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CONTENT


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EFFECT OF SOLVENT AND EXTRACTION CONDITIONS ON ANTIOXIDATIVE ACTIVITYOF SAGE (*SALVIA OFFICINALIS L.*) EXTRACTS OBTAINED BY MACERATION

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: In this paper, the extraction of phenolics from sage (Salvia Officinalis L.) which was grown in the plantations of MP Ljekobilje Trebinje, was carried out by maceration at different extraction time periods (30, 60, 90 , 120, 150 and 180 min), using different organic solvents (40%, 50% and 60% ethanol, 40%, 50% and 60% methanol) and water. The influence of each solvent on the extraction of total phenolics, flavonoids and antioxidative activity of obtained extracts was evaluated. The effect of solid-to-solvent ratio (1:7, 1:10 and 1:15) on the extraction yield of total phenolics was investigated. The influence of different extraction temperatures (room temperature, 30, 40, 50 and 60 ºC) on the content of total phenolics and flavonoids and antioxidative activity of the extract was also investigated. The content of total phenolics and flavonoids in the obtained sage extracts was determined spectrophotometrically. The radical scavenging capacity was determined by the DPPH method, wherein the extract concentration required to neutralize 50% of the initial DPPH radical concentration was also determined. The aqueous solutions of ethanol gave the highest yield of the extract, ie. the highest content of total phenolics and flavonoids in extracts obtained at room temperature and optimal extraction time of 60 minutes. Increasing the time of extraction has increased the content of the total phenolics and flavonoids in extracts, while the excessive time and temperature of the extraction had a negative effect on total phenolics, flavonoids and antioxidative activity of sage extracts.

KEYWORDS: solvent extraction, sage extract, total phenolics, flavonoids, antioxidative activity.

INTRODUCTION

A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital [1]. The presence of unpaired electrons makes these species unstable and very reactive to the interaction with other molecules, because they need to pair the electrons and to make up a more stable compound [2]. Free radicals are produced either from normal cell metabolisms in situ, or from external sources (pollution, cigarette smoke, radiation, medication) [3] and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission [4].

Because radicals have the capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defences, both endogenous and exogenous, are present to protect cellular components from free radical induced damage [5]. An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate delays or prevents oxidation of that substrate.[6]. A balance between free radicals and antioxidants is necessary for proper physiological function [7]. The human body naturally produces antioxidants, but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age [8]. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues [7]. Oxidative stress carries harmful effects to all the body systems and is implicated in the pathogenesis of various diseases including hypertension, atherosclerosis, diabetes mellitus and cancer [9].

Strategies such as diet and exogenous antioxidant supplementation may have a potential role in combating oxidative stress caused as a result of environmental factors[10]. Among food components fighting against chronic diseases, great attention has been paid to phyto-chemicals, plant derived molecules endowed with steady antioxidant power[11]. There are a number of epidemiological studies that have shown inverse correlation between the levels of established antioxidants/phytonutrients present in tissue/blood samples and occurrence of cardiovascular disease, cancer or mortality due to these diseases [12]. Sources of natural antioxidants are primarily plant phenolics that may occur in all parts of plants [13]. Plant phenolic compounds are secondary natural metabolites that commonly possess an aromatic ring bearing one or more hydroxyl substituents. These compounds are often reffered to as „polyphenols“ [14]. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators [15].

The most common classification of phenolic metabolites distinguishes the flavonoid and non-flavonoid compounds [16]. Among phenolic compounds found in plants, flavonoids are the most widely studied, with respect to their antioxidant and biological activities [17]. Owing to their omnipresence and impressive biological functions/activities they continue to be thoroughly investigated as potential drugs or food supplements [18].

The increasing consumers demands to acquire healthier fruits and vegetables as well as the urgency in looking to natural compounds with antioxidant activity and enhanced antimicrobial activity against antibiotic-resistant pathogenic bacterial strains have encouraged a quick expansion of research studies about enhanced phenolic extraction and identification methods [19]. In spite of the development of new extraction techniques, classic extraction dominates in many laboratories, mainly due to its simplicity and low economic outlay [20]. The existing techniques of classic extraction, like Soxhlet extraction, maceration and hydrodistillation use solvent, often coupled with elevated temperature and/or agitation. The efficiency of the process can be widely regulated by the selection of suitable solvents and application of possibly effective terms and conditions of extraction under special pressure on its duration and applied temperature [20]. Antioxidant power is usually related to the phenolic content, and the recovery of phenolic compounds from plant materials is influenced by the extraction technique, the extraction time and temperature, the solvents used, the solvent to solid ratio, however, many phenolic compounds are subject to degradation or undergo undesirable oxidation [18].

In this paper an experimental study was conducted to investigate the influence of various solvents and extraction conditions of maceration on total phenolics, flavonoids and antioxidative activity of sage extracts.

EXPERIMENTAL

For the experimental part of the work, plant material was provided - dried leaves of sage (*Salvia officinalis* L.), that has been planted at the plantations of MP Ljekobilje Trebinje. Plant material was grinded in the electric coffee grinder.

In this study were used: ethanol (95-96%), methanol (99.5%), chlorogenic acid (99%), distilled water, catechin hydrate, Folin Ciocalteu reagent, $AlCl_3$, 0.135 mM solution of 2,2-diphenyl-picylhydrazil (DPPH), an aqueous solution of Na_2CO_3 (20%), sodium hydroxide and sodium nitrite (5%). Different concentrations of aqueous solutions of ethanol and methanol (40%, 50% and 60%) were prepared by dilution of organic solvents with distilled water.

Determination of the degree of plant material fragmentation was performed using vibratory sieves and using the equation:

$$\frac{100}{d} = \sum \left(\frac{m_i}{d_i}\right)$$

Where: m_i is the mass fraction presentage, d_i is the intermediate ratio of i – fraction and d is the degree of fragmentation.

Extraction of plant material by maceration method was performed using 40%, 50% and 60% aqueous ethanol and methanol, as well as distilled water. For each solvent, a 10 g of grinded plant material was introduced into the Erlenmeyer of 250 ml, and then poured with a solvent, whereby solid-to-solvent ratio was 1:10.

The procedure was carried out at different time intervals (30, 60, 90, 120, 150 and 180 minutes) at room temperature, stirring the mixture occasionally.Upon expiration of the required time of extraction, the content of the Erlenmeyer was filtered through Whatman No. 40 filter paperusing a Buchner funneland the volume of the filtrate was recorded. The liquid extract was supplemented with the same solvent to the initial volume (100 ml) and left in the refrigerator until analysis. The content of total phenolics in obtained extracts was determined in order to evaluate their yield depending on the time of extraction, and estimate the optimum extraction time and solvent. The total phenolic content (TPC) was determined quantitatively using the Folin Ciocalteu reagent [21], with chlorogenic acid as standard and expressed as milligrams of chlorogenic acid equivalents per gram of dry sage (mg CAE/g).

Total flavonoid content (TFC) in extracts obtained by using different solvents at optimum extraction time was also examined. TFC was measured using the aluminium chloride colorimetric assay, according to Markham [22] and expressed as milligrams of catechin equivalents per gram of dry sage (mg CE/g). The effect of solid-to-solvent ratio on the extraction yield (%) of total phenolics was also investigated.

Further extraction procedure was carried out at optimum time and using the optimum solvent at higher temperatures (30, 40, 50 and 60ºC), to evaluate the effect of extraction temperature on the content of total phenolics and flavonoids, whereby the heating was provided by using water bath.

To evaluate the antioxidant activity of extracts obtained by using different extraction solvents and temperatures, the radical scavenging capacity (RSC) for each extract wereassessed by the method described described in [23] and calculated according to the following equation:

$$RSC = \frac{A_c - A_s}{A_s} \cdot 100$$

%

Where: A_c is is the absorbance of control and A_s is the absorbance of sample.

Based on the known RSC value of each extract, the IC50 value (mg/ml), which presents the concentration of extract required to inhibit 50% of the initial free radical (DPPH) concentration, was estimated from the %RSC versus concentration plot, using a non-linear regression algorithm.

RESULTS AND DISCUSSION

Grinded dry plant material was separated into fractions by particle size, using the vibratory sieves. The calculated degree of fragmentation was 0.206 mm.

For each individual concentration of an aqueous solution of methanol and ethanol (40%, 50% and 60%) as well as distilled water as a solvent, six samples of dry grinded sage were subjected to maceration at different extraction time (30, 60, 90, 120, 150 and 180 min) and room temperature (25.6°C). The obtained values of the extraction yield of total phenolicsfrom *Salvia officinalis* are shown in Figure 1.

As seen in Figure 1, the highest yield of total phenolics in sage extracts was obtained using a 40% aqueous ethanol solution, which confirms that water-ethanol solvents are probably the most suitable for extraction of phenolic compounds from the sage due to the different polarity of the bioactive constituents, and the acceptability of this solvent system for human consumption [24]. Since the highest yield of phenolic extracts by using 40% ethanol (13.711%), 60% ethanol (7.0256%), 40% methanol (6.3196%), 60% methanol (6.734%) and distilled water (4.2199%) was obtained at extraction time of 60 minutes, the aforementioned was selected as the optimum extraction time in the continuation of the study.

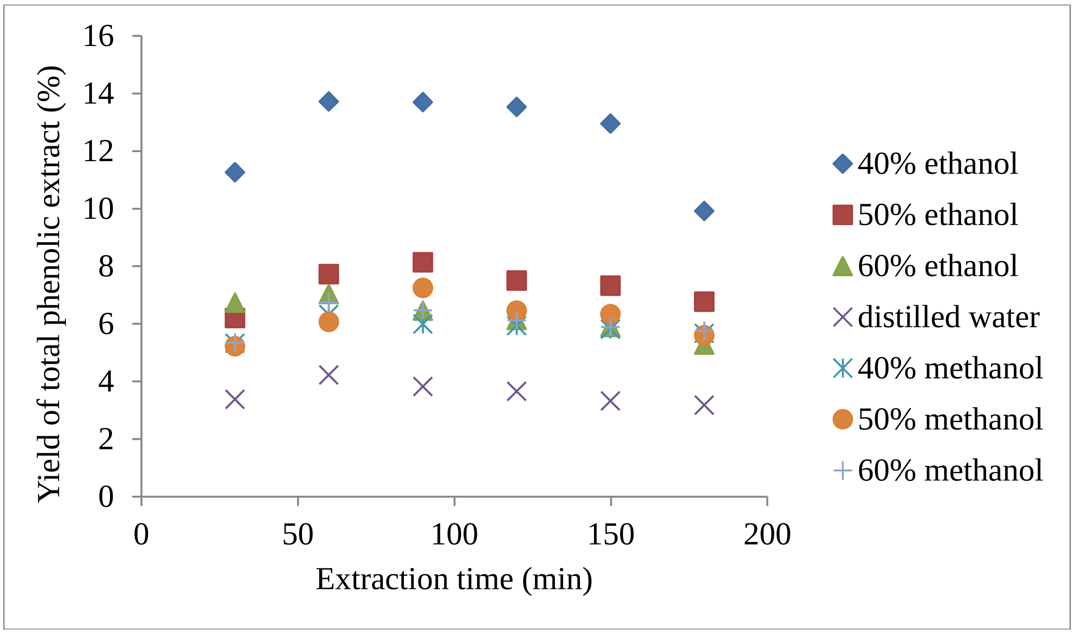

Figure 1. Effect of extraction time on the yieldof total phenolics in sage extract

The extraction yield of total phenolics obtained by using 40% and 60% ethanol, 40% and 60% methanol and distilled water was reduced at extraction time above 60 minutes. This phenomenon can be explained as hydrolysis and oxidation of phenolic compounds which may occur due to a longer time of extraction [25]. In case of 50% ethanol and 50% methanol, the yield increased during the first 90 minutes of extraction, indicating differences in the structure of phenolic compounds that also determine their solubility in solvents of different polarity [26], and decreased by extending the extraction beyond that time period. The highest yields of phenolic extract by using 50% ethanol (13.711%) and 50% methanol (7.0256%) were obtained at extraction time of 90 minutes.

To evaluate the effect of solid-to-solvent ratio on the extraction yield of total phenolics, 40%ethanol was selected as solvent. The extraction procedure was carried out at solid-to-solvent ratio of 1:7, 1:10 and 1:15 for 60 minutes at room temperature. The yield of phenolic extract at solid-to-solvent ratio of 1:7 was 7.8952%, while at solid-to-solvent ratio of 1:10 was 13.7110%. The obtained values are in accordance with the laws of mass transfer, according to which the increase of solvent volume affects the increase of the concentration gradient between the plant material and the surrounding liquid phase (solvent), thereby increasing the diffusion of dissolved matter from plant material into liquid mass. However, the lowest phenolic yield was obtained at solid-to-solvent ratio of 1:15 (5.5116%).Sampath [27] obtained similar results in the phenolics extraction study and explained that excessive solvent volume promotes the extraction of undesired compounds from the plant material that may affect the quality of the desired compounds and decrease the yield also.

In further research, the total flavonoid content was determined in the extracts obtained using 40%, 50% and 60% ethanol, 40%, 50% and 60% methanol and distilled water as solvents and at solid-to-solvent ratio of 1:10 during a 60 minute extraction time, and obtained values are compared with the previously obtained values of the total phenolic content (Figure 2).

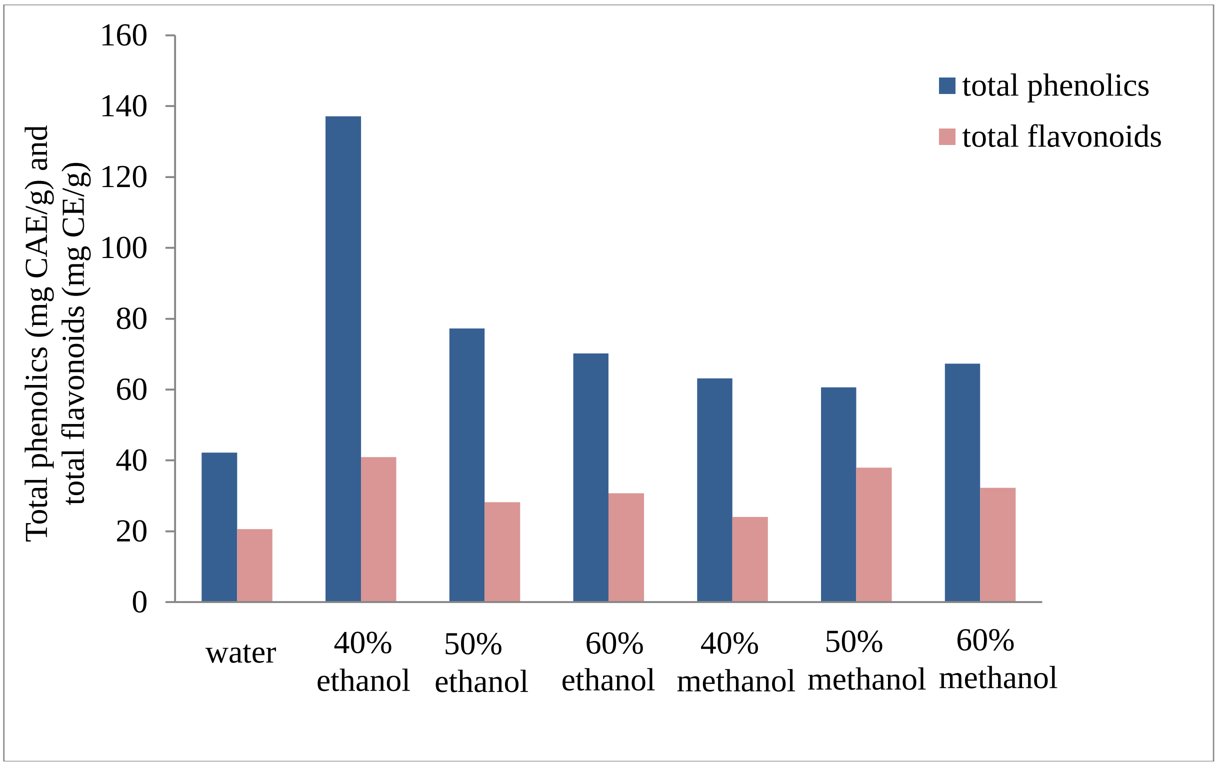

Figure 2. Effect of extraction solvent on the content of total phenolics and flavonoids in sage extract

The highest contents of phenolics and flavonoids in sage extracts were obtained by using 40% ethanol as a solvent (137.11 mg CAE/g and 40.912 mg CE/g), and the smallest by application of water as a solvent (42.199 mg CAE/g and 20.62mg CE/g). The obtained results confirmed ethanol as an optimal solvent for extraction of both total phenolics and flavonoids. Therefore, in further research, the influence of the temperature regime on the content of total phenolics and flavonoids in extract obtained by extraction with 40% ethanol as solvent was investigated.

The research of the effects of different temperature regimes (25.6, 30, 40, 50 and 60°C) was performed by extraction procedure of 60 minutes and solid-to-solvent ratio of 1:10. The obtained results are given in Figure 3.

The results indicate that increasing the extraction temperature reduced the content of total phenolics and flavonoids in sage extracts.

Gradually increasing the extraction temperature from 25.6°C to 60°C the content of total phenolics was reduced from 137.11 mg CAE/g to 65.728 mg CAE/g. Total flavonoid content was reduced from 40.912 mg CE/g to 22.452 mg CE/g. This can be explained by the temperature degradation of phenolic compounds [24].

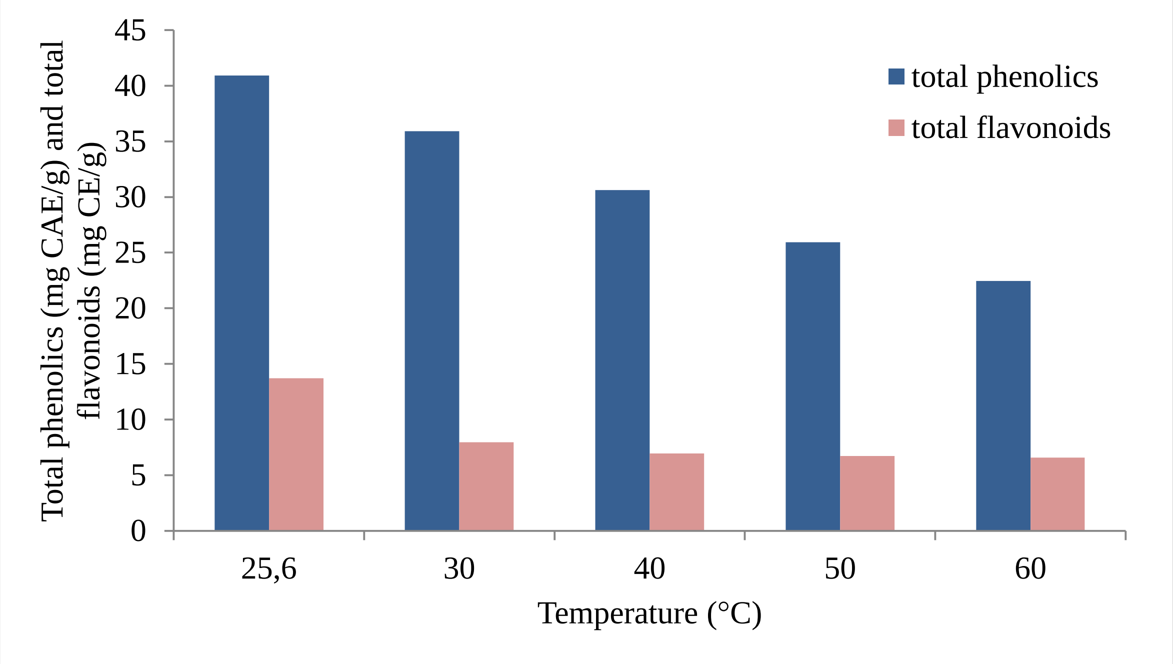

Figure 3. Effect of extraction temperature on the content of total phenolics and flavonoids in sage extract

The research of the effects of different solvents on the antioxidative activity of extracts was conducted onextracts obtained by maceration at room temperature during 60 minutes and solid-to-solvent ratio of 1:10.

The obtained concentrations of extracts needed to inhibit 50% of the initial DPPH radical concentration (IC50) are given in Figure 4.

The results show that the extract obtained by 40% ethanol as solvent has the highest antioxidant activity (0.073 mg/ml), while the extract obtained by distilled water exhibit the lowest antioxidant activity (0.355 mg/ml). The observed difference ofantioxidant activitiesis incorelation to the difference of total phenolic contents in mentioned extracts. By increasing the concentration of aqueous ethanol solution ie. by using 50 and 60% aqueous solution of ethanol, extracts of a lower antioxidant activity were obtained (0.075 and 0.078 mg/ml). The extracts obtained by using methanol as solvent showed a fairly uniform antioxidant activity, with the lowest antioxidant activity (0.101 mg/ml) shown by the extract obtained using 40% methanol.

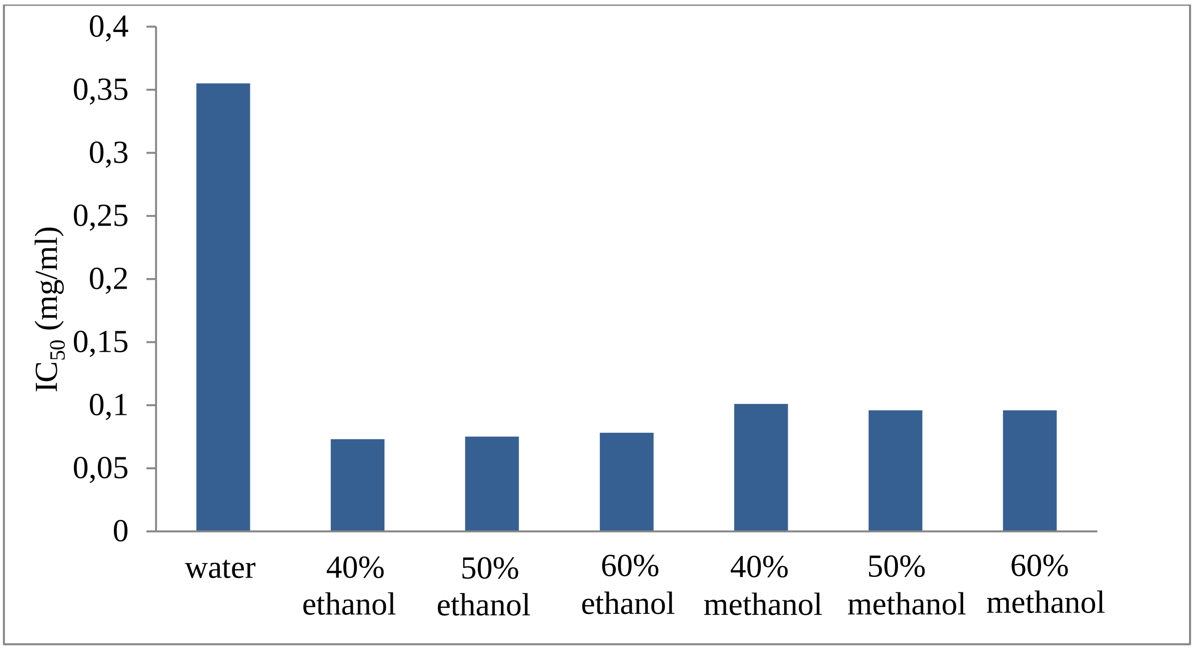

Figure 4. Effect of extraction solvent on antioxidant activity of sage extract

The effect of extraction temperature on the antioxidant activity of the extract obtained using 40% ethanol as a solvent was investigated at extraction time of 60 minutes and solid-to-solvent ratio of 1:10. Figure 5 shows IC50 values of extracts obtained at different extraction temperatures. The lowest value of IC50 was determined for the extract obtained at room temperature (0.073 mg/ml), meaning that the extract exhibited the highest antioxidant activity, i.e. the highest ability of free DPPH radicals inhibition. Further increase of extraction temperature resulted in an increase in IC50 values of extracts, which varied at different temperature values (0.101 mg/ml at 30°C; 0.084 mg/ml at 40°C; 0.096 mg/ml at 50°C and 0.088 at 60°C).

Heating might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions, therefore more polyphenols would migrate into the solvent [28], but some unwanted components can also be extracted, which may reduce the antioxidant activity of the extract.

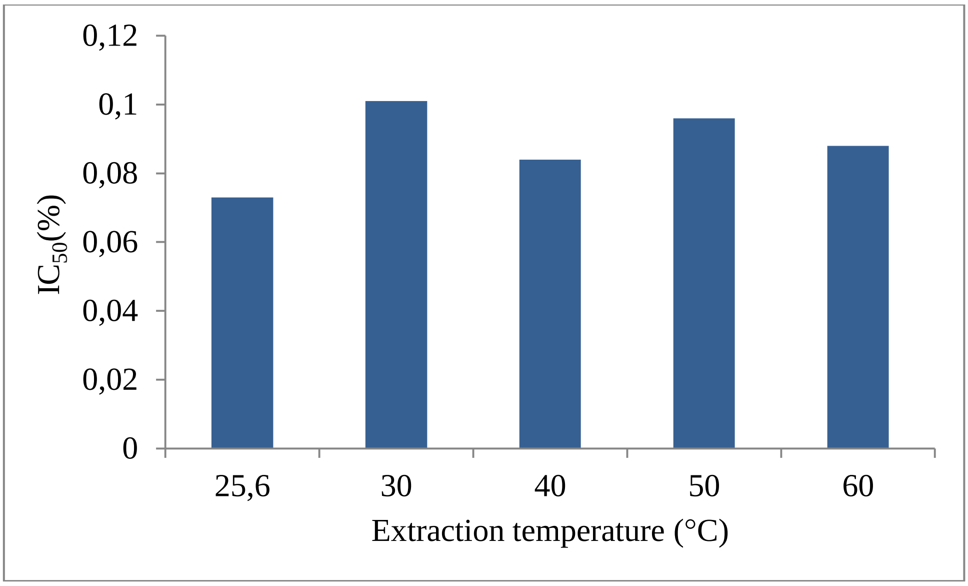

Figure 5. Effect of extraction temperature on antioxidant activity of sage extract

CONCLUSION

The results of the present research on extraction of antioxidants from Salvia officinalis by maceration showed that type of solvent, solid-to-solvent ratio, extraction time and temperature influence the content of total phenolics, flavonoids and the antioxidative activity of phenolic extracts. By extraction of sage using distilled water, 40%, 50%, 60% aqueous ethanol, 40%, 50% and 60% aqueous methanol at room temperature, the phenolic extract yield increased by extraction time, for all solvents used. Most of the solvents gave the highest phenolic extract yield after 60 minutes of extraction. The deviations showed 50% ethanol and 50% methanol, which achieved maximum yield after 90 minutes.Longer extraction time leads to hydrolysis and oxidation of phenolic compounds, which results in a reduction in the phenolic extract yield. The aqueous solutions of ethanol have been shown to be the most suitable solvents for the extraction of phenolics from sage, wherein the solvents with a higher water content showed better extractive properties. Solid-to-solvent ratio can significantly affect the phenolic extract yield, where the increase in solvent volume increases the extraction of phenolics but can also promote the extraction of compounds that affect the quality and the yield of the extract. Increasing the extraction temperature has a negative effect on the content of total phenolics and flavonoids in extracts due to their thermal degradation.

It has been shown that the content of total phenolics and flavonoids in sage extract contributes to increasing its antioxidant activity. Extract obtained by using 40% ethanol as solvent at room temperature and 60 minutes of extraction time had the highest value of IC50, due to the highest content of phenolic compounds.

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LOCAL AND GLOBAL SENSITIVITY ANALYSIS OF MODEL PARAMETERS FOR COMPOSTING PROCESS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: In this paper, a local sensitivity analysis was performed using one at time technique (OAT) on the parameters of the mathematical model for the composting process. An integrated mathematical model for composting process was used, in which kinetic parameters and the reaction order was estimated. The values of the absolute and relative sensitivity of the specified parameters are calculated. The following dynamic variables were selected as the objectives functions for sensitivity analysis: the mass of organic matter at the end of the process, the minimum amount of oxygen, the maximum amount of carbon dioxide and the maximum substrate temperature. The sensitivity analysis showed that the variations of the parameters mostly affect the amount of carbon dioxide, and at least the substrate temperature, and that the most sensitive parameter is the reaction order. ANOVA analysis (one-way and two-way) showed a statistically significant difference between experimental data.

KEYWORDS: mathematical modeling, model parameters, composting, sensitivity analysis, ANOVA.

INTRODUCTION

Composting process is the process of organic matters degradation in aerobic conditions under the influence of microorganisms. The rate and duration of the process are influenced by several parameters, which most important are: initial moisture content, content of organic matter (OM), amount of oxygen, temperature, pH value, C/N ratio, etc. The process parameters that are monitored during the composting process and are important for the matematical modeling of process are: content of organic matters, moisture content, the amount of carbon dioxide generated, then the consumption of oxigen and the temperature of the substrate.

In order to develop a process that would lead to more efficient degradation of organic matter and reduction of the negative impact of waste on the environment, mathematical modeling provides great opportunities for simulation and optimization of the process, which greatly facilitates the work in designing reactor and in situ systems for the composting process. The possibilities of applying numerical simulations influence the reduction of the need for performing expensive experiments, better understanding, control and optimization of the process. The verified mathematical model predict, within certain limits, the characteristics of the process in a laboratory, pilot and full scale [1]. Mathematical modeling of the composting process dates back to 1976, and since then several dozen models have appeared, with researchers which are used different approaches. Most of the models were based on a deterministic approach, and only a few authors dealt with the stochastic approach [2]-[5]. In most models with a deterministic approach, elements of a stochastic approach are also built in. For the past forty years researchers also worked on corrective functions for temperature, free air space, moisture content and oxygen concentration. The review of the literature showed that the most significant and most modeled corrective function is related to temperature. The first corrective function for temperature is based on the modification of the Arrhenius expression [6].

A general review of corrective functions for temperature was given by Mason[1] in his work. In order to verify the stability and reliability of the model, it is necessary to perform the sensitivity analysis of the model parameters. Sensitivity analysis of model parameters can be done before and after model development. Most authors performed a sensitivity analysis of the model after the development, investigating sensitivity of model parameters and their influence on the stability of the model [6]-[9]. In this paper a corrective function for the temperature based on the modification of the Arrhenius expression is used. In the integrated model, three kinetic parameters were evaluated [10]. The sensitivity analysis seeks to determine how the model depends on the assigned values, structure of the model, and the assumptions on which it is set up. Also, sensitivity analysis represents an important method for checking the quality of the proposed model.

The aim of this paper is to assess the relative importance of the selected model parameters by sensitivity analysis and ANOVA analysis of experimental data in two different experiments.

MATERIALS AND METHODS

MATERIALS

The experiments were conducted in a pilot scale reactors (57 liters of volume). During experiments (23 and 15 day), three reactors were used, with mixtures of different initial composition. In both experiments, the organic fraction of municipal solid waste (OFMSW), poultry manure, sawdust, waste yeast and kiselguhr from the beer industry were used. The composition of OFMSW and characterization of the initial mixtures of the first experiment are given in the paper of Paprаćanin & Petric [10]. Composition of OFMSW used in second experiment is shown in Table 1.

Table 1. The composition of the OFMSW used for the second experiment

Waste	Composition (*mass*%)
Food waste	63.6
Paper and cardboard	25.6
Garden waste	10.8

Table 2. Percentage composition of initial mixtures (*mass*%) in reactors (second experiment)

Reactor	OFMSW	PM	S	WY	K
1	67.8	9.2	4.6	9.2	9.2
2	66.6	8.9	6.7	-	17.8
3	73.2	4.9	7.3	-	14.6

PM-Poultry manure,
S-Sawdust, WY-Waste yeast,
K- kiselguhr

Table 3. Characterization of initial mixtures in reactors (second experiment)

Reactor	Moisture (% w.b.)	OM (% d.b.)	pH	C/N
1	67.13	83.09	7.10	43.70
2	59.53	79.30	7.36	40.40
3	62.35	82.35	7.32	34.50

w.b. - wet base,
d.b. - dry base

The percentage composition of the initial composting mixtures in the second experiment is shown in Table 2, and their basic physical and chemical characteristics are given in Table 3. The prepared mixtures took up about 90% of the total volume of the reactors. For the first and second experiment, the reactors was filled with: 26.1 kg, 19.5 kg, 24.4 kg, 19.1 kg, 18 kg and 18.7 kg of the compost mass, respectively. Other details about eksperimental procedure can be found in literature [7], [10]. Sheme of experimental set-up is shown on figure 1.

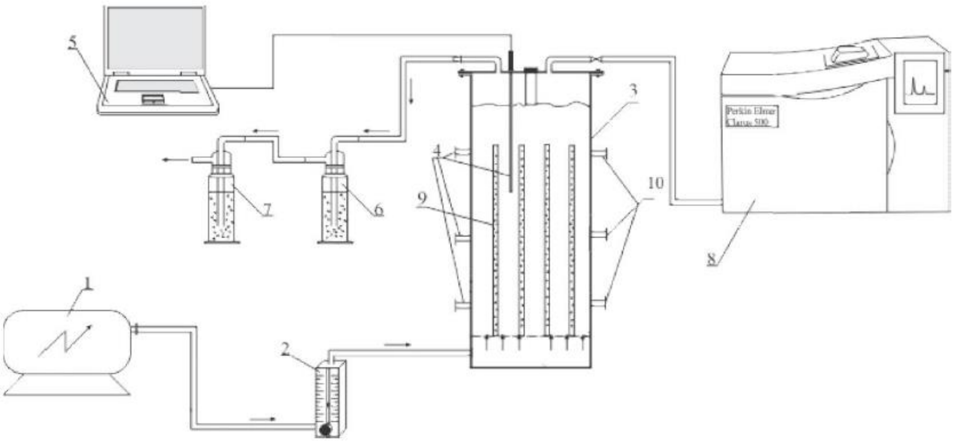


Figure 1. Schematic diagram of the reactor system: 1-compressor, 2-flow meter, 3-reactor, 4 thermocouples, 5-port computer with acquisition module, 6-rinse with sodium hydroxide solution, 7-bottle wash with boric acid solution, 8- chromatograph-gas analyzer, 9-perforated tubes, 10-holes for sampling.

SAMPLING AND ANALYSIS

After daily mixing of the composting mixtures, samples were taken from different places in the reactor (top, middle and bottom, three samples from each places) in order too obtain a representative sample. Moisture content, OM content and pH value was measured daily. Measurment and used methods for composting proces, are standard methods, and can be found in the literature [6], [9], [10], [11]. For measurment of concentrations of carbon dioxide and oxygen concentrations, Infrared Gas Analyzer MGA5, VarioPlus Industrial (MRU GmbH, Germany) was used. Concentrations of CO_2 and O_2 are measured at three heights in the reactor and at the top of the reactor (gas outlet).

Measurement of the air flow was done by rotameters (Cole-Parmer, USA). The temperature in the reactors was measured automatically every 30 minutes for the entire duration of the experiment by thermocouple (type T, Digi-Sense, Cole-Parmer, USA), which are connected to a notebook via the acquisition module (Nomadics, USA). The ambient air temperature in the laboratory was 22.5 ± 2.5 ° C during the experiments.

MATHEMATICAL METHODS

The mathematical model that is used for performing local and global sensitivity analaysis of model parameters, was presented in a previous work [7], [10] also as mathematical methods.

SENSITIVITY ANALYSIS METHODOLOGY

In this study, local sensitivity was defined as the relative change in the system output when a small perturbation (±1%) was imposed on a single kinetic parameter.

The sensitivity analysis was done in order to assess the relative importance of the selected model parameters. For the numerical simulation of the process, developed program were implemented in Matlab [12] while MS Excel used for graphical representation of the results of the sensitivity analysis. The sensitivity analysis of the parameters was done in two ways [7], [9], [10]. Investigated sensitivity functions of model are: the minimum weight of organic matters, the maximum amount of carbon dioxide and the maximum substrate temperature. Absolute and relative sensitivity of the model was calculated in MS EXCEL, based on the data obtained from simulations and experimental data. The numerical simulation data were obtained in Matlab [12] (ODE23s solver). The absolute sensitivity was obtained by minimizing the variation of the optimized kinetic parameters by creating a "noise" [13]. Obtained kinetic parameters [10] are varied by +1% of their optimum values. Absolutely Parametric Sensitivity (APS) and Relative Parametric Sensitivity (RPS) is calculated as described in literature

$$APS = \frac{\partial f}{\partial k_i} \approx \frac{\Delta f}{\Delta k_i} \quad \text{...(1)}$$

where in:
f – optimization function,
k_i – parameter.

Since sensitivity can not be expressed analytically for nonlinear dynamical models, it is possible to use differential approximation. Relative Parametric Sensitivity (RPS) is calculated from the following expression:

$$RPS = \left| \frac{k_i \partial f}{f \partial k_i} \right| \approx \left| \frac{k_i \Delta f}{f \Delta k_i} \right| \quad \text{....................................(2)}$$

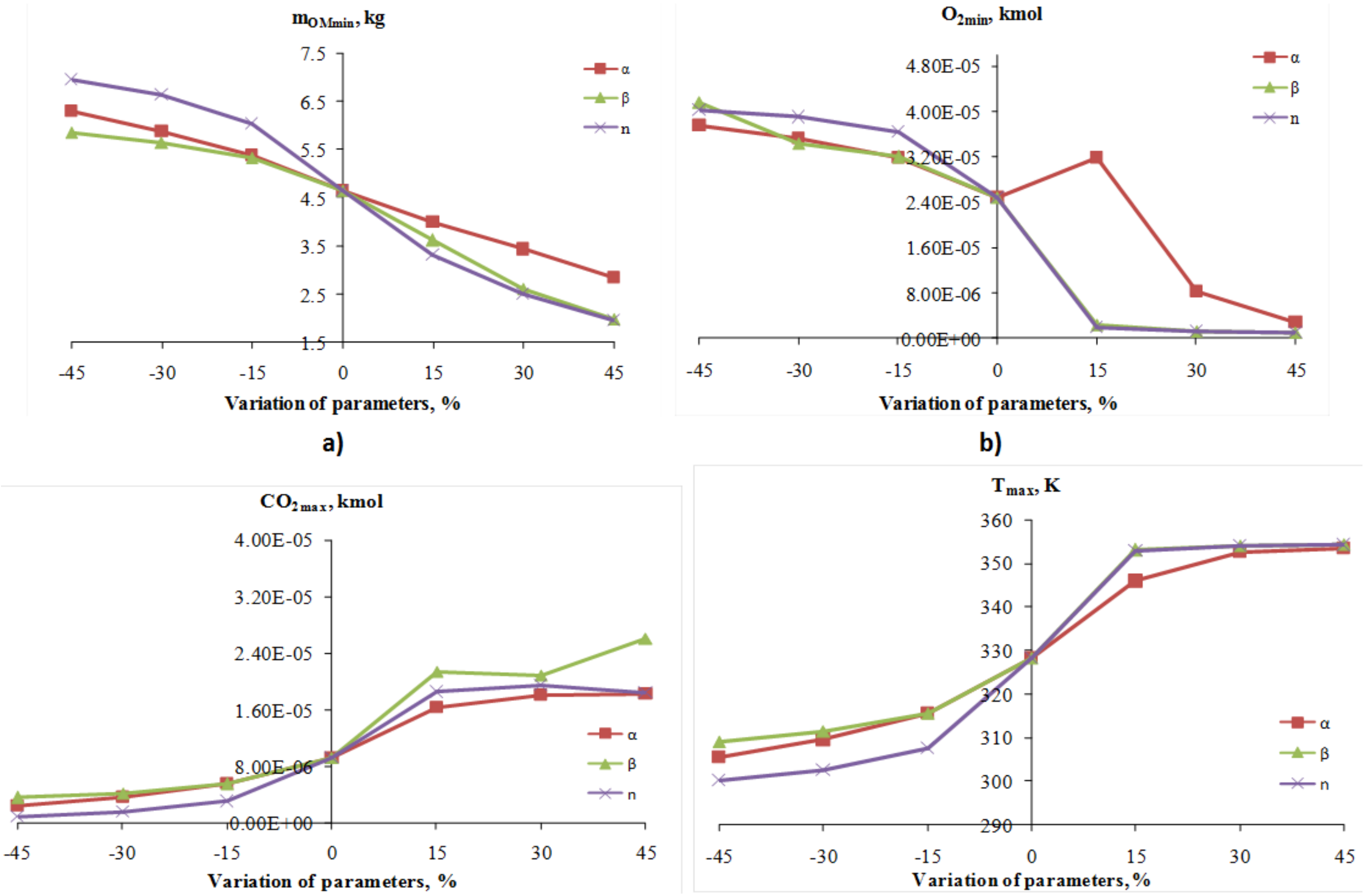
Similar to the absolute sensitivity of the parameters, the differential approximation of the equation (1) can also be used to express the relative sensitivity of the parameters. More details can be found in the literature [9].

In order to determine statistical differences between treatments in individual reactors, ONE-WAY ANOVA (variance analysis) was performed. Statistical analysis of data related to organic matter loss, carbon dioxide concentration and substrate temperature was performed. This data relates to the mean height in the reactor. TWO-WAY ANOVA analysis (multi comparison test) was carried out in order to determine statistically significant differences in data obtained at different heights (spatial gradients) in reactors and different treatments in experiments. In both cases (one-way and two-way ANOVA), a T (*Tukey*) statistical test was used. Statistical analysis was performed in Matlab [12].

RESULTS AND DISCUSSION

SENSITIVITY ANALYSIS

Sensitivity analysis is the method of variation of the input parameters of the model within the permitted area and observation of variations of the dependent variables as output of the model. Generally, the sensitivity analysis can be defined as the study of uncertainty in the output of models that can be attributed to different sources of uncertainty in the input model [14]. The sensitivity analysis is used to increase the reliability of the model and its prediction, so that it allows understanding how model variables respond to changes in input parameters [15]. In essence, the sensitivity analysis is performed before optimizing the parameters to determine which parameter affects the reliability and stability of the model, but it is possible to perform the analysis after optimization. One approach to sensitivity analysis is local sensitivity analysis or one-at-one (OAT) techniques. OAT technique analyzes the effect of changing one parameter of model, while the values of other parameters are fixed, which is also applied in this paper. The influence of individual parameters on the objective functions is shown in Figure 2.



a)

b)

c)

d)

Figure 2. The influence of variation of parameters on:
a) the mass of organic matter at the exit; b) a minimum amount O_2;
c) the maximum amount CO_2; d) the maximum substrate temperature

Figure 2 shows that the parameter that most affects the selected objective functions is the reaction order *n*, that is, the parameter that shows the largest amplitude of the deviation. Negative variations of parameters significantly increase the values of the mass of organic matters at the output and the minimum amount of oxygen. Since the mass of organic matters and the amount of oxygen are practically reactants in the reaction of degradation, it can be concluded that, due to less degradation of organic matter and less oxygen consumption, a smaller amount of carbon dioxide is generated and a lesser amount of heat due to the biological reaction is distinguished. Also, it can be seen that positive variation of parameters, or their increase, results in higher production of carbon dioxide and maximum substrate temperature during the process. Positive variations of all three parameters show an "unusual" trend of changing the function of the target, because the first drop is seen, and then the value is increased. Negative variations of parameters show a "proper" behavior trend that can be explained and linked to the actual process. After the reaction order *n*, a significant influence is given by the parameter *β*, especially when it comes to the minimal amount of organic matter and the minimum amount of oxygen as a objective function. Obtained values of kinetic parameters of the model can be found in paper

Papraćanin & Petric [10]. The α parameter has the least effect on the selected objective function, especially when it comes to small temperature changes. For all three parameters variations of the objective functions are greater in the case of positive variations of the parameters. Increasing the value of kinetic parameters and the reaction order significantly influences on the stability and reliability of the data obtained as output from the model. The smallest influence of parameter variation has on substrate temperature values, while the greatest influence is observed in the amount of carbon doxide. The variations of +30% and +45% give the values of the objective functions, which have no physical meaning.

Local sensitivity analysis was also carried out in previous work [7]. Several authors performed the sensitivity analysis in the same way [9], [16], [17], [18], whereby the came to the conclusion that only one of several parameters has a significant influence on the model output. The absolute sensitivity of the parameters is characterized by the direction in which the observed parameter is changed. Its positive value leads to an increase in the difference between the model and the experimental data, while its negative value reduces the difference between the model and the experimental data.

Part of the results of performed sensitivity analysis was presented in the paper Papraćanin & Petric [10]. The difference in the order of the size of the APS value is related to the order of the size of the model parameters. The lower value of the parameter gives a higher APS value and The sensitivity analysis was performed for a slight deviation of the model parameters from their optimal values (+1%) [10]. Therefore, increasing the value of the parameters leads to an increase in the difference between the experimental and the results obtained by the mathematical model as shown in the paper Papraćanin & Petric [10].

The F-distribution results show that the model is acceptable for the given conditions with a degree of significance $\alpha=0.05$ for seven dynamic state variables[10].

Most authors have tested the influence of one fundamental parameters on the model, using a variety of methods and techniques for sensitivity analysis [19]. Researchers performed sensitivity analysis in models with microbiological kinetics [20], [21] or the sensitivity analysis were limited to only one segment of the process [19], [22], [23]. Very few researchers performed the sensitivity analysis in the integrated model [7], [24] so that there is enough space for future research in this direction. More precisely, it is possible to perform a sensitivity analysis before optimizing the kinetic and process parameters in order to obtain more reliable simulation results. Also, special attention should be paid to the optimization of the reaction order, which has the greatest influence on the differences between model and experimental data.

STATISTICAL ANALYSIS

The first part of the statistical analysis refers to data obtained from two experiments (three reactors in each experiment) which were measured in the middle height of the pilot scale reactor. The data were statistically analyzed by one-way ANOVA ($p<0.05$).

Measured and statistically analyzed variables are: mass of organic matter, the amount of carbon dioxide and substrate temperature.

The second part of the statistical analysis refers also to the data obtained from the same experiments but measured at different heights in the reactor (50 mm, 270 mm, 490 mm). For the second part of the analysis, "two-way" ANOVA was performed, with multiple data comparison.

The first part of the analysis was done separately, due to the fact that optimization of the kinetic parameters, as well as the verification of the proposed model using on these data [10] (used date were measured at medium height). For a better understanding data obtained from each reactor are numbered from 1 to 6. The first three groups from 1 to 3 refer to the data obtained from first experiment, while groups 4, 5, and 6 refer to data from second experiment.

Table 4 summarizes the results of the p-value for: mass of organic matter, amount of carbon dioxide and substrate temperature in all six reactors, measured at middle height. It can be said that there is a statistically significant difference ($p<0.05$) between the reactors for mass of organic matters and amount of carbon dioxide.

Table 4. Results of one-way ANOVA analysis

Measured variable	p
Mass of organic matter	$\mathbf{3.86 \cdot 10^{-6}}$
Amount of carbon dioxide	**0.007**
Substrate temperature	0.3905

Since the organic matters and the temperature of the substrate were measured at three heights in the reactors, and the carbon dioxide concentration was additionally measured at the top (free air space), a two-way ANOVA analysis with multiple comparison was performed. Statistical data processing for the mass of organic matters and substrate temperature from six reactors to three heights, and data analysis for the concentration of carbon dioxide in six reactors at four heights.

Analysis of the data for the mass of organic matters showed that the data by reactors statistically significantly differ (p=8.65·10^{-6}), while the data in height do not statistically differ (p=0.631873). Results of multiple comparison test are showed in Table 5 (only groups that are statistically significantly differ) .

Table 5. Results of multiple comparison test for mass of organic matters

Groups		Lower value for 95%	Mean	Upper value for 95%
1	**2**	-0.2586	-0.1436	-0.0285
2	**3**	0.1038	0.2188	0.3338
2	**6**	0.0024	0.1174	0.2324
3	**4**	-0.2500	-0.1350	-0.0200

Since data from different heights are not statistically significantly, results are not showed. Based on these it could be concluded that the material is well homogenized since there are no statistically significant differences in heights.

Comparison of data for the amount of carbon dioxide by reactors at four levels (three heights and the top of the reactor) showed that, in opposite to organic matter, the data by reactors do not statistically differ, while they differ significantly in heights (p=1.5·10^{-28}). Multiple comparison showed that the data per reactor differ significantly only in the case of reactors 4 and 6 (lower limit of 95% is 0.2924 and upper limit for 95% is 2.2858), but when it comes to heights, five significant statistical differences can be noticed. The results of multiple comparison test for groups of heights that are statistically different are shown in Table 6. Since the measurements are made at different heights and that it is a gaseous phase, it is logical that there are significant statistical differences. Considering facts that the gas phase flow through the material in the reactor (tree heights) relative to the data from group 4 (free air space, where the concentration is highest) and between each other

Table 6. Results of multiple comparison test for data by height for amount CO2

Heights		Lower value for 95%	Upper value for 95%	The difference in mean values
1	**2**	1.8174	3.2847	2.5510
1	**3**	-1.5305	-0.0632	-0.7968
2	**3**	-4.0816	-2.6143	-3.3478
2	**4**	-3.4368	-1.9695	-2.7031

Two way ANOVA for substrate temperature by reactors and hights are showed that there is a statistically significant difference in both cases (Table 7). Results of multiple comparison test showed that the all three groups (three heights) different from each other. Results of multiple comarison test of six groups (reactors), for groups that are statistically different are shown in Table 8.

Table 7. Results of two-way ANOVA analysis for substrate temperature

	SS^a	dF^b	SS/dF^c	F	p	Fc
Height	12059	2	6029	102.5	4.3·10^{-42}	3.00
Reactor	16051	5	3210	54.6	1.4·10^{-51}	2.22
Interaction	2725	10	272	4.6	1.6·10^{-6}	1.87

a-sum of squares;
b-degrees of freedom;
c-mean square deviation

The Table 7 shows, beside that the substrate temperature data are statistically significantly different both in reactors and in height, and that there is strong interaction between the reactors and the heights.

Such results can be explained by the fact that substrate mixing was not carried out, so in some parts of the reactor there was a mass overheating because the temperature of the substrate has the highest value in the center of the mass. Other authors also performed a one-way and two-way analysis of the variance for different reactor treatments. For example, Schloss and Walker [25] have investigated the effect of the active sludge addition as an inoculum, resulting in significant deviations in substrate temperature. Some authors investigated the effects of various additives, the influence of mixing, particle size and various types of inoculum, on the substrate temperature, amount of carbon dioxide, organic matter content [26], [27]. Rebollido [28] have studied the influence of temperature, pH, electrical conductivity and moisture content on the concentration of microorganisms, and concluded that pH and temperature significantly influence (p <0.01) on bacteria, fungi and actinomycetes.

Table 8. Results of multiple comparison test for data by reactors for substrate temperature

Groups		Lower value for 95%	Mean	Upper value for 95%
1	**3**	2.6489	4.7366	6.8244
1	**5**	0.3310	2.1187	4.2065
1	**6**	7.1188	9.2066	11.2943
2	**3**	4.0368	6.1245	8.2123
2	**4**	0.4187	2.5065	4.5942
2	**5**	1.4189	3.5066	5.5942
2	**6**	8.5067	10.5945	12.6822
3	**6**	-5.7058	-3.6181	-1.5303
3	**5**	-4.7056	-2.6179	-0.5301
3	**6**	2.3822	4.4699	6.5577
4	**6**	6.0002	8.0880	10.1758
5	**6**	5.0001	7.0878	9.1756

Komilis and Tziouvaras [29] investigated various types of vegetable seed supplements, concluding that compost on some seed species may have a phytotoxic effect, while other species can influence this to accelerate germination and growth. Kalamdhad [30] analyzed the results of the ANOVA, concluding that the reduction of carbon dioxide production significantly varied over the time ($p<0.0001$) and treatments (different C/N ratio), ($p=0.0013$). Wang [31] also carried out an equivariant analysis of the variance, but in order to examine the effects of different treatments on the maturity and composition of the finished compost.

CONCLUSION

Local sensitivity analysis has shown that variation of parameters mostly affects the amount of carbon dioxide, and at least the substrate temperature, and that the most sensitive kinetic parameter is the reaction order. A global sensitivity analysis has shown that the variations of all three parameters influence the increase in the differences in the agreement between the model and the experiment, and that the reaction order is the parameter that affects the stability and the sensitivity of the model to the greatest extent.

The analysis of the variance (ANOVA) for the mass of organic matters and aomunt of carbon dioxide, showed statistically significant difference between the reactors. Two-way ANOVA for the mass of organic matter showed that the data by reactors statistically significantly differ ($p=8.65\cdot10^{-6}$), while the data in heights do not statistically differ ($p=0.631873$). Comparison of data for the amount of carbon dioxide by reactors at four levels (three heights and the top of the reactor) showed that, in opposite to organic matter, the reactor data does not statistically significantly differ, while they differ significantly in heights ($p=1.52\cdot10^{-28}$). Substrate temperature data are statistically significantly different in both cases, reactors and heights, and there is strong interaction between reactors and heights.

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PHOTOCATALYTIC DEGRADATION OF ORGANIC COMPOUNDS IN WASTEWATERS

SCIENTIFIC REVIEW PAPER

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ABSTRACT: Due to the toxicity effects and endocrine disrupting properties of many organic compounds, their removal from water and wastewater has gained widespread global attention. This review summarizes photocatalytic degradation of different organic molecules present in wastewaters. This is an overview of photocatalytic degradation with the goal of presenting the technique as an attractive and viable process unit. This process has great potential for replacing other conventional methods for treatment of wastewaters and can be used at the advanced treatment stage. Photocatalytic degradation techniques should be more used in wastewater treatment because with this technique it is possible to decrease contaminants to certain acceptable discharge limits. However, the technique is still not being utilised on an industrial scale. This is mainly due to focus of researchers to study singular contaminants such as alcohols, carboxylic acid, phenol and its derivate, chlorinated aromatic compounds, colours, active pharmaceutical ingredients, and different type of surface active agents. TiO_2 can be used as a photocatalyst in water purification to degrade organic pollutants.

KEYWORDS: photocatalytic degradation, organic compounds, wastewater decontamination

INTRODUCTION

Water pollution is now an acute problem worldwide, and in developing countries in particular. Demand for high quality water (i.e., water that is free of toxic chemicals and pathogens) has increased due to population growth, more stringent health based regulations and economic development (Ali Ayati et al., 2014) [1]. The rapid development of manufacturing technology after the industrial revolution has improved the standards of living significantly but it is becoming a factor that is threatening human health and the environment (Seul-Yi Lee and Soo-Jin Park, 2013) [2].

The reuse of treated water is amongst the most suitable solutions to achieve a really sustainable use of water, especially in water-deprived countries. One of the most relevant issues in wastewater recycling is the presence of micro contaminants, also known as emerging contaminants (ECs). Wastewater from industries and municipal treatment plants can be recycled after the proper treatment. Amongst the different advanced oxidation processes, the use of photocatalysis based upon TiO_2 for water decontamination is receiving an increasing interest in recent years due to its high photostability, non-toxicity and cost effectiveness (Miranda-García et al., 2014) [3].

However, though it is a good catalyst, its wide band gap (3.2eV) limits the use of visible light as the light source. This has consequent implications for the use of titania materials as solar or room-light activated catalysts, because the majority of sunlight consists of visible light and only a 3–5% of UV light. Hence, increasing the efficiency of visible photocatalysis is important for the practical application of this technique (K. Maeda and K. Domen), [4]. Various attempts have been made to develop the visible light activity to TiO_2 such as doping with metal and non-metal ions (H.E. Chao et al.,[5], (D. Chatterjee et al., 2005)[6], dye sensitization (G. Zhao et al. 1996) [7], and semiconductor coupling (D.L. Liao et al., 2008)[8], (B. Pant et al., 2014) [9] etc.

Among physical, chemical or biological waste treatment methods, chemical route sand, in particular, the photocatalytic procedures are considered the most interesting ones thanks to their wide applicability, low cost and environmental compatibility. Physical treatments mainly operate to concentrate the pollutants rather than remove them; on the other hand, biological processes are of great importance in many applications, but the operational conditions need to be strictly controlled to preserve the active bacterial colonials. Conventional chemical routes require the addition of dangerous or expensive chemical reagents (A. Galenda et al., 2014) [10].

ORGANIC COMPOUNDS

Wastewater becomes a serious hazard to ecological and human health due to increasing sewage discharge generated from industrial manufacture and agriculture irrigation like dyes, pesticides, plasticizers.

AROMATIC COMPOUNDS

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that are mostly colourless, white, or pale yellow solids. They are a ubiquitous group of several hundred chemically related compounds, environmentally persistent with various structures and varied toxicity. The mechanism of toxicity is considered to be interference with the function of cellular membranes as well as with enzyme systems which are associated with the membrane. It has been proved that PAHs can cause carcinogenic and mutagenic effects and are potent immune suppressants. PAHs are commonly detected in air, soil, and water (Hussein I. et al., 2016) [11]. Endocrine disrupting chemicals (EDCs) such as bisphenol A (BPA), estradiol, and estrone are typical emerging pollutants commonly found in water resources and effluents from wastewater treatment plants. Bisphenol A has been widely used in the plastic industry for the production of polycarbonate plastics and epoxy resin (Li-Fen Chiang and Ruey-an Doong, 2014, [12].

PESTICIDES AND HERBICIDES

Pesticides are biologically active compounds produced for use in agricultural production to prevent or limit the adverse effects of biological agents such as insects, grafts, plant pathogens, undesirable plant species (weeds) etc. However, pesticide application often monitors the risk of undesirable consequences for the environment. Pesticides can contaminate surface and groundwater, may adversely affect harmful crops, beneficial soil organisms, tiny siblings and birds, can be found as residues in food and can cause resistance to biological agents. It is therefore essential to understand the destiny of pesticides in the environment and to assess the potential exposure and risks to human health and the quality of the environment. Some persistent pollutants, including several pesticides, are carried in air and water over several hundred miles affecting the wildlife and general populace. These chemicals are non-biodegradable in nature and have been known to have carcinogenic, mutagenic or chronic toxic effects (Mukesh Goel et al., 2010) [13].

Chlorinated organic compounds are found to be resistant to biochemical degradation. Large quantities of higher chlorophenols (pentachlorophenol, tetrachlorophenol, etc.) are used in the wood preservation industry. Monochlorophenols and dichlorophenols serve as intermediates in the production of pesticides (S. Zuzana et al., 2008) [14]. They are also used as additives to inhibit microbial growth in a wide array of products such as adhesives, oils, textiles, and pharmaceutical products (M. Contrerasa et al., 2003) [15].

Examples of herbicides include RS-2- (4-chloro-o-tolyloxy) propionic acid, (4-chloro-2-methylphenoxy) butyric acid and 3,6-dihydropyridine-2-carboxylic acid, whose photodegradation was studied with TiO_2 doped with nitrogen and iron as well as comparing their efficacy with the most commonly used TiO_2 Degussa P25 [16]. Among pesticides and herbicides, the following should be mentioned: DDT (dihloro-diphenyl-trichlorethane)-organohloric insecticide, s-triazine herbicide (2,4,5-trichlorophenoxyacetic acid). Quinmerac (7-chloro-3-methylquinoline-8-carboxylic acid) has been commercially used over the last ten years as a new class of highly selective herbicides. Clomazone (2- (2-chlorobenzyl) -4,4-dimethyl-1,2-oxazolidin-3-one) is a selective herbicide used to control weeds in soy, corn, tobacco, rice, sugar beet and other vegetables [17].

Increased use of pesticides and inadequate methods of wastewater disposal are of particular importance for freshwater (surface and groundwater), coastal and marine waters (Abdennouri, M. et al., 2011) [18]. Low level of pesticide residues in water generally does not produce acute toxic problems, but chronic effects are of great importance (Smith, C.N., et al., 1987)[19]. Pesticides contribute to the onset of cancer (Younes, M. and Galal-Gorchev, H. (2000) [20], Parkinson's disease, affect reproduction, foetal damage, delayed neurological event and possible immune disorders (Doull, J. (1989)) [21].

DYE

The toxicity level of a particular dye is very important due to its diverse effects on the environment and living organisms. Among all the techniques for dye removal, adsorption and photocatalysis are two important processes which have been gaining much attention in recent years. Some of the important ancient natural dyes used for textile dyeing were: Tyrian purple (6, 6'-dibromoindigo), kermes, indigo (5,7,3', 4'-tetrahydroxyflavone, C.I. Vat Blue 1) [22, 23, 24].

The first two colours were of animal origin, while the last colour was obtained from plants. According to the Colour Index classification, about 8,000 chemically different types of synthetic dyes are currently registered [25]. The increase in the production of synthetic colours is inextricably linked with the textile industry. Experts estimate that they produce about $7x10^5$ ton colour per year for this type of industry only [26].

From the standpoint of the market, azo colours represent the largest and most significant class of organic colours. More than 50% of commercial dyes and pigments belong to azo compounds. The popularity of azo dye is most influenced by strong colour intensity; the molar extinction of the azo dye coefficient is about twice as high as the anthraquinone, the second largest colour group, which means that the double the amount of paint is needed to obtain the same colour intensity. Azo dye synthesis procedures are relatively simple. Also, there is a wide range of cheap petrochemical raw materials that can be used to synthesize a variety of azo dyes of specific characteristics. Most azo colours have good stability to light. Another advantage of azo colours is that they cover a whole spectrum of colours [27]. Azo dyes are most used in the textile industry for the dyeing of cotton, paper, silk, leather, wool, and dyeing of polyamide, acrylic, polyolefin, polyester, viscose and cellulose acetate fibers [28, 29]. In addition to the textile industry, azo dyes have been used in the past decades for other purposes: in the pharmaceutical and food industry, in the cosmetics industry and in the manufacturing industry, in photo making, photo/video filter dyeing, printer mugs and photocopiers, solar cells, etc. [30]. Heterocyclic azo dyes are important not only because of excellent textile dyeing properties but also for good application in other branches such as photodynamic therapy, lasers, reprographic technology, nonlinear optical systems. They are well known for their use on LCD screens and in inkjet printing [31]. The first monoation pyridone dyes were synthesized by Burkhard and associates of diazotized anilines and 3-cyano-6-hydroxy-4-methyl-2-pyridone in tartaric acid at 0-5 °C and at pH 4.5 [32]. The resulting dyes were used to stain synthetic materials, mostly in yellow [33, 34] or in green-yellow shades [35]. Subsequent modification of the amino component (e.g., 5-amino-4,6-dicyanindane) yielded red and orange shades [36]. Photomineralization of organic dyes to H_2O and CO_2 is a much researched area and of particular interest for the textile industry, which produces liquid dye waste in large quantities. Azo dyes continue to be heavily used, and disposing of them is difficult (Jussi Kasanen et al., 2011) [37].

PHOTOCATALYTIC DEGRADATION

Titanium dioxide (TiO_2) exhibits excellent photocatalytic activity and it has been extensively used in various applications, e.g., photovoltaics devices, pollutant decomposition and antibacterial materials (Warapong Krengvirat et al., 2013) [38]. TiO_2 can be used as a photocatalyst in water purification to degrade organic pollutants. Semiconductor mediated heterogeneous photocatalysis is considered as a promising alternative for the removal of organic pollutants and pathogens from wastewaters, as it can potentially work under solar irradiation without generating harmful by-products. Due to its high efficiency, low toxicity, excellent physical–chemical stability, and relative low costs, nanosized TiO_2 is the most widely used photocatalyst for environmental purifications (Lei Liu et al., 2013) [39]. Conventional water treatment technologies have limitations in complete decontamination of those emerging anthropogenic organic pollutants or disinfection of bacteria without harmful disinfection byproducts.

MECHANISM OF THE PHOTOCATALYTIC DEGRADATION WITH TIO2

The photocatalytic mechanism starts when a photon with energy, hν matches or exceeds the band gap energy, $E \geq E_g$, of the semiconductor. Conduction electrons, e_{cb}^-, are promoted from the valence band into the conduction band (CB), leaving a hole, h_{vb}^+, behind. The lifetime of e^-h^+ pairs is only a few nanoseconds, but it is long enough to initiate redox reactions with semiconductor material in solutions or gaseous phases. The resulting e^-h^+ pairs within the semiconductor particle can be separated, diffused to the surface of the semiconductor and thus participate in the oxidation and reduction reactions of the organic and inorganic compounds or are subjected for the recombination process by decreasing the quantum yield of the reaction. The hole can either oxidize a compound directly or react with electron donors like water to form OH radicals, which in turn react with pollutants such as chlorophenols, dyes, and organic compounds resulting in the total mineralization of most of these compounds (A. Aguedach et al., 2005) [40]. The electrons in conduction band can react with oxygen, O_2 to yield super oxide anion, then in reaction with water can form hydrogen peroxide, $O_2^{\boxed{?}-}$ and then give a hydroxyl radical, $^{\bullet}OH$ (Figure 1). The resulting OH radicals are very strong oxidizing agents (standard redox potential +2.8 V). The most important species for photocatalytic processes of degradation of pollutants is hydroxyl radical. The hydroxyl radical is a non-selective oxidant with a high oxidation potential which leads to complete mineralization, to CO_2 and H_2O of the most of the organic molecules present in wastewater by oxidation reaction.

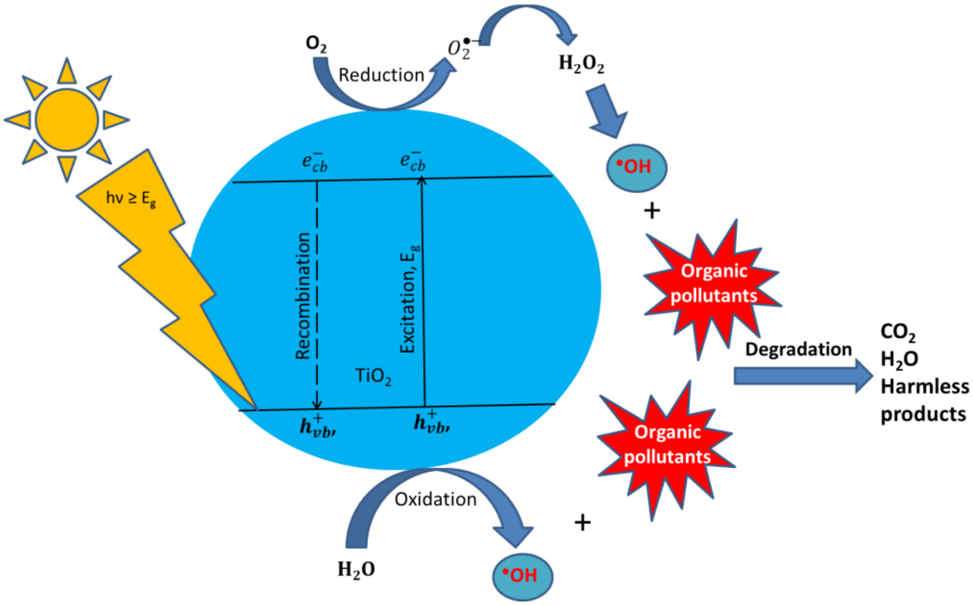

Figure 1. Mechanism of the photocatalytic degradation with TiO_2.

TYPE OF WASTEWATERS

From industrial wastewaters where the most common organic molecules are found are:

1. synthetic colours/textile wastewaters
2. oil industry/refinery wastewaters
3. pesticides and herbicides/agricultural wastewaters
4. medicaments/pharmaceutical wastewaters.

TEXTILE WASTEWATERS

All textile colours are synthetized so that they are persistent during washing, chemical and microbiological activity and on the effects of light. Thanks to the properties of durability, textile colours are extremely persistent in the environment and prone to bioaccumulation.

Since textile azo is dyed by its xenobiotic nature and is hardly biodegradable, their removal from waste water attracts more and more attention of environmental protection researchers. For these reasons, azo dyes must be removed from the waste waters before discharge into natural watercourses. Removing paint does not always mean removing toxicity. Incomplete degradation and formation of degradation ingredients can increase toxicity.

REFINERY WASTEWATERS

Wastewater generated by petroleum industries is very complex, and includes several inorganic and organic components, such as Ca^{2+}, Mg^{2+}, S^{2-}, Cl^-, PO_4^{3-}, Fe^{2+}, Fe^{3+}, SO_4^{2-}, emulsified oil, sulphides, ammonia, cyanides and especially phenol and phenolic derivatives that are the most important contaminants (A.M. Mansouri et al., 2014) [41].

Wastewater from petroleum refinery has the characteristics of high concentration of aliphatic and aromatic petroleum hydrocarbons, which could lead to heavy pollution on the surface of soil and rivers. Refineries generate polluted wastewater, containing COD (the amount of organic matter that can be chemically oxidised) levels of approximately 200–600 mg/l; 20–200 mg/l phenol; 1–100 mg/l benzene; 0.1–100 mg/l chrome and 0.2–10 mg/l lead; and other pollutants (F. Shahrezaei et al., 2012) [42].

Industrial wastewater containing dyes, such as indigo carmine, are discharged without previous treatment into the environment, causing a change in water colour, aquatic life disturbance and generation of toxic aromatic amines (F.A. Rodríguez et al., 2012) [43].

AGRICULTURAL WASTEWATERS

In intensive agricultural practice, repeated use of pesticides results in frequent occurrence of pesticides in European raw water resources. Pollution sources from agricultural activities are well known: unused treatment solutions, spray machine and pesticide container washing. Small volumes and high concentrations characterize these effluents. Numerous studies have demonstrated that the heterogeneous photocatalysis of pesticides is an effective process to degrade pesticides and to mineralize some of them (L. Lhomme et al., 2008) [44]. The study of Lhomme and co-workers has shown that by photocatalytic degradation are possible to remove 70% to 85% for an irradiation time ranging from 15 to 20 h. The results of this research also have shown that for the degradation of the target compounds and mineralization is very important nature of the additives in the commercial pesticide solutions. For example, in pure water the two pesticides were completely degraded by photocatalysis, whereas, in the case where additives were present, the degradation rate and the mineralization of the pesticide could be significantly decreased.

PHARMACEUTICAL WASTEWATERS

The presence of polluting substances such as: medicines and personal hygiene products, dietary products, fragrance components, sun protection agents, are detected in many waste- and surface-waters [45, 46, 47]. These compounds, as well as their biologically active metabolites, continuously enter the aquatic environment through various pathways and primarily through untreated or inadequately treated wastewaters. The ever-increasing health and environmental problem in the world is medicine and personal hygiene, due to the fact that long-term exposure to low concentrations of these substances can have negative consequences for water and land ecosystems and/or human health. Uncontrolled discharge of drug-laden wastewater into natural streams can lead to the development of resistant bacteria, slowing oxidation of nitrite and methanogenesis, and potential increase in toxicity due to the synergistic effect of various drugs and their metabolites.

Active pharmaceutical ingredients are complex molecules with different functionalities, physical-chemical and biological properties. Mostly, they are polar compounds, molecular mass ranging from 200 to 500 or 1000 Da. They belong to the group of pollutants also referred to as micropollutants, since they are present in the aquatic environment in microgrammic ($\mu g\ dm^{-3}$) or nanogram concentrations ($ng\ dm^{-3}$).

Pharmaceuticals can be divided based on their purpose and biological activity on antibiotics, analgesics, antipyretics, antihistamines, antineoplastic agents, anti-inflammatory drugs. Classification of molecules of the active component of drugs based on the chemical structure is mainly used within a particular subgroup of drugs. Thus, antibiotic groups differ from subgroups: ß-lactams, cephalosporins, penicillins or quinolones. Even small changes in the chemical structure can have a significant effect on their solubility and polarity, as well as on other traits relevant to their destiny in the environment. Active drug components generally have basic or acid functional groups, sometimes both within the same molecule. Under conditions that dominate the drug molecule's environment, they can be neutral, cationic, anionic or in the form of zwitterion. All of this makes the behaviour of drugs in the environment extremely complex.

Over the last few years, it has been discovered that from the point of view of ecological risk assessment are not only active components of drugs, but also compounds that arise as a result of their structural changes in the environment. Once they get into the environment, active drug components are subject to various structural changes under the influence of biotic and abiotic processes. Also, structural changes occur during the treatment of wastewaters [48, 49, 50].

Studies in different countries have confirmed the presence of drugs in surface and municipal sewage waters in the concentration range of several $ng\ dm^{-3}$ to several $\mu g\ dm^{-3}$. On the other hand, little is known about the appearance, destiny, and metabolite activity, which can also be detected in the environment [51].

It is known that some antibiotics (tetracyclines) tend to bind to particles or form complexes with ions present in the water [52, 53, 54]. Antibiotic sorption depends on the amount and nature of free and suspended particles in the aqueous phase and natural organic matter, of the present minerals and the distribution coefficient [52].

CONCLUSION

The classic methods of wastewater treatment include mechanical, chemical and biological treatment. Since chemical treatment implies the introduction of chemicals such as ferric chloride or aluminum sulphate and they remain in the environment for a long time and can decrease pH values as well they may have a harmful effect on the environment. Also, classical methods imply constant use of chemicals, while photocatalytic treatment can be carried out several times in mild conditions and the end products are environmentally acceptable. Since that, in photocatalytic processes for the fotocatalyst activation is used UV/Vis light (sun light) as well as the oxygen found in air and these processes can operate at room temerature and preassure conditions they can be classified as more green processes then classic one. In recent decades, an increasing interest has been devoted to the development of photocatalytic processes both in homogeneous and heterogeneous phases. Especially in terms of heterogeneous systems, the use of photo-saturated semiconductors as a catalyst for organic processes has been of great interest due to its ease of use, recycling and low environmental impact. Photocatalytic semiconductors are promising technologies that have a large number of applications in environmental protection systems such as water and air purification, water disinfection, and hazardous waste remediation. Photocatalytic degradation techniques should be more used in wastewater treatment because with this technique it is possible to decrease contaminants to certain acceptable discharge limits. Numerous studies have demonstrated that the heterogeneous photocatalysis of organic compounds is an effective process to degrade and to mineralize them.

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CONTROLLED RELEASE OF RANITIDINE FROM CONDUCTIVE POLYPYRROLE FILMS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: Incorporation and controlled release of active substances from the conductive polypyrrole films by electric stimulation were investigated. Change of the redox state of the conductive polymer was induced by this stimulation, which allowed the incorporation and release of the drug at different rates. Polymerization of pyrrol on a stainless steel substrate was performed by cycling the potential 40 times in predetermined potential window, after which uniform polymeric film was formed and used as a medium for incorporation and release of the active substance. Stability of obtained films, as well as the electrochemical behaviour of ranitidine hydrochloride was investigated by cycling the potential of the film electrode in 0.9 % NaCl in the same potential window used for the polymerization. Uptake and release of the active substance was performed at constant potential and monitored by chronoamperometry. Although incorporation of the ranitidine hydrochloride was not obvious from the measured infrared spectra, incorporation and release was confirmed and quantified by monitoring the concentration of the active substance in the electrolyte. It was determined that maximum reversible uptake was 351 µg cm^{-2}. However, successive potential stimuli did not result in the equal released quantity, as expected for the ideal controlled-release system based on conducting polymers.

KEYWORDS: conductive polymers, ranitidine hydrochloride, controlled release

INTRODUCTION

It has always been considered that any substance can be seen either as a medicine or as a poison. It is important in all this to have a specific measure or dosage, by which we can manipulate the amount of active substance we need. Dosage, subjectively speaking, has different effects for each person, but each one has different visions and experiences. There are various variations on this subject, namely that the dosage alone depends on the physicochemical characteristics of the drug, the organism and its metabolism, to what extent the drug is dosed and in general how many times a day the drug is administered.

A special concept of administration and release of the drug into the organism has been developed through indirect "vectors", which will allow the release of the required amount of medication at a given time interval. In addition, apart from having a long-term effect and controlling the release rate, multiple administration of the drug is avoided.

In order to achieve such an effect, it is necessary to pack the drug in a particular substance, which will be compatible with it, and which will exhibit such traits that will enable it to be released at the given location. More and more considerations come from polymers, where the drug is incorporated into their structure and as such release into the organism. In recent years, polypyrrole (PPy) is in the focus of many studies compared to other conductive polymers due to its high chemical stability and air stability and simple preparation. Oxidation of pyrrole into polypyrrole can be achieved by using an electrochemical or chemical method, with the addition of strong oxidizing agents ([1] Anuar et al., 2004). It can be easily modified to be more suitable for biomedical applications through the incorporation of bioactive molecules. Polipyrrole also responds to stimuli, enabling a dynamic control of its properties by applying electric potential ([2] Ateh et al., 2006). The need to prepare these materials in various forms in order to enable integration or linkage with other structures, including living beings, is of vital importance ([3] Wallace et al., 2009). Polypyrrole is an electronically conductive polymer with conductivity up to 1000 Scm^{-1}, demonstrating its versatile application in batteries, electronic devices, functional electrodes, electromagnetic devices, sensors, etc. It can be manufactured with a large surface, with different porosity or can be easily modified to be more suitable for biomedical applications through the incorporation of bioactive molecules. Polypyrrole also responds to stimuli, enabling dynamic control of its properties by applying electric potential ([2] Aten et al., 2006). These properties are well exploited in potential controlled-release systems based on PPy ([4] Geetha et al., 2006; [5] Alshammary et al., 2016; [6] Thompson et al., 2006; [7] Kontturi et al., 1998) and derivatives ([8] Krukiewicz et al., 2016), while some authors claimed the preparation of PPy-based chip for controlled release ([9] Ge et al., 2009). Also, some authors addressed biocompatibility of PPy and PPy-based materials, essential for their potential application in biological systems ([2] Aten et al., 2006).

The dosage represents the amount of active substance (drug) administered to a human or an animal. The dose-response and drug effect can be graded and quantal. Drug concentration in body after oral administration can be simplified and presented as concentration-time profile (Figure 1). The rate at which the drug appears in the blood is defined by $t_{\max}$, the time at which the drug achieves maximum concentration, and $c_{\max}$ or maximum concentration ([10] Mehmedagić, 2002).

In this paper, incorporation of ranitidine hydrochloride into the electropolymerized polypyrrole matrix, and its controlled release was performed by electrochemical transformation of polymeric film. Ranitidine is a non-imidazolyl-blocker of histamine receptors that competitively antagonizes the action of histamine on all H_2 receptors, but is mainly used to inhibit gastric acid secretion. Ranitidine is used in the treatment of the following diseases: intestinal sores and stomach ulcers; gastro-oesophageal reflux; erosive esophagitis (http: 1). Ranitidine is administered orally and is well absorbed.

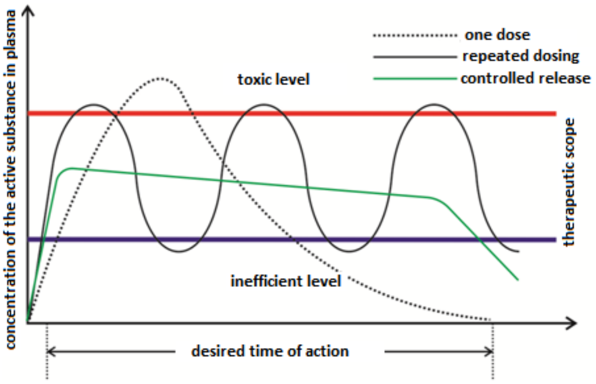

Figure 1. Level of active substance concentration in plasma after: one dose, repeated dose, controlled release ([11] Babić, 2015)

EXPERIMENTAL

Pyrrole, lithium perchlorate and camphorsulfonic acid (CSA) were of analytical grade and obtained from Sigma-Aldrich, USA. Pyrrole was distilled under the reduced pressure prior to use. Ranitidine hydrochloride was of analytical grade and was obtained from Hemofarm, Serbia. Phosphate buffer was prepared as following solution, with analytical grade chemicals: NaCl 8 gL^{-1}, KCl 0.2 gL^{-1}, Na_2HPO_4 1.42 gL^{-1}, KH_2PO_4 0.24 gL^{-1} + redistilled water.

The substrate was made of stainless steel with high content of nickel and chrome. It was used in the form of plates which were coated with Teflon tape in order to achieve the geometric surface area of 2 cm^2 exposed to the electrolyte (Figure 2).

Figure 2. Stainless steel plate (in the middle)

The polymerization of pyrrole was performed using cyclic voltammmetry in a three electrode system with $Ag/AgCl/KCl_{sat}$ as the reference (0.197 V vs. SHE) and platinum foil (2 cm^2) as auxiliary electrode, by cycling the potential 40 times between -0.6 and 0.6 V in 0.05 mol dm^{-3} pyrrole solution with 0.1 mol dm^{-3} camphorsulfonic acid or lithium perchlorate as the inert electrolyte. Stainless steel plate was the working electrode.

Chronoamperometry was employed in order to incorporate the active substance, as well as to release it from the polypyrrole film. All electrochemical experiments were performed with potentiostat/galvanostat PAR 263A controlled by PowerSTEP interface. The argon stream was passed through the cell several minutes prior to the start as well as during the measurement to remove oxygen from the electrolyte. The camphorsulfonate anions were used as large anions, which can be incorporated into the polymer to balance the positive charge caused by oxidation of the polymer during synthesis. After polymerization, the obtained film was washed with redistilled water and left in water until the next treatment. Characterization of polymer films was performed by infrared spectroscopy with Fourier transform using Perkin Elmer Spectrum BX. UV spectrophotometry was used to determine the concentration of ranitidine hydrochloride in the buffered electrolyte after release. In this case a phosphate buffer was used as a blind test. The absorption spectrum in the ultraviolet and visible area was recorded for the active component dissolved in phosphate buffer. Molar absorption coefficient of the active substance is determined from the slope of absorbance-concentration dependence at the maximum absorbance. Measurements were performed on the UV-Vis ultrasonic spectrophotometer 2000. The cyclic voltammmetry of the active component in the sodium chloride solution was performed to determine the electrochemical behaviour of this substance. Measurements were performed in the previously described three electrode system, where the glassy carbon electrode was used as the working electrode. Measurements were made within the limits of the potential electrolyte window.

Figure 3. The prochrome plate after using cyclic voltammmetry

Loading of the conductive polymer film with the active component was performed using a potentiostatic method of highly concentrated aqueous solution (100 g/L) of the active component in the standard three electrode system. Charging of the polymer was performed at a potential of +0.60 V and monitored using chronoamperometry.

Controlled release was performed in the same cell in a small volume of phosphate buffer solution (10 cm^3), where the excitation signal was at the potential of +0.55 V. After each signal from the cell, small volume was sampled and analyzed by means of UV spectrophotometry, after which analyzed solution was transferred back to the electrochemical cell for subsequent stimuli of PPy matrix. In addition, another sample of the charged polymer was left for 24 hours in the buffer solution, after which the buffer was analyzed for the content of the active component, in order to determine spontaneous release.

RESULTS AND DISCUSSION

Figure 4 shows the voltammograms recorded during polymerization of pyrrole in two electrolytes: lithium perchlorate and camphorsulfonic acid. In both cases, voltammograms show the gradual growth of polymer film.

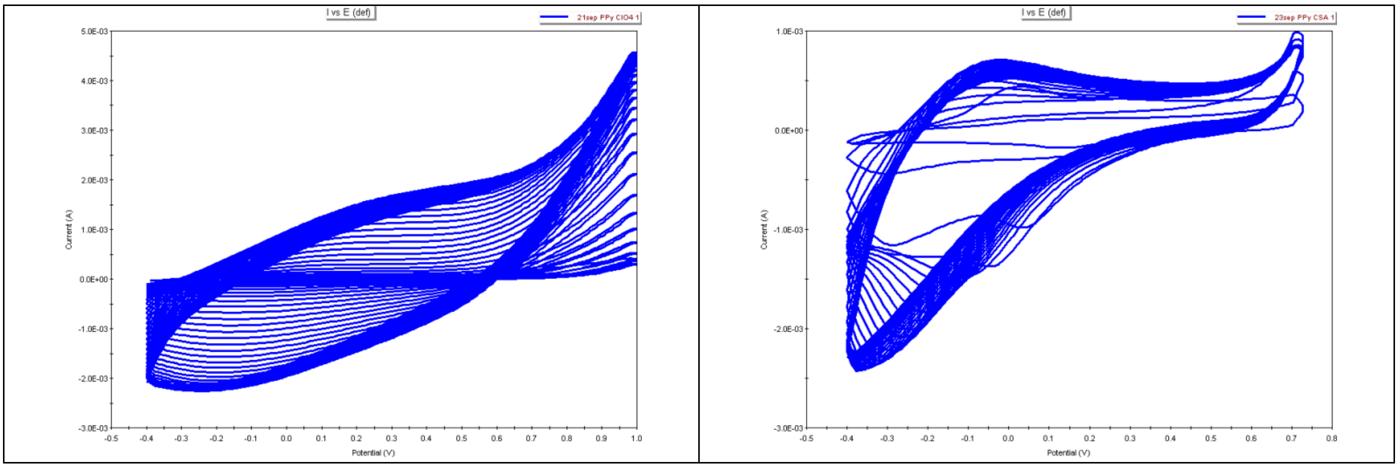

Figure 4. Cyclovoltammograms of pyrrol polymerization on stainless steel;
left: with $LiClO_4$; right: with CSA as auxiliary electrolyte

However, in the case where the supporting electrolyte is camphorsulfonic acid, it is noticeable that the current peaks induced by the redox transformation of the polypyrrole are considerably sharper and better defined, which is a result of significantly better kinetics of these processes than those in polypyrrole obtained with lithium perchlorate. Since the electronic transfer is a fast process and therefore cannot limit the speed, the reason for the different kinetics of redox transformation in the polypyrrole film lies at different diffusion rates of counter ions (perhlorate or camphor sulfonate) in the mass of the polymer, which is in accordance with different sizes of these ions. Namely, using a relatively larger camphorsulphonate anion, the formed polymer structure is looser, but also the anion is captured in the polymer structure. For this reason, during the redox transformation of this system, the cation diffusion (in the case of camphorsulfonic acid, H^+ ions) becomes the dominant process. This fact represents an important precondition for the incorporation of cationic active components, such as ranitidine.

Figure 5 shows an infrared spectrum of electrochemically prepared polypyrrole with marked characteristic vibration strings.

At 1422 cm^{-1} there are vibrations of C=C elongation of the pyrrol ring. At 1035 cm^{-1} there are N-H vibrations. At 1460 cm^{-1}, the asymmetric stretching of the C-C bond was noticeably observed. At 1545 cm^{-1} there is a characteristic peak of the pyrrol ring.

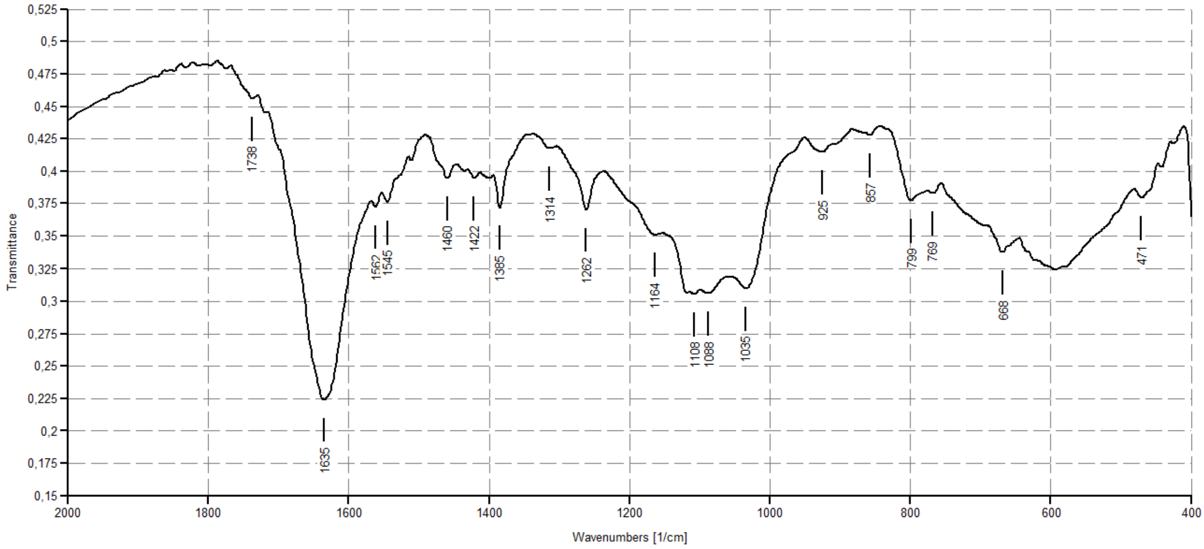

Figure 5. Infrared spectrum of polypyrrole

SPECTROSCOPIC CHARACTERIZATION AND ELECTROCHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Figure 6 shows the absorption spectrum of the active component. Ranitidine hydrochloride has two absorption maxima, at 230 nm and 310 nm.

Figure 7 shows the standard calibration curve, respecting Beer's law in a given range of 0.04 to 0.2 g dm^{-3}. When subjected to a regression analysis, the value of the coefficient of regression in ranitidine hydrochloride is the value of coefficient 0.942.

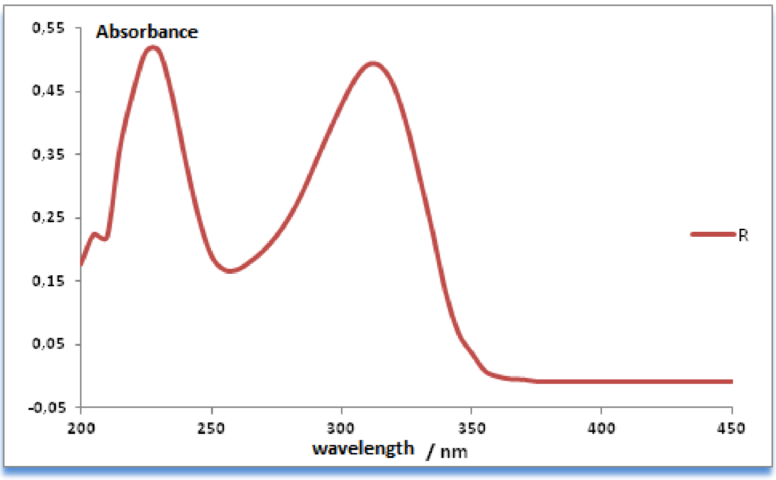

Figure 6. The absorption spectrum of ranitidine in the ultraviolet and visible area

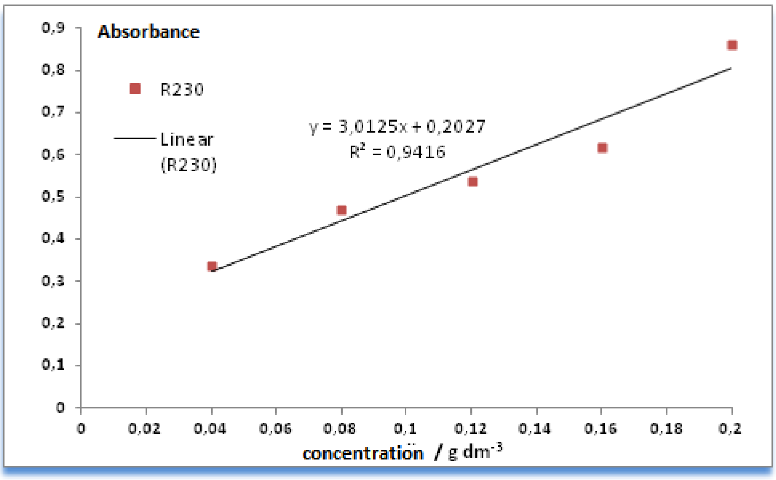

Figure 7. The standard calibration curve of ranitidine hydrochloride

Figure 8 shows the infrared spectrum of ranitidine hydrochloride. For ranitidine hydrochloride the following peaks are characteristic: from 3260 cm^{-1} to 3100 cm^{-1} for OH group stretching and symmetrical stretching of N-H peaks, bands around 3066 and 3017 cm^{-1} are attributed to C-H bond of furan which is a part of the chemical structure of the active substance. 2970, 2950 and 2908 cm^{-1} bands are present in the C-H aliphatic group.

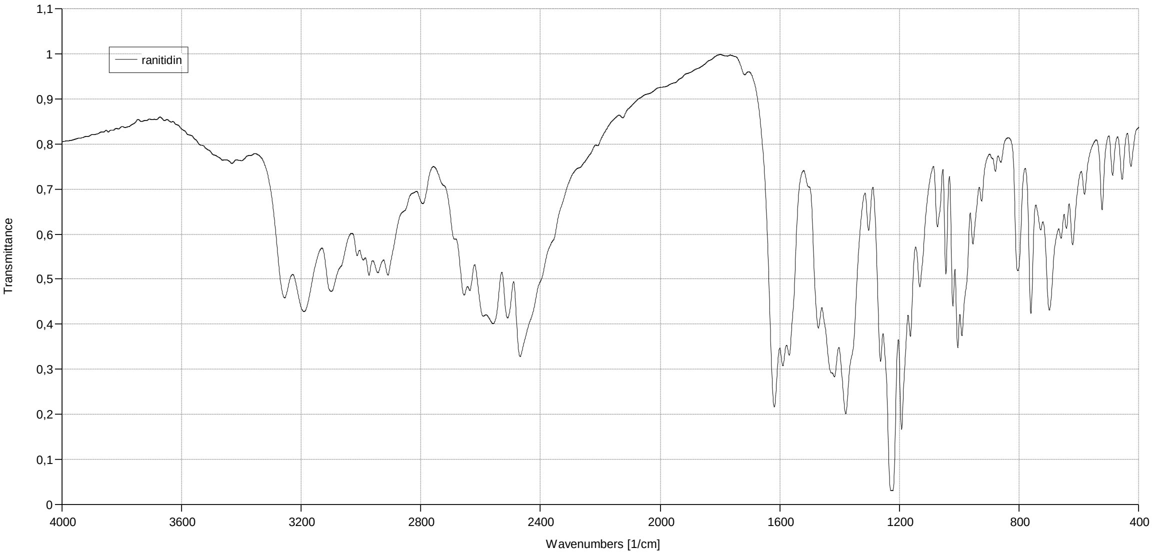

Figure 8. Infrared spectrum of ranitidine

Figure 9 shows the infrared spectrum of ranitidine incorporated in a PPy film. Based on this IR spectrum, it is not possible to establish with certainty the presence of ranitidine hydrochloride, and the reason for this may be the low concentrations of the active substances in the conductive PPy film. However, our conclusion that the substance is actually absorbed into the film is based on the fact that the polymer film was oxidized in the solution containing only the active substance (no other counter ion to compensate the charge introduced by potentiostat), as well observation that it is released after the opposite stimuli.

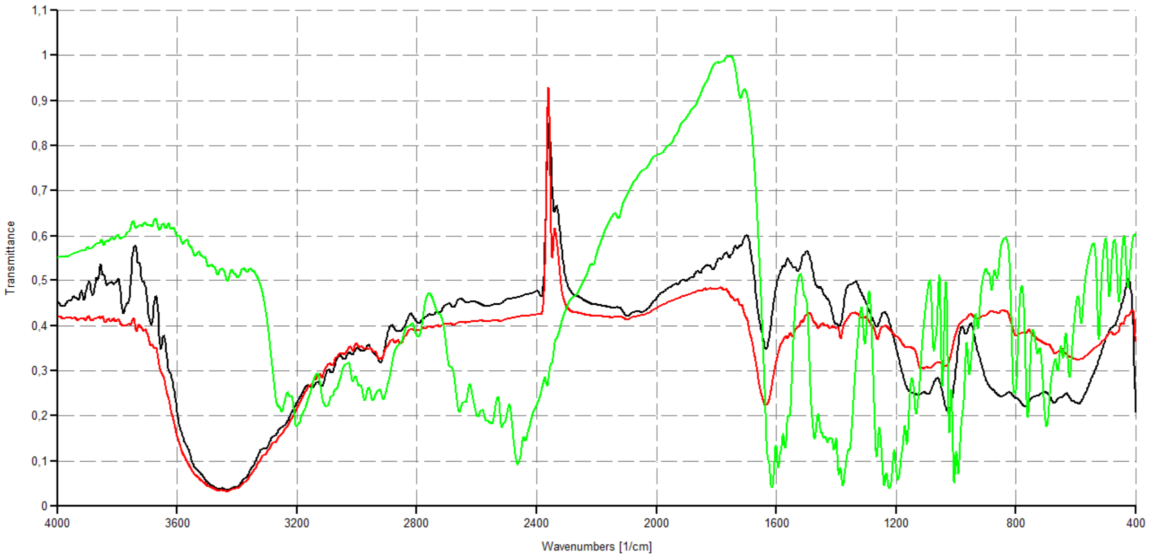

Figure 9. Infrared spectrum of ranitidine hydrochloride incorporated in polypyrrole film; the black spectrum - ranitidine hydrochloride and polypyrrole, the red spectrum - polypyrrole spectrum, the green spectrum - ranitidine hydrochloride

CONTROLLED RELEASE

Figure 10 shows the cyclovoltammogram of the active component in 0.9% NaCl. Given that the measurements worked in a potential window, it is concluded from this data that the active component in this potential range cannot be electrochemically dissolved.

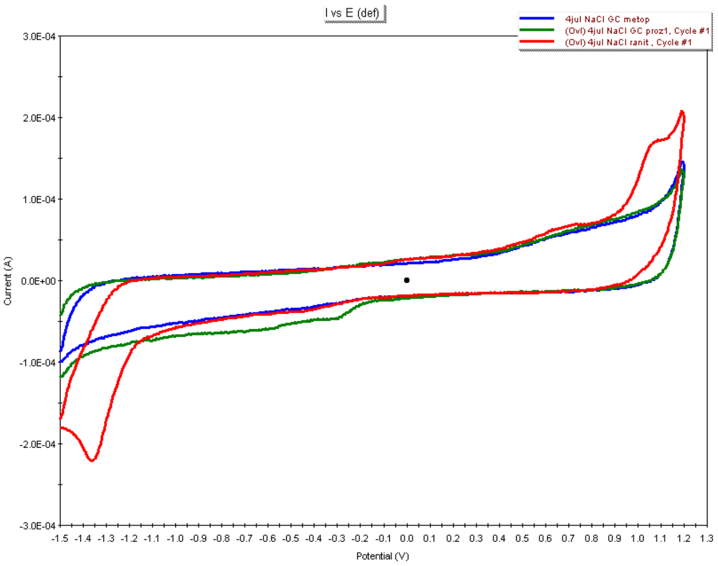

Figure 10. Cyclovoltammogram of ranitidine hydrochloride in 0.9% NaCl

Since the loading of polymer film implies its redox transformation, whereby the counter-ions of the electrolyte (in this case, the ions of the active substance) are incorporated into the polymer to maintain its electroneutrality, the amount of incorporated active substance can be estimated from the total charge passed through the electrochemical cell during the charging of the film. This charge is obtained from the chronoamperamograms scanned during film loading (Figure 11), and it amounts to 96.53 mC for ranitidine hydrochloride. Based on Faraday's law:

$$Q = znF\text{, i.e. } n = \frac{Q}{zF},$$

where z=1, F=96500 C mol^{-1} and Q the above mentioned charge, the estimated incorporated amount of the active substance is 1.00 × 10^{-6} mol or 351 µg ranitidine hydrochloride.

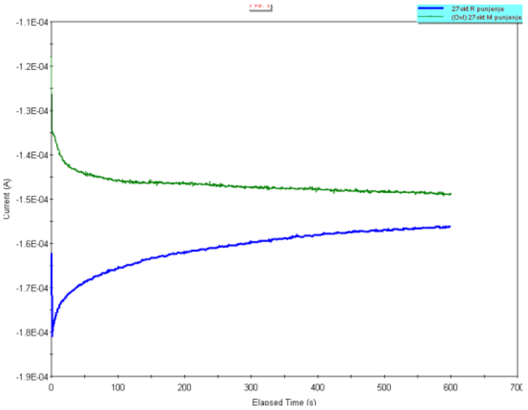

Figure 11. Chronoamperogram showing the incorporation of ranitidine hydrochloride into a polypyrrole film at a given time

The release was carried out in a small volume of phosphate buffer (10 cm^3) to obtain a concentrated solution for spectrophotometric analysis. Table 1 shows the abundance absorbance values after each excitation signal (1 second), as well as the calculated values of the concentration of the released active substance in the buffer. By measuring absorbance of buffer solution with control samples that were left for 24 h without the excitation signal, it was found that no measurable release of the active substance had occurred. Based on this, using the estimated value of the weight of the drug incorporated, the relative amount of drug release was calculated in percentages, which is also shown in the table. Figure 12 gives graphical representation of data from the table above.

Table 1. Controlled release of ranitidine

Number of excitation signal	1	2	3	4	5
A	0.011	0.029	0.046	0.052	0.063
c / mmol dm^{-3}	3.65	9.63	15.27	17.26	20.91
n_{uk} / µmol	36.5	96.3	152.7	172.6	209.1
n_{dif} / µmol	36.5	59.8	56.4	19.9	36.5
% released in total	10.40	30.00	47.57	53.77	65.14

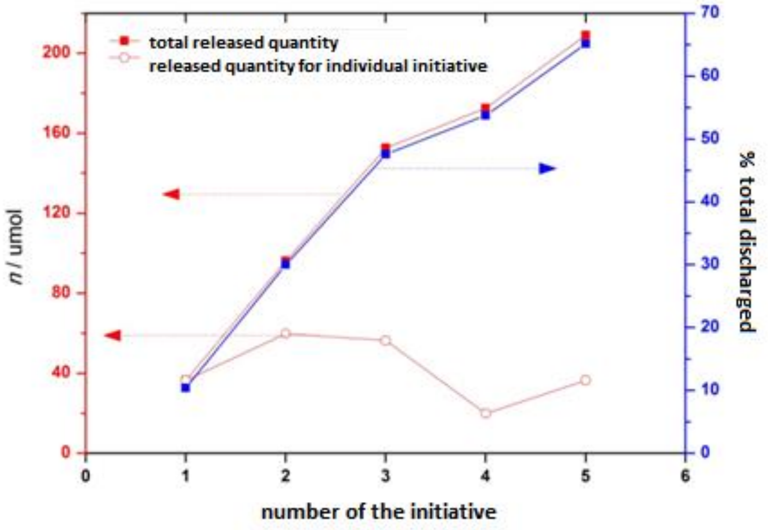

Figure 12. Total and individual amounts of released ranitidine and percentage of total released amount in the function of the stimulus

From the data shown above, it is apparent that at each excitation potential signal, a controlled release of the active substance is achieved. It is noteworthy, however, that the released amount varies during successive stimuli, which would ideally be the same (open circles in Figure 12), whereas the total released amount has a steady trend of growth.

CONCLUSION

From the results of the research it can be concluded:

- that after cyclic voltammmetry, the polymerization of polypyrrole on a thin plate was successfully performed. After 40 cycles over a period of 45 minutes, a unique film was formed, which was used as a medium for incorporating and releasing active substances.
- The use of camphorsulfonic acid as an electrolyte has proven to be a better choice because the bulk anion creates a looser polypyrrole structure, which will allow the recipience of a greater number of anions, which will later be important in the

ion exchange with the active substance (in this case cations).

- Through infrared spectroscopy it was possible to obtain infrared spectra for polypyrrole and the active substance, but after the incorporation of the active substance into the polypyrrole film, spectral spikes could not be read clearly. The reason may be insufficient concentration of the active substance, which on the other hand means a small amount of incorporated active substance in the conductive polypyrrole film.
- Cyclovoltammmetric analysis of ranitidine was also performed in 0.9% NaCl solution within the potential window (potential range in which there is no electrolyte degradation). Based on this it can be concluded that the active substance in the range of these potentials will not be electrochemically dissolved.
- Chronoamperometry can be used to determine the amount of active substance incorporated into a conductive polypyrrole film, following Faraday's law, where for ranitidine hydrochloride amounts to 1.00×10^{-6} mole or 351 μg.
- In addition to loading, chronoamperometry was also used for the release of the active substance from the conductive polypyrrole film. Low values of absorbances may indicate low concentrations of the active substance, instrument failure, or much more advanced techniques and materials are needed to enable higher concentrations of active component, as well as their loading and release from the polypyrrole film.
- It is noteworthy, however, that the released amount varies with successive stimuli, which should ideally be the same as the total released quantity has a steady growth trend.

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NUTRITIONAL VALUE, SENSORY ANALYSIS AND HEALTH SAFETY OF DO-DO SPICE

PROFESSIONAL PAPER

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ABSTRACT: DO-DO spice is a nutrient of exceptional quality based on salt intended for final consumer consumption. It is used to enhance the taste of food, and it is added to dishes to enrich the taste. The process of producing DO DO spice is a batch process, and all raw materials used in the production process meet the prescribed specifications. According to the adopted recipe of Solana dd (a joint stock company) Tuzla, the same components are mixed until complete homogenization of the mixture is achieved. Constant monitoring of the production and packaging process is performed with application of the implemented standards ISO 9001: 2015, ISO 22000: 2005, HACCP, HALAL BAS 1049: 2010, KOSHER. The nutritive value of substances used in DO DO spice is 494 kJ / 116 kcal, and due to the presence of vegetable seasoning and the possibility of losing their nutritive properties, it is best to add it at the end of cooking. The aim of Solana dd Tuzla is to produce the spice which is health safety, nutritiously valuable and which has satisfying sensory properties, because what we eat has an impact on our health.

KEYWORDS: DO-DO spice, nutritional value, health safety, sensory characteristics.

INTRODUCTION

Spices are used to improve the organoleptic characteristics of foodstuffs, dishes or finished food products. Spices are parts of plants which by their content change the smell, taste and colour of food, and give it its recognizability, as does the good, domestic DO DO spice. Spice trade has enriched many countries. European expansion began in the 14th century by opening the Spice Route (a name for Malaysian and the Indochinese island). In addition to the economic impact, some spices also have psychological properties which improve the mood; and even in the 12th century, they were used medicinally. One of the products of Solana dd Tuzla based on salt is DODO spice, a mixture of spices which gives traditional dishes of Bosnia and Herzegovina a distinctive taste. Apart from its quality, DO DO spice is a nutritionally valuable foodstuff.

The process of producing DO DO spice is a batch process, and all the raw materials that are used meet the quality. According to the adopted recipe of Solana dd Tuzla, the same ingredients are mixed until complete homogenization of the mixture is achieved.
Sensory evaluation is a scientific discipline that analyzes, measures and interprets the reactions of food characteristics that are perceived by senses of sight, smell, and taste. In order to define sensory properties, sensor parameters have often been used: smell, taste, texture, appearance. The smell of the product is felt when the fragrant volatile molecules reach the nose. The optimal smell sensation lasts for one to two seconds. The taste is defined as the sum of perceptions that results from stimulation of the ends of sensations grouped at the entrance of the digestive and respiratory system. The receptors for taste are taste cells inside the taste buds that are located within the oral cavity. There are three kinds of buds: *papilae vallatae*, *papilae fungiformes* and *papilae foliate*. The taste intensity depends on the concentration of dissolved substance, a place which is being stimulated, duration of stimulation, viscosity, chemical state of saliva and temperature. Texture is a feature definded by the way in which the various structural elements are incorporated into the micro and macro structure, and by the effect of their external manifestations in terms of flow and deformation. During the test, the toughness, elasticity, hardness, softness and tenderness of the product are most often evaluated. Appearance is an optical property that is related to the purchase and consumption. The main characteristics are colour (important sensory characteristic), shape and size, clarity and foaming.

EXPERIMENTAL

The assessment of nutrients in foodstuffs using chemical analyzes is carried out by different methods that are not practical due to a wide range of nutrients. Common methods for evaluating values include: using values from different (but similar) food, calculating values from different forms of the same food, calculating values from other components in the same food, calculating values from domestic recipes or formulations of commercial products for multicomponent foodstuff, converting values from information on the label of commercial food product nutrients, calculation of values from product standards, and assumption of zero value. Evaluation of the nutritive value of DO DO spice is based on analytical methods for determining the proportion of proteins, carbohydrates, fats and oils. The results obtained are compared with the values calculated from other components in the same food. Evaluation of the nutritional value of DO DO spice, which is produced according to a Solana dd Tuzla recipe, was done.

Table 1. Nutritive / energy value of DO-DO spice

100 g of the product contains an average of	
Energy value 494 kJ / 116 kcal	494kJ/116 kcal
Proteins	5.50 gr
Carbohydrates	23.0 gr
Fat	0.25 gr

The sensory evaluation of two samples of DO DO spice of 500 g was done at the Faculty of Technology, University of Tuzla. Thirteen testers participated in the sensory evaluation, and the parameters that were examined were the smell, distribution of chopped parts, appearance and colour. The evaluation was done using points from 1 to 5, so that the maximum number of points for the smell was 3,93, and for the other properties was under 3.

Table 2. Results of organoleptic assessment of DO DO spice

Quality property	Importance factor	Grade	Points
Smell	0.8	4.92	3.93
Distribution of crushed parts	0.6	3.62	2.17
Appearance, colour	0.6	4.54	2.72
TOTAL			8.82

RESULTS AND DISCUSSION

The nutritional value of substances in the DO-DO spice is 494 kJ (116 kcal), and due to the presence of vegetable seasoning and the possibility of losing their nutritional properties, it is best to add it at the end of cooking. If the theoretical knowledge and the obtained nutritional value of DO DO spice are compared, it can be noticed that its quantity is negligible when preparing meals. Furthermore, this spice mixture can be used to reduce the salt intake, and at the same time it contributes to enhancement and enrichment of flavours of prepared dishes. Comparing characteristics of salt based spice mixtures by other manufacturers, it can be noticed that DO DO is a product with lower amounts of fat and carbohydrates, and as such is part of a healthy diet. The sensory evaluation of DO DO spice showed a high score of 8.82 points. The maximum scent score of 3.93 points was related to the peculiarity of the raw materials, which are used in the production, proving the high quality of the raw materials, while the appearance and the colour had a score of 2.72 points. The worst results were observed in chopped parts, 2.17 points, owing to the granulometric composition of the salt which is an essential ingredient of this product.

CONCLUSION

DO DO spice adds flavour to food, and gives it a distinctive taste of Bosnian and Herzegovinian traditional cuisine. Nutritional value of spice per 100 g is 116 kcal, therefore it is negligible in one dish, but it makes the difference over a longer period of consuming. The production of mixed spices marks its growth. DO DO spice is a product of natural ingredients with a reduced share of flavour enhancers, and with its quality stands out in the market and is incorporated into the trend of healthy nutrition. The sensory evaluation of DO DO spice showed a high score of 8.82 points. The maximum scent score of 3.93 points was related to the peculiarity of the raw materials, which are used in the production, proving the high quality of the raw materials, while the appearance and the colour had a score of 2.72 points. The worst results were observed in chopped parts, 2.17 points, owing to the granulometric composition of the salt which is an essential ingredient of this product.The evaluation results have shown that the sensory properties of DO DO spice, i.e. smell, distribution of chopped parts, colour and appearance, are at a satisfactory level. The quality of DO DO spice meets health safety and nutritional value of the product taking into account the recipe. The results obtained provide the basis for taking further steps in order to improve the product quality, thereby moving it from a quality product group into a premium product group. The aim of this paper was to confirm and present the quality of DO DO spice by scientific methods.

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