



Review

Salivary Calcium as a Biomarker: Analytical Challenges and Clinical Interpretation—A Narrative Review

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Abstract

Salivary calcium is a biologically plausible but methodologically challenging biomarker because of its central role in enamel demineralization/remineralization, dental calculus formation, and oral mineral homeostasis. This narrative review summarizes the analytical challenges and clinical interpretation of salivary calcium measurements in oral and systemic disease. Evidence from periodontal research generally indicates higher salivary calcium concentrations in periodontitis, although interpretation is complicated by calculus formation, gingival crevicular fluid contribution, smoking, inflammation, and variation in saliva collection protocols. In dental caries, findings are heterogeneous, and calcium appears more meaningful when interpreted together with salivary flow rate, pH, phosphate, buffering capacity, and fluoride exposure than as an isolated diagnostic marker. Studies in osteoporosis, chronic kidney disease, hemodialysis, and diabetes suggest possible associations with systemic disturbances, but salivary calcium does not currently provide sufficient specificity or predictive value to replace established serum, imaging, or clinical biomarkers. Overall, salivary calcium should be regarded as a context-dependent component of broader salivary biomarker panels. Its clinical translation requires standardized sampling, clear distinction between total and ionized calcium, appropriate analytical validation, and careful control of pre-analytical and biological confounders.

Keywords: salivary calcium; salivary biomarkers; oral diseases; systemic diseases; periodontal disease; dental caries



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1. Introduction

The increasing demand for non-invasive, patient-friendly diagnostic approaches has positioned saliva as a highly attractive biological specimen in both clinical and research settings. As a complex biological fluid, saliva is produced primarily by the three major salivary gland pairs, parotid, submandibular, and sublingual, with additional contributions from numerous minor glands distributed throughout the oral mucosa [1,2]. Its composition reflects not only glandular secretory activity but also inputs from gingival crevicular fluid, mucosal transudate, and serum-derived components, making whole saliva a composite mirror of both local oral and systemic physiological states [3,4].

1.1. Saliva as a Diagnostic Fluid

From a practical standpoint, saliva offers distinct advantages over conventional biological matrices such as blood or urine. Collection is non-invasive, requires no specialised clinical procedure, and can be repeated at frequent intervals without patient burden, attributes that are particularly valuable for longitudinal monitoring and large-scale epidemiological studies [5,6]. These features have driven a sustained and growing interest in the identification of salivary biomarkers across a wide spectrum of conditions, including periodontal disease, diabetes mellitus, cardiovascular disease, and malignancies [7,8]. Advances in high-throughput analytical platforms, encompassing proteomics, transcriptomics, metabolomics, and microbiomics, have substantially expanded the catalogue of candidate salivary biomarkers and have given rise to the emerging field of salivaomics [6,8]. Despite this scientific momentum, the translation of salivary biomarkers into routine clinical practice remains limited. Saliva is an inherently dynamic and heterogeneous fluid, and its composition is sensitive to a range of physiological variables including salivary flow rate, circadian rhythms, hydration status, dietary intake, and oral hygiene practices [2]. These sources of pre-analytical variability, combined with the absence of widely adopted standardised collection and processing protocols, continue to hamper the reproducibility and clinical applicability of salivary biomarker research [8]. Inorganic ions, in particular, are highly susceptible to such confounders, and their interpretation requires careful contextualisation within the broader physicochemical environment of the oral cavity.

1.2. Why Calcium Deserves Attention

Among the inorganic constituents of saliva, calcium holds a position of particular biological significance. Together with phosphate and other electrolytes, salivary calcium is a principal determinant of the mineral equilibrium at the tooth surface, governing the dynamic interplay between enamel demineralisation and remineralisation [9,10]. Under normal physiological conditions, whole saliva remains supersaturated with calcium and phosphate relative to hydroxyapatite, a state that is essential for the integrity of dental hard tissues and the continuous repair of incipient carious lesions [11,12]. Salivary calcium is also a key determinant of dental calculus formation, as supersaturation of the oral environment promotes the precipitation of calcium phosphate salts within dental plaque, a process directly relevant to the pathogenesis of periodontal disease [6].

Beyond the oral cavity, salivary calcium concentrations have attracted interest as a potential non-invasive proxy for systemic mineral metabolism. Disturbances in calcium homeostasis accompanying conditions such as osteoporosis, chronic kidney disease, and hyperparathyroidism have been investigated in relation to changes in salivary calcium levels, reflecting the broader hypothesis that saliva may provide complementary information on selected systemic metabolic disturbances [13,14]. This combination of clear biological relevance and analytical accessibility makes salivary calcium an intuitively appealing biomarker candidate across multiple clinical contexts.

However, its diagnostic utility is substantially constrained by a number of interrelated limitations. Salivary calcium concentration is not a disease-specific signal; rather, it reflects the net outcome of glandular secretion, protein binding, pH-dependent speciation, and the contribution of gingival crevicular fluid—all of which vary considerably both within and between individuals [6,15]. Consequently, the same observed shift in calcium concentration may arise from entirely different biological processes depending on the clinical context, fundamentally limiting its specificity as a standalone diagnostic marker.

1.3. Aim and Scope of the Review

This narrative review critically appraises the current state of evidence on salivary calcium as a biomarker in oral and systemic disease. Our aim is to provide a balanced, evidence-based assessment that serves both researchers engaged in salivary biomarker discovery and clinicians seeking to contextualise the growing body of literature in this field.

2. Methods: Literature Search and Selection Strategy

This narrative review was informed by structured literature searches conducted between March and the end of May 2026 in PubMed/MEDLINE, Scopus, and Web of Science. The searches were designed to identify original research articles, methodological studies, systematic reviews, and narrative reviews addressing salivary calcium measurement, biological determinants of salivary calcium concentration, and its potential role as a biomarker in oral and systemic diseases.

The search combined terms related to saliva and calcium, including “salivary calcium”, “saliva calcium”, “ionized calcium”, “total calcium”, “calcium activity”, and “salivary electrolytes”, with terms related to analytical methodology and clinical conditions, including “colorimetric assay”, “ion-selective electrode”, “atomic absorption spectrometry”, “ICP-MS”, “ICP-OES”, “periodontitis”, “gingivitis”, “dental caries”, “calculus”, “osteoporosis”, “bone mineral density”, “chronic kidney disease”, “hemodialysis”, and “diabetes mellitus”.

Articles were considered eligible if they provided data or conceptual insight relevant to one or more of the following domains: salivary calcium physiology and speciation; pre-analytical and analytical determinants of calcium measurement; associations between salivary calcium and oral diseases; associations between salivary calcium and systemic conditions; or methodological recommendations for salivary biomarker research. Studies were excluded when calcium was not reported as a distinct salivary analyte, when the biological matrix was not saliva or oral fluid, when the analytical method or clinical context could not be determined, or when the article was not available in English. Reference lists of relevant reviews and primary studies were additionally screened to identify further studies of historical or methodological importance.

Because of substantial heterogeneity in saliva collection protocols, calcium fractions measured, analytical platforms, study populations, and clinical outcomes, no formal meta-analysis was attempted. Instead, the evidence was synthesized narratively, with emphasis on methodological comparability, biological plausibility, consistency of findings, and limitations affecting clinical interpretation.

3. Physiology and Biological Role of Salivary Calcium

3.1. Sources of Calcium in Saliva

Saliva is produced in a daily volume ranging from 500 mL to 1.5 L, with approximately 90% secreted by the three major salivary glands and the remainder by minor glands [16]. Acinar cells within the salivary glands, activated by neural stimulation and regulated by intracellular calcium and electrolyte balance, produce primary saliva by driving water movement from the systemic circulation through aquaporin channels and tight junctions, forming an isotonic, plasma-like fluid [17]. The ductal system subsequently modifies this secretion through selective electrolyte transport, producing final hypotonic saliva that remains supersaturated with calcium and phosphate before entering the oral cavity [1,17–19]. The major salivary glands provide most of the saliva volume and electrolytes, while minor glands contribute relatively little fluid but are rich in blood-group substances [20].

Salivary calcium levels are influenced by both glandular secretion and components derived from the bloodstream that enter the oral cavity via gingival crevicular fluid, mu-

cosal transudate, and intraoral bleeding, thereby contributing to the composition of whole saliva and reflecting both systemic and local influences [3].

3.2. Regulation of Salivary Calcium Concentration

The regulation of salivary calcium concentration reflects a complex interaction among salivary flow rate, pH, ionic balance, and salivary protein composition [2]. Flow rate is a key determinant of electrolyte composition and depends on autonomic stimulation of the salivary glands [21,22]. As flow increases under stimulation, the concentrations of sodium, bicarbonate, chloride, calcium, and proteins rise, whereas potassium and phosphate levels decrease only slightly [23]. These changes are accompanied by an increase in salivary pH, mainly due to rising bicarbonate levels during stimulation [12,21–23].

Salivary pH is closely linked to calcium stability and mineral balance. At pH values above 5.3, saliva remains supersaturated with calcium and phosphate relative to hydroxyapatite, promoting enamel remineralization [11,24]. However, when pH decreases, hydrogen ions interact with phosphate and hydroxyl ions, reducing their availability and shifting the equilibrium toward demineralization [12,25].

A critical pH threshold, most commonly cited at approximately 5.5, marks the point below which saliva becomes undersaturated with respect to hydroxyapatite and net demineralization begins [11,12,26]. It should be emphasized, however, that critical pH is not a fixed physicochemical constant but varies between individuals depending on the prevailing calcium and phosphate concentrations of saliva; higher salivary calcium and phosphate raise the degree of supersaturation and lower the effective critical pH, whereas lower concentrations raise it [11,12].

A substantial proportion of salivary calcium exists in the free ionized form, with the remainder protein-bound or complexed with phosphate and carbonate, indicating a dynamic equilibrium rather than a fixed concentration. Reported estimates of the ionized fraction vary widely (on the order of 45–85%), and this variability reflects genuine differences between saliva types and collection conditions rather than measurement error alone: the ionized proportion differs between parotid and whole saliva and between resting and stimulated states, with resting parotid saliva showing less calcium complexation than stimulated saliva [15]. Because these early estimates derive largely from gland-specific saliva under defined conditions, they should be applied cautiously to whole-saliva studies, and the collection type and stimulation status should always be specified when the ionized fraction is reported.

Salivary phosphoproteins such as statherin and proline-rich proteins bind calcium ions and regulate their availability by preventing the crystallization of calcium phosphate on the tooth surface [27–29]. The salivary pellicle that is formed post-absorption of proteins into enamel acts as a reservoir for calcium ions and regulates their diffusion toward the enamel surface [30,31].

Physiological factors such as circadian rhythms, reflex stimulation, age, stress, inflammation, and hormonal changes also contribute to normal fluctuations in both protein composition and electrolyte levels in saliva [2,23]. Overall, salivary calcium concentration reflects a dynamic interaction between flow rate, pH, ionic balance, and protein binding. This complexity, far from being merely an analytical inconvenience, has direct biological consequences: the same factors that regulate calcium availability in saliva also determine whether the oral environment favours mineral preservation, repair, or pathological deposition—processes explored in the sections that follow.

3.3. Role in Enamel Demineralization and Remineralization

The integrity of dental enamel is maintained through a dynamic equilibrium between demineralization and remineralization, governed by the saturation state of saliva and plaque fluid with respect to tooth mineral [11,32]. Although the critical pH for enamel dissolution is often cited at approximately 5.5, this threshold depends on local calcium and phosphate availability, fluoride exposure, ionic strength, and buffering capacity, as discussed above [12,25]. Therefore, the biological relevance of salivary calcium in caries is best understood through its contribution to mineral saturation rather than through total concentration alone. The role of calcium in this context is therefore not merely quantitative: it is the availability of free ionic calcium, rather than total calcium concentration, that determines the effective degree of salivary supersaturation and the capacity to remineralise. Remineralisation is a crystallisation process in which calcium and phosphate ions from saliva are deposited into the subsurface enamel matrix, reconstituting the mineral phase lost during acid attack [10,32]. Critically, remineralisation depends on an adequate supply of both calcium and phosphate ions at the enamel surface; a reduction in either, whether through decreased salivary flow, altered secretion, or buffering capacity failure, compromises the repair capacity of saliva [21,32]. Salivary proteins also participate actively in this process. Statherin and proline-rich proteins stabilise the supersaturated state of saliva by inhibiting spontaneous calcium phosphate precipitation in the fluid phase, thereby preserving calcium availability for controlled deposition at the enamel surface rather than allowing its loss through uncontrolled precipitation [27,29]. The acquired salivary pellicle, formed by selective adsorption of these proteins onto enamel, further acts as a diffusion barrier and a reservoir for calcium ions, modulating their access to the mineral surface during both acidic challenges and recovery phases [30,31]. Taken together, these mechanisms underscore why salivary calcium concentration is closely monitored in studies of caries risk. Deviations from the normal supersaturated state, whether due to reduced secretion, altered protein composition, or changes in pH, shift the balance towards demineralisation and may serve as early indicators of compromised mineral homeostasis. However, as discussed in subsequent sections, the relationship between salivary calcium concentration and clinical caries experience is far from straightforward, and its interpretation must account for the full physicochemical context in which calcium operates.

3.4. Calcium and Dental Plaque Mineralization

The same physicochemical principles that govern enamel remineralization also drive the pathological mineralization of dental plaque, a process that culminates in the formation of dental calculus. Dental calculus represents mineralized bacterial plaque composed predominantly of calcium phosphate salts, including brushite, octacalcium phosphate, hydroxyapatite, and whitlockite, embedded within an organic matrix of proteins, polysaccharides, and microbial remnants [11,33]. Its formation is relevant not merely as a local oral hygiene issue but as a key pathogenetic factor in periodontal disease, where calculus deposits provide a scaffold for continued microbial colonization and impede effective mechanical debridement.

The driving force for plaque mineralization is salivary supersaturation with calcium phosphate salts [11,33]. Once bacteria adhere to the acquired pellicle and form a mature plaque biofilm, calcium and phosphate ions from the surrounding saliva, and, in the case of subgingival calculus, from gingival crevicular fluid, are progressively absorbed onto the biofilm matrix. Initial mineral deposition occurs as amorphous calcium phosphate, which subsequently matures into more crystalline phases over time. The rate and extent of this process are modulated by salivary flow rate, local pH, and the concentration and activity of calcium ions in the plaque fluid [23,33].

Salivary proteins play a dual and somewhat paradoxical role in this context. While statherin and proline-rich proteins inhibit spontaneous calcium phosphate crystallization in saliva, thereby preventing uncontrolled precipitation, the same ions that are held in a metastable supersaturated state in free saliva become available for mineralization once they are incorporated into the plaque biofilm environment, where protein inhibitors are less effective and local pH conditions may differ significantly [27,33]. Elevated salivary calcium concentrations have consistently been associated with greater calculus formation, and individuals classified as heavy calculus formers tend to show higher total and ionic calcium levels in their saliva compared with light formers [6].

This relationship between salivary calcium and calculus burden is of direct clinical relevance to the interpretation of salivary calcium as a biomarker in periodontal disease. Elevated salivary calcium in a patient with periodontitis may not reflect a direct inflammatory signal but rather an oral environment that is inherently more conducive to calculus formation and, consequently, to the progression of periodontal tissue destruction. Distinguishing between these mechanistic pathways— inflammatory versus mineralogenic—is one of the central interpretive challenges addressed in subsequent chapters of this review.

4. Why Salivary Calcium Is a Difficult Biomarker

Despite its well-established biological role in oral homeostasis, salivary calcium presents considerable challenges from a biomarker perspective. For an analyte to be clinically useful, it must demonstrate sufficient stability, specificity, and reproducibility to distinguish disease-related changes from physiological variation. In the case of salivary calcium, this distinction is frequently obscured by the complex interplay of local, systemic, and methodological factors that influence its concentration. Contemporary reviews of salivary diagnostics consistently highlight that variability, rather than absolute concentration, represents the primary limitation of many salivary analytes, including inorganic ions such as calcium (Table 1) [6,8].

Table 1. Saliva collection approaches relevant to salivary calcium measurement.

Saliva Type/Collection Approach	Main Advantages	Main Limitations	Specific Relevance for Salivary Calcium	Minimum Reporting Recommendation
Unstimulated whole saliva	Non-invasive; simple; reflects resting oral conditions; clinically feasible	Strongly affected by hydration, circadian rhythm, medications, oral inflammation and passive GCF contribution; lower flow may concentrate analytes	Often most relevant for basal oral mineral homeostasis, but elevated calcium may reflect low flow or inflammatory/GCF contribution rather than true glandular output	Time of day, fasting interval, oral hygiene restrictions, collection duration, flow rate, oral inflammatory status
Stimulated whole saliva	Provides larger sample volume; easier collection; useful when unstimulated flow is low	Mechanical or gustatory stimulation changes flow rate, pH, bicarbonate, phosphate and electrolyte composition; not interchangeable with unstimulated saliva	Stimulation can alter calcium concentration and secretion rate; interpretation requires reporting of stimulation type and flow rate	Type of stimulation, duration, collection volume, flow rate/secretion rate, pH

Table 1. Cont.

Saliva Type/Collection Approach	Main Advantages	Main Limitations	Specific Relevance for Salivary Calcium	Minimum Reporting Recommendation
Passive drool	Minimal external contamination; avoids absorbent materials; suitable for ions and proteins	May be slow in xerostomic patients or children; requires participant cooperation	Preferred for minimizing artefactual ion binding or contamination from collection devices	Collection time, total volume, flow rate, pre-collection restrictions
Spitting method	Practical and widely used; suitable for larger volumes	Can introduce bubbles, variable CO ₂ loss, mucosal debris, and mechanical stimulation; may alter pH during collection	CO ₂ loss and pH shifts may affect calcium speciation, especially when ionized calcium is measured	Collection frequency, duration, storage tube type, time to pH measurement/processing
Absorbent devices/swabs	Useful in children, elderly patients, patients with low salivary flow, or field settings	Potential ion binding, dilution effects, variable recovery, contamination from device material	Generally less suitable for quantitative calcium unless recovery is validated for the device	Device type, recovery validation, extraction protocol, sample volume estimate
Parotid saliva	Gland-specific; reduces contamination from GCF and oral debris; useful for secretion physiology	Requires special collection devices; lower clinical feasibility; does not represent whole-mouth environment	Useful for distinguishing glandular calcium secretion from whole-saliva calcium influenced by GCF/plaque/oral disease	Collection device, stimulation status, gland side, flow rate, collection duration
Submandibular/sublingual saliva	Gland-specific; relevant because these glands contribute substantially to resting saliva	Technically demanding; often mixed ductal secretions; less common in clinical studies	May help clarify gland-specific differences in calcium secretion and protein binding	Collection device, gland/source definition, stimulation status, flow rate
Gingival crevicular fluid contribution to whole saliva	Clinically relevant in gingivitis and periodontitis; captures inflammatory oral environment	Not a standardized saliva type; contribution varies with inflammation, pocket depth, bleeding and sampling conditions	May increase whole-saliva calcium through serum-derived ions and inflammatory exudate; major confounder in periodontal studies	Periodontal status, bleeding on probing, calculus index, pocket depth, exclusion of visible blood contamination
Plaque fluid/plaque-associated sampling	Directly relevant to enamel saturation, caries and plaque mineralization	Technically difficult; small volumes; not comparable to routine whole saliva	More mechanistically relevant for local mineral saturation than whole saliva, but limited feasibility	Sampling site, plaque status, pH, method of calcium fraction measurement

4.1. Pre-Analytical Variability

Pre-analytical variability is among the most consequential sources of inconsistency in salivary calcium measurement. Collection method, stimulation status, time of day, recent food intake, hydration, smoking, oral hygiene, inflammation, and sample handling can all affect measured concentrations [5,8]. Because whole saliva is a composite fluid that includes glandular secretions, gingival crevicular fluid, desquamated cells, microorganisms, and possible blood contamination, collection protocols must be reported in sufficient detail. At minimum, studies should specify saliva type, stimulation status, collection duration, flow rate or secretion rate, time of day, pre-collection restrictions, oral inflammatory status, storage conditions, and time to analysis [34].

Across collection approaches, salivary calcium studies should report concentration together with flow rate or secretion rate whenever possible (Figure 1). This is particularly important because a higher concentration may result from reduced salivary volume rather than increased calcium secretion. For studies focused on mineral saturation or ionized calcium, pH should be measured immediately or under controlled conditions, and the time between collection and analysis should be stated. Visible blood contamination should be excluded or explicitly reported, especially in periodontal studies.

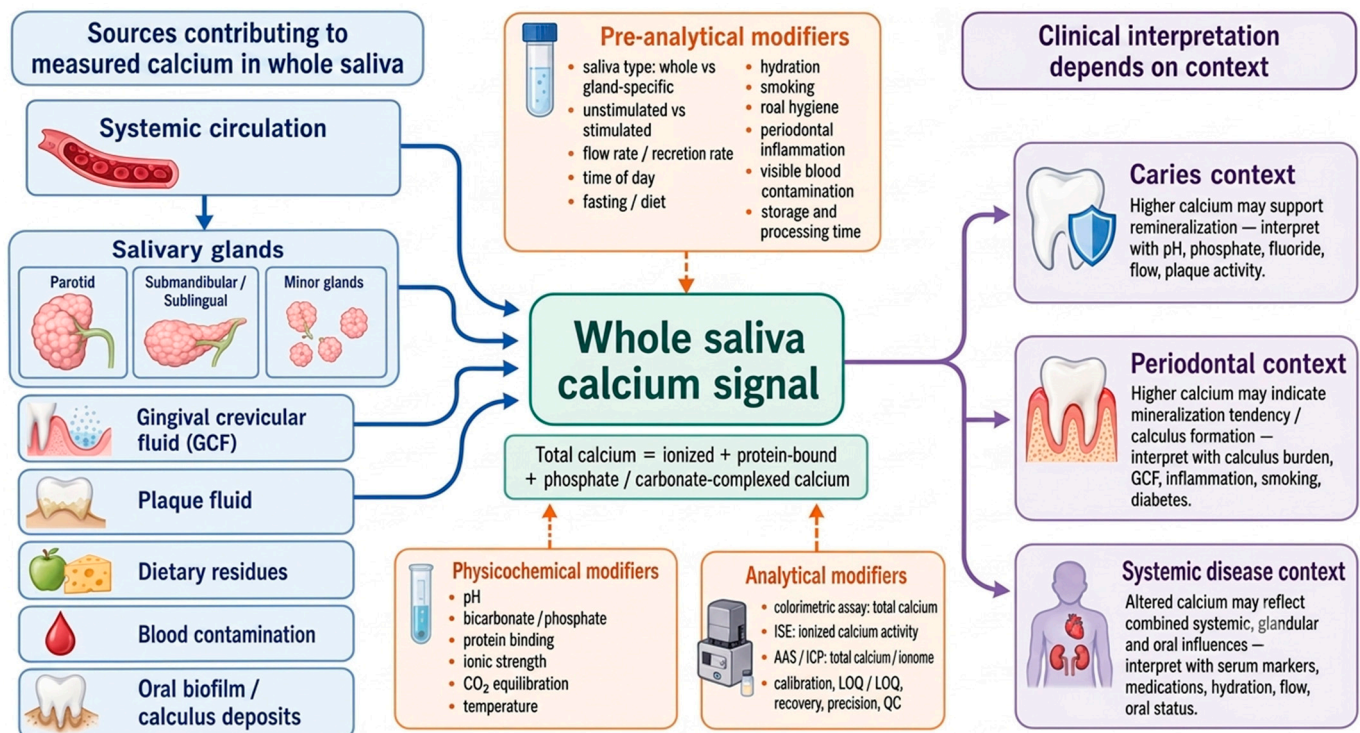


Figure 1. Determinants and context-dependent interpretation of salivary calcium measurements. Measured salivary calcium reflects the combined contribution of glandular secretion, gingival crevicular fluid, plaque fluid, dietary residues, oral biofilm, and possible blood contamination. The measured value is further modified by saliva type, stimulation status, flow rate, pH, phosphate/bicarbonate, protein binding, ionic strength, CO₂ equilibration, temperature, sample handling, and analytical method. Because total calcium and ionized calcium activity represent different biological quantities, the clinical meaning of salivary calcium depends on both the fraction measured and the disease context. Salivary calcium is therefore best regarded as a context-dependent component of multivariable biomarker panels rather than a standalone diagnostic marker.

4.2. Flow Rate and Dilution Effects

Salivary flow rate is a major confounder in the interpretation of calcium concentration. A higher measured concentration may reflect reduced salivary volume rather than increased calcium secretion, while stimulation may alter both dilution and electrolyte composition [35,36]. Therefore, calcium concentration should, whenever possible, be reported together with flow rate or secretion rate to distinguish true changes in calcium output from dilution artefacts [8].

4.3. pH, Buffering Capacity, and Ionic Interactions

The interpretation of salivary calcium is inseparable from the physicochemical properties of saliva as a fluid. Calcium exists in multiple forms, including free ionised calcium and forms bound to proteins or complexed with phosphate and carbonate, and the distribution between these fractions is strongly dependent on salivary pH, buffering capacity, and ionic

interactions [37]. Shifts in pH can alter this equilibrium and thereby affect both the total and biologically active fractions of calcium. Saliva is ordinarily supersaturated with calcium phosphates, a state that is essential for enamel protection; however, this equilibrium is dynamic and sensitive to local environmental changes [11]. The interpretation of salivary calcium is inseparable from saliva pH, buffering capacity, phosphate, carbonate, and protein binding. These factors determine the distribution between free ionized calcium and complexed or protein-bound fractions [8]. Because pH shifts can substantially alter calcium speciation and mineral saturation, calcium should not be interpreted as an isolated analyte, particularly in studies of caries risk, calculus formation, or periodontal disease.

4.4. Analytical Issues and Measurement Heterogeneity

Analytical heterogeneity presents an additional challenge in salivary calcium research. The literature encompasses a range of measurement approaches, including colorimetric assays, ion-selective electrodes, atomic absorption spectrometry, and inductively coupled plasma methods, which differ in sensitivity, specificity, and susceptibility to matrix interference, contributing to variability in results across studies [38]. An important distinction also exists between total and ionised calcium. Although total calcium is more frequently measured due to analytical simplicity, ionised calcium represents the biologically active fraction and is arguably the more relevant parameter. Its measurement, however, requires strict control of analytical conditions, including pH and temperature, which is not consistently implemented across studies [39]. Furthermore, saliva is a complex biological matrix containing proteins, enzymes, and mucins that may interfere with analytical methods. Many studies provide insufficient detail regarding sample processing, calibration procedures, and quality control measures, making it difficult to assess the reliability of reported results. Recent methodological literature has called for the standardisation of analytical protocols as a prerequisite for improving the comparability and translational value of salivary calcium data [8].

4.5. Biological Non-Specificity

Perhaps the most fundamental limitation of salivary calcium as a biomarker is its biological non-specificity. Unlike biomarkers that reflect discrete pathophysiological mechanisms, salivary calcium represents a general indicator of mineral balance and glandular function. Its concentration may be altered across a wide range of oral and systemic conditions, including periodontal disease, dental caries, and metabolic disorders, without reliably pointing to any single underlying aetiology [6]. This non-specificity complicates clinical interpretation considerably: similar shifts in calcium concentration may arise from fundamentally different biological processes. Elevated concentrations may indicate an increased propensity for mineralisation and calculus formation, while reduced concentrations may reflect diminished remineralisation potential or altered salivary flow, yet without additional contextual data, such as clinical parameters or complementary biomarkers, these changes cannot be reliably interpreted in isolation. The available evidence suggests that salivary calcium is unlikely to possess sufficient diagnostic value as a standalone marker. Its most plausible clinical role lies within a multimarker framework that integrates biochemical, microbiological, and clinical data [7], an approach consistent with contemporary trends in biomarker research and a more realistic path toward clinical implementation.

5. Analytical Methods for Calcium Determination in Saliva

The selection of an appropriate analytical method for salivary calcium quantification is not a trivial decision. Calcium exists in saliva in multiple physicochemical forms and different methods capture these fractions selectively or in combination [15,40]. This distinc-

tion has direct implications for the biological interpretation of results. Total calcium and ionised calcium are not equivalent measures and may yield divergent findings in the same population. The analytical literature encompasses four principal approaches, colorimetric methods, ion-selective electrodes (ISE), atomic absorption spectrometry (AAS), and inductively coupled plasma-based methods (ICP-OES/ICP-MS), each with distinct characteristics in terms of sensitivity, throughput, cost, and suitability for salivary matrix [36,39].

In the context of salivary calcium research, analytical validity refers not only to the ability of a method to detect calcium, but also to the reliability with which it measures the relevant calcium fraction in a complex and variable salivary matrix. Key parameters include analytical accuracy, precision, reproducibility, linearity and working range, limits of detection and quantification, recovery, matrix effects, sample volume requirements, calibration strategy, quality control procedures, and inter-laboratory comparability. These parameters are particularly important because the physiological range of salivary calcium is relatively narrow and because saliva composition varies substantially with flow rate, stimulation, pH, protein content, and oral inflammatory status. Therefore, analytical performance cannot be interpreted independently of pre-analytical conditions or of the calcium fraction measured. For this reason, future studies should report, at minimum, the saliva type and collection protocol, calcium fraction measured, analytical platform, calibration procedure, LOD/LOQ or working range, intra- and inter-assay precision, recovery or method-comparison data, sample storage conditions, and quality-control procedures.

5.1. Colorimetric Methods

Colorimetric techniques represent the most widely applied approach for calcium determination in biological fluids in routine clinical and research settings. Their operating principle is based on the formation of a coloured complex between calcium ions and a metallochromic reagent, the absorbance of which is measured spectrophotometrically and related to calcium concentration via a calibration curve [41]. The most commonly used reagents in the context of salivary calcium include o-cresolphthalein complexone (CPC), methylthymol blue (MTB), and arsenazo III, each with distinct pH optima, sensitivity ranges, and susceptibility to matrix interferences. Although colorimetric assays are often grouped together, they are not analytically interchangeable. The most frequently encountered approaches are based on dye-binding reactions, particularly o-cresolphthalein complexone/cresolphthalein complexone (OCPC/CPC), methylthymol blue (MTB), and arsenazo III. OCPC/CPC-based assays are widely used in automated clinical chemistry and have also been adapted to paper-based point-of-care formats. MTB-based methods provide stable calcium–dye complexes under alkaline conditions and have been used both in conventional spectrophotometric assays and in μ PAD systems. Arsenazo III is another established metallochromic reagent, but its performance is also affected by pH, matrix composition, and potential interference by other divalent cations. Because most colorimetric assays measure total calcium, their results should not be interpreted as equivalent to ionized calcium activity. A principal limitation of colorimetric methods is their non-selective interaction with the salivary matrix. Magnesium, phosphate, and salivary proteins compete with calcium for binding to the chromogenic reagent, leading to underestimation of the true calcium concentration unless appropriate masking agents, most commonly 8-quinolinol for magnesium, are incorporated into the assay protocol [41,42]. The MTB method offers particular advantages in this regard: MTB binds calcium tightly and with sufficient affinity to resist competition from matrix components, and the addition of reducing agents such as sodium sulphite further stabilises the coloured complex, permitting analysis over extended time windows without significant signal drift [41]. These properties have made MTB the preferred reagent for both manual and automated calcium assays in

biological fluids. A notable methodological development is the adaptation of colorimetric calcium detection to paper-based microanalytical devices (μ PADs). These systems exploit wax-patterned chromatographic paper to direct sample flow, enabling colour formation and quantification by flatbed scanner or smartphone camera, without specialised laboratory equipment [42,43]. Point-of-care calcium determination in saliva using μ PAD platforms has demonstrated acceptable analytical performance, with detection limits suitable for the physiological concentration range and good agreement with AAS reference values [43]. Such approaches may hold promise for future chairside diagnostics, though their clinical validation in diverse patient populations remains at an early stage. Colorimetric methods measure total calcium, the sum of all ionic, complexed, and protein-bound forms present in the sample, and cannot distinguish between biologically active and inactive fractions. This is both a practical advantage (simplicity, no special sample preparation) and an interpretive limitation, particularly in a matrix as compositionally variable as saliva.

5.2. Ion-Selective Electrodes

Ion-selective electrodes offer a fundamentally different approach: rather than measuring total calcium, ISE methods directly detect free ionised calcium activity (Ca^{2+}) through a selective membrane that generates a potential proportional to ion activity [39]. This is clinically significant because Ca^{2+} , not total calcium, is the physicochemically active fraction that governs mineral saturation at the tooth surface and directly influences enamel demineralisation and remineralisation dynamics [39,40]. ISE measurements in saliva are technically demanding. The response of the electrode is to ion activity, not concentration, making the result dependent on the ionic strength of the sample, a parameter that varies considerably across individuals and collection conditions. Furthermore, the distribution between ionised and complexed calcium in saliva is strongly pH-dependent, meaning that even minor changes in CO_2 equilibration or temperature between collection and measurement can shift the measured Ca^{2+} activity substantially [39,44]. For this reason, Carey and Vogel emphasised that salivary and plaque fluid samples should ideally be analysed under controlled pCO_2 conditions immediately after collection [39]. Protein adsorption onto the electrode membrane, so-called electrode poisoning, represents an additional practical obstacle in salivary applications that has not been fully resolved by current ISE designs. Despite these challenges, ISE methods provide more physiologically relevant information than total calcium assays when the research question concerns the actual driving force for mineralisation or demineralisation. A single early study reported a correlation ($r = 0.84$) between ionised calcium in whole saliva and plaque fluid [40], tentatively suggesting that salivary ISE measurements could serve as a proxy for plaque fluid calcium activity, although this rests on limited evidence and awaits replication.

A further limitation of ISE-based calcium measurement is that the electrode responds to ion activity rather than concentration. Consequently, Ca^{2+} readings are influenced by sample ionic strength, which itself varies with salivary flow rate, stimulation, bicarbonate concentration, disease status, and sample handling. In addition, equilibration with atmospheric CO_2 , changes in pH, and temperature shifts between collection and analysis can alter calcium speciation and produce drift in measured Ca^{2+} activity. These factors are particularly problematic because many clinical studies do not report whether samples were analysed immediately, whether temperature was controlled, whether CO_2 exposure was minimized, or whether ionic strength was measured or corrected. For studies using ISE, future reporting should therefore include sample pH, temperature, time from collection to measurement, saliva type, whether samples were stored aerobically or anaerobically, calibration conditions, and, where feasible, ionic strength or the method used for ionic strength adjustment.

5.3. Atomic Absorption Spectrometry and ICP-Based Methods

For research requiring high analytical accuracy and sensitivity, particularly when calcium concentrations are low or when multi-elemental profiling is intended, atomic absorption spectrometry and inductively coupled plasma-based methods represent the analytical gold standard [36]. Both techniques require sample preparation involving digestion or dilution under controlled conditions, and both offer excellent accuracy and precision across the concentration ranges relevant for salivary calcium.

Flame AAS (FAAS) is the established reference method for total calcium determination in biological fluids and remains widely used due to its relatively low cost and well-characterized performance. Graphite furnace AAS (GFAAS) provides markedly improved sensitivity, enabling detection at lower concentrations but at the cost of lower throughput. ICP-optical emission spectrometry (ICP-OES) and ICP-mass spectrometry (ICP-MS) extend analytical capability considerably further, permitting simultaneous quantification of multiple elements, including calcium alongside trace metals of clinical interest, from small sample volumes, with detection limits that far exceed those achievable by AAS [36]. ICP-MS in particular is now considered the method of choice for comprehensive elemental profiling of saliva in research settings, combining high sensitivity, isotopic selectivity, and multi-element capability in a single analytical run [45,46].

The primary constraints on wider clinical adoption of ICP-based methods are practical. Equipment costs are substantially higher than for colorimetric or ISE approaches, and the requirement for specialized instrumentation and trained personnel limits their use to dedicated analytical laboratories. Consequently, ICP-MS and ICP-OES have found their primary application in research studies examining the salivary ionome in disease, rather than in routine clinical calcium monitoring.

5.4. Methodological Comparability Across Studies

The coexistence of multiple analytical methods, each measuring a different calcium fraction under different matrix conditions, represents one of the most significant but underappreciated sources of heterogeneity in the salivary calcium literature. A study using colorimetric total calcium measurement and one using ISE-based ionised calcium determination are, in effect, measuring different biological quantities in the same fluid. Direct comparison of their numerical results, or their clinical conclusions, is therefore methodologically inappropriate without explicit acknowledgement of this distinction [39,40].

This problem is compounded by inconsistency in sample processing. Salivary matrix components can interfere with all analytical platforms to varying degrees, and their removal through centrifugation, filtration, or dialysis alters the sample in ways that may not be equivalent across methods [47]. Many published studies provide insufficient procedural detail to allow replication or meaningful inter-laboratory comparison, a deficiency consistently noted in systematic reviews of salivary ionomics literature [8,36]. The absence of certified reference materials for salivary calcium, and the lack of consensus on whether total or ionised calcium should serve as the primary outcome measure in clinical research, further erode the comparability of existing data. Addressing these gaps through method harmonisation and transparent reporting is a prerequisite for advancing salivary calcium from a research curiosity to a clinically actionable biomarker.

The summary and comparison of different analytical methods for salivary calcium determination are presented in Table 2.

Table 2. Analytical methods for salivary calcium determination: measured fraction, representative performance, and methodological limitations.

Method	Calcium Fraction Measured	Representative Analytical Performance/Range	Main Advantages	Main Limitations	Best Use
Colorimetric assays: OCPC/CPC, MTB, arsenazo III, other dye-binding assays	Total calcium	Method- and kit-dependent; conventional colorimetric assays are generally suitable for the physiological salivary calcium range, but LOD/LOQ, linear range, recovery, and CV should be reported for the salivary matrix	Simple, inexpensive, high throughput, suitable for routine laboratories	Matrix interference from Mg^{2+} , phosphate, proteins and mucins; measures total rather than ionized calcium; reagent- and pH-dependent	Screening, routine research, larger clinical studies
MTB-based μ PAD	Usually total calcium	Linear range 5–40 mg/L; LOD 2.9 mg/L; LOQ 8.9 mg/L; intra-day RSD 4.2–5.1%; inter-day RSD 5.8–6.4%	Low-cost, portable, minimal instrumentation, potential chairside use	Early validation stage; dilution may be needed; matrix effects remain relevant	Point-of-care and field applications after further validation
CPC-based μ PAD	Total calcium	Range 0.27–4.50 mmol/L/ 11–180 mg/L; LOD 80 μ mol/L/3.2 mg/L; relative deviation from AAS < 10% in saliva samples	Disposable, portable, saliva-specific validation, compatible with POC testing	Limited clinical validation; performance based on small sample sets; requires standardised handling	POC-oriented calcium monitoring and method-development studies
Ion-selective electrode	Ionized calcium activity	Analytical performance depends strongly on calibration, temperature, pH, pCO_2 and ionic strength control; should be reported as activity, not simply concentration	Measures biologically active Ca^{2+} ; directly relevant to mineral saturation and de-/remineralization	Sensitive to pH, CO_2 loss, temperature, ionic strength, protein fouling and electrode drift	Mineral saturation studies, plaque fluid/saliva comparisons, caries mechanistic research
AAS	Total calcium	Study-specific; requires reporting of calibration, digestion/dilution, recovery and precision in saliva	Accurate reference method; useful for method comparison	Laboratory-based, sample preparation required, usually single-element analysis	Validation studies and comparison with simpler assays
ICP-OES/ICP-MS	Total calcium and multi-element profiles	Highly sensitive and suitable for multi-element salivary ionomics; performance depends on instrument and matrix preparation	Multi-element analysis, high sensitivity, small sample volumes possible	Expensive, specialized instrumentation, digestion/dilution and matrix correction required	Salivary ionomics, trace element profiling, research settings

6. Salivary Calcium in Oral Diseases

The oral cavity represents the primary biological context in which salivary calcium exerts its most direct and well-characterised physiological functions. It is therefore not surprising that salivary calcium has been most extensively investigated as a potential biomarker in oral conditions, particularly those in which disturbances of mineral home-

ostasis, calcification, or enamel integrity are central to the disease process. The following sections critically examine the evidence across the principal oral disease categories in which salivary calcium has been studied, with particular attention to the consistency, interpretive challenges, and clinical relevance of available findings.

6.1. Periodontal Diseases

Periodontal diseases represent the most extensively studied oral disease context for salivary calcium research. The biological rationale is strong, because salivary supersaturation with calcium phosphate salts contributes to dental calculus formation, and calculus is a well-established local factor that facilitates plaque retention and periodontal inflammation [33]. However, salivary calcium should not be interpreted as a direct inflammatory biomarker of periodontitis. Rather, it may reflect a mineralogenic oral environment, the contribution of gingival crevicular fluid, altered salivary flow and pH, or the combined effect of inflammation, plaque retention, and calculus accumulation.

6.1.1. Gingivitis and Early Inflammatory Changes

Evidence regarding salivary calcium in gingivitis is comparatively limited and less consistent than in periodontitis. The biological basis for a possible calcium increase in gingivitis is the enhanced contribution of gingival crevicular fluid to whole saliva during gingival inflammation, which may introduce serum-derived ions into the oral fluid [3]. Nevertheless, the available studies do not consistently demonstrate a clear or diagnostically useful elevation of salivary calcium in gingivitis alone. When gingivitis groups are included together with periodontitis and periodontally healthy controls, salivary calcium values often appear to fall between health and periodontitis, but differences from healthy controls are frequently not statistically significant or are not reported with sufficient effect-size detail for direct comparison [36]. Therefore, gingivitis should be interpreted as a transitional and methodologically difficult category rather than as a condition in which salivary calcium has established diagnostic value.

6.1.2. Periodontitis Severity and Disease Progression

The association between elevated salivary calcium and periodontitis is among the most consistently reported findings in the salivary calcium literature. Multiple cross-sectional studies have reported significantly higher salivary calcium concentrations in patients with periodontitis than in periodontally healthy controls [48–51]. This pattern has also been supported by broader ionomics evidence, in which calcium is among the elements most frequently elevated in periodontitis, and by a recent meta-analysis reporting higher salivary calcium concentrations in periodontitis patients, with possible association with disease severity [36,52].

At least two overlapping mechanisms may explain higher salivary calcium in periodontitis. Elevated calcium may reflect a mineralogenic oral environment that favours calculus formation and plaque retention. Also, periodontal inflammation may increase gingival crevicular fluid flow and thereby enrich whole saliva with serum-derived ions. Additional modifiers, including smoking, diabetes, medications, salivary flow, and pH, may further influence the measured calcium signal [53,54]. For this reason, salivary calcium should be interpreted as an adjunctive marker of periodontal mineralization tendency and oral environmental risk, rather than as a disease-specific inflammatory biomarker.

The relationship between salivary calcium and clinical periodontal parameters is therefore not straightforward. Some studies have reported associations with probing pocket depth, clinical attachment loss, bleeding on probing, or calculus indices, whereas others have found weak or non-significant relationships [36,55]. Comorbidities such as diabetes and hypertension may further confound the association, and smoking appears

to modify salivary calcium levels in patients with periodontitis [55–57]. These findings suggest that salivary calcium may capture aspects of the mineralizing and inflammatory oral environment, but it does not provide a disease-specific signal.

From a clinical perspective, salivary calcium is therefore best interpreted as an adjunctive marker of periodontal mineralization tendency and oral environmental risk rather than as a diagnostic biomarker of periodontitis itself. Its potential value is likely greatest when combined with periodontal clinical parameters, calculus assessment, salivary flow rate, pH, smoking status, glycemic status, and established inflammatory or microbial biomarkers. Longitudinal studies are needed to determine whether elevated salivary calcium predicts future calculus accumulation or periodontal progression, or whether it primarily reflects already established disease.

6.2. Dental Caries

6.2.1. Salivary Calcium and Caries Experience

The relationship between salivary calcium and dental caries is biologically plausible but clinically complex. Calcium is an essential constituent of hydroxyapatite, and the availability of calcium ions in saliva contributes to the degree of saturation of the oral fluid with respect to enamel mineral. In this context, higher calcium availability may support remineralization and resistance to acid-induced demineralization, whereas reduced calcium availability may contribute to a less protective oral environment [10–12,32]. However, the clinical evidence linking salivary calcium concentration to caries experience is heterogeneous and should not be interpreted as indicating a simple linear relationship.

Several studies have reported inverse associations between salivary calcium concentrations and caries experience, with lower calcium levels observed in caries-active individuals than in caries-free controls [10,11]. In a long-term follow-up of children within a dietary-intervention trial, those who remained caries-free tended to show higher stimulated salivary calcium than those who developed caries, although the concurrent dietary modification limits interpretation of this association [58]. These findings are consistent with the concept that reduced calcium availability may compromise the maintenance of hydroxyapatite supersaturation and limit the remineralization of early enamel lesions. Nevertheless, salivary calcium does not operate independently. Its protective effect depends on the simultaneous presence of phosphate, appropriate pH and buffering capacity, fluoride exposure, adequate salivary flow, and the dynamics of plaque biofilm acid production [12,32,39]. Therefore, total salivary calcium concentration alone is an incomplete indicator of remineralization potential.

This is particularly important because most caries-related studies report total calcium rather than ionized calcium activity. Total calcium includes ionized, protein-bound, and complexed fractions, whereas the free ionized fraction is more directly relevant to saturation state and mineral exchange at the enamel surface. Consequently, studies measuring total calcium may not fully capture the biologically active calcium available for enamel remineralization. This distinction may partly explain why some studies report no significant association between salivary calcium and caries indices, including Decayed, Missing, and Filled Teeth (DMFT) scores, especially in populations where fluoride exposure, diet, oral hygiene, and bacterial acid production are dominant determinants of caries risk [36].

The available evidence therefore supports the interpretation of salivary calcium as one component of the physicochemical caries-risk environment. Its value is likely greatest when assessed alongside salivary flow rate, pH, buffering capacity, phosphate, fluoride exposure, diet, plaque activity, and clinical caries indices, rather than as an isolated diagnostic marker.

6.2.2. Children, Adolescents, and Paediatric Populations

Salivary calcium has been frequently investigated in children and adolescents because caries risk is strongly influenced by developmental, dietary, behavioural, and salivary factors during this period. The eruption of primary and permanent teeth, post-eruptive enamel maturation, variable oral hygiene practices, dietary sugar exposure, and fluoride availability all influence the balance between demineralization and remineralization. These factors make paediatric populations biologically relevant but methodologically challenging for evaluating salivary calcium as an independent biomarker.

Several paediatric studies suggest that caries-active children may have lower salivary calcium concentrations than caries-free peers, but the magnitude of this difference is often modest, and overlap between groups is considerable [36]. Age, dentition stage, salivary flow, stimulation status, pH, diet, fluoride exposure, and sampling protocol are important sources of variability. Therefore, salivary calcium should not be interpreted as a stand-alone marker of caries risk in children, but rather as one parameter within a broader caries-risk assessment model.

6.2.3. Orthodontic Patients and White Spot Lesions

Fixed orthodontic treatment provides a clinically relevant model for studying salivary calcium in relation to enamel demineralization. Orthodontic appliances promote plaque retention and make oral hygiene more difficult, thereby increasing the risk of white spot lesions around brackets [59,60]. In this setting, salivary calcium may plausibly function as one component of the host-related remineralization capacity.

A prospective study by Balda et al. [61] reported that higher pretreatment salivary calcium concentrations were inversely associated with the development of active carious lesions during orthodontic treatment. This finding supports the hypothesis that adequate salivary calcium availability may contribute to enamel protection under plaque-retentive conditions. However, the evidence remains limited, and salivary calcium has not been shown to provide independent predictive value beyond established risk factors such as baseline caries experience, plaque control, fluoride exposure, salivary flow rate, pH, and buffering capacity.

Therefore, pretreatment salivary calcium measurement may be considered an exploratory risk-stratification adjunct in orthodontic patients, but not a validated predictor of white spot lesion development. Future prospective studies should evaluate calcium together with phosphate, pH, buffering capacity, fluoride exposure, plaque indices, and standardized clinical lesion assessment.

6.3. Other Oral Conditions

Beyond periodontal disease and caries, salivary calcium has been examined in a small number of additional oral conditions, though the evidence base is considerably less developed. In oral squamous cell carcinoma (OSCC), which is associated with profound alterations in salivary composition, calcium has been reported as one of numerous analytes that differ between cancer patients and healthy controls, though inorganic ions represent a relatively minor focus of OSCC biomarker research compared to nucleic acids, proteins, and metabolites [62]. The existing studies on salivary mineral profiles in OSCC are limited by small sample sizes, cross-sectional designs, and failure to control for the numerous confounders that influence salivary composition in oncological patients, including xerostomia, radiation therapy, and altered nutrition.

In conditions characterised by salivary gland dysfunction, such as Sjögren's syndrome or radiation-induced hyposalivation, salivary calcium concentrations may be altered through changes in glandular secretory capacity and electrolyte transport, rather than

through processes directly linked to disease pathogenesis [2]. Interpreting calcium values in these populations requires careful distinction between primary changes in mineral secretion and secondary effects of reduced flow.

Across these additional oral conditions, the available evidence does not support salivary calcium as a reliable or specific biomarker. Its alterations are inconsistent, mechanistically ambiguous, and have not been studied in adequately designed trials. Its potential utility, if any, is likely to be confirmatory or adjunctive rather than primary, and only within multimarker frameworks that integrate additional clinical and biochemical data.

7. Salivary Calcium in Systemic Diseases

Systemic conditions may alter salivary composition through changes in glandular secretion, hydration status, medication use, inflammation, autonomic regulation, and systemic mineral metabolism, leading to changes in electrolyte components, including calcium ions [63–65]. Although saliva contains serum-derived components and selected studies have reported correlations between serum and salivary calcium levels [66], whole saliva should not be regarded as a simple ultrafiltrate of serum or as a direct substitute for blood-based calcium assessment. Its composition is further shaped by ductal modification, salivary flow rate, pH, protein binding, gingival crevicular fluid, oral inflammation, and local mineral exchange. Accordingly, salivary calcium has been investigated as a potential non-invasive biomarker in systemic diseases beyond oral health [14,67,68], but its interpretation in these settings is complex and requires careful consideration of both systemic and local oral confounders.

7.1. Osteoporosis and Postmenopausal Bone Loss

Osteoporosis is characterized by reduced bone mass, deterioration of bone microarchitecture, and increased fracture risk [69,70]. In the context of salivary diagnostics, research has focused mainly on postmenopausal women, in whom estrogen deficiency accelerates bone loss and may also influence oral health, salivary flow, and periodontal status [67,71]. Several studies have reported higher salivary calcium concentrations in postmenopausal women with osteoporosis or lower bone mineral density (BMD), and some have described inverse associations between salivary calcium and BMD at skeletal sites such as the hip, femur, or spine [67,68,72–75]. These observations have led to the hypothesis that salivary calcium might provide a non-invasive indicator of altered systemic mineral metabolism or osteoporosis risk.

The biological interpretation of this association, however, remains uncertain. A direct causal pathway from increased bone resorption to elevated salivary calcium is not firmly established. Serum total calcium is normally maintained within a narrow physiological range through parathyroid hormone, vitamin D, calcitonin, renal handling, and skeletal buffering mechanisms. Therefore, in uncomplicated osteoporosis, systemic bone loss does not necessarily produce a proportional or sustained increase in circulating calcium that would be expected to translate directly into saliva. This limits the plausibility of interpreting salivary calcium simply as a salivary surrogate of bone resorption.

Alternative explanations are at least equally plausible. Postmenopausal women may have reduced salivary flow, medication-related xerostomia, altered pH and buffering capacity, calcium or vitamin D supplementation, changes in dietary intake, and a higher prevalence of periodontal disease or dental calculus, all of which can influence salivary calcium independently of BMD [76,77]. In particular, periodontal inflammation and gingival crevicular fluid may enrich whole saliva with serum-derived ions, while low salivary flow may increase measured calcium concentration through a concentration effect rather than

increased secretion. These factors are not consistently controlled across available studies and may partly explain the heterogeneity of reported associations.

Consequently, salivary calcium should not be interpreted as a standalone marker of osteoporosis or as a substitute for dual-energy X-ray absorptiometry (DEXA), FRAX-based risk assessment, serum calcium/phosphate, vitamin D status, parathyroid hormone, or established bone turnover markers. Its most defensible role, based on current evidence, is as a potential adjunctive parameter in exploratory screening panels, particularly when combined with age, menopausal status, oral health assessment, salivary flow rate, pH, and other biochemical markers [78]. Larger, well-controlled prospective studies are required to determine whether salivary calcium adds predictive value beyond established osteoporosis risk indicators.

7.2. Chronic Kidney Disease and Hemodialysis

Chronic kidney disease (CKD) is associated with progressive loss of renal function and broad disturbances in fluid balance, acid–base status, uremic toxin accumulation, and mineral metabolism [79,80]. CKD-related mineral and bone disorder affects calcium–phosphate balance through reduced calcitriol synthesis, secondary hyperparathyroidism, altered renal phosphate handling, and changes in bone turnover [14,80]. These systemic disturbances provide a rationale for investigating salivary electrolytes, including calcium, in patients with CKD and those undergoing hemodialysis.

Unlike osteoporosis, CKD is more directly associated with systemic biochemical disturbances that may plausibly influence salivary composition. Patients with advanced CKD frequently present with reduced salivary flow, xerostomia, altered pH, elevated salivary urea and creatinine, halitosis, calculus formation, and mucosal changes [81–83]. Several studies have reported altered salivary calcium, phosphate, sodium, potassium, albumin, urea, and creatinine concentrations in CKD or hemodialysis populations [14,81,84–90]. However, the direction and magnitude of salivary calcium changes are inconsistent.

The inconsistent findings in pre- and post-hemodialysis studies should be interpreted in light of methodological and biological heterogeneity. Hemodialysis acutely alters hydration status, serum electrolytes, acid–base balance, urea concentration, and salivary gland perfusion. Some studies have reported increased salivary flow after dialysis with reductions in urea, creatinine, chloride, and potassium, while calcium remained relatively unchanged [84,89]. Other studies observed post-dialysis decreases in salivary calcium, potassium, and pH [90]. These discrepancies may reflect differences in dialysis modality and dialysate composition, timing of sampling, residual renal function, secondary hyperparathyroidism, serum phosphate and calcium levels, medication use, fluid restriction, and whether stimulated or unstimulated saliva was collected.

From a clinical perspective, the key question is not whether salivary calcium changes in CKD, but whether it provides information beyond established renal and mineral–bone markers. At present, salivary urea and creatinine appear more directly aligned with renal clearance than salivary calcium, while serum calcium, phosphate, parathyroid hormone, vitamin D, and alkaline phosphatase remain central to the evaluation of CKD-related mineral and bone disorder. Salivary calcium may nevertheless contribute to a broader salivary panel describing oral and systemic consequences of CKD, particularly when interpreted together with salivary flow rate, pH, urea, creatinine, phosphate, and oral findings such as calculus and xerostomia. Current evidence does not support salivary calcium as an isolated monitoring marker for CKD or hemodialysis response.

These examples illustrate a broader principle relevant to systemic diseases: salivary calcium becomes clinically interpretable only when the systemic disorder has a plausible

pathway to alter oral fluid composition and when local oral and pre-analytical confounders are measured rather than assumed to be negligible.

7.3. Diabetes Mellitus

Diabetes mellitus increases oral health issues, particularly periodontal disease, which is more common when the glycemic control is poor, emphasizing the necessity for comprehensive management strategies [91,92]. It is linked to alterations in salivary composition, affecting both biochemical and immunological components, including glucose, amylase, calcium, phosphorus, immunoglobulins, total protein, and certain electrolytes [93,94]. Recent studies indicate that glycemic control is a primary determinant of these alterations, as higher HbA1c and fasting blood glucose levels are consistently associated with higher salivary glucose concentrations and poorer oral health outcomes [95,96]. Additionally, individuals with diabetes frequently exhibit reduced salivary flow and increased xerostomia, with a negative association between salivary flow rate and the severity of dry mouth symptoms [97,98].

This reduction in salivary flow appears more pronounced in patients with poor glycemic control, as decreased salivary flow has also been reported in individuals with uncontrolled glucose levels and higher HbA1c values [95]. Reduced salivary secretion in diabetes may result from dehydration due to hyperglycemia, as well as glandular, vascular, and neural alterations [97,99].

These mechanisms can significantly alter salivary electrolyte concentrations and complicate the interpretation of laboratory results. Particular attention should be directed toward salivary calcium, as findings remain inconsistent across studies. Some studies have reported significantly elevated salivary calcium levels in diabetic patients compared to healthy individuals [94,100], while others found no significant elevation [93]. Conversely, lower salivary calcium and magnesium levels have been observed in diabetic patients with periodontitis, whereas greater periodontal disease severity was associated with lower magnesium levels and higher salivary calcium levels [55].

This heterogeneity suggests that salivary calcium concentrations are influenced by multiple interacting factors beyond diabetes alone. Therefore, salivary calcium levels in diabetic patients should be interpreted in the context of glycemic control, salivary flow rate, hydration status, and periodontal condition, rather than as an isolated biochemical measure.

7.4. Other Systemic Conditions

Research has shown inconsistent changes in saliva among hypertensive patients, with some studies indicating an increase [101] and others reporting a decrease in salivary calcium levels [55,63]. Biochemical analyses of saliva in cystic fibrosis (CF) have consistently reported alterations in electrolyte composition, most prominently elevated chloride and sodium concentrations, together with a reduced salivary flow rate [102,103]. Findings for salivary calcium are less consistent: several studies have reported increased calcium in gland-specific and whole saliva [104], whereas others found no significant difference between CF patients and controls [105]. Reflecting this variability, a systematic review concluded that salivary electrolyte changes in CF are driven chiefly by chloride and sodium, while calcium and phosphate findings remain inconsistent across studies [106]. Overall, these observations position salivary calcium as a non-specific and unreliable indicator in CF, consistent with the broader theme of this review.

Overall, the evidence from hypertension, cystic fibrosis, and other systemic conditions remains too sparse and inconsistent to support disease-specific interpretation of salivary calcium outside exploratory multimarker settings.

A structured summary of reported salivary calcium changes across oral and systemic conditions is presented in Table 3. Because most available studies are cross-sectional, use different saliva collection protocols, and measure predominantly total rather than ionized calcium, the table emphasizes clinical interpretation rather than diagnostic performance. Where the calcium fraction was not consistently specified across studies, this is indicated as a limitation. The aim is not to establish disease-specific thresholds, but to clarify the direction, consistency, and interpretive constraints of the available evidence.

Table 3. Clinical interpretation of reported salivary calcium changes across oral and systemic conditions.

Condition	Reported Salivary Calcium Pattern	Biological Interpretation	Main Limitations	Clinical Interpretation
Periodontitis	Often increased	May reflect calculus-forming tendency, GCF contribution, inflammation-related changes	Mostly cross-sectional; confounding by smoking, diabetes, oral hygiene	Possible adjunctive marker, not diagnostic alone
Gingivitis	Inconsistent or intermediate values	Mild inflammation may not produce stable calcium shift	Limited evidence	Low current utility
Dental caries	Heterogeneous; sometimes lower in caries-active individuals	Lower calcium may reduce remineralization capacity	Strong confounding by pH, fluoride, diet, flow rate	Use only with pH, flow, phosphate, caries-risk profile
Orthodontic white spot lesions	Higher pretreatment calcium may be protective	Better remineralization potential	Limited prospective evidence	Potential risk-stratification marker
Osteoporosis/postmenopausal bone loss	Often increased	Association biologically uncertain; may reflect salivary flow, xerostomia, periodontal status, supplementation, or oral confounders rather than bone resorption itself.	Small studies; limited predictive value alone	Exploratory adjunct only; not a substitute for DEXA, FRAX, serum mineral markers, or bone turnover markers.
CKD/hemodialysis	Altered; often increased but variable after dialysis	Reflects renal mineral-bone disorder, salivary gland changes, dialysis effects	Complex systemic confounding	Possible component of renal salivary panels; not supported as an isolated CKD or hemodialysis monitoring marker
Diabetes mellitus	Inconsistent; increased, reduced, or unchanged	Influenced by glycemic control, xerostomia, periodontal disease, hydration	Strong confounding by periodontal status and flow rate	Not useful alone
Other conditions	Variable	Disease-specific mechanisms unclear	Sparse evidence	Research only

Across conditions, the evidence suggests that salivary calcium rarely functions as a disease-specific signal. Increased calcium may indicate a mineralizing oral environment in periodontitis, enhanced remineralization potential in caries-related contexts, or altered electrolyte balance in systemic disease. Conversely, reduced calcium may indicate impaired remineralization capacity, altered glandular secretion, or dilution/flow-related effects. This bidirectional and context-dependent meaning supports the interpretation of salivary calcium as a component of multivariable salivary panels rather than as a standalone diagnostic biomarker.

8. Critical Appraisal of the Available Evidence

The body of literature on salivary calcium as a biomarker is extensive in its breadth but limited in its depth. Across oral and systemic conditions, studies have consistently demonstrated that salivary calcium concentrations differ between patient groups and controls—yet the clinical meaning of these differences remains difficult to establish. A rigorous appraisal of the available evidence reveals recurring methodological and interpretive shortcomings that substantially constrain the conclusions that can be drawn.

8.1. Study Design Limitations

The overwhelming majority of studies examining salivary calcium in disease are cross-sectional in design. While cross-sectional studies are appropriate for generating hypotheses and describing prevalence, they are fundamentally limited in their capacity to establish causality, directionality, or the prognostic utility of a biomarker [6,7]. In the context of salivary calcium, this is a particularly consequential limitation: it remains unknown, for nearly all conditions studied, whether the observed changes in calcium concentration precede disease onset, reflect active disease, or arise as secondary consequences of treatment, medication, or altered oral function. Sample sizes across the salivary calcium literature are frequently small, often below fifty participants per group, and many studies recruit from single clinical centers, limiting the representativeness of findings. With small samples, the risk of type I and type II errors is substantial, and statistically significant differences may reflect sampling variability rather than genuine biological signals. The absence of power calculations in many published studies further undermines confidence in their conclusions. Longitudinal cohort studies, the design most suited to evaluating whether salivary calcium predicts disease onset or progression, remain rare, and the few that exist are limited by short follow-up periods and modest sample sizes [36,58].

8.2. Population Heterogeneity

A recurring obstacle to the synthesis of salivary calcium data across studies is the marked heterogeneity of study populations. Salivary composition is influenced by a wide range of individual characteristics, including age, sex, hormonal status, body mass index, hydration, smoking, medications, and oral health status, all of which can independently alter calcium concentrations and confound the relationship between calcium and the disease of interest [2,8]. Many published studies neither stratify nor adequately control for these variables, making it impossible to determine whether observed differences in salivary calcium reflect the target condition or one of many unmeasured confounders. This problem is compounded in populations with systemic comorbidities. Studies of salivary calcium in patients with diabetes, chronic kidney disease, or osteoporosis frequently enroll participants who are concurrently taking medications, antidiabetics, calcium channel blockers, bisphosphonates, or diuretics, that may independently alter glandular secretion or systemic mineral balance [2]. Similarly, the presence of periodontal disease as a comorbidity in studies primarily designed to examine a systemic condition, or vice versa, introduces additional sources of variation that are rarely accounted for in the analysis. The result is a literature in which findings from different populations are frequently incomparable, and in which effect sizes are difficult to interpret in isolation.

8.3. Lack of Standardized Sampling and Reporting

One of the most consistent criticisms in systematic reviews of salivary biomarker research is the absence of standardised protocols for sample collection, processing, and reporting [8,36]. In the salivary calcium literature specifically, studies vary widely in whether they collect stimulated or unstimulated saliva, the time of day at which samples

are obtained, the fasting interval required before collection, the centrifugation protocol applied, and the method used to measure calcium. Each of these variables can meaningfully influence the result, yet few studies describe their protocols with sufficient detail to allow replication.

The distinction between stimulated and unstimulated saliva is particularly important: unstimulated whole saliva better reflects resting conditions and is more clinically accessible, but it is also more susceptible to contamination and compositional variability, while stimulated saliva, typically of higher volume and more consistent composition, may not capture pathological changes in resting glandular function. Without explicit specification and consistent application of collection type, pooling data across studies or comparing absolute calcium values is methodologically unsound [8,35]. Reference intervals for salivary calcium have not been established through adequately powered, demographically representative studies, and in the absence of such data, the categorisation of individual values as “elevated” or “reduced” rests on highly variable comparator groups.

8.4. Inconsistent Interpretation of Elevated or Reduced Levels

Perhaps the most striking feature of the salivary calcium literature is that the same directional finding, elevated salivary calcium, can be interpreted in diametrically opposite ways depending on the clinical context in which it is reported. In the context of dental caries, elevated salivary calcium has been interpreted as a protective factor, reflecting a mineral-rich environment that favours remineralisation and resists enamel dissolution [11,58]. In the context of periodontal disease, the same elevation has been interpreted as a risk indicator, reflecting supersaturation conducive to calculus formation and plaque mineralisation [33,36]. In the context of osteoporosis, elevated salivary calcium has been interpreted as a consequence of accelerated bone resorption, releasing mineral into the systemic circulation and thereby increasing salivary calcium as an ultrafiltrate of serum [67]. These interpretations are not mutually exclusive, and in individual patients with comorbid conditions, more than one mechanism may operate simultaneously, but the fact that a single finding can carry such disparate biological meanings fundamentally limits its diagnostic specificity of salivary calcium as a standalone marker.

This interpretive ambiguity extends to reduced calcium values. Low salivary calcium may reflect diminished glandular secretory capacity, reduced dietary calcium intake, altered salivary flow, or changes in protein binding, any of which may or may not be clinically relevant depending on the condition under study. Without a clearly defined biological model that specifies which mechanism is expected to dominate in a given disease context, and without biomarker panels that provide complementary information, the interpretation of isolated calcium measurements remains speculative. This observation is not unique to calcium; it reflects a broader limitation of single-analyte salivary biomarker approaches, but it is particularly pronounced for a physiologically ubiquitous ion whose concentration is shaped by so many interacting factors [6,7]. This context-dependent and bidirectional interpretation supports the conclusion that salivary calcium should be interpreted as part of multivariable salivary panels rather than as a unidirectional disease marker.

9. Advantages and Limitations of Salivary Calcium as a Biomarker

9.1. Advantages

Salivary calcium possesses several attributes that make it an appealing candidate biomarker, particularly in the context of oral health monitoring. Its collection is non-invasive, painless, and repeatable without patient burden, characteristics that are especially valuable in longitudinal studies, paediatric populations, and point-of-care settings where venepuncture is impractical [5,6]. The analytical methods available for its quantification

span a wide range of complexity and cost, from simple colorimetric assays suitable for routine laboratory use to high-sensitivity ICP-MS platforms capable of multi-elemental profiling from small sample volumes [36,43]. This analytical accessibility, combined with the emerging availability of paper-based microanalytical devices, positions salivary calcium as a realistic component of future point-of-care or chairside diagnostic workflows [42]. From a biological standpoint, salivary calcium has a well-characterised physiological role in oral mineral homeostasis—its involvement in enamel remineralisation, dental plaque mineralisation, and calculus formation provides a clear mechanistic rationale for its investigation across a range of oral conditions [32,33]. Furthermore, its potential as a non-invasive proxy for systemic mineral metabolism, particularly in conditions such as osteoporosis and chronic kidney disease where calcium homeostasis is disrupted, aligns with the broader goals of salivary diagnostics as a complement to blood-based monitoring [13,14].

9.2. Limitations

Against these advantages must be weighed a set of fundamental limitations that currently preclude salivary calcium from serving as a reliable standalone diagnostic or prognostic marker. Its most significant weakness is biological non-specificity: salivary calcium concentration reflects the net outcome of multiple simultaneous processes, glandular secretion, protein binding, pH-dependent speciation, flow rate, and gingival crevicular fluid contribution, none of which is disease-specific [6,15]. The same measured concentration may arise from entirely different biological contexts, rendering interpretation highly dependent on clinical information that is rarely incorporated into study designs. Taken together, these limitations mean that salivary calcium as an isolated analyte is unlikely to meet the criteria for a robust clinical diagnostic test.

9.3. Salivary Calcium in Relation to Established Biomarkers

The clinical interpretation of salivary calcium should be considered in relation to established diagnostic and biomarker frameworks rather than in isolation. In periodontal disease, several salivary biomarkers, including matrix metalloproteinase-8, interleukin-1 β , interleukin-6, tumour necrosis factor- α , and microbial markers, more directly reflect inflammatory tissue destruction and dysbiosis. Salivary calcium does not capture these inflammatory pathways directly, but may provide complementary information on mineralization tendency, calculus formation, and the physicochemical environment that favours plaque retention.

In dental caries, salivary calcium should be interpreted alongside established clinical and biochemical indicators of caries risk, including salivary flow rate, pH, buffering capacity, phosphate, fluoride exposure, dietary sugar intake, plaque accumulation, and caries indices such as DMFT or ICDAS. In this setting, calcium is most relevant as part of the mineral saturation system rather than as an independent marker of caries activity.

In systemic diseases, the limitations are even more pronounced. In osteoporosis, dual-energy X-ray absorptiometry, FRAX-based fracture risk assessment, serum calcium and phosphate, vitamin D, parathyroid hormone, and bone turnover markers remain more clinically established than salivary calcium. In chronic kidney disease, serum creatinine, estimated glomerular filtration rate, urea, calcium, phosphate, parathyroid hormone, vitamin D, and alkaline phosphatase remain central to diagnosis and monitoring. Salivary calcium currently has no evidence-based role as a replacement for these established markers. Its potential value lies in non-invasive, repeated sampling and in integration with broader salivary panels that include flow rate, pH, phosphate, urea, creatinine, inflammatory markers, and oral clinical findings.

Therefore, the most realistic clinical role of salivary calcium is not as a standalone diagnostic test, but as a context-dependent adjunct that may improve interpretation when combined with disease-specific biomarkers and standardized clinical assessment. Therefore, the most realistic clinical role of salivary calcium is not as a standalone diagnostic test, but as a context-dependent adjunct that may improve interpretation when combined with disease-specific biomarkers and standardized clinical assessment.

10. Clinical Relevance and Future Perspectives

10.1. Current Clinical Applicability

The available evidence is dominated by cross-sectional studies with small, heterogeneous samples, inconsistent protocols, and contradictory findings, a combination that precludes the establishment of validated reference intervals, clinically meaningful cut-off values, or robust sensitivity and specificity estimates for any specific condition. Its present utility is primarily investigative: salivary calcium contributes to the characterisation of oral mineral environments in research settings and may serve as an adjunctive parameter in studies examining mineralogenic risk or salivary gland function. Clinicians seeking a non-invasive screening tool for conditions such as osteoporosis or chronic kidney disease should exercise caution in interpreting salivary calcium in isolation, pending further validation.

10.2. Need for Protocol Harmonization

Advancing the clinical relevance of salivary calcium will require, as a prerequisite, the adoption of standardised and widely agreed-upon protocols for sample collection, processing, and analysis. Future studies should specify, and adhere to, clearly defined pre-collection fasting and oral hygiene intervals, time of day, collection method (stimulated versus unstimulated), centrifugation parameters, and storage conditions [8,36]. Equally important is consensus on whether total or ionised calcium should serve as the primary outcome measure, given that these fractions differ in their biological relevance and analytical requirements [39,40]. Without harmonised reporting, the aggregation of data across studies, and the development of population-level reference values, will remain unachievable.

10.3. Directions for Future Research

The most pressing research priorities in this field are longitudinal study designs and adequately powered cohorts. Prospective studies following participants over time, ideally from health to disease, are needed to determine whether salivary calcium changes precede clinical manifestations and whether it can predict disease onset or progression. Cohorts should be large enough to permit stratification by age, sex, medication use, and comorbidity status, and should incorporate concurrent measurement of complementary salivary parameters to enable multivariate modelling. The distinction between total and ionised calcium should be explicitly addressed, and studies should report secretory rates alongside concentrations to separate dilution effects from genuine changes in glandular output [35].

10.4. Potential Role in Multimarker and Chairside Approaches

Looking ahead, salivary calcium is most likely to find a clinical role as a component of integrated diagnostic panels, rather than as a sole analyte. The development of chairside and point-of-care platforms capable of simultaneously quantifying multiple salivary parameters, including calcium alongside inflammatory cytokines, pH, and microbial biomarkers, would substantially increase the clinical information obtainable from a single non-invasive sample [6,42,43]. Such multimarker approaches, informed by machine learning-based data integration, represent the most plausible trajectory toward a diagnostically actionable role

for salivary calcium, not as a primary signal, but as one informative element within a richer biological profile.

11. Conclusions

Salivary calcium is a biologically meaningful but clinically context-dependent biomarker. Its central role in enamel mineral balance, remineralization, and calculus formation provides a strong rationale for investigation in oral diseases, while systemic conditions may influence its concentration through combined effects on glandular secretion, hydration, inflammation, medication use, mineral metabolism, and oral health. However, current evidence does not support salivary calcium as a standalone diagnostic marker.

Across periodontal disease, dental caries, osteoporosis, chronic kidney disease, hemodialysis, and diabetes, reported changes in salivary calcium are often statistically detectable but mechanistically non-specific. The same directional change may have different implications depending on the clinical setting: higher calcium may support remineralization in caries-related contexts, indicate mineralization tendency and calculus formation in periodontal disease, or reflect mixed systemic and oral influences in systemic disease. Interpretation therefore requires concurrent assessment of salivary flow rate, pH, phosphate, calcium fraction measured, oral inflammatory status, and relevant systemic biomarkers.

Future progress depends on standardized saliva collection and processing protocols, explicit reporting of total versus ionized calcium, analytical validation in the salivary matrix, and adequately powered longitudinal studies. The most realistic clinical role of salivary calcium is as one component of multivariable salivary biomarker panels rather than as an isolated diagnostic test.

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