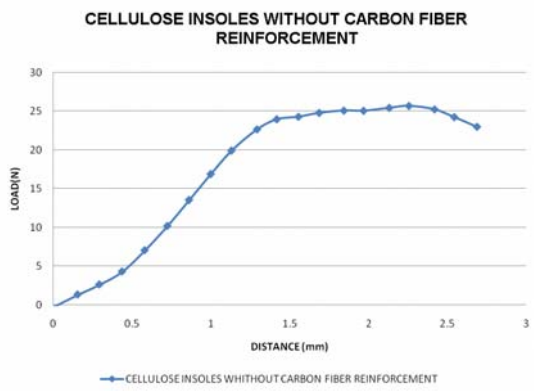
The paper presents a new polymer-bioactive compound system based on regenerated cellulose and hydrazone derivatives, with very promising use in alternative treatments of diabetic foot diseases.

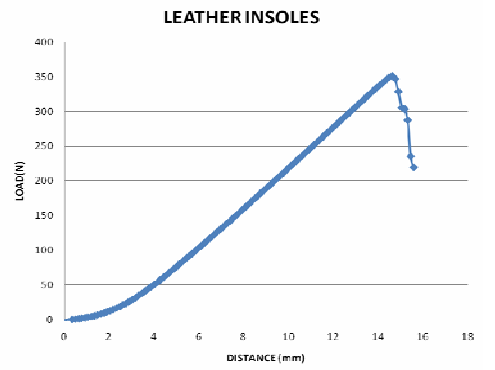
Such treatments are necessary in most foot diseases, once they provide the only opportunity to wear apparently regular shoes and, at the same time, to benefit from continuous onsite treatment. This eliminates the ingestion of various drugs, which could determine some secondary effects, and long periods of external foot treatment.

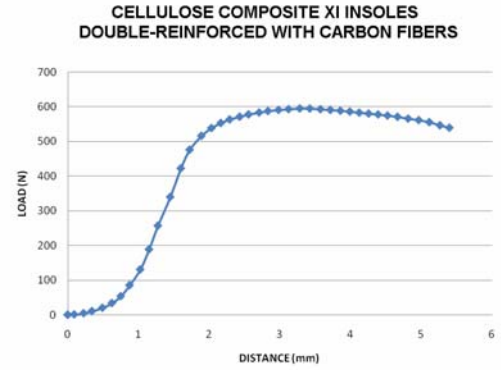
The significantly higher values of water sorption are due to the highly porous and hydrophilic regenerated cellulose present in the composite structure.

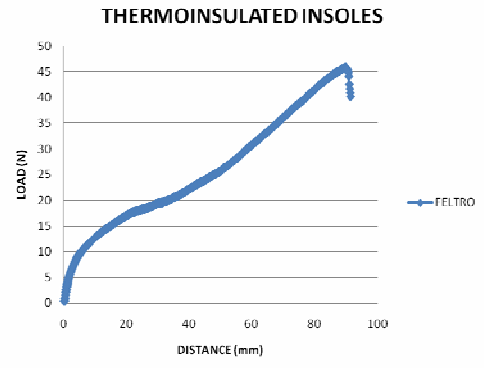
In a worn-out state, when the samples were pressed at maximum pressure levels, the sorption capacity remained within the standard range, proving very good characteristics of the composite for insole fabrication.

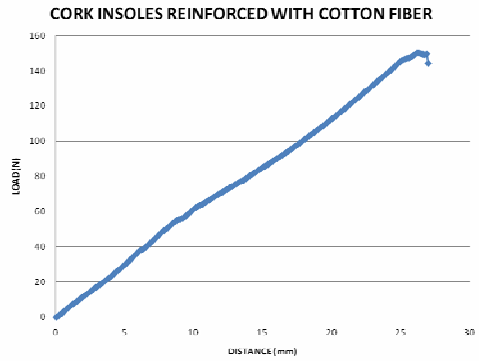












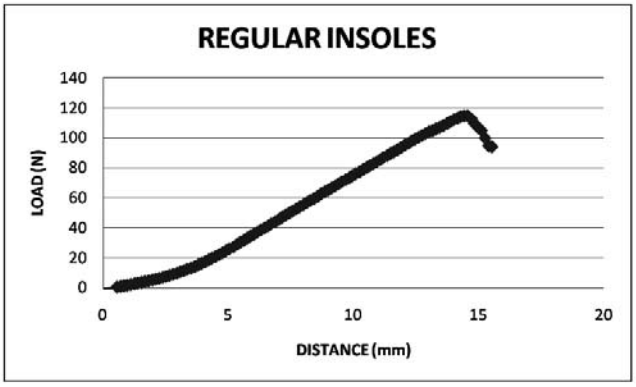


Figure 6: Load (N) of materials *vs*. distance (mm) in tensile tests

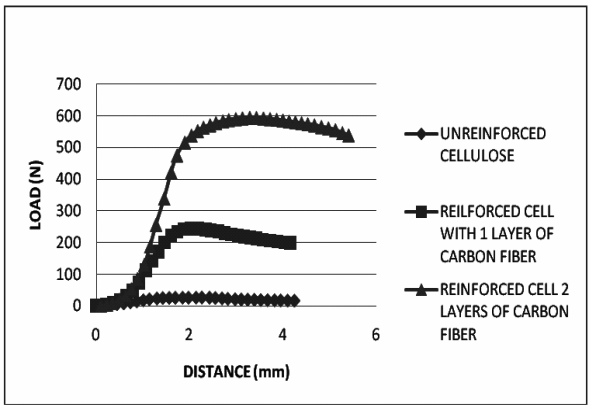


Figure 7: Comparison between non-reinforced and reinforced celluloses

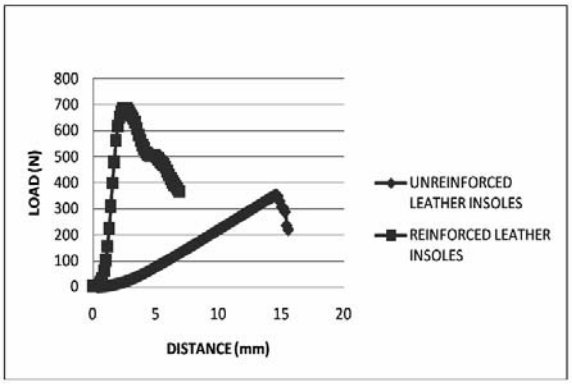


Figure 8: Comparison between non-reinforced and reinforced leather insoles

The immobilization of hydrazone derivatives was done to obtain a low level of toxicity of the bioactive compound and also of its aseptic, germicide and fungicide properties. The porosity of the cellulose matrix and the size of the bioactive compound molecule were the major factors that influenced the immobilization kinetics.

The composite material was tested and proved to be a suitable candidate for medical insole production.

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