UNIVERSITY POLITEHNICA OF BUCHAREST
Faculty of Applied Chemistry and Materials Science

PhD THESIS
Abstract

Obtaining of materials based on natural chromophores and biopolymers with optoelectronic properties

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To my husband and daughter,
with all my love.

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CONTENTS

INTRODUCTION

In the recent years, intense efforts have been made to develop photonic devices based on organic materials. The research is inspired by the richness of organic materials, mainly biomaterials, which are a special class, because they are a renewable source and they are biodegradable. Deoxyribonucleic acid (DNA) is the most popular and the most important biomaterial, because it is the polymeric molecule that carries the genetic code in all living organisms. The interest of scientific communities is increasingly focused on building photonic devices from the "molecule of life" due to its interesting, unique and special properties resulting from its double helical structure.

At the same time, there has been a growing interest in natural sources of dyes due to the growing awareness of the impact of environmental pollution and impact on human health and on that of all living beings. Spices, along with their ability to give aroma and flavor to food have important nutraceutical properties, among which the best known are the ability to "hunt" free radicals responsible for damage to the human body, as well as other benefits such as anti-inflammatory properties, anti - obesity, anti-carcinogenic, anti-inflammatory, etc. The existing chromophore groups grafted on the structures of the compounds that are presented in spices also have dyeing capacities that can form together with different biopolymers new material with photonic / optoelectronic properties.

Starting from these premises the thesis is focused on the elaboration of DNA-based materials and colored extracts obtained from four spices: turmeric, black pepper, paprika and curry leaves. The PhD thesis is structured in two parts, and consists of eight chapters. The first part contains 3 chapters which are dedicated to the documentary study, while the second part contains 5 chapters presenting the original contributions.

Chapter 1 presents possible sources of natural dyes and their classification according to several criteria. This chapter presents a short history of the spices as well as a brief presentation of the DNA. The literature study highlighted possible chromophore groups existing in the four spices studied as well as the beneficial effects they can bring to consumers health.

Chapter 2 presents some methods of extraction of existing dyes in spices such as: maceration, Soxhlet technique, microwave extraction, supercritical fluid extraction, ionic liquid extraction and enzymatic extraction, while **Chapter 3** describes the principles of nonlinear optics with the presentation of techniques for harmonics generation and optical damage threshold.

Chapter 4 describes the reagents, apparatus and methods used to obtain and to characterize the colored extracts based on spices as well as the new materials.

Chapter 5 is devoted to the spice powders characterization as well as to the presentation of the extraction methods employed and developed (maceration and Soxhlet extraction).

Chapter 6 presents the characterization of the obtained extracts (by the two methods) by UV-Vis spectral analysis, spectrofluorimetry, and ultra-performing liquid chromatography coupled with high-resolution mass spectrometry.

DNA functionalization with natural chromophores extracted from spices was performed through three methods presented in **chapter 7**. The obtained materials were spectrally characterized (UV – Vis and fluorescence).

Chapter 8 is structured in two subchapters. The first one is devoted to the thin film obtaining while the second one to the preparation of the colored membranes. For the thin films the spectral and nonlinear optical properties were studied, while the membranes were analyzed from thermal stability and structural morphology point of view.

The PhD thesis ends with the presentation of the general conclusions, the original contributions, the further development perspectives, the list of published papers and conferences, as well as the references.

The PhD thesis includes 174 figures, 52 tables and 194 bibliographical references.

The results of this PhD thesis were capitalized through 4 papers, out of which 1 article in the UPB Bulletin and 3 articles in ISI-listed journals. The results were also presented at 8 international scientific events.

In the following are presented briefly the main results obtained during the PhD study. In this abstract the chapters, subchapters, figures, tables and references numbers are kept as in the PhD thesis.

DOCUMENTARY STUDY

CHAPTER 1. BIOPOLYMERS AND COLORED NATURAL EXTRACTS

Biopolymers are known as materials with outstanding physical, chemical and mechanical properties, with numerous applications in medicine [1], in biomedical, biotechnological [2], food or pharmaceutical industries, being used in microencapsulation, immobilization and controlled release of many active compounds [3]. Biopolymers are polymers produced in living organisms, and there are 3 renewable sources of natural polymers: plants (soy proteins, cellulose), animals (deoxyribonucleic acid - DNA, chitosan, keratin, silk) and bacterial products (poly-hdroxy-butyrate - PHB and polyhydroxial) under nutrient limitation and / or carbon excess [1]. Dyes, derived from natural sources have emerged as an alternative to synthetic dyes. Given the disadvantages of synthetic dyes today there is a growing worldwide interest in the production and use of dyes mainly from two natural sources (plants and microorganisms). According to the literature study, it can be appreciated that the natural extracts of the spices studied, due to their complexity, can be used in gastronomy, to promote health, but they can also represent natural sources of chromophores.

CHAPTER 2. COLORED EXTRACTS

2.1. METHODS OF EXTRACTION OF NATURAL DYES

Over the last 50 years significant progress has been made in the extraction processes of natural pigments from plant materials. Conventional extraction methods, such as maceration, Soxhlet technique and hydrodistillation have been supplemented with new unconventional methods [65] such as: microwave assisted extraction [66], extraction with supercritical fluids [67 - 72], accelerated solvent extraction [66, 73], hydrotropic extraction, enzymatic digestion [74], pulsed electric field extraction [75], ultrasound extraction [76, 77].

Table 2.1. The advantages and disadvantages of natural dye extraction methods

Technology	**Advantages**	**Disadvantages**
Maceration	-small solvent volume -concentrated extract	-high extraction time (several days) -unselective -the need to filter the extract
Soxhlet technique	-short extraction time (few hours), -does not require filtration after completion of extraction	-high solvent volume, -obtaining diluted extract, the need to concentrate the extract with another apparatus, -exposure of the extract to the boiling point of the solvent and degradation of the labile compounds at elevated temperatures, -continuous supervision of the installation
Microwave assisted extraction	-selective, -reduced extraction time - speed, -good performance, -low solvent volume	-special installation / equipment
Supercritical fluid extraction	-fast -selective -pure extracts, without traces of solvents -supercritical fluids have solvation power similar to that of organic solvents, but with higher diffusion power, -non-toxic solvents -the working temperature is low, it avoids the distortion of	-expensive installation to obtain supercritical solvents, -supercritical fluids have lower viscosity and surface tension than organic solvents

Technology	Advantages	Disadvantages
	thermally sensitive products	
Extraction with ionic liquids	-low toxicity -non-volatility and non-flammability -selective	- high cost
Enzymatic extraction	-ecological solvents -non-volatility -non-flammability	- high cost of enzymes

Given the advantages and disadvantages of extraction methods (table 2) were chosen as methods for obtaining colored extracts: maceration and Soxhlet technique, and the results are presented in the chapters belonging to the original contributions part.

CHAPTER 3. NON-LINEAR OPTICAL PROPERTIES OF SOME MATERIALS BASED ON BIOPOLYMERS FUNCTIONLIZED WITH NATURAL DYES

Natural extracts are receiving more and more attention due to their rapid degradation rate and low toxicity. Manea et al. also studied the nonlinear optical properties of natural blueberry-based extracts and functionalized DNA. THG measurements for films showed that a third-order susceptibility of 25 - 34 times higher than the susceptibility of the reference silica is obtained. For an all bio material with nonlinear optical properties it is a remarkable value [129].

Based on the literature data, it can be stated that biopolymers can form with natural chromophores, materials with nonlinear optical properties comparable to those based on synthetic dyes.

II. ORIGINAL CONTRIBUTIONS

RESEARCH OBJECTIVES

The main objective of the PhD thesis was to obtain new organic materials based on natural dyes and biopolymers, namely dyes extracted from spices and DNA obtained from waste, as well as the characterization of the materials obtained. This main objective was achieved by fulfilling the following specific objectives:

- characterization of four spices, namely turmeric, black pepper, paprika and curry leaves in powder form, by:
  - vibrational absorption spectrometry (FT - IR),
  - electronic absorption spectrometry (UV - Vis) including the color analysis,
- obtaining colored extracts in different solvents by two methods:
  - maceration,
  - Soxhlet technique,
- characterization of colored extracts by:
  - electronic absorption spectrometry (UV - Vis),
  - fluorescence spectrometry,
  - high performance liquid chromatography coupled with high resolution mass spectrometry (UPLC - HRMS),
- doping of DNA with colored extracts by the following methods:
  - method A: 10 g / L DNA - CTMA solution and colored extracts in 2 - propanol and n - butanol,
  - method B: DNA – CTMA (solid) and colored extracts in n - butanol,
  - method C: 6 g / L DNA solution in water and colored extracts in 2 - propanol and n - butanol
- characterization of totally "bio" materials by:
  - electronic absorption spectrometry (UV - Vis),
  - fluorescence spectrometry,
  - analysis of the hydrophilic - hydrophobic balance,
  - analysis of photo, thermal and chemical stability,
- thin films obtaining,
- characterization of thin films by:
  - electronic absorption spectrometry (UV - Vis),
  - fluorescence spectrometry,
  - scanning electron microscopy (SEM),
  - analysis of the hydrophilic - hydrophobic balance,
  - analysis of photo, thermal and chemical stability,
  - determination of nonlinear optical properties:
    - determination of the optical damage threshold,
    - third harmonic generation,
- membranes based on biopolymers and colored extracts obtaining
- characterization of the totally "bio" membranes:
  - membrane stability analysis,
  - morphological analysis by scanning electron microscopy (SEM).

CHAPTER 4. EXPERIMENTAL MATERIALS AND METHODS

4.2.1. Maceration

Maceration is the simplest solid-liquid extraction technique. Solid-liquid extraction involves the contact of the solid material with the solvent. It is based on the principle of similarity, the classes of compounds of a certain polarity being extracted by solvents of the same polarity. A number of solvents of different polarity were used to obtain a more complete profile of the classes of compounds in the extracts of interest. The plant material (4 g) was placed in glass containers/jars figure 4.14, over which the solvent (20 mL) was added. The containers/jars were covered with aluminum foil and subjected to magnetic stirring at room temperature. After 48 hours they were filtered, by simple filtration, and the extracts were kept in the refrigerator to avoid degradation of the compounds of interest.

Fig. 4.14. Maceration and filtration of spice samples

4.2.1. Soxhlet technique

The plant material (4 g) was introduced into a paper cartridge and placed in the Soxhlet extractor, Figure 4.15. The samples were extracted until the solvent (150 mL) in the extractor became colorless, an average of 10 siphons were required. The extracts were kept at 4-6 °C for the same reasons as in the case of maceration [142].

Fig. 4.15. The experimental set-up used for Soxhlet extraction

The extracts obtained both by the Soxhlet technique and by maceration were spectrally analyzed and used to obtain new materials based on DNA and colored extracts.

CHAPTER 5. CHARACTERIZATION OF SPICE POWDERS AND OBTAINING OF THE COLORED EXTRACTS

5.1. CHARACTERIZATION OF SPICE POWDERS

5.1.1. Vibration absorption spectrometry (FT-IR) of spice powders

Figures 5.1 (b), 5.2, 5.3, 5.4 (b) show the FT - IR spectra of spice powders, while tables 5.1 - 5.4 list the bands observed in the recorded spectra.

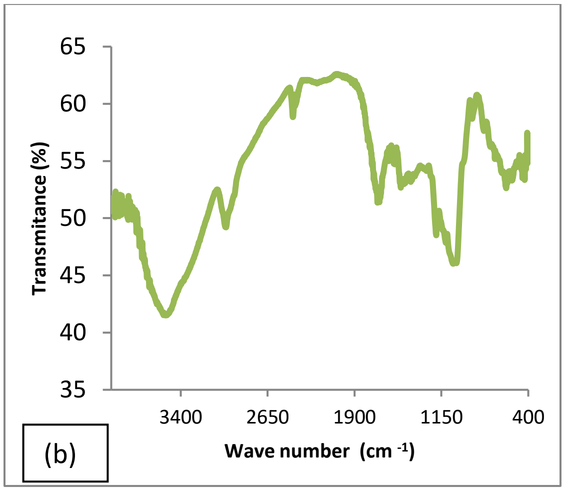


Fig.5.1.(b)FT-IR spectrum of turmeric powder

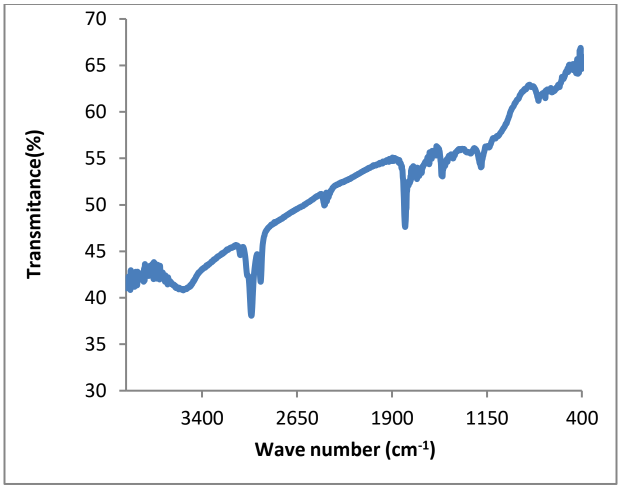


Fig.5.2.FT-IR spectrum of sweet paprika powder

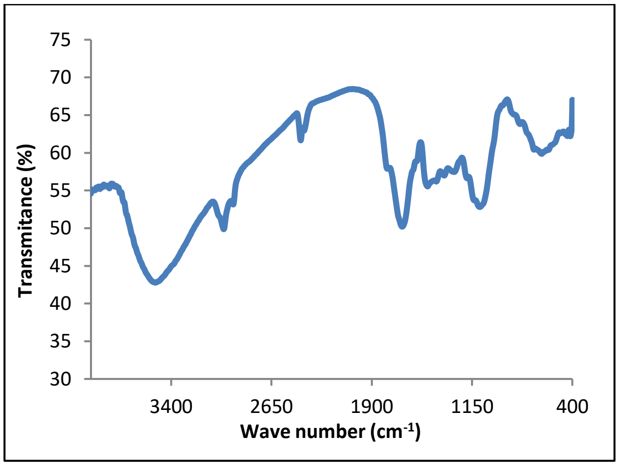


Fig. 5.3. FT - IR spectrum of curry leaves powder

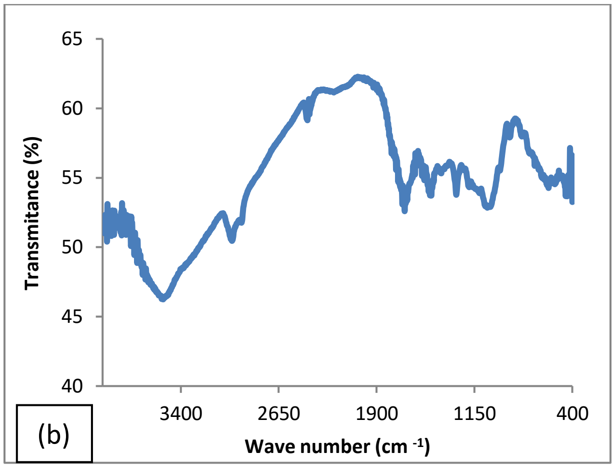


Fig. 5.4. (b) FT - IR spectrum of black pepper

Table 5.1. Spectral assignment for turmeric / standard curcumin / curcumin

Frequency observed in the curcumin spectrum [162] (cm^{-1})	**Frequency observed in the standard curcumin spectrum (cm^{-1})**	**Frequency observed in the spectrum of turmeric powder (cm^{-1})**	**Assigned vibration types of the bonds in the studied matrices**
3508	3603	3508	Stretching vibration of the O - H bond (ν_{OH})
3293	3058.3018	-	Stretching vibration of the C - H bond (ν_{CH})
2972	-	2999	Asymmetric stretching vibration of the C - H bond (ν_{CH}) in the CH_3 group
2945	-	2937	Asymmetric stretching vibration of the C - H bond (ν_{CH}) in the –O - CH_3 group
1626	1676	1702	Bond stretching vibration (C = O) ($\nu_{C = H}$) and Cring – C = C ($\nu_{Cring-C = C}$)
1601	1645	1605	Stretching vibration of the C = C bond of the aromatic ring ($\nu_{C = C}$)
1508	1552	1500	Stretching vibration of the C = O bond (ν C = O), plane deformation vibration of the CCC bonds (γ_{CCC}), CC = O ($\gamma_{CC = O}$)
1272	1237	1194	Stretching vibration of the aromatic C - O bond (ν c-$_{Oaromatic}$), plane deformation vibration of the C - H bond (γ_{CH}) from the group C = CH
959	986	1007	Tensile vibration of the CO bond (ν_{CO}), plane deformation of the CCH group (δ_{CCH})
852	879	868	Out-of-plane deformation vibration of the C-H bonds of the aromatic ring ($\gamma_{C\text{-}H \text{ of the aromatic ring}}$)
713	734	688	Plane deformation vibration of the C - H bonds of the aromatic ring ($\gamma_{C - H \text{ of the aromatic ring}}$)

Table 5.2. Spectral assignment for sweet paprika powder

Frequency observed in the spectrum of sweet paprika powder (cm^{-1})	**Assigned vibration types of the different bonds present in the sweet paprika powder compounds**
3500	Stretching vibration of the O - H bond ($\nu_{O - H}$)
3079	Asymmetric stretching vibration of the C - H bond in the unsaturated group ($\nu_{C - H}$)
3010	Symmetrical stretching vibration of the C - H bond in the unsaturated group ($\nu_{C - H}$)

2937	stretching vibration of the C - H bond in the saturated group (ν_{C-H})
1795	Stretching vibration of the C = O bond ($\nu_{C=O}$)
1680, 1665	Stretching vibration of the C = C bond with trans isomers ($\nu_{C=C \text{ with trans isomers}}$)
1660, 1630	Stretching vibration of the C = C bond with cis isomers ($\nu_{C=C \text{ with cis isomers}}$)
743	Deformation vibration of the C – H bond in the plane (δ_{C-H})

Table 5.3. Spectral assignment for curry leaves

Frequency observed in the spectrum of curry leaves (cm $^{-1}$)	**Assigned vibration types of the different bonds present in curry leaf powder compounds**
3494	O - H bond stretching vibration (ν_{O-H})
3023	Stretching vibration of the C - H bond in the aromatic / unsaturated group ($\nu_{C-H \text{ in the unsaturated group}}$)
2994	Asymmetric CH_2 stretching vibration aliphatic CH ($\nu_{C-H \text{ asymmetric}}$)
2927	Stretching symmetrical vibration of aliphatic CH (symmetric ν_{C-H})
1764	Stretching vibration of the C - O - C bond (ν_{C-OC})
1667	Stretching vibration of the C = O bond ($\nu_{C=O}$), stretching vibration of the primary N - H bond (ν_{NH})
1482, 1410	Deformation vibration of the C – H bond in the plane, from the aromatic ring ($\delta_{C-H \text{ from the aromatic ring}}$)
1062	Out-of-plane C – H bond deformation vibration in the aromatic ring ($\gamma_{C-H \text{ in the aromatic ring}}$)

Table 5.4. Spectral assignment for black pepper / standard piperine / piperine [43]

Piperine literature[43] (cm^{-1})	**standard piperine (cm^{-1})**	**Black pepper powder (cm^{-1})**	**Assigned vibration types of the bonds in the studied matrices**
-	3548	3535	Stretching vibration of the O - H bond (ν_{OH})
3000	3025	3008	Stretching vibration of the aromatic CH bond ($\nu_{\text{aromatic CH}}$)
2925	2928	2935	Asymmetric stretching vibration of the C -

			H bond ($\nu_{\text{asymmetric CH}}$) in the CH_2 ($_{\text{aliphatic}}$) group
1635	1656	1633 f mic	Symmetrical stretching vibration of the C = C (diene) bond ($\nu_{\text{C = C diene}}$)
1635	1656	1665	Stretching vibration - CO - N (ν -$_{\text{CO-N}}$)
1608	1628	1605 f mic	Asymmetric stretching vibration of the C = C (diene) bond ($\nu_{\text{C = C}}$) diene
1608 1580 1495	1628 1553 1490	1605 f mic 1543 1485	Stretching vibration of the C = C bond in the benzene ring ($\nu_{\text{C = C in the benzene ring}}$)
1250 1190	1228 1165	1229 1191	Bond Stretching vibration = C - O - C - asymmetric ($\nu_{\text{= asymmetric COC}}$)
1132	1135	1028	Deformation vibration of the C - H phenyl bond in the plane (δ_{CH})
1030	1025	1028	Bond stretching vibration = C - O - C – symmetric ($\nu_{\text{= symmetrical COC}}$)
930	953	947	Stretching vibration C - O (ν_{COC})
850 830 805	869 826 809	870 830 794	Deformation vibration of the C - H bond outside the plane in the 1,2,4 trisubstituted phenyl group ($\gamma_{\text{CH in the 1,2,4 trisubstituted phenyl group}}$)

5.1.2. Chromatic characterization of spice powders

Table 5.5 shows the values of the trichromatic parameters for the studied spice powders. It is found that all samples have a low brightness, and the values of the parameters a * and b * are consistent with the colors observed visually (red - brick for paprika, yellow - red for turmeric, yellow - green for curry leaves, yellow for black pepper).

Table 5.5. Color parameters for UV-Vis spectra for the studied spices

Spice	**L***	**a ***	**b ***	**C ***	**h ***	**White degree (%)**
Sweet paprika	36.26	11.26	7.8	13.7	34.53	34.8
Turmeric	54.35	13.05	46.7	44.2	72.55	33.4
Curry leaves	48.5	-0.85	13.45	13.45	93.55	46.8
Black pepper	46.53	3.8	14.73	15.23	75.53	44.4

5.3. PARTIAL CONCLUSIONS

The FT - IR spectra, recorded for turmeric and black pepper, confirmed the presence of specific bonds of the chromophore groups from curcumin and piperine.

In the FT –IR spectrum recorded for sweet paprika powder and in the one for curry leaves, bands specific to the C = C and C = O type bonds were observed. These bond present π type electrons and usually belong to chromophore groups.

Based on the color analysis, it was found that all samples had a low brightness. The values of the parameters a * and b * were in agreement with the visually observed colors for all spices as follows: for paprika red-brick, for turmeric yellow-red, for curry leaves yellow-green, for black pepper yellow.

In Soxhlet extraction, the sample is frequently in contact with the fresh solvent which accelerates the mass transfer and extraction of the dissolved substance. Thus, although the basic equipment of Soxhlet extraction is simple, a considerable amount of solvent is required (approximately 7.5 times higher), compared to the maceration method. Moreover, the Soxhlet technique involves a special installation and supervision throughout the extraction. The advantage of Soxhlet extraction, however, is that it does not require filtering.

Since maceration is a simpler technique that does not involve a special installation with supervision during the extraction and since concentrated extracts with low volume of solvent are obtained, this method was further chosen as the process for obtaining the extracts.

CHAPTER 6. CHARACTERIZATION OF COLORED EXTRACTS

The natural extracts obtained on the basis of spices by the two methods, maceration and the Soxhlet technique were characterized by: UV-Vis spectrometry, fluorescence spectrometry and liquid chromatography coupled with mass spectrometry, and the results obtained are presented below.

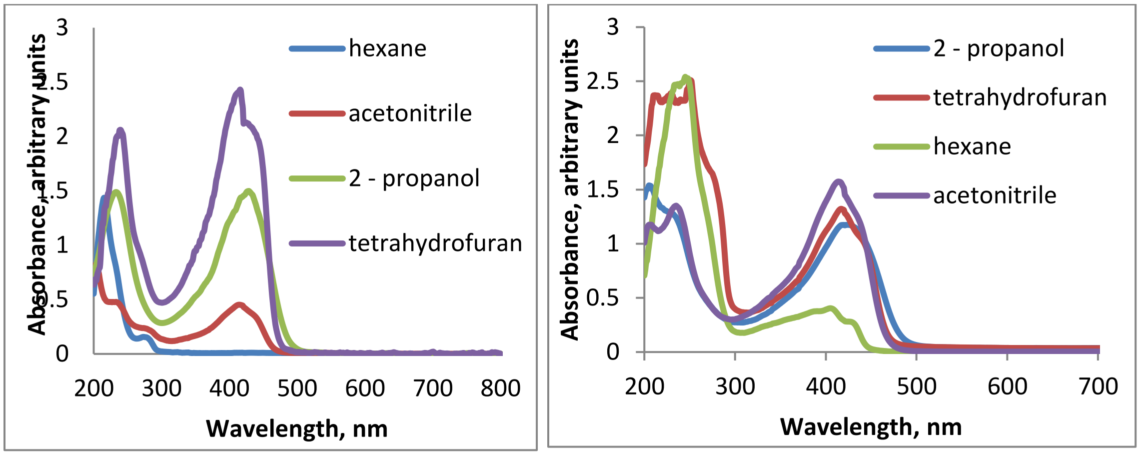
6.1. TURMERIC EXTRACT

6.1.1. UV - Vis and fluorescence spectral characterization

Curcumin is soluble in both polar and non-polar solvents, but is insoluble in water at neutral and acidic pH values. However, it is soluble in alkaline solutions, due to the ionization of phenolic groups [168]. For this reason, turmeric extracts were obtained from turmeric powder in various solvents. In order to be characterized the obtained extracts were subjected to successive dilution because they were highly concentrated.

In table 6.1 are synthetically presented the colors of the extracts and the wavelengths at which the maximum absorption of turmeric extracts was recorded in the following solvents: acetonitrile, tetrahydrofuran, 2 - propanol, n - hexane and n - butanol, for extracts obtained by both maceration and Soxhlet techniques.

Figure 6.1 (a) and (b) show the overlaped spectra of turmeric extracts in the following solvents: acetonitrile, tetrahydrofuran, hexane and 2-propanol. The extracts were obtained by (a) maceration and (b) the Soxhlet technique. The overlaps were made for solutions with the same dilution factor, for each technique. It is known from the literature that turmeric extract in methanol has 2 maxima, one at 240 nm and one at 420 nm [169]. These maxima undergo shifts to shorter or longer wavelengths depending on the polarity of the solvent [170].



(a) (b)

Fig. 6.1. Overlaped spectra of turmeric extracts
(a) maceration (1:400 dilution) and (b) Soxhlet technique (1:100 dilution)

Table 6.1. Color and wavelengths at which absorption maxima were recorded for turmeric extracts

Nr. Crt.	**Extraction technique**	**Solvent**	**µ [Db]**	**Color**	**λ max [nm]**
1	maceration	hexane	0.08	pale yellow	215, 413
2		2 - propanol	1.66 (30 °C)	orange	231, 424
3		n - butanol	1.75	orange	233, 425
4		tetrahydrofuran	1.75	orange-red	238, 429
5		acetonitrile	3.44 (20 °C)	orange	235, 414
1	Soxhlet technique	hexane	0.08	yellow - green	243, 413
2		2 - propanol	1.66 (30 °C)	orange	227, 421
3		n - butanol	1.75	orange	232, 427
4		tetrahydrofuran	1.75	yellow - red	250, 425
5		acetonitrile	3.44 (20 °C)	orange	241, 415

In the emission spectrum shown in figure 6.3. (b) an increase in fluorescence intensity can be observed as the concentration of the sample increases up to the C3 sample or 1: 600 dilution. Starting with the 1: 500 dilution it can be seen that a fluorescence quenching occurs probably due to the formation of chromophore aggregates. The samples are diluted as follows: C1 (1: 200), C2 (1: 500) C3 (1: 600) and C4 (1: 700).

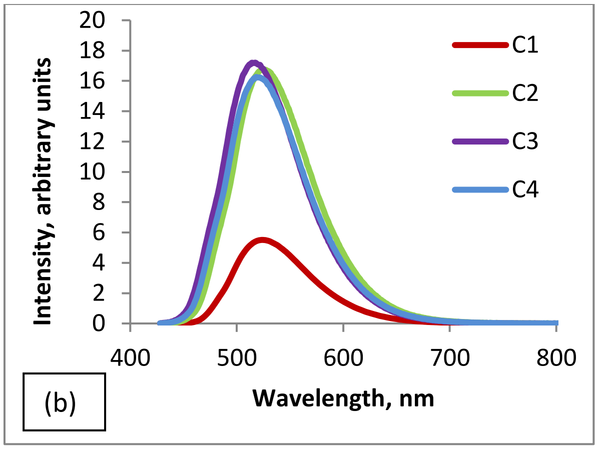


Fig. 6.3.(b) Emision spectra of turmeric extract in 2 - propanol (λ_{ex} = 448nm)

6.1.2. Chromatographic characterization

In table 6.6. the attribution of the compounds identified in the turmeric extract in n - butanol is presented. The ions specific to the following compounds were identified: curcumin, demethoxycurcumin and bisdemethoxycurcumin.

Table 6.6. Assignment of turmeric extract compounds

Compound	**Retention time [min]**	**m / z measured**	**Ion formula**	**The total formula**	**m / z**	**adduct**	**z**
Bisdemethoxicurcumin	11.4	309.1123	$C_{19}H_{17}O_4$	$C_{19}H_{16}O_4$	309.1121	M+H	1+
Demethoxi curcumin	11.5	339.1227	$C_{20}H_{19}O_5$	$C_{20}H_{18}O_5$	339.1227	M+H	1+
Curcumin	11.8	369.1331	$C_{21}H_{21}O_6$	$C_{21}H_{20}O_6$	369.1333	M+H	1+

Based on the spectral characterization and the results obtained by the analysis of UPLC - HRMS it can be stated that turmeric extracts contain the chromophores of interest.

6.2. SWEET PEPPER EXTRACT

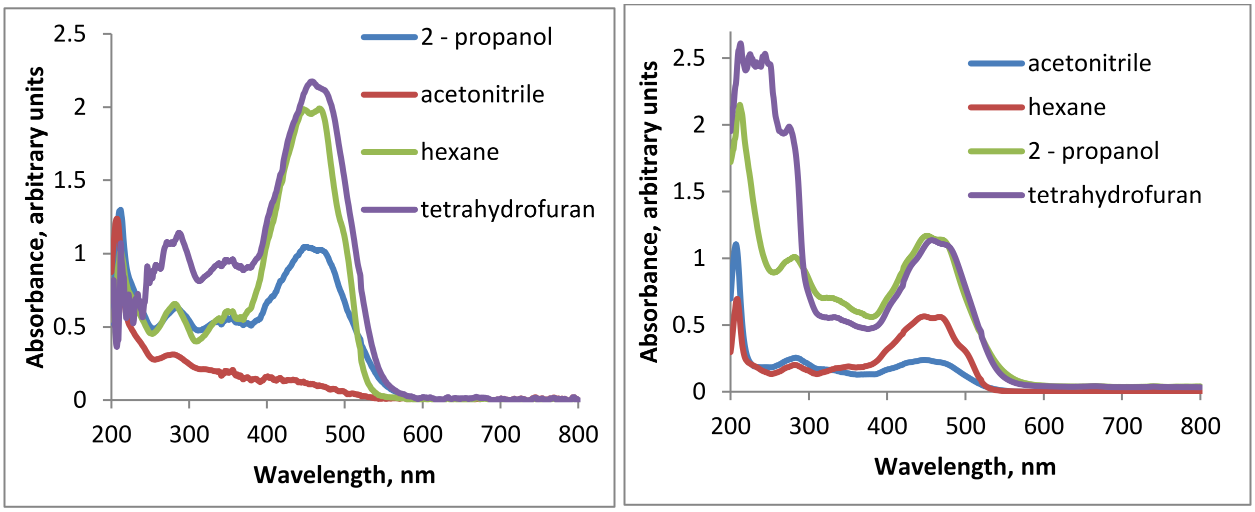
6.2.1. UV - Vis and fluorescence spectral characterization

In table 6.7. both the absorption maxima and the colors of the extracts obtained by the two techniques: maceration and the Soxhlet technique are synthetically presented.

Table 6.7. Color and wavelengths at which absorption maxima were recorded for sweet paprika extracts

Extraction technique	**Solvent**	**μ [Db]**	**Color**	**λ max [nm]**
maceration	hexane	0.08	red - brick	210, 275, 351, 451, 470
	2 - propanol	1.66 (30 ° C)	red - brick	209, 275, 351, 451, 470

	n - butanol	1.75	red - brick	210, 276, 350, 452, 474
	tetrahydrofuran	1.75	red - brick	213, 291, 351, 456, 481
	acetonitrile	3.44 (20 °C)	orange	204, 279
Soxhlet technique	hexane	0.08	brick-red	208, 278, 450
	2 - propanol	1.66 (30 °C)	red - orange	210, 281, 451, 483
	n - butanol	1.75	red - orange	211, 282, 453, 480
	tetrahydrofuran	1.75	yellow - green	209, 275, 452, 485
	acetonitrile	3.44 (20 °C)	orange	211, 279, 450



(a) (b)

Fig. 6.11. Overlaped spectra of sweet paprika extracts
(a) maceration (1:50 dilution) and (b) Soxhlet technique (1:10 dilution)

The complex appearance of the spectra on the UV-Vis wavelength range may be due to the complexity of the extract (figure 6.11).

In figure 6.12. are shown the chemical structures of the compounds of the most important classes of flavonoids that can exist in the extracts studied for sweet paprika powder.

Analyzing, the spectra recorded for the extracts, obtained by the maceration method, figure 6.11 (a) it can be seen that there are similar to the spectra recorded for the extracts obtained by the Soxhlet method figure 6.11 (b). The peaks observed in all extracts at wavelengths of approximately 210 nm and 280 nm can be attributed to the A ring from the flavones, wihle the shoulder observed at about 330 nm to the B ring. The band observed at 280 nm may also be due to the presence of flavonols. The wide peak observed in the range of 400 - 500 nm hides two peaks at about 450 and 470 nm due to the presence of anthocyanins [174] and carotenoids [175, 176].

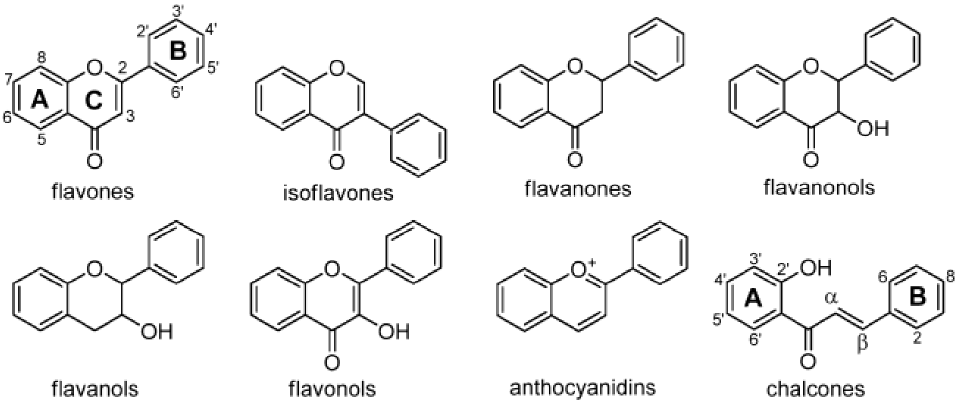


Fig. 6.12. The structures of the compounds of the most important classes of flavonoids

It can be seen from fig. 6.31 (b) that, as the concentration of fluorescent compounds decreases, there is an increase in fluorescence intensity suggesting the formation of aggregates as the number of fluorescent molecules increases. The samples were diluted and marked as B1 (1:15), B2 (1:30) and B3 (1:50).

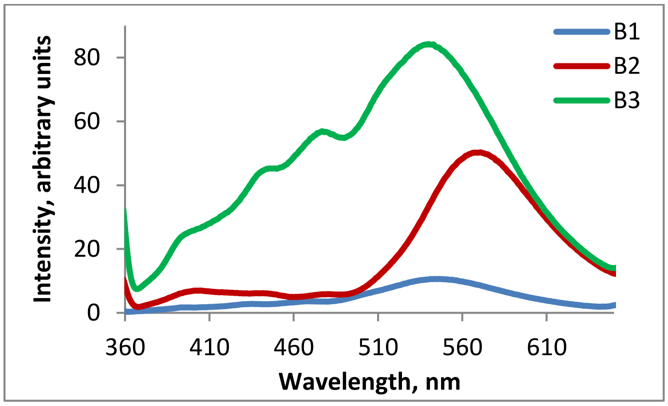


Fig. 6.13. (b) Overlaped emission spectra λ_{ex} = 340 nm of sweet paprika extract in 2 - propanol for different dilutions

6.2.2. Chromatographic characterization

According to the UPLC - HRMS analysis, the major compound in the sweet paprika extract in n - butanol is capsanthin, Table 6.10.

Table 6.10. Assignment of the sweet paprika extract compound

Compound	**Retention time [min]**	**m/z measured**	**Ion formula**	**The total formula**	**m / z**	**adduct**	**z**
Capsanthin	20.6	585.4307	$C_{40}H_{57}O_3$	$C_{40}H_{56}O_3$	585.4302	M+H	1+

The UPLC - HRMS analysis corroborated with the spectral characterization (the presence of absorption maxima in the range of 400 - 500 nm specific for carotenoids) confirms the presence of capsanthin in the studied extract.

6.3. CURRY LEAVES EXTRACT

6.3.1. UV - Vis and fluorescence characterization

In table 6.11. it can be seen the maximum absorption of curry leaves extracts obtained by maceration and Soxhlet technique, as well as their colors. The extracts had the same color with slightly different shades for all solvents used.

Table 6.11. Color and wavelength at which absorption maxima were recorded for curry leaves extracts

Nr. Crt.	Extraction technique	Solvent	μ [Db]	Color	λ max [nm]
1	maceration	hexane	0.08	yellow - brown	242, 292, 354, 408, 668
2		2 - propanol	1.66 (30 °C)	yellow - brown	243, 290, 352, 417, 677
3		n - butanol	1.75	yellow - brown	245, 289, 361, 415, 674
4		tetrahydrofuran	1.75	yellow - brown	200 - 300, 415, 500, 672
5		acetonitrile	3.44 (20 °C)	yellow - brown	242, 269 - 277, 326, 352, 425, 660
1	Soxhlet technique	hexane	0.08	yellow - brown	242, 289, 344, 362, 416, 677
2		2 - propanol	1.66 (30 °C)	yellow - brown	200, 300, 361, 414, 678
3		n - butanol	1.75	yellow - brown	243, 291, 363, 416, 676
4		tetrahydrofuran	1.75	yellow - brown	225, 263, 302, 346, 416, 675
5		acetonitrile	3.44 (20 °C)	yellow - brown	242, 289, 344, 362, 417, 676

6.3.2. Chromatographic characterization

In table 6.15. are presented the assignments resulting from the UPLC - HRMS analysis. The specific ions of the following compounds have been identified: myricetin - 3 - glucoside and quercetin. These two compounds together with campferol, fisetin, galagin belong to the subclass of flovonols, the class of flavonoids that are found in the large group of polyphenols [178].

Based on the UV - Vis spectra of the studied extracts, where the absorption maxima characteristic of quercetin and myricetin are presented at 260 nm and 360 nm [179, 180] on the

UPLC - HRMS analysis it can be concluded that the two compounds were successfully identified in the extracts obtained.

Table 6.15. Assignment of curry leaf extract compounds

Compound	**Retention time [min]**	**m/z measured**	**Ion formula**	**The total formula**	**m/z**	**adduct**	**z**
myricetin-3-glucoside	7.2	479.0829	$C_{21}H_{19}O_{13}$	$C_{19}H_{20}O_{13}$	479.0831	M-H	1-
quercetin	10.4	301.0353	$C_{15}H_9O_7$	$C_{15}H_{10}O_7$	301.0354	M-H	1-

6.4. BLACK PEPPER EXTRACT

6.4.1. UV – Vis and fluorescence spectral characterization

In table 6.16. the absorption maxima of black pepper extracts in different solvents can be observed. The extracts and their colors are of different shades of yellow (table 6.16).

Table 6.16. Colors and wavelength at which absorbtion maxima were recorded for black pepper extract

Nr. Crt.	**Extraction technique**	**Solvent**	**μ [Db]**	**Color**	**λ max [nm]**
1	maceration	glacial acetic acid	0.08	yellow - green - dark	260, 349
2		2 - propanol	1.66 (30 °C)	pale yellow	207, 258, 344
3		n - butanol	1.75	pale yellow	207, 261, 305, 344
4		ethanol	1.75	yellow - greenish - pale	220, 267, 345

In the emission spectrum a maximum can be observed at the wavelength of 426 nm, the increase of the dilution determines the increase in intensity of this peak suggesting that at high concentrations in solution, aggregates could appear. The dilution ratios and the corresponding notations are as follows: B41 (1: 300), B42 (1: 500), B43 (1: 700), B44 (1: 1000) and B45 (1: 1500).

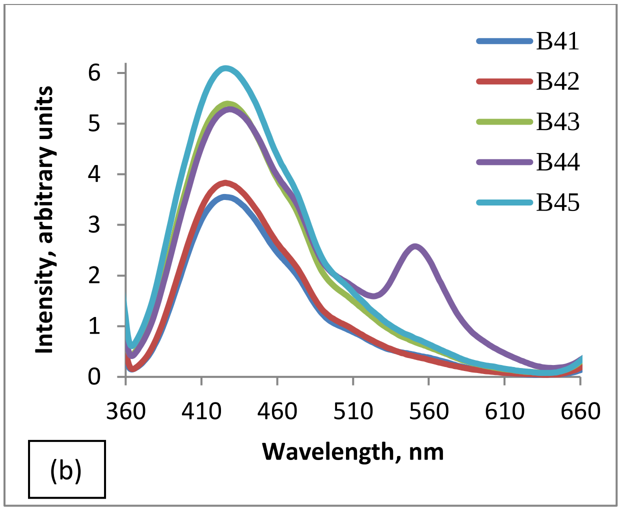


Fig. 6.29.(b). Overlaped emission spectra (λex = 342 nm) of black pepper extract in 2 - propanol for different dilutions

6.4.2. Chromatographic characterization

Table 6.19 shows the attribution of the UPLC-HRMS determination. The identified compound was piperiline.

In the black pepper extract in n - butanol was identified, on the basis of the mass spectrum, piperiline or trichostachine [42]. This compound has a piperine – like structure [40].

The results obtained on the basis of mass spectrometry as well as spectral characterization show that black pepper extract contains piperiline.

Table 6.19. Assignment of the black pepper extract compound to n - butanol

Compound	**Retention time [min]**	**m/z measured**	**Ion formula**	**The total formula**	**m / z**	**adduct**	**z**
Piperiline	13.2	272.1283	$C_{16}H_{18}NO_3$	$C_{16}H_{17}NO_3$	272.1281	M+H	1+

6.4.3. Qualitative determination of the presence of piperine in black pepper extract

Figure 6.33 shows the samples of black pepper extract tested according to the procedure described in Chapter 2. It can be estimated that the extracts contain piperine.

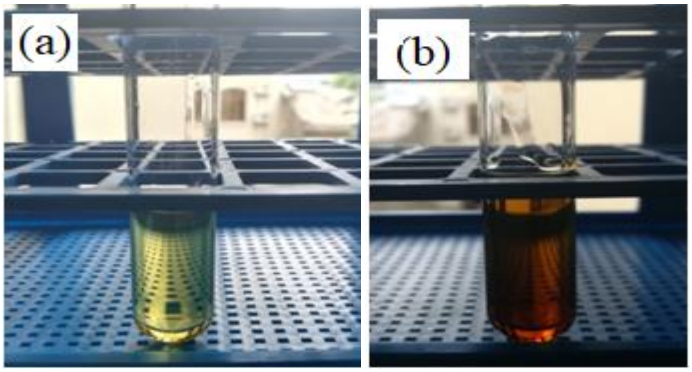


Fig.6.33. Reaction of piperine with sulfuric acid (a) pepper extract in glacial acetic acid, (b) pepper extract in glacial acetic acid after the addition of few drops of H_2SO_4 (98%).

6.5. PARTIAL CONCLUSIONS

In this chapter, the spice-based extracts in different solvents were characterized spectrally and chromatographically. It was found that for each spice were obtained complex extracts containing chromophores with fluorescent properties.

Thus, for turmeric along with curcumin, its isomers bisdemethoxycurcumin and demethoxycurcumin were also identified.

In the case of sweet paprika, extract which is known to be rich in carotenoids, capsanthin, a compound belonging to this class, has been identified.

Following the analysis of the extracts of the curry leaves, the compounds miricetin - 3 - glucoside and quercetin were identified, and in the black pepper extract was identified piperiline, a compound belonging to the same class as piperine.

The solvents chosen to continue the experiments were: 2 - propanol and n - butanol.

CHAPTER 7.OBTAINING AND CHARACTERIZING THE SOLUTIONS BASED ON BIOPOLYMERS AND COLORED EXTRACTS

In this chapter will be presented the methods of obtaining solutions based on DNA and colored extracts, as well as their spectral characterization. There were chosen only the extracts obtained by maceration and using as extraction solvents n - butanol and 2 - propanol. Several methods based on DNA or DNA-CTMA were used.

In order to obtain solutions based on DNA and colored extracts, 3 methods were considered:

- method A: 10g / L solution of DNA - CTMA in n - butanol and colored extracts in 2 - propanol and n - butanol, the schematic presentation is shown in figure 7.3,
- method B: DNA - CTMA (solid) and colored extracts in n - butanol, the schematic presentation is shown in figure 7.5,
- method C: 6g / L DNA solution in water and colored extracts in 2 - propanol and n - butanol, the schematic presentation is shown in figure 7.7.

In figure 7.4. the samples obtained by method A are presented, the DNA - CTMA solutions and colored extracts were miscible and clear solutions whith different colors depending on the colored extract used were obtained. The samples obtained by method B (figure 7.6) were also clear, the DNA-CTMA complex being completely dissolved in the colored extracts. These solutions were more intense colored than those obtained by method A.

The samples obtained by method C behaved differently depending on the solvent used as it can be seen in Figure 7.8. The samples based on n - butanol extracts showed the formation of two immiscible layers: a lower one opaque, white when sweet paprika extract was used, yellow when turmeric extract was used and green when curry leaves extract was used. The top layer was clear with intense colors specific to the extracts used. The samples obtained from the extracts in 2 - propanol were miscible/homogeneous (from the point of view of the liquid phase) but they were not clear, sign that a part of the DNA had solidified.

Tables 7.1 - 7.3 show the plant materials used to obtain the solutions based on biopolymer and colored extracts, as well as the solvents used and the coding of the samples obtained by the 3 methods: A, B and C.

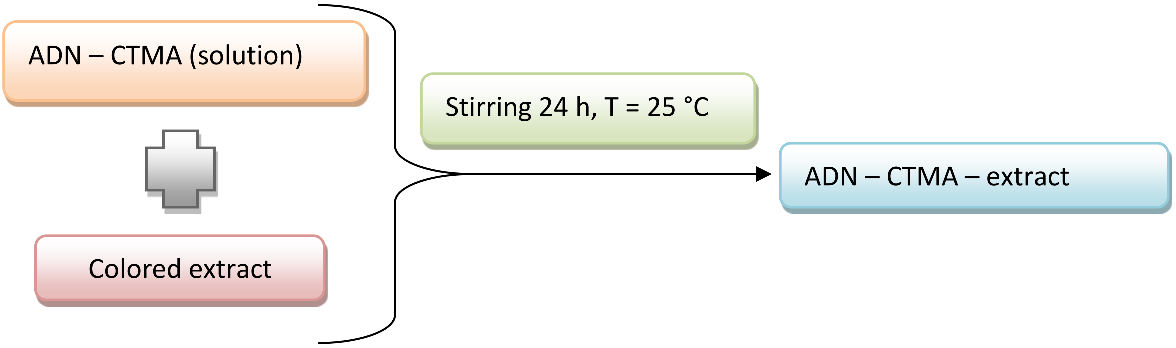


Fig. 7.3. Scheme for obtaining the DNA - CTMA - colored extracts solutions (method A)

Table 7.1. Solutions obtained from DNA - CTMA (solution) and colored extracts (method A)

Plant material	**Solvent**	**Sample code**	**Extraction method**
sweet paprika	2 - propanol	ACA1	maceration
	n - butanol	ACA2	
turmeric	2 - propanol	ACA3	
	n - butanol	ACA4	
curry leaves	2 - propanol	ACA5	
	n - butanol	ACA6	
black pepper	2 - propanol	AB3	
	n - butanol	AB4	
turmeric	n - butanol	ACA7	Soxhlet
	2 - propanol	ACA8	

Fig. 7.4. DNA - CTMA - colored extracts solutions, obtained by method A

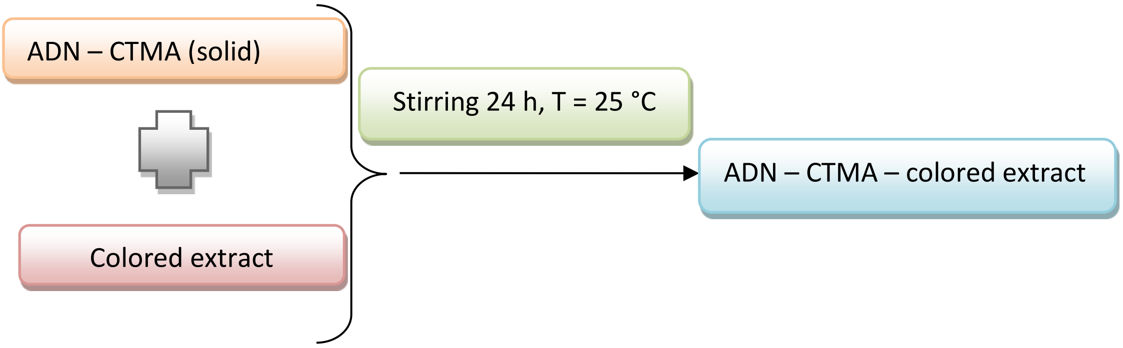


Fig. 7.5. Schematic presentation of obtaining DNA - CTMA and colored extracts solutions (method B)

Table 7.2. Solutions obtained from DNA - CTMA (solid) and colored extracts (method B)

Plant material	**Solvent**	**Sample coding**	**Extraction method**
Sweet paprika	2 - propanol	A1AC	maceration
	n - butanol	A2AC	
Turmeric	2 - propanol	A3AC	
	n - butanol	A4AC	
Curry leaves	2 - propanol	A5AC	
	n - butanol	A6AC	
Turmeric	n - butanol	A7AC	Soxhlet
	2 - propanol	A8AC	

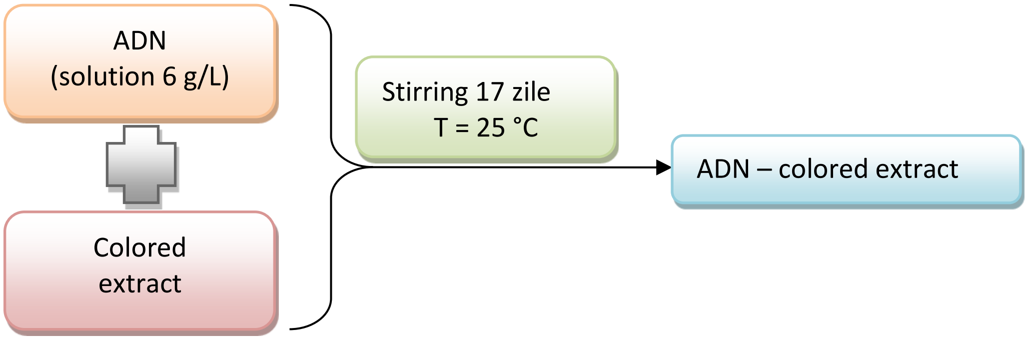


Fig. 7.7. Schematic presentation of the DNA - colored extracts solutions (method C)

Table 7.3. Solutions obtained from DNA solution in water and colored extracts (method C)

Plant material	**Solvent**	**Sample code**	**Extraction method**
Sweet paprika	2 - propanol	AA1	maceration
	n - butanol	AA2	
Turmeric	2 - propanol	AA3	
	n - butanol	AA4	
Curry	2 - propanol	AA5	

leaves	n - butanol	AA 6	
Turmeric	n - butanol	AA7	Soxhlet
	2 - propanol	AA8	

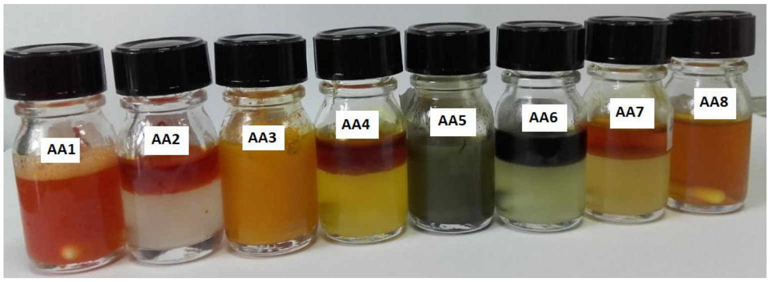


Fig. 7.8. Samples AA1 - AA8, obtained by method C, for sample code see table 7.3

7.2. CHARACTERIZATION OF SOLUTIONS BASED ON NATURAL BIOPOLYMERS AND COLORED EXTRACTS

7.2.1. UV - Vis spectral characterization

Similar to the spectra recorded for solutions based on DNA - CTMA (solution) and extracts, the spectra of the solutions of solid - CTMA doped with colored extracts were also obtained. As expected in all spectra are found the specific absorption maxima of DNA - CTMA [124, 183] and the absorption maxima specific to extracts.

In all cases, the presence of both the biopolymer and the chromophore is clearly observed.

7.3. PARTIAL CONCLUSIONS

Three methods were used to obtain new materials based on colored extracts and biopolymers (DNA and DNA - CTMA).

The new solutions were characterized spectrally and following the electronic spectra it was possible to observe the presence of both chromophores and DNA, which means that the biopolymer was successfully doped.

The fluorescence spectra led to the idea that both method A, i.e. mixing two solutions, and method B, which consists in dissolving DNA - CTMA in the extract, are optimal to obtain materials with higher optical properties.

The materials obtained have been used to obtain thin films and membranes which are presented and characterized in Chapter 8.

CHAPTER 8. OBTAINING AND CHARACTERIZATION OF FILMS AND MEMBRANES BASED ON BIOPOLYMERS AND NATURAL EXTRACTS

This chapter will present the methods of obtaining thin films and membranes based on DNA and colored extracts. The films and membranes obtained have been characterized spectrally and by a number of other techniques that are presented in detail throughout the chapter.

8.1. THIN FILMS

8.1.1. Thin films obtaining

Figure 8.1 shows the scheme for obtaining thin films. These films were obtained on the glass slides by the spin-coating procedure using the solutions presented and characterized in Chapter 7. After the solutions were deposited on the glass slides, the thin films were kept in the oven to remove traces of solvent.

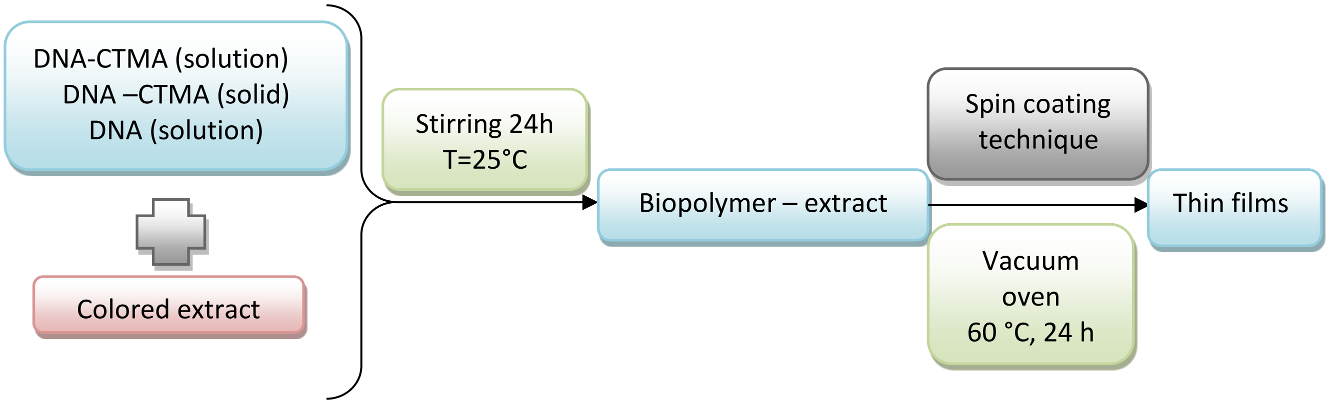


Fig. 8.1. Scheme for obtaining thin films

8.1.2. Spectral characterization of thin films

The thin films were characterized spectrally by recording the UV - Vis spectra. In all cases it can be seen that the films have absorption maxima at wavelengths characteristic of both DNA and chromophores specific to each extract that formed the basis of the solutions. The appearance of the spectra is similar regardless the method used to obtain the solutions (A, B or C).

Absorption spectra were recorded for thin films obtained from solutions that were made with different volumes of colored extracts. The allure of the spectra is similar to that observed for the spectra of the solutions. In generally, the absorbance intensity increases with increasing the cromophore concentration, but anomalies were also found, probably due to the different thickness of the films.

8.1.3. Thickness of thin films

In order to analyze the surface morphology, several DNA-based films doped with colored extracts were analyzed by scanning electron microscopy. The thin films were obtained by spin coating technique on a glass or copper support. Following the analysis of the obtained images, it can be concluded that the technique of obtaining the films, the support on which the films are deposited as well as the colored extract used influence their morphology. It should be noted, however, that these images were obtained as a result of a compromise between energy and image, as these films are very sensitive being burned during recording.

Figures 8.23 - 8.25 show the SEM images obtained on the prepared films. Figures 8.23 and 8.24 show both an ordering of the DNA strands and an agglomeration of chromophore molecules. In the case of DNA - CTMA films doped with black pepper extract deposited on the glass (figure 8.25) no regular ordering is observed.

Table 8.3 shows the film thicknesses obtained for the films of DNA and DNA-CTMA-doped with various colored extracts, depending on the method of obtaining the stock solutions, the extract and on the spin speeds used to deposit the film.

Table 8.3. Variation of the film thickness with the method of film preparation, the method of obtaining the stock solution and the method of obtaining the colored extract. Thin films deposition programs: A = 1500 rot / 40 sec, 2000 rot / min – 1 min, C = 1200 rot / 40 sec, 1800 rot / min – 1 min, E: 500 rot / min – 20 sec, 800 rot / min – 40 sec. Methods A, B, C of solutions obtaining are described in subchapter 7.1

Nr. Crt.	**Thin film**	**Method of obtaining/ technique (M-maceration, S-Soxhlet)**	**Thickness [nm]**	**Thin film deposition program**
1.	ADN – CTMA – sweet paprika in 2 – propanol	A/M	36±4	C
2.	ADN – CTMA – sweet paprika in 2 – propanol	A/M	50±5	E
3.	ADN – CTMA – turmeric in n – butanol	A/S	20±3	E
4.	ADN – turmeric in n – butanol	C/S	NA	A
5.	ADN – CTMA – sweet paprika in n – butanol	A/M	50±5	E
6.	ADN – CTMA – turmeric in n – butanol	A/M	32±4	E
7.	ADN – CTMA – turmeric in n – butanol	B/S	65±5	E
8.	ADN – CTMA – curry leaves in n – butanol	A/M	25±3	E
9.	ADN – CTMA – turmeric in 2 – propanol	A/M	80±8	E
10.	ADN – CTMA – curry leaves in 2 – propanol	A/M	19±2	E
11.	ADN – CTMA – turmeric in 2 – propanol	A/S	26±3	E
12.	ADN – CTMA – sweet paprika in 2 – propanol	B/M	79±8	E
13.	ADN – CTMA – sweet paprika in n – butanol	B/M	28±3	E
14.	ADN – CTMA – turmeric in 2 – propanol	B/M	78±8	E
15.	ADN – CTMA – turmeric in n – butanol	B/M	168±12	E

Nr. Crt.	Thin film	Method of obtaining/ technique (M-maceration, S-Soxhlet)	Thickness [nm]	Thin film deposition program
16.	ADN – CTMA – curry leaves in n – butanol	B/M	35±4	E
17.	ADN – CTMA – turmeric in 2 – propanol	B/S	50±4	E
18.	ADN – sweet paprika in 2 – propanol	C/M	30±4	A
19.	ADN – sweet paprika in n – butanol	C/M	30±3	A
20.	ADN – turmeric in 2 – propanol	C/M	30±3	A
21.	ADN – turmeric in n – butanol	C/M	30±4	A
22.	ADN – curry leaves in 2 – propanol	C/M	30±4	A
23.	ADN – curry leaves in n – butanol	C/M	30±4	A
24.	ADN – turmeric in 2 – propanol	C/S	NA	A
25.	ADN – CTMA –black pepper in n – butanol	A/M	58±5	E
26.	ADN – CTMA – black pepper in 2 – propanol	A/M	48±4	E
27.	ADN – CTMA – black pepper in n – butanol	B/M	64±5	E
28.	ADN – CTMA – black pepper in 2 – propanol	B/M	56±5	E

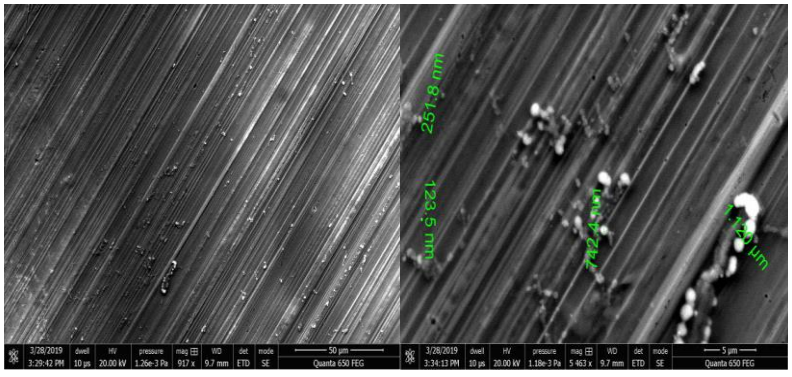


Fig. 8.23. SEM images of a DNA-CTMA turmeric film deposited on copper, at different magnifying powers (a) 917 x and (b) 5463 x

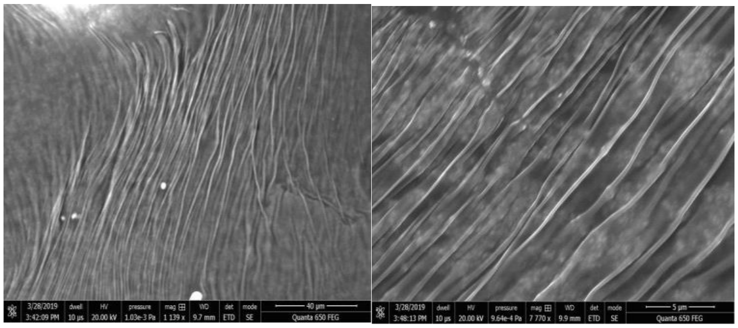


Fig. 8.24. SEM images of a DNA – CTMA turmeric film deposited on glass at different magnifying powers (a) 1139 x and (b) 7770 x

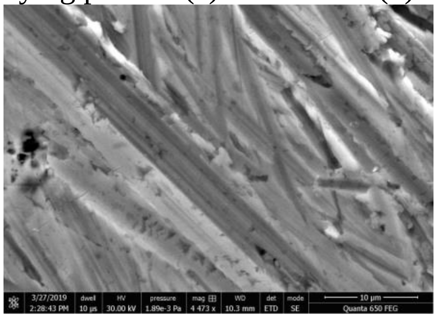


Fig. 8.25. SEM image of a DNA - CTMA - black pepper film deposited on glass at a magnification of 4473 x

8.1.4. Hydrophilic - hydrophobic balance

Figure 8.26 graphically represents the contact angle values for each liquid sample (black pepper, turmeric and sweet paprika extract) on different solid materials (steel, Teflon or glass).

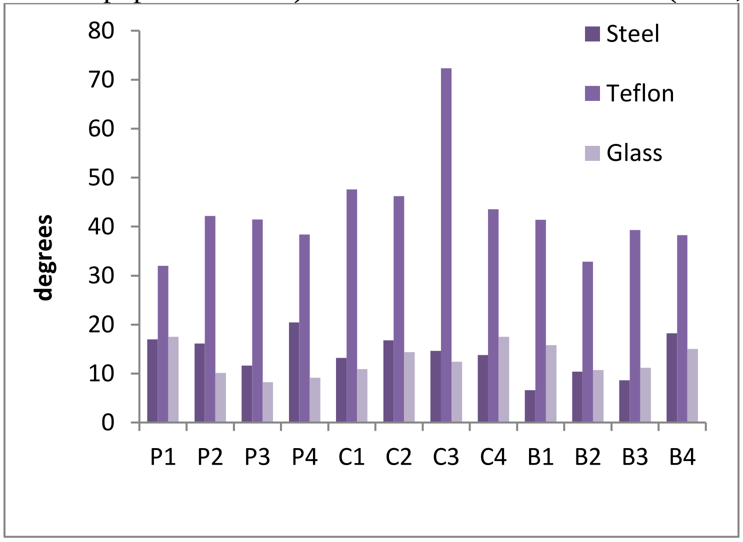


Fig.8.26. Graphical representation of the contact angle for each liquid sample (colored extract of black pepper, turmeric and sweet paprika) on different solid materials (steel, Teflon or glass), P1-P4 black pepper extract different concentration (P1 the smallest, P4 the highest), C1-C4 turmeric extract different concentration (C1 the smallest, C4 the highest), B1-B4 sweet paprika extract different concentration (B1 the smallest, B4 the highest)

From the results obtained it can be seen that the values of the contact angles measured for Teflon are higher (> 30 degrees) compared to those measured on materials such as steel or glass. The data obtained show that natural extracts are more adherent to the surface of steel and glass.

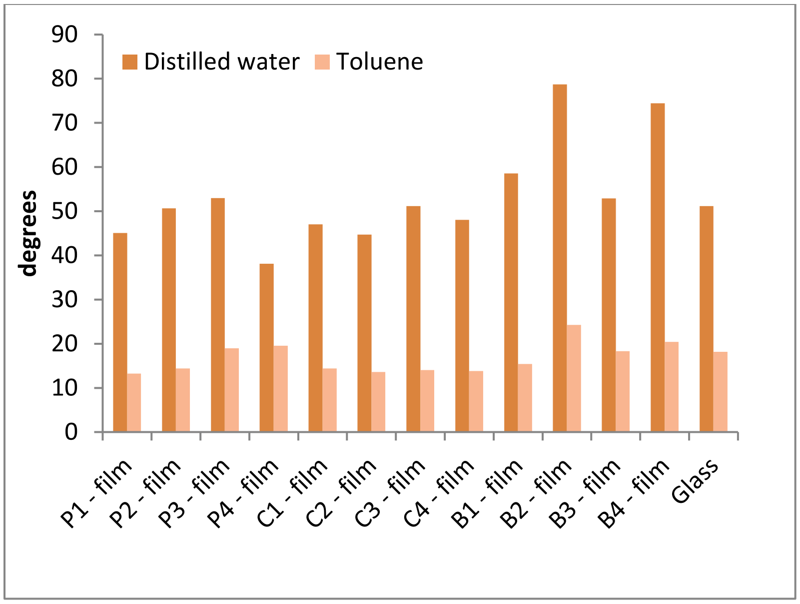


Fig. 8.27. Graphical representation of the contact angle of a drop of distilled water or toluene on the surface of thin films based on natural extracts, notations according to figure 8.26

Figure 8.27 shows that in the case of thin films, the highest values of the contact angle are recorded in the case of distilled water (> 30 degrees), and the lowest values (<24.27 degrees) in the case of toluene, suggesting a hydrophobic character of the films.

8.1.5. Photo, thermal and chemical stability of solutions and thin films

The chemical degradation of the studied films was monitored by the temporal variation of the absorption spectra (figure 8.28 - example).

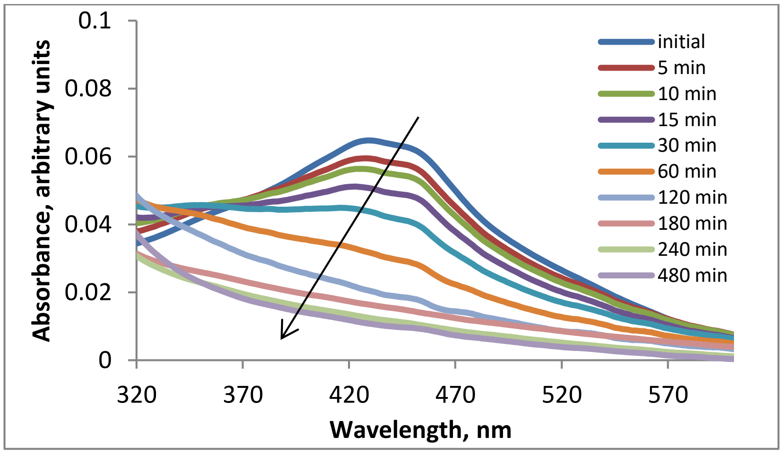


Fig. 8.28. Variation of the absorption spectrum for a DNA - CTMA turmeric extract film subjected to UVB degradation

Table 8.7 presents the data obtained for solutions and films based on DNA - CTMA and colored extracts for degradations at room temperature and at high temperatures.

Analyzing the data in Table 8.7, it is found that unlike synthetic chromophores, the degradation of colored extracts at high temperatures is less pronounced. The composition of colored extracts also plays an important role in the value of activation energy. Thus, it is found that for turmeric and sweet paprika extracts the activation energy is higher in the case of solutions compared to the activation energy of the degradation of the extracts in the form of thin films. In all cases, however, the values obtained for the activation energy show that the degradation of the chromophores contained in the natural extracts is not a thermally activated reaction.

Table 8.7. Kinetic data on chemical and thermal degradation of DNA-CTMA colored extracts materials [185]

ADN – CTMA – Turmeric					
Solution			Thin film		
Temperature,°C	k, min^{-1}	Ea, J	Temperature,°C	k, min^{-1}	Ea, J
25	0.50 x 10^{-4}	28292	25	1.22 x 10^{-4}	13650
40	1.14 x 10^{-4}		40	-	
60	3.86 x 10^{-4}		60	1.95 x 10^{-4}	
80	4.44 x 10^{-4}		80	3.21 x 10^{-4}	
100	4.61 x 10^{-4}		100	3.46 x 10^{-4}	
ADN – CTMA – Sweet paprika					
Solution			Thin film		
Temperature,°C	k, min^{-1}	Ea, J	Temperature,°C	k, min^{-1}	Ea, J
25	negligible	35877	25	1.16 x 10^{-4}	19646
40	negligible 0.794 x 10^{-4} (after 20h)		40	1.78 x 10^{-4}	
60	-		60	5.70 x 10^{-4}	
80	negligible 0.779 x 10^{-4} (after 20h)		80	7.94 x 10^{-4}	
100	6.02 x 10^{-4} 1.24 x 10^{-4}		100	1.04 x 10^{-4}	
ADN – CTMA – Black pepper					
Solution			Thin film		
Temperature,°C	k, min^{-1}	Ea, J	Temperature,°C	k, min^{-1}	Ea, J

25	4.12 x 10^{-4}	5921	25	1.28 x 10^{-4}	19630
40	5.17 x 10^{-4}		40	2.51 x 10^{-4} 0.296 x 10^{-4}	
60	4.18 x 10^{-4}		60	-	
80	4.65 x 10^{-4}		80	3.21 x 10^{-4}	
100	5.26 x 10^{-4}		100	8.74 x 10^{-4}	
ADN – CTMA – DR1 10%[a]					
			Thin film		
			Temperatur,°C	k, min^{-1}	
			25	1.5 x 10^{-5}	
			85	5.5 x 10^{-5}	
ADN – CTMA – Rh 10%[a]					
			Thin film		
			Temperature,°C	k, min^{-1}	
			25	2.7 x 10^{-6}	
			85	4.0 x 10^{-5}	
PC Rh 5%[a]					
			Thin film		
			Temperature,°C	k, min^{-1}	
			25	3.1 x 10^{-6}	
			85	1.1 x 10^{-2}	

a - [186]

Although the degradation of dyes in natural extracts is slightly faster compared to synthetic chromophores, the results obtained are promising and suggest that the colored extracts can be used to replace synthetic pigments.

Table 8.8. Degradation kinetic data of the materials based on DNA - CTMA and colored extracts under UV irradiation

Material	**Solution**		**Thin film**	
	k, min $^{-1}$		**k, min $^{-1}$**	
	$\lambda_{irradiance}$=312nm	**$\lambda_{irradiance}$=365nm**	**$\lambda_{irradiance}$=312nm**	**$\lambda_{irradiance}$=365nm**
ADN – CTMA – Turmeric	8.16 x 10^{-3}	1.61 x 10^{-2}	6.73 x 10^{-3}	3.13 x 10^{-2} after 30 min the degradation is negligible
ADN – CTMA – Black pepper	5.62 x 10^{-4}	2.83 x 10^{-3}	1.90 x 10^{-2} 2,31 x 10^{-2}	2.77 x 10^{-2} after 30 min the degradation is negligible
ADN – CTMA – Sweet paprika	1.41 x 10^{-2}	5.91 x 10^{-3}	-	-

ADN – CTMA – DR 10% [186]	-	-	8 x 10^{-4}	2.2 x 10^{-4}
ADN – CTMA – Rh 10% [186]	-	-	8 x 10^{-4}	2.2 x 10^{-4}
PC Rh 5% [186]	-		1.0 x 10^{-3} 1.1 x 10^{-3}	2.3 x 10^{-3} 2.0 x 10^{-3}

Table 8.8 compares the kinetic results obtained for both solutions and for thin films based on DNA - CTMA and colored extracts of turmeric, paprika and black pepper subjected to the action of ultraviolet rays. In all cases there is a more pronounced degradation under the action of UVA rays compared to the degradation under the action of UVB rays. This degradation, as expected, is faster than thermal degradation. Another observation that deserves to be emphasized in the case of thin films based on DNA - CTMA and extracts of turmeric and black pepper is that the degradation under the action of UVA rays takes place only in the first 30 minutes.

8.1.6. Determining some optical parameters of the studied materials

Study of nonlinear optical properties of colored extracts

The β and β_0 values obtained are given in table 8.14. Analyzing these data it can be stated that all extracts have second nonlinear optical properties. The highest value of the first hyperpolarizability, $\beta = 221 \times 10^{-30}$ esu, is observed for curry leaves extract. It is a very high value, indicating that the colored extracts can have very interesting NLO properties and therefore can find applications in photonics.

Table 8.14. Integral (I) of the absorption spectrum, transition moment, μeg, first hyperpolarizability β at 1064.2 nm, static value of the first hyperpolarizability β_0 for the studied molecules

Colored extract colrat	**Integral I, (cm^{-2})**	$\lvert\mu_{eg}\rvert$**, Db**	**β, 10^{-30} esu**	**β_0, 10^{-30} esu**
Curry leaves	18.8	10.2	221	143
Sweet paprika	26.4	4.2	3.88	1.23
Turmeric	98	1.5	16.6	2.9
Black pepper	126.8	3.7	15.5	8.3

Optical damage threshold

Figure 8.35 compares the results obtained for the films obtained from solutions in which the DNA-CTMA complex was first dissolved in n-butanol and then mixed with the colored extract (method A) or dissolved directly in the colored extract. Figure 8.36 allows the comparison of the results of the DNA-CTMA complex with those of DNA. Therefore the method A was chosen for comparison, because in method C, the DNA is first dissolved in water and then mixed with the colored extract.

Figure 8.36 shows the measured optical damage thresholds as a function of the matrix. In all cases this threshold is higher, the best results being observed for films with turmeric extract. The improvement of the optical damage threshold may be the result of possible interactions (physical or even chemical) between the main compounds of the extract and the polar functions of the DNA. For example, curcumin can participate to a physico – chemical interaction between the two species with an acidic enol moiety and DNA with a basic amino function. The physical interaction could be an electrostatic attraction between the negative enolate C=C-O^- and the positive ammonium –$NH_3{}^+$ functions, but also could go as far as the formation of a condensation reaction product, an imine with a C=N double bond.

In all cases, the optical damage thresholds of DNA and DNA - CTMA doped with natural extracts are higher than those of the pure matrix and significantly higher than those of synthetic polycarbonate (PC) [141].

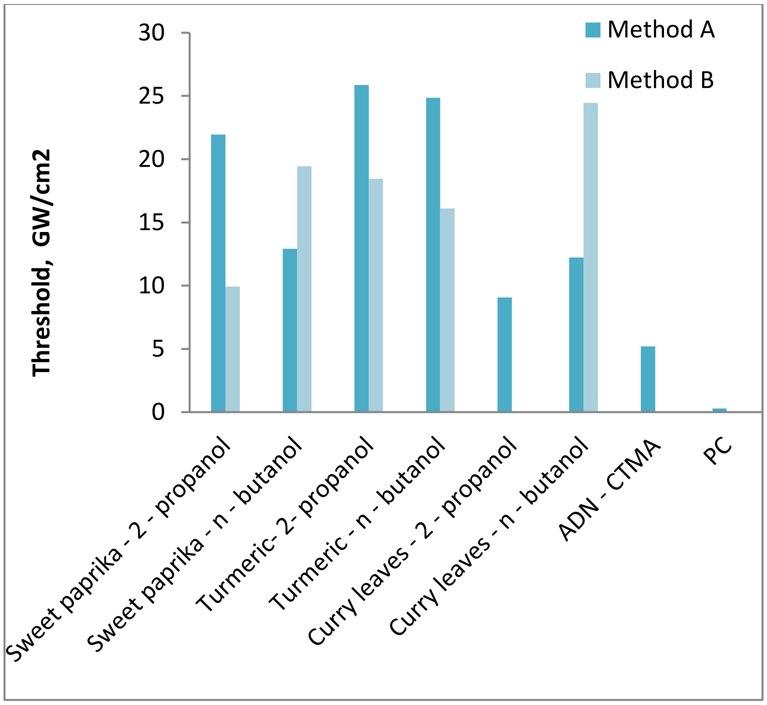


Fig. 8.35. Optical damage thresholds for DNA – CTMA – colored extracts of thin films obtained from solutions prepared by the methods A and B, compared to those of DNA-CTMA and PC

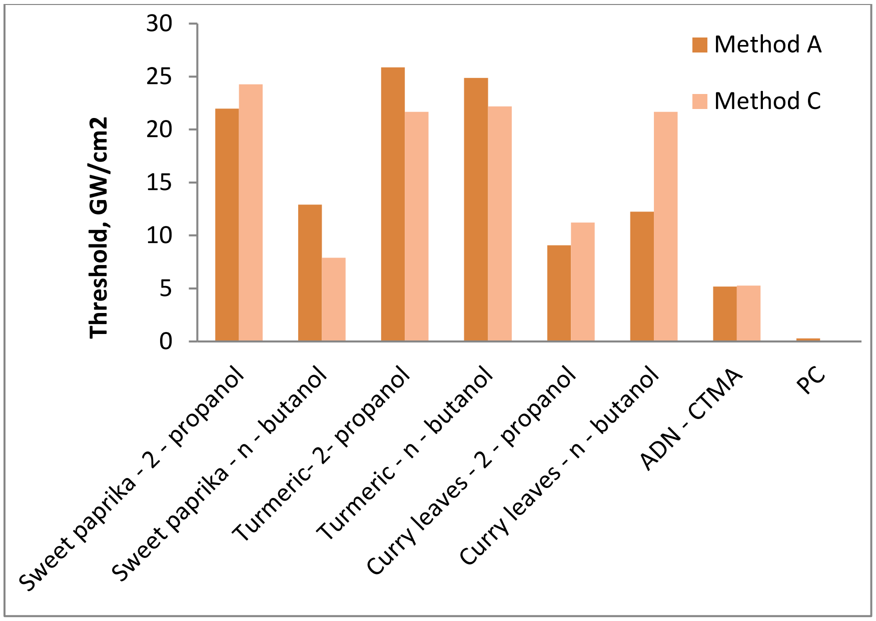


Fig. 8.36. Optical damage thresholds for thin films prepared from DNA and DNA - CTMA solutions doped with colored extracts compared to those of DNA and PC

Third harmonic generation

Table 8.16. Refractive indices at fundamental frequencies n_{ω} and harmonic frequencies $n_{3\omega}$, the ratio of cubic susceptibilities and optical susceptibilities THG $\chi^{(3)}$ (- 3ω; ω, ω, ω) for the studied films [192].

Material	**Reference index n_{ω}**	**Reference index $n_{3\omega}$**	$\chi^{(3)}$**material**/$\chi^{(3)}$**glass**	$\chi^{(3)}_{\text{material}}(-3\omega;\omega,\omega,\omega)$ ***10^{-14} esu**
ADN – CTMA – sweet paprika	1.488	1.512	74.7±7	156.9 ± 16
ADN – sweet paprika	1.488	1.512	94±9	197.4 ± 20
ADN – CTMA – turmeric	1.488	1.512	99.5±10	208.9 ± 21
ADN – turmeric	1.488	1.512	80±8	168.0 ± 17
ADN – CTMA – curry leaves	1.488	1.512	189±19	396.9 ± 40
ADN – curry leaves	1.488	1.512	90.7±9	190.5 ± 19
ADN – CTMA – DR1 (5%)	1.488	1.512	325.5±33	155 ± 16[a]
ADN – CTMA – DR1 (10%)	1.488	1.512	144.9±15	69 ± 7
ADN – CTMA – DR1 (15%)	1.488	1.512	178.5±18	85 ± 9

Material	**Reference index n_{ω}**	**Reference index $n_{3\omega}$**	**$\chi^{(3)}$material/$\chi^{(3)}$glass**	**$\chi^{(3)}_{material}(-3\omega; \omega, \omega, \omega)$ $*10^{-14}$ esu**
ADN – CTMA	1.488 [b]	1.512	5.48 ± 0,5	11.5 ± 0.1[c]
PMMA	1.4795	1.5009	1.52	3.2[d]
Glass	1.50664	1.53820	1	2.1 ± 0.2[d]
Silica	1.44967	1.4761	0.68	1.43 ± 0.14[e]

a- [193], b- [194], c- [137], d- [138], e- [139]

The values calculated for the susceptibilities $\chi^{(3)}$ (-3ω; ω, ω, ω) of the studied materials are presented in table 8.16 and compared with values from the literature [137 - 139, 193, 194], for thin films of doped DNA - CTMA with standard Disperse Red 1 chromophore and poly-methyl-methacrylate (PMMA) thin films.

From table 8.16 it can be seen that for all the thin films studied the values $\chi^{(3)}$ (-3ω; ω, ω, ω) calculated are by two orders of magnitude larger than those obtained for silica [139] and by more than one order higher than those obtained for DNA - CTMA [137]. The highest value was recorded for DNA - CTMA and curry leaves extract: $\chi^{(3)}$ (-3ω; ω, ω, ω) = (396.9 ± 40) * 10^{-14} esu. The corresponding values for the other two extracts are: (156.9 ± 16) * 10^{-14} esu and (208.9 ± 21) * 10^{-14} esu for DNA - CTMA and paprika extract respectively DNA - CTMA and turmeric extract.

8.2. MEMBRANES BASED ON BIOPOLYMERS AND COLORED EXTRACTS

8.2.1. Obtaining membranes based on biopolymers and colored extracts

The membranes were obtained from a solution of DNA in water (10 g / L) and colored extracts: curry leaves, turmeric, sweet paprika, black pepper in 2 - propanol. The solutions obtained were transparent and much more viscous than those used to make the films. Figure 8.37 shows the scheme for obtaining colored membranes, while figure 8.38 the membranes obtained.

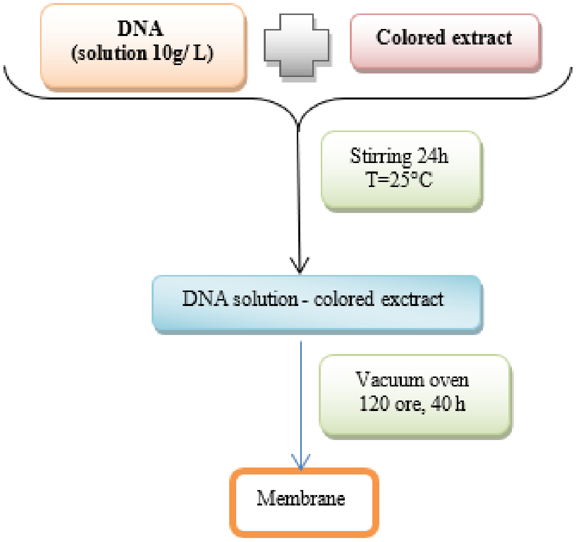


Fig. 8.37. Scheme for obtaining membranes based on DNA and colored extracts

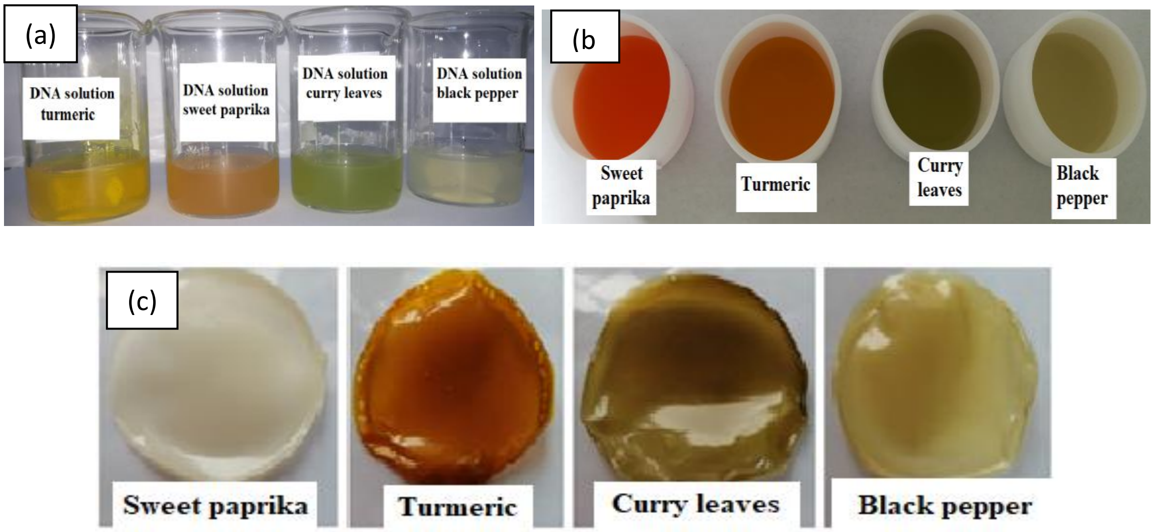


Fig. 8.38. a) Solutions based on DNA - solution (10 g / L) and colored extracts, b) Solutions based on DNA - solution (10 g / L) and colored extracts after being subjected to magnetic stirring, c) membranes based on solutions based on DNA - solution (10 g / L) and colored extracts after they were taken out of the oven

8.2.2. Membrane stability

The recording of UV-Vis spectra in time showed only the changes that the DNA molecule undergoes. Changes in the visible range, given by the chromophores contained in the extracts could not be detected due to the high concentration of chromophores and consequently the spectra in the visible range were supersaturated.

8.2.3. Membrane morphology

As in the case of thin films, scanning electron microscopy was used in order to analyze the morphology of the surfaces of DNA-based membranes doped with colored extracts.

After analyzing the images obtained for membranes compared to those of thin films, where one could see both an ordering of DNA strands and an agglomeration of chromophore molecules, in the case of membranes the ordering of chains is not observed (figure 8.41), suggesting that this takes place after centrifugation. On the other hand, it is observed that in the case of membranes the agglomerations of chromophores are higher.

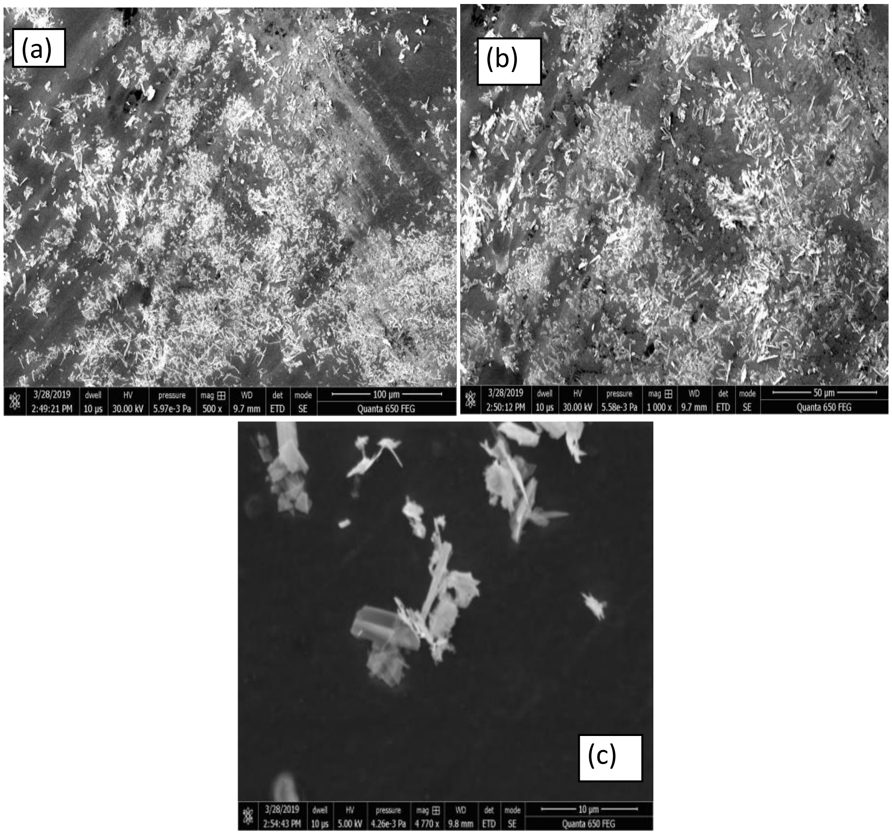


Fig. 8.41. SEM images of a DNA and turmeric membrane, at different magnifications (a) 500 x, (b) 1000 x, (c) 4770 x

8.3. PARTIAL CONCLUSIONS

The thin films obtained on the basis of DNA / DNA - CTMA solutions and colored extracts were characterized by UV - Vis spectrometry. In the obtaining spectra the presence of absorption bands of both DNA and chromophore groups were successfully identified. Based on the fluorescence spectra recorded for the thin films, it was found that these films show low intensity fluorescence compared to the solutions fluorescence.

The films presented a thickness between 13 and 100 nm and there were no remarkable differences in this respect between the three obtaining methods.

Based on the study concerning the hydrophilic-hydrophobic balance and the degradation of thin films, it can be concluded that they have a hydrophobic character and an increased thermal stability compared to the degradation under the action of UVA and UVB rays.

By studying the nonlinear optical properties of thin films, it can be stated that they have a higher damage threshold than the biopolymer itself and synthetic polycarbonate, and the values obtained for third harmonic generation showed that the new materials are comparable to materials containing synthetic chromophores.

The analysis of the morphology of the membranes, allows to visualize an agglomeration of chromophores, and based on the study regarding the degradation under the influence of UVA and UVB rays it can be stated that the membranes have different behavior which depend on the colored extract used to obtain them.

CONCLUSIONS

C.1. GENERAL CONCLUSIONS

In recent years there has been a great increase in interest in the environment and thus a new branch of chemistry had been developed, namely "green chemistry". Today, more and more studies are being carried out trying to replace polluting materials with "environmentally friendly" compounds. Starting from this idea, in the present thesis was used the most widespread biopolymer, namely DNA and four spices, which are probably found in many kitchens, in the desire to obtain totally "organic" materials. The spices used were the following: turmeric, sweet paprika, black pepper and curry leaves. The studied research has following conclusions:

- ✓ Based on the literature study the four spices are natural sources of chromophores.
- ✓ FT - IR spectral analysis revealed the presence of chromophore groups in spice powders, and the study of the parameters L * a * b * confirmed the colors they presented, respectively: turmeric - yellow, sweet paprika - red, leaves curry - greenish yellow and black pepper - yellow.
- ✓ UV - Vis and fluorescence spectral analysis of colored natural extracts in different solvents highlighted both the chromophore groups and their fluorescent properties.
- ✓ Based on the electronic and fluorescence spectral analysis of the new totally "bio" materials, it can be concluded that the DNA was successfully doped with the chromophoric compounds present in the colored extracts and showed fluorescent properties.
- ✓ For doping the DNA with the chromophore molecules from the obtained extracts, three methods were proposed and used:

- method A: 10 g / L DNA - CTMA solution and colored extracts in 2 - propanol and n - butanol,
- method B: DNA – CTMA (solid) and colored extracts in n - butanol,
- method C: 6 g / L DNA solution in water and colored extracts in 2 - propanol and n - butanol

- ✓ Thin films obtained by using the solutions prepared by the three methods A, B and C were analyzed by UV – Vis and Fluorescence spectrometry and reveal similar behavior to that of solutions.
- ✓ The hydrophilic - hydrophobic balance of thin films was studied by recording the contact angles for a drop of liquid (water or toluene) on their surface and it turned out that they have a hydrophobic character.
- ✓ The study of the hydrophilic - hydrophobic balance of DNA - CTMA - extract solutions showed that they were more adherent to the surface of steel and glass.
- ✓ The photo, thermal and chemical stability of the solutions / thin films based on biopolymer and natural colored extracts was investigated compared to the stability of films containing synthetic chromophores. The results showed that at high temperatures the degradation of natural chromophores is less pronounced, and the values obtained for the activation energy revealed that the degradation of chromophores contained in natural extracts is not a thermally activated reaction.
- ✓ Both the solutions and the films based on DNA and colored extracts were subjected to UVA and UVB irradiation, and the degradation is much more accentuated under the action of UVA rays and much faster than the thermal degradation.
- ✓ The nonlinear optical properties of the studied extracts were studied by performing the electronic and fluorescence spectra in several solvents with different polarities. Based on the analysis of the spectra, a solvatochromic effect was observed, which leads to the idea

that the colored extracts have second-order nonlinear optical properties. The most significant effect was observed for curry leaves, and the least for black pepper.

- ✓ The nonlinear properties tests consisted in determining the threshold of optical damage on the one hand, and on the other hand determining the parameters corresponding to the third harmonic generation (THG). The values of the optical damage thresholds of thin films (DNA / DNA - CTMA - colored extracts) were compared with those of the biopolymer itself or biopolymer - synthetic chromophore. Higher values were obtained for materials containing natural chromophores and the best results were obtained for the film containing turmeric extract. Also, the THG values obtained for the studied materials were compared with those obtained for films based on DR1 synthetic chromophore and thin films based on polymethylmethacrylate – PMMA. Better results were obtained when natural chromophores were used, and the films with extract of curry leaves showed the highest value $(396, 9 \pm 40) * 10^{-14}$ esu, followed by films with turmeric extract $[(208, 9 \pm 21) * 10^{-14}$ esu] and films with sweet paprika extract $[(156, 9 \pm 16) * 10^{-14}$esu].
- ✓ The study of the morphology of thin films / membranes was performed using scanning electron microscopy and after analyzing the obtained images it can be concluded that the obtaining technique, the support on which they were deposited as well as the colored extract used influence their morphology. SEM images of the films showed an ordering of the DNA strands and an agglomeration of chromophores, except for the pepper extract where no regular ordering was observed. In the case of membranes, they showed an increased agglomeration of chromophores compared to films, without ordering of the DNA chains.
- ✓ The study of membrane stability revealed different behaviors. Thus, for the membrane doped with black pepper extract, it was observed that the intensities of the primary colors increase under the action of rays with a wavelength of 365 nm and return to the initial values after exposure to sunlight, this behavior indicating a reversibility of the color of this membrane. In the case of membranes doped with turmeric extract and paprika, it was found that after exposure to UV rays with a wavelength of 365 nm there is a decrease in the intensity of primary colors, after this value the intensities of the primary colors no longer are influenced by exposure to UV radiation or sunlight. For the membrane doped with curry leaves extract, no significant changes were recorded at any exposure to neither UV nor sunlight.

Given all the results obtained, it can be concluded that, although at this moment, ″all – bio″ materials based on DNA and natural extracts from spices are difficult to obtain and reproduce, they have non-linear spectral and optical properties that can make them useful for various applications, leaving the hope that in the future these materials could replace synthetic materials.

C.2. ORIGINAL CONTRIBUTIONS

This PhD thesis brings original contributions in obtaining ″all – bio″ materials based on DNA and chromophores present in four spices (turmeric, sweet paprika, black pepper and curry leaves) materials which can be used in the development of devices with applications in optoelectronics. The elements of originality that are distinguished in this thesis are the following:
- doping the biopolymer both in the simple state and in complex combination with CTMA with colored natural extracts,

- obtaining new materials with second-order nonlinear optical properties,
- obtaining ″all – bio″ materials with values of the optical damage threshold much higher than those for material using synthetic chromophores,
- obtaining "all – bio" materials with values of cubic susceptibility higher than those recorded for DR1 and PMMA-based materials.

C.3. PERSPECTIVES FOR FURTHER DEVELOPMENT

The results obtained during the doctoral stage open new directions of research in photonics and especially in biophotonics, through the generosity of nature to provide inexhaustible sources of biopolymers and chromophores such as: chitosan, collagen and many classes of compounds with chromophoric groups: anthocyanins, flavonoids, flavonols that are found in abundance in colored fruits / flowers, etc.

Another perspective would be the optimization of the biopolymer: dye ratio to obtain materials with important nonlinear optical properties.

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1. **A.M. Anton**, I. Rau, F. Kajzar, A.-M. Simion, C. Pirvu, N. Radu, C. Simion, “Natural materials with enhanced optical damage threshold”, Optical Materials, 2018, 86, 1– 6. **FI (2018) = 2,779**
2. **A.M. Anton**, I. Rau, F. Kajzar, A.-M. Simion, C. Simion, “Third order nonlinear optical properties of DNA-based biopolymers thin films doped with selected natural chromophores”, Optical Materials, 2019, 88, 181 – 186. **FI (2019) = 2,779**
3. **A.M. Anton**, C.-.C Pădurețu, C. A. Marin, A.- M. Simion, C. Simion, F. Kajzar, I. Rău, “Photoresponsive Natural Materials”, Molecular Crystal Liquid Crystal, 2019, vol. 695, no. 1, pp. 37–44. **FI (2019) = 0,512**.
4. **A.M. Anton**, I. Rau, F. Kajzar, A.-M. Simion, C. Simion, “Stability Studies of Some DNA Based Materials Doped with Natural Extracts”, UPB Scientific Bulletin – series B Chemistry and Materials Science, 2019, vol. 81, no. 4, pp. 121 – 130.

FI cumulat = 6,07

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1. **A.M. Anton**, I. Rău, Rapid assessment of spice extracts composition using UV-Vis and fluorescence spectra (poster presentation), RICCCE, Brașov , România, 2017.
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4. **A.M. Anton**, I. Rău, F. Kajzar, C. Simion, A. Simion, DNA based materials doped with natural extracts: obtaining, characterization and stability study (oral presentation), 6th International Workshop on Advanced, Nano- and Biomaterials and Their Device Applications (6th NABM), Cluj Napoca, Romania, mai 2019.
5. I. Rau, **A.M. Anton**, C. Paduretu, M. Apietroaei, F. Kajzar, Photoresponsive Natural Materials, Malaezia, 15 International Conference on Frontiers of Polymers and Advanced Materials, Penang, Malaiezia, iunie 2019
6. I. Rău, **A.M. Anton**, F. Kajzar, J. Mysliewicz, C. Pirvu, A. Simion, C. Simion, All Bio Materials with multiple applications, RICCCE, Constanța, Romania, Septembrie, 2019.
7. **A.M. Anton**, I. Rău, A. Simion, C. Simion, On the Stability and Degradation of DNA Based Materials Doped with Natural Extracts (poster presentation), RICCCE, Constanța, Romania, Septembrie, 2019.
8. I. Rau, **A.M. Anton**, F. Kajzar, C. Paduretu, M. Apetroaei, All-Bio materials with different Aplications, 5th International Workshops on Nano and Bio-Photonics, St. Nectaire, Franta, septembrie, 2019.

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