

# NEW HORIZONS IN CHEMISTRY:

From Fundamental Principles To Applications

Editor:

**Prof. Dr. Hülya ÇELİK**



**BİDGE Yayınları**

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## ÖNSÖZ

Kimya bilimi, maddenin yapısını, özelliklerini ve dönüşümünü açıklayan temel ilkeleri ile bilimsel ilerlemenin merkezinde yer almaktadır. Atomik etkileşimlerin anlaşılmasından karmaşık moleküler sistemlerin tasarımına kadar uzanan geniş kapsamıyla kimya; fizik, biyoloji ve uygulamalı bilimler arasında köprü kuran bütüncül bir disiplin niteliği taşımaktadır. Malzeme bilimi, çevre sürdürülebilirliği, biyoteknoloji ve enerji araştırmaları gibi pek çok alanda sağladığı katkılar, kimyanın modern dünyanın karşı karşıya olduğu sorunların çözümündeki vazgeçilmez rolünü açıkça ortaya koymaktadır.

Bu geniş çerçeve içerisinde farmasötik kimya, biyolojik olarak aktif moleküllerin rasyonel tasarımı, sentezi ve optimizasyonuna odaklanarak kimya biliminin insan sağlığına en doğrudan katkı sunduğu alanlardan biri olarak öne çıkmaktadır. Moleküler modelleme, modern sentez yöntemleri ve ileri analitik tekniklerdeki gelişmeler, yeni terapötik ajanların keşif ve geliştirme süreçlerini önemli ölçüde hızlandırmıştır. Bununla birlikte, küresel ölçekte artan sürdürülebilirlik bilinci, yeşil kimya yaklaşımlarını bilimsel araştırmaların merkezine taşımış; daha güvenli, daha verimli ve çevre dostu kimyasal süreçlerin geliştirilmesini öncelikli hale getirmiştir.

Bu eser, kimya biliminin birbirleriyle ilişkili bu çok boyutlu alanlarını bütüncül bir bakış açısıyla ele almayı amaçlamaktadır. Temel prensiplerden modern sentez stratejilerine ve sürdürülebilir yaklaşımlara uzanan geniş bir perspektif sunarak, hem teorik bilgiyi hem de uygulamaya yönelik yaklaşımları bir araya getirmektedir. Bu çalışmanın, araştırmacılar, akademisyenler ve lisansüstü öğrenciler için değerli bir başvuru kaynağı olması ve kimya biliminin yenilikçi ve sürdürülebilir yönlerine katkı sağlaması temennisiyle...

**Prof. Dr. Hülya ÇELİK**  
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# CHAPTER 1

## GAS CHROMATOGRAPHY–MASS SPECTROMETRY IN OMICS, ARTIFICIAL INTELLIGENCE AND CLINICAL RESEARCH

MERVE KILIÇ<sup>1</sup>

### Introduction

Gas chromatography–mass spectrometry (GC–MS) is widely recognized as a robust and reliable analytical platform for the separation and identification of small molecules in complex biological systems. By combining chromatographic separation with mass spectral detection, GC–MS enables both qualitative and quantitative analysis of volatile and semi-volatile compounds with high reproducibility. This capability makes GC–MS particularly suitable for metabolomics studies, especially in the analysis of primary metabolites such as organic acids, amino acids, sugars, and fatty acids (Frolova et al., 2025; Ioannidis et al., 2025).

Recent developments in analytical workflows have significantly enhanced the performance of GC–MS in biological and clinical research. Advances in derivatization techniques and sample preparation strategies have improved the detection of low-

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abundance metabolites, while the expansion of spectral libraries has increased identification accuracy. In addition, the reproducibility of electron ionization (EI) fragmentation patterns allows reliable compound matching across studies, which is essential for large-scale metabolomic investigations (Bajo-Fernández et al., 2024; Ioannidis et al., 2025).

Moreover, the integration of computational approaches has transformed GC–MS data analysis. Modern workflows increasingly incorporate chemometric tools and machine learning algorithms to improve peak resolution, metabolite annotation, and pattern recognition. These approaches are particularly important in clinical research, where GC–MS-based metabolomics is used to identify disease-related metabolic signatures and potential biomarkers (Nafie et al., 2025; Wang et al., 2026).

Despite the growing use of complementary platforms such as LC–MS, GC–MS remains a cornerstone technique due to its robustness, well-established spectral libraries, and cost-effectiveness. Its ability to generate highly reproducible fragmentation patterns continues to support reliable metabolite identification in diverse research fields (Frolova et al., 2025).

Overall, GC–MS continues to play a central role in analytical biochemistry, bridging classical analytical chemistry with emerging fields such as metabolomics, artificial intelligence–based data analysis, and precision medicine.

## **Analytical Principles of Gas Chromatography–Mass Spectrometry**

GC–MS operates on a relatively simple idea: compounds in a mixture are first separated in space and time, and then identified based on how they fragment under controlled conditions. Although the technique is often introduced as a combination of two

independent systems, in practice, its analytical strength emerges from how seamlessly these two components interact.

The first stage, GC, is responsible for separating compounds before they reach the detector. In this step, the sample is vaporized and transported by an inert carrier gas—typically helium—through a capillary column coated with a stationary phase. As molecules move along the column, they interact differently with the stationary phase depending on their volatility and polarity. Compounds that interact weakly elute faster, while those with stronger interactions are retained longer. This results in a temporal separation, where each compound exits the column at a characteristic retention time (Fiehn, 2016).

However, retention time alone is rarely sufficient for confident identification, particularly in complex biological samples. This is where MS becomes essential. As compounds elute from the GC column, they enter the ionization chamber of the mass spectrometer. The most commonly used ionization method in GC–MS is EI, which involves bombarding molecules with high-energy electrons. This process produces charged fragments in a highly reproducible manner, generating a pattern that can be considered a molecular “fingerprint” (Kind & Fiehn, 2010).

Once ionized, the fragments are separated according to their mass-to-charge ( $m/z$ ) ratios using a mass analyzer, most commonly a quadrupole system. By scanning across a defined  $m/z$  range, the instrument records a mass spectrum for each compound. These spectra can then be compared with reference libraries such as NIST, enabling reliable identification even in the absence of authentic standards. The reproducibility of EI fragmentation patterns is one of the key reasons why GC–MS remains widely used in metabolomics and analytical chemistry (Dunn et al., 2005).

An important aspect of GC–MS is that the quality of the analytical output depends not only on instrument performance but also on the physicochemical properties of the analytes. Because GC requires analytes to be volatile and thermally stable, many biological compounds—especially polar metabolites—must undergo derivatization prior to analysis. This chemical modification improves volatility and chromatographic behavior, allowing a broader range of metabolites to be analyzed using GC–MS (Fiehn, 2016; Patti et al., 2012)

GC–MS data are often interpreted by considering both retention behavior and mass spectral patterns together. This combined approach increases confidence in compound identification and reduces the likelihood of false positives. As a result, GC–MS continues to serve as a dependable analytical platform, particularly in studies where reproducibility and structural information are critical.

### **Sample Preparation Strategies in Omics Studies**

Sample preparation is often treated as a preliminary step, yet in practice it plays a decisive role in the quality and interpretability of omics data. In GC–MS-based workflows, where analytes must be volatile and thermally stable, preparation steps are not only about extraction but also about making compounds analytically compatible with the system. Small variations introduced at this stage can easily propagate through the analysis and influence both qualitative identification and quantitative outcomes (Dunn et al., 2005).

In metabolomics, sample preparation strategies are typically designed with two competing priorities in mind: preserving the native biochemical state of the sample and achieving efficient extraction of chemically diverse metabolites. Biological matrices such as plasma, urine, or tissue contain compounds with widely different polarities and concentrations. As a result, no single

extraction method is universally optimal. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are commonly used approaches, each offering advantages depending on the target metabolite group. While LLE is relatively simple and suitable for broad metabolite coverage, SPE provides improved selectivity and cleaner extracts, which can be particularly useful when dealing with complex clinical samples (Dettmer et al., 2007).

For GC–MS specifically, derivatization is often an essential step rather than an optional refinement. Many biologically relevant metabolites—especially amino acids, sugars, and fatty acids—are not sufficiently volatile in their native form. Chemical modification, such as silylation or methylation, improves volatility and reduces polarity, enabling better chromatographic separation and peak shape. In lipidomics applications, fatty acids are commonly converted into fatty acid methyl esters (FAMES), a process that enhances both detectability and reproducibility (Fiehn, 2016).

Another critical consideration is the stability of metabolites during sample handling. Enzymatic activity and oxidation reactions can alter metabolite levels rapidly if samples are not processed under controlled conditions. Rapid quenching, low-temperature storage, and minimizing freeze–thaw cycles are simple but important measures to reduce analytical variability (Patti et al., 2012).

In addition to chemical preparation, reproducibility across samples is essential, especially in clinical and comparative studies. The use of internal standards and quality control samples helps monitor extraction efficiency and instrument performance. These practices are particularly valuable in large-scale omics studies, where subtle differences between groups may otherwise be masked by technical variability (Dunn et al., 2005).

Sample preparation in GC–MS-based omics studies should be viewed as an integral part of the analytical process rather than a

routine preliminary step. Thoughtful selection of extraction methods, careful handling of biological samples, and appropriate derivatization strategies together determine the reliability of downstream data and the validity of biological interpretation.

## **GC–MS in Metabolomics and Lipidomics**

Metabolomics focuses on capturing small molecules that reflect the current physiological state of a biological system. Unlike genomic or proteomic data, which provide more static information, metabolite profiles tend to change rapidly in response to environmental and pathological conditions. Because of this dynamic nature, analytical platforms used in metabolomics must be both sensitive and consistent. GC–MS has remained one of the most dependable techniques in this field, particularly for low-molecular-weight metabolites that can be volatilized or chemically modified prior to analysis (Frolova et al., 2025; Hajnajafi & Iqbal, 2025).

One of the key strengths of GC–MS in metabolomics lies in its ability to generate highly reproducible fragmentation patterns. These patterns allow metabolites to be identified with relatively high confidence through comparison with established spectral libraries. In untargeted metabolomics workflows, this feature becomes especially valuable, as large numbers of metabolites need to be screened simultaneously without prior selection. In addition, the separation efficiency of gas chromatography reduces signal overlap, making it easier to distinguish structurally similar compounds within complex biological matrices (Ioannidis et al., 2025).

Despite these advantages, GC–MS does not capture the entire metabolome. Its applicability is largely restricted to volatile compounds or those that can be derivatized. For this reason, it is often used alongside complementary techniques such as LC–MS or NMR to achieve broader metabolome coverage. Multi-platform approaches are increasingly preferred, as they allow different

chemical classes of metabolites to be analyzed more comprehensively and interpreted within the same biological context (Frolova et al., 2026).

Within metabolomics, lipidomics has emerged as a rapidly expanding subfield that focuses specifically on lipid species and their biological roles. Lipids are not only structural components of cell membranes but also active participants in signaling pathways and energy metabolism. Changes in lipid composition are closely linked to metabolic disorders, inflammation, and chronic diseases, making lipidomics particularly relevant for clinical research (Liao et al., 2025).

GC–MS plays a distinct role in lipidomics, especially in the analysis of fatty acids. Through derivatization into fatty acid methyl esters (FAMES), GC–MS enables accurate profiling of fatty acid composition with high reproducibility. This approach is widely used in studies investigating membrane lipid alterations, oxidative stress, and metabolic adaptations. Compared to other lipid analysis techniques, GC–MS offers robust quantification and well-established reference databases, which support reliable comparisons across studies (Frolova et al., 2025).

In clinical metabolomics, GC–MS-based approaches have been increasingly applied to identify disease-related metabolic signatures. For example, metabolic profiling studies have demonstrated that alterations in amino acid, carbohydrate, and fatty acid metabolism can be associated with specific disease states. These findings highlight the potential of GC–MS in biomarker discovery and early diagnosis, particularly when combined with multivariate statistical analysis and pattern recognition methods (Bajo-Fernández et al., 2024; Pak et al., 2025).

GC–MS continues to hold a central position in metabolomics and lipidomics, not because it captures everything, but because it produces consistent and interpretable data for a well-defined subset of metabolites. When integrated with complementary analytical platforms and modern data analysis approaches, it contributes to a more complete understanding of metabolic networks and their role in health and disease.

### **Integration with Artificial Intelligence in Data Analysis**

As analytical technologies have become more sensitive, the volume and complexity of data generated by platforms such as GC–MS have increased substantially. Interpreting these datasets is no longer limited to identifying peaks and matching spectra; instead, it requires recognizing patterns across hundreds or even thousands of variables. Traditional statistical approaches remain useful, yet they can struggle to capture non-linear relationships or subtle interactions within high-dimensional metabolomics data. In this context, AI-based methods have started to play a more prominent role in data analysis (Johnson et al., 2016; Libbrecht & Noble, 2015).

One of the areas where AI contributes most effectively is peak detection and deconvolution. In GC–MS data, co-eluting compounds and background noise can complicate spectral interpretation. Machine learning algorithms can be trained to distinguish true signals from noise, improving both sensitivity and reliability. These approaches are particularly useful in untargeted metabolomics, where the goal is not only to identify known compounds but also to detect previously uncharacterized features (Smith et al., 2006).

Beyond preprocessing, AI methods are increasingly used to support metabolite identification and classification. Spectral matching remains a central step in GC–MS workflows, but incomplete libraries and structural similarities between compounds

can limit accuracy. Machine learning models, including supervised classification techniques, can incorporate additional information—such as retention indices and fragmentation patterns—to improve annotation confidence. In recent years, deep learning approaches have also been explored for predicting mass spectra and assisting compound identification, especially when reference data are limited (Goodacre et al., 2007).

AI-driven analysis is particularly valuable in clinical metabolomics, where datasets often involve multiple patient groups and heterogeneous biological variability. Multivariate techniques such as principal component analysis (PCA) and partial least squares–discriminant analysis (PLS-DA) have long been used to identify group differences. However, machine learning methods—including random forests and support vector machines—offer greater flexibility in handling complex datasets and can improve classification performance. These models can reveal metabolic signatures associated with disease states, supporting biomarker discovery and patient stratification (Libbrecht & Noble, 2015).

Despite these advantages, the use of AI in metabolomics is not without challenges. Model interpretability remains a key concern, particularly in clinical applications where understanding the biological relevance of findings is essential. In addition, overfitting can occur when models are trained on limited datasets, leading to results that may not generalize well to independent cohorts. Careful validation, transparent reporting, and integration with domain knowledge are therefore critical when applying AI-based methods in GC–MS data analysis (Johnson et al., 2016)

The integration of artificial intelligence into GC–MS workflows does not replace traditional analytical reasoning but rather complements it. When applied thoughtfully, AI-based approaches can enhance data interpretation, uncover hidden patterns,

and contribute to a more comprehensive understanding of metabolic changes in both research and clinical settings.

### **Clinical Applications and Biomarker Discovery**

Clinical metabolomics aims to translate complex biochemical data into information that can support diagnosis, prognosis, and treatment decisions. In this context, GC–MS has remained a reliable analytical tool, particularly for the measurement of small, well-defined metabolites. Its strength lies not only in analytical sensitivity but also in the consistency of spectral data, which allows findings to be compared across different studies and patient populations (Dettmer et al., 2007; Fiehn, 2016).

In clinical settings, GC–MS has been widely used to investigate metabolic alterations associated with various diseases. Changes in amino acid metabolism, organic acid profiles, and fatty acid composition have been linked to conditions such as metabolic syndrome, cancer, and chronic kidney disease. These metabolic shifts often reflect underlying pathophysiological mechanisms, including oxidative stress, inflammation, and altered energy metabolism. Because GC–MS can quantify these metabolites with relatively high precision, it provides a practical platform for exploring disease-related biochemical changes (Johnson et al., 2016; Patti et al., 2012).

One of the most active areas of research involves the identification of biomarkers—measurable indicators that can distinguish between physiological and pathological states. In metabolomics, biomarker discovery rarely depends on a single compound. Instead, patterns of multiple metabolites are evaluated together to improve diagnostic performance. GC–MS is particularly suited for this approach, as it can generate reproducible profiles of metabolite panels, especially in untargeted or semi-targeted analyses (Dunn et al., 2005).

Lipidomics has contributed significantly to biomarker discovery, especially in diseases where membrane composition and lipid signaling are affected. Fatty acid profiling using GC–MS has been used to examine alterations in membrane structure, oxidative damage, and inflammatory processes. For instance, shifts in saturated and unsaturated fatty acid ratios have been associated with metabolic disturbances and chronic disease states. These changes are not only descriptive but may also provide insight into disease progression and response to treatment (Quehenberger et al., 2010).

In the context of chronic kidney disease (CKD), metabolomic studies have revealed disruptions in energy metabolism, amino acid turnover, and lipid composition. Such alterations are often linked to complications including anemia, inflammation, and cardiovascular risk. Although conventional clinical markers remain essential, metabolomics offers an additional layer of information that may help refine patient stratification and identify subclinical changes that are not captured by routine tests (Rhee et al., 2016)

Despite its potential, translating metabolomic findings into clinically usable biomarkers remains challenging. Biological variability, differences in sample handling, and the need for validation across independent cohorts can limit reproducibility. For this reason, biomarker discovery is increasingly approached as a multi-step process, combining robust analytical methods, appropriate statistical modeling, and biological interpretation.

Overall, GC–MS continues to support clinical research by providing consistent and interpretable metabolic data. While it may not capture the full biochemical complexity of human disease on its own, its contribution to biomarker discovery remains substantial, particularly when integrated with complementary analytical platforms and clinical data.

## **Future Directions in Precision Medicine**

Precision medicine is gradually shifting clinical practice away from generalized treatment strategies toward more individualized approaches that consider biological variability between patients. In this context, metabolomics has gained attention as it captures biochemical changes that reflect both genetic background and environmental influences. Among analytical platforms, GC–MS continues to play a meaningful role by providing reproducible and structurally informative data for a defined subset of metabolites (Johnson et al., 2016; Patti et al., 2012).

Looking ahead, one of the main directions in precision medicine involves integrating metabolomics with other layers of biological information, including genomics, transcriptomics, and proteomics. This multi-omics perspective allows a more comprehensive understanding of disease mechanisms, moving beyond single biomarkers toward network-based interpretations. Rather than focusing on isolated metabolic changes, future studies are increasingly expected to examine how metabolic pathways interact with gene expression and protein activity in specific disease contexts (Hasin et al., 2017)

Another important development is the growing emphasis on longitudinal data. Many current metabolomics studies rely on cross-sectional designs, which provide only a snapshot of metabolic status. However, repeated measurements over time can reveal dynamic changes in metabolism, offering insights into disease progression and treatment response. GC–MS, with its relatively high reproducibility, is well suited for such longitudinal applications, where consistency across measurements is essential (Wishart, 2016).

Advances in computational approaches are also expected to shape the future of precision medicine. The combination of metabolomics data with machine learning models can support risk

prediction, patient stratification, and treatment optimization. However, the success of these approaches will depend not only on algorithm performance but also on data quality, standardization, and interpretability. Ensuring that analytical pipelines are transparent and reproducible remains a key challenge, particularly in clinical settings where decisions must be justified and validated (Libbrecht & Noble, 2015).

In addition, there is increasing recognition that translating metabolomics findings into clinical practice requires more than analytical accuracy. Large-scale validation studies, standardized protocols, and integration with routine laboratory workflows are necessary before metabolite-based biomarkers can be widely adopted. Regulatory considerations and cost-effectiveness will also influence how quickly these approaches become part of standard care (Hasin et al., 2017).

Overall, the future of precision medicine will likely depend on the ability to integrate high-quality analytical data with meaningful biological interpretation. GC–MS, while not comprehensive on its own, will remain a valuable component of this framework due to its robustness and reproducibility. When combined with complementary technologies and carefully designed clinical studies, it has the potential to contribute to more personalized and data-driven healthcare approaches.

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## CHAPTER 2

# BIOACTIVE GLASS-CERAMICS FOR BONE REGENERATION AND CANCER THERAPY: FROM FUNDAMENTALS TO MULTIFUNCTIONAL SYSTEMS

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### 1. Introduction

Glass and glass-ceramic materials constitute two closely related yet intrinsically different categories of inorganic solids. Conventional glasses are amorphous in nature, characterized by the absence of long-range atomic order, whereas glass-ceramics are obtained through the deliberate and controlled crystallization of precursor glasses. This process yields a heterogeneous microstructure composed of crystalline phases uniformly distributed

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within a residual glassy matrix. Owing to this dual-phase architecture, glass-ceramics typically exhibit superior mechanical performance, improved thermal resistance, and adjustable functional characteristics compared to fully amorphous glass systems. These advantageous attributes have rendered glass-ceramics highly suitable for a wide range of advanced technological applications, particularly within the biomedical domain (Höland & Beall, 2019; Fu et al., 2020; Deubener et al., 2018; Schneider & Komatsu, 2013; Rahaman et al., 2011; Jones, 2013; She et al., 2023; Cao et al., 2025; He et al., 2026; Jiang et al., 2024). design (Pope, 1983; Müller & et al., 1998).

Over the past few decades, there has been an increasing emphasis on the development of biomaterials that actively interact with biological environments rather than remaining passive or inert. Conventional implant materials, including metals and dense ceramics, provide adequate mechanical strength; however, they generally lack the capacity to establish strong biological bonding with surrounding tissues (Chen & Thouas, 2021). In contrast, specific glass and glass-ceramic formulations have demonstrated the ability to chemically bond with bone tissue, a phenomenon defined as bioactivity (Hench, 2006; Hoppe et al., 2020; Baino et al., 2021). This distinctive feature has elevated glass-ceramics to a prominent position in applications such as bone repair, tissue regeneration, and implant surface modification (Zhang et al., 2022; Kaur et al., 2022; Ribas et al., 2019; Safaei et al., 2026; Gupta et al., 2026).

The notion of bioactive materials was first systematically proposed by Hench in the late 1960s, representing a transformative milestone in the field of biomaterials science (Hench, 2006). Unlike bioinert materials, which are engineered to minimize biological interactions, bioactive materials are designed to trigger controlled biological responses at the material-tissue interface (Dash et al., 2023; Pantulap et al., 2022). Upon exposure to physiological environments, bioactive glasses and glass-ceramics undergo a sequence of surface

reactions, including ionic exchange processes, partial dissolution of the glass network, and the formation of a silica-rich layer (Kaou et al., 2023; Mîrț et al., 2024). These processes ultimately lead to the nucleation and growth of a hydroxycarbonate apatite (HCA) layer. Due to its chemical and structural similarity to the mineral component of bone, this layer facilitates strong interfacial bonding and enhances tissue integration. Furthermore, the capacity to tailor ionic release profiles enables these materials to modulate cellular responses such as proliferation, differentiation, and extracellular matrix production (Gabriel et al., 2025; Pantulap et al., 2022).

Bone tissue is a complex, hierarchical, and continuously remodeling system, which imposes stringent requirements on biomaterials intended for its repair or replacement. Such materials must provide adequate mechanical support while simultaneously promoting biological functions including tissue regeneration, vascularization, and biochemical signaling (Aldhaher et al., 2023; Li et al., 2025). Consequently, an ideal bone substitute should integrate mechanical compatibility with controlled degradation and intrinsic bioactivity. Glass-ceramic materials are particularly well-suited to meet these criteria. Through careful control of composition and crystallization behavior, it is possible to fine-tune their mechanical properties, dissolution kinetics, and ion release characteristics. In addition, the incorporation of biologically active ions such as strontium, zinc, lithium, and fluoride has further expanded their functional capabilities, enabling enhanced osteogenic activity, improved angiogenesis, and antibacterial effects (Rajendran et al., 2024; Ranmuthu et al., 2021).

In addition to their well-established role in bone tissue regeneration, glass and glass-ceramic biomaterials have recently attracted considerable attention in the context of bone-related cancer applications. Primary bone tumors and metastatic bone diseases often result in severe bone defects that require simultaneous tumor treatment and tissue reconstruction. In this regard, glass-ceramic

systems offer a unique advantage by combining structural support with therapeutic functionality (Hussein et al., 2025; Vallet-Regí et al., 2020; Li et al., 2025). Through the incorporation of specific therapeutic ions (e.g.,  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Ga}^{3+}$ , or rare earth elements), these materials can exhibit anticancer, antibacterial, and angiogenic properties (Wu et al., 2019). Furthermore, certain glass-ceramic compositions have been explored for localized drug delivery and photothermal therapy, enabling targeted cancer treatment while minimizing systemic side effects (Amani et al., 2024; Borges et al., 2022). Their tunable degradation behavior and ion release kinetics allow for controlled therapeutic action, which is particularly advantageous in post-tumor resection scenarios where both tumor suppression and bone regeneration are required (Mobeen et al., 2026). Consequently, multifunctional glass-ceramic biomaterials are emerging as promising platforms for integrated cancer therapy and bone tissue engineering applications (Moeni et al., 2022).

Nevertheless, the design and development of glass-ceramic biomaterials are governed by complex interdependencies among composition, processing conditions, microstructure, and biological performance (Vallet-Regí & Ruiz-Hernández, 2011; Wu & Chang, 2012). A comprehensive understanding of these relationships is essential for optimizing material functionality and broadening their range of applications. Moreover, recent advancements in the field, including multifunctional systems, controlled ion delivery platforms, and hybrid materials that combine glass-ceramics with polymers or electrically conductive phases, are redefining conventional approaches to biomaterial design.

This chapter provides a focused yet comprehensive overview of glass-ceramic materials in biomedical applications. Initially, the fundamental aspects related to their structure, crystallization mechanisms, and processing techniques are outlined. Subsequently, the mechanisms of bioactivity and the influence of ion release on biological responses are discussed. The interplay between

composition, microstructure, and functional properties is then analyzed to elucidate key design considerations. Following this, major application areas, particularly in bone tissue engineering and implant technologies, are presented. Finally, recent developments in functional and ion-modified glass-ceramic systems are highlighted, along with current challenges and future research directions in this evolving field.

## **2. Fundamentals of Glass and Glass-Ceramic Materials**

Glass and glass-ceramic materials constitute a fundamental class of inorganic solids whose structural characteristics directly govern their physical and functional properties (Zachariasen, 1932; Varshneya and Mauro, 2019; Fraser and Girtan, 2023). Unlike crystalline materials, conventional glasses lack long-range atomic order and instead exhibit a disordered network structure formed through the rapid cooling of a melt (Greaves and Sen, 2007; Vinay et al., 2025). This amorphous arrangement results in unique features such as isotropy, optical transparency, and compositional flexibility, making glass systems highly versatile for both structural and functional applications (Doremus, 1994; Zanotto and Mauro, 2020).

At the atomic scale, the structure of glass is commonly interpreted through the random network model originally proposed by Zachariasen, which describes a continuous three-dimensional network formed by interconnected structural units without periodicity (Zachariasen, 1932; Greaves and Sen, 2007). Within this framework, oxide glasses are typically described in terms of network formers, intermediates, and network modifiers (Varshneya and Mauro, 2019; Zanotto and Mauro, 2020). Network formers such as  $\text{SiO}_2$ ,  $\text{B}_2\text{O}_3$ , and  $\text{P}_2\text{O}_5$  establish the backbone of the glass structure through strong covalent bonding, while modifiers like  $\text{Na}_2\text{O}$  or  $\text{CaO}$  disrupt the network by generating non-bridging oxygens. These structural disruptions significantly influence macroscopic properties including viscosity, glass transition temperature, chemical durability,

and dissolution kinetics (Varshneya and Mauro, 2019; Fraser and Girtan, 2023).

In addition to compositional effects, processing conditions play a critical role in defining glass structure and properties. Parameters such as cooling rate, melting temperature, and thermal history can significantly influence structural relaxation phenomena, which in turn affect the physical properties of glass-forming systems (Hodge, 1995; Málek, 2010). These relaxation processes govern the evolution of the glass structure toward equilibrium and are strongly dependent on the thermal path followed during processing (Hodge, 1995; Debenedetti and Stillinger, 2001). Consequently, even glasses with identical compositions may exhibit markedly different physical behaviors depending on their thermal history, highlighting the importance of processing-structure-property relationships in glass science (Debenedetti and Stillinger, 2001).

Glass-ceramics, on the other hand, are produced through the controlled crystallization of precursor glasses via carefully designed heat-treatment schedules. This process, commonly referred to as ceramming, involves two primary stages: nucleation and crystal growth (Zanotto, 1998; Höland and Beall, 2012). The use of nucleating agents promotes the formation of a high density of nuclei, enabling the development of fine and homogeneous microstructures (Höland and Beall, 2012). As a result, glass-ceramics consist of crystalline phases uniformly distributed within a residual glassy matrix, distinguishing them from both fully amorphous glasses and conventional polycrystalline ceramics (Zanotto, 1998; Höland and Beall, 2012).

One of the most significant advantages of glass-ceramics lies in their microstructural tunability. By adjusting parameters such as composition, nucleating agents, and thermal treatment conditions, it is possible to control crystal size, morphology, orientation, and volume fraction (Höland and Beall, 2012; Zanotto, 2010). These microstructural features directly influence key properties such as

mechanical strength, fracture toughness, thermal expansion, and resistance to thermal shock (Höland and Beall, 2012). In many cases, glass-ceramics exhibit superior mechanical performance compared to their parent glasses due to the reinforcing effect of finely dispersed crystalline phases (Zanotto, 2010).

Furthermore, the presence of both crystalline and amorphous phases enables glass-ceramics to combine desirable characteristics from each component. While the crystalline phases contribute to strength, stiffness, and thermal stability, the residual glassy phase facilitates processing and can play a crucial role in functional behavior, particularly in terms of ion mobility, chemical reactivity, and surface interactions (Hench and Jones, 2015; Höland and Beall, 2012). This dual-phase architecture is especially advantageous in applications where controlled degradation, surface activity, or interaction with biological environments is required (Hench and Jones, 2015).

Another important aspect of glass-ceramic systems is their ability to be engineered across multiple length scales. At the atomic level, compositional design governs bonding and network connectivity; at the nanoscale, phase separation and nucleation phenomena determine crystal formation; and at the microscale, grain size and distribution influence macroscopic properties (Micoulaut, 2016; Smedskjaer, 2014; James, 1974; Fokin et al., 2006; Höland and Beall, 2012). This hierarchical control provides a powerful platform for designing materials with highly tailored performance characteristics (Raghavan et al., 2010; Zanotto, 2017).

In summary, the fundamental distinction between glasses and glass-ceramics lies in their structural organization and degree of crystallinity. The ability to manipulate composition, processing conditions, and microstructure enables the development of materials with precisely controlled physical, chemical, and functional properties. This versatility forms the foundation for their widespread use in advanced technological applications, particularly in areas

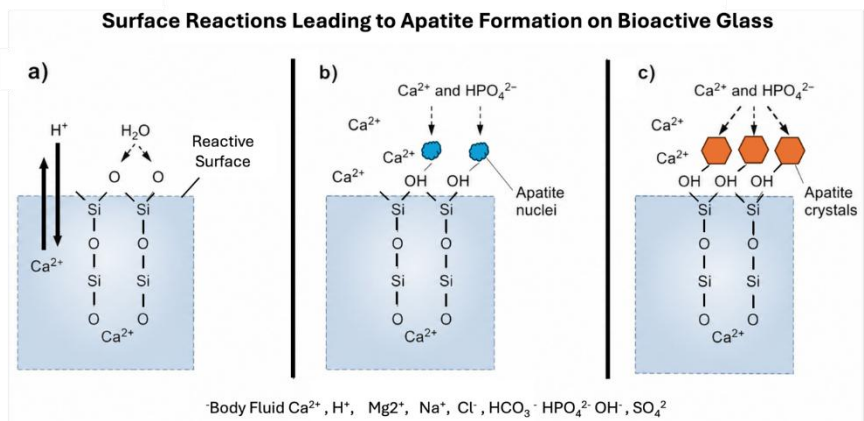
where a combination of structural integrity and functional performance is required.

### **3. Bioactivity Mechanisms of Glass Ceramics**

Bioactive glass-ceramics represent a unique class of materials capable of eliciting specific biological responses when in contact with physiological environments. Unlike inert ceramics, these materials actively interact with surrounding tissues, leading to the formation of a stable bond with bone and, in some cases, soft tissues. The bioactivity of glass-ceramics is primarily governed by their surface reactions, ionic dissolution behavior, and the subsequent formation of biologically active layers (Piatti et al., 2024; Berbecaru et al., 2010). As shown in Figure 1, these reactions proceed through a sequence of stages including ion exchange between modifier cations and physiological fluids, the formation of a reactive silica-rich surface layer, apatite nucleation, and the subsequent growth of apatite crystals on the material surface. These processes occur through a series of well-defined physicochemical and biological mechanisms, including ion exchange, network dissolution, and hydroxycarbonate apatite (HCA) formation, which are strongly influenced by composition, crystallization behavior, and microstructure (Peitl Filho et al., 1996; Zhou et al., 2026). Specifically, Figure 1 illustrates the progressive development of a bioactive surface, beginning with  $\text{Ca}^{2+}/\text{H}^{+}$  exchange and hydroxylation of the glass surface (Figure 1a), followed by calcium-phosphate accumulation and apatite nuclei formation (Figure 1b), and culminating in the growth of apatite crystals that promote bonding with surrounding bone tissue (Figure 1c). In particular, recent studies have emphasized that compositional tuning and structural modification-such as the incorporation of network formers or modifiers like zirconium or variations in oxide ratios-can significantly alter dissolution kinetics and biological response both in vitro and in vivo (Piatti et al., 2024; Milewczyk et al., 2025). These

mechanisms span multiple length scales, from atomic-level ion exchange processes to macroscopic tissue integration, ultimately governing the performance of glass-ceramic biomaterials.

*Figure 1. Surface reactions leading to hydroxycarbonate apatite formation on bioactive glass and glass-ceramic surfaces a) ion exchange and formation of a reactive silica-rich layer, b) nucleation of apatite, and c) growth of apatite crystals resulting in a bioactive surface.*



**Surface Reactions and Initial Ion Exchange:** The bioactivity of glass-ceramics begins immediately upon exposure to body fluids. The first stage involves rapid ion exchange between modifier cations in the glass (such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , or  $\text{K}^+$ ) and hydrogen ions ( $\text{H}^+$  or  $\text{H}_3\text{O}^+$ ) from the surrounding physiological solution. This exchange leads to the formation of silanol ( $\text{Si-OH}$ ) groups on the material surface and results in a localized increase in pH (Taye, 2022; Yadav et al., 2024). Figure 2. An example for POM-doped polymeric scaffolds (Zhao & et al., 2022). Simultaneously, the breakdown of the silicate network occurs through hydrolysis reactions, particularly affecting  $\text{Si-O-Si}$  bonds. This process leads to the release of soluble silica species (e.g.,  $\text{Si(OH)}_4$ ) into the surrounding medium. The dissolution

behavior is strongly influenced by the glass composition, network connectivity, and the presence of modifying oxides (Madival, 2026; Furko, 2025). Glass-ceramics with lower network connectivity generally exhibit higher dissolution rates, which can enhance bioactivity but may compromise long-term stability (Piatti et al., 2024; Milewczyk et al., 2025).

**Formation of the Silica-Rich Layer:** Following initial dissolution, a silica-rich layer begins to form on the material surface due to the condensation and repolymerization of silanol groups. This layer is typically amorphous and depleted in alkali and alkaline earth elements. It acts as a critical intermediate phase that facilitates further reactions leading to biological bonding (Omidian et al., 2022; Krishnamoorthy & Subramanian, 2026). The formation of this layer is essential because it provides a chemically active and structurally suitable surface for subsequent calcium phosphate nucleation. Its high surface area and abundance of silanol groups promote the adsorption of calcium and phosphate ions from the surrounding environment. This step represents a transition from purely chemical processes to biologically relevant mineralization mechanisms (Zăvoi et al., 2025; Taye, 2022).

**Nucleation and Growth of Hydroxycarbonate Apatite (HCA):** One of the defining features of bioactive glass-ceramics is their ability to form a hydroxycarbonate apatite (HCA) layer on their surface. This layer is chemically and structurally similar to the mineral phase of natural bone, enabling strong interfacial bonding (Larry L. Hench, 1998; Piatti et al., 2024). The formation of this biologically active apatite-like layer is widely recognized as the key mechanism underlying the bioactivity of these materials. The formation of HCA begins with the migration and accumulation of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions at the silica-rich surface. These ions initially form an amorphous calcium phosphate (ACP) phase, which subsequently crystallizes into HCA. The kinetics of this transformation depend on several factors, including ion release rates,

surface energy, and the presence of nucleation sites (Jones, 2013; De Micco et al., 2025). Recent studies further emphasize that surface reactions leading to silica gel formation play a critical intermediate role in enabling apatite nucleation. In glass-ceramics, the presence of crystalline phases can significantly influence apatite formation. Certain crystalline phases act as preferential nucleation sites, enhancing the rate and uniformity of HCA layer formation. This controlled crystallization is one of the key advantages of glass-ceramics over purely amorphous bioactive glasses (Piatti et al., 2024; Ellakwa et al., 2025). Moreover, recent findings suggest that compositional tuning and surface modification strategies can further optimize apatite-forming ability and biological performance.

#### **Role of Ionic Dissolution Products in Biological Response:**

Beyond surface mineralization, the ions released from bioactive glass-ceramics play a crucial role in stimulating cellular responses. Dissolved species such as Si, Ca, P, and other therapeutic ions (e.g., Sr, Zn, Mg) can regulate gene expression and influence cellular activities including proliferation, differentiation, and extracellular matrix production (Julian R. Jones, 2013; Aldo R. Boccaccini et al., 2020). Silicon ions, for example, are known to enhance osteoblast activity and promote collagen synthesis, while calcium ions are involved in signaling pathways associated with bone mineralization. Phosphate ions, on the other hand, are essential for apatite formation. The synergistic and controlled release of these ions creates a favorable biochemical microenvironment that supports tissue regeneration (Larry L. Hench, 1998; Aldo R. Boccaccini et al., 2020). Importantly, the concentration and release kinetics of these ions must be carefully controlled. Excessive ion release can induce cytotoxic effects, whereas insufficient release may result in limited biological stimulation. Therefore, tailoring composition and microstructure remains critical for optimizing bioactivity and ensuring a balanced cellular response (Julian R. Jones, 2013; Hoppe et al., 2011; Zheng et al., 2021).

**Influence of Microstructure on Bioactivity:** The bioactivity of glass-ceramics is strongly dependent on their microstructural characteristics, including crystal size, distribution, and the volume fraction of crystalline phases. A fine and homogeneous microstructure generally enhances bioactivity by providing a larger surface area and more nucleation sites for apatite formation. Phase assemblage also plays a crucial role. For instance, certain crystalline phases may be bioactive, while others are relatively inert. The coexistence of crystalline and residual glassy phases allows for a balance between mechanical strength and bioactivity. The glassy phase typically governs ion release, whereas crystalline phases contribute to structural integrity. Additionally, porosity-particularly interconnected porosity-can significantly improve biological performance by facilitating fluid transport, nutrient diffusion, and tissue ingrowth. However, increased porosity often comes at the expense of mechanical strength, necessitating careful optimization depending on the intended application.

**Biological Integration and Tissue Response:** The final stage of bioactivity involves the interaction between the material surface and living tissue. Once the HCA layer forms, it serves as a biologically active interface that enables the adsorption of proteins and the attachment of osteogenic cells. This leads to the formation of a strong bond between the implant and bone tissue, often referred to as osseointegration. Cellular responses are influenced not only by chemical composition but also by surface topography and roughness. Micro- and nanoscale features can enhance cell adhesion and proliferation by providing favorable physical cues. Over time, the material may either remain stable or undergo controlled degradation, depending on its composition and structure. In applications such as bone regeneration, the ability of glass-ceramics to support new tissue formation while gradually integrating into the biological environment makes them highly valuable. Their multifunctionality-

combining mechanical support with biological activity-distinguishes them from traditional biomaterials

**Summary of Bioactivity Mechanisms:** In summary, the bioactivity of glass-ceramics is governed by a sequence of interconnected processes:

1. Ion exchange and network dissolution
2. Formation of a silica-rich surface layer
3. Nucleation and growth of hydroxycarbonate apatite
4. Release of biologically active ions
5. Cellular response and tissue integration

These mechanisms are highly sensitive to composition, processing conditions, and microstructure. The ability to control these parameters enables the design of advanced glass-ceramic materials with tailored biological performance, making them suitable for a wide range of biomedical applications, particularly in bone repair and regeneration.

#### **4. Applications in Bone Tissue Engineering**

Bone tissue engineering has emerged as a promising strategy to address the limitations of conventional treatments for bone defects, such as autografts and allografts, which are often associated with limited availability, donor site morbidity, and risk of immune rejection (Battafarano et al., 2021). In this context, bioactive glass-ceramics have gained significant attention due to their unique ability to combine structural support with biological functionality. Their capacity to bond directly to bone, stimulate cellular activity, and release biologically active ions makes them highly suitable for applications in bone regeneration and repair (Shearer et al., 2022; He, 2023). The broad range of biomedical applications of bioactive glass-ceramics in bone tissue engineering is summarized in Figure 2, including bone defect repair, spinal fusion, dental and maxillofacial reconstruction, drug delivery systems, osteochondral

repair, bone cancer reconstruction, and scaffold-based tissue regeneration.

*Figure 2. Applications of bioactive glass-ceramics in bone tissue engineering.*



### **Role of Bioactive Glass-Ceramics in Bone Regeneration:**

Bioactive glass-ceramics play a multifunctional role in bone tissue engineering by acting as both structural scaffolds and bioactive stimulators. Upon implantation, these materials undergo surface reactions that lead to the formation of a hydroxycarbonate apatite (HCA) layer, which closely resembles the mineral phase of natural bone. This layer serves as a biologically active interface that promotes osteoblast attachment, proliferation, and differentiation (Shearer et al., 2022; Fu et al., 2021). In addition to surface mineralization, the controlled release of ionic dissolution products (such as silicon, calcium, and phosphorus) plays a crucial role in regulating cellular responses. These ions can activate signaling pathways involved in osteogenesis, angiogenesis, and extracellular matrix formation. As a result, bioactive glass-ceramics not only

support bone growth but actively participate in the healing process by enhancing tissue regeneration at the molecular level (Baino et al., 2021; He, 2023).

**Scaffold Design and Structural Requirements:** A key requirement in bone tissue engineering is the development of scaffolds that mimic the hierarchical structure of natural bone. Bioactive glass-ceramics can be engineered into porous three-dimensional scaffolds with controlled architecture, enabling them to provide both mechanical support and biological functionality (Baino & Fiume, 2020). Porosity is one of the most critical design parameters. Interconnected macropores are essential for cell migration, vascularization, and nutrient transport, while micropores contribute to increased surface area and enhanced bioactivity. The ability to tailor pore size distribution and overall porosity allows for optimization of both mechanical strength and biological performance. Moreover, advances in fabrication techniques, such as sol–gel processing, foam replication, and additive manufacturing (3D printing), have enabled precise control over scaffold geometry and internal architecture. These technologies allow for the creation of patient-specific implants with optimized structural and functional properties (Cui et al., 2024; El-Rashidy et al., 2021).

**Mechanical Performance and Load-Bearing Applications:** While bioactive glass-ceramics exhibit excellent bioactivity, their relatively brittle nature poses challenges for load-bearing applications. To address this limitation, various strategies have been developed to enhance their mechanical properties without compromising bioactivity. One common approach involves the design of glass-ceramic composites, where reinforcing phases such as crystalline domains or secondary materials (e.g., polymers or ceramics) are introduced to improve toughness and strength. Controlled crystallization within the glass matrix can also enhance mechanical performance by creating a fine microstructure that resists crack propagation. Additionally, functionally graded materials (FGMs)

have been explored to achieve a balance between mechanical stability and biological activity. In such systems, the composition and structure vary gradually across the material, allowing for a strong core combined with a bioactive surface layer. This approach is particularly useful for implants that must withstand mechanical loads while maintaining bioactive functionality at the tissue interface.

**Ion Doping and Functionalization:** One of the most powerful advantages of bioactive glass-ceramics is their compositional flexibility, which allows for the incorporation of therapeutic ions to enhance biological performance. Ion doping has been widely used to tailor the properties of these materials for specific biomedical applications. For example, the incorporation of strontium (Sr) has been shown to stimulate osteoblast activity and reduce osteoclast-mediated bone resorption, making it particularly useful in the treatment of osteoporosis. Zinc (Zn) contributes to bone formation and exhibits antibacterial properties, while magnesium (Mg) plays a role in cellular metabolism and enhances mechanical performance. Copper (Cu) and cobalt (Co) ions have been investigated for their ability to promote angiogenesis by stimulating vascular endothelial growth factor (VEGF) expression. Beyond biological effects, ion doping can also influence the glass network structure, dissolution behavior, and crystallization kinetics. This provides an additional level of control over both material properties and biological responses, enabling the design of highly specialized biomaterials for targeted applications.

**Drug Delivery and Controlled Release Systems:** Bioactive glass-ceramics have also been extensively explored as platforms for drug delivery in bone tissue engineering. Their porous structure and surface chemistry enable the adsorption and controlled release of therapeutic agents, including antibiotics, growth factors, and anti-inflammatory drugs. This dual functionality—combining structural support with localized drug delivery—offers significant advantages

in clinical applications. For instance, the incorporation of antibiotics into bioactive scaffolds can help prevent post-surgical infections, while the delivery of growth factors such as bone morphogenetic proteins (BMPs) can accelerate bone regeneration. The release kinetics of these agents can be tailored by modifying the composition, porosity, and surface properties of the material. This allows for sustained and controlled delivery profiles that align with the different stages of the healing process.

**Angiogenesis and Vascularization:** Successful bone regeneration requires not only osteogenesis but also the formation of new blood vessels to supply nutrients and oxygen to the growing tissue. Bioactive glass-ceramics have demonstrated a strong ability to promote angiogenesis through the release of specific ions and the formation of bioactive surfaces. Ions such as silicon, copper, and cobalt have been shown to stimulate angiogenic signaling pathways, leading to increased expression of angiogenic factors and enhanced vascularization. The formation of a biologically active surface layer further supports endothelial cell attachment and proliferation, contributing to the development of functional vascular networks. The coupling of osteogenesis and angiogenesis is a critical factor in successful bone tissue engineering, and bioactive glass-ceramics offer a unique platform for achieving this synergy.

**Clinical Applications and Future Perspectives:** Bioactive glass-ceramics have already been applied in various clinical settings, including bone defect repair, spinal fusion, dental implants, and maxillofacial reconstruction. Their ability to bond with bone and support tissue regeneration has led to the development of several commercially available products. Looking forward, future research is focused on improving the performance of these materials through advanced design strategies, including nanostructuring, hybrid materials, and smart biomaterials capable of responding to environmental stimuli. The integration of bioactive glass-ceramics with emerging technologies such as bioprinting and tissue-

engineered constructs is expected to further expand their applications. Moreover, a deeper understanding of structure-property-biological response relationships will enable the development of next-generation materials with enhanced functionality and reliability. As a result, bioactive glass-ceramics are expected to play an increasingly important role in the advancement of regenerative medicine and bone tissue engineering.

## **5. Emerging Applications in Bone Cancer Therapy**

Translating Bone cancer is a type of cancer in which tumors form, destroying bone tissue, due to the abnormal division and multiplication of cells in the bones. Cancers that originate in the bone itself are called primary bone cancers. Primary bone cancers are cancers that originate directly from the cells of the bone tissue itself. They are rarer and tend to occur especially during childhood, adolescence, or young adulthood [Hussein et al., 2025]. Three types of tumors are observed within the bone structure: osteosarcoma (OS), Ewing sarcoma, and chondrosarcoma. Osteosarcoma is the most common type of primary bone cancer. It most often occurs in children and young adults under the age of 20. It develops in the femur, tibia, and the ends of the humerus, particularly around the knee and shoulder. It can be aggressive and has the potential to metastasize even in the early stages. Despite making up less than 1 % of all malignancies diagnosed each year, these diseases have significant morbidity and fatality rates. Among adolescents and teenagers, osteosarcoma is the most frequent primary bone cancer, and the third most common type of cancer [Simpson and Brown, 2018]. Even after the development of adjuvant treatment in the 1970s, which increased the 10-year survival rate from 30 % to roughly 50 %, patients with osteosarcoma still face a weak prognosis. In the study conducted by Deliormanlı and his group, bioactive glass powders containing superparamagnetic maghemite (2%, 5%, 10%, 20% by weight) were prepared for the treatment of osteosarcoma.

For this purpose, maghemite nanoparticles were synthesized using the co-precipitation technique, and bioactive glass-ceramic composites containing maghemite were produced by the sol-gel process. The structural, morphological, thermal, and magnetic properties and in vitro bioactivity of the prepared bioactive glasses were investigated. In vitro cytotoxicity was examined using SaOS-2 and MC3T3-E1 cells. The fluorouracil (5-FU) release behavior of the studied bioactive glass powders was also monitored in phosphate-buffered saline as a function of time. The results revealed that the synthesized maghemite nanoparticles and bioactive glass-ceramic composites containing maghemite possess superparamagnetic properties. They have high bioactivity with up to 5% maghemite content. The prepared bioactive glass composites did not exhibit cytotoxicity against osteosarcoma and pre-osteoblast cells at low concentrations. The drug-loaded bioactive glass powder showed sustained release behavior. Overall results indicate that the prepared glass composites have high potential for use in magnetic hyperthermia and anticancer drug delivery [Deliormanlı et al., 2022]. Chondrosarcoma is a type of cancer that originates from cells that produce cartilage tissue. It most commonly occurs in the pelvis, shoulder, ribs, thigh, and upper arm bones. It is more common in adults, particularly those aged 40 and older, and some subtypes can be quite aggressive. While the overall 5-year survival rate is approximately 70–75 %, high-grade tumors and aggressive chondrosarcoma subtypes are associated with a particularly poor prognosis. Agulnik et al. Reported a review about clinical behavior, molecular mechanisms, and emerging therapeutic strategies of chondrosarcoma. They explored chondrosarcoma subtypes, diagnostic approaches, prognostic factors, and molecular alterations, followed by a discussion of current and emerging treatment strategies [Agulnik et al., 2026]. Ewing sarcoma, commonly seen in children and young adults, is an aggressive tumor that can originate from bone or surrounding soft tissue. It is most frequently found in

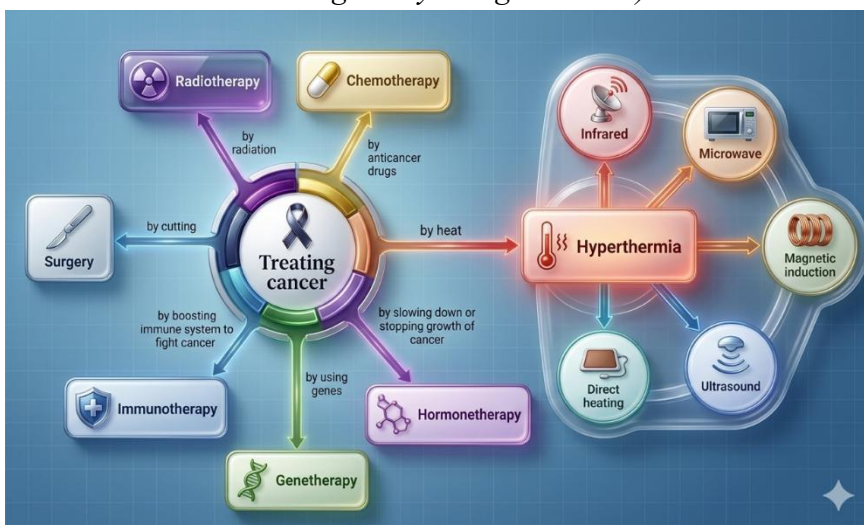
the ribs, pelvis, scapula, and hip bones. A recent review article published in 2026 reported that Ewing sarcoma is an aggressive bone and soft-tissue malignancy affecting adolescents and young adults, and that the development of in vitro and in vivo models has significantly contributed to a better understanding of the disease biology. These models have become essential tools for evaluating novel therapeutic approaches, particularly in metastatic or relapsed cases. Furthermore, the review summarized current preclinical modeling strategies, including cell lines, tumor organoids, and xenograft models, and discussed factors that may influence experimental outcomes [Bailey et al., 2026].

Bone cancer that starts in other tissues or parts of the body and spreads to the bones, known as metastasis, is considered a secondary type of cancer. Secondary bone cancers (bone metastases) occur when a cancer spreads to the bones via the bloodstream or lymphatic system. They develop when cancers from different organs of the body, such as breast, lung, prostate, kidney, and thyroid cancers, reach the bone and form new tumor metastases. Metastatic bone tumors, on the other hand, are much more common. Between 65 and 80 % of people with late-stage breast and prostate cancers have bone metastases, which is a common sign of advanced disease [Jakob et al., 2014], as do 35 to 42 % of people with advanced renal, lung, or thyroid cancers [Griffin et al., 2011; McCauley et al., 2011].

A single mechanism to fully cure cancer has not yet been developed. The most commonly used techniques include radiotherapy (treating cancer cells with radiation), chemotherapy (treating cancer cells with chemicals/drugs), and hyperthermia (treating cancer cells with heat) (Figure 3). Hyperthermia is considered one of the promising techniques because of its high potential for destroying cancer cells. For this purpose, interest in bioactive glass ceramics has increased due to their heat-generating capabilities. In addition to their heat-generating ability, these materials, when properly selected glass compositions react with

physiological fluids, can in some cases exhibit a bioactive response towards natural bone and even soft tissues. In this way, besides eliminating cancer cells, bioactive glass ceramics can also aid in the regeneration of affected bone portions [Danewalia and Singh, 2021; Zeisberger et al., 2006; Verne et al., 2015; Miola et al., 2017; Bairo et al., 2018; Bruno et al., 2014; Gerhardt and Boccaccini, 2010; Fernandes et al., 2018].

*Figure 3. Various techniques for treatment of cancer (This figure was designed by Google Gemini).*



## 6. Multifunctional and Hybrid Glass-Ceramic Systems and Biological Applications

In recent years, glass-ceramic systems have evolved from being purely structural biomaterials into multifunctional platforms capable of addressing complex biological requirements. This shift has been largely driven by the integration of organic and inorganic components, as well as the incorporation of functional additives that enable electrical, chemical, and biological responsiveness. Such

developments have positioned hybrid glass-ceramic systems at the forefront of advanced biomedical applications (Cui et al., 2024; Yang et al., 2025; Deubener et al., 2018; Baino et al., 2021; Jones, 2022; Kargozar et al., 2023).

**Polymer-Glass-Ceramic Hybrids:** Polymer-glass-ceramic hybrids are designed by combining organic polymer networks with inorganic glass-ceramic phases at the molecular or nanoscale level. This intimate integration allows the material to benefit simultaneously from the bioactivity of the inorganic phase and the flexibility of the polymeric component. Compared to conventional composites, these hybrids often exhibit improved interfacial compatibility and more uniform structural organization (Wu et al., 2021; Rizwan et al., 2022). From a biological standpoint, these materials are particularly attractive for tissue engineering applications. The inorganic phase can stimulate mineralization and support osteogenic activity, while the polymeric network provides mechanical compliance and reduces brittleness. Additionally, their degradation behavior can be tailored by adjusting composition and crosslinking density, allowing synchronization with tissue regeneration rates. This tunability is a key advantage in applications such as bone scaffolds, where both mechanical integrity and gradual resorption are required (Nommeots-Nomm et al., 2021; Sharifi et al., 2023; Li et al., 2022).

**Electrically Conductive Systems (e.g., MXene-Based Composites):** The incorporation of electrically conductive components into glass-ceramic matrices has opened new possibilities, especially in the context of neural tissue engineering and bioelectronic interfaces. Among emerging materials, MXenes have attracted considerable attention due to their unique combination of high electrical conductivity, hydrophilicity, and surface functionalization capability (Rasool et al., 2022; Szuplewska et al., 2023). When integrated into glass-ceramic systems, MXene nanosheets can form conductive networks that facilitate electrical signaling at the cell–material interface. This is particularly relevant

for electrically active tissues, such as nerve and muscle, where external or endogenous electrical cues influence cellular behavior. Preliminary studies suggest that such conductive hybrids can enhance cell proliferation, differentiation, and signal transmission, although their long-term biological stability and safety remain under investigation (Zha et al., 2022; Kang et al., 2023; Liu et al., 2024).

**Stimuli-Responsive (Smart) Materials:** Another important direction in this field involves the development of stimuli-responsive glass-ceramic systems. These materials are capable of altering their properties in response to environmental triggers such as pH, temperature, light, or magnetic fields. Rather than acting as passive scaffolds, they function as dynamic systems that interact with their surroundings in a controlled manner (Cui et al., 2024; Wei et al., 2022). For instance, pH-sensitive materials can exploit the acidic microenvironment of diseased tissues, enabling localized activation of therapeutic functions. Similarly, thermally or photo-responsive systems can be used for controlled drug release or combined therapies, such as photothermal treatment. The inherent stability of the glass-ceramic phase provides a robust framework, while the responsive component introduces adaptability, resulting in a synergistic effect that enhances overall performance (Liu et al., 2023; Zhang et al., 2021; Kargozar et al., 2022).

**Controlled Ion Release Platforms:** One of the defining features of bioactive glass-ceramics is their ability to release therapeutic ions in a controlled manner. Ions such as calcium, silicon, strontium, and zinc are known to play critical roles in cellular signaling pathways related to bone formation, angiogenesis, and antibacterial activity (Baino et al., 2021; Hoppe et al., 2022). In hybrid systems, the kinetics of ion release can be finely tuned through compositional design and structural modification. The presence of a polymeric phase, for example, can act as a diffusion barrier or degradation-controlled reservoir, allowing for more sustained and predictable release profiles. This level of control is particularly important in

regenerative medicine, where the timing and concentration of ionic cues can significantly influence treatment outcomes (Miguez-Pacheco et al., 2023; Jones et al., 2021; Zheng et al., 2024).

## **7. Challenges and Future Perspectives**

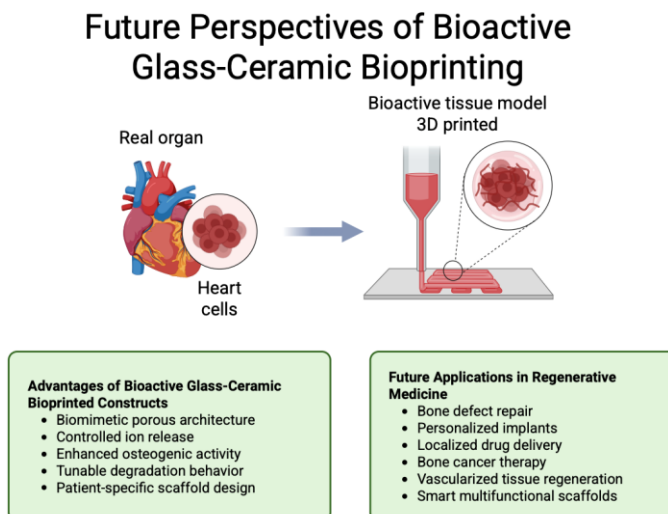
Despite the promising capabilities of multifunctional glass-ceramic systems, several challenges must be addressed before their full clinical potential can be realized. These challenges span material design, processing, and biological evaluation. Current challenges associated with hybrid bioactive glass-ceramic systems remain a major focus of ongoing research. One of the primary limitations lies in the complexity of fabrication processes. The synthesis of hybrid systems often requires precise control over multiple parameters, including composition, phase distribution, and microstructure. While such control is achievable at the laboratory scale, translating these methods to industrial production remains a significant hurdle (Fu et al., 2021). Another critical issue is the balance between mechanical properties and biological functionality. Enhancing bioactivity or introducing additional functionalities may compromise structural integrity, particularly in load-bearing applications. Achieving an optimal balance between these competing requirements continues to be a key research focus (Boccaccini et al., 2022; Deliormanlı & Rahaman, 2021). Long-term biocompatibility is also a concern, especially for systems incorporating novel nanomaterials. Although short-term studies often demonstrate promising results, the long-term effects of these materials within the human body are not yet fully understood. This uncertainty poses challenges for regulatory approval and clinical adoption (Gaharwar et al., 2022;). Furthermore, achieving precise control over ion or drug release profiles remains technically demanding. Small variations in material structure or environmental conditions can lead to significant changes in release behavior,

potentially affecting therapeutic efficacy (Mouriño & Boccaccini, 2021; Wu et al., 2022).

Future Perspectives, looking ahead, the development of personalized biomaterials is expected to play a central role in this field. Advances in additive manufacturing technologies, particularly 3D printing, are enabling the fabrication of patient-specific implants with tailored structural and functional properties (Bose et al., 2021; Tarafder et al., 2022). As illustrated in Figure 4, bioactive glass-ceramic bioprinting offers the potential to combine living cells with bioactive scaffold materials, enabling the fabrication of tissue-mimicking constructs that more closely replicate the architecture and biological function of native tissues. Furthermore, the biomimetic porous architecture and tunable ion-release characteristics of bioactive glass-ceramic systems make them particularly attractive for next-generation tissue engineering platforms. Combined with advances in stem cell technology, biofabrication, and artificial intelligence-assisted design, these materials may enable the production of highly customized regenerative therapies in the future. The integration of bioactive glass-ceramics with bioprinting technologies is expected to expand their application beyond conventional bone substitutes toward advanced regenerative medicine strategies. Future developments may include patient-specific implants, localized drug delivery systems, vascularized tissue constructs, smart multifunctional scaffolds, and novel approaches for bone cancer reconstruction, as summarized in Figure 4. These innovations aim to improve therapeutic outcomes while providing greater control over tissue regeneration and functional recovery. There is also a growing interest in designing truly multifunctional platforms that can simultaneously provide mechanical support, deliver therapeutic agents, and modulate biological responses. Such integrated systems could significantly improve treatment efficiency and reduce the need for multiple interventions (Zheng et al., 2026). The incorporation of

nanotechnology will likely continue to expand, offering new ways to control material behavior at the molecular level. Nanostructured additives can enhance surface interactions, improve mechanical performance, and enable more precise control over biological processes (Liu et al., 2022; Gao et al., 2021). In addition, the concept of autonomous or self-regulating materials is gaining attention. These systems would be capable of sensing changes in their environment and responding accordingly without external intervention, representing a significant step toward intelligent biomaterials (Zheng et al., 2026). Finally, efforts to standardize fabrication methods and establish comprehensive biological evaluation protocols will be essential for accelerating clinical translation. Bridging the gap between laboratory research and real-world application will require interdisciplinary collaboration and a strong focus on reproducibility and safety.

*Figure 4: Future perspectives of bioactive glass-ceramic bioprinting, highlighting the transition from cell-based biofabrication toward personalized regenerative medicine applications.*



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# CHAPTER 3 PHENOBARBITAL: CHEMICAL STRUCTURE, SYNTHESIS, PHARMACOLOGICAL PROPERTIES, AND CLINICAL APPLICATIONS

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## Introduction

Phenobarbital is one of the oldest antiseizure medications and has remained an important therapeutic agent in clinical practice for more than a century. Since its introduction in 1912, it has been widely used for the management of generalized tonic–clonic seizures, focal seizures, neonatal seizures, and status epilepticus. Despite the development of newer antiseizure drugs, phenobarbital continues to play a significant role in epilepsy treatment owing to its proven efficacy, low cost, extensive clinical experience, and widespread availability. Consequently, it remains included in the World Health Organization (WHO) Model List of Essential Medicines and is still recommended for selected seizure disorders, particularly in low- and middle-income countries.

The clinical effectiveness of phenobarbital is closely related to its well-defined chemical structure and pharmacological properties. The molecule possesses a hybrid architecture consisting of a non-aromatic barbiturate nucleus and an aromatic phenyl ring. The ethyl and phenyl substituents at the C<sub>5</sub> position enhance lipophilicity and facilitate penetration across the blood–brain barrier, whereas the carbonyl and imide groups of the barbiturate ring play a crucial role in molecular interactions with the GABA<sub>A</sub> receptor. These structural features have established phenobarbital as one of the classical examples for understanding structure–activity relationships (SAR) in medicinal chemistry (Perucca, 2018; Katzung, 2024).

Pharmacologically, phenobarbital acts as a positive allosteric modulator of the GABA<sub>A</sub> receptor, enhancing inhibitory neurotransmission within the central nervous system. By prolonging the opening time of chloride channels, it promotes neuronal hyperpolarization and suppresses the propagation of epileptic discharges. At higher concentrations, inhibition of glutamatergic neurotransmission further contributes to its broad-spectrum anticonvulsant activity (Rang et al., 2011; Brunton et al., 2023).

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Despite its clinical benefits, long-term phenobarbital therapy is associated with several limitations. As a potent inducer of hepatic cytochrome P450 enzymes, it may alter the metabolism of numerous co-administered drugs, resulting in clinically significant drug interactions. In addition, prolonged treatment may cause sedation, cognitive impairment, physical dependence, and metabolic disturbances. Nevertheless, phenobarbital remains an essential therapeutic option for neonatal seizures, refractory epilepsy, and status epilepticus because of its well-established efficacy and extensive clinical experience (Brunton et al., 2023; Pressler et al., 2023).

In recent years, research on phenobarbital has extended beyond its clinical applications to include crystal structure analysis, electronic properties, pharmacogenomics, structure–activity relationships, computational drug design, and advanced pharmaceutical formulations. Emerging approaches such as controlled-release drug delivery systems and artificial intelligence-assisted molecular design are expected to improve therapeutic outcomes while reducing adverse effects.

This chapter provides a comprehensive overview of the chemical structure, synthesis, molecular properties, structure–activity relationships, mechanism of action, pharmacokinetic profile, clinical applications, safety considerations, and recent advances related to phenobarbital. Collectively, these topics highlight the continuing importance of phenobarbital as both a classical anticonvulsant and a valuable model compound in medicinal chemistry and clinical pharmacology.

## Chemical Structure and Molecular Properties

Phenobarbital (5-ethyl-5-phenylbarbituric acid) is a classical barbiturate derivative with the molecular formula  $C_{12}H_{12}N_2O_3$  and a molecular weight of 232.24 g/mol. It belongs to the pyrimidine-2,4,6-trione family and consists of a barbiturate ring substituted with ethyl and phenyl groups at the C<sub>5</sub> position. This molecular architecture largely determines its physicochemical properties, pharmacokinetic profile, and anticonvulsant activity (Patrick, 2017; Brunton et al., 2023).

The molecule contains two distinct structural domains that contribute to its biological activity. The barbiturate nucleus functions as the pharmacophore responsible for interaction with the GABA<sub>A</sub> receptor, whereas the ethyl and phenyl substituents enhance lipophilicity and facilitate penetration across the blood–brain barrier. In addition, the three carbonyl groups and two imide nitrogen atoms provide hydrogen-bond donor and acceptor sites that stabilize receptor binding and contribute to the pharmacological activity of phenobarbital (Macdonald & Olsen, 1994; Olsen & Sieghart, 2008; Brunton et al., 2023).

Unlike the phenyl ring, which exhibits classical aromaticity, the barbiturate ring is non-aromatic because conjugation is interrupted by the tetrahedral C<sub>5</sub> carbon atom. Consequently, the aromatic phenyl group primarily contributes to hydrophobic interactions, whereas the heterocyclic barbiturate nucleus provides the functional groups responsible for receptor

recognition and molecular stability. X-ray crystallographic studies have confirmed this structural organization and its relationship to molecular conformation (Williams, 1973; Balaban et al., 2004).

Phenobarbital behaves as a weak acid with a  $pK_a$  of approximately 7.3, allowing the molecule to exist in both ionized and non-ionized forms under physiological conditions. This acid-base equilibrium directly influences aqueous solubility, plasma protein binding, membrane permeability, and distribution within the central nervous system. Although keto-enol tautomerism may occur under specific experimental conditions, the keto form predominates under physiological conditions and is considered responsible for the drug's pharmacological activity (Anderson, 2002; Vanderah, 2024).

The structural and physicochemical characteristics of phenobarbital have been extensively investigated using spectroscopic, crystallographic, and computational techniques. Modern molecular modeling studies indicate that receptor affinity depends not only on individual functional groups but also on the overall three-dimensional molecular geometry, electronic distribution, hydrogen-bonding capacity, and lipophilicity. These properties have established phenobarbital as one of the classical model compounds for understanding structure–activity relationships (SAR) and rational drug design in medicinal chemistry (Patrick, 2017; Masiulis et al., 2019; Brunton et al., 2023).

**Table 1.** Physicochemical properties of phenobarbital

| Property                       | Value                                           |
|--------------------------------|-------------------------------------------------|
| Chemical name                  | Phenobarbital (5-ethyl-5-phenylbarbituric acid) |
| Molecular formula              | $C_{12}H_{12}N_2O_3$                            |
| Molecular weight               | 232.24 g/mol                                    |
| Chemical class                 | Barbiturate derivative                          |
| IUPAC name                     | 5-Ethyl-5-phenyl-1,3-diazinane-2,4,6-trione     |
| $pK_a$                         | ~7.3                                            |
| logP                           | ~1.4–1.5                                        |
| Hydrogen-bond donors           | 2                                               |
| Hydrogen-bond acceptors        | 3                                               |
| Topological polar surface area | ~75 Å <sup>2</sup>                              |

The molecular architecture of phenobarbital provides an excellent example of the relationship between chemical structure and biological activity. Its balanced combination of hydrophobic substituents, hydrogen-bonding functional groups, and moderate lipophilicity determines receptor affinity, pharmacokinetic behavior, and therapeutic efficacy. These structural characteristics have established phenobarbital as a reference compound in medicinal chemistry and continue to guide the design of novel barbiturate derivatives with improved pharmacological properties.

## Synthesis

Phenobarbital is a classical synthetic barbiturate whose development represents one of the landmark achievements in medicinal chemistry. Following the discovery of the sedative properties of barbital by Emil Fischer and Joseph von Mering in 1903 and the subsequent synthesis of phenobarbital in 1911, numerous synthetic strategies have been developed. Nevertheless, all reported methods are based on constructing the characteristic 5-ethyl-5-phenyl-substituted barbiturate nucleus, which is essential for its anticonvulsant activity (López-Muñoz et al., 2005; Patrick, 2017).

The conventional synthesis begins with the preparation of substituted malonic acid esters, followed by sequential alkylation to introduce the ethyl and phenyl substituents at the C<sub>5</sub> position. The resulting intermediate subsequently undergoes cyclization with urea under basic conditions, leading to the formation of the pyrimidine-2,4,6-trione ring characteristic of barbiturates. Owing to its simplicity, reproducibility, and high yield, this route remains the preferred method for both laboratory synthesis and industrial production (Nantachit et al., 2007; Wilson & Gisvold, 1998).

Several alternative synthetic approaches have also been investigated to improve reaction efficiency, reduce processing time, or simplify purification. These include modified cyclization strategies, catalytic protocols, and the use of alternative nitrogen-containing precursors. Although these methods differ in reaction design, they ultimately generate the same pharmacologically active barbiturate framework (Patrick, 2017; Brunton et al., 2023).

Growing interest in sustainable pharmaceutical manufacturing has encouraged the development of greener synthetic methodologies. Microwave-assisted synthesis, solvent-reduced reactions, catalytic transformations, and continuous-flow technologies have demonstrated the potential to shorten reaction times, decrease energy consumption, and minimize chemical waste. Although these approaches have not been widely adopted for industrial phenobarbital production, they illustrate how green chemistry principles can be integrated into pharmaceutical synthesis (Anastas & Warner, 1998; Brunton et al., 2023).

Beyond the production of phenobarbital itself, synthetic modification of the C<sub>5</sub> substituents has provided a versatile platform for developing structurally related barbiturates with distinct pharmacological properties. These derivatives have significantly contributed to structure–activity relationship studies and continue to support the design of novel anticonvulsant agents with improved efficacy and safety profiles (Patrick, 2017; Perucca, 2018).

## **Structure–Activity Relationships (SAR)**

The pharmacological activity of phenobarbital is closely associated with its molecular structure. As with other barbiturates, the anticonvulsant efficacy of phenobarbital is primarily determined by the chemical modifications at the C<sub>5</sub> position of the barbiturate ring. These substituents influence lipophilicity, receptor affinity, blood–brain barrier permeability, and overall pharmacokinetic behavior. Consequently, the C<sub>5</sub> carbon has long been recognized as

the principal site for structural optimization in barbiturate chemistry (Patrick, 2017; Brunton et al., 2023).

The ethyl and phenyl substituents present at the C<sub>5</sub> position provide an optimal balance between hydrophobicity and molecular flexibility. The phenyl ring promotes hydrophobic interactions within the receptor-binding pocket, whereas the ethyl group contributes to molecular stability and appropriate lipophilicity. This structural combination enhances central nervous system penetration while maintaining sufficient receptor selectivity for effective anticonvulsant activity (Macdonald & Olsen, 1994; Perucca, 2018).

The barbiturate nucleus itself represents the essential pharmacophore responsible for biological activity. Its three carbonyl groups and two imide nitrogen atoms participate in hydrogen-bonding interactions that stabilize binding to the GABA<sub>A</sub> receptor. The spatial arrangement of these functional groups is critical, as even minor structural modifications may significantly alter receptor affinity and pharmacological potency (Olsen & Sieghart, 2008; Masiulis et al., 2019).

Lipophilicity is another major determinant of biological activity. Molecules with balanced lipophilic properties generally exhibit improved absorption, efficient penetration across the blood–brain barrier, and prolonged central nervous system exposure. However, excessive lipophilicity may increase nonspecific tissue distribution and toxicity. Therefore, the physicochemical properties of phenobarbital represent an optimized compromise between therapeutic efficacy and safety (Anderson, 2002; Vanderah, 2024).

Modern computational approaches have substantially expanded the understanding of phenobarbital SAR. Molecular docking, molecular dynamics simulations, and quantum chemical calculations have demonstrated that receptor recognition depends on the combined effects of molecular geometry, electronic distribution, hydrogen-bonding capacity, and hydrophobic interactions rather than on a single structural feature. These computational techniques have become valuable tools for predicting biological activity and guiding the rational design of novel barbiturate derivatives (Masiulis et al., 2019; Zhavoronkov et al., 2019).

Recent advances in artificial intelligence (AI)-assisted drug discovery have further accelerated the identification of new anticonvulsant candidates. Machine learning algorithms integrated with molecular modeling and virtual screening platforms enable rapid prediction of structure–activity relationships, optimization of physicochemical properties, and prioritization of compounds with improved therapeutic potential. These emerging technologies are expected to play an increasingly important role in the future development of safer and more selective barbiturate analogues (Stokes et al., 2020; Zhavoronkov et al., 2019).

**Table 3.** Major structural determinants influencing the biological activity of phenobarbital

| Structural feature               | Pharmacological significance                   |
|----------------------------------|------------------------------------------------|
| C <sub>5</sub> ethyl substituent | Improves lipophilicity and molecular stability |

|                                   |                                                                |
|-----------------------------------|----------------------------------------------------------------|
| C <sub>5</sub> phenyl substituent | Enhances receptor affinity and blood–brain barrier penetration |
| Barbiturate nucleus               | Core pharmacophore responsible for anticonvulsant activity     |
| Carbonyl groups                   | Hydrogen-bond acceptors involved in receptor binding           |
| Imide nitrogen atoms              | Contribute to hydrogen bonding and acid–base properties        |
| Molecular lipophilicity           | Determines CNS penetration and pharmacokinetic behavior        |

This section demonstrates that the pharmacological profile of phenobarbital results from the coordinated contribution of its structural components rather than from a single functional group. Understanding these relationships continues to provide valuable guidance for the rational design of next-generation barbiturate derivatives with improved efficacy and reduced adverse effects (Patrick, 2017; Brunton et al., 2023).

## Mechanism of Action

Phenobarbital exerts its anticonvulsant activity primarily by enhancing  $\gamma$ -aminobutyric acid (GABA)-mediated inhibitory neurotransmission within the central nervous system. As a positive allosteric modulator of the GABA<sub>A</sub> receptor, phenobarbital binds to a regulatory site distinct from the GABA binding site, thereby increasing receptor responsiveness without directly activating the receptor at therapeutic concentrations (Macdonald & Olsen, 1994; Olsen & Sieghart, 2008).

Upon GABA binding, phenobarbital prolongs the opening duration of receptor-associated chloride channels, leading to increased chloride influx into neurons. This influx produces membrane hyperpolarization, reduces neuronal excitability, and suppresses the initiation and propagation of epileptic discharges. Unlike benzodiazepines, which primarily increase the frequency of chloride channel opening, phenobarbital predominantly extends the channel open time, resulting in sustained inhibitory neurotransmission (Twyman et al., 1989; Brunton et al., 2023).

Although potentiation of GABAergic signaling represents its principal mechanism, phenobarbital also reduces excitatory neurotransmission at higher concentrations. Experimental studies have demonstrated that the drug suppresses glutamate-mediated neuronal excitation, particularly through inhibition of AMPA-type glutamate receptors. This additional effect contributes to its broad-spectrum anticonvulsant activity and may explain its effectiveness in severe seizure disorders, including status epilepticus (Rho et al., 1996; Perucca, 2018).

The therapeutic activity of phenobarbital reflects the coordinated modulation of inhibitory and excitatory neuronal pathways. By enhancing GABAergic inhibition while attenuating glutamatergic excitation, the drug restores the balance between neuronal excitation and inhibition that is disrupted during epileptic seizures. This dual mechanism has been extensively supported by electrophysiological, pharmacological, and structural studies and remains the basis for its clinical use (Masiulis et al., 2019; Brunton et al., 2023).

Recent advances in structural biology have provided detailed insights into the interaction between phenobarbital and the GABA<sub>A</sub> receptor. High-resolution cryo-electron microscopy and molecular modeling studies have demonstrated that barbiturates stabilize specific receptor conformations, thereby enhancing chloride channel function and improving inhibitory synaptic transmission. These findings have refined the molecular understanding of barbiturate

pharmacology and support the rational design of next-generation GABAergic modulators with improved selectivity and safety (Masiulis et al., 2019; Olsen & Sieghart, 2008).

**Table 4.** Molecular mechanisms underlying the anticonvulsant activity of phenobarbital

| <b>Mechanism</b>                                              | <b>Pharmacological consequence</b>                      |
|---------------------------------------------------------------|---------------------------------------------------------|
| Positive allosteric modulation of GABA <sub>A</sub> receptors | Enhances inhibitory neurotransmission                   |
| Prolongation of chloride channel opening                      | Increases neuronal hyperpolarization                    |
| Reduction of neuronal excitability                            | Suppresses seizure initiation and propagation           |
| Inhibition of glutamatergic neurotransmission                 | Decreases excitatory signaling at higher concentrations |
| Stabilization of receptor conformation                        | Improves receptor-mediated inhibitory activity          |

The anticonvulsant efficacy of phenobarbital arises from its ability to strengthen inhibitory neurotransmission while simultaneously limiting excessive neuronal excitation. Advances in receptor structural biology and computational pharmacology continue to improve our understanding of these mechanisms and provide valuable guidance for the development of safer and more selective antiseizure agents (Brunton et al., 2023; Masiulis et al., 2019).

## Pharmacokinetics

The pharmacokinetic profile of phenobarbital has been extensively characterized and is one of the principal reasons for its long-standing clinical use. Following oral administration, phenobarbital is almost completely absorbed from the gastrointestinal tract, with an oral bioavailability generally exceeding 90%. Peak plasma concentrations are typically reached within 8–12 h, although this interval may vary depending on the pharmaceutical formulation, dosage, and patient characteristics. Food has little influence on the extent of absorption, contributing to the predictable pharmacokinetic behavior of the drug (Anderson, 2002; Brunton et al., 2023).

After absorption, phenobarbital is widely distributed throughout body tissues and readily penetrates the central nervous system because of its moderate lipophilicity. Approximately 40–60% of circulating phenobarbital is bound to plasma proteins, while the remaining free fraction is responsible for pharmacological activity. The drug readily crosses both the placental barrier and the blood–brain barrier and is also excreted into breast milk, factors that should be considered during pregnancy and lactation (Vanderah, 2024; Perucca, 2018).

Phenobarbital undergoes hepatic metabolism primarily through the cytochrome P450 enzyme system, particularly CYP2C9 and, to a lesser extent, CYP2C19. A distinctive characteristic of phenobarbital is its potent enzyme-inducing capacity, which accelerates the metabolism of numerous co-administered drugs, including oral anticoagulants, corticosteroids, hormonal contraceptives, and several antiseizure medications. Consequently, clinically significant drug–drug interactions are common during long-term therapy and should be carefully monitored (Brunton et al., 2023; Patsalos et al., 2018).

Approximately 25–50% of an administered dose is eliminated unchanged by the kidneys, whereas the remaining fraction is excreted as hepatic metabolites. Because phenobarbital is a weak acid, urinary alkalinization enhances renal elimination by reducing tubular reabsorption,

a strategy that may be beneficial in cases of overdose. The elimination half-life ranges from 2 to 6 days in adults but may vary according to age, hepatic function, renal function, and concomitant medications. This prolonged half-life allows once-daily dosing in many patients but also increases the risk of drug accumulation during chronic therapy (Anderson, 2002; Brunton et al., 2023).

Therapeutic drug monitoring plays an important role in optimizing phenobarbital therapy because of considerable interindividual variability in drug disposition. The generally accepted therapeutic plasma concentration ranges from 15 to 40 µg/mL, whereas concentrations exceeding this range are associated with an increased risk of excessive sedation, respiratory depression, and neurotoxicity. Individual dose adjustment based on serum drug concentrations is therefore recommended, particularly in pediatric patients, older adults, and individuals receiving multiple antiseizure medications (Patsalos et al., 2018; Perucca, 2018).

Recent pharmacogenomic studies have demonstrated that genetic polymorphisms affecting CYP2C9 and CYP2C19 activity contribute to variability in phenobarbital metabolism and therapeutic response. These findings support the growing role of precision medicine in antiseizure therapy and suggest that genotype-guided dosing strategies may further improve treatment efficacy while reducing adverse effects in selected patient populations (Perucca, 2018; Brunton et al., 2023).

**Table 5.** Pharmacokinetic characteristics of phenobarbital

| Parameter                                             | Characteristics                                  |
|-------------------------------------------------------|--------------------------------------------------|
| Oral bioavailability                                  | >90%                                             |
| Time to peak plasma concentration (T <sub>max</sub> ) | 8–12 h                                           |
| Plasma protein binding                                | 40–60%                                           |
| Primary metabolic enzymes                             | CYP2C9, CYP2C19                                  |
| Major route of elimination                            | Renal excretion (unchanged drug and metabolites) |
| Elimination half-life                                 | 2–6 days                                         |

The favorable pharmacokinetic characteristics of phenobarbital, including excellent oral absorption, extensive tissue distribution, prolonged elimination half-life, and predictable plasma concentrations, continue to support its widespread clinical use. Nevertheless, its potent enzyme-inducing properties and substantial interindividual variability necessitate careful dose individualization and therapeutic drug monitoring to maximize efficacy while minimizing toxicity (Brunton et al., 2023; Patsalos et al., 2018).

## Clinical Applications, Safety, and Drug Interactions

Phenobarbital has been used in clinical practice for more than a century and continues to be an important antiseizure medication owing to its well-established efficacy, predictable pharmacokinetic profile, and relatively low cost. Although the introduction of newer antiseizure drugs has reduced its routine use in many high-income countries, phenobarbital remains an essential therapeutic option in resource-limited settings and is still included in the World Health Organization (WHO) Model List of Essential Medicines (World Health Organization [WHO], 2025; Brunton et al., 2023).

Clinically, phenobarbital is effective in the management of generalized tonic–clonic seizures, focal seizures, neonatal seizures, and status epilepticus. It is considered the first-line treatment for neonatal seizures in many clinical guidelines because of its rapid onset of action, well-documented efficacy, and extensive clinical experience. In refractory status epilepticus, phenobarbital is commonly administered after benzodiazepines and other second-line antiseizure medications have failed to achieve seizure control (Perucca, 2018; Pressler et al., 2023).

Despite its clinical benefits, phenobarbital is associated with several dose-dependent adverse effects. The most frequently reported reactions include sedation, dizziness, impaired concentration, cognitive slowing, and psychomotor dysfunction, particularly during treatment initiation or dose escalation. Long-term therapy has also been associated with behavioral changes, depression, osteoporosis secondary to vitamin D deficiency, and, in some patients, physical dependence. These adverse effects often limit prolonged use, especially when safer therapeutic alternatives are available (Vanderah, 2024; Brunton et al., 2023).

One of the most important clinical limitations of phenobarbital is its ability to induce hepatic cytochrome P450 enzymes, resulting in numerous clinically significant drug interactions. Enzyme induction accelerates the metabolism of several medications, including oral anticoagulants, corticosteroids, hormonal contraceptives, immunosuppressants, and other antiseizure drugs, thereby reducing their therapeutic efficacy. Consequently, careful evaluation of concomitant medications and appropriate dose adjustment are essential during phenobarbital therapy (Patsalos et al., 2018; Brunton et al., 2023).

Special consideration is required when phenobarbital is prescribed to pregnant women, older adults, and pediatric patients. The drug readily crosses the placenta and is excreted into breast milk; therefore, potential fetal and neonatal risks should be carefully balanced against the benefits of seizure control. In older adults, prolonged elimination and increased sensitivity to central nervous system depressants may enhance the risk of excessive sedation, falls, and cognitive impairment. These patient populations often require individualized dosing and close therapeutic monitoring (Perucca, 2018; Vanderah, 2024).

Therapeutic drug monitoring remains an important component of phenobarbital treatment, particularly in patients receiving long-term therapy or multiple concomitant medications. Regular measurement of serum drug concentrations, together with clinical assessment, facilitates dose optimization, minimizes toxicity, and improves long-term treatment outcomes. Current clinical practice increasingly emphasizes individualized therapy based on pharmacokinetic characteristics, comorbidities, and patient-specific risk factors (Patsalos et al., 2018; Brunton et al., 2023).

**Table 6.** Major clinical applications, adverse effects, and drug interaction

| Clinical aspect         | Description                                                                              |
|-------------------------|------------------------------------------------------------------------------------------|
| Primary indications     | Generalized tonic–clonic seizures, focal seizures, neonatal seizures, status epilepticus |
| Common adverse effects  | Sedation, dizziness, cognitive impairment, psychomotor slowing                           |
| Long-term complications | Dependence, osteoporosis, vitamin D deficiency, behavioral changes                       |

|                                     |                                                                                                                                   |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Major drug interactions             | CYP450 enzyme induction affecting anticoagulants, corticosteroids, oral contraceptives, immunosuppressants, and antiseizure drugs |
| Patients requiring close monitoring | Neonates, pregnant women, older adults, patients receiving multiple medications                                                   |
| Therapeutic monitoring              | Serum drug concentration measurement and individualized dose adjustment                                                           |

Overall, phenobarbital remains an effective and accessible antiseizure medication despite the availability of newer therapeutic alternatives. Its well-established efficacy, affordability, and broad clinical experience continue to justify its use in selected patient populations. However, appropriate patient selection, careful monitoring, and awareness of potential adverse effects and drug interactions are essential to maximize therapeutic benefit while minimizing treatment-related risks (WHO, 2025; Brunton et al., 2023; Perucca, 2018).

## Recent Advances and Future Perspectives

Although phenobarbital has been used clinically for more than a century, it continues to attract considerable scientific interest owing to its well-characterized pharmacology and its value as a model compound in antiseizure drug research. Recent advances in structural biology, computational chemistry, pharmacogenomics, and pharmaceutical technology have substantially improved our understanding of its molecular mechanism and therapeutic potential (Brunton et al., 2023; Masiulis et al., 2019).

One of the most significant developments has been the application of high-resolution structural biology to the study of GABA<sub>A</sub> receptors. Cryo-electron microscopy and molecular modeling have provided detailed insights into the interaction between phenobarbital and receptor subunits, revealing the structural basis of allosteric modulation and chloride channel regulation. These findings have facilitated the rational design of new GABAergic modulators with improved selectivity and reduced adverse effects (Masiulis et al., 2019; Olsen & Sieghart, 2008).

Another rapidly expanding research area is pharmacogenomics, which aims to optimize antiseizure therapy according to an individual's genetic profile. Variations in genes encoding drug-metabolizing enzymes, particularly CYP2C9 and CYP2C19, influence phenobarbital metabolism, plasma concentrations, and treatment response. Incorporating pharmacogenomic information into clinical decision-making may improve therapeutic efficacy while reducing toxicity and interindividual variability (Perucca, 2018; Brunton et al., 2023).

Recent progress in artificial intelligence (AI) and computer-aided drug design has further transformed anticonvulsant drug discovery. Machine learning algorithms, molecular docking, molecular dynamics simulations, and virtual screening techniques enable the rapid identification and optimization of compounds with favorable pharmacological and physicochemical properties. These computational approaches reduce experimental costs and accelerate the development of next-generation antiseizure agents inspired by classical barbiturate scaffolds (Zhavoronkov et al., 2019; Stokes et al., 2020).

Pharmaceutical formulation research has also focused on improving the safety and therapeutic performance of phenobarbital. Controlled-release formulations, nanotechnology-

based drug delivery systems, and targeted delivery strategies are being investigated to maintain stable plasma concentrations, reduce dosing frequency, and minimize dose-related adverse effects. Although many of these technologies remain in the experimental stage, they represent promising approaches for future clinical applications (Brunton et al., 2023; Patrick, 2017).

Despite the availability of numerous newer antiseizure medications, phenobarbital remains an indispensable reference compound in medicinal chemistry and clinical pharmacology. Future research is expected to integrate structural biology, precision medicine, computational modeling, and advanced pharmaceutical technologies to develop safer and more selective GABAergic therapeutics while preserving the proven efficacy that has characterized phenobarbital for decades (Brunton et al., 2023; Perucca, 2018).

**Table 7.** Emerging research directions for phenobarbital

| Research area                  | Future contribution                                               |
|--------------------------------|-------------------------------------------------------------------|
| Structural biology             | Improved understanding of GABA <sub>A</sub> receptor interactions |
| Pharmacogenomics               | Personalized dosing and optimized therapeutic response            |
| Artificial intelligence        | Accelerated drug discovery and molecular optimization             |
| Molecular modeling             | Prediction of receptor binding and biological activity            |
| Advanced drug delivery systems | Controlled drug release and reduced adverse effects               |
| Precision medicine             | Individualized treatment strategies                               |

The future of phenobarbital research extends beyond its traditional clinical use. The integration of computational drug design, pharmacogenomics, structural biology, and innovative drug delivery technologies is expected to enhance therapeutic precision while supporting the development of safer and more effective antiseizure medications. Consequently, phenobarbital is likely to remain not only an established therapeutic agent but also a valuable platform for future pharmaceutical innovation (Brunton et al., 2023; Zhavoronkov et al., 2019).

## Conclusion

Phenobarbital remains one of the most influential and extensively studied antiseizure medications in the history of clinical pharmacology. Despite the introduction of numerous newer antiseizure drugs, its well-established efficacy, predictable pharmacokinetic profile, affordability, and broad clinical experience continue to support its use in the management of epilepsy, particularly in neonatal seizures, status epilepticus, and resource-limited healthcare settings (Brunton et al., 2023; Perucca, 2018).

The therapeutic success of phenobarbital is closely linked to its molecular architecture. The combination of a barbiturate nucleus with ethyl and phenyl substituents at the C<sub>5</sub> position provides an optimal balance between receptor affinity, lipophilicity, and central nervous system penetration. These structural characteristics, together with its ability to enhance GABA-mediated inhibitory neurotransmission, explain its sustained anticonvulsant efficacy and its continuing importance as a model compound for structure–activity relationship studies (Macdonald & Olsen, 1994; Olsen & Sieghart, 2008).

Although long-term therapy may be associated with adverse effects and clinically significant drug–drug interactions due to hepatic enzyme induction, these limitations can be effectively managed through individualized dosing, therapeutic drug monitoring, and careful patient selection. The increasing integration of pharmacogenomics into clinical practice is

expected to further improve treatment safety and optimize therapeutic outcomes by enabling more personalized dosing strategies (Patsalos et al., 2018; Vanderah, 2024).

Recent advances in structural biology, computational chemistry, artificial intelligence, and pharmaceutical technology have substantially expanded the scientific understanding of phenobarbital beyond its traditional clinical applications. High-resolution receptor structures, molecular modeling, virtual screening, and AI-assisted drug discovery are providing valuable opportunities for the development of novel GABAergic modulators with improved efficacy and reduced toxicity. At the same time, innovative drug delivery systems may further enhance therapeutic performance by improving bioavailability and minimizing adverse effects (Masiulis et al., 2019; Zhavoronkov et al., 2019; Stokes et al., 2020).

In conclusion, phenobarbital remains not only an established antiseizure medication but also a cornerstone of medicinal chemistry and neuropharmacology. Its historical significance, well-defined molecular properties, and continuing contribution to modern drug discovery ensure that phenobarbital will remain an important reference compound for future research and therapeutic innovation (Brunton et al., 2023; Perucca, 2018).

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