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Obtaining & Analysis of the Binary Systems of Juglone with β-Cyclodextrin

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Abstract: This study evaluates the ability of obtaining and of analysis of the binary systems of juglone with β-cyclodextrin using the simplest method of complexation - the kneading method. The purpose of this paper is to obtain and to investigate the binary systems of juglone with β-cyclodextrin. The obtained systems were analyzed with the spectrometric method in IR. In its turn, the given purpose gave the formulation of the following research objectives: a) the creation of a binary system that could reduce the toxic properties of juglone and enhance its antiseptic properties; b) the analysis of the influence of the quantity of water in the obtaining of the complex compounds by the method of kneading; c) the applying of the spectrometric method in IR in order to evaluate the efficiency of the research. Thus, it was found that the complexation process of juglone with β-cyclodextrin is more efficient with the use of water acting as facilitator of the formation of the proposed complex system, both substances being hydrophobic they tend to form a common complex.

Keywords: Analysis, Binary Systems, β-Cyclodextrin, Complexation Reaction, Juglone

1. Introduction

A 2005 study published in the American Journal of Toxicology and Applied Pharmacology proves through a series of experiments on human cells grown *in vitro* with juglone, which was inducing their death. The study shows that the juglone produces two types of cell responses: necrosis and apoptosis (programmed cell death).

Thus, artificial induction of apoptosis was attempted through which the destruction of dangerous cells for the human organism (e.g. cancer cells) would be possible.

In 2009, in Cell Biology International, after an experiment in which juglone caused the planned destruction of an *in vitro* created tumor, this substance was categorized by the National Cancer Institute as an anti-cancer agent.

Another recent study demonstrates the antiseptic properties of juglone against *Acanthamoeba*, protozoa that causes granulomatous amoebic encephalitis (infection of the brain and spine that affects the immune system). In addition, juglone destroys the protein *1a8g*, enzyme in the HIV virus.

Juglone affects the human fibroblasts, specialized cells found in connective tissue that are responsible for the synthesis of the connective fibers and of the collagen. Specifically, the researchers found that the juglone drastically reduced the level of a protein called *p53*, this damaged the DNA of these cells and triggered allergies to some subjects, to others - necrosis. [3]

The difference in juglone concentrations required for sedation and which causes death is quite insignificant. According to another study, the general toxic effects of juglone in large quantities resemble to those of cyanide. There was experimentally determined the toxicity of juglone, which is DL_{50} i.e. approx. 0.25 mg juglone/100 g muscle mass of mice. [5]

Therefore, creating complexes in which the toxicity of juglone can be mitigated and the antiseptic properties highlighted, in our opinion, presents a particular scientific and practical interest. Although the authors of the sources reviewed and presented in the bibliography have approached the theme of the antiseptic properties of juglone, we have tried to present a new method through which these properties can be exploited without being affected by its toxicity.

2. Materials and Methods

2.1. Materials

Juglone - extracted from nuts in the Laboratory of Organic and Biopharmaceutical Synthesis of the Institute of Chemistry of the Academy of Sciences of Moldova by the method [7]; β-cyclodextrin (βCD) - manufacturer: Cyclolab R&D Ltd; distilled water; analytical balance; Micropipette 100-1000µl; mortar and pestle; IR Jasco FTIR 6100 IR Spectrometer.

Juglone is a naphthoquinone with the molecular formula $C_{10}H_6O_3$ (5-hydroxy-1,4-naphthalenedione). In the food industry, the juglone is known as C.I. Natural Brown 7 or C.I. 75500. The juglone occurs naturally in the leaves, roots, husks, fruit (the epicarp), and bark of plants in the *Juglandaceae* family, particularly the black walnut (*Juglans nigra),* and is toxic or growth-stunting to many types of plants. It is sometimes used as an herbicide, as a dye for cloth and inks, and as a coloring agent for foods and cosmetics.

The juglone is an allelopathic compound, a substance that is produced by a plant to stunt the growth of another. Juglone exerts its effect by inhibiting certain enzymes needed for metabolic function. This in turn inhibits the effects of respiration of mitochondria and inhibits photosynthesis found in common crops at juglone concentrations that are at or below those common in nature.

In addition to these inhibitions, juglone has been shown to alter the relationship between plants and water because of its effect on stomatal functioning. [6]

Physical properties of juglone:

a) Molecular weight: 174.16 g/mol.
b) Solubility in water: Slightly soluble (52 mg/l).
c) Melting temperature: 162-163˚C (435-436K).
d) Appearance: solid, yellow, photodegradable.
e) Density: 1.5 g/cm^3.

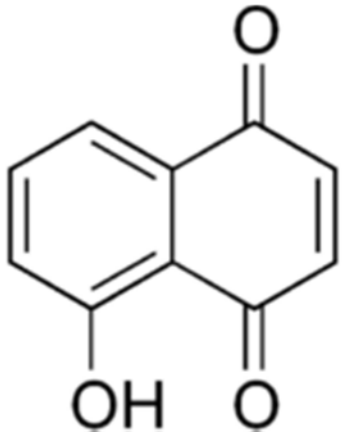


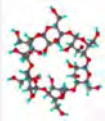
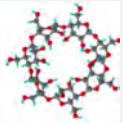
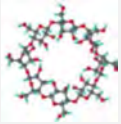
Figure 1. *The chemical structure of juglone.*

The cyclodextrins are a family of natural cyclic oligosaccharides and their semi-synthetic derivatives, which are used to create the pharmaceutical forms of biologically active substances [2, 8]. The most common naturally occurring α-, β- and γ- cyclodextrins are macro-rings made from 6, 7 and respectively 8 units of glucopyranose (Table 1). In the aqueous solution, the cyclodextrin cavity is occupied by water molecules, which are energetically disadvantaged due to the polar-non-polar interaction and thus can be easily substituted by other less polar molecules added to the solution.

The main causes of using the cyclodextrins as a complexation agent for different substances are:

a) are produced from a natural regenerable material (starch) by applying environmentally friendly technologies (enzymatic conversions);
b) are relatively inexpensive and are produced in quantities of thousands of tonnes per year;
c) are non-toxic in small quantities and toxic sequential effects can be easily eliminated with appropriate chemical modification. [4]

Table 1. *Types of cyclodextrins.*

			
Parameters	**α-cyclodextrin**	**β-cyclodextrin**	**γ-cyclodextrin**
Number of fragments of glucopyranose	6	7	8
Molecular weight (g/mol)	973	1135	1297
Internal cavity diameter (nm)	0.47–0.53	0.60–0,66	0.75–0.83
External cavity diameter (nm)	1.46±0.04	1.54±0.04	1.75±0.04
Cavity Volume (ml/mol)	104	157	256
Solubility in water (g/100 ml at 25°C)	14,5	1,85	23,2

2.2. Methods

The preparation of juglone-βCD mixtures: equimolar mixtures (1: 1) of juglone with β -cyclodextrin were prepared by milling in an agar mortar for 90 minutes of the respective quantities of substances accurately weighed at the analytical balance, the method used is called the kneading method. Kneaded products were obtained from blends of substances by adding volumes of distilled water during the kneading.

Thus, we analysed four cases of obtaining junction-βCD binary systems:

a) 0,3 mmol juglone: 0,3 mmol βCD;
b) 0,3 mmol juglone: 0,3 mmol βCD: 10 mmol H_2O;
c) 0,3 mmol juglone: 0,3 mmol βCD: 20 mmol H_2O;
d) 0,3 mmol juglone: 0,3 mmol βCD: 40 mmol H_2O.

Calculations:

m (βCD) = 0,0003 mol · 1135 g/mol = 0,340 g
m (juglone) = 0,0003 mol · 174 g/mol = 0,052 g
$V_1(H_2O)$ = 0,01 mol · 18 g/mol: 1 g/ml = 0,18 ml = 180 µl
$V_2(H_2O)$ = 0,02 mol · 18 g/mol: 1 g/ml = 0,36 ml = 360 µl
$V_3(H_2O)$ = 0,04 mol · 18 g/mol: 1 g/ml = 0,72 ml = 720 µl

Figure 2. *Obtaining binary systems (juglone and βCD) by the kneading method.*

After mixing and kneading the four systems, the mass of the systems has been weighed and the losses of substances in the working process have been calculated:

m_1 = 0,340 g; 0,340 g / 0,392 g = 86,73%;
100% - 86,73% = 13,27%

m_2 = 0,352 g; 0,352 g / 0,392 g = 89,79%;
100% - 89,79% = 10,21%

m_3 = 0,356 g; 0,356 g / 0,392 g = 90,81%;
100% - 90,81% = 9,19%

m_4 = 0,359 g; 0,359 g / 0,392 g = 91,58%;
100% - 91,58% = 8,42%

In addition to the kneading method, for obtaining complex compounds other methods are used, such as:

a) Co-precipitation method;
b) Lyophilization method;
c) Co-evaporation method, etc.

2.3. Characterization Methods in IR Spectrometry

Samples for IR absorption spectrometry investigations were obtained by the compression technique with KBr. IR spectra were recorded using a Jasco FTIR 6100 spectrometer in the frequency range of 4000-315 cm^{-1}, with a resolution of 2 cm^{-1}.

3. Results and Discussions

In order to study the efficiency of the complexation process, the peak shifts of the binary systems from the IR spectra are analyzed in relation to the peaks of the simple substances - juglone and β-cyclodextrin:

a) the peak intensities are compared;
b) the displacements of the peaks are compared;
c) the appearance / disappearance of the peaks is analyzed.

By analyzing the shift of the peak from the wavelength 3275 cm^{-1} for β-cyclodextrin and from the wavelength 3298 cm^{-1} for systems obtained, there were observed the hydrogen bonds which were formed between juglone and β-cyclodextrin.

The absence of the peak at the wavelength 3676 cm^{-1}, as well as the 2990 cm^{-1} peak of the binary systems, demonstrates that probably the functional group OH- of the juglone was incorporated into the internal cavity of β-cyclodextrin, so it was formed an inclusion complex compound (ICC). Other methods involving Roentgen radiation will be used to confirm this.

Examining the IR spectra of the binary systems 1, 2, 3, 4 it was observed that generally at the wavelength 3299 cm^{-1} in the kneading process, the peak intensity tends to decrease.

The same thing was noticed at the wavelength 1022 cm^{-1}, so as the amount of water increases, peak intensity decreases. Interaction and formation of complex systems is stronger as the amount of water increases in the formation process.

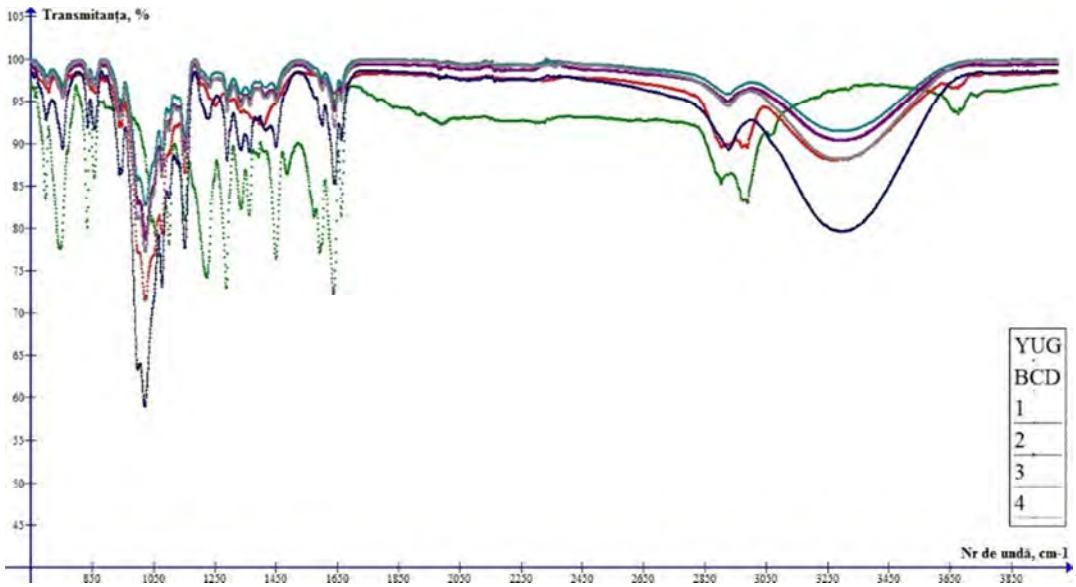


Figure 3. *IR spectra of binary systems juglone - βCD (1: 1).*

4. Conclusions

Following the analysis of the specialized literature and the experimental data obtained, the following conclusions were made:

a) The use of β-cyclodextrin may reduce the toxicity of juglone in the case of complex compounds;
b) The complexation process of juglone with β-cyclodextrin is more efficient with the use of water acting as a facilitator of the formation of the proposed complex system, both substances being hydrophobic they tend to form a common complex;
c) The analysis of the positions and of the form of absorption bands in IR allowed us to highlight the different types of interactions that occur at the molecular level between the antiseptic substance used in the study and the β-cyclodextrins in the binary products obtained;
d) These analyzes could contribute to the development of further research on obtaining and analyzing the binary systems of juglone with β-cyclodextrin, as well as on determining the efficiency of the action of the obtained systems.

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