National University of Science and Technology POLITEHNICA Bucharest

DOCTORAL THESIS

APPLICATIONS OF OZONE IN HEALTH AND ENVIRONMENT

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Content

APPLICATIONS OF OZONE IN HEALTH AND ENVIRONMENT
Summary

Introduction

The development of this doctoral thesis was made out of the desire to research and deepen a the action of ozone in the biological system, on the one hand knowing its strong oxidation capacity and potential toxic effect on the human body, but on the other hand having remarkable clinical results in the practice of ozone therapy. In the 180 years since the discovery of ozone, numerous researches have been carried out, discovering its molecular formula, structure, physical and chemical properties, role and applications in various fields. The strong oxidative potential of ozone underlies all its applications and uses, but is also the cause of concern regarding its potential toxicity. These aspects were considered insufficiently studied, hence the skepticism related to the benefit and especially the risks of applying ozone in medicine.

The second direction of research addressed in the thesis is related to the use of ozone in environmental protection, i.e. in the removal of synthetic dyes from industrial or urban wastewater, the increasing industrial use of these compounds leading to their accumulation in different real water systems (sewage network, water of surface etc) – with significant toxic potential on aquatic organisms. In these conditions, the possibility of using ozone has increased. Moreover, in the last decades, most treatment plants use ozone in at least one of the processing stages.

Main objectives - In this context, this doctoral thesis addresses two distinct themes (medicine and environmental protection) of topicality and novelty, both nationally and worldwide, with new research perspectives for maintaining and increasing the quality of life. The main objectives of the original part are:

• Ozone in medicine - Highlighting the efficiency and safety of medical treatment with ozone in serious, potentially disabling pathologies: diabetic neuropathy and trigeminal neuralgia. In order to achieve this objective, two groups of patients treated with medical ozone were studied in the period 2015-2020, presenting these conditions, whose etiopathogenic mechanisms are chronic oxidative stress, chronic inflammation, nociception disorders, etc. Based on the knowledge of the chemical behavior of ozone, its interactions with the biological system, an important objective was to understand / explain the mechanisms of its action in the human body in the researched pathology.

• Ozone in the protection of the environment – Studying the applicability of the ozonation process to the discoloration of some dyes refractory to conventional treatment methods. Brilliant Blue (BB), a synthetic dye commonly used in the pharmaceutical, food, textile, cosmetic, and even medicine industries, was selected.

The study of influencing factors was carried out: pH, initial concentration of dye and applied ozone, contact time, molar ratio [O3:BB], stirring time, in order to establish the optimal decolorization parameters to obtain a non-hazardous effluent. The removal of color by ozonation does not lead to a complete mineralization of the dye, and the intermediate products formed can have a greater toxicity than the initial dye, which led to the establishment of two other objectives:

• Identification of the intermediate oxidation products, obtained during the degradation of BB by ozonation, under the given conditions, by 1H – NMR spectroscopy;

• Comparative study of the ecotoxicity of BB dye solutions before and after ozonation by following the effects on some aquatic organisms (planktonic crustaceans, green micro-algae and freshwater fish);

• The process dynamics approach - the verification of the classical kinetic models, of order 1 and 2, by the integral method, and of those of pseudo order 1 and 2 did not lead to

interpretable results. Not knowing the stoichiometry of the reactions during ozonation and the dependence on the previously mentioned factors, but corroborating the information provided by the mass spectrum analysis of the dye, the study of the toxicity of the products and the sequence of possible reactions and the multiple possibilities of splitting the dye, a complex kinetic model for O3 decolorization of BB solutions.
The results obtained in the conducted research constitute the scientific basis for evaluating the technical, economic and environmental feasibility, the advantages and disadvantages of using ozone in the removal of this dye.
The parallel study of the chemical behavior of ozone in aqueous solutions and its action in biological systems represents the original contribution. Important contributions were also made in the research topics addressed by obtaining new information on the ecotoxicity of the BB dye and a first attempt to make a modeling of the BB discoloration process by ozonation taking into account both mass transfer and the system of successive, chain reactions.
Through the assumed objectives, the doctoral thesis is part of the current issues of the two areas addressed:
1. Ozone applied in medicine as a safe and effective complementary treatment in multiple pathologies - it has experienced significant development in recent years, but requires a scientific explanation of the mechanisms of action, benefits and application limits, considering its toxicity.
2. Ozone as an oxidant applied in the protection of the environment, respectively in the removal of some chemical and biological pollutants (dyes, additives, drugs, microorganisms) - allows the expansion of its area of applicability, provided that the aspects of efficiency and limitation of the processes are highlighted.
This doctoral thesis was structured in two parts and includes seven representative chapters for the topics covered: Part I - Bibliographic study (3 chapters) and Part II - Own contributions (4 chapters). The thesis has a total number of 169 pages written in 1.15 lines, of which 53 pages are Part I and 116 pages are Part II. The thesis includes 75 tables, 61 figures/diagrams. At the end of the paper, 308 bibliographic references (of which 183 from 2014-2024) are presented in the order of their citation in the text, as well as the works published during the development of the thesis.
The first part of the thesis (chapters 1-3) presents literature data regarding the current state of knowledge in the field of ozone applications in health and the environment. The bibliographic study was focused on information from the literature related to the chemistry of ozone, to its action in the biological system and in the environment. Aspects regarding the correlation between the structure, properties and applications of ozone in medicine and environmental protection were considered.
Chapter 1, entitled "Aspects of ozone chemistry", includes notions related to the structure of the ozone molecule, the chemical and physical properties of ozone, especially the behavior of ozone in aqueous solutions, as well as studies on ozone toxicity.
Chapter 2, entitled "Ozone Applications in Health", includes recent information on redox homeostasis, as well as characteristic aspects of the action of ozone in the biological system, modern concepts of current ozone therapy and clinical applications of medical ozone.
Chapter 3 entitled "Applications of ozone in the environment" is dedicated to the applications of ozone in environmental protection and industry - through direct and indirect reactions (due to the ability of O_3 to induce radical chain reactions).
The second part of the thesis (chapters 4-6) presents own contributions to applications of ozone in medicine and environmental protection. **Chapter 4,** entitled "Applications of ozone therapy in diabetes mellitus complicated with diabetic neuropathy" presents the development and results of a medical study in which the action of medical ozone in diabetic neuropathy - a serious, potentially disabling pathology - is highlighted. **Chapter 5** entitled "Ozone therapy

applications in trigeminal neuralgia. Neurophysiopathological mechanisms and results" presents the stages and results of the research on the effect of ozone therapy (O3T) in the treatment of a debilitating pain condition - trigeminal neuralgia - difficult to treat, whose etiopathogenic mechanisms are still partially known. **Chapter 6** entitled "Research on the discoloration by ozonation of aqueous media" is dedicated to some studies on the applicability of the ozonation process to the discoloration of Brilliant Blue dye (BB FCF), refractory to conventional treatment methods. The optimal parameters for decolorization by ozonation (with an emphasis on the influence of pH), the kinetics of the reactions underlying the decolorization of BB FCF, the identification of oxidation products by ozonation, as well as the study of the ecotoxicity of pre- and post-ozonation BB FCF solutions were investigated. **Chapter 7** includes the general conclusions of the work and the possibilities for further development along with own contributions. The thesis ends with a Bibliography chapter containing 308 bibliographic references and the annex of published works.
The present summary includes in a concise form the content of chapters 4-6 of own contributions. The numbering of chapters, subchapters and tables/figures corresponds to that in the thesis.

Chapter 4
Applications of ozone therapy in diabetes mellitus complicated with diabetic neuropathy

The specific objective of this study was to evaluate and quantify the effect of O3T, applied complementary to some patients (N=73) suffering from diabetes mellitus (DM) complicated with diabetic neuropathy (ND), this pathology constituting a serious public health problem, through the alarming increase in the number of cases, the development of chronic complications and the aggravation of co-morbidities (cardiovascular, etc.).
Patient selection (inclusion/exclusion), clinical and biological evaluation, monitoring of the evolution under treatment were performed according to strict criteria, in accordance with international regulations in the field [103,106,191,196,197,200-203]. The patients performed biological investigations specific to DZ, ND [203]. For the clinical evaluation, the following were used: the ND screening and the neuropathic disability score (after the Michigan test) [201,203], the Dyck scale to assess the ND stage pre- and post-O3T, the numerical pain evaluation scale (NPES) to quantify pain but and assessing the impact of neuropathic pain on patients' functionality and mental/emotional state (QoL parameters) [204]. Evaluation of sensitivity (epicritic-protopathic, thermal, nociceptive, proprioceptive-vibratory) was performed using semi-quantitative tests (sQST) [205-207].
Treatment administration - Patients were treated with medical ozone in addition to conventional treatment of the underlying disease / co-morbidities. Ozone was obtained using an Ozonosan Alpha Plus Photonik Boardcase generator (using high purity oxygen) specific to the general and topical/local application modalities of O3T. The concentrations used varied between 5 and 35 mcg O3/100 mL mixture, depending on the route of administration. The doses/concentrations of ozone applied followed the "start low, go slow" rule - with the intention of balancing the oxidative status and inducing a tolerance to ozone [81,92,272]. Ozone was produced and used only at the time of application of the treatment, with strict compliance with the rules regarding the safety of the patient, the user and the environment [29]. Consumable, sterile, single-use devices and materials were used, most of which were made of ozone-resistant materials (glass, silicone, polypropylene, stainless steel, etc.).
Results of DM+ND patients treated with medical O3 – after 5 weeks of O3T, significant improvement was observed in most of the clinical and biological criteria followed.

Table 4.4: Evolution of some clinical and biological parameters in DM and ND patients after O3T

Evolution			Difference* (in %)	p Value (*t*-Student test)
Parameter	**Average of numerical variables ***			
	Before O_3T*	**After O_3T***		
BMI (Kg/cm^2)	29,44	27,60	6,24	0,004
Fasting Blood Glucose (mg/dL)	174	153	12,07	0,069
HbA1c (%)	8,12	7,24	10,78	0,081
Total Cholesterol (mg/dL)	262,37	240,31	8,40	0,053
TGL (mg/dL)	188,51	174,82	9,38	0,041
Creatinine (mg/dL)	1,35	1,15	14,81	0,063
Uric Acid uric (mg/dL)	7,17	6,54	8,76	0,038
CRP (mg/dL)	6,06	4,95	12,16	0,057
Fibrinogen (mg/dL)	407,28	369,76	9,21	0,092
GGT (U/L)	42,45	39,12	7,84	0,028

Note: *Numerical variables with Gaussian distribution

Table 4.5: Evolution of neuropathic pain and quality of life (QoL) parameters in DM and ND patients after O3T

Parameter	Average of numerical variables *		Difference* (in %)	P Value
	Before O_3T	**After O_3T**		
Neuropathic pain (NPES scale)	2,16	1,06	50,63	0,028
Paresthesia	2,05	0,97	52,67	0,014
General condition altered	3	1.39	53,42	0,050
Motor/Mobility disorders	2,59	1,46	43,49	0,009
Decreased functionality-autonomy/self-care	1,03	0,38	62,65	0,027
Decreased functionality - household/daily	1,97	1,07	45,84	0,016
Decreased social functioning	2,52	1,28	48,91	0,031
Insomnia	1,93	1,04	46,10	0,042
Anxiety	2,16	1,12	48,10	0,038
Despondency, depressive disorder	2,14	1,01	52,55	0,017

Note: *Numerical variables with Gaussian distribution

Table 4.6: Evolution of sensitivity disorders in DM and ND patients after O3T

Parameter	Average of numerical variables*		Difference* (in %)	P value (*t*-Student test)
	Before O_3T	**After O_3T**		
Hypoesthesia	0,93	0,60	35,18	0,038
Vibration sensitivity disorder	0,86	0,52	39,69	0,004
Nociceptive sensitivity disorder	0,55	0,28	47,51	0,039
Thermal sensitivity disorder	0,35	0,16	6,83	0,085

Statistical data processing - The analysis of influencing factors took into account different variables: patient age, diabetes mellitus (DM), body mass index (BMI), blood glucose values, HbA1c, TGL, total cholesterol, CRP, fibrinogen, GGT , but also the general condition, motor impairment, functional impairment, the presence of sensitivity disorders, etc. The database

contained 73 records (N=73), each with 26 evaluation parameters, estimated before and after O_3T (Appendix 6). Analysis of variance was performed using the ANOVA test.
The statistical processing - through the method of multiple regressions - allowed complex correlations between biological and clinical parameters, between advanced age and the prolonged evolution of the disease, etc. Figs 4.2 and 4.3.

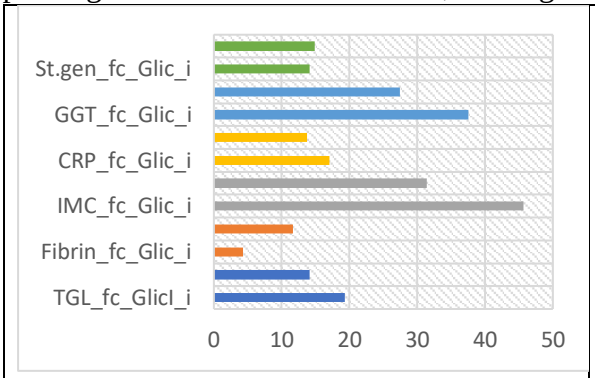


Figura 4.2: Analysis of the influence of Glucose (independent factor) on TGL, Fibrinogen, BMI, CRP, GGT, General condition (dependent factors) - before (i) and after treatment (f)

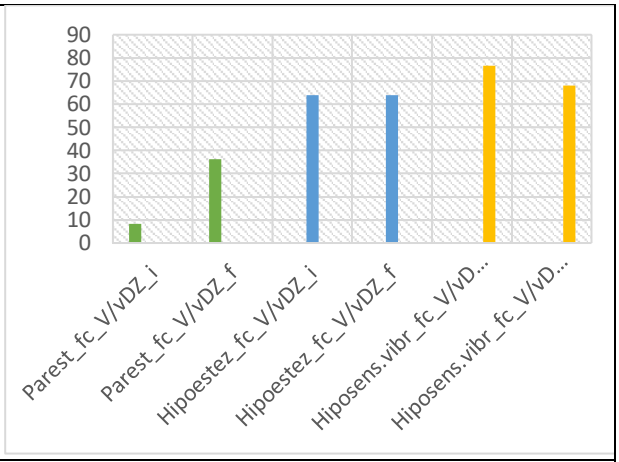


Figura 4.3: Analysis of the influence of the patients' Age + DM Age (independent factors) on paresthesias, hypoaesthesia and decreased vibratory sensitivity (dependent factors) - before (i) and after O3T (f) - by the method of multiple regressions

Conclusion:

• The direct link between the application of medical ozone and the complex improvement (biochemical, metabolic, neuropathic pain and its impact on the quality of life) of the patients selected in the study was found;

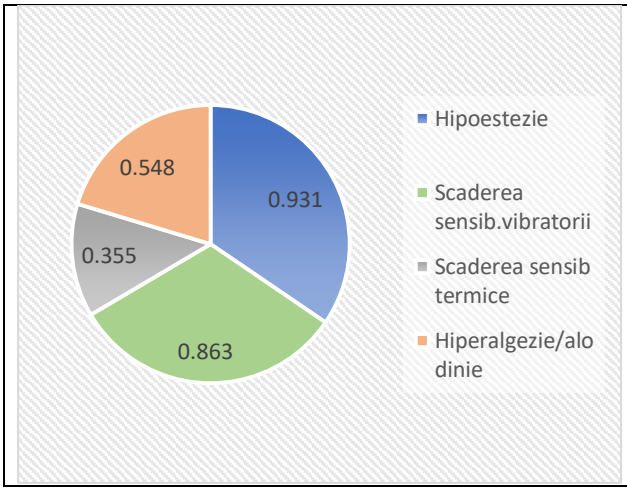
• It was possible to decrease the therapeutic need - in agreement with the attending physician - through the synergistic effects of O3 and the basic treatment and by improving the more active metabolism of the pathogenic +/- symptomatic treatment, under the influence of O3T (detoxifying effect);

• Reducing the morbidity caused by this pathology, by preventing the progression to diabetic foot and amputations, etc.; positive impact on cardiovascular, renal, ophthalmological complications and the aging process;

• O3T applied correctly - accurate diagnostics, low doses/concentrations, standardized and safe procedures - complementary to the specific treatment, respecting the correct diet and healthy lifestyle, acted beneficially on the target pathology, improving the general condition, correcting biological imbalances, stimulating the healing-scarring process, practically without adverse effects.

Elements of originality of the study:

- The complex way of evaluating patients before and after O3T;
- The association of medical ozone application types: topical, local and loco-regional applications and CDL, combined with general applications - which seems to be the most effective therapeutic strategy, acting synergistically and cumulating the benefits of the local and general effects of ozone;



-The description of the sensory phenotypes in the study patients (Fig 4.4) – allows specifying the anatomical substrate of the neuropathic pain and explaining the variability of the treatment response.

Figure 4.4: Sensory typology of study patients

Limitations of the study:
- Absence of a control lot;
-Insufficiency of assessment of oxidative stress and antioxidant defense.
Research perspectives - Organization and conduct of national/international multicenter studies with unitary criteria and procedures, as well as randomized controlled trials in this pathology.

Chapter 5
Applications of ozone therapy in trigeminal neuralgia. Neurophysiopathological mechanisms and outcomes

The specific objective of the study on the application of O3T in trigeminal neuralgia was to evaluate and quantify the effect of ozone therapy (O3T) on a group of patients suffering from trigeminal neuralgia (NT), a debilitating painful condition that is very difficult to treat. Thirty-three patients of both sexes, aged between 28-83 years, presented at the Medical Center Dr. Tiron between 2015 and 2020 with primary TN. The study was approved by the Ethics Committee of the Medical Center.
Diagnosis and study inclusion were based on the ICHD-3 criteria for TN [235].
The evaluation of the patients – was carried out by anamnesis, general and local examination, evaluation of sensitivity disorders – similarly to the previous study.
Application and monitoring of treatment - patients were fully informed about the purpose/procedure of treatment and all signed an informed consent. Patients were given 12-15 treatment sessions with a frequency of 2-3 sessions/week.
Patients were considered responders based on improvement/disappearance of pain, overall satisfaction, resumption of usual activities, etc. Quantitative and qualitative changes in pain and associated symptoms/signs were considered.
The obtained results showed that, following the treatment, there were significant improvements ($P<0.05$) regarding: pain relief (decrease in pain intensity/frequency of painful paroxysms - PD, attenuation/disappearance of the painful background - FD); decrease in medication consumption; improving the general condition, eating disorders, sleep disorders, mental/emotional condition; resumption of usual activities (domestic, professional, social); improvement/disappearance of sensitivity disorders - there was a statistically significant decrease in hyperesthesia, mechanical and thermal hyperalgesia post-treatment, and the differences were significant.
Through the statistical processing carried out, the study allowed correlations between old age and the prolonged evolution of the disease, affecting the quality of life, the presence of anxiety

and depression, the presence of insomnia, etc. – Fig 5.17. However, older age was not significantly correlated with pain intensity.

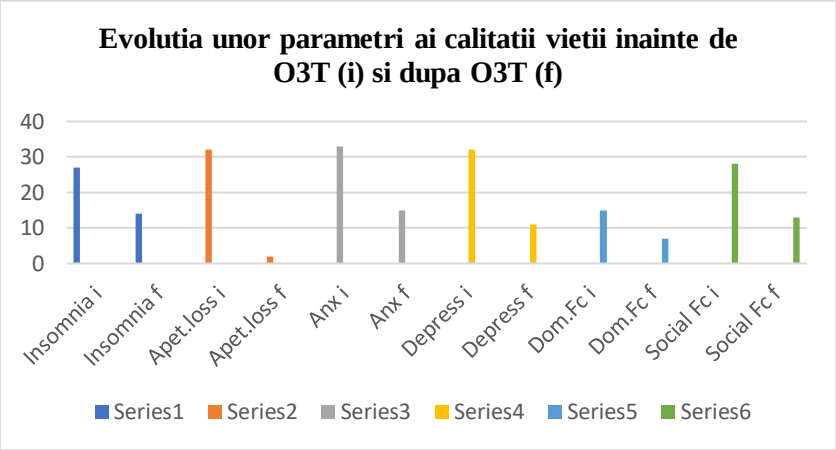


Figure 5.17: The influence of O3T on the evolution of some parameters of the quality of life (QoL)

The obtained results - processed by the Wilcoxon Signed Ranks Test method - are presented in Table 5.4.

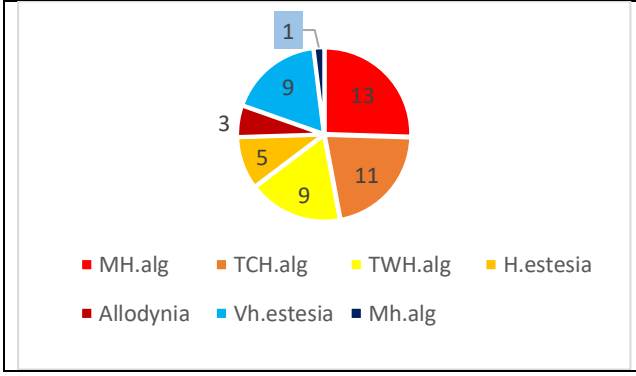
Table no. 5.4: Results obtained and statistical analysis

	Variabila	Initial	Final	Valoarea P
1	**Parox.D (NPRS=0-10)**	9.00[8.00, 10.00]	2.00 [1.00, 3.00]	0.000001
2	**Frecv Parox.D**	3.00 [2.00, 5.00]	13.00 [9.00, 17.00]	0.000000
3	**FD (NPRS=0-10)**	7.00 [6.00, 8.00]	2.00 [1.00, 3.00]	0.007110
4	**Consum de analgezice/ (0-4)**	3.00 [2.00, 3.00]	1.00 [1.00, 1.00]	0.000003
5	**Insomnie (0-4)**	2.00 [1.00, 2.50]	0.00 [0.00, 1.00]	0.000021
6	**Anxietate (0-4)**	3.00 [2.00, 3.00]	1.00 [0.00, 1.00]	0.000001
7	**Descurajare (0-4)**	2.00 [1.00, 3.00]	0.00 [0.00, 1.00]	0.000001
8	**Viz.catastrof.**	0.00 [0.00, **1.50**]	0.00 [0.00, **0.50**]	0.002700
9	**Scadere a apetitului alimentar**	1.00 [1.00, 2.00]	0.00 [0.00, 0.00]	0.000001
10	**Funcționalitate - (autoîngrijire/casnică)**	1.00 [0.00, 1.00]	0.00 [0.00, 0.00]	0.000162
11	**Funcționalitate - (aprovizionare)**	1.00 [0.00, 2.00]	0.00 [0.00, 1.00]	0.000011
12	**Funcționalitate - (activitate profesională)**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.002551
13	**Funcționalitate - (activitate socială)**	2.00 [1.00, 2.00]	0.00 [0.00, 1.00]	0.000003
14	**Hiperestezie (1 - 5)**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.038434
15	**Hipoalgezie mecanică (-1) – (-5)**	0.00 [0.00,**1.00**]	0.00 [0.00, **0.00**]	0.317311
16	**Hiperalgezie mecanică (+1) – (+5)**	0.00 [0.00, **2.00**]	0.00 [0.00, **1.00**]	0.000311
17	**Hipoalgezie la cald (-1) – (-5)**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.157299
18	**Hiperalgezie la cald**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.002700

	(+1) – (+5)			
19	**Hiperalgezie la rece (1 - 5)**	0.00 [0.00, **2.00**]	0.00 [0.00, **0.50**]	0.001341
20	**Alodinie (0 - 5)**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.083265
21	**Hipoestezie vibratorie (-1) – (-5)**	0.00 [0.00, **1.00**]	0.00 [0.00, **0.00**]	0.002700

The conclusions of the study regarding the application of O3T in trigeminal neuralgia:
• The research provides evidence for the application of O3T as an effective pain modulation tool, through the causal link between the multimodal actions of medical O3 and the neuropathogenic processes at the origin of NT;
• The drug treatment previously used by the patients was without results or difficult to tolerate due to the existence of adverse reactions. O3T increased the tolerability of neurotropic treatment, through the synergy of effects and through its more efficient metabolism, making it possible to lower the necessary doses and even to abandon this treatment [81];
• Medical O3 acted regardless of the age of the disease - recent or long-standing - and despite unfavorable factors (age of patients, comorbidities, etc.), and its effects persisted over time.

Elements of originality:



1. The use of simple, easily reproducible tests "at the patient's bedside" (sQST) for sensitivity disorders, allowing the outline of a characteristic sensory phenotype of selected patients, useful for specifying the anatomical substrate and predicting the response to treatment (Figure 5.18). This paper appears to be the first study on O3T applied in TN using these tests;

Figure 5.18: Sensory disturbances in the NT study

2. The particular application of ozone - through subcutaneous injections performed not only in the painful area, but also symmetrically in other cephalic and cervico-dorso-lumbar points; these loco-regional applications acted on: local oxidative homeostasis, inflammatory and hypoxic substrate, peripheral sensitization, nociceptive modulation, nerve tissue regeneration;
3. A comparative presentation of the etiopathogenic mechanisms involved in NT and the multifactorial effect of medical ozone was presented in Table 5.22.
Limitations of the study - small single-center comparative study, with selected patients, (so not representative of the general population), no control group, no single/double-blind design; longer follow-up was not systematic. Spontaneous remission of NT may occur, in this situation it must be clarified whether the patient is in remission or has benefited from therapy.
-Assessment of the patient's pain / condition, the effectiveness of treatment based on questionnaires and the assessment of sensitivity with the help of sQST tests can be influenced by subjective factors that are potential sources of error (bias);
Research perspectives - Required:
- Multicenter studies with strict diagnostic and selection criteria and unitary procedures, randomized, controlled studies with large patient sample sizes, possibly with single-double-blind application and with longer post-study follow-up periods;
- Application of valid, reliable and scalable sensory profiling methods;
- Deepening the studies on the medical O3 interactome and in other relevant pathologies.

Chapter 6
Research on decolorization by ozonation of aqueous media

The increase in the use of food additives (including synthetic dyes) has led to their presence in wastewater, with an impact on the quality of surface/deep water and related ecosystems, some dyes being refractory to conventional bleaching methods [145,147,148]. Thus, the in-depth study of the ozone bleaching process was considered appropriate, for this purpose the Brilliant Blue FCF (BB FCF) dye used in the food industry, pharmaceuticals, even in medicine, was selected. The information on the discoloration of BB FCF by ozonation is in small numbers, incomplete, sometimes contradictory, these being the reasons for the orientation of the experimental research, within the thesis, towards the following objectives:

• the study of the optimal parameters for decolorization by ozonation - without pH change
• the study of the influence of pH on the discoloration process;
• the kinetic study of the reactions underlying the discoloration of BB FCF;
• identification of oxidation products by ozonation;
• the study of the ecotoxicity of BB FCF solutions pre- and post-ozonation on some invertebrate organisms and fish specific to the fresh waters of the country.

6.3.2 Study of optimal parameters for decolorization by ozonation, without pH change - followed the yield of decolorization by ozonation of some dye solutions of different concentrations, subjected to ozonation with different concentrations of ozone, at different values of the contact time, with or without mechanical agitation.

Ozonation decolorization without pH change of BB FCF dye solution – confirmed that decolorization efficiency (%R) is closely related to initial dye concentration (CiBB) and applied ozone concentration. The experimental data (Table 6.2) led to the following observations:

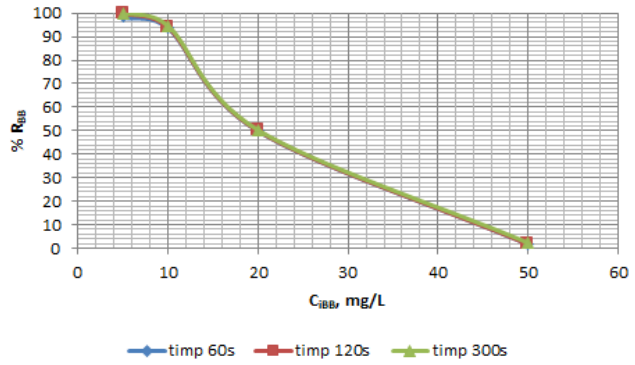
- the efficiency of the bleaching process (%R) increases with the ozone concentration applied at each of the dye concentration values and at the same contact time value (300 s);
- at low dye concentrations (5 mg/L), the %R was significant (99.2 – 99.9%) at all applied O3 concentrations, but the decolorization efficiency decreases at higher concentrations of BB FCF, requiring higher concentrations greater than O3;
- the mechanical factor (stirring) has a favorable influence on the ozonation and implicitly on the bleaching efficiency;
- It can be stated that an increase greater than 200 mg O3 /L has no economic justification considering the fact that the yield increased satisfactorily until the application of this concentration, this argument also being valid for higher dye concentrations (20 - 50 mg /L) ;
- The research results also emphasized the influence of ozone purity on the oxidation process, ozone being obtained from 99.5% pure oxygen.

Table 6.2. Decolorization efficiency by ozonation of studied aqueous systems

C_{iBB} (mg / L)	Fără agitare		Cu agitare	
	C_{fBB} (mg / L)	%R	C_{fBB} (mg / L)	%R
Ozonizare cu 100 mg O_3 /L				
5	0,08	98,4	0,04	99,2
10	4,18	58,2	1,46	85,4
20	12,20	39,0	6,32	68,4
50	49,2	1,6	46,00	8,0
Ozonizare cu 150 mg O_3 /L				
5	0,06	98,8	0,03	99,4
10	3,30	67,0	0,66	93,4
20	10,00	50,0	5,10	74,3
50	49,00	2,0	45,50	9,0
Ozonizare cu 200 mg O_3 /L				

5	0,01	99,8	0,01	99,8
10	0,58	94,2	0,12	98,8
20	10,00	50,0	2,88	85,6
50	48,90	2,2	45,50	9,0
Ozonizare cu 250 mg O_3 /L				
5	0,007	99,9	0,007	99,9
10	0,01	99,9	0,01	99,9
20	9,40	53,0	1,96	90,2
50	48,60	2,8	44,5	11,0

Contact time - In the time interval 0 - 300 s a bleaching efficiency (%R = 98.8) is obtained for the dye solution (10 mg/L) under the action of an ozone dose (200 mg O3/L), when the graph presents a plateau aspect... the positive influence of agitation, which, in the concentration range (Ci BB = 5 - 10 mg / L), at the contact time of 300 s, a increased bleaching efficiency (% R > 90). The experiments were carried out with the same BB FCF solutions of different concentrations (5, 10, 20 and 50 mg / L), which were ozonized, in turn, with doses of 200 mg O3 / L a. g., at different contact times (60, 120 and 300 s). The obtained results are presented in Fig. 6.6.a and 6.6.b...at increasing ozone doses, the contact time required for discoloration shortens.



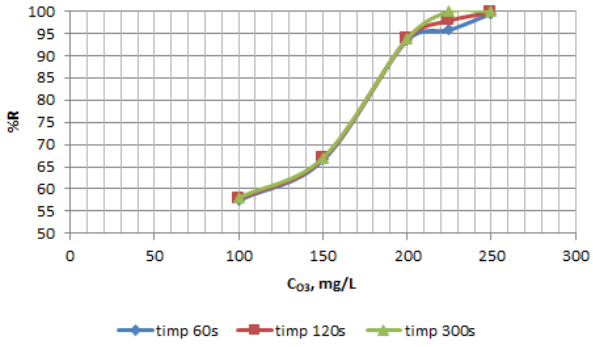
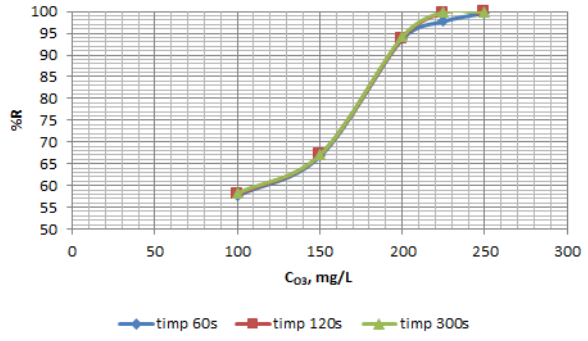


Fig. 6.6.a: % R = f(Ci BB) without stirring, Ci O_3 = 200 mg O3 / L [145]	Fig. 6.6.b: % R = f(Ci BB) with stirring, Ci O_3 = 200 mg O3 / L [145]

The next step was to optimize the ozone concentration used to decolorize the BB FCF. To carry out this stage, we continued to work with the solution Ci BB = 10 mg / L, which reacted well to ozonation, with different concentrations of ozone (100, 150, 200 and 250 mg O3 / L a. g.), at a time contact time of 300 s (Fig. 6.7.a and 6.7.b).



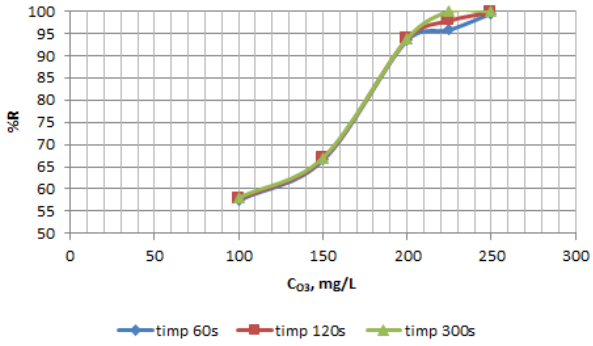


Fig.6.7.a: $\%R_{BB} = f(C_{i\,O3})$, without stirring, $C_{i\,BB}$ = 10 mg/L [145]

Fig. 6.7.b: $\%R_{BB} = f(C_{i\,O3})$ with stirring, $C_{i\,BB}$ = 10 mg/L [145]

Preliminary conclusion - The bleaching efficiency was maximum in the case of low dye concentrations (10 mg/L), to which high concentrations of ozone (200 mg O3 / L a. g.) were applied, under conditions of mechanical agitation (200 rot / min), at a pH value = 7.03.

6.4 The influence of pH on the discoloration process

This research is of particular interest because the specialized literature shows that in aqueous solutions the BB FCF molecule is found in acid-base forms in an equilibrium state depending on the acidity or alkalinity of the medium [277,292]; this is how the particular mechanism of ozonation discolouration can be explained, oxidation taking place at predominantly acidic pH through a direct, selective mechanism, attributed to molecular ozone (most authors attribute it to oxygen giving birth), and at alkaline pH through an indirect, non-selective mechanism, due to hydroxyl radicals [291,292].

6.4.1. Discoloration at acidic pH

The bleaching efficiency was studied for different concentrations of BB FCF (2.5, 5.0, 7.5 and 10.0 mg/L) at the lowest studied ozone concentration of 100 mg O3/L a. g.), pH = 4.00, for 90 s, in the two variants: without stirring and with stirring at 200 rpm.

From the experimental data, a maximum decolorization yield (%R >99.9) can be observed at the concentration of CiBB = 2.5 mg / L, both in the variant with stirring and in the one without stirring, at a contact time of 90 s. Thus, the values determined experimentally, at low concentrations of BB FCF, are in good agreement with those mentioned in the literature.Table 6.4 shows the values obtained under the mentioned conditions.

Table 6.4. Decolorization efficiency by ozonation of aqueous systems studied at pH = 4 and different contact times

$\tau_{contact}$ (s)	Fără agitare		Cu agitare	
	C_{fBB} (mg / L)	%R	C_{fBB} (mg / L)	%R
C_{iBB} (2,5 mg / L)				
15	0,52	79,2	0,14	94,4
30	0,30	88,0	0,09	96,4
45	0,22	91,2	0,06	97,6
60	0,15	94,0	0,03	98,8
75	0,02	99,2	ND	>99,9
90	ND	>99,9	ND	>99,9
C_{iBB} (5,0 mg / L)				
15	1,09	78,2	0,32	93,6
30	0,74	85,2	0,23	95,4
45	0,48	90,4	0,13	97,4
60	0,30	94,0	0,06	98,8
75	0,05	99,0	0,01	99,8
90	0,01	99,8	0,01	99,8
C_{iBB} (7,5 mg / L)				
15	1,78	76,3	0,94	81,2
30	1,25	83,3	0,62	91,7
45	1,06	85,9	0,35	95,0
60	0,68	91,0	0,12	98,4
75	0,44	94,1	0,07	99,1
90	0,18	97,6	0,03	99,6
C_{iBB} (10,0 mg / L)				
15	2,64	73,6	1,92	80,8
30	2,25	77,5	1,12	88,88
45	1,98	80,2	0,77	92,3
60	1,57	84,3	0,34	96,6
75	1,28	87,2	0,12	98,8
90	1,08	89,2	0,07	99,3

6.4.2 Decolorization by ozonation at neutral pH

The bleaching efficiency was studied for different concentrations of BB FCF (2.5, 5.0 , 7.5 and 10.0 mg/L) at the ozone concentration of 100 mg O3/L a. g.), pH = 7.00, for 900 s, in the two variants: without stirring and with stirring at 200 rpm. In Table 6.5. the values obtained under these conditions are presented.

Table 6.5. Decolorization efficiency by ozonation of aqueous systems studied at pH = 7 and different contact times

$\tau_{contact}$ (s)	Fără agitare		Cu agitare	
	C_{fBB} (mg / L)	%R	C_{fBB} (mg / L)	%R
C_{iBB} (2,5 mg / L)				
15	1,27	49,2	0,34	86,4
30	0,95	62,0	0,20	92,0
45	0,82	67,2	0,12	95,2
60	0,72	71,2	0,09	96,4
75	0,55	78,0	0,08	96,8
90	0,34	86,4	0,07	97,2
105	0,15	94,0	0,06	97,6
120	0,06	97,6	0,03	98,8
300	0,04	98,4	0,02	99,2
600	0,02	99,2	0,01	99,6
900	0,01	99,6	ND	>99,9
C_{iBB} (5,0 mg / L)				
15	2,32	53,6	0,68	86,4
30	1,83	63,4	0,38	92,4
45	1,51	69,8	0,22	95,6
60	1,23	75,4	0,16	96,8
75	1,06	78,8	0,12	97,6
90	0,62	87,6	0,10	98,0
105	0,33	93,4	0,09	98,2
120	0,12	97,6	0,06	98,8
300	0,08	98,4	0,04	99,2
600	0,04	99,2	0,02	99,6
900	0,02	99,6	0,01	99,8
C_{iBB} (7,5 mg / L)				
15	3,24	56,8	2,54	66,1
30	3,17	57,7	2,44	67,5
45	3,05	59,3	2,12	71,7
60	2,88	61,6	1,76	76,4
75	2,67	64,4	1,45	80,7
90	2,34	68,7	1,17	84,4
105	2,11	71,9	0,88	88,3
120	1,83	75,6	0,57	92,4
300	1,53	79,6	0,27	96,4
600	1,04	86,1	0,16	97,9
900	0,76	90,0	0,08	98,9
C_{iBB} (10,0 mg / L)				
15	4,24	57,6	3,22	67,8
30	4,23	57,7	3,12	68,8
45	4,23	57,7	3,04	69,6
60	4,22	57,8	2,76	72,4
75	4,22	57,8	2,52	74,8

90	4,21	57,9	2,38	76,2
105	4,21	57,9	2,14	78,6
120	4,20	58,0	1,72	82,8
300	4,18	58,2	1,46	85,4
600	4,18	58,2	1,14	88,6
900	4,18	58,2	0,98	90,2

From the experimental data, a maximum decolorization yield (%R >90.0) is observed at CiBB concentration = 2.5 mg / L and 5.0 mg / L, both in the variant with stirring and in the one without stirring, at a contact time of 105 s (higher than in the version with acidic pH). At concentrations higher than 5 mg/L BB FCF (CiBB = 7.5 mg/L and 10.0 mg/L) maximum bleaching yields (%R >90.0) are observed only at a contact time of 900 s and only in the version with stirring. In the specialized literature, no studies were found at this pH, being part of the originality of the thesis.

6.4.3. Decolorization by ozonation at alkaline pH

In Table 6.6. the bleaching efficiency values for different concentrations of BB FCF (2.5, 5.0, 7.5 and 10.0 mg/L) are presented at the lowest ozone concentration studied, of 100 mg O3/L a. g.), pH = 10.00, for 60 s in the two variants: without stirring and with stirring at 200 rpm.

Table 6.6. Decolorization efficiency by ozonation of aqueous systems studied at pH = 10 and different contact times

	Without stirring		**With stirring**	
$\tau_{contact}$ **(s)**	C_{fBB} **(mg / L)**	**%R**	C_{fBB} **(mg / L)**	**%R**
C_{iBB} (2,5 mg / L)				
15	0,42	83,2	0,03	98,8
30	0,28	88,8	0,02	99,2
45	0,09	96,4	0,01	99,6
60	ND	>99,9	ND	>99,9
C_{iBB} (5,0 mg / L)				
15	0,90	82,0	0,08	98,4
30	0,64	87,2	0,04	99,2
45	0,18	96,4	0,02	99,6
60	0,09	98,2	0,01	99,8
C_{iBB} (7,5 mg / L)				
15	1,38	81,6	0,78	89,6
30	0,83	89,0	0,33	95,6
45	0,38	95,0	0,10	98,7
60	0,25	96,7	0,06	99,2
C_{iBB} (10,0 mg / L)				
15	2,08	79,2	1,42	85,8
30	1,63	83,7	0,64	93,6
45	0,95	90,5	0,25	97,5
60	0,43	95,7	0,08	99,2

At pH=10, maximum yields (%R >99.9) are found at CiBB concentration=2.5 mg/L, in both variants, but at a contact time much shorter than 60 s. And these experimentally determined values are in good agreement with those mentioned in the literature. pH values of 4.0; 7.0 and 10.0 favored BB FCF decolorization. This was due to the predominant effects of molecular ozone, at acidic pH, and the effects of hydroxyl radicals, at alkaline pH. At neutral pH, a balanced effect of molecular ozone and hydroxyl radicals is assumed, but discoloration requires a longer contact time (up to 900 s).

6.4.4 Validation of parameters (Reproducibility of discoloration)

The reproducibility of the discoloration was verified for each system studied (Table 6.7).

Table 6.7. Reproducibility of BB FCF decolorization by ozonation

Nr. Sample	Characteristics Discolored sample (ozonized)	C_{fBB}, mg / L		Statistical Probability	
		With stirring	Without stirring	Ozonized sample with stirring	Ozonized sample without stirring
1	C_{iBB} = 5 mg / L $V_{probă}$ = 100 cm^3 pH ≅ 4,0 $\tau_{contact}$ = 90 s	0,01	0,02	$\overline{X}$ =0,0128 S=0,0002 $S_{\overline{X}}$ =0,0049	$\overline{X}$ =0,0180 S=0,0005 $S_{\overline{X}}$ =0,0049
2		0,02	0,03		
3		0,009	0,02		
4		0,01	0,01		
5		0,02	0,01	P=95%, t $S_{\overline{X}}$ = 0,0111 C_{fBB}=0,0128 ± 0,0111	P=95%, t $S_{\overline{X}}$ =0,0111 C_{fBB}=0,0180 ± 0,0111
6		0,01	0,02		
7		0,01	0,01		
8		0,009	0,03	P=99%, t $S_{\overline{X}}$ = 0,0158 C_{fBB}=0,0128 ± 0,0158	P=99%, t $S_{\overline{X}}$ =0,0158 C_{fBB}=0,0180 ± 0,0158
9		0,01	0,02		
10		0,02	0,01		
1	C_{iBB} = 5 mg / L $V_{probă}$ = 100 cm^3 pH ≅ 7,0 $\tau_{contact}$ = 900 s	0,04	0,08	$\overline{X}$ =0,0340 S=0,0012 $S_{\overline{X}}$ =0,0051	$\overline{X}$ =0,0800 S=0,0004 $S_{\overline{X}}$ =0,0052
2		0,03	0,07		
3		0,03	0,08		
4		0,05	0,09		
5		0,02	0,08	P=95%, t $S_{\overline{X}}$ = 0,0115 C_{fBB}=0,0340 ± 0,0115	P=95%, t $S_{\overline{X}}$ = 0,0115 C_{fBB}=0,0800 ± 0,0115
6		0,02	0,08		
7		0,03	0,07		
8		0,04	0,08	P=99%, t $S_{\overline{X}}$ = 0,0164 C_{fBB}=0,0340 ± 0,0164	P=99%, t $S_{\overline{X}}$ = 0,0164 C_{fBB}=0,0800 ± 0,0164
9		0,04	0,09		
10		0,05	0,08		
1	C_{iBB} = 5 mg / L $V_{probă}$ = 100 cm^3 pH ≅ 10,0 $\tau_{contact}$ = 60 s	0,01	0,09	$\overline{X}$ =0,0118 S=0,0002 $S_{\overline{X}}$ =0,0052	$\overline{X}$ =0,0820 S=0,0006 $S_{\overline{X}}$ =0,0052
2		0,009	0,08		
3		0,02	0,07		
4		0,01	0,09		
5		0,01	0,08	P=95%, t $S_{\overline{X}}$ = 0,0115 C_{fBB}=0,0118 ± 0,0115	P=95%, t $S_{\overline{X}}$ = 0,0115 C_{fBB}=0,0820 ± 0,0115
6		0,01	0,08		
7		0,009	0,09		
8		0,01	0,07	P=99%, t $S_{\overline{X}}$ = 0,0164 C_{fBB}=0,0118 ± 0,0164	P=99%, t $S_{\overline{X}}$ = 0,0164 C_{fBB}=0,0820 ± 0,0164
9		0,02	0,08		
10		0,01	0,09		

6.5. Aspects of the kinetics of the bleaching process of BB FCF with ozone

In the experimental research to estimate the ozonation kinetics, two work variants were chosen, at room temperature, for drawing the CBB = f(t) curves:

1. BB FCF aqueous solution of constant concentration (5 mg / L) and variable concentration of ozone over the concentration range (100 - 250 mg O3 / L a.g.) at three average pH levels (acidic, neutral and basic) with and without stirring, at variable dye-ozone contact times (0 - 10 min at pH = 7, 0 - 1.5 min at pH = 4 and 0 - 1 min at pH = 10);
2. constant ozone concentration (100 mg O3 / L a.g.) and BB FCF aqueous solution of variable concentration, in the concentration range (2.5 - 10 mg / L), under the same conditions as in point 1.

6.5.1. Preliminary research

The reaction kinetics of the decolorization of aqueous BB solution by ozone was studied by estimating the variation of dye concentration during mutual interaction at the three pH values, four ozone concentration values, and 5 mg/L dye. To determine the likely order of reaction, the experiment was conducted with four ozone concentrations (100, 150, 200 and 250 mg/L), three pH values (pH = 4.0, pH = 7.0 and pH = 10 ,0), with / without stirring.

In Fig. 6.8.a, 6.8.b, 6.9.a, 6.9.b, 6.10.a and 6.10.b show the time variations of BB FCF concentrations during ozonation, variant a) being with stirring and variant b) without stirring, at all three pH values studied.

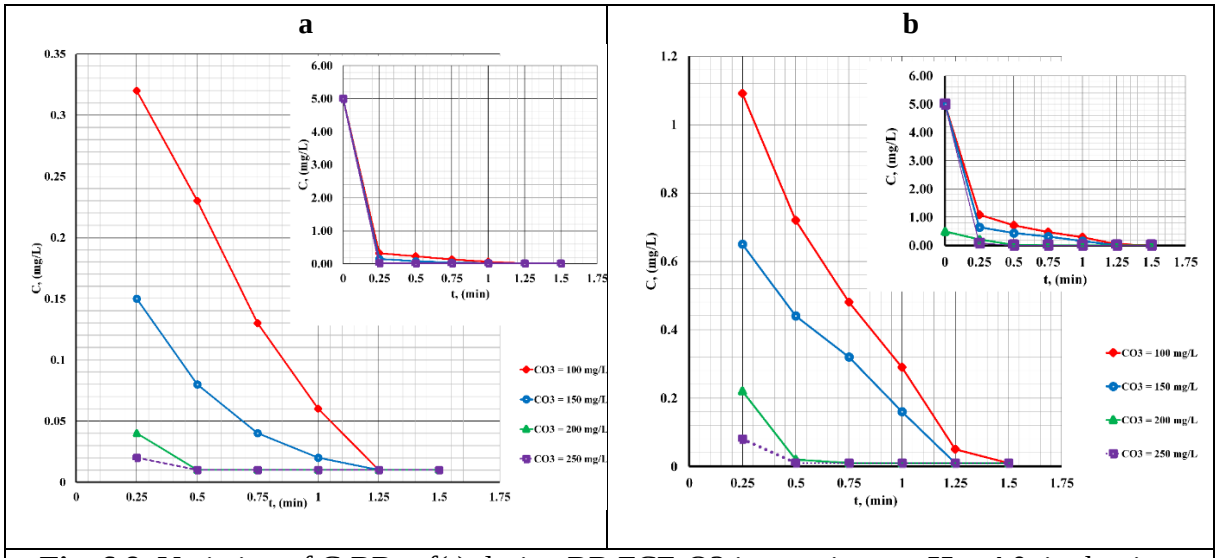


Fig. 6.8: Variation of CtBB = f(t) during BB FCF-O3 interaction at **pH = 4.0**, in the time interval 0 – 1.5 min, CiBB = 5 mg/L; a) with stirring (200 rpm), b) without stirring [297]

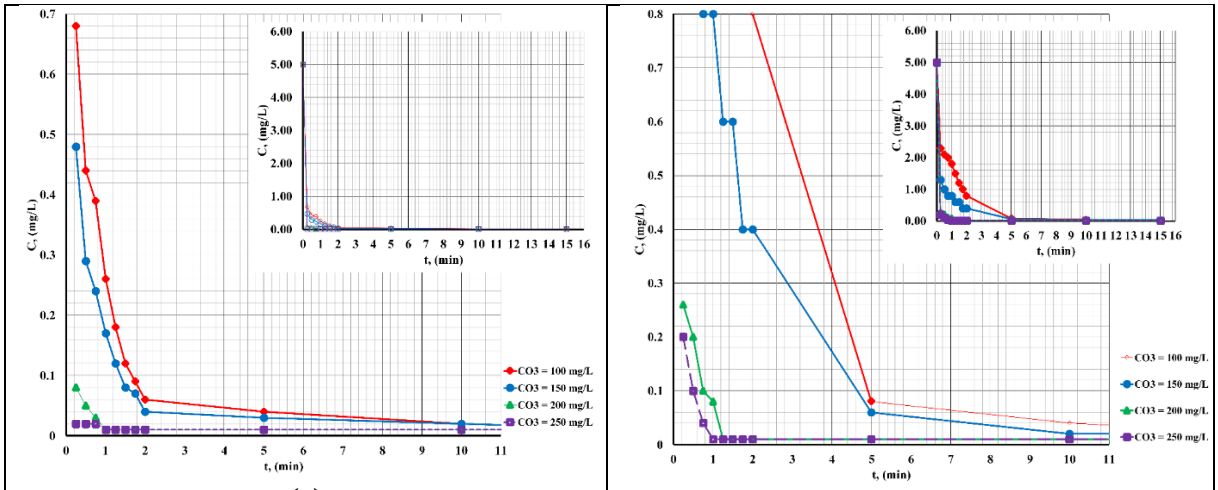


Fig. 6.8: Variation of CtBB = f(t) during BB FCF-O3 interaction at **pH = 7.0**, in the time interval 0 – 1.5 min, CiBB = 5 mg/L; a) with stirring (200 rpm), b) without stirring [297]

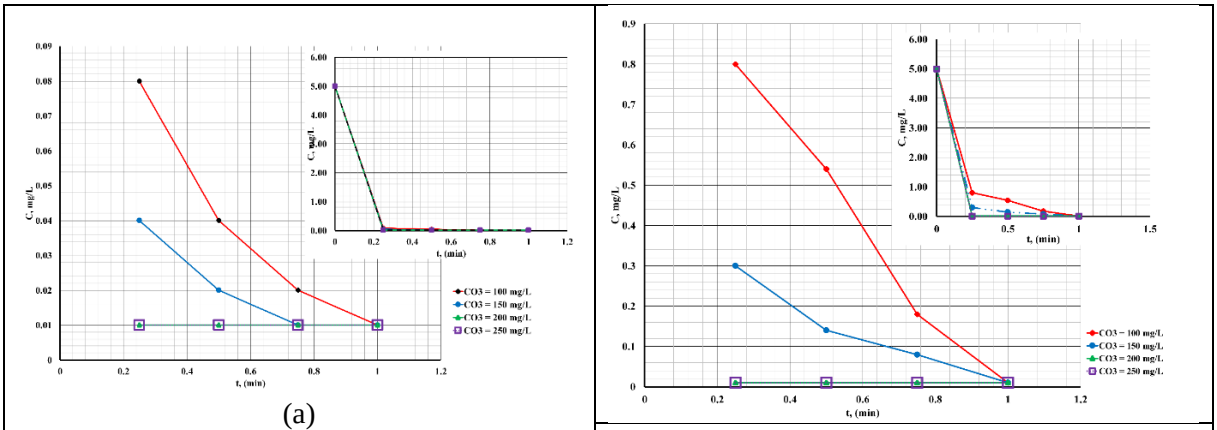


Fig. **6.10**. Variation of CtBB = f(t) during BB FCF-O3 interaction at **pH = 10.0**, in the time interval 0 – 1.5 min, CiBB = 5 mg/L; a) with stirring (200 rpm), b) without stirring [297]

In all variants (with or without stirring) the reaction rate is higher at higher concentrations of ozone. Classical 1st order kinetic models were applied to these experimental results and 2, using the integral method. The results obtained are presented in Table 6.9.

Table 6.9. Kinetic parameters calculated for the reaction order by the integral method, at different pH values and different ozone concentrations, CiBB= 5mg/L

$C_{i,O3}$ mg/ L	N	pH = 4				pH = 7				pH = 10			
		k, min^{-1}		R^2		k, min^{-1}		R^2		k, min^{-1}		R^2	
		*	**	*	**	*	**	*	**	*	**	*	**
100	1	-3,14	-3,65	0,93	0,89	-0,25	0,35	0,78	0,90	-2,77	-5,70	1	0,86
	2	89,18	63,16	0,76	0,57	6,24	3,29	0,97	0,98	115	119,98	0,92	0,64
150	1	-2,34	-3,76	0,97	0,87	-0,22	0,31	0,73	0,88	-1,94	-4,31	0,89	0,91
	2	86,19	90,13	0,91	0,71	6,08	3,97	0,96	0,94	110	4,32	0,90	0,91
200	1	-0,79	-2,00	0,43	0,57	0,07	0,14	0,16	0,24	-6×10^{-15}	-6×10^{-15}	N/A	N/A
	2	0,79	2,00	0,43	0,57	3,20	8,21	0,17	0,27	0	-6×10^{-15}	N/A	N/A
250	1	-0,40	-1,19	0,43	0,43	-0,03	0,09	0,17	0,16	-6×10^{-15}	-6×10^{-15}	N/A	N/A
	2	28,57	50,00	0,43	0,43	2,04	3,55	0,17	0,17	0	-6×10^{-15}	N/A	N/A

*with stirring, **without stirring

The following were observed from the variation Ct,BB= f(t):
- the version with mixing is more efficient than the one without mixing;
- at pH = 4.0 complete discoloration occurs after 1.25 min (for Ci,O3 = 100 mg/L and 150 mg/L) and respectively after 0.50 min (for Ci,O3 = 200 mg/L and 250 mg/L);

- at pH = 7.0 complete discoloration occurs after 10 min (for Ci,O3 = 100 mg/L and 150 mg/L) and respectively after 1 min (for Ci,O3 = 200 mg/L and 250 mg/L) ;

- at pH = 10.0 the final concentration of BB is much lower in a shorter time (0.2 min) compared to the pH values previously studied, probably due to the increased efficiency of oxidation of OH radicals.

In acidic and alkaline media, respectively, the reactions are fast and cannot be interpreted by classical 1st and 2nd order kinetic models (Table 6.9, negative rate constants).

These conclusions confirm the fact that factors such as: pH value, ozone concentration, BB FCF concentration, agitation with effect on the mass transfer component of

the whole process are important in the ozonation process. Furthermore, considering that the discoloration of BB FCF by ozonation occurs with multiple possibilities of attack (mainly at chromophoric groups), it is important to take this aspect into account as well; for this purpose, the 1H-NMR mass spectrum (Fig. 6.11) of a sample was recorded.

An oxidation variant is also presented according to the results obtained from the mass spectrum analysis.

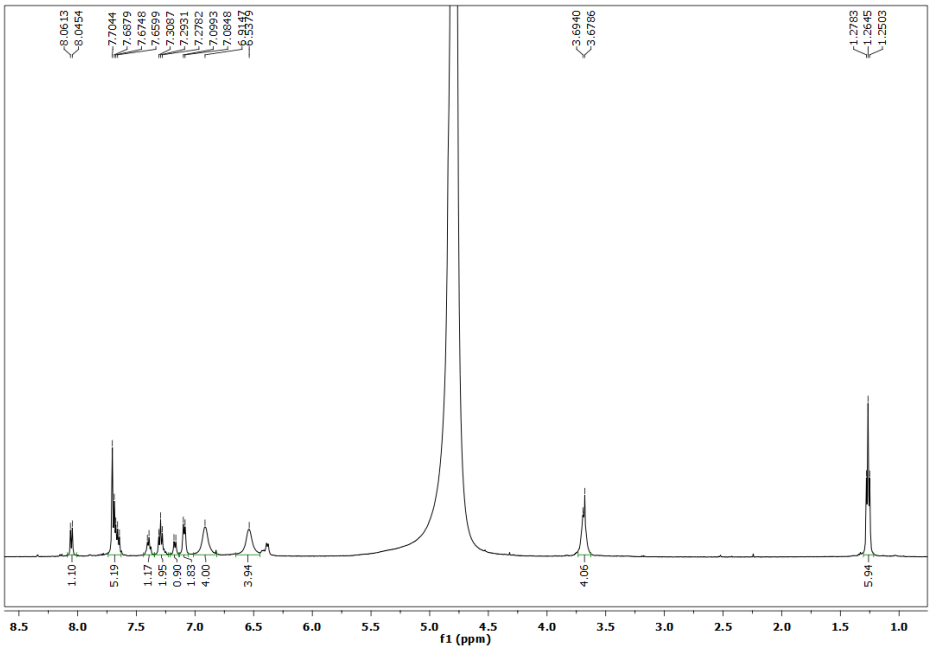


Fig. 6.11: 1H-NMR spectrum of BB FCF dye [297]

Ozone can oxidize BB in two ways:
1) Direct oxidation - Compounds treated with ozone form ozonides, which, in the presence of water, form aldehydes and ketones;
2) Generation of intermediate free radicals, such as the OH· radical, which is a powerful, efficient, and nonselective oxidizing agent.

To study the reaction kinetics of the ozonation process of the BB FCF dye, only the radical mechanism was considered.

A proposed degradation pathway of BB FCF in aqueous solution using ozone is illustrated in Fig. 6.12 and confirms the data from the literature [148].

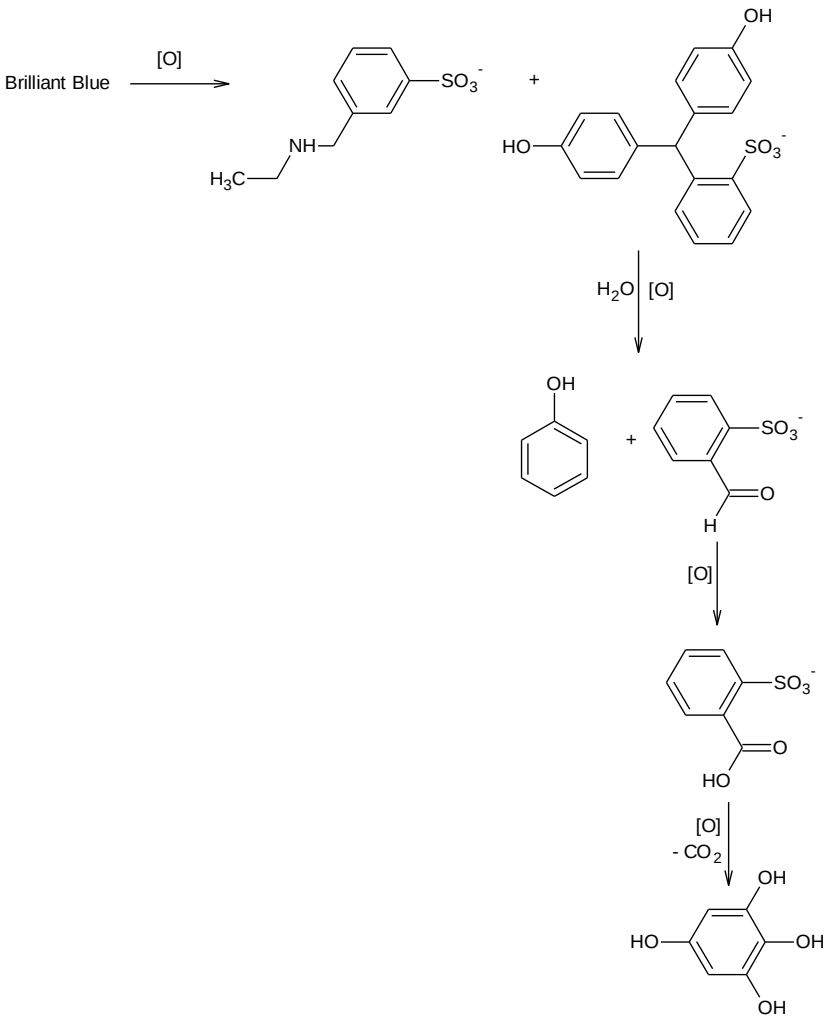
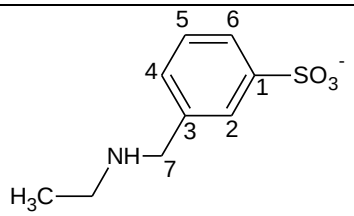
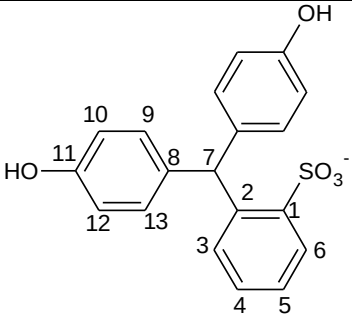


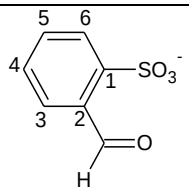
Fig. 6.12: Degradation of BB from aqueous solutions by ozonation [297]

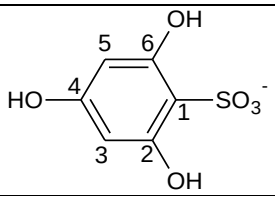
It is noted that, based on the initial conditions, the last reaction step is not kinetically observable.

Corroborating the data obtained by NMR spectroscopy with the data from the literature [148] **confirms the existence of four secondary degradation products in the ozonated mixture**:

	^{1}H-NMR (500,13 MHz, D_2O, δ pmm, J Hz): 8,14 (m, 1H, H-2), 7,90-7,89 (m, 2H, H-4 and H-6, superimposed with other aromatic signals from compounds resulting from BB degradation), 7,78 (t, 1H, H-5, 7,85 Hz), 4,32 (s, 1H, H-7), 3,05 (q, 2H, C**H**$_2$ from etil, 7,40 Hz), 1,28 (t, 3H, C**H**$_3$ from etil, 7,40 Hz)

	^{1}H-NMR (500,13 MHz, D_2O, δ pmm, J Hz): 7,90-7,89 (m, 1H, H-6, superimposed with other aromatic signals from compounds resulting from BB degradation), 7,63-7,62 (m, 3H, H-3, H-4 și H-5), 7,30-7,26 (m, 4H, H-9 and H-13), 7,08-7,06 (m, 4H, H-10 and H-12), 5.35 (s, 1H, H-7)

	^{1}H-NMR (500.13 MHz, D_2O, δ pmm, J Hz): 10,03 (s, 1H, C**H**O), 8,00 (m, 1H, H-4), 7,95 (m, 1H, H-5), 7,90-7,89 (m, 1H, H-3 and H-6, superimposed with other aromatic signals from compounds resulting from BB degradation)

	^{1}H-NMR (500,13 MHz, D_2O, δ pmm, J Hz): 6,39 (s, 2H, H-3 and H-5)

Several studies have reported that the secondary oxidation products are more toxic than the original dye solution [148, 282,294, 296].

Thus, the idea of kinetic modeling is motivated both by the complexity of the dye structure and by its multiple cleavage possibilities depending on the attack conditions, since there are too many unknowns and the stoichiometry of the reactions is not known either.

6.5.3. Kinetic modeling

Due to the complexity of the oxidation process and the lack of knowledge of the stoichiometry of the reactions, an attempt was made, based on the experimental data obtained, to propose a kinetic model based on some simplifying assumptions:

1. a finite number of reaction centers in the BB molecule;
2. The reactivities of these centers are sufficiently different so that they are attacked successively. Based on these assumptions, it follows that the ozone molecule reacts with the initial chromophore at its most reactive center, then with the first ozonation secondary product at its most reactive center, etc.;
3. The first attack destroys the chromophore, so the rate of discoloration is proportional to the rate of BB decomposition;
4. Each ozonation step follows apparent 2nd order kinetics (1st order for both ozone and substrate).

According to these simplifying assumptions, the global ozonation process can be described by the following chain of reactions:

$$B_0 + O_3 \xrightarrow{k_0} B_1$$

$$B_1 + O_3 \xrightarrow{k_1} B_2$$

$$\vdots \qquad (1)$$

$$B_n + O_3 \xrightarrow{k_n} B_{n+1}$$

...

Mass transfer modeling is based on double layer theory...

...

As an additional simplifying assumption, the ratio

$$\alpha = \frac{k_i}{k_{i-1}}$$

is assumed to be independent of i. This means that the difference between the activation energies, in the successive steps of the chain (1), is approximately constant. Under this assumption, the reaction-diffusion model finally becomes:

$$t = \beta_0 ln\frac{C_0}{C_{B_0}} + \beta_1(C_0 - C_{B_0}) + \sum_{n=1}^{N} \gamma_n \left[1 - \left(\frac{C_{B_0}}{C_0}\right)^{\alpha_n}\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

To validate model (6), experimental data should contain sufficient kinetic information.
Upon checking the available data, it was decided to select the following sets of BB decay data measured at pH = 7, with stirring, with different concentrations of BB and ozone:
1. C_0 = 2.5 mg/L, C_{O3} = 100 mg/L; 2. C_0 = 5.0 mg/L, C_{O3} = 100 mg/L; 3. C_0 = 7.5 mg/L, C_{O3} = 100 mg/L; 4. C_0 = 10.0 mg/L, C_{O3} = 100 mg/L; 5. C_0 = 5.0 mg/L, C_{O3} = 150 mg/L.
Parameter estimation was performed based on model (6) by ordinary linear regression, and the results (in the form of a calculated kinetic curve, represented by the black solid line curve) plotted against the experimental data (in the form of a dotted curve red color), can be viewed in Fig.6.13.

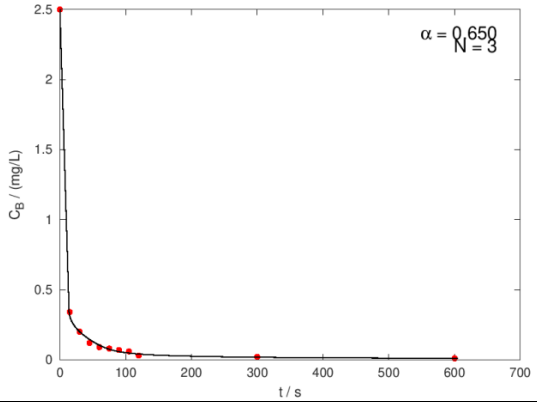


Fig. 6.13: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 2.5 mg/L and gas-phase ozone concentration 100 mg/L, on a time interval (0 - 600 s) [297]

Fig. 6.13 shows the experimental and calculated kinetic curves over the entire time interval of the experiment. It can be seen that model (6) fits the experimental data set very well. However, it should be noted that the data set itself does not provide information about the first 15 seconds of the process, where approximately 85% of the initial chromophore apparently decays. Therefore, the regression model is based only on data from the time interval (15 s, 600 s), which covers the decay of less than 15% of the original chromophore. Fig.6.14 provides a closer look at the relationship between model (6) and the experimental data over the relevant time interval. As can be clearly seen, even at this much smaller scale, the agreement between model and data is very good.

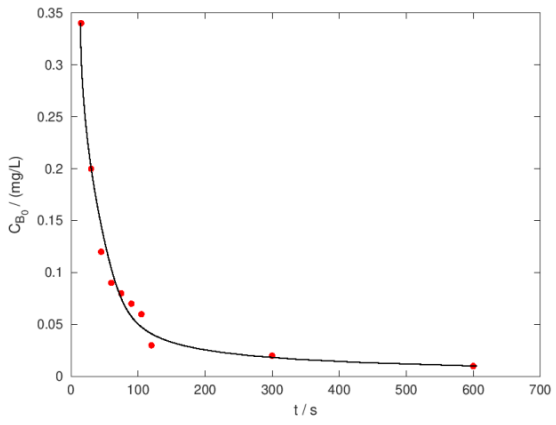


Fig. 6.14: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 2.5 mg/L and gas-phase ozone concentration 100 mg/L, on a time interval (15 - 600 s) [297]

Similar observations can be made about all other data sets.

Figures 6.15, 6.16 and 6.17 show the relationships between the calculated and experimental kinetic curves, measured at pH = 7, the initial ozone concentration in the gas phase 100 mg/mL and the initial chromophore concentrations 5 mg/L, 7.5 mg/L and 10 mg/L. In all cases, it is clear that model (6) and the experimental data fit very well. Also represented on these graphs are the reactivity meta-parameter values and the chain length (α and N, respectively).

As can be seen, both optimal values for these parameters change slightly with the initial chromophore concentration. More specifically, the chain length parameter decreases from 3 (at initial chromophore concentration C_0 = 2.5 mg/mL) to 2 (at higher initial chromophore concentrations). A possible explanation for this is that as the initial substrate concentration increases, less and less ozone is left available for further attacks on primary and secondary ozonation by-products, so that the number of consecutive chain reactions (1) decreases. The change in the reactivity parameter, on the other hand, may indicate a corresponding change in the reaction pathway itself, which can be explained by a change in the chemical identity for the intermediates B_1, B_2, ..., B_n in the reaction chain (1).

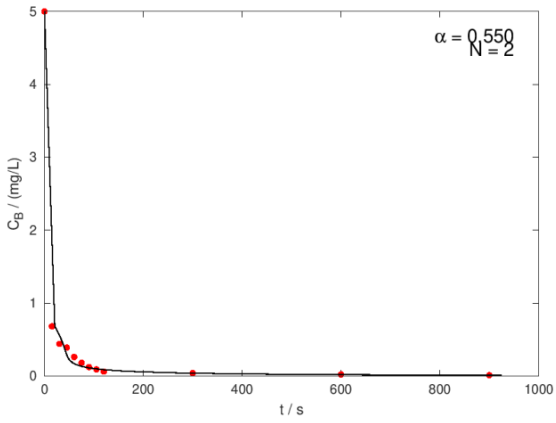


Fig. 6.15: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 5 mg/L, and gas-phase ozone concentration 100 mg/L, over a range of time (0 - 600 s) [297]

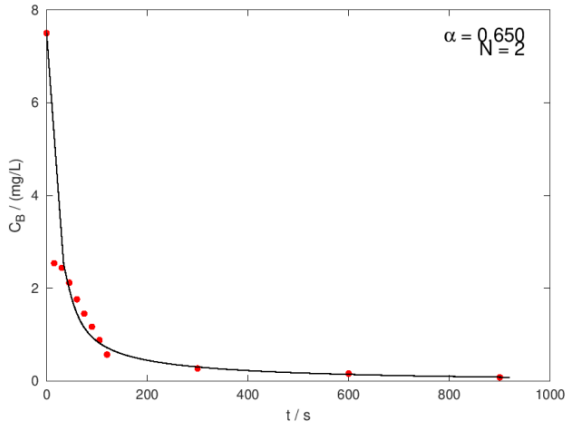


Fig. 6.16: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 7.5 mg/L, and gas-phase ozone concentration 100 mg/L, on a time interval (0 - 600 s) [297]

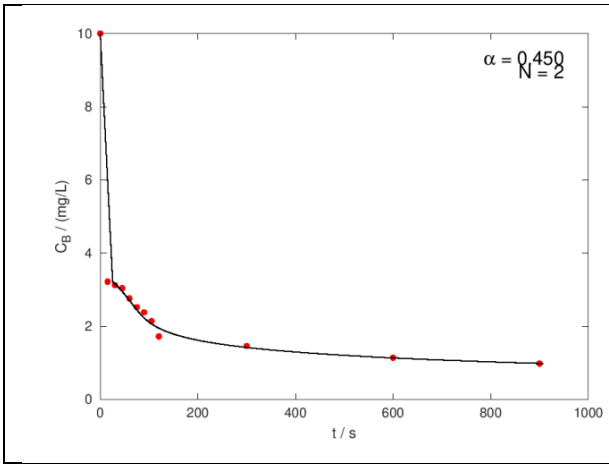


Fig. 6.17: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 10 mg/L, and gas-phase ozone concentration 100 mg/L, over a range of time (0 - 600 s) [297]

Finally, Fig.6.18 shows the model-vs-data relationship for the data set measured at C0 = 5 mg/mL, pH = 7, and gas-phase ozone concentration 150 mg/L.

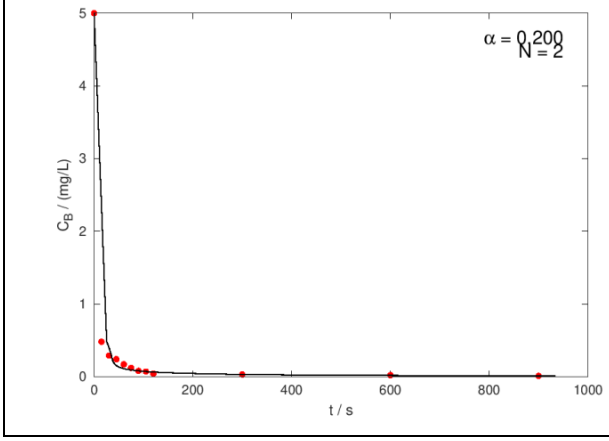


Fig. 6.18: Experimental (red dots) and calculated (solid black line) kinetic curve for BB decay at pH = 7, initial chromophore concentration 5 mg/L, and gas-phase ozone concentration 150 mg/L, over a range of time (0 - 900 s) [297]

In Figure 6.18 it is clearly observed that the model (6) and the experimental data are in very good agreement. The chain length parameter is as low as before (excess ozone in the liquid phase is still insufficient to promote further reactions in the chain (1)), but the reactivity parameter is even lower than before. A possible explanation for its decrease (which implies larger differences between activation energies in consecutive chain reactions (1)) is that, at higher ozone concentrations, more attack centers on the initial chromophore can be attacked simultaneously , which leads to an apparent increase in its reactivity relative to that of other secondary products, which react with ozone in fewer (possibly only one) attack centers.

Not here, at Conclusions? - The results obtained in this chapter of the doctoral thesis allowed the following evaluations to be made for the process of discoloration of BB FCF by ozonation:

1. The ozonation process of BB dye can produce several compounds as a result of oxidation, depending on the initial reaction conditions, due to the structural complexity of the substrate;
2. Classical kinetic models are not applicable because the initial oxidation products may be subsequently ozonized, so the actual process involves a sequence of chain reactions;
3. 1H-NMR spectroscopy analysis was performed at the time of decolorization. The results clearly show that discoloration does not indicate complete mineralization. Consequently, the process must be continued, after the initial discoloration, to avoid new, colorless but toxic secondary products remaining in the system;
4. The discoloration of BB FCF takes place through successive reactions that offer a possibility to model the process taking into account both the diffusion of ozone from the gas to the liquid and the chain reaction system in the liquid phase.

6.6. Toxicity of BB FCF dye and oxidation products

As the complex structure and toxic potential of the BB FCF dye on aquatic organisms are known, the need to remove it from aqueous environments (industrial or urban wastewater) is obvious. Different dye removal methods have been studied over time, ozonation and advanced oxidation processes (AOP) being successfully applied (Chap. 3.2, page 48). Most of the studies carried out in this field, however, emphasized the obtaining of intermediate (secondary) products in the conditions of an incomplete mineralization of the dye, compounds that can be more toxic than the initial dye.

In Chap. 6.5.2 the presence of four reaction products following the ozonization of the studied dye was reported. In this context, a comparative study of the ecotoxicity of the aqueous solutions of BB FCF dye, before ozonation, and of the resulting aqueous solutions after ozonation of the dye was required.

The study presents experiments to determine the toxicity expressed by the median lethal concentration for 50% of the test population (LC50) and the median effective concentration for 50% of the test population (EC50) on Daphnia magna (planktonic crustaceans), Selenastrum capricornutum (green microalgae) and Cyprinus carpio (freshwater carp fingerlings) for researching non-ozonized BB FCF solutions, and for ozonized solutions, only Daphnia magna. In the research, lethal or inhibitory concentrations were used for 50% of the organisms tested (LC50 / EC50). In the first step, all organisms were exposed to 0.01- 100 mg/L BB FCF aqueous solutions . The toxic potential of neutral and acidic solutions of BB FCF (1 to 50 mg /L) treated with 200 mg O_3 / L was studied only on Daphnia magna.

The aqueous solution of BB FCF showed no toxicity to fish and crustaceans (LC50 / EC50 > 100mg/L). However, toxicity effects have been observed in algae. Oxidized solutions results showed a high toxicity to crustaceans compared to aqueous solution of the original dye.

Evaluation of decolorization efficiency (%R) - All solutions were treated with the same concentration of ozone (200 mg/L g.m) for a contact time of 300 seconds, under continuous stirring (200 rpm) with obtaining the maximum decolorization efficiency of the solutions experienced (Table 6.10). The experiment was performed at the pH of the BB FCF solution (pH = 7.05), except where the influence of pH was also evaluated when the tests were performed at pH = 4.03.

Table 6.10: Decolorization efficiency (%R) of BB FCF solutions used in toxicity tests

BB FCF Concentration (mg/L)	pH-of the tested solution	%R
1	7,05	99,92
	4,03	99,94
2,5	7,05	99,81
	4,03	99,87
5	7,05	99,68
	4,03	99,74
10	7,05	98,80
	4,03	99,50
30	7,05	97,66
	4,03	98,94
50	7,05	96,82
	4,03	97,04

Toxicity tests - The toxicity experiments were carried out in two stages:
1) initial testing of BB FCF dye in different concentrations prepared in dilution with water or growth medium;

2) final testing of the BB FCF solutions obtained postozonation.
To evaluate the aquatic toxicity of BB FCF, experiments were initially performed on invertebrates (planktonic crustaceans Daphnia magna and freshwater green algae Selenastrum capricornutum or Pseudokirchneriella subcapita) to evaluate EC50 data. Bioassays with Cyprinus carpio fish were subsequently applied to evaluate the LC50 data.
BB FCF testing was performed with different concentrations in the range of 0.01 - 100 mg/L. Ozonated solutions were tested in the range of 2.5 – 50 mg/L BB FCF. The tests were carried out both on undiluted solutions and on 50% diluted solutions, with or without adjusted pH. The organisms were directly exposed to BB FCF solutions, under static conditions, for up to 96h. The tests performed are presented in Table 6.11.

Table 6.11: Experimental toxicity tests [300]

Species	Test	Test type	Final effect	Testing/ Incubation period
Cyprinus carpio	OECD 203 OECD 202	Static, acute	Mortality, LC_{50}, Clinical signs	96h, 21-22^{o}C
Daphnia magna	DAPTOXKIT F	Static, acute	Mortality/immobilization, LC_{50}	24-48h, 20^{o}C
Selenastrum capricornutum/ Pseudokirchneriella subcapita	OECD 201/ SR EN ISO 8692:2012 ALGALTOXKIT F	Static, acute	Growth rate inhibition, ECr_{50}	72h, 21-25^{o}C

All acute toxicity tests were performed for BB FCF dye solutions in the concentration range 0.01–100 mg/L. Aqueous BB FCF solutions showed no toxicity to fish, having only minor lethal/inhibitory effects (5-20%) on Daphnia magna after 48 hours of incubation (Table 6.12).

Table 6.12: Acute toxicity results on aquatic organisms of aqueous solutions of BB FCF

Tested solutions (mg/L BB FCF)	pH	Dissolved Oxygen mg/L	Acute effects %	Toxicity class (LC_{50}/EC_{50} - mg/L)/ REACH clasiffication
Fish test				
100	7,99	8,12	0	LC_{50} >100 mg/L
10	7,94	7,36	0	Nontoxic
1	7,64	8,49	0	
Blanck	7,64	7,60	0	
Algae test				
100	7,74	8,63	116,71	ECr_{50} = 24 mg/L
50	-	-	98,05	Harmful??
20	-	-	9,01	Tests were influenced
10	-	-	8,50	by color (cannot be
5	7,69	8,63	-6,27	considered valid data)
Blanck	7,49	8,74	-	
Crustacean test				
100	7,60	8,62	20	EC_{50} >100 mg/L
50	7,03	-	20	Nontoxic
20	7,35	-	15	
10	7,35	-	10	
5	7,42	-	10	
1	7,38	-	5	

0,1	7,32	-	0	
0,01	7,28	8,66	0	
Blanck	7,14	8,91	0	

Note: value marked with "- " stimulation of algal growth; ECr_{50} refers to the concentration that effects on the growth rate of 50% of the algae

EC_{50} >100 mg/l indicates no harmful impact of the dye on these organisms. Similar results for Daphnia magna have been reported for BB FCF between 97 mg/L and >1000 mg/L by international databases for chemical registration such as the Ecotoxicology Knowledge Base (ECOTOX) [306] and other environmental organizations [307]. According to the Regulation on the categories of classification of hazardous substances for the aquatic environment [305], BB FCF is classified as non-toxic to crustacean species (Daphnia) (EC_{50}>100 mg/L).

The concentration of BB FCF with a toxic effect on algal growth (ECr50) after 72 h incubation was 24 mg/L. Significant effects were also observed at BB FCF concentrations of 50 mg/L and 100 mg/L, respectively.

Figure 6.19 shows the results of the acute toxicity effects of ozonated solutions on daphnia. Ozone treatment combined with sample acidification increased the decolorization yield but led to an increase in the toxicity of the solutions thus treated, due to the high oxidizing capacity of ozone, which leads to secondary compounds more toxic than the initial ones [152].

Ozonated samples exerted 5-10 times more toxicity than untreated samples.

The same level of toxicity was observed in ozonated samples to which pH correction was applied.

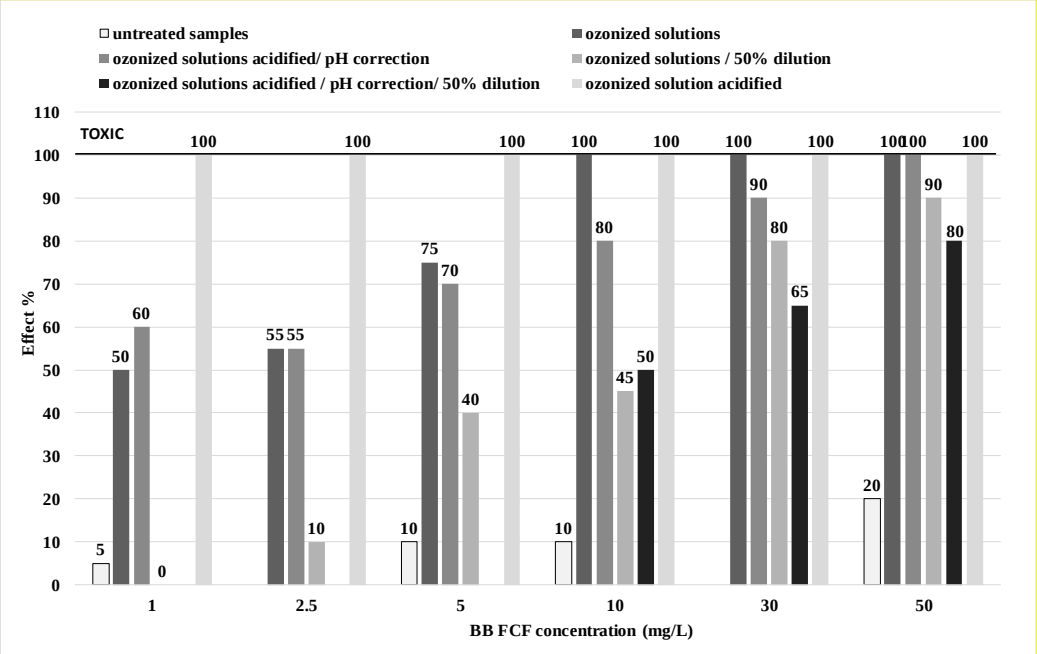


Fig. 6.19: Acute toxicity of ozonated BB FCF solutions on planktonic crustaceans

The toxicity of the ozonized samples decreased with their 50% dilution, but even in these cases the toxicity was superior to the untreated BB FCF solutions, having concentrations of 5, 10, 30 and 50 mg/L.

The same toxicity trend was observed with ozonated and acidified, pH corrected and 50% diluted solutions.

The acidification of the ozonized solutions (pH=4) produced a more effective discoloration but at the cost of a high toxicity (100%), incompatible with the survival of the studied species, which required the correction of the pH to 6.5 – 8.5.

The 50% diluted ozonized BB FCF solutions had between 0 and 100% effects on daphnia, with the level of toxicity being lower than that of the undiluted samples. At an EC50(48h) calculated for a 50% diluted sample, the toxic value was 4.8 mg/L BB FCF (fig. 6.20). This value indicates that solutions of BB FCF ozonized in order to decolorize cause very toxic effects (50-100%).

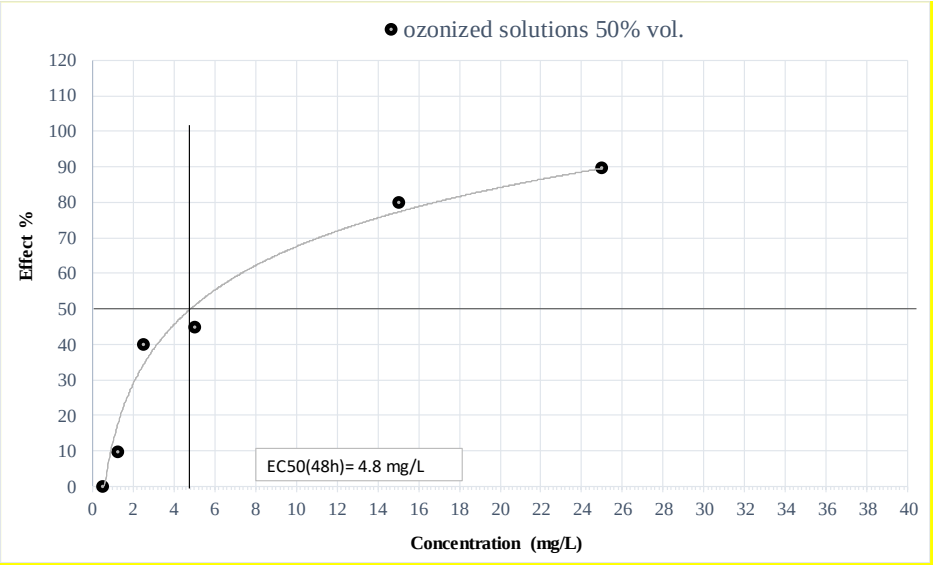


Fig. 6.20: Estimated EC50 (48h) for 50% diluted BB FCF solutions

Previous studies have shown a decolorization yield of over 90% in the case of BB FCF solutions of 5 mg/L and 10 mg/L respectively ozonized (200mg O3/L), under stirring (200 rmp) and acidified (pH4) conditions [145,147], but the toxic impact on organisms was significant (80%). Diluting these solutions by 50% decreased the toxic effects to 45 – 50%.
We can thus conclude that the toxicity of ozonized samples (especially those with concentration ≤ 10 mg/L) can be reduced by diluting them by >50%, at a pH of 6.5 – 8.5 suitable for survival. The use of even lower concentrations of BB FCF (2.5 or 5 mg/L) ozonized and then diluted will ensure a much lower toxicity (10 – 40%).
Taking into account the non-toxic concentrations prescribed for BB FCF (PEC) for surface waters (< 0.1 - 626 µg/L), according to the economic sector (industrial, food, cosmetic) [307], the estimated level of toxicity can be reduced by approximately 100 times.

Chapter 7
Conclusions

The general and specific objectives assumed in the doctoral work were achieved through an extensive bibliographic study, by carrying out two clinical studies and by carrying out a complex environmental study.
The comparative clinical study on the application of O_3T in diabetes complicated with diabetic neuropathy (chapter 4) was carried out in order to evaluate and quantify the effect of O3T, applied complementary to 73 patients suffering from diabetes mellitus (DM) complicated with diabetic neuropathy (ND), pathology constituting a serious public health problem, through the alarming increase in the number of cases, the development of chronic complications (e.g. diabetic foot, with disastrous evolution towards amputations) and through aggravation of co-morbidities (cardiovascular, etc.). After 5 weeks of O_3T, significant improvement was found in most clinical and biological parameters monitored (biochemical, metabolic, neuropathic pain and quality of life). The statistical processing of the obtained data allowed complex correlations

between biological and clinical parameters, between advanced age and the prolonged evolution of the disease, etc.
In the study on the application of O_3T in trigeminal neuralgia (chapter 5), the effect of medical ozone was followed on a group of 33 patients suffering from trigeminal neuralgia (NT), a debilitating painful condition, very difficult to treat. Patients were given 12-15 treatment sessions with a frequency of 2-3 sessions/week.
Patients were considered responders based on improvement/disappearance of pain, overall satisfaction, resumption of usual activities, etc. Quantitative and qualitative changes in pain and associated symptoms/signs were considered.
Patients were fully informed about the purpose/procedure of the treatment and all signed an informed consent. The studies were approved by the Ethics Committee of the Medical Center.

Conclusions of the medical studies - The beneficial effects of O_3T presented in the two medical studies were due to its multimodal action: restoring redox homeostasis, immunomodulation, fighting neuroinflammation, cell apoptosis/senescence, endothelial damage and improving tissue oxygenation , re-balancing energy, carbohydrate and lipid metabolism, involvement in the mechanism of nociception. It also positively influenced psycho-emotional status (reducing anxiety/depression).
The use of low concentrations of ozone (5 – 35 μg O_3/mL) led to hormetic action on the biological substrate, without toxic effects. Side effects of O_3T are minimal: the World Federation of Ozone Therapy (WFOT) estimates the incidence of complications at 0.0007% [77]. In the presented studies, O3T acted regardless of the age of the disease - recent or long-standing and despite the aggravating factors (patient age, comorbidities, etc.). These conclusions are in agreement with the vast literature developed over the last 30-40 years on ozone therapy [49,50,81,87,92,94,98,103,105,106,214-216,245,272].
Considering the oxidative potential of ozone (including the medical one), this treatment must meet certain cumulative criteria: to be applied by well-trained doctors, in small doses, spaced out and in carefully selected cases; concentrations of O3 must be tailored according to the existing pathology and the condition of the patient – especially taking into account the severity of oxidative distress and the effectiveness of the patient's antioxidant defenses. Empiricism in O3T may raise fundamental concerns regarding the efficacy and safety of this treatment [132].]. If ozone therapy is applied correctly - precise diagnosis, adequate doses, standard procedures, associated with the recommended treatment/diet - it will act favorably on the disease, improving the clinical condition, stimulating the healing process, practically without side effects [272]. Medical ozone is a safe and effective complementary therapy, but further research is needed to confirm the effectiveness and limitations of this treatment.

Elements of originality of medical studies:
- The complex way of evaluating patients before and after O3T;
- The use of simple, easily reproducible tests "at the patient's bedside" (sQST) for sensitivity disorders, allowing the outline of a characteristic sensory phenotype of selected patients, useful for specifying the anatomical substrate and predicting the response to treatment. This paper appears to be the first study on O3T applied in trigeminal neuralgia (NT) using these tests;
- Comparative presentation of etiopathogenic mechanisms involved in NT and the multifactorial effect of medical ozone (Table 5.22);
- The association of medical ozone application types: topical, local and loco-regional applications and CDL, combined with general applications - which seems to be the most effective therapeutic strategy, acting synergistically and cumulating the benefits of the local and general effects of ozone;
-Particular application of medical ozone- through subcutaneous injections performed not only in the painful area, but also symmetrically in other cephalic and cervico-dorso-lumbar points;

these loco-regional applications acted on: local oxidative homeostasis, inflammatory and hypoxic substrate, peripheral sensitization, nociceptive modulation, nerve tissue regeneration;
- Description of the sensory phenotypes in the study patients – the existence of these phenotypes allowed specifying the anatomical substrate of the neuropathic pain and explained, to a large extent, the variability of the response to the treatment.
Research Perspectives - In-depth studies are needed to demonstrate the actions and limitations of O3T:
- National/international multicenter studies with well-specified diagnostic and selection criteria and uniform procedures. Ideal – randomized, controlled studies with large patient sample sizes, possibly with single-double-blind application and longer post-study follow-up periods;
-Valid, reliable and scalable sensory profiling methods are needed;
-Specific laboratory analyzes and imaging are recommended;
- Deepening the studies regarding the medical O3 interactome;
-Studies of O3T and in other pathologies presenting OS pathogenesis, chronic inflammation, chronic pain, premature senescence, etc.

In chapter 6 ("Research on the discoloration by ozonation of aqueous media"), the following were followed:
• Applicability of the ozonation process to the discoloration of some dyes refractory to conventional treatment methods. Brilliant Blue (BB), a synthetic dye commonly used in the pharmaceutical, food, textile, cosmetic, and even medicine industries, was selected;
• Study of influencing factors: pH, initial concentration of dye and applied ozone, contact time, molar ratio [O3:BB], stirring time, in order to establish the optimal decolorization parameters to obtain an effluent with quality indicators according to the legislation in force;
• Studying discoloration by ozonation at neutral pH;
• The removal of color by ozonation does not lead to a complete mineralization of the dye, so the identification of the intermediate oxidation products, obtained during the degradation of BB by ozonation, under the given conditions, was carried out by 1H – NMR spectroscopy;
• The comparative study of the ecotoxicity of the BB dye and the solutions after ozonation - by following the effects on some aquatic organisms - revealed that the intermediate products formed showed a higher toxicity than the initial dye;
• Approaching the dynamics of the process by checking the classic kinetic models did not lead to interpretable results, the stoichiometry of the reactions during ozonation being unknown, so a complex kinetic model was proposed for the discoloration of BB solutions with ozone - model based on the information provided by the analysis of the mass spectrum of the dye, corroborated with data provided by the study of the toxicity of the products and taking into account the sequence of possible reactions and the multiple possibilities of cleavage of the dye;
• The results obtained in the research carried out allowed a correct evaluation of the advantages and disadvantages of the action of ozone on this dye.

The conclusions of the study "Preliminary data on the behavior of ozone in aqueous solutions" (chapter 6.3) - Ozonation of aqueous solutions of BB FCF dye without changing the pH led, under certain reaction conditions, to its rapid and effective discoloration. Oxidation processes (only by ozonation or within AOP) prove to be promising alternatives for the removal of this dye, leading to discolorations of more than 90% under certain physico-chemical conditions. The bleaching efficiency was maximum in the case of low dye concentrations (10 mg/L), to which ozone concentrations (200 mg O3 / L a.g.) were applied, under mechanical stirring conditions (200 rpm), at a pH value = 7.03.
The research results also emphasized the influence of ozone purity on the oxidation process, ozone being obtained from 99.5% pure oxygen.

The results of this preliminary study were established as premises for further research, regarding the mechanisms and efficiency of ozonation in acidic and alkaline environments, respectively, on the kinetics of the reactions. The data were also useful for the identification of secondary products and for the evaluation of their toxicity.

Conclusions of the study on the influence of pH on the decolorization of aqueous systems by ozonization (chapter 6.4) - The experimental results showed that both the pH of the aqueous solutions and the ozone concentration favored the decolorization efficiency. Depending on these two important factors, it was also possible to determine the dye-ozone contact times.

pH values of 4.0; 7.0 and 10.0 favored BB FCF decolorization. This was due to the predominant effects of molecular ozone, at acidic pH, and the effects of hydroxyl radicals, at alkaline pH. At neutral pH, a balanced effect of molecular ozone and hydroxyl radicals is assumed.

From the interpretation of the experimental results, the following conclusions can be drawn:

- At pH = 4, the discoloration yields are lower compared to those at pH = 10, at the same contact times;
- At pH=10, the bleaching efficiency being better, it occurs in a shorter dye-ozone contact time (60 s compared to 90 s at pH = 4);
- At pH=7, the bleaching efficiency is low (between %R = 49.2 and %R = 99.6) in the version without stirring and (between %R = 66.1 and %R > 99.9) in the version with agitation, probably through an equilibrium effect between the two predominant species at acidic and alkaline pH. This was also the reason for a longer contact time, reaching up to 900 s;
- In conditions of mechanical agitation (200 rpm) the bleaching yields had higher values (between %R = 66.1 and %R >99.9) compared to the variant without agitation (between %R = 49.2 and %R >99.9).

Conclusions of the study on aspects of the kinetics of the process of discoloration of BB FCF with ozone (chapter 6.5) - The results obtained in this chapter of the doctoral thesis allowed the following evaluations to be made for the process of discoloration of BB FCF by ozonation:

-The ozonation process of BB dye can produce several compounds as a result of oxidation, depending on the initial reaction conditions, due to the structural complexity of the substrate;

- Classical kinetic models are not applicable, because the initial oxidation products can be subsequently ozonized, so that the actual process involves a sequence of chain reactions;

- 1H-NMR spectroscopy analysis was performed at the time of discoloration. The results clearly show that discoloration does not indicate complete mineralization. Consequently, the process must be continued, after the initial discoloration, to avoid new, colorless but toxic secondary products remaining in the system;

- The discoloration of BB FCF takes place through successive reactions that offer a possibility to model the process taking into account both the diffusion of ozone from the gas to the liquid and the chain reaction system, in the liquid phase.

Conclusions of the study on the toxicity of BB FCF dye and oxidation products (chapter 6.6) - This sub-chapter presented a comparative study of the ecotoxicity of BB FCF solutions before and after ozonation. Ozone was very effective in decolorizing the solutions, however not strong enough to ensure complete mineralization of the dye, moreover leading to toxic oxidation products.

Three species of aquatic organisms (Daphnia magna, Cyprinus carpio, Selenastrum capricornutum) were used for the ecotoxicity study.

Acute toxicity tests of aqueous solutions of BB FCF demonstrated the lack of toxicity on fish and daphnia (LC/EC50>100 mg/L), but also the negative influence on the growth process of green algae (ECr50=24 mg/L).

Based on the experimental results, the dye was included in Category 3 of acute toxicity.

Solutions treated with ozone at a concentration of 200 mg O3/L showed significant toxic effects on Daphnia magna, most likely through the appearance of oxidation products containing groups that cause toxicity. These compounds were identified and described in Chap. 6.5.2, page 147.

The yield of discoloration after ozonation at these concentrations was high (90%), but the toxic impact on organisms cannot be neglected.

Therefore, the reaction products resulting from the ozonation of aqueous solutions of BB FCF show much higher acute aquatic toxicity than the dye in solutions up to 100 mg/L, which requires other additional treatments in the case of post-ozonated solutions, such as: corrections of pH, water dilutions in different proportions (50 – 60 %), with the use of lower concentrations of BB FCF (≤10mg/L).

Perspectives of studies on other applications of O_3 in the environment – the removal by ozonation of micropollutants (estrogen hormones, NSAIDs, methotrexate, etc.) from aqueous solutions / waste water.

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