National University of Science and Technology POLITEHNICA

Bucharest

Doctoral School of the Faculty of Chemical Engineering and Biotechnology

DOCTORAL THESIS
EXTENDED SUMMARY

HYDROGEL-BASED SYSTEMS FOR CONTROLLED RELEASE OF ANTIDOTES AND DECONTAMINANTS

PhD Candidate

Chem. Adriana-Elena BRATU

PhD Supervisor

Prof. Eng. Izabela-Cristina STANCU, PhD

Buchares

2026

Acknowledgement

I would like to express my sincere gratitude to Prof. Isabela-Cristina Stancu for her guidance, support, and trust throughout the five years of my studies. Her patience, calmness, and understanding, as well as the rigor and honesty with which she introduced me to the realities of the research environment, have greatly contributed to my professional and personal development. I would also like to extend my sincere thanks to AndradaSerafim for her constant support, willingness to explain and clarify complex concepts, and for her patience and dedication to my learning process. Furthermore, I would like to thank Elena Olăreț for her assistance, collaboration, and support throughout the activities carried out, contributing to the successful completion of this scientific work.

I would like to express my sincere appreciation to the team of the Research and Innovation Center for CBRN Defense and Ecology, particularly the Chemical Analysis Laboratory, for the excellent collaboration, continuous support, and sustained effort throughout this journey. The professional and friendly environment has provided an essential framework for my development, and I hope that the experiences gained represent only the beginning of a long-term collaboration, allowing us to continue exploring this vast and challenging field of scientific research. In particular, I would like to thank Dr. Eng. GinghinaRaluca for her guidance, support, and the professional partnership we have built over time. Beyond her role as a mentor, she has been a constant and genuine source of support, contributing significantly to my growth in this field. I sincerely hope that this collaboration will continue in the long term, maintaining the same enthusiasm, rigor, and openness to innovation that have guided us so far.

I would like to express my sincere thanks to my colleagues from the Military Technical Academy 'FERDINAND I' for their support and for the constructive collaboration throughout this journey. In particular, I would like to thank Dr. Eng. Gabriela Toader and Dr. Eng. Aurel Diacon for their openness, professionalism, and dedication. Their collaboration has been a valuable support in the development of this scientific work, and I hope that the experience gained will serve as a foundation for a long-term collaboration, aimed at strengthening and further developing the research outcomes.

I express my gratitude to the members of my doctoral advisory committee for their scientific support, observations and recommendations during the development of the thesis, which contributed to its improvement. I also express my sincere thanks to all the co-authors of the papers that formed the basis of this thesis, for their efficient collaboration, exchange of ideas and valuable contributions during the research activity.

Last but not least, I would like to thank my parents and my brother, for their unconditional support, trust, and strength they have given me throughout these years. They have always been by my side, encouraging me to move forward and providing me with stability and confidence on my own path. I am also grateful to my friends for their support, understanding, and for the moments in which they brought balance and positive energy during this demanding period.

Summary

PART I. CRITICAL STUDY OF LITERATURE DATA 7
CHAPTER 1
CHEMICAL WARFARE AGENTS: MECHANISMS OF ACTION, BIOMARKERS AND METHODS OF IDENTIFICATION 7
1.1. Chemical warfare agents: classification, properties and general characteristics 7
1.2. Mechanisms of biological action of vesicant and nerve agents 8
1.3. Specific biomarkers following exposure to chemical warfare agents 9
1.4. Analytical methods for identification specific biomarkers formed following exposure to chemical warfare agents 10
1.5. Treatment and prophylaxis of exposure to chemical warfare agents 11
CHAPTER 2
DECONTAMINATION STRATEGIES AND DEVELOPMENT OF ADVANCED MATERIALS FOR CBRN AND BIOMEDICAL APPLICATIONS 13
2.1. General principles of chemical warfare decontamination 13
2.2. Operational management and conventional methods of decontamination of chemical warfare agents 14
2.3. Hydrogels and advanced biomaterials 15
2.4. Modified hydrogels for decontamination of surfaces exposed to chemical warfare agents 16
2.5. Biocompatible hydrogels and biomedical applications 17
PART II – ORIGINAL CONTRIBUTIONS
EXPERIMENTAL STUDY 20
CHAPTER 3.
EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF ANALYTICAL STRATEGIES FOR THE IDENTIFICATION OF BIOMARKERS OF EXPOSURE TO CHEMICAL WARFARE AGENTS 20
3.1. The necessity of development integrated analytical strategies for identifying biomarkers of exposure to chemical warfare agents 20
3.2. Preparation methods for biological samples (urine and plasma) 20
3.3. Development and optimization of the analytical method for identification of specific biomarkers to exposure to chemical warfare agents 23
3.4. Results and Discussion 26
3.4.1. Identification of specific biomarkers of exposure to blister and nerve agents in plasma and urine samples by LC-MS/MS 26
3.4.2. Identification of specific biomarkers of exposure to blister and nerve agents in plasma and urine samples by GC-MS/MS 29
3.5. Conclusions 31
CHAPTER 4
EXPERIMENTAL STUDY ON THE EVALUATION OF THE DECONTAMINATION EFFICIENCY OF CHEMICAL WARFARE AGENTS – SOMAN AND MUSTER - USING REACTIVE ORGANIC SUSPENSIONS CONTAINING NANOADSORBANTS OF ZINC OXIDE (ZnO), TITANIUM DIOXIDE (TiO_2) AND ZEOLITE 32
4.1. The necessity of development of modern decontamination strategies for chemical warfare agents 32
4.2. Methods for synthesis of decontamination solutions, physicochemical characterization of the organic component and verification of the compatibility of the organic component with nanoparticles. Testing of decontamination efficiency with real chemical warfare agents 32

4.3. Characterization 33
4.4. Results and discussions 34
4. 5. Conclusions 36
CHAPTER 5
DEVELOPMENT OF PEELABLE FILM-FORMING HYDROGELS BASED ON BIOPOLYMERS INCORPORATING METAL-ORGANIC FRAMEWORKS FOR THE IMMEDIATE DECONTAMINATION OF SULFUR MUSTARD 38
5.1. The necessity of development functionalized hydrogels with metal-organic frameworks for the decontamination of vesicant agents 38
5.2. Preparation methods for formulations and testing decontamination efficiency 38
5.3. Characterizations 39
5.4. Results and discussions 39
5.5. Conclusions 41
CHAPTER 6
HYDROGELS FOR CONTROLLED RELEASE OF ATROPINE IN CHEMICAL WARFARE AGENTS THREAT SCENARIOS 43
6.1. The necessity to develop hydrogels for the controlled release of therapeutic agents in chemical warfare agent exposure scenarios 43
6.2. Methods for the synthesis of functionalized hydrogels for the controlled release of atropine sulfate 44
6.3. Characterizations 44
6.4. Results and discussions 45
6.5. Conclusions 44
CHAPTER 7
CONCLUSIONS AND PERSONAL CONTRIBUTIONS 47
7.1. Importance and relevance of the study 47
7.2. Overall conclusions 47
7.3. Integrative synthesis of experimental results on identification, decontamination and treatment in chemical warfare agent exposure scenarios 48
7.4. Author’s contributions 50
7.5. Future research directions 53

PART I. CRITICAL STUDY OF LITERATURE DATA

CHAPTER 1

CHEMICAL WARFARE AGENTS: MECHANISMS OF ACTION, BIOMARKERS AND METHODS OF IDENTIFICATION

1.1. Chemical warfare agents: classification, properties and general characteristics

Chemical warfare agents (CWA) are a category of toxic substances with lethal or incapacitating effects on living organisms, being included in the CBRN (chemical, biological, radiological and nuclear) threats. Due to their high toxicity, rapid effects and difficulty in detection under certain conditions, these compounds are a subject of major interest in the field of security and defense[1, 2].

From a classification point of view, chemical warfare agents can be grouped according to the mechanism of action in the body. The most important categories include nerveagents, vesicants, suffocating agents, irritant agents and psychochemical agents[2, 3]. Nerve agents, such as sarin (GB), soman (GD), VX or A series compounds, act by inhibiting the enzyme acetylcholinesterase, leading to the accumulation of acetylcholine and severe dysfunction of the nervous system[3, 4]. Blistering agents, such as sulfur mustard and its analogues, cause skin and eye damage through mechanisms of alkylation of biomolecules[4, 5], while suffocating agents affect the respiratory system by destroying lung tissues.

In terms of physicochemical properties, these compounds show high variability, which influences their behavior in the environment and in the body. Some substances are volatile and persist for short periods of time (sarin), while others are less volatile and can contaminate surfaces for an extended period of time (VX, mustard gas)[2, 6]. Chemical stability, solubility and reactivity determine both the mode of dispersion and the mechanisms of degradation and transformation in the biological environment[5, 6].

An important characteristic of chemical warfare agents is their ability to rapidly interact with biomolecules, leading to the formation of degradation products and biological adducts. These transformations complicate the direct identification of the intact compound in biological samples, requiring the use of specific biomarkers to confirm exposure[3, 5, 7]. Thus, the analysis of chemical agents is not limited to the detection of the initial substance, but involves a complex approach, based on the identification of the products resulting from reactions in the body.

In conclusion, chemical warfare agents represent a diverse class of compounds, with distinct properties and mechanisms of action, which require the development of adapted analytical and operational strategies. Understanding their classification, properties and behavior is a key element for the development of detection, protection and intervention methods in the field of chemical security[1, 7].

The classification of chemicals under the Chemical Warfare Convention (CWC) is based on criteria related to toxicity, legitimate uses and potential for military use. Thus, compounds are organized into three main categories (Schedules 1, 2 and 3)26, each reflecting the level of risk and the applicable control regime. A synthetic representation of these categories and the main associated characteristics is presented in Figure 1.1.

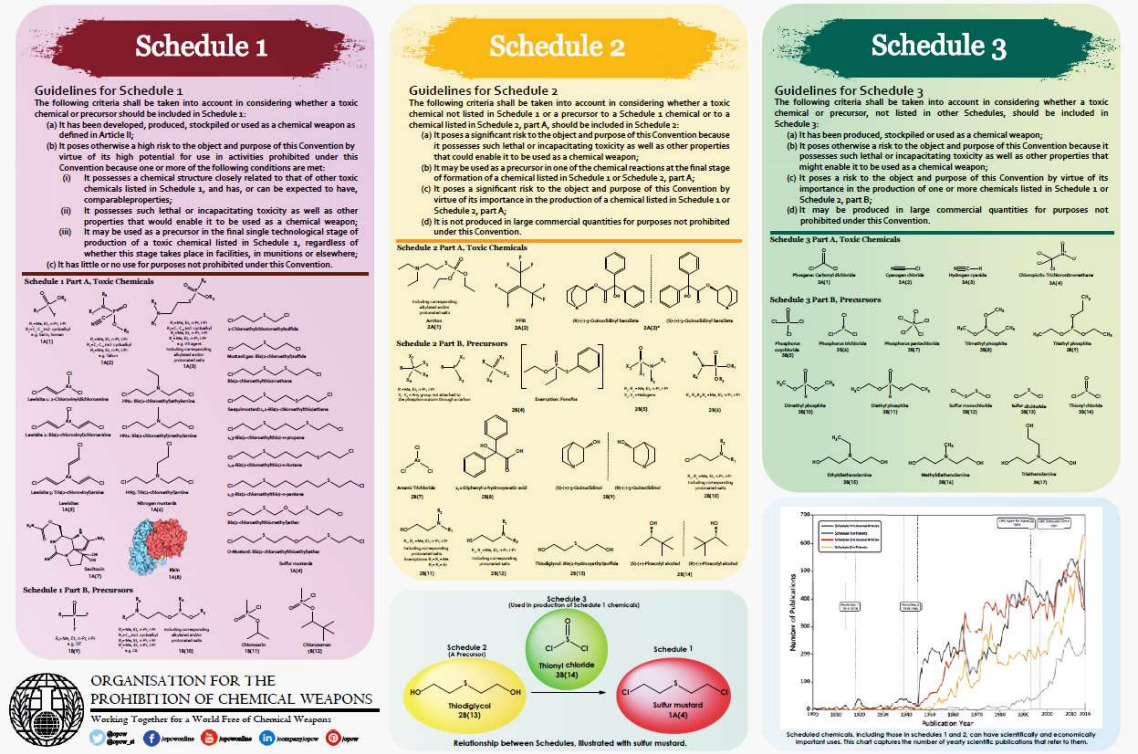


Figure 1.1. Classification of chemicals according to the Chemical Weapons Convention[8] (CWC)

1.2. Mechanisms of biological action of vesicant and nerve agents

The analysis of biological mechanisms of action is important not only for understanding toxic effects, but also for substantiating the experimental approaches adopted in this thesis. The particularities of chemical reactions and interactions with biological systems directly influence the choice of analytical methods for identifying exposure biomarkers, as well as the design of advanced materials intended for decontamination and therapeutic intervention. Thus, the correlation of molecular mechanisms with detection and response strategies allows the development of integrated solutions, adapted to the complexity of real exposure scenarios. Next, the main mechanisms of action of vesicant and nerve agents are presented, with emphasis on the processes relevant to the experimental approaches investigated.

Nerve agents, especially organophosphorus compounds, act by irreversibly inhibiting the enzyme acetylcholinesterase (AChE), which is essential in regulating nerve impulse transmission. By phosphorylating the enzyme's active site, these compounds cause excessive accumulation of acetylcholine at the synapse, leading to continued stimulation of cholinergic receptors. This hyper activation is manifested by severe muscarinic and nicotinic effects, including bronchospasm, hypersecretion, convulsions, and ultimately respiratory paralysis. Over time, the enzyme–inhibitor complex can undergo a cellular aging process, which stabilizes the bond and makes enzyme reactivation extremely difficult, reducing the effectiveness of treatment if intervention is not rapid.

In contrast to blister agents, for nerve agents there are well-established therapeutic regimens, based on the injectable administration of atropine, oximes and anticonvulsants, which can counteract the toxic effects if applied promptly. However, the effectiveness of the treatment critically depends on the rapidity of the intervention and the type of agent involved.

Thus, although there are established antidotes, the complexity of the mechanisms of action and the variability of the therapeutic response highlight the need for continuous development of detection and intervention strategies. At the same time, in the case of vesicants, for which there is no well-defined specific antidote, research must be intensified towards the development of effective response solutions, capable of completing the lack of well-defined therapies.

Blister agents are reprezented by a class of toxic substances characterized by the ability to produce severe lesions in the skin, eyes and respiratory system, mainly through the alkylation mechanism of cellular components. Sulfur mustard and its analogs are the most studied and toxicologically relevant compounds, benefiting from analogies of their historical use and the complex biological effects they induce.

Since their use during World War I, blister agents have posed a major threat to individuals and have caused approximately two million casualties[8]. A clear understanding of these agents and their methods of action is essential to develop an effective decontamination strategies and therapeutic actions. Due to their high toxicity and restricted use, research has been conducted and many mechanisms remain to be studied in terms of identifying the mechanism of biological action of these compounds. Various decontamination solutions and detection technologies have been developed, which have proven to be effective in mitigating the time. However, a major obstacle seems to be the lack of adequate development of the toxicological mechanism of action of these compounds.

1.3. Specific biomarkers following exposure to chemical warfare agents

Understanding the mechanisms of biological action of blister and nerve agents represents the first step for interpreting toxic effects at the molecular and systemic levels, also constituting the basis for identifying specific markers of exposure, used in the analytical confirmation of contact with these compounds.

Starting from these mechanisms of action, the identification of biomarkers specific to exposure to chemical warfare agents represents a first measure for confirming exposure and assessing the toxicological impact on the body. In the present thesis, the development and optimization of analytical strategies for the detection of these biomarkers constitutes a central element, allowing the correlation of biochemical processes induced by toxic agents with modern analysis methods, as well as with the strategy for managing decontamination procedures and prophylactic response to combat the toxic effects of these classes of compounds.

Identification of chemical warfare agent exposure is based on the detection of specific biomarkers in biological matrices, such as plasma and urine. Due to the high reactivity and rapid transformations undergone by these compounds in the body, direct detection of the intact substance is often difficult, which is why their analysis is directed at their degraded products and adducts formed with biomolecules[9, 10].

In the case of nerve agents, the main biomarkers are represented either by intact compounds or by hydrolysis products, such as alkylmethylphosphonic acids (EMPA, IMAP, PMPA), detectable especially in urine samples[10, 11]. An important category of primary biomarkers is represented by adducts formed with plasma proteins, especially with butyrylcholinesterase (BChE) or albumin, where they can be highlighted as modified peptides

following enzymatic digestion[12, 13]. These provide precise information on exposure, having greater stability compared to free compounds.

For blister agents such as sulfur mustard and its analogues, biomarkers include hydrolysis products such as thiodiglycol (TDG) and its derivatives, which are frequently identified in urine, as well as adducts formed with plasma proteins, particularly at the nucleophilic groups in the polypeptide chains. Alkylation reactions form stable compounds that can be subsequently identified by sensitive analytical methods, providing clear evidence of exposure. Biomarker analysis requires the use of advanced analytical techniques such as LC-MS/MS and GC-MS/MS, which are capable of providing high selectivity and sensitivity[9, 14]. The choice of biomarker type depends on several factors, including the elapsed time of exposure, the stability of the compound, and the biological matrix being analyzed.

1.4. Analytical methods for identification specific biomarkers formed following exposure to chemical warfare agents

The identification of specific biomarkers formed following exposure to chemical warfare agents is achieved by using advanced analytical methods, capable of ensuring high sensitivity, selectivity and reproducibility, in the conditions of complex biological matrices, such as plasma and urine. The complexity of these samples, as well as the low concentrations of target compounds, require the development of integrated analytical strategies, which include both appropriate preparation steps and high-performance instrumental techniques[15].

Among the most common analytical methods are chromatography-mass spectrometry techniques, especially high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS). These techniques allow the identification and quantification of biomarkers based on specific precursor ion–product ion transitions, offering a high degree of selectivity by using the MRM (Multiple Reaction Monitoring) mode of operation.

LC-MS/MS is predominantly used for the analysis of polar or thermolabile compounds, being suitable for the identification of hydrolysis products and biological adducts, modified peptides resulting from the interaction of neurotoxic or vesicant agents with plasma proteins[16]. The advantages of this technique include the direct analysis of compounds under mild ionization conditions, as well as compatibility with a wide range of biomarkers.

On the other hand, GC-MS/MS is preferred for the analysis of volatile or semi-volatile compounds, as well as for their determination by derivatization reactions if the compounds are not sufficiently volatile. This technique provides efficient separation and characteristic fragmentation, being particularly useful for the confirmation of real compounds and for the analysis of hydrolysis products following derivatization reactions[17]. Although fragmentation under electron impact ionization (EI) conditions is more complex compared to LC-MS/MS, this method provides valuable structural information, useful in the identification process.

An important role in these identification methods is represented by the sample preparation step, which may include the extraction of compounds from the biological matrix, their purification, concentration and, in some cases, their derivatization. The choice of preparation method depends on the nature of the biomarker and the type of matrix analyzed, being important for obtaining reliable results[15].

1.5. Treatment and prophylaxis of exposure to chemical warfare agents

The development of analytical methods for the identification of biomarkers specific to exposure to chemical warfare agents is an essential step not only for confirming exposure, but also for substantiating decisions regarding medical intervention. The information obtained through advanced analytical techniques allows the assessment of the degree of exposure and the type of agent involved, decisive aspects for the choice and efficiency of treatment and prophylaxis strategies. In this sense, the correlation of analytical data with biological action mechanisms contributes to the optimization of the therapeutic response and the development of solutions adapted to real exposure scenarios. In the present thesis, this integrated approach highlights the importance of the connection between detection and intervention, justifying the detailed analysis of the treatment and prophylaxis options presented below. The management of exposure to chemical warfare agents is not limited to their identification and decontamination, but also involves the implementation of effective treatment and prophylaxis strategies, adapted to the toxic mechanisms specific to each class of compounds. The complexity of these interventions derives from the diversity of induced biological effects, as well as from the absence, in some cases, of specific antidotes, which requires differentiated and multidisciplinary therapeutic approaches.

A distinct category of nerve agents are the Novichok series, developed by Soviet military programs during the Cold War. These new-generation organophosphorus agents were designed to be extremely toxic and to evade detection by standard methods used at the time.

Chemically, the Novichoks are acetylcholinesterase inhibitors, similar to G- and V-series agents, but may be as toxic as or even more[18, 19]. Exposure results in the rapid onset of severe cholinergic symptoms, including miosis, convulsions, and respiratory failure.

Interest in this class of compounds has increased following recent incidents, including the poisoning of political opponent Alexei Navalny in 2020, the use of an agent from the Novichok family was confirmed[20]. This event highlighted both the persistence of these substances in a geopolitical context and the need to develop advanced detection methods and effective countermeasures.

Regarding to nerve agents, treatment of acute poisoning with this class of compounds includes decontamination, respiratory support, and administration of specific antidotes. Decontamination is achieved by removing contaminated clothing and washing the skin with water and appropriate solutions, including sodium hypochlorite. Atropine is used to antagonize cholinergic effects and is administered repeatedly until secretions are controlled. Oximes, such as pralidoxime (2-PAM), reactivate acetylcholinesterase and contribute to the restoration of neuromuscular function[21, 22]. Convulsions are treated with benzodiazepines, with midazolam being preferred for its rapid action. For prophylactic purposes, pyridostigmine bromide can be administered before exposure to reversibly protect the enzyme from irreversible inhibition[23]. Recent research has highlighted the use of bioscavengers, such as butyrylcholinesterase, as well as the potential of some neuroprotective agents, including ketamine and dexanabinol[24].

For a better understanding of the mechanisms involved in the toxicity of nerve agents, as well as the mode of action of the antidotes used in treatment, Figure 1.2 schematically presents the inhibition of acetylcholinesterase and the intervention of atropine at the level of cholinergic transmission. The figure shows both the accumulation of acetylcholine following enzymatic inhibition and the antagonistic effects of atropine on muscarinic receptors. Under physiological conditions, acetylcholinesterase rapidly hydrolyzes acetylcholine into choline

and acetate, ensuring the termination of the nerve signal. In the presence of organophosphorus agents, the enzyme is phosphorylated at the active site, which determines its inhibition and the accumulation of acetylcholine at the synaptic level, leading to excessive cholinergic stimulation. This phenomenon is manifested by severe muscarinic and nicotinic effects, such as hypersecretion, bronchoconstriction, bradycardia and muscle paralysis. Atropine intervenes through competitive antagonism at muscarinic receptors, reducing the peripheral effects of excess acetylcholine, without significantly influencing nicotinic receptors, which is why it is used in combination with oximes in the treatment of poisoning with neurotoxic agents.

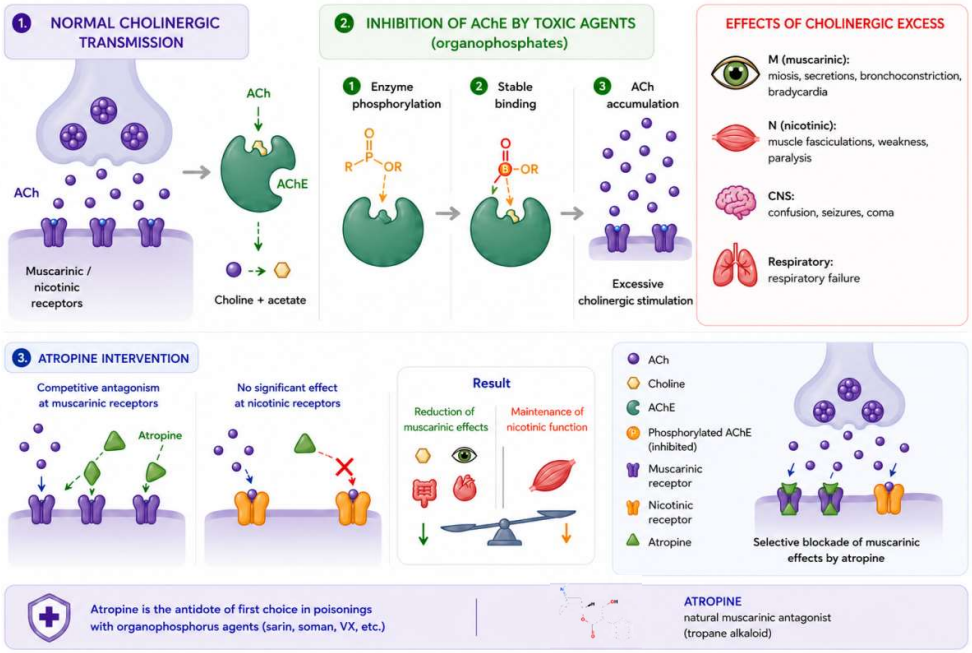


Figure 1.2. *Schematic representation of the mechanism of acetylcholinesterase inhibition and the mode of action of atropine at the level of cholinergic transmission (BioRender, accessed March 2026)*

The importance of atropine in the treatment of poisoning with nerve agents, as well as the limitations associated with conventional systemic administration, has led to the exploration of alternative antidote delivery systems. In this context, the use of hydrogels for the controlled release of atropine represents an innovative direction, allowing for sustained and localized administration of the active substance, with the potential to improve therapeutic efficacy and reduce adverse effects. The integration of these systems into chemical incident response strategies reflects the transition from reactive approaches to advanced solutions, with both therapeutic and prophylactic roles.

Treatment of sulfur mustard exposure is mainly symptomatic, in the absence of a specific antidote. Intervention includes evacuation from the contaminated area, decontamination[25] by removing clothing and washing the skin, followed by treatment of skin lesions with corticosteroids and antimicrobial agents[27, 28]. In the case of extensive involvement, the approach is similar to the management of burns. In the case of ocular exposure, eye irrigation, topical antibiotics and steroids are necessary[27]. Chemical pneumonia is characterized by productive cough, dyspnea and fever and occurs 12–24 hours after inhalation[29]. Infections generally occur between the third and fifth days, starting with high fever, pulmonary infiltrates, increased sputum secretion and change in its color. The

formation of seudomembranes may require bronchoscopy[30]. Bronchodilators and glucocorticoids are beneficial in the case of bronchospasm. Bone marrow suppression begins on the third day after exposure and reaches a maximum at 7–14 days; in this case, stimulation of hematopoiesis (by granulocyte growth factors) or, in severe cases, bone marrow transplantation may be necessary[27]. In experimental animal studies, the beneficial effect of vanilloid-type compounds and N-acetylcysteine has also been observed[31].

In the case of other blister agents, such as lewisite, the therapeutic approach has common elements, but is differentiated by the existence of a specific antidote. Treatment consists of rapid removal of victims from the contaminated area by appropriately equipped personnel, followed by decontamination by removing clothing and washing the skin. Unlike mustard gas, there is a specific antidote for lewisite, dimercaprol (BAL), which is used to prevent systemic toxicity. Chelating agents such as 2,3-dimercaptopropane-1-sulfonate (DMPS), meso-2,3-dimercaptosuccinic acid (DMSA) and its derivatives may also have beneficial effects following oral or intravenous administration[2].

CHAPTER 2

DECONTAMINATION STRATEGIES AND DEVELOPMENT OF ADVANCED MATERIALS FOR CBRN AND BIOMEDICAL APPLICATIONS

2.1. General principles of chemical warfare decontamination

Decontamination of chemical warfare agents is the first countermeasure in the management of CBRN exposure situations, with the main objective of reducing or eliminating contamination from surfaces, equipment, the environment or living organisms. The decontamination process aims to limit the toxic effects of toxic agents, prevent the spread of contamination and protect personnel involved in the intervention.

The general principles of decontamination are based on a combination of physical and chemical mechanisms, adapted according to the nature of the chemical agent and the type of contaminated surface. From a physical point of view, decontamination involves the mechanical removal of the contaminant by washing, dilution or adsorption on porous materials. These methods are particularly effective for persistent agents, reducing the amount of contaminant available for absorption or volatilization.

Chemical decontamination involves the transformation of toxic agents into less dangerous compounds, through hydrolysis, oxidation or neutralization reactions. The choice of decontamination agent plays an important role and depends on the chemical structure of the target compound. For example, organophosphorus agents can be degraded by hydrolysis or oxidation reactions, while blister agents, such as sulfur mustard, can undergo oxidation or nucleophilic substitution reactions. In this context, the efficiency of the process is influenced by factors such as pH, temperature, contact time and the nature of the environment in which the reaction takes place[26].

An important aspect of decontamination is the classification of chemical agents according to persistence. Non-persistent agents, such as sarin, evaporate quickly and require rapid interventions to limit exposure, while persistent agents, such as VX or sulfur mustard,

can remain on surfaces for long periods, requiring more intensive and durable decontamination methods.

Recently, research has focused on the development of advanced functional materials, capable of combining adsorption and degradation processes in a single system. These include nanostructured materials, metal oxides, zeolites or metal-organic frameworks, which offer extended active surfaces and improved reactive properties. The integration of these materials into application systems, such as suspensions or hydrogels, allows the development of efficient solutions for rapid and controlled decontamination[27, 28].

Decontamination of chemical warfare agents is a complex process, which requires an approach adapted to the type of agent and the intervention conditions. Understanding the mechanisms involved and developing innovative solutions significantly contribute to increasing the efficiency of the intervention and reducing the risks associated with exposure to toxic compounds.

2.2. Operational management and conventional methods of decontamination of chemical warfare agents

The general principles of decontamination provide the conceptual framework necessary for understanding the processes involved in reducing chemical contamination, but their effective application in real conditions requires integration into a rigorous operational system. Managing chemical incidents involves the spatial organization of the affected area, establishing intervention stages and using validated decontamination methods, adapted to the type of agent and environmental conditions. In the present thesis, this point has an important role in substantiating subsequent experimental directions, allowing the correlation of operational requirements with the development of advanced materials intended for decontamination. Next, the main operational management strategies and conventional methods used in the decontamination of chemical warfare agents are presented.

The operational management of chemical warfare agent decontamination is a set of procedures and organizational measures designed to limit the effects of exposure and prevent the spread of contamination in CBRN emergencies. It involves the rapid coordination of resources, risk assessment and the implementation of standardized intervention protocols, adapted to the type of chemical agent and the environment.

A main element of operational management is the establishment of intervention zones, delimited according to the degree of contamination: the hot zone, where contamination is maximum and access is strictly controlled; the warm zone, intended for decontamination operations; and the cold zone, where support and monitoring activities are carried out. This organization allows for the reduction of the risk of secondary contamination and the protection of the personnel involved[29].

Conventional decontamination methods include physical and chemical procedures, applied depending on the type of chemical agent and the contaminated surface. Chemical decontamination involves the use of reactive agents that transform toxic compounds into less dangerous products. The most commonly used decontamination agents include solutions based on hypochlorite, peroxides or alkaline compounds, which act through oxidation or hydrolysis mechanisms. These methods are particularly effective for neutralizing organophosphorus agents and blistering agents, but may have limitations related to compatibility with the treated surfaces or the generation of secondary degradation products[30].

Another important aspect is the intervention time, which directly influences the efficiency of the decontamination process. Rapid interventions can significantly reduce the

degree of contamination and limit the absorption of chemical agents in the body or in materials. At the same time, environmental conditions, such as temperature, humidity and type of surface, can affect the efficiency of the methods applied.

Although conventional decontamination methods are the basis for emergency management, they have limitations related to efficiency in the case of persistent agents, high resource consumption and environmental impact. In this context, current research is oriented towards the development of alternative solutions, based on functional materials and reactive systems, capable of providing more efficient and sustainable decontamination[31].

2.3. Hydrogels and advanced biomaterials

The limitations of conventional decontamination methods, highlighted both from the perspective of efficiency in real conditions and compatibility with different types of surfaces and materials, have led to the orientation of research towards the development of advanced material systems, capable of meeting current requirements in the field of chemical safety. In this context, polymeric biomaterials, especially hydrogels, have attracted considerable interest due to their specific properties, such as the ability to retain significant amounts of liquid, to adhere to surfaces and to allow the integration of active compounds involved in adsorption and chemical degradation processes. These characteristics allow the simultaneous approach of several stages of the decontamination process, contributing to reducing the risk of contaminant redistribution and improving control over the process.

In the present thesis, the exploration of these materials has the role of responding to the limitations identified at the level of conventional methods, by developing systems capable of combining the retention and neutralization of chemical agents in a controlled manner and adapted to real exposure scenarios. Next, the main characteristics of hydrogels and polymeric biomaterials are presented, as well as their potential in decontamination applications.

Hydrogels are an important class of functional biomaterials, characterized by the ability to retain significant amounts of water in their three-dimensional crosslinked structure. Due to this property, hydrogels show a structural and functional similarity to the extracellular matrix, which gives them high biocompatibility and recommends them for biomedical and protective applications in the CBRN field.

From a structural point of view, hydrogels are formed by polymer networks stabilized by physical or chemical bonds, which determine the mechanical properties, the degree of swelling and the transport behavior of substances. The polymers used can be of natural origin (alginate, gum arabic, gelatin) or synthetic, each category presenting specific advantages in terms of biocompatibility, stability and control of the final properties of the material[32].

An important aspect in the development of functional hydrogels is the possibility of functionalization, by integrating active compounds or nanostructures into the polymer network. This approach allows the modification of material properties, such as increasing mechanical strength, adjusting rheological behavior or introducing additional functions, such as adsorption, degradation or controlled release of active substances.

In the context of CBRN applications, hydrogels present great interest due to their ability to act simultaneously as barrier and decontamination systems. Through their swelling and adhesion properties, they can be applied directly to contaminated surfaces, facilitating the capture and retention of chemical agents. At the same time, functionalization with reactive materials, such as metal oxides or metal-organic frameworks, allows the initiation of

degradation processes of toxic compounds, contributing to the rapid reduction of contamination[33].

In addition to applications in decontamination, hydrogels are also investigated as systems for the controlled release of active substances, having the ability to transport and release therapeutic compounds in a time-controlled manner. This property is particularly important in chemical agent exposure scenarios, where the effective administration of antidotes can significantly influence the clinical outcome[34].

Recently, research has focused on the development of composite and nanostructured hydrogels, capable of combining the structural properties of polymers with the functionality of advanced materials. This approach allows for the creation of multifunctional systems, adaptable to different operational and biomedical requirements[35].

Functional hydrogels and biomaterials represent a versatile platform for the development of innovative solutions in the field of decontamination and chemical protection, as well as in biomedical applications, offering the possibility of integrating multiple functions into a single system.

2.4. Modified hydrogels for decontamination of surfaces exposed to chemical warfare agents

Although hydrogels and polymeric biomaterials show promising properties in the context of biomedical and environmental applications, their use in the field of chemical warfare agent decontamination requires the adaptation of their composition and structure to meet the specific requirements of exposure scenarios. The integration of active components, such as inorganic particles or porous structures with a role in adsorption and degradation, allows the performance of these systems to be extended beyond simple contaminant retention, towards efficient chemical neutralization processes.

In the context of the development of modern decontamination strategies, functionalized hydrogels have attracted increased interest due to their ability to combine favorable structural properties with active chemical functionalities. These systems represent an advanced class of materials, capable of acting simultaneously through mechanisms of adsorption, retention and degradation of chemical warfare agents[36].

Functionalized hydrogels are obtained by integrating active components into the polymer network, such as nanoparticles, metal oxides or metal-organic frameworks (MOFs). This functionalization allows for the increase of the active surface area and the introduction of reactive centers capable of interacting directly with toxic agents. Depending on the nature of the introduced components, these materials can favor hydrolysis, oxidation or catalysis processes, contributing to the transformation of chemical agents into less dangerous products[35].

A major advantage of hydrogels in decontamination applications is their swelling behavior and the ability to adhere to surfaces, including the skin. This property allows the formation of a protective layer that limits the dispersion of the contaminant and facilitates its capture in the structure of the material. At the same time, the peelable nature of certain formulations allows the rapid removal of the contaminant by simply mechanically removing the hydrogel, reducing the risk of secondary contamination.

The integration of nanostructured materials, such as metal oxides (ZnO, TiO_2) or MOF structures, leads to the development of multifunctional systems, in which adsorption processes

are complemented by catalytic degradation mechanisms. These materials show high efficiency in the interaction with organophosphorus or blister agents, due to their extensive active surfaces and the ability to generate reactive species involved in the degradation of toxic compounds[37, 38].

In addition, functionalized hydrogels can be designed to respond to external stimuli, such as pH, temperature or UV radiation, which allows the control of decontamination processes and the adaptation of the material to specific conditions of use. This flexibility opens up important perspectives for the development of intelligent systems, capable of acting effectively in various exposure scenarios[39, 40].

Compared to conventional decontamination methods, functionalized hydrogels offer significant advantages, such as reducing the consumption of chemical solutions, limiting the environmental impact and increasing efficiency by combining several mechanisms in a single system. In this context, these materials represent a promising direction for the development of modern and efficient solutions in the field of chemical protection.

In conclusion, functionalized hydrogels constitute a versatile and innovative platform for the decontamination of chemical warfare agents, offering the possibility of integrating multiple functionalities and adapting to the specific requirements of CBRN scenarios.

2.5. Biocompatible hydrogels and biomedical applications

In the context of exposure to chemical warfare agents, especially blister agents, skin damage is one of the main consequences, characterized by the appearance of skin lesions, inflammation and delayed healing processes. In these conditions, the decontamination approach cannot be separated from the need to manage the resulting tissue damage, which requires the development of material systems capable of combining the properties of contaminant removal with those of support for tissue regeneration. Biocompatible hydrogels, frequently used in wound healing applications, present relevant advantages in this regard, due to their ability to maintain a moist environment, protect the affected area and facilitate tissue repair processes. In addition, these systems can be adapted to allow the controlled release of active substances, contributing not only to tissue protection and regeneration, but also to localized therapeutic intervention. In the present thesis, this direction is capitalized on by developing hydrogels capable of ensuring the controlled release of atropine, highlighting the potential of these materials to integrate multiple functions in a single system, from decontamination to treatment. The main characteristics of biocompatible hydrogels and their applications in the biomedical field are presented, with an emphasis on their relevance in the management of injuries associated with exposure to chemical warfare agents.

Biocompatible hydrogels represent an advanced class of biomaterials, characterized by the ability to interact favorably with biological systems, without inducing significant adverse reactions. Due to their hydrated three-dimensional structure, these materials exhibit properties similar to the extracellular matrix, which gives them high potential for biomedical applications, including tissue engineering, regeneration and controlled delivery systems of active substances[41].

The biocompatibility of hydrogels is influenced by the nature of the polymers used, the degree of crosslinking and the presence of any functional components. Polymers of natural origin, such as gelatin, alginate or gum arabic, are frequently used due to their biodegradability and compatibility with biological tissues. At the same time, their

combination with synthetic polymers or nanostructured materials allows the adjustment of the mechanical and functional properties of the final system.

A major area of interest is the use of hydrogels as systems for the controlled release of drugs. Their porous structure allows the incorporation of active substances and their release in a controlled manner, influenced by factors such as diffusion, degradation of the polymer network or external stimuli. This feature is essential in scenarios of exposure to chemical warfare agents, where the efficient and controlled administration of antidotes can significantly contribute to reducing toxic effects[42].

Biocompatible hydrogels are also being investigated for applications in wound healing and skin protection, due to their ability to maintain a moist environment favorable to tissue regeneration and to act as a barrier against contaminants. In this context, the integration of bioactive compounds or antimicrobial agents can improve the performance of these materials, contributing to the prevention of infections and the acceleration of the healing process.

In addition, the development of smart hydrogels, capable of responding to stimuli such as pH, temperature or the presence of specific compounds, opens new perspectives in the biomedical field. These systems can adapt the release of active substances according to local conditions, offering increased control over therapeutic processes[43].

Biocompatible hydrogels represent a versatile platform for biomedical applications, offering the possibility of developing efficient systems for protection, regeneration and treatment. The integration of these materials into complex response strategies to exposure to chemical warfare agents highlights their potential in developing innovative and tailored solutions to current requirements.

Conclusions

The critical analysis of the literature data highlights the complexity and multidisciplinary nature of the field of chemical warfare agents, both from the perspective of biological mechanisms of action and of the strategies required for identification, decontamination and exposure management. The diversity of these compounds, reflected in varied physicochemical properties, different levels of persistence and distinct toxic mechanisms, requires the development of integrated and adaptable approaches, capable of responding to increasingly complex exposure scenarios.

From an analytical point of view, the identification of specific biomarkers in biological matrices such as plasma and urine is a central element in confirming exposure, especially in conditions in which the initial compounds undergo rapid transformations in the body. The use of advanced analysis techniques, in particular LC-MS/MS and GC-MS/MS, allows obtaining results with a high degree of sensitivity and selectivity, contributing to an unambiguous identification of toxic agents and their degradation products or biological adducts. The complementarity of these methods highlights the need for an integrated analytical approach, in which the selection of the technique is dictated by the nature of the biomarker and the context of the analysis.

In parallel, decontamination strategies have evolved significantly, from conventional methods based on physical and chemical processes, to modern solutions that capitalize on the properties of advanced functional materials. Although classical methods remain basic for rapid interventions in the field, they present limitations related to environmental impact, which has led to the orientation of research towards innovative systems, capable of combining multiple mechanisms of action.

In this context, hydrogels and functionalized biomaterials emerge as particularly promising platforms, due to their ability to integrate structural and functional properties in a single system. Through the possibility of functionalization with nanoparticles, metal oxides or metal-organic frameworks, these materials can simultaneously ensure the adsorption, retention and degradation of chemical agents, offering an efficient and controlled approach to the decontamination process. At the same time, the biocompatible nature of certain formulations opens up important prospects for biomedical applications, including in skin protection and in the controlled release of active substances.

Therefore, the theoretical framework presented highlights the need to develop multifunctional systems, capable of simultaneously meeting the requirements of detection, decontamination and protection. The integration of high-performance analytical methods with advanced materials and innovative intervention strategies represents an essential direction for strengthening capacities in the field of chemical security and for aligning with international standards. This integrated approach constitutes the foundation of the experimental research presented in this thesis, oriented towards the development of efficient solutions applicable in real scenarios of exposure to chemical warfare agents.

PART II – ORIGINAL CONTRIBUTIONS

EXPERIMENTAL STUDY

CHAPTER 3.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF ANALYTICAL STRATEGIES FOR THE IDENTIFICATION OF BIOMARKERS OF EXPOSURE TO CHEMICAL WARFARE AGENTS

The results presented in this chapter were evaluated in the context of participation in international interlaboratory proficiency testing programs conducted under the aegis of the OPCW, during the period 2024-2026, where compounds in unknown samples were identified and reported correctly. In parallel, the developed analytical methods were applied and verified in international exercises conducted in collaboration with VERIFIN, University of Helsinki, Finland, active collaboration during the period 2022-2026.

3.1. The necessity of development integrated analytical strategies for identifying biomarkers of exposure to chemical warfare agents

The identification of exposure to chemical warfare agents in biological matrices represents a major analytical challenge, determined by their complexity, the low concentrations of target compounds and the rapid transformations they undergo in the body. In most cases, the initial compounds are no longer present in their intact form, being transformed into hydrolysis products or adducts with biomolecules, which requires the analysis to be oriented towards the identification of specific and stable biomarkers. In this context, analytical methods must ensure a high level of sensitivity and selectivity, as well as the ability to differentiate between compounds with similar structures or present in complex matrices, such as plasma and urine. Also, the requirements imposed at international level regarding the confirmation of exposure require the use of rigorous analytical strategies, based on multiple and correlated identifications. Therefore, the development of integrated analytical strategies, combining complementary techniques and appropriate sample preparation methods, becomes extremely important to obtain reliable results and to ensure unambiguous identification of specific biomarkers. This chapter addresses these issues by developing and optimizing analytical methods adapted to different classes of compounds, in order to highlight biomarkers in relevant biological matrices.

3.2. Preparation methods for biological samples (urine and plasma)

a) Preparation of urine samples

The urine samples used in this study underwent a controlled spiking process with different classes of chemical warfare agents, including nerve and blister agents, in order to study the evaluation of biomarkers formed depending on the type of compound. In general, in the spiked matrix, mainly hydrolysis products are identified, resulting both from the reactivity of the agents in the aqueous environment and as a result of metabolic processes. These metabolites are related to biomarkers relevant to exposure and are eliminated through excretion. An exception is the A series compounds, which offer high chemical stability and can also be detected in unmodified form.

For a better understanding of the analytical strategies applied, a conceptual scheme was developed (Figure 3.1), which illustrates the main transformation pathways of chemical agents in the biological environment and the types of biomarkers resulting, correlated with the analytical techniques used. In order to control interferences, the samples were analyzed together with blank samples, prepared under the same conditions, to check the background signal.

The preparation of urine samples was carried out by applying multiple strategies, adapted to the class of compounds analyzed and the type of biomarker pursued. These included:

✓ solid phase extraction (SPE), used to isolate and concentrate alkylphosphonic acids and specific metabolites, for analysis by LC-MS/MS;

✓ chemical derivatization (with PFB-Br or HFBI), applied to increase the volatility and detectability of the compounds in GC-MS/MS analysis;

✓ solvent exchange procedures, for adapting the samples between the two analytical techniques (GC-MS/MS and LC-MS/MS);

✓ direct dilution of the sample, used as a rapid for screening by LC-MS/MS;

✓ specific chemical treatment (reduction with $TiCl_3$), applied to highlight β-lyase metabolites in the case of vesicant applications.

Generally, the preparation procedures involved extraction, purification, concentration and, where appropriate, derivatization steps, depending on the analytical nature and the analytical technique used. The choice of the preparation method was made in such a way as to ensure an optimal compromise between analyte recovery, elimination of matrix interferences and compatibility with the instrumental method.

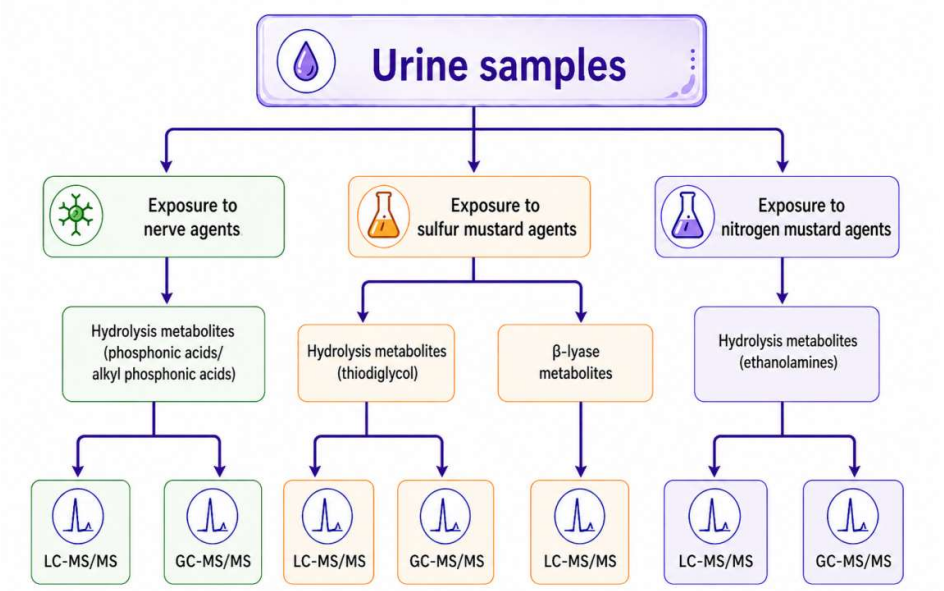


Figure 3.1. *Flowchart of biomarkers of exposure to nerve agent, sulfur mustard and nitrogen mustard, and analytical methods for detection (BioRender, accessed April 2026)*

b) Preparation of plasma samples

The handling of plasma samples was carried out in strict compliance with biosafety regulations, as they are considered potentially infectious. Since the concentrations of

unreacted chemical warfare agents are generally low in biological samples, the analysis is predominantly based on the identification of biomarkers formed following their interaction with plasma components. To maintain the stability of the compounds of interest, the samples were handled under controlled conditions and stored at low temperatures (−20 °C or lower) to prevent degradation and growth of microorganisms. The analytical strategies applied to plasma samples were based on the nature of the specific biomarkers formed following exposure to nerve and blister agents. In the case of nerve agents, the identification was based on three main types of biomarkers: hydrolysis metabolites, protein adducts and regenerated compounds. Protein adducts, resulting from the direct reaction of agents with enzymes or plasma proteins, represent primary biomarkers of exposure, offering a high degree of certainty. In certain situations, agents can also be identified in intact or regenerated form, by applying specific techniques, such as refluorination, which allow the release of the original compound from biological structures.

For blister agents, the identification of biomarkers included hydrolysis metabolites, products resulting from β-lyase reactions and protein adducts, specific to each class of compounds (sulfur mustards and nitrogen mustards). The correlation of these biomarkers with appropriate analytical techniques (LC-MS/MS and GC-MS/MS) allowed an integrated approach to the identification process.

In order to obtain specific biomarkers, plasma samples were subjected to a controlled spiking process, followed by incubation under conditions close to physiological ones, to allow the development of binding reactions and the formation of derivative compounds. To control for interferences, the samples were analyzed together with blank samples, prepared under the same conditions.

The preparation of plasma samples involved the application of multiple strategies, adapted to the type of biomarker pursued and the analytical technique used, including:

✓ enzymatic digestions (pepsin, pronase), used to highlight protein adducts in the form of modified peptides or amino acids;

✓ reactivation reactions (refluorination), applied to regenerate nerve agents from protein adducts and identify them by GC-MS/MS;

✓ solid phase extraction (SPE), used to isolate and concentrate hydrolysis metabolites;

✓ chemical derivatization (PFB-Br, HFBI), to increase the volatility and detectability of compounds in GC-MS/MS analysis;

✓ protein precipitation and extraction with organic solvents, to separate compounds of interest from the biological matrix;

✓ hydrolysis procedures and specific chemical treatments, used to release compounds from adducts or transform them into detectable forms;

✓ direct dilution of the sample, used as an alternative method in situations of very low concentrations, for rapid analysis by LC-MS/MS.

Figure 3.2.describes the flow chart of biomarkers of exposure to the neurotoxic agent, sulfur mustard and nitrogen mustard, and analytical methods for detection.

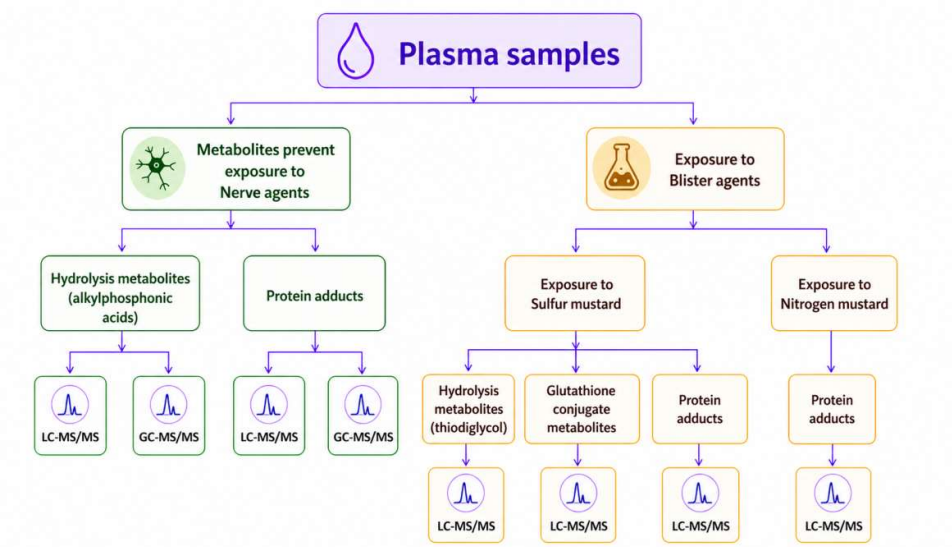


Figure 3.2. *Flowchart of biomarkers of exposure to nerve agent, sulfur mustard and nitrogen mustard, and analytical methods for detection (BioRender, accessed April 2026)*

3.3. Development and optimization of the analytical method for identification of specific biomarkers to exposure to chemical warfare agents

Chromatographic analyses were performed using gas chromatography (GC) and ultra-high performance liquid chromatography (UHPLC) techniques, both coupled with tandem mass spectrometry (MS/MS). For GC-MS/MS determinations, a TRACE 1310 system coupled with a TSQ 9000 mass spectrometer (Thermo Scientific) was used, operated in EI and CI ionization modes, while LC-MS/MS analyses were performed with an ACQUITY I-Class Plus UPLC system coupled with a Xevo TQ Absolute mass spectrometer (Waters).

GC-MS/MS analysis

The chromatographic separation was performed on a TG-5MS capillary column (30 m × 0.25 mm × 0.25 µm), using helium as the carrier gas. The temperature program was optimized to ensure efficient separation of the compounds, with a heating ramp up to high temperatures (300°C).

Detection was performed in the following ionization modes:

- EI (Electron Ionization) – characterized by extensive fragmentation, used to obtain detailed structural spectra;
- CI (Chemical Ionization) – characterized by reduced fragmentation and increased sensitivity, used to highlight molecular or quasi-molecular ions.

Tandem mass spectrometry was performed in SRM mode, using argon as collision gas.

GC-MS/MS method optimization

Method optimization was performed in stages, aiming to obtain an optimal compromise between sensitivity and selectivity:

- initial analysis in full scan mode, to identify characteristic mass spectra and evaluate chromatographic separation;
- selection of precursor ions, preferably molecular or high-abundance ions;

o identification of product ions and optimization of collision energies (CE) to maximize response;

o definition of SRM transitions, selecting 2–4 transitions/compound, depending on the ionization mode;

o method verification on test samples in relevant matrix, to evaluate analytical performance.

In general, the CI mode generated a lower number of transitions, due to more limited fragmentation compared to the EI mode.

Identification criteria

Compound identification was performed based on:

✓ retention time;

✓ selected MRM transitions;

✓ ion ratio between confirmation and quantification transitions.

At least two transitions were used for each compound, a main transition (Q) for quantification, one or more secondary transitions (q) for confirmation. The q/Q ratio was compared between samples and reference standards, ensuring confirmation of the identity of the compounds under conditions of high selectivity.

Figure 3.3 shows the operating diagram of a GC-MS/MS and the fragmentation mode of the analysts.

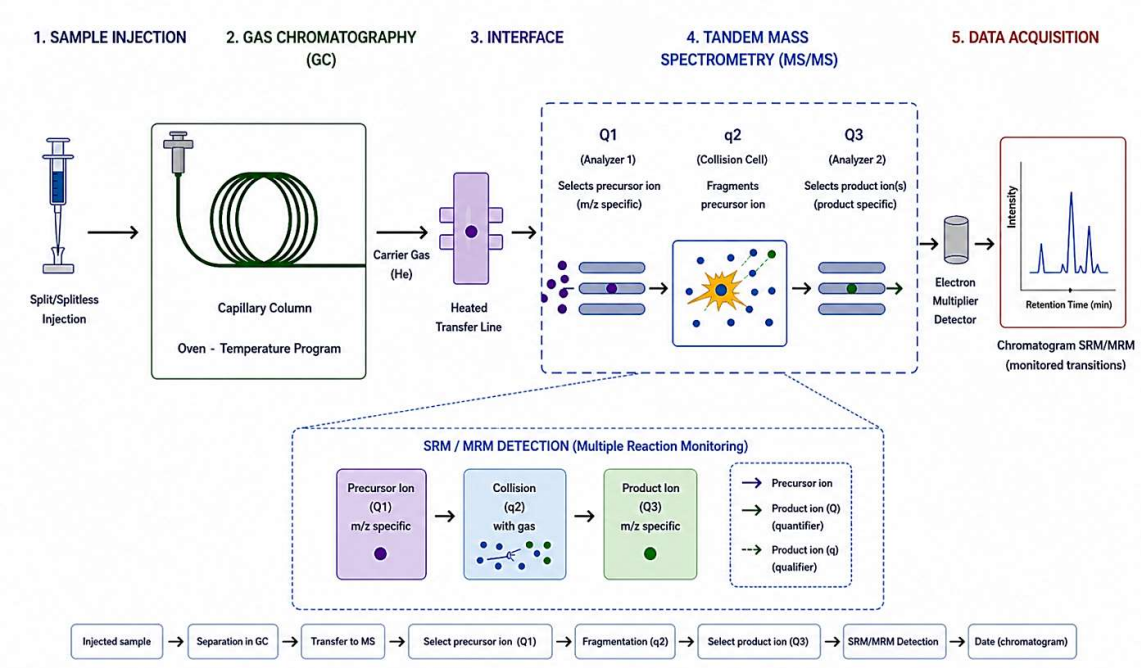


Figure 3.3. GC-MS/MS analysis scheme (BioRender, accessed April 2026)

LC-MS/MS analysis

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used for the analysis of polar, non-volatile or thermolabile compounds, difficult to determine by GC-MS/MS without derivatization. The technique offers high sensitivity and selectivity, being suitable for the identification of target compounds in complex biological matrices.

Chromatographic conditions

The separation was performed on a C18 column (100 mm × 2.1 mm, 1.7 μm), using mobile phases based on water and acetonitrile, both acidified with 0.1% formic acid. An elution gradient was applied, used as a general method for most compounds, ensuring an efficient and reproducible separation.

The chromatographic conditions were generally kept constant (column type, mobile phases, gradient profile), which allowed the analysis of a wide spectrum of compounds without major modifications of the method. For compounds with high retention, the analysis time was extended (up to 30 min), preserving the gradient shape, but adjusting the duration of the steps. The column temperature was maintained at 45°C, and the autosampler at 15°C.

MS/MS detection

Detection was performed by electrospray ionization (ESI), in positive or negative mode, depending on the chemical nature of the compounds:

- ESI(+): for basic compounds;
- ESI(–): for acidic compounds.

The analysis was performed in MRM (Multiple Reaction Monitoring) mode, monitoring the transitions between the precursor ion and the product ions, which ensures high selectivity and reduces matrix interferences.

LC-MS/MS method optimization

The method optimization was performed in stages:

- determination of the precursor ion and the optimal ionization mode (ESI+/–) using reference standards;
- identification of the product ions through fragmentation experiments;
- adjusting collision energies (CE) to maximize the intensity and reproducibility of MRM transitions;
- optimizing chromatographic conditions for efficient separation of compounds and reduction of interferences;
- comparing retention times between samples and standards analyzed under the same conditions.

Identification criteria

The identification of compounds was performed based on:

- ✓ retention time;
- ✓ specific MRM transitions;
- ✓ the ion ratio between the quantification and confirmation transitions.

At least two transitions were used for each compound, a main transition (Q) for quantification and one or more secondary transitions (q) for confirmation. The q/Q ratio was compared with that of the reference standard, and falling within the accepted tolerance limits allowed reliable confirmation of the identity of the compounds.

In figure 3.4.the operating scheme of an LC-MS/MS and the fragmentation mode of the analysts are presented.

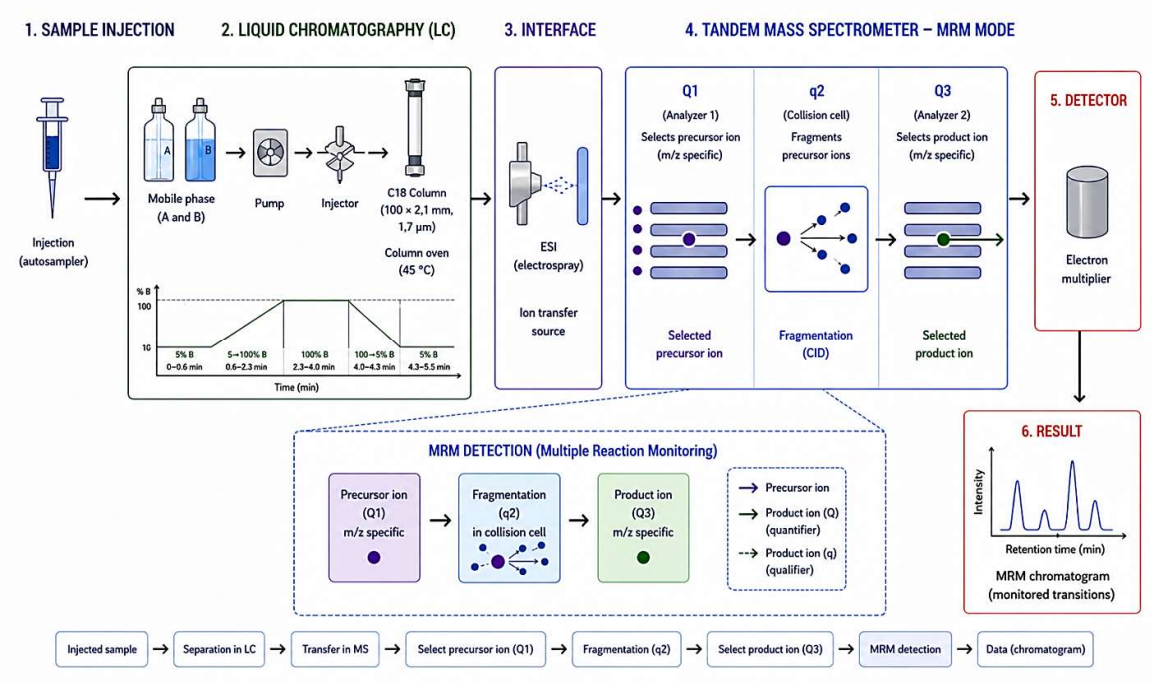


Figure 3.4. LC-MS/MS analysis scheme (BioRender, accessed April 2026)

3.4. Results and Discussion

3.4.1. Identification of specific biomarkers of exposure to blister and nerve agents in plasma and urine samples by LC-MS/MS

Several categories of specific biomarkers of exposure to blister and nerve agents were investigated by LC-MS/MS analysis, including hydrolysis products, intact compounds and adducts formed with biomolecules. The identification strategy was based on the development of MRM methods, by selecting characteristic precursor and product ions, optimizing collision energies and comparing retention times and ion ratios with those obtained for reference standards.

In the case of blister agents, LC-MS/MS analysis targeted both hydrolysis products and peptide adducts formed following the interaction of the compounds with plasma proteins. Hydrolysis products, such as thiodiglycol and its analogues, were used as relevant biomarkers of exposure, especially due to their stability in aqueous media. For TDG, although the theoretical ionization should have led to the formation of the protonated ion $[M+H]^+$with m/z 123, under the experimental conditions used the ion m/z 105, assigned to an episulfonium-type structure, which is more energetically stable. This ion was used as a precursor for the development of the MRM method, the transition m/z 105 → 45 being selected for quantification, and the transitions m/z 105 → 61 and m/z 105 → 87 for confirmation.

Hydrolyzed sesquimustard could be identified using the same analytical approach, the differentiation from sulfur mustard being made mainly on the basis of the retention time, influenced by the different molecular mass and polarity. The 1,3-bis(2-hydroxyethylthio)propane analog showed a more stable ionization behavior, being detected by the precursor ion m/z 197, and the transition m/z 197 → 123 was used as the quantitation transition. For triethanolamine, a hydrolysis product associated with nitrogen mustards, the

precursor ion m/z 150 was monitored in positive ESI, and the transition m/z 150 → 70 was selected as the main transition, due to the high signal intensity.

Peptide adducts of the blister agents were monitored as CPF-type fragments. For sulfur mustard, the S-HETE-CPF adduct was identified by the precursor ion m/z 470.17, and the transition m/z 470.17 → 105 was selected for quantitation, the product ion m/z 105 being characteristic of the sulfur fragment derived from mustard. The adducts corresponding to sesquimustard and the propyl-bridged analogue showed higher molecular masses, m/z 530.2 and m/z 544.2, respectively, requiring similar or slightly higher collision energies. In the case of the propyl-bridged analogue, the higher collision energy indicates increased structural stability, probably due to the extended aliphatic chain. For nitrogen mustard, the HN3-CPF adduct was identified by the precursor ion m/z 497.2, the transition m/z 497.2 → 364.1 being used for quantification.

For nerve agents, LC-MS/MS analysis allowed the identification of compounds in multiple forms: intact compounds, hydrolysis products, and protein adducts. Compounds in the A series were analyzed exclusively by LC-MS/MS, due to their low volatility and reduced compatibility with GC-MS/MS. A-230 and A-232 were detected in intact form, via precursor ions m/z 195.1 and m/z 211.1, and the common transition to m/z 74.1 was used as the quantitation transition. This observation supports the high chemical stability of the A-series compounds under LC-MS/MS conditions.

For A-234, the tyrosine adduct was identified, monitored by the precursor ion m/z 386.2. Identification of tyrosine adducts was possible only for Sarin and A-234, suggesting a more favorable formation or better stability of these biomarkers under the preparation conditions used. For A-230 and A-232, nonapeptide adducts were also identified, observed as doubly charged ions. This behavior is specific to high-mass peptide fragments, which contain multiple protonable sites and can simultaneously stabilize two positive charges.

The hydrolysis products of the A series compounds were also identified by LC-MS/MS. A-230-OH, A-232-OH and A-234-OH showed characteristic transitions to the product ion m/z 74, confirming the common nature of the fragmentation of organophosphorus compounds of this class. In addition, the PFB derivatives of these products, although initially prepared for GC-MS/MS analysis, could also be detected by LC-MS/MS, demonstrating the complementary and versatile nature of this technique.

In the case of VX, Soman and Sarin, the identification was based on the combination of nonapeptide adducts and hydrolysis products. For VX-NP and GD-NP, the doubly charged forms were preferred due to the better analytical response and efficient fragmentation. For GB-NP, the monocharged form was sufficiently stable and allowed the obtaining of characteristic transitions. The hydrolysis products EMPA, PinMPA and IpMPA were analyzed in negative ESI mode, as $[M-H]^-$ ions, and the transition to m/z 95 was used in all cases as the quantification transition, being characteristic of methylphosphonic acids.

Table 3.1 presents the main identified biomarkers, the collision energies and the main transitions for their identification by LC-MS/MS.

Table 3.1. *Main biomarkers and their characteristics by LC-MS/MS analysis*

Class	Compound / biomarker	Precursor ion (m/z)	Q transition	q transitions	CE for Q	Obs.
Blister	TDG	105	105 → 45	105 → 61; 105 → 87	13 eV	Episulfonium ion, not $[M+H]^+$
Blister	1,3-Bis(2-hydroxyethylthio)propane	197	197 → 123	197 → 136; 197 → 75	15 eV	Stable protonated molecular ion
Blister	TEA	150	150 → 70	150 → 114; 150 → 88	20 eV	Hydrolysis product nitrogen mustard
Blister	S-HETE-CPF	470,17	470,17 → 105	470,17 → 263,1; 470,17 → 137	35 eV	CPF adduct of sulfur mustard
Blister	Et-[S-HETE]-CPF	530,2	530,2 → 105	530,2 → 263,1; 530,2 → 137	35 eV	CPF adduct of sesquimustard
Blister	Pro-[S-HETE]-CPF	544,2	544,2 → 151	544,2 → 263,1; 544,2 → 119	40 eV	More stable structure
Blister	HN3-CPF	497,2	497,2 → 364,1	497,2 → 301; 497,2 → 152,1	40 eV	CPF adduct of nitrogen mustard
Serie A	A-230	195,1	195,1 → 74,1	195,1 → 81; 195,1 → 122	14 eV	Intact compound
Serie A	A-232	211,1	211,1 → 74,1	211,1 → 97; 211,1 → 138	14 eV	Intact compound
Serie A	A234-Tyr	386,2	386,2 → 244,3	386,2 → 285,3	20 eV	Tyrosine adduct
Serie A	A-232-NP	493,7	493,7 → 209,1	493,7 → 673,3; 493,7 → 778,3	23 eV	Double charged ion
Serie A	A-230-NP	485,7	485,7 → 193,1	485,7 → 673,3; 485,7 → 778,3	16 eV	Double charged ion
Serie A	A-230-OH	193,0	193,0 → 74,0	193,0 → 115; 193,0 → 120	15 eV	Hydrolysis product
Serie A	A230-OH-PFB	373,1	373,1 → 74,1	373,1 → 300	23 eV	PFB derivative also detected by LC
Serie A	A-232-OH	209,1	209,1 → 74,2	209,1 → 136	14 eV	Interference checked by blank
Serie A	A-232-OH-PFB	389,1	389,1 → 74,1	389,1 → 316	23 eV	Non-specific secondary signals excluded
Serie A	A-234-OH	223	223 → 74	223 → 150	14 eV	Hydrolysis product
Serie A	A-234-OH-PFB	403,1	403,1 → 74,1	403,1 → 330	14 eV	PFB derivative also detected by LC
Serie A	VX-NP	451,7	451,7 → 602	451,7 → 673; 451,7 → 778	25 eV	Double charged ion
Serie V	EMPA	123	123 → 95	123 → 79; 123 → 77	8 eV	Negative ESI
Serie G	GD-NP	497,7	497,7 → 673,3	497,7 → 778,3	15 eV	Double charged ion
Serie G	PinMPA	179	179 → 95	179 → 79; 179 → 77	15 eV	Negative ESI
Serie G	GB-NP	916	916 → 778	916 → 673; 916 → 602	30 eV	Monocharge ion
Serie G	GB-Tyr	302	302 → 214	302 → 260; 302 → 197	25 eV	Tyrosine adduct
Serie G	IpMPA	137	137 → 95	137 → 79; 137 → 77	17 eV	Negative ESI

The set of LC-MS/MS methods developed allowed the selective identification of specific biomarkers of exposure to blister and nerve agents in biological samples. The results revealed that hydrolysis products represent stable and easy-to-monitor biomarkers, while protein adducts offer a high level of specificity, but require more elaborate preparation strategies. For the A series compounds, LC-MS/MS analysis proved essential, due to their high stability and low volatility. The use of the MRM mode, together with the selection of quantification and confirmation transitions, allowed the differentiation of compounds and reduced the risk of unambiguous identification, including in the presence of matrix interferences. Thus, LC-MS/MS has proven to be a versatile and robust technique for the characterization of biomarkers formed following exposure to chemical warfare agents.

3.4.2. Identification of specific biomarkers of exposure to blister and nerve agents in plasma and urine samples by GC-MS/MS

In addition to LC-MS/MS analyses, GC-MS/MS was used to confirm specific biomarkers of expouser to blister and nerve agents in biological samples. This approach allowed the identification of compounds in intact form, when stability and volatility allowed, as well as of hydrolysis products transformed into more volatile derivatives by derivatization reactions with PFB-Br or HFBI.

Unlike LC-MS/MS, where ESI ionization favors the formation of molecular or pseudomolecular ions, GC-MS/MS frequently involves more energetic fragmentation processes, especially in EI mode. Under these conditions, the ions selected as precursors may represent primary fragments generated from the ionization source, and the SRM transitions reflect secondary fragmentations of these fragments. Therefore, the interpretation of GC-MS/MS data was based on the selection of stable, intense and reproducible fragments, correlated with retention time and specific ion ratios.

In the case of the blister agents, GC-MS/MS analysis allowed the identification of mustard gas, sesquimustard and the chlorinated analogue 1,3-bis(2-chloroethylthio)propane, as well as the hydrolysis products derivatized with HFBI. Sulfur mustard was identified in the EI-SRM mode by the transitions m/z 109 → 73 and m/z 141 → 86, with the chromatographic signal observed at approximately 10.59 min. The transition m/z 109 → 73 was used as the main transition due to its high intensity and stability of the signal, and the transition m/z 141 → 86 provided confirmation of the compound identification.

The main hydrolysis product of sulfur mustard, thiodiglycol, was analyzed after derivatization with HFBI, a step necessary to increase volatility and thermal stability. The TDG-HFBI derivative was identified by the transitions m/z 301 → 240 and m/z 241 → 169, at a retention time of approximately 12.16 min. This analysis complemented the LC-MS/MS identification, providing further confirmation of exposure to mustard gas.

Sesquimustard was identified in EI-SRM mode by the precursor ion m/z 182, with the transitions m/z 182 → 123 and m/z 182 → 63, both optimized at 15 eV. The transition m/z 182 → 123 was selected as the quantitation transition, and m/z 182 → 63 as the confirmation transition. The hydrolysis product of sesquimustard was identified after derivatization with HFBI, in CI mode, by the transitions m/z 301 → 241 and m/z 301 → 169, both at 8 eV. The use of CI allowed for a milder and more controlled fragmentation, suitable for polar derivatives.

The analogue 1,3-bis(2-chloroethylthio)propane was identified in the EI-SRM mode at a retention time of approximately 18.71–18.72 min, by the transitions m/z 169 → 73 and m/z

171 → 107. The transition m/z 169 → 73 was used for quantification, and m/z 171 → 107 for confirmation. The difference in collision energies reflects the different stability of the monitored fragments, with the fragment m/z 73 requiring a more thorough fragmentation process.

For nerve agents, GC-MS/MS was used both to identify the forms regenerated by refluorination and to confirm the derived hydrolysis products. In the case of VX, the refluorination process does not regenerate the intact compound, because the S-2-diisopropylaminoethyl residue, involved in binding to biomolecules, remains associated with biological structures. Refluorination reconstitutes only the phosphonic moiety, resulting in an ethyl methylphosphonofluoridate derivative, different from the original VX structure. This was identified in the EI-SRM mode by the transitions m/z 99 → 81 and m/z 111 → 81, at a retention time of approximately 4.41–4.42 min.

The hydrolysis product of VX, EMPA, was analyzed after derivatization with PFB-Br, in the CI mode. Derivatization was necessary due to the high polarity and acidic character of EMPA. The EMPA-PFB derivative was identified by the transitions m/z 123.1 → 95 and m/z 123.1 → 79, at approximately 14.34 min. Identification of both the regenerated form and the derivatized hydrolysis product provided complementary confirmation for VX exposure.

Soman was identified in the EI-SRM mode by the transitions m/z 126 → 82 and m/z 99 → 81. The presence of two close chromatographic signals, in the interval 8.45–8.52 min, was attributed to the existence of specific isomers of Soman, partially separated under the gas chromatography conditions. The presence of both peaks on the same transitions confirms that they belong to the same compound in different isomeric forms. Sarin was identified in intact form by the transitions m/z 125 → 99 and m/z 99 → 47, in the EI-SRM mode, at approximately 5.11–5.14 min. The transition m/z 125 → 99 was used for quantification, and m/z 99 → 47 for confirmation. Its hydrolysis product, IpMPA, was analyzed after derivatization with PFB-Br in CI mode, through the transitions m/z 137 → 95 and m/z 137 → 79. The simultaneous identification of Sarin and IpMPA-PFB supports the confirmation of exposure, and the correlation with LC-MS/MS results increases the degree of analytical certainty.

Table 3.2 presents the main identified biomarkers, the collision energies and the main transitions for their identification by GC-MS/MS.

Table 3.2. *Main biomarkers and their characteristics by GC-MS/MS analysis*

Class	Compound / biomarker	Ionization mode	Approx. RT	Transition Q	CE Q	Transition q	CE q	Obs
Blister	Sulfur mustard	EI	10,59 min	109 → 73	5 eV	141 → 86	15 eV	Formă clorurată; fragmente EI stabile
Blister	TDG-HFBI	EI	12,16 min	301 → 240	6 eV	241 → 169	20 eV	Produs de hidroliză derivatizat
Blister	Sesquimustard	EI	17,51 min	182 → 123	15 eV	182 → 63	15 eV	Formă clorurată
Blister	SQ-OH-HFBI	CI	7,72 min	301 → 241	8 eV	301 → 169	8 eV	Produs de hidroliză derivatizat
Blister	1,3-Bis(2-chloroethylthio)propane	EI	18,71–18,72 min	169 → 73	12 eV	171 → 107	6 eV	Analog clorurat

Serie V	Refluorinated VX / phosphonofluorinated derivative	EI	4,41–4,42 min	99 → 81	16 eV	111 → 81	10 eV	Reconstituire fragment fosfonic, nu VX intact
Serie G	EMPA-PFB	CI	14,34 min	123,1 → 95	18 eV	123,1 → 79	36 eV	Produs de hidroliză VX derivatizat
Serie G	Soman	EI	8,45–8,52 min	126 → 82	12 eV	99 → 81	20 eV	Două vârfuri atribuite izomerilor
Serie G	Sarin	EI	5,11–5,14 min	125 → 99	10 eV	99 → 47	28 eV	Formă intactă
Serie G	IpMPA-PFB	CI	14,62 min	137 → 95	20 eV	137 → 79	38 eV	Produs de hidroliză Sarin derivatizat

3.5. Conclusions

The study developed an integrated LC-MS/MS–GC-MS/MS approach for the identification of biomarkers of nerve and blister agents in plasma and urine, highlighting both intact compounds and hydrolysis products and biological adducts. The methods were adapted to the chemical peculiarities of each class, including for A series compounds, demonstrating their stability and detectability. The results were validated through OPCW interlaboratory tests, confirming the robustness and applicability of the methods for the analysis of complex biological samples.

CHAPTER 4

EXPERIMENTAL STUDY ON THE EVALUATION OF THE DECONTAMINATION EFFICIENCY OF CHEMICAL WARFARE AGENTS – SOMAN AND MUSTER - USING REACTIVE ORGANIC SUSPENSIONS CONTAINING NANOADSORBANTS OF ZINC OXIDE (ZnO), TITANIUM DIOXIDE (TiO2) AND ZEOLITE

The results of this study were published in full durring the doctoral studies and can be consulted in the article:

Raluca Elena Ginghina, Adriana Elena Bratu, Gabriela Toader, Andreea Elena Moldovan, Tudor Viorel Tiganescu, Ramona Elena Oncioiu, Panaghia Deliu, Razvan Petre, Gabriel Epure, Munizer Purica, ***Reactive Organic Suspensions Comprising ZnO, TiO_2, and Zeolite Nanosized Adsorbents: Evaluation of Decontamination Efficiency on Soman and Sulfur Mustard***, publicat în revista Toxics (MDPI), 2021, Volume 9, Issue 12, 334, ISSN 2305-6304, https://doi.org/10.3390/toxics9120334; WOS: 000738377700001, IF2021 = 4.472, Q2 (Toxicology; Environmental Sciences).

4.1. The necessity of development of modern decontamination strategies for chemical warfare agents

The need to develop strategies based on reactive organic suspensions containing nanometric adsorbents (ZnO, TiO_2, zeolites) derives from the limitations of conventional decontamination methods, which are often non-specific, slow or difficult to apply on sensitive surfaces and in real conditions. Chemical warfare agents, such as soman and sulfur mustard, present high toxicity and can form stable interactions with surfaces or biomaterials, requiring solutions that efficiently combine adsorption and chemical degradation processes. Nanostructured materials offer significant advantages due to their large specific surface area and increased reactivity, allowing both the rapid capture of toxic agents and their transformation into less toxic compounds. Thus, the development of such multifunctional systems represents an extremely important direction for obtaining rapid, efficient and applicable decontamination methods in real scenarios.

4.2. Methods for synthesis of decontamination solutions, physicochemical characterization of the organic component and verification of the compatibility of the organic component with nanoparticles. Testing of decontamination efficiency with real chemical warfare agents

Organic decontamination solutions (SD) were obtained by controlled mixing of the components in a properly equipped reaction system at 30 °C. The formulation included ethylene glycol monoethyl ether, monoethanolamine, NaOH and surfactant (sodium lauryl sulfate in isopropyl alcohol), added sequentially. In the final step, nanometric adsorbents (ZnO, TiO_2 and zeolite) were dispersed by sonication, obtaining suspensions with variable concentrations of nanoparticles (0.1–2% m/m).

The organic component was characterized from a physicochemical point of view (density and alkalinity), and the compatibility with surfaces was evaluated by corrosion and stability tests, according to relevant standards. Also, the stability of the solution was tested

under extreme temperature conditions, demonstrating the maintenance of decontamination properties. The performance of SD was preliminarily verified on chemical warfare agents, confirming the effectiveness even after exposure to severe environmental conditions.

The decontamination efficiency of the formulations was evaluated on real agents (HD and GD), by controlled contamination followed by decontamination under stiring at room temperature. The samples were analyzed at variable time intervals, using dichloromethane extraction and GC-MS, and the degradation products were identified after derivatization. The performance was expressed by the decontamination factor (DF), calculated based on the difference between the initial and residual concentration. All determinations were performed in triplicate, and the results reported as mean values.

4.3. Characterization

FT-IR spectra of the decontamination solutions were obtained at a resolution of 4 cm−1, from 550 to 4000 cm−1, using a Perkin Elmer Spectrum Two instrument, ATR mode. Gas chromatography mass spectrometry analyses were performed on a Thermo Scientific Trace 1310–TSQ 9000 triple quadrupole MS GC. The analytical method conditions for GC-MS for quantifying the decontamination efficiency and identifying the degradation products are described in Table 4.1.

Table 4.1. *GC-MS analysis conditions for quantifying decontamination efficiency and identifying degradation products*

Parameter	Conditions
Carrier gas	Helium
Carrier gas flow rate	1,5 mL/min (constant flow)
Injector temperature	270 °C
Injection mode	Splitless
Chromatographic column	TR5MS Gold
Stationary phase	5% phenyl – 95% dimethylpolysiloxane
Column dimensions	30 m × 0,25 mm × 0,25 µm
Temperature program	40 °C → 300 °C, ramp 10 °C/min
Solvent delay time	2,5 min (standard) / 8,5 min (derivatisation)
MS ionization mode	Electron impact (EI)
Electron energy	40 eV
Scan interval	40–650 m/z

Qualitative analyses and identification of toxic compounds and their degradation products were performed by comparing the mass spectra of the chemicals with the NIST (National Institute of Standards and Technology) and OCAD (OPCW Central Analytical Database) spectral libraries. Quantitative analyses were performed by the internal standard method.

4.4. Results and discussions

The first objective of the study was to develop an organic decontamination solution (SD), subsequently used as a dispersion medium for nanoadsorbents. The solution obtained presented a density of 0.91 ± 0.02 g/cm^3 and an alkalinity of 2.47 ± 0.5 cm^{3}HCl 1 N/g,

without causing degradation of the tested metal surfaces. It also maintained its decontamination efficiency even after exposure to extreme temperature and humidity conditions, demonstrating stability and operational compatibility.In the next stage, nanoadsorbents of the ZnO, TiO_2 and zeolite type were integrated to evaluate the impact on the decontamination efficiency. Morphological analysis revealed nanometric dimensions for ZnO and TiO_2 (approx. 60–100 nm) and a zeolite-specific porous structure, with larger dimensions (100–500 nm), which may influence the adsorption behavior.Chemical characterization of the solutions confirmed the presence of functional groups specific to the organic components, highlighting a complex system in which interactions between components and chemical agents directly influence the decontamination process. Overall, the results indicate that both the chemical composition and the nature of the nanoadsorbents play a particularly important role in the efficiency of the process and in the formation of degradation products.

The interaction of reactive organic suspensions with the chemical warfare agents HD and GD was evaluated by monitoring the decrease in the concentration of the toxic agent and by identification the degradation products formed over time. The agents were identified by GC-MS based on characteristic ions and by comparing the spectra obtained with the NIST and OCAD libraries.

In the case of sulfur mustard (HD), the organic solution without nanoadsorbents showed a rapid decontamination reaction, reaching an efficiency of approximately 76% after 2 minutes and 99.99% after 24 hours. The addition of nanoadsorbents significantly improved the efficiency of the process in the first minutes, an important aspect from an operational point of view. The best results were obtained for the suspensions with 0.1% TiO_2 and 0.1% zeolite, which determined an efficiency of approximately 95% after only 2 minutes and almost 100% after 5 hours. For ZnO, the optimal efficiency was observed at a concentration of 1%, while the concentration of 2% led to lower performance, most likely due to agglomeration of nanoparticles and reduction of the available active surface area.

In the case of Soman (GD), decontamination was much faster than in the case of HD. The pure organic solution reached an efficiency of 99.83% after 2 minutes and over 99.9% after 1 hour. Suspensions with nanoadsorbents accelerated the process even more, especially the formulations with 0.1% TiO_2 and 0.1% zeolite, for which the reaction was practically complete after about 10 minutes. In the case of ZnO, the efficiency increased with concentration up to 1%, but at 2% a decrease in performance was observed, attributed to particle agglomeration.

Table 4.2. *Summary of decontamination efficiency for HD and GD*

Toxic agent	Formulation	Initial efficacy	Relevant final efficacy	Obs
HD	SD pure	76,42% after 2 min	99,99% after 24 h	Fast initial reaction but slow completion
HD	SD + 1% ZnO	66,96% after 2 min	97,42% after 5 h	Optimal efficiency for ZnO at 1%; 2% reduces efficiency through agglomeration
HD	SD + 0,1% TiO_2	94,95% after 2 min	99,90% after 5 h	Major increase in initial efficiency over SD

HD	SD + 0,1% zeolite	95,64% after 2 min	99,92% after 5 h	Best efficiency for HD
GD	SD pure	99,83% after 2 min	99,91% after 1 h	Very fast decontamination
GD	SD + 1% ZnO	99,91% after 2 min	99,97% after 10 min	Optimal efficiency for ZnO at 1%
GD	SD + 0,1% TiO_2	99,95% after 2 min	complete reaction after 10 min	Significant reduction in decontamination time
GD	SD + 0,1% zeolite	99,96% after 2 min	complete reaction after 10 min	Best performance for GD

Compared to the pure organic solution, suspensions with 0.1% TiO2 and 0.1% zeolite significantly reduced the time required for GD decontamination, from approximately 1 hour to 10 minutes. In the case of HD, these suspensions reached efficiencies comparable to those obtained by the pure solution after 5 hours in 2–10 minutes. This result confirms the hypothesis that the addition of small amounts of nanoadsorbents can improve the decontamination efficiency, by increasing the active surface area and by facilitating the interaction between the toxic agent and the decontamination medium.

In the final stage, the main degradation products formed during the decontamination process were identified. For HD, four major products were identified, and for GD, five main products. In the case of HD, the predominant product was 2-chloroethyl vinyl sulfide, formed from the first minutes and subsequently transformed into secondary compounds, such as 1,2-bis(vinylthio)ethane. The presence of 2-thiomorpholinoethanol indicates the reaction of HD with monoethanolamine from the organic solution composition. In the case of GD, the dominant product was the di(2-ethoxyethyl) ester of methylphosphonic acid, resulting from the reaction of the agent with 2-ethoxyethanol, the major component of the formulation. Pinacolylmethylphosphonic acid was the second major product, showing an increase up to approximately 3 hours, followed by a decrease, suggesting its subsequent transformation into other degradation products.

Table 4.3. *Main degradation products identified for HD and GD*

Agent	Degradation product	Formation observation
HD	2-Chloroethyl vinyl sulfide	Major product; formed at the beginning of the process and increases until about 3 h, then decreases
HD	1,4-dithiane	Present in low concentrations; increases in suspensions with nanoadsorbents
HD	1,2-bis(vinylthio)ethane	Increases over time, especially after 5 h, possibly through the transformation of other intermediates
HD	2-thiomorpholinoethano	Results from the reaction of HD with monoethanolamine; reaches a maximum at the beginning of the process, then decreases
GD	Methylphosphonic acid	Appears rapidly, especially in the first minutes; then decreases
GD	Isopropyl pinacolyl methylphosphonate	Formed by the reaction of GD with isopropyl alcohol; increases slightly over time
GD	Pinacolyl methylphosphonic acid	Second product in abundance; increases until about 3 h, then decreases
GD	Dipinacolyl methylphosphonate	Forms from the beginning and increases slightly over time
GD	Methylphosphonic acid, di(2-ethoxyethyl) ester	Major product; results from the reaction of GD with 2-ethoxyethanol, formed rapidly and in large quantities

The identified degradation products have an estimated lower toxicity than the initial agents, generally falling into lower acute toxicity classes. For some compounds, complete toxicological data are not available, but the results indicate the transformation of highly toxic agents into compounds with significantly reduced hazard. Thus, the decontamination process should not be viewed only as the quantitative elimination of the initial agent, but also as its chemical transformation into less hazardous products. Overall, the results confirm that the developed organic solution has high decontamination capacity for HD and GD, and the integration of small amounts of nanoadsorbents, especially TiO_2 and zeolite at 0.1%, significantly improves the speed of the process. These formulations thus have potential applications for operational scenarios, where rapid reduction of contamination is essential.

4. 5. Conclusions

In this chapter, an organic decontamination solution was synthesized and characterized, subsequently used as a dispersion medium for three types of nanoadsorbents: ZnO, TiO_2 and zeolite. The basic solution was evaluated from a physicochemical point of view, by determining the density, pH and total alkalinity, and preliminary tests demonstrated the maintenance of decontamination performance after exposure to extreme temperature and humidity cycles, according to the requirements of NATO standards applicable to the CBRN field.

The obtained formulations were characterized by SEM and FT-IR, highlighting the morphology of the nanoadsorbents and their compatibility with the organic matrix. Subsequently, the efficiency of the reactive suspensions was evaluated on real chemical warfare agents, soman (GD) and sulfur mustard (HD), by monitoring the initial and residual concentration of the toxic agent at different time intervals, using GC-MS.

The results demonstrated that the basic organic solution has a high decontamination efficiency, and the integration of nanoadsorbents contributes to the acceleration of the process, especially in the case of GD. For HD, the best results were obtained for suspensions with TiO_2 and zeolite, which determined higher decontamination factors and a faster reaction compared to ZnO. In the case of ZnO suspensions, the optimal concentration was 1% m/m, while higher concentrations led to a slight decrease in efficiency, most likely due to particle agglomeration and reduction of the available active surface. Compared to HD, which required longer intervals for complete decontamination, GD was degraded much faster, the process being completed in a maximum of 60 minutes. This difference reflects the distinct chemical characteristics of the two agents and the different reactivity towards the components of the decontamination solution. Monitoring of degradation products allowed the identification and quantification of four main products resulting from HD decontamination and five main products resulting from GD decontamination, providing a detailed picture of the chemical transformation mechanisms involved. Overall, the results obtained confirm the efficiency of reactive organic suspensions functionalized with ZnO, TiO_2 and zeolite nanoadsorbents for the decontamination of HD and GD agents. By combining a reactive organic matrix with nanostructured materials, these formulations allow both the rapid reduction of contamination and the transformation of toxic agents into low-hazard degradation products, highlighting their potential application in CBRN operational scenarios.

CHAPTER 5

DEVELOPMENT OF PEELABLE FILM-FORMING HYDROGELS BASED ON BIOPOLYMERS INCORPORATING METAL-ORGANIC FRAMEWORKS FOR THE IMMEDIATE DECONTAMINATION OF SULFUR MUSTARD

The results of this study were published in full durring the doctoral studies and can be consulted in the article:

Adriana Elena Bratu, Aurel Diacon, Izabela-Cristina Stancu, Mihail Munteanu, Raluca Elena Ginghina, Gabriela Toader, ***Development of alginate–hydroxyethyl cellulose–gum arabic ternary peelable hydrogels for sulfur mustard decontamination***, acceptat pentru publicare în Carbohydrate Polymer Technologies and Applications (Elsevier), 2026, Volume 15, 101197, https://doi.org/10.1016/j.carpta.2026.101197, IF2026 = 7.6, Q1.

5.1. The necessity of development functionalized hydrogels with metal-organic frameworks for the decontamination of vesicant agents

Peelable hydrogels represent an emerging class of materials with high potential for decontamination applications in CBRN scenarios, due to their ability to combine favorable physical and chemical properties in a single functional platform. The necessity to develop such systems stems from the limitations of conventional decontamination methods, which often involve the use of aggressive liquid solutions, difficult to control on sensitive surfaces or in direct contact with the skin. In the case of blister agents, such as sulfur mustard, rapid intervention is essential to reduce absorption and toxic effects, which requires the use of materials capable of acting simultaneously by adsorption, immobilization and chemical degradation. In this context, peelable hydrogels based on biopolymers offer significant advantages, including biocompatibility, controlled adhesion to the surface and the possibility of mechanical removal of the contaminant by forming a continuous film. Their functionalization with metal-organic frameworks (MOFs) introduces an additional level of efficiency, due to the high specific surface area and the ability of these materials to adsorb and transform toxic compounds. Thus, the development of functionalized hydrogels represents a promising strategy for obtaining fast, efficient and easy-to-apply decontamination systems under operational conditions.

5.2. Preparation methods for formulations and testing decontamination efficiency

Nine distinct biopolymer-based hydrogel formulations, functionalized with metal-organic frameworks (MOFs) or CeO_2nanoparticles, were developed in order to identify the optimal composition for decontamination. The preparation involved the initial dispersion of the nanoparticles by homogenization, followed by the gradual incorporation of the biopolymers and their dissolution in deionized water at 60 °C, obtaining homogeneous, stable systems suitable for crosslinking.

The formation of the hydrogel films was achieved by ionic crosslinking, by spraying a 10% $CaCl_2$ solution over the formulations applied to the surface, leading to the obtaining of cohesive and peelable films. For physicochemical (SEM, XRD, FT-IR) and mechanical

characterizations, the films were obtained under controlled conditions, on clean supports, to ensure the uniformity and reproducibility of the analyses.

The decontamination efficiency was evaluated on polycarbonate model surfaces spiked with sulfur mustard, applied as microdroplets and kept in contact with the substrate before treatment. The hydrogels were applied directly to the contaminated surface, and after crosslinking, the films were maintained for short contact times (2.5 and 10 minutes), then mechanically removed. The quantitative analysis of sulfur mustard was performed by separate extraction of the hydrogel and the surface, followed by GC-MS determination.The results allowed the evaluation of the decontamination efficiency by comparing the amount of toxic agent retained in the hydrogel with that remaining on the surface, demonstrating the potential of these peelable systems for rapid and efficient removal of contaminants under operationally relevant conditions.

Table 5.1. *Reagents and compositional details for formulation preparation*

Sample	2-Hydroxyethyl cellulose (HEC) (Weight %)	Gum Arabic (GA) (Weight %)	Alginate (ALG) (Weight %)	Adsorbent
F1	0	0	3	none
F2	2	0	3	none
F2_0.5% MOF-1	2	0	3	0.5 % (weight %) Basolite Z1200
F2_1% MOF-1	2	0	3	1 % (weight %) Basolite Z1200
F2_1.5% MOF-1	2	0	3	1.5 % (weight %) Basolite Z1200
F3	2	1	3	none
F3_0.5% MOF-1	2	1	3	0.5 % (weight %) Basolite Z1200
F3_1% MOF-1	2	1	3	1 % (weight %) Basolite Z1200
F3_1.5% MOF-1	2	1	3	1.5 % (weight %) Basolite Z1200
F3_0.5% MOF-2	2	1	3	0.5 % (weight %) Basolite C300
F3_0.5% MOF-3	2	1	3	0.5 % (weight %) Basolite Z377
F3_0.5% CeO_2	2	1	3	0.5 % (weight %) CeO_2 (d<25 nm)
F4	0	1	3	none

5.3. Characterizations

The hydrogel formulations were characterized by a complex set of physicochemical, morphostructural and mechanical methods. Initially, the viscosity of the uncrosslinked formulations was determined using a rotary viscometer, to assess their flow behavior and suitability for application on contaminated surfaces. The swelling degree of the crosslinked hydrogels was determined gravimetrically, by relating the difference between the mass of the swollen hydrogel at equilibrium and the mass of the xerogel to the mass of the dry xerogel.

The morphological and compositional characterization of the crosslinked hydrogels was performed by SEM-EDS, after washing, swelling, lyophilization and conductive coating with a thin layer of gold. This analysis allowed the evaluation of the surface architecture and cross-

section, as well as the elemental distribution of the inorganic components in the hydrogel matrix.

The crystal structure and the presence of functional materials in the hydrogels were investigated by X-ray diffraction (XRD), using dried and ground samples, compared with reference materials. The chemical interactions between the hydrogel components and the characteristic functional groups were evaluated by FT-IR/ATR, in the spectral range 4000–600 cm^{-1}.

The mechanical and viscoelastic properties of the crosslinked hydrogels were determined by dynamic mechanical analysis (DMA). Uniaxial tensile tests allowed the evaluation of the mechanical strength of the films, and oscillatory shear tests were used to determine the linear viscoelastic region, network stability and frequency-dependent behavior. This integrated approach provided a complete picture of the relationship between composition, structure and functional performance of the developed hydrogels.

5.4. Results and discussions

In this chapter, peelable hydrogels based on biopolymers, functionalized with metal-organicframeworks and nanoparticles, were developed and evaluated for the rapid decontamination of sulfur mustard. The concept pursued was to obtain hybrid films capable of combining the physical capture of the toxic agent with its immobilization and partial degradation, thus reducing the risk of secondary contamination after removal of the material from the contaminated surface.

The formulations were made starting from matrices based on alginate, hydroxyethylcellulose and gum arabic, ionic crosslinked with calcium ions. In their structure, different type of metal-organic frameworks and CeO_2 nanoparticles were integrated, in order to increase the adsorption capacity and to introduce additional reactive functional groups. The rheological analysis of the uncrosslinked formulations revealed a non-Newtonian shear-thinning behavior, favorable for application on contaminated surfaces, as it allows easy stretching of the material under the action of mechanical force, followed by maintaining stability at rest. The formulations containing HEC, especially the F2 and F3 systems, presented more favorable rheological properties and were selected for further investigations.

Morphological characterization showed that the structure of the hydrogels is influenced by both the composition of the polymer matrix and the type and concentration of the filler. The systems without filler presented relatively uniform surfaces, while the introduction of MOFs determined the increase in roughness and structural heterogeneity. The HEC–GA–Alg ternary matrix demonstrated a better particle integration capacity compared to simpler systems, and MOF-1 presented the best compatibility with the polymer network. EDS analyses confirmed the incorporation of fillers, and XRD results revealed the maintenance of the crystalline structure of MOF-1 in the hydrogels, without major phase transformations. The changes observed in the diffractograms suggest the existence of interfacial interactions between the MOF particles and the polymer matrix. Similarly, FT-IR analysis indicated that the interactions between the components are predominantly physical, based on hydrogen bonds and surface interactions.

The swelling degree was strongly influenced by the architecture of the polymer network and the nature of the filler. The alginate-based formulation showed the highest water absorption, while the systems containing HEC and gum arabic had a lower swelling degree, as a result of the formation of a denser network. The introduction of MOFs further modified the

swelling capacity, depending on the concentration and the compatibility with the matrix. These results confirm that the balance between network density, filler dispersion and water retention capacity are essential for obtaining effective and stable films.

The mechanical properties highlighted the characteristic behavior of soft polymer networks, capable of supporting significant deformations. The integration of MOF-1 led to the most important improvement in mechanical strength and ductility, especially at intermediate concentrations, where a favorable balance between rigidity and flexibility was achieved. Shear tests confirmed the viscoelastic nature of the hydrogels, with a response dominated by the elastic component, an important aspect for maintaining the integrity of the film during application and removal from the contaminated surface.

The evaluation of the decontamination efficiency demonstrated that the biopolymer matrices without fillers have a moderate capacity for removing sulfur mustard, with similar values between the formulations. The introduction of MOF-1 significantly increased the performance, especially in the ternary HEC–GA–Alg matrix, where the decontamination efficiency exceeded 80% after 10 minutes for the formulations with 1–1.5% MOF-1. In comparison, MOF-3 and CeO_2 mainly favored the migration of sulfur mustard into the film, without a significant reduction in the total amount of intact agent recovered. In contrast, MOF-1 formulations led to a significant decrease in total recovered sulfur mustard, suggesting not only physical capture of the agent, but also strong retention or partial degradation of it.

The results demonstrate that ternary HEC–GA–Alg hydrogels functionalized with MOF-1 are the most efficient systems among the investigated formulations, due to the combination of processability, mechanical stability, capture capacity and sulfur mustard degradation potential. This approach highlights the potential of functionalized peelable hydrogels as modern rapid decontamination platforms, applicable in CBRN scenarios where efficient and controlled removal of the contaminant is essential.

5.5. Conclusions

In this chapter, a peelable Ca^{2+} ionic crosslinked hydrogel platform was developed, based on a ternary biopolymer matrix (HEC–GA–Alg), capable of efficiently integrating metal-organic frameworks and ensuring rapid decontamination of sulfur mustard from solid surfaces. The ternary architecture proved to be essential for obtaining an optimal combination of viscosity, film-forming capacity and mechanical cohesion, while allowing uniform dispersion of MOF-type particles at higher concentrations, without the occurrence of agglomeration phenomena observed in binary systems. These characteristics are supported by the rheological behavior of viscoelastic dominant elastic type ($G' > G''$), the hydrogels presenting resistance to deformation in the range of 30–35%.

The results of the decontamination tests revealed a clear advantage of MOF-1 (Basolite Z1200) within the ternary system F3 (HEC–GA–Alg). While the exclusively polymeric matrices achieved decontamination efficiencies of approximately 67–72% after 10 minutes, the formulations containing 1–1.5% MOF-1 showed significantly higher values of 80.1–82.9%, also showing rapid absorption from the first minutes of contact. In comparison, the systems based on MOF-3 and CeO_2 only resulted in moderate improvements. An important aspect is the reduction in the total amount of mustard gas recovered in the case of formulations with MOF-1 (approximately 41–45%), compared to the higher values observed for the other fillers (≥54–65%), indicating that these systems not only transfer the toxic agent into the film, but also contribute to its retention or partial transformation. The identification of

degradation compounds, such as 1,4-oxathiane and 1,4-dithiane, confirms this mixed mechanism of adsorption and chemical conversion.

The analysis of the structure–property particularity highlights the role of the polymer matrix composition and the filler concentration. In the binary system F2, the mechanical properties show a “volcano” dependence, with a maximum around the concentration of 1% MOF-1, while the ternary system F3 demonstrates a progressive increase in performance up to 1.5% MOF-1, suggesting that the presence of gum arabic favors both dispersion stabilization and efficient network stress transfer. Morphological observations confirm these trends, highlighting a more uniform particle distribution and a more homogeneous granular structure in the case of the ternary formulations.

The swelling behavior reflects, in turn, the influence of the network architecture and the fillers, with values varying significantly between formulations. The introduction of MOF-1 initially causes an increase in the swelling capacity at low concentrations, followed by a limitation of the network expansion at higher concentrations, due to the constraint effect induced by the dispersed particles.

The HEC–GA–Alg system functionalized with MOF-1 stands out as a high-performance peelable decontamination platform, capable of combining rapid removal of sulfur mustard with a reduction in the amount of recovered agent. This approach offers a clear advantage over systems based solely on adsorption and opens perspectives for the development of advanced materials for CBRN applications. Future studies will aim to further optimize the MOF loading, extend the scope of application to other chemical agents, and improve the decontamination efficiency above 90%, while maintaining the mechanical properties and biological compatibility of the materials.

CHAPTER 6

HYDROGELS FOR CONTROLLED RELEASE OF ATROPINE IN CHEMICAL WARFARE AGENTS THREAT SCENARIOS

The results of this study were published in full durring the doctoral studies and can be consulted in the article:

Adriana Elena Bratu, Izabela-Cristina Stancu, Aurel Diacon, Gabriela Toader, Ana Mihaela Gavrila, Raluca Elena Ginghina, Sebastian Stefan Pricop, Tudor-Viorel Tiganescu, ***Hydrogels for controlled release of atropine in chemical warfare agents threat scenarios***, publicat în Sci. Bull., Series B, Vol. 88, Iss. 2, 2026, ISSN 1454-2331, IF2026=0.5, Q4.

6.1. The necessity to develop hydrogels for the controlled release of therapeutic agents in chemical warfare agent exposure scenarios

In the context of exposure to chemical warfare agents, especially to nerve agents from the organophosphorus series, rapid and effective intervention is essential to reduce acute toxic effects and increase the chances of survival. Atropine is one of the main antidotes used in such situations, acting as an antagonist of muscarinic receptors and counteracting the effects of excess acetylcholine generated by inhibiting acetylcholinesterase. However, conventional administration of atropine, by injection, presents significant limitations, including the need for rapid intervention by specialized personnel, difficulties in maintaining constant therapeutic concentrations and the risk of adverse reactions associated with overdose. In this context, the development of alternative delivery systems, capable of ensuring a controlled and sustained release of the active substance, is becoming a research direction of major interest. Hydrogels represent a promising platform for such applications, due to their three-dimensional structure, biocompatibility and ability to incorporate and release active molecules in a controlled manner. By adjusting the composition of the hydrogel, the degree of crosslinking and the interactions between the matrix and the active substance, it is possible to modulate the release profile of atropine, allowing the achievement of systems adapted to the specific requirements of emergency scenarios.

Furthermore, the use of hydrogels in this context can contribute to the development of easy-to-administer therapeutic solutions, including topical or transdermal forms, reducing the dependence on parenteral administration and facilitating use in difficult operational conditions. In addition, these systems can provide local protection and can be integrated into multifunctional materials, capable of combining decontamination and treatment roles, thus representing an integrated approach in the management of exposure to chemical agents.

Therefore, the development of hydrogels for the controlled release of atropine is part of a strategic research direction, aimed at increasing the efficiency of medical interventions in CBRN situations, by obtaining safe, efficient and easy-to-use systems, adapted to real conditions of use.

6.2. Methods for the synthesis of functionalized hydrogels for the controlled release of atropine sulfate

In this study, functionalized hydrogels were developed for the controlled release of atropine sulfate, using a polymer system obtained by UV-induced radical photopolymerization. The hydrogel matrix was based on HEAA and NVP, in the presence of PVA, using DVE as a crosslinking agent and Irgacure 2959 as a photoinitiator. To optimize the functional properties, the formulations were modified by introducing specific additives, such as titanium dioxide and halloysite nanotubes, to improve mechanical strength, respectively β- and γ-cyclodextrins, to facilitate the interaction with the atropine molecule and obtain a sustained release profile.

The synthesis process involved the prior dissolution of PVA in water, followed by the incorporation of additives and monomers, obtaining a homogeneous mixture of prepolymers. Photopolymerization was performed under controlled conditions, in an inert environment, to avoid inhibition of the reaction by oxygen, leading to the formation of hydrogel films with well-defined structural properties. The samples were subsequently processed into suitable shapes for mechanical and swelling testing and for the evaluation of the loading and release capacity of the active substance.

The loading of atropine sulfate was performed by immersing the lyophilized hydrogels in an aqueous solution, a process based on the diffusion of the active substance into the porous network of the material. The loading conditions were optimized to allow uniform impregnation, and the amount of atropine incorporated was determined indirectly by analyzing the residual solution, using spectrofluorometric methods. Subsequent drying of the samples ensured the stabilization of the system and its preparation for release studies.

The evaluation of the release kinetics was performed in phosphate buffer solution (PBS, pH 7.4), at physiological temperature, to simulate relevant biological conditions. Rehydration of the hydrogels allowed the controlled diffusion of atropine sulfate from the matrix, a process influenced by parameters such as the degree of crosslinking, the porosity of the network and the nature of the polymer–active substance interactions. The samples were analyzed at regular intervals by UV-Vis and spectrofluorometric methods, ensuring the monitoring of the release profile over time.

The results obtained demonstrate that these hydrogels represent efficient systems for the controlled delivery of atropine, offering the possibility of adjusting the release profile by modifying the composition and structure of the polymer network. Thus, the developed platform presents significant potential for therapeutic applications in scenarios of exposure to nerve agents, where a rapid but controlled administration of the antidote is required.

6.3. Characterizations

The characterization of functionalized hydrogels for the controlled release of atropine sulfate was performed by a set of physicochemical and mechanical methods, aimed at revealing both the structure of the materials and their functional behavior. The morphological analysis of the lyophilized hydrogels was performed by scanning electron microscopy, highlighting the porous architecture of the polymer network and the uniform distribution of the components within the matrix.

The chemical structure and interactions between the components were investigated by FT-IR spectroscopy, which allowed the identification of characteristic functional groups and confirmation of the formation of the crosslinked polymer network. The structural stability of

the hydrogels was evaluated by determining the gel fraction, calculated based on the resistance of the material to extraction in water, this parameter reflecting the degree of crosslinking and the integrity of the network formed.

The degree of swelling was determined using the gravimetric method, the results indicating the ability of the hydrogels to absorb water and rehydrate under physiological conditions. This behavior is essential for controlled delivery applications, as it directly influences the diffusion of the active substance from the matrix.

Mechanical properties were evaluated by compression and tensile tests, highlighting the resistance of the materials to deformation and their ability to maintain their structural integrity under load. In addition, the viscoelastic behavior was investigated by frequency-dependent shear and deformation tests, providing information on the stability of the network and its response under dynamic conditions.

The evaluation of the release kinetics of atropine sulfate was performed by spectrophotometric and spectrofluorometric methods, using UV-Vis and fluorescence measurements to determine the concentration of active substance released over time. Calibration curves allowed for the precise quantification of atropine, the fluorescence-based method being preferred due to its high sensitivity and reproducibility.

These characterization methods provided a complete picture of the structural, mechanical, and functional properties of the hydrogels, highlighting the key parameters that influence their performance as controlled-release systems for atropine.

6.4. Results and discussions

The results obtained highlight that the type of additive used directly influences the morphology and functional properties of hydrogels intended for the controlled release of atropine sulfate. Morphological analyses showed that hydrogels containing cyclodextrins present well-organized and interconnected porous structures, favorable for controlled release processes, while systems including halloysite or TiO_2 develop more fragmented and irregular networks, associated with faster release mechanisms. The control hydrogel revealed a more disorganized structure, confirming the essential role of additives in controlling the internal architecture.

FT-IR analysis confirmed the formation of the polymer network and the successful integration of the additives into the matrix, without major changes in the basic structure, but with subtle variations in the spectral fingerprint region, associated with specific interactions between the components. From a structural point of view, all hydrogels presented high values of the gel fraction, indicating an advanced degree of crosslinking. The sample containing halloysite showed the highest gel fraction and the lowest swelling degree, suggesting a denser and stiffer network, while the hydrogels with cyclodextrins showed a more pronounced swelling behavior, favorable to the controlled diffusion of the active substance.

The mechanical properties revealed significant differences between the formulations. The hydrogel with halloysite showed the highest mechanical strength and stiffness, while the systems with β- and γ-cyclodextrins demonstrated an optimal balance between flexibility and structural stability. In contrast, the hydrogel with TiO_2 and the control sample showed inferior mechanical performances, being characterized by a predominantly viscous behavior and a reduced structural integrity, which is why the formulation with TiO_2 was excluded from further studies.

Release studies showed that all formulations followed a diffusion-dominant mechanism, best described by the Korsmeyer–Peppas model, with a non-Fickian behavior, suggesting the simultaneous involvement of diffusion and relaxation of the polymer network. Among all the formulations, the β-cyclodextrin-containing hydrogel emerged as the most promising, due to its ability to combine favorable release kinetics with structural stability and molecular compatibility. This behavior is attributed to the formation of stable inclusion complexes between β-cyclodextrin and atropine, which allow for a more controlled release and improved bioavailability.

6.5. Conclusions

IPN-type hydrogels were obtained by photopolymerization, incorporating functional additives (halloysite, TiO2, β- and γ-cyclodextrins), to control the release of atropine sulfate. FT-IR analysis confirmed their integration into the polymer network. Halloysite led to an increase in mechanical strength and a reduction in the degree of swelling, indicating the formation of a more compact structure. Hydrogels with cyclodextrins showed good mechanical strength and high G′ values, suggesting a stable and flexible network.

The β-cyclodextrin based hydrogel showed the slowest release of atropine, due to the formation of stable inclusion complexes. The Korsmeyer–Peppas model indicated a non-Fickian diffusion, involving both diffusion and relaxation of the polymer network. The results demonstrate that the choice of additives allows for the adjustment of mechanical properties and release kinetics, highlighting the potential of semiIPN hydrogels for biomedical and defense applications.

CHAPTER 7

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

7.1. Importance and relevance of the study

The topic of this doctoral thesis is part of a field of major international interest, located at the intersection of advanced analytical chemistry, materials science and CBRN (chemical, biological, radiological and nuclear) security. In the current context, characterized by complex geopolitical developments and the persistence of risks associated with the use of chemical warfare agents (CWA), the development of effective methods for identification, decontamination and medical protection is a strategic necessity.

The importance of the topic is supported primarily by the need for rapid and unambiguous identification of chemical agents and their biomarkers in complex biological matrices. In real exposure scenarios, detection is not limited to the initial compounds, but also involves the identification of hydrolysis products and biological adducts, which requires the development of integrated, robust and sensitive analytical methodologies. In this sense, the combined use of LC-MS/MS and GC-MS/MS techniques, specifically optimized for different classes of compounds, becomes essential to ensure a high degree of analytical certainty, in accordance with international requirements, including those imposed by the OPCW.

On the other hand, the topic addresses a critical issue related to the limitations of conventional decontamination methods, which can be aggressive for surfaces or can generate secondary contamination. The development of efficient, rapid and field-compatible decontamination solutions is an essential objective in the field of chemical defense. In this context, the use of nanoadsorbents and hybrid materials, such as reactive suspensions or functionalized hydrogels, offers an innovative direction, based on combined mechanisms of adsorption and chemical degradation.

The importance of the topic is also amplified by the integration of hydrogel-type components for dual applications, both in decontamination and in the controlled delivery of antidotes. In the case of exposure to nerve agents, rapid and controlled administration of atropine is essential for survival. Thus, the development of controlled release systems based on hydrogels represents a relevant contribution to both the medical and defense fields.

The thesis responds to real and current needs, contributing to the development of integrated solutions that cover the entire intervention chain: identification - decontamination - treatment. Through the multidisciplinary approach and the use of modern technologies, the work makes a significant contribution to the consolidation of analytical and operational capacities in the field of chemical security.

7.2. Overall conclusions

The results obtained in this thesis demonstrate the efficiency of an integrated approach in the investigation, identification and neutralization of chemical warfare agents, as well as in the development of advanced platforms for protection and treatment.

In the first stage, analytical methods based on LC-MS/MS and GC-MS/MS were developed and optimized for the identification of specific biomarkers of nerve and blister agents in biological samples of plasma and urine. The differentiated approach of matrices and the use of complementary techniques allowed the identification of compounds both in intact form and in the form of hydrolysis products or biological adducts. The study particularly

highlighted the behavior of compounds from the A series, demonstrating their increased stability and providing analytical methods adapted for their detection.

Next, reactive organic suspensions containing ZnO, TiO_2 and zeolite nanoadsorbents were developed, intended for the decontamination of real chemical agents, such as soman (GD) and sulfur mustard (HD). The results showed that these systems exhibit high decontamination efficiency, especially in the case of GD, where degradation was almost instantaneous. For HD, the process was slower, but significantly accelerated by using certain types and concentrations of nanoadsorbents. The study highlighted the existence of optimal concentrations, as well as the influence of agglomeration phenomena on performance.

An important contribution was represented by the development of peelable hydrogels functionalized with metal-organic frameworks (MOF), capable of combining physical capture of the toxic agent with partial chemical degradation. The systems based on the ternary matrix HEC–GA–Alg and MOF-1 demonstrated the best performances, achieving high decontamination efficiencies in a short time, simultaneously with reducing the amount of intact agent recovered.

In the last stage, hydrogels for the controlled release of atropine sulfate were developed, highlighting the role of additives in controlling the morphology, mechanical properties and release kinetics. Hydrogels containing β-cyclodextrin showed the best results, offering an optimal balance between structural stability and controlled release.

The results confirm that the use of hybrid materials and nanostructures allows the development of efficient and versatile systems for the management of exposure to chemical warfare agents.

7.3. Integrative synthesis of experimental results on identification, decontamination and treatment in chemical warfare agent exposure scenarios

The experimental data obtained in the thesis demonstrate the achievement of the proposed objectives by developing integrated strategies for identification, decontamination and protection in chemical warfare agent exposure scenarios. The first experimental direction aimed at the development and application of LC-MS/MS and GC-MS/MS analytical methods for the identification of biomarkers specific to nerve and blister agents in plasma and urine samples. Depending on the matrix and the nature of the compound, distinct preparation strategies were applied, including solid phase extraction, derivatization with PFB-Br or HFBI, enzymatic digestion, refluorination, protein precipitation and direct dilution. This approach allowed the identification of compounds in intact form, as hydrolysis products or as biological adducts, providing multiple analytical confirmation of exposure.

By LC-MS/MS, relevant biomarkers for exposure to blister agents have been identified. Compounds such as hydrolysis products of mustard gas, the analogue 1,3-bis(2-hydroxyethylthio)propane, triethanolamine, as well as CPF-type adducts of intact products have been reported. For TDG, although the theoretical protonated ion would have been $[M+H]^+$ with m/z 123, under the experimental conditions the ion m/z 105 was observed, assigned to an episulfonium-type structure, and subsequently used as a precursor ion. The transition 105 → 45 was selected as the quantification transition, and 105 → 61 and 105 → 87 as confirmation transitions. For the peptide adducts of mustard, sesquimustard and the propyl-bridged analogue, precursor ions m/z 470.17, 530.2 and 544.2 were monitored, with characteristic transitions to specific sulfur or peptide fragments. For nitrogen mustard, the

HN3-CPF adduct was identified by the precursor ion m/z 497.2, with the transition 497.2 → 364.1 being used for quantification.

In the case of nerve agents, the LC-MS/MS technique allowed the identification of A-series compounds in intact form and as derived biomarkers. A-230 and A-232 were detected by precursor ions m/z 195.1 and 211.1, both using the transition to m/z 74.1 as the quantitation transition, confirming a common fragmentation behavior for this class. The hydrolysis products A-230-OH, A-232-OH and A-234-OH also showed characteristic transitions towards m/z 74, and the corresponding PFB derivatives were also detected by LC-MS/MS, although the derivatization method had been initially used for GC-MS/MS. For the peptide biomarkers of compounds A-230 and A-232, doubly charged ions were observed at m/z 485.7 and 493.7, behavior specific to nonapeptide adducts. Also, for Sarin and A-234, tyrosine adducts were identified, GB-Tyr at m/z 302 and A234-Tyr at m/z 386.2.

In addition, intact compounds or volatile derivatives of the hydrolysis products were confirmed by GC-MS/MS. Intact mustard was identified in the EI mode by the transitions 109 → 73 and 141 → 86, at a retention time of approximately 10.59 min, and HFBI-derivatized TDG was identified by the transitions 301 → 240 and 241 → 169, at approximately 12.16 min. Sesquiiperite was identified by the transitions 182 → 123 and 182 → 63, at approximately 17.51 min, and its derivatized hydrolysis product by the transitions 301 → 241 and 301 → 169. For Sarin, the identification of the intact molecule was achieved by the transitions 125 → 99 and 99 → 47, at 5.11–5.14 min, and the hydrolysis product IpMPA-PFB by the transitions 137 → 95 and 137 → 79. Soman presented two close signals, at 8.45–8.52 min, attributed to isomers, monitored by the transitions 126 → 82 and 99 → 81. Thus, by correlating LC-MS/MS with GC-MS/MS, the identification of biomarkers was achieved based on retention times, Q/q transitions and ion ratios, reducing the risk of misattribution.

The second experimental direction aimed at the development of reactive organic suspensions for the decontamination of real HD and GD agents. The basic organic solution presented a density of 0.91 ± 0.02 g/cm^3 and an alkalinity of 2.47 ± 0.5 cm^3 HCl 1 N/g, without degrading the tested metal surfaces and maintaining efficiency after exposure to extreme temperature and humidity conditions. By integrating ZnO, TiO_2 and zeolite nanoadsorbents, suspensions with concentrations of 0.1–2% m/m were obtained. For HD, the pure organic solution reached 76.42% efficiency after 2 minutes and 99.99% after 24 hours. In comparison, the suspension with 0.1% TiO2 reached 94.95% after 2 minutes and 99.90% after 5 hours, and the suspension with 0.1% zeolite reached 95.64% after 2 minutes and 99.92% after 5 hours, demonstrating a major acceleration of the process. For ZnO, the optimal concentration was 1%, with 97.42% efficiency after 5 hours, while 2% led to a decrease in performance, attributed to particle agglomeration.

For GD, the decontamination process was considerably faster. The pure organic solution reached 99.83% after 2 minutes and 99.91% after 1 hour, while the suspensions with 0.1% TiO2 and 0.1% zeolite led to practically complete reaction after 10 minutes. The 1% ZnO suspension reached 99.91% after 2 minutes and 99.97% after 10 minutes. Monitoring of degradation products confirmed the transformation of toxic agents into compounds with reduced hazard. For HD, four main products were identified: 2-chloroethyl vinyl sulfide, 1,4-dithiane, 1,2-bis(vinylthio)ethane and 2-thiomorpholinoethanol. For GD, five main products were identified: methylphosphonic acid, isopropyl pinacolyl methylphosphonate, pinacolyl methylphosphonic acid, dipinacolyl methylphosphonate and di(2-ethoxyethyl) ester of methylphosphonic acid. These results demonstrate that the developed formulations act not

only by removing the initial agent, but also by converting it into less hazardous degradation products.

The third experimental direction aimed at the development of removable film-forming hydrogels HEC–GA–Alg incorporating metal-organic frameworks for rapid decontamination of mustard gas. Formulations based on alginate, HEC and gum arabic, ionically cross-linked with Ca^{2+} and loaded with MOF-1, MOF-2, MOF-3 or CeO_2 were developed. The formulations exhibited shear-thinning rheological behavior, suitable for application on contaminated surfaces, and the viscoelastic response dominated by the elastic component supported the integrity of the films during application and removal. The polymer hydrogels without filler had decontamination efficiencies of approximately 67–72% after 10 minutes, while the integration of MOF-1 into the F3 ternary matrix increased the efficiency to 80.1–82.9% for concentrations of 1–1.5% MOF-1. Furthermore, the total amount of mustard gas recovered decreased to approximately 41–45% for these formulations, compared to ≥54–65% for MOF-3 and CeO_2, indicating a dual mechanism: physical capture and partial transformation. The identification of 1,4-oxathiane and 1,4-dithiane confirms the contribution of chemical degradation processes.

The fourth experimental direction consisted in the development of sIPN hydrogels for the controlled release of atropine sulfate. The matrix was obtained by UV photopolymerization of HEAA and NVP in the presence of PVA, using DVE as a crosslinking agent and Irgacure 2959 as a photoinitiator. The additives used were halloysite, TiO_2, β-cyclodextrin and γ-cyclodextrin. Hydrogels with cyclodextrins presented organized porous structures, favorable for controlled release, while halloysite led to the highest gel fraction, 90.85%, and the lowest degree of swelling indicating a denser and more rigid network. Formulations with β- and γ-cyclodextrin presented a favorable combination of flexibility, structural stability and diffusion capacity. The release studies were best described by the Korsmeyer–Peppas model, with $R^2 > 0.97$, indicating a non-Fickian mechanism, based on diffusion and relaxation of the polymer network. Among the formulations, the β-cyclodextrin hydrogel stood out as the most promising for the controlled release of atropine, due to the ability to form stable inclusion complexes with the active molecule.

By correlating these results, the thesis demonstrates the achievement of the proposed experimental objectives: the development of robust analytical methods for the identification of biomarkers in biological samples, the formulation of efficient reactive suspensions for the decontamination of HD and GD, the obtaining of removable hydrogels capable of rapidly reducing mustard gas contamination and the development of hydrogel-based systems for the controlled release of atropine. The totality of the experimental data highlights the integrated nature of the research, oriented towards identification, intervention and protection in real CBRN scenarios.

7.4. Author's contributions

Developing an integrated approach to managing exposure to chemical warfare agents

The most significant original contribution of the present doctoral thesis lies in the conception and demonstration of the feasibility of an integrated approach to the management of chemical warfare agent exposure, built upon the principle of continuity between analytical identification, decontamination, and therapeutic intervention. In the existing scientific literature, these components are generally addressed as independent research fields and are

typically developed separately by distinct scientific communities. In contrast, the research presented in this thesis proposes a unified perspective on the entire CBRN response process, in which each stage is intrinsically linked to the preceding one and directly influences the effectiveness of the subsequent stage.

Thus, the identification of specific biomarkers is the primary step for confirming exposure and characterizing the agent involved, providing the information necessary to initiate response measures. Decontamination aims to limit absorption and prevent secondary contamination, and the controlled administration of antidotes completes the intervention by diminishing the pathophysiological consequences of exposure. By integrating these components into a single experimental and conceptual flow, the thesis goes beyond the classical approach based on individual studies and demonstrates that the efficiency of the overall system is the result of optimizing each step.

The originality of the work is not limited only to the development of new analytical methods and materials, but also to the definition of a multidisciplinary platform that reflects the real way of conducting interventions in CBRN scenarios. This integrative perspective constitutes one of the main contributions of the thesis and may represent a model for the future development of operational strategies dedicated to protection against chemical threats.

Development of integrated analytical strategies for the identification of specific biomarkers to chemical warfare agents

A major personal contribution of this thesis consists in the optimization of complementary analytical strategies based on LC-MS/MS and GC-MS/MS techniques for the identification of biomarkers specific to neurotoxic and vesicant agents in complex biological matrices, such as plasma and urine. The originality of this direction does not consist exclusively in the use of the two analytical techniques, but in the design of an integrated methodology, adapted to the particularities of each class of compounds and each type of biomarker.

Distinct sample preparation procedures, such as solid phase extraction, enzymatic digestion, derivatization with PFB-Br and HFBI, refluorination, protein precipitation and direct dilution, were developed and optimized, depending on the nature of the analyte of interest. This approach allowed the simultaneous identification of intact compounds, hydrolysis products and biological adducts, providing multiple analytical confirmation and reducing the risk of misidentifications.

By correlating the information obtained by LC-MS/MS and GC-MS/MS, robust analytical confirmation strategies have been developed, based on retention time, ion ratios and precursor-product transitions, compatible with current requirements for the identification of chemical warfare agents. The results obtained demonstrate that the complementary use of the two techniques leads to increased selectivity and confidence of identification, representing a relevant contribution for laboratories involved in the analysis of CBRN samples.

Contributions on the investigation of A-series agents (Novichok)

One of the most valuable original contributions of this thesis is represented by the investigation of the A-series compounds (Novichok), a category of nerve agents that has attracted the attention of the scientific community in recent years, following recent international incidents and the inclusion of these substances in the OPCW monitoring programs. Compared to classical organophosphorus agents, the available information on the

behavior of these compounds in biological fluids, their stability and the resulting biomarkers is still limited.

In this context, the research aimed at developing dedicated analytical methods for the identification of compounds A-230, A-232 and A-234 both in intact form and as hydrolysis products and biological adducts. Experimental data on their behavior under LC-MS/MS and GC-MS/MS analysis conditions were obtained, highlighting the high chemical stability of these agents and the possibility of their direct identification in biological samples.

A novel element is the demonstration that PFB derivatives, traditionally used in GC-MS/MS analysis, can be successfully integrated into LC-MS/MS strategies, obtaining intense, stable and reproducible signals. The relevance and robustness of this approach were confirmed in the interlaboratory tests organized under the aegis of the OPCW (Organization for the Prohibition of Chemical Weapons), where the sample preparation methods and analytical strategies developed in the present thesis demonstrated a high level of reliability and accuracy in the identification of biomarkers specific to chemical warfare agents, in accordance with the international standards applicable to designated laboratories. These results confirm not only the innovative nature of the proposed methodology, but also its potential for implementation in specialized laboratories involved in verifying compliance with the Chemical Weapons Convention and in investigating CBRN incidents.

This approach expands existing analytical possibilities and contributes to the development of methodologies adapted to a class of agents for which the specialized literature is still in a consolidation stage.

Development of reactive organic suspensions for the decontamination of chemical warfare agents

An important original contribution consists in the development and optimization of reactive organic suspensions incorporating ZnO, TiO_2 and zeolite nanoadsorbents, intended for the decontamination of real chemical agents, such as soman (GD) and sulfur mustard (HD). The research aimed to overcome the limitations of conventional decontamination systems by designing formulations capable of combining adsorption and chemical degradation processes in a single material.

By systematically investigating the type and concentration of nanoadsorbents, the existence of optimal concentrations of use was demonstrated and the impact of agglomeration phenomena on decontamination efficiency was highlighted. Unlike numerous studies that report exclusively the degree of contaminant elimination, the research carried out also included the identification of degradation products resulting from detoxification reactions, providing additional information on the mechanisms involved.

The results demonstrate that the developed formulations act not only by physically removing the toxic agent, but also by transforming it into compounds with reduced toxicity, a particularly important aspect in evaluating the performance of a decontamination system intended for CBRN applications.

Development of peelable hydrogels incorporating metal-organic frameworks

A contribution with a pronounced innovative character is represented by the development of a new generation of peelable hydrogels based on HEC–GA–Alg, capable of incorporating metal-organic frameworks for the rapid decontamination of blister agents. The design of these materials was based on the limitations identified in existing formulations,

which provided either adequate mechanical properties or chemical activity on the contaminant, but rarely combined these characteristics in a single system.

Starting from the experience accumulated in the development of ionic crosslinked hydrogels without metal-organic frameworks, previously presented in the specialized literature, in the present thesis a new architecture based on the integration of MOFs in a ternary matrix of biopolymers was proposed. This approach allowed the obtaining of materials that combine film-forming properties, rapid detachment capacity, efficient adsorption and partial chemical degradation of mustard gas.

The rheological, mechanical and morphological characterization demonstrated the existence of a synergistic effect between the polymer matrix and the metal-organic structures, reflected by the improvement of decontamination performances and the reduction of the amount of toxic agent recovered after the application of the hydrogel. Therefore, the research not only proposes a new formulation, but also introduces a new concept of multifunctional material intended for immediate intervention in CBRN scenarios.

- **Development of hydrogels for controlled release of atropine**

The last experimental direction extended the applicability of the developed hydrogels to the field of medical intervention, by designing controlled release systems of atropine sulfate. This approach goes beyond the classic concept of hydrogels used exclusively for decontamination and demonstrates their potential to contribute to the therapeutic stage of the CBRN response.

By using different additives, such as halloysite, β-cyclodextrin, γ-cyclodextrin and TiO_2, the relationships between the architecture of the polymer network, the mechanical properties and the release profile of the active substance were investigated. The results demonstrated that the controlled modification of the composition allows the adjustment of the release kinetics and the development of systems capable of ensuring a sustained administration of atropine.

Beyond the characterization of the materials, the research proposes an alternative direction of antidote administration, with potential for use in situations where access to injectable administration is limited or delayed. In this sense, the developed hydrogels represent more than simple drug delivery systems, constituting a possible platform for the development of modern technologies intended for rapid intervention in scenarios of exposure to neurotoxic agents.

7.5. Future research directions

The results obtained open multiple future research directions. In the analytical field, extended methods can be developed for the identification of other classes of chemical agents and for the integration of rapid screening techniques.

Regarding decontamination materials, future research can aim at further optimizing the compositions, exploring other types of nanomaterials and evaluating the performance under real field conditions.

For functionalized hydrogels, future directions include increasing the MOF loading, improving stability and evaluating biocompatibility for direct skin applications.

In the case of controlled release systems, other active substances, stimuli-response release mechanisms and integration into portable devices for use in emergency situations can be investigated.

Overall, the thesis creates a solid basis for the development of advanced technologies in the field of protection against chemical agents, with potential for application in both the civilian and military fields.

List of published works and conference participations

7 ISI articles resulted (cumulative IF – 28.447, 4 Q1 and Q2) of which 3 ISI articles as lead author (cumulative IF – 11,272, Q1 and 1 Q2) and 9 presentations at national/international conferences of which 3 as lead author.

Research activities were funded through 5 research and development projects (2 national projects and 3 international projects), of which the author is project director in 1 national project.

Publications indexed in the Web of Science Core Collection resulting from the research of the doctoral thesis

Lead author (cumulative IF – 12.572)

1. **Adriana Elena Bratu**, Aurel Diacon, Izabela-Cristina Stancu, Mihail Munteanu, Raluca Elena Ginghina, Gabriela Toader, *Development of alginate–hydroxyethyl cellulose–gum arabic ternary peelable hydrogels for sulfur mustard decontamination*, Carbohydrate Polymer Technologies and Applications (Elsevier), 101197 pre-proof online; **(IF 2026 – 7.6, Q1)**

2. **Adriana Elena Bratu**, Izabela-Cristina Stancu, Aurel Diacon, Gabriela Toader, Ana Mihaela Gavrila, Raluca Elena Ginghina, Sebastian Stefan Pricop, Tudor-Viorel Tiganescu, *Hydrogels for controlled release of atropine in chemical warfare agents threat scenarios,* publicat în Sci. Bull., Series B, Vol. 88, Iss. 2, 2026, WoS, ISSN 1454-2331; **(IF 2026 – 0.5, Q4)**

3. **A. Bratu**, R.E. Ginghina, G. Toader, A. Moldovan, T.V. Tiganescu, R. Oncioiu, P. Deliu, R. Petre, G. Epure, M. Purica, *Reactive organic suspensions comprising ZnO, TiO2 and zeolite nanosized adsorbents: evaluation of decontamination efficiency on soman and sulfur mustard*, Toxics 2021, 9 (12), 334; **(IF 2022 - 4.472, Q2)**

Co-autor (IF cumulat – 15.875)

4. G. Toader, R.E. Ginghina, **A.E. Bratu,** A.I. Podaru, D. Pulpea, T. Rotariu, A.M. Gavrilă, A. Diacon, *Ionic Crosslinked Hydrogel Films for Immediate Decontamination of Chemical Warfare Agents,* Gels **2024**,10, 428; **(IF 2024 – 5.3, Q1)**

5. G. Toader, R.E. Ginghina, A. Diacon, E. Rusen, **A.E. Bratu,** A. Podaru, T. Rotariu, *Design and Application of Photocrosslinkable Hydrogel Films for Fast and Efficient Decontamination of Chemical Warfare Agents*, ACS Appl. Polym. Mater. 2022; **(IF 2022 – 4.7, Q1)**

6. R.E. Ginghina, G. Toader, M. Purica, **A. Bratu**, C. Lazaroaie, T.V. Tiganescu, R. Oncioiu, O. Iorga, F. Zorila, M. Constantin, G. Craciun, F. Comanescu, C. Romanitan, *Antimicrobial activity and degradation ability study on nanoparticle-enriched formulations specially designed for the neutralization of real and simulated biological and chemical warfare agents*, Pharmaceuticals 2022, 15 (1), 97; **(IF 2022 - 5.575, Q1)**

7. T.V. Țigănescu, R.E. Ginghină, N. Grigoriu, A. Pretorian, R. Petre, **A. Bratu**, O. Hoza, *Decontamination Efficiency Of Nerve Agent Soman (GD) With A New Amino-Alcoholic Solution,* U.P.B. Sci. Bull., Series B, 83 (4), 2021, 75-84; **(IF 2021 – 0.3)**

International conferences / proceedings / Conference volumes

8.**A. Bratu,** I.C. Stancu, R.E. Ginghină, G. Toader, O. Hoza, F.L. Zorila, T.V. Tigănescu, *Hydrogels for wounds caused by blister agents,* PRIOCHEM 18th Edition, Bucharest 2022; **(poster presentation)**

9. **A.E. Bratu,** R.E. Ginghina, A.E. Modovan, T.V. Tiganescu, G. Toader, R.E. Oncioiu, P. Deliu, G. Epure, *Zinc oxide nanoparticles for the decontamination of chemical warfare nerve agents and blister agents*, Chem. Proc. 2022, 7, 78; **(articol în volum de conferință)**

10. **A. Bratu**, R. Ginghina, A. Moldovan, T.V. Tiganescu, G. Toader, R. Oncioiu, P. Deliu, G. Epure, *The influence of the zinc oxide nanoparticles over decontamination efficiency of soman and sulfur mustard,* PRIOCHEM 17th Edition, Bucharest 2021, ISSN 2601 - 4203 and ISSN-L 2601 - 4181; **(poster presentation)**

11. R. Ginghina, G. Toader, **A. Bratu,** I. A. Stoian, *Emerging Technologies and Solutions for Chemical*

Warfare Agents Decontamination, The 28th International Scientific Conference Knowledge-Based Organization KBO 2022, Conference proceedings, ISSN: 1843-682X, 28 (3), 2022, 39 – 43;**(articol în volum de conferință)**

12. R.E. Ginghină, **A.E. Bratu,** G. Toader, T.V. Tigănescu, *Nanoparticles - Enriched Compositions with Applications in Chemical Warfare Agents Neutralization*, 9th International Conference on Materials Science and Technologies – RoMAT 2022, Bucharest 2022; **(oral presentation)**

13. O. Hoza, A. Podaru, R. Ginghina, G. Toader, M. Alexandru, **A. Bratu**, A.M. Gavrila, B. Trica, *Hydrogel coatings for CBRN applications,* PRIOCHEM 18th Edition, Bucharest 2022; **(poster presentation)**

14. R.E. Ginghină, G. Toader, T.V. Tigănescu, M. Purica, **A. Bratu**, C. Lazaroaie, F. Zorila, M. Constantin, *Antimicrobial activity of the natural zeolites and titanium dioxide nanoparticles embedded in organic decontaminants*, 9th International Conference "Biomaterials, Tissue Engineering & Medical Devices" BIOMMEDD'2022, Bucharest, 2022, p. 200; **(poster presentation)**

15. R.E. Ginghină, T.V. Țigănescu, A.E. Moldovan, R. Oncioiu, O. Hoza, **A. Bratu,***Zinc oxide nanoparticles – amino alcoholic solution for biological decontamination*, 2nd Bucharest Polymer Conference, Bucharest, 2021; **(poster presentation)**

16. T. V. Țigănescu, R.E. Ginghină, N. Grigoriu, A. Pretorian, R. Petre, **A. Bratu**, O. Hoza, *Decontamination efficiency of nerve agent soman (GD) with amino-alcoholic solution*, The 8th International Conference on Materials Science and Technologies – RoMat 2020, Bucharest, 2020; **(poster presentation)**

Projects in which doctoral thesis research activities were carried out.

1. *National capabilities for chemical warfare agents specific biomarkers identification from biomedical samples*, MoD R&D plan**, 2025 –2027– project director;**

2. *Ultrasonic continuous flow technology catalyzed by advanced nanomaterials for waste-free destruction of chemical warfare agents and highly toxic compositions***,** PN-IV-P7-7.1-PED-2024-0980, **2025-2026 – team member;**

3.*Hybrid supramolecular hydrogels for CBRN (chemical, biological, radiological and nuclear) fast decontamination applications,* PN-IV-P2-2.1-TE-2023-0806, **2025-2026** – **team member;**

4.*Antimicrobial patches with bacterial cellulose and plant extracts-emerging technologies for controlled release medical devices*, PN-IV-P7-7.1-PTE-2024-0305, **2025 – 2027** – **team member;**

5. *Deterring the Russian Federation's further use of chemical weapons through enhancing romania's sophisticated detection capabilities of environmental samples*, US Grant **2025-2026 – team member;**

6. *Deterring WMD Assassinations by Developing State of the Art Detection Capabilities for CWC Related Analysis on Biotoxins and New Schedule 1 Chemicals from Environmental Samples in Romania*,US Grant,**2024-2025– team member;**

7. *Deterring State-Based WMD assassinations by Developing Detection and Analysis Capabilities from Biomedical samples in Romania*, US Grants SISNCT21CA3040, 2022-2023; SISNCT22CA0059, **2023-2024– team member;**

8. *Active gels: eco-friendly tools for immediate chemical and biological decontamination of sensitive surfaces*, PN-III-P2-2.1-PED-2021-3415, **2022 – 2024– team member.**

The PhD thesis outlines 222 pages

References

1. Chauhan, S.; Chauhan, S.; D'Cruz, R., Chemical Warfare Agents. *Environmental Toxicology and Pharmacology* **2020**.
2. Marrs, T. C.; Maynard, R. L.; Sidell, F. R., *Chemical Warfare Agents: Toxicology and Treatment*. 2 ed.; Elsevier: 2007.
3. John, H.; Thiermann, H., Analysis of Chemical Warfare Agents. *Journal of Chromatography A* **2021**.
4. Franjesevic, A. J., Mechanisms of Sulfur Mustard Toxicity. *Toxics* **2020**.
5. Balali-Mood, M., Vesicant Agents: Toxicity and Mechanisms. *Toxics* **2021**.
6. Gupta, R. C., *Handbook of Toxicology of Chemical Warfare Agents*. Elsevier: 2020.
7. Noort, D.; Hulst, A. G., Biomarkers of Exposure to Chemical Warfare Agents. *Toxics* **2021**.
8. Nair, A.; Yadav, P.; Behl, A.; Sharma, R. K.; Kulshrestha, S.; Butola, B. S.; Sharma, N., Toxic blister agents: Chemistry, mode of their action and effective treatment strategies. *Chemico-Biological Interactions* **2021,** *350*, 109654.
9. John, H.; Thiermann, H., Recent advances in analysis of chemical warfare agent biomarkers. *Journal of Chromatography A* **2021**.
10. Noort, D.; Hulst, A. G., Biomarkers of exposure to chemical warfare agents: a review. *Toxics* **2022**.
11. Li, B.; Schopfer, L. M.; Lockridge, O., Mass spectrometry detection of organophosphate adducts to proteins. *Chemical Research in Toxicology* **2021**.
12. Balali-Mood, M., Sulfur mustard toxicity and biomarkers. *Toxics* **2021**.
13. Black, R. M.; Read, R. W., Biomarkers of exposure to sulfur mustard. *Archives of Toxicology* **2021**.
14. Gunderson, E. W., Advances in LC-MS/MS analysis of chemical warfare agents. *Trends in Analytical Chemistry* **2022**.
15. van der Schans, M. J., Sample preparation and analysis of chemical warfare agent biomarkers. *Journal of Chromatography B* **2022**.
16. Zurer, P., LC-MS/MS approaches for biomarker identification in toxicology. *Analytical Chemistry* **2021**.
17. Petrovic, M., Recent developments in GC-MS/MS for toxic chemical detection. *Toxics* **2023**.
18. Opravil, J.; Pejchal, J.; Finger, V.; Korabecny, J.; Rozsypal, T.; Hrabinova, M.; Muckova, L.; Hepnarova, V.; Konecny, J.; Soukup, O.; Jun, D., A-agents, misleadingly known as "Novichoks": a narrative review. *Archives of Toxicology* **2023,** *97* (10), 2587-2607.
19. Charejoo, A.; Arabfard, M.; Jafari, A.; Hasani Nourian, Y., A complete, evidence-based review on novichok poisoning based on epidemiological aspects and clinical management. *Frontiers in Toxicology* **2023,** *4*, 1004705.
20. Organisation for the Prohibition of Chemical, W. OPCW Provides Technical Assistance to Germany Regarding Allegations of Chemical Weapons Use Against Alexei Navalny. https://www.opcw.org/media-centre/news/2020/09/opcw-provides-technical-assistance-germany-regarding-allegations-chemical.
21. Gupta, R.; Parmar, M., Pralidoxime. In *StatPearls*, StatPearls Publishing: Treasure Island (FL), 2023.
22. Schaffer, D. H.; Hayoun, M. A., Nerve Agents. In *StatPearls*, StatPearls Publishing: Treasure Island (FL), 2025.
23. Food, U. S.; Drug, A. FDA Approves Pyridostigmine Bromide as Pretreatment Against Nerve Gas. https://www.fda.gov/drugs/bioterrorism-and-drug-preparedness/fda-approves-pyridostigmine-bromide-pretreatment-against-nerve-gas.

24. Li, H.; Lu, C.; Liu, Z.; Xiang, F.; Liu, B.; Wang, H.; Chang, J.; Pan, L.; Chen, Y.; Chen, J., Advancements in bioscavenger mediated detoxification of organophosphorus poisoning. *Toxicol Res (Camb)* **2024,** *13* (3), tfae089.
25. Organisation for the Prohibition of Chemical, W. Chemical Agents and Medical Response. https://www.opcw.org.
26. Snider, V. G.; Hill, C. L., Functionalized reactive polymers for the removal of chemical warfare agents. *Journal of Hazardous Materials* **2023**.
27. Huang, T., MOF-based materials for degradation of chemical warfare agents. *Nanomaterials* **2024**.
28. Ginghina, R. E.; Bratu, A. E., Reactive organic suspensions with ZnO, TiO2 and zeolite for chemical warfare agent decontamination. *Toxics* **2021**.
29. Sura, P.; Popiel, S., Selected decontamination methods of chemical warfare agents. *Biuletyn Wojskowej Akademii Technicznej* **2023**.
30. Shumeiko, A. E., Chemical warfare agents: structure, properties and decontamination. *Journal of Organic and Pharmaceutical Chemistry* **2023**.
31. Snider, V. G.; Hill, C. L., Functionalized reactive polymers for removal of chemical warfare agents. *Journal of Hazardous Materials* **2023**.
32. Segneanu, A.-E.; Bejenaru, L. E.; Bejenaru, C.; Blendea, A.; Mogoşanu, G. D.; Biţă, A.; Boia, E. R., Advancements in Hydrogels: A Comprehensive Review of Natural and Synthetic Innovations for Biomedical Applications. *Polymers* **2025,** *17* (15), 2026.
33. Gao, M.; Yang, J.; Zhang, Y.; Gao, M.; Zhang, M.; Wang, X.; Xu, L.; Wang, Z.; Shen, B., Adsorption of chemical warfare agents and their simulants by metal-organic frameworks: GCMC and DFT studies. *Journal of Environmental Chemical Engineering* **2025,** *13* (3), 116628.
34. Dwivedi, R.; Singh, A. K.; Dhillon, A., pH-responsive drug release from dependal-M loaded polyacrylamide hydrogels. *Journal of Science: Advanced Materials and Devices* **2017,** *2* (1), 45-50.
35. Durán-Egido, V.; García-Giménez, D.; Martínez-López, J. C.; Pérez-Vidal, L.; Carretero-González, J., Metal–Organic Frameworks as Fillers in Porous Organic Polymer-Based Hybrid Materials: Innovations in Composition, Processing, and Applications. *Polymers* **2025,** *17* (14), 1941.
36. DeFalco, A. P., Chemical warfare agents and delivery systems. In *Encyclopedia of Toxicology (Fourth Edition)*, Wexler, P., Ed. Academic Press: Oxford, 2024; pp 761-768.
37. Esposito, R.; Musteata, V.; Chisca, S.; Nunes, S. P., Rheology of Polytriazole/ZIF-8 Solutions and Dynamics of Mixed-Matrix Composite Films. *ACS Applied Polymer Materials* **2021,** *3* (12), 6045-6055.
38. Feng, K.; Zhang, J. A.; Shen, W. T.; Leng, T.; Zhou, Z.; Yu, Y., Recent Development of Nanoparticle Platforms for Organophosphate Nerve Agent Detoxification. *Langmuir* **2025,** *41* (4), 2124-2140.
39. Toader, G.; Diacon, A.; Rotariu, T.; Alexandru, M.; Rusen, E.; Ginghină, R. E.; Alexe, F.; Oncioiu, R.; Zorila, F. L.; Podaru, A.; Moldovan, A. E.; Pulpea, D.; Gavrilă, A. M.; Iordache, T. V.; Șomoghi, R., Eco–Friendly Peelable Active Nanocomposite Films Designed for Biological and Chemical Warfare Agents Decontamination. *Polymers* **2021,** *13* (22), 3999.
40. Toader, G.; Ginghina, R.-E.; Bratu, A. E.; Podaru, A. I.; Pulpea, D.; Rotariu, T.; Gavrilă, A. M.; Diacon, A., Ionic Crosslinked Hydrogel Films for Immediate Decontamination of Chemical Warfare Agents. *Gels* **2024,** *10* (7), 428.
41. Olteanu, G.; Neacșu, S. M.; Joița, F. A.; Musuc, A. M.; Lupu, E. C.; Ioniță-Mîndrican, C. B.; Lupuliasa, D.; Mititelu, M., Advancements in Regenerative Hydrogels in Skin Wound Treatment: A Comprehensive Review. *Int J Mol Sci* **2024,** *25* (7).

42. Peppas, N. A.; Hoffman, A. S., 1.3.2E - Hydrogels. In *Biomaterials Science (Fourth Edition)*, Wagner, W. R.; Sakiyama-Elbert, S. E.; Zhang, G.; Yaszemski, M. J., Eds. Academic Press: 2020; pp 153-166.
43. Rafieian, S.; Mirzadeh, H.; Mahdavi, H.; Masoumi, M. E., A review on nanocomposite hydrogels and their biomedical applications. *Science and Engineering of Composite Materials* **2019,** *26* (1), 154-174.

43. Esposito, R.; Musteata, V.; Chisca, S.; Nunes, S. P., Rheology of Polytriazole/ZIF-8 Solutions and Dynamics of Mixed-Matrix Composite Films. *ACS Applied Polymer Materials* **2021,** *3* (12), 6045-6055.
44. Feng, K.; Zhang, J. A.; Shen, W. T.; Leng, T.; Zhou, Z.; Yu, Y., Recent Development of Nanoparticle Platforms for Organophosphate Nerve Agent Detoxification. *Langmuir* **2025,** *41* (4), 2124-2140.
45. Toader, G.; Diacon, A.; Rotariu, T.; Alexandru, M.; Rusen, E.; Ginghină, R. E.; Alexe, F.; Oncioiu, R.; Zorila, F. L.; Podaru, A.; Moldovan, A. E.; Pulpea, D.; Gavrilă, A. M.; Iordache, T. V.; Șomoghi, R., Eco–Friendly Peelable Active Nanocomposite Films Designed for Biological and Chemical Warfare Agents Decontamination. *Polymers* **2021,** *13* (22), 3999.
46. Toader, G.; Ginghina, R.-E.; Bratu, A. E.; Podaru, A. I.; Pulpea, D.; Rotariu, T.; Gavrilă, A. M.; Diacon, A., Ionic Crosslinked Hydrogel Films for Immediate Decontamination of Chemical Warfare Agents. *Gels* **2024,** *10* (7), 428.
47. Olteanu, G.; Neacșu, S. M.; Joița, F. A.; Musuc, A. M.; Lupu, E. C.; Ioniță-Mîndrican, C. B.; Lupuliasa, D.; Mititelu, M., Advancements in Regenerative Hydrogels in Skin Wound Treatment: A Comprehensive Review. *Int J Mol Sci* **2024,** *25* (7).
48. Peppas, N. A.; Hoffman, A. S., 1.3.2E - Hydrogels. In *Biomaterials Science (Fourth Edition)*, Wagner, W. R.; Sakiyama-Elbert, S. E.; Zhang, G.; Yaszemski, M. J., Eds. Academic Press: 2020; pp 153-166.
49. Rafieian, S.; Mirzadeh, H.; Mahdavi, H.; Masoumi, M. E., A review on nanocomposite hydrogels and their biomedical applications. *Science and Engineering of Composite Materials* **2019,** *26* (1), 154-174.