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# Decentralized vitamin C & D dual biosensor chip: Toward personalized immune system support

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ABSTRACT

Combating the ongoing COVID-19 pandemic has put the spotlight on nutritional support of the immune system through consumption of vitamins C and D. Accordingly, there are urgent demands for an effective on-the-spot multi-vitamin self-testing platform that monitors the levels of these immune-supporting micronutrients for guiding precision nutrition recommendations. Herein, we present a compact bioelectronic dual sensor chip aimed at frequent on-the-spot simultaneous monitoring of the salivary vitamin C and D dynamics. The new bioelectronic chip combines a new electrocatalytic vitamin C amperometric assay along with competitive vitamin D immunoassay on neighboring electrodes, to perform selective and cross-talk free detection of both vitamins in a 10-μL saliva sample within 25 min. The distinct vitamin C or D temporal profiles obtained for different individuals after vitamin supplementation indicate the potential of the new bioelectronic chip strategy for enhancing personalized nutrition towards guiding dietary interventions to meet individual nutrition needs and promote immune system health.

## 1. Introduction

Major organizations, such as the Academy of Nutrition and Dietetics and the American Nutrition Association, have recognized the importance of personalized nutrition to support physical, mental, and emotional resilience, as well as the need for further research to better understand the impact of individual variability on the development of possible personalized approaches toward improved public health and wellness (Bush et al., 2020; Rozga et al., 2020). Personalized (or precision) nutrition, which aims at the assessment of individual nutritional status regarding intake or metabolism of dietary constituents, requires tight nutrition-related biomarker monitoring in body fluids. Such monitoring is essential to get direct feedback and guidance for supporting dietary behavior change (Picó et al., 2019). Precision nutrition can be used to tailor nutrient intake based on the individual metabolic system to ensure a balanced diet, and to prevent diseases (Ates et al., 2021; Micha et al., 2017; Picó et al., 2019). With the increasing emphasis on precision nutrition there are growing demands for rapid reliable low-cost home self-testing to guide dietary and behavioral changes (Semptionato et al., 2021; Yu et al., 2020). In this sense, electrochemical sensors play the leading role in transferring biosensing approaches to portable meters for tracking nutrition in decentralized settings, reflecting their distinct advantages such as speed, inherent miniaturization, scalable fabrication, low cost and low power requirements (Semptionato et al., 2021). The rapid data acquisition associated with such frequent nutrition measurements can be coupled with advanced analytical tools, such as machine learning, and related simulations to support timely predictions necessary to implement precision nutrition (Verma et al., 2018). The absence of effective drug treatments and limited access to vaccines for the ongoing crisis of the COVID-19 pandemic have further emphasized the need for well-balanced nutrition to support the immune system for ensuring protection against harmful substances and pathogens, while reducing the risk of chronic or infectious diseases (Ayseli et al., 2020; Calder et al., 2020; Chowdhury et al., 2020; Gombart et al., 2020; Iddir et al., 2020; Im et al., 2020; Jayawardena et al., 2020). In this regard, the intake of vitamins C (VC) and D (VD) has been particularly encouraged, since their benefits have been closely associated with the improvement of

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antioxidant status, limitation of oxidative stress, regulation of immune functions, and reduction of viral replication (Aranow, 2011; Beard et al., 2011; Carr and Maggini, 2017; Im et al., 2020; Maggini et al., 2017; Padayatty et al., 2003). In fact, there is a growing literature associating low body levels of VC and VD with relative immune deficit, higher risk of infection, and unfavorable outcomes during viral infections (Arvinte et al., 2020; Meltzer et al., 2020; Name et al., 2020; Patterson et al., 2021; Rondanelli et al., 2018). Accordingly, acute supplementation of these micronutrients to high-risk patients or those diagnosed with COVID-19 has been used as medical approach to boost the immune defense (Abobaker et al., 2020; Ebadi and Montano-Loza, 2020; Hoang et al., 2020; Murdaca et al., 2020). VC is the most common vitamin associated with prevention and alleviation of viral infections (Padayatty et al., 2003). It can reduce the inflammatory mediator cascade manifested during COVID-19 infection (Feyaerts and Luyten, 2020). VD has received tremendous recent attention and has been established as a key player in the function of the immune system by improving the physical barrier against viruses and stimulating the production of antimicrobial peptides (Bae and Kim, 2020; Jolliffe et al., 2021; Rhodes et al., 2021). However, despite the substantial demands for vitamin-fortified products and supplements, there are only general dietary guidelines and medical recommendations for their proper and personal consumption.

Considering the importance and need of maintaining balanced levels of VC and VD, in general, there are urgent demands for developing effective bioelectronic sensors for their rapid and reliable self-monitoring in decentralized settings. Recently, efforts related to decentralized multi-marker self-monitoring have led to the development of a dual-analyte biosensor platform for simultaneous detection of key diabetes biomarkers in undiluted body fluids (Vargas et al., 2019; Vargas et al., 2020). Several electrochemical and optical devices for individual measurements of VC or VD have been reported (see Table S1 in Supplementary Information), yet the challenge of single-device simultaneous VC-VD has not been addressed. Among the electrochemical approaches, these include flexible wearable and point-of-care enzymatic amperometric sensors for dynamic changes of VC in sweat, tears, saliva, urine, or blood (Sempionatto et al., 2020; Zhao et al., 2021). Similarly, electrochemical VD immunosensors were recently described based on electrodes modified with different nanomaterials (Carlucci et al., 2013; Chauhan et al., 2018; Chauhan et al., 2019; Kaur et al., 2020; Sarkar et al., 2018), with only one study involving an assay of body fluid (serum) (Kaur et al., 2020). The sophistication of transduction has limited the use of optical approaches for direct VD detection in biological fluids (Prante et al., 2019; Di Meo et al., 2020), with only few examples described smartphone-assisted optical approaches applied solely in diluted (Walter et al., 2020) or filtered serum samples (Lee et al., 2014). Therefore, the development of integrated bioelectronic devices for reliable and rapid decentralized multiplexed VC and VD assessment in body fluids remains an unmet challenge. A selective and reliable dual marker sensor system, capable of conveying timely information to the consumer or patient by concurrently monitoring both vitamins, is essential to address VC and VD deficiencies while optimizing nutrition care for preventing illness and improved recovery. Such simultaneous self-testing would benefit from the use of non-invasive biofluids, such as saliva, and provide convenience for the user.

This paper reports the first disposable VC-VD bioelectronic sensor chip capable of fast on-the-spot simultaneous vitamin detection in a 10-μL saliva sample in less than 25 min. This VC-VD strip detection system was realized by integrating different (electrocatalytic and immunoassay) detection principles on a single chip platform (Fig. 1). The rational chip design and optimized dual sensing protocol resulted in reliable and rapid simultaneous detection of VC and VD in a single saliva sample droplet, with no apparent cross-talks between the neighboring electrocatalytic and bioaffinity sensors. This novel dual sensing approach combines in a single device and protocol the simultaneous

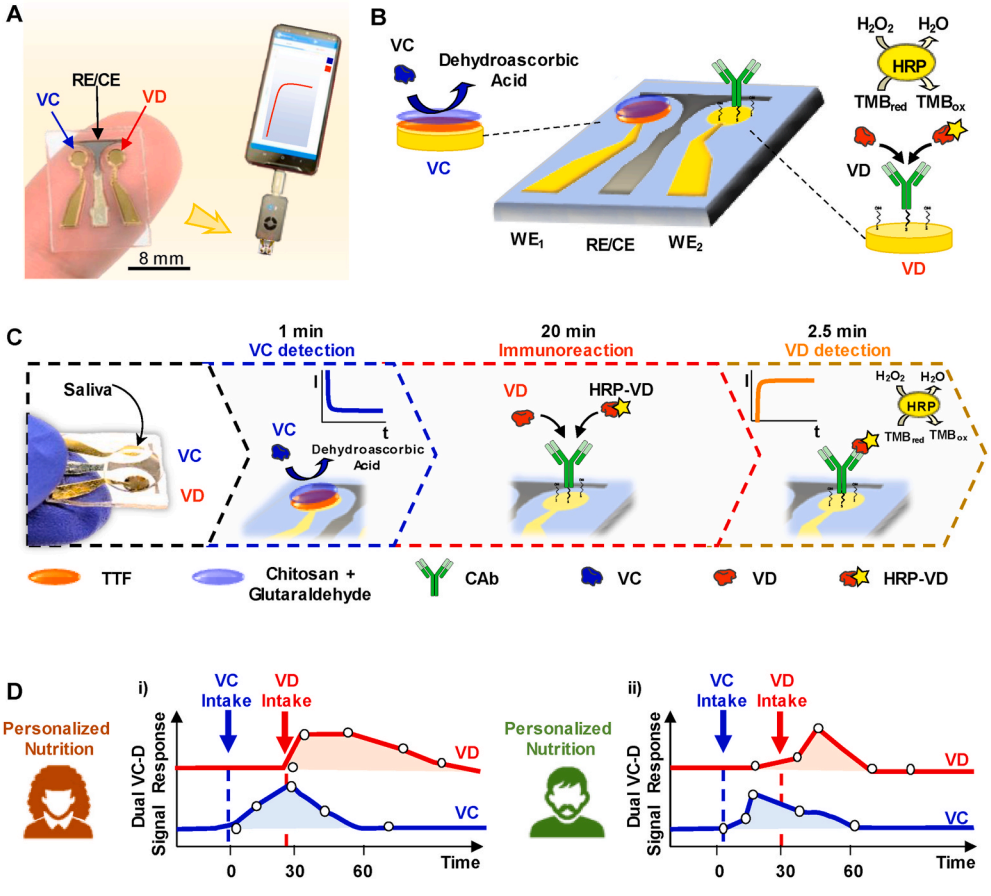


**Fig. 1. The concept of VC-VD Dual Chip for personalized nutrition monitoring. A)** Images of the lab made sputtered chip consisting of two Au-sputtered electrodes, employed as working electrodes (WE), and an Ag/AgCl electrode acting as a joint reference/counter (RE/CE), along with the digital monitoring. **B)** Schematic diagram of the localized VC and VD detection on a single sensor chip, showing the immobilized TTF and anti-VD antibody for direct competitive immunoassay allowing selective VC and VD detection, respectively. **C)** Analysis protocol: VC is first measured (+0.1 V, 60 s), followed by 20 min incubation of the sample supplemented with the HRP-labeled VD and electrochemical detection by chronoamperometry (−0.1 V, 2.5 min) using the $H_2O_2$/HRP/TMB redox probe. **D)** The conceptual idea of interindividual variability in responding to VC and VD micronutrients administration: distinct VC and VD temporal variation profiles for two different subjects (i, ii).

detection of both vitamins, simplifying dramatically the operation and instrumentation involved in existing individual VC and VD assays. Ascorbic acid (VC) and 25-Hydroxyvitamin D3 (VD) were selected in this work as well-established markers to evaluate the dynamic profiles of these vitamins (Heureux, 2017). The main goal of this work focused on the successful monitoring of VC-VD physiological changes in undiluted saliva, in order to establish personalized temporal profiles after intake of dietary supplements. The distinct temporal profiles recorded for different subjects illustrate the interpersonal variability based on metabolism, background, and environmental exposure. By replacing existing lab-based blood vitamin assays, and corresponding sample-to-answer time delays, the new biochip provides attractive on-the-spot assessment of dose-response temporal vitamin profiles. Such timely assessment of the nutrition status can facilitate personalized recommendations and interventions to meet individual nutrition needs and enable optimal nutrition status towards enhanced immune system health.

## 2. Material and methods

### 2.1. Equipment and instruments

All electrochemical characterization and data acquisition was performed with electrochemical EmStat3 Blue potentiostat (Palmsens, The Netherlands) controlled by PSTrace software provided by PalmSens. A Barnstead Thermolyne (Type 16,700 Mixer) vortex for homogenization of the solutions, pH/ISE meter (Orion Star A214 model) and IEC CL31R Multispeed centrifuge from Thermo-Scientific were used.

### 2.2. Chemicals and reagents

All the reagents used were of the highest available grade. Commercial phosphate buffer solution (1.0M), potassium chloride (KCl), 11-mercaptoundecanoic acid (MUA), 6-mercapto-1-hexanol (MCH), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), ethanolamine hydrochloride (≥%99), 2-(N-morpholino)ethanesulfonic acid (MES) and sodium dodecyl sulfate (SDS), tetrathiafulvalene, glutaraldehyde solution (50%), low molecular weight chitosan, acetone, uric acid, L (+)-ascorbic acid (VC), acetaminophen, 25-Hydroxyvitamin D3 (VD), retinol (vitamin A), thiamine hydrochloride (B1), riboflavin (B2), pyridoxine hydrochloride (B6) and vitamin $B_{12}$ were obtained from Sigma-Aldrich. HPLC grade 2-propanol, sodium hydroxide (NaOH) and Tween-20 were acquired from Fisher Scientific. Pure ethanol was purchased from KOPTEC. 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was obtained from Neogen (Enhanced KBlue TMB Substrate, Neogen Life Sciences, USA). Recombinant human IgG1 25-OH vitamin D2&D3 antibody (D23HC, V00202) (CAb) and 25-OH vitamin D3-HRP Conjugate (T00102) (HRP-VD) were purchased from GenScript.

Details about the solutions preparation, thin film Au-sputtered chips fabrication, VD immunostrip fabrication and immunoasay protocols, VC-VD Dual chip fabrication and analysis protocols, human saliva samples and commercial vitamins table samples used are described in detail in Supporting information.

## 3. Results and discussion

### 3.1. Simultaneous vitamin C-D dual biosensor chip: design and operation

The realization of a compact, low-cost and user-friendly dual bioelectronic microchip with integrated catalytic and immunochemical sensing approaches enabling fast, on-the-spot simultaneous detection of VC and VD, performed in a 10-μL saliva sample droplet, requires careful attention to fabrication, operational and detection challenges. These challenges include: i) the distinctive surface chemistries requirements of catalytic and the immunoassay-based sensors; ii) the distinctly different principles and time scales of the 2 assays; iii) the largely different physiological concentrations of VC (μM) and VD (nM), and their distinct pharmacological dynamics, and related sensitivity, selectivity and speed requirements; iv) the requirements for reproducibility and miniaturization of a sensor platform capable of multiplexed detection in a 10-μL saliva sample; v) and potential cross-talks between the adjacent sensors. These challenges have been successfully addressed by judicious optimization of the fabrication and analytical protocols. The working principle of the resulting dual VC-VD sensor chip is illustrated in Fig. 1. The metal-sputtered bioelectronic chip consists of two Au working electrode (WE) transducers for detecting VC and VD analytes, along with a central joint Ag/AgCl reference/counter electrode (RE/CE) (Fig. 1A). The specific VC and VD assays rely on different surface modification strategies of the 2 neighboring gold transducers, and involve precise spatial separation of the neighboring electrodes and use of ultra-low reagent volumes. The VC electrode fabrication involved the modification of the $WE_1$ surface with the electron transfer-mediator TTF, confined within a chitosan and glutaraldehyde film for highly selective enzyme-free VC detection. The VD electrode fabrication involved the immobilization of a VD-specific capture antibody (CAb) onto $WE_2$ through covalent attachment to the carboxylic groups of the 11-mercaptoundenoid acid (MUA)/6-mercapto-1-hexanol (MCH) mixed self-assembled monolayer (SAM) for VD molecule recognition (Fig. 1B). Lastly, the dual monitoring of both vitamins in a single saliva sample drop was performed through convenient temporal separation of the transduction steps toward cross-talk free measurements and VD immunoreaction accomplishment. Fig. 1C illustrates the sequential analysis protocol applied, including the different recognition reactions and electrochemical reactions involved in the simultaneous detection of VC and D in the same saliva sample within 25 min. For this, a 10 μL-drop of saliva containing HRP-tagged VD (HRP-VD) was placed on the sensor chip covering the entire electrode area. VC was measured amperometrically at +0.1 V on $WE_1$ by direct oxidation using TTF as catalyst within the first 60 s, followed by an additional 20 min incubation for the competition reaction of the target VD and the HRP-VD for the binding sites of the CAb immobilized on the $WE_2$ surface. The subsequent chronoamperometric readout (−0.1 V, 2.5 min), via $H_2O_2$/HRP/TMB redox probe, produced a cathodic current corresponding to the HRP-catalyzed reduction of $H_2O_2$, which is inversely proportional to the VD concentration in the sample. Such simple protocol ensures frequent and accurate simultaneous tracking of vitamins C and D levels in the same saliva droplet, towards establishing personal vitamin metabolism profiles and formulating personalized dietary interventions that meet individual nutritional needs (Fig. 1D).

### 3.2. Vitamin D immunostrip: analytical performance

In order to ensure the successful implementation of the VD biosensor for reliably tracking the dynamically-changing salivary status of this key nutrition marker, we initially tested and developed the immunoassay for VD detection using a single analyte chip design shown in Fig. 2A. This VD immunosensor consisted of two Au-sputtered electrodes operating as the working (WE) and joint reference/counter (RE/CE) electrodes. Factors affecting the performance of the VD immunostrip were systematically optimized, with most favorable conditions obtained using an antibody concentration of 50 μg $mL^{-1}$, immobilization time of 60min, a HRP-VD concentration of 200 ng $mL^{-1}$, and a competition reaction time of 20 min (Fig. S1; Table S2).

The sensing performance of the resulting immunostrip was demonstrated for detecting 50 ng $mL^{-1}$ VD increments in PBT buffer (0.1 M phosphate buffer solution supplemented with 0.02% Tween-20 (PBT), pH 7.4) (Fig. 2B) and saliva (Fig. 2C). Low VD concentration changes resulted in well-defined current signals along with highly linear calibration plots (shown in the corresponding insets; $R > 0.99$). The limits of detection (LODs) of 29 ng $mL^{-1}$ (290 pg/1.20x$10^{-2}$ IU) and 12 ng $mL^{-1}$ (120 pg/4.80x$10^{-3}$ IU) estimated in PBT buffer and saliva, respectively (Table S3), indicate the suitability of this VD biosensing approach for

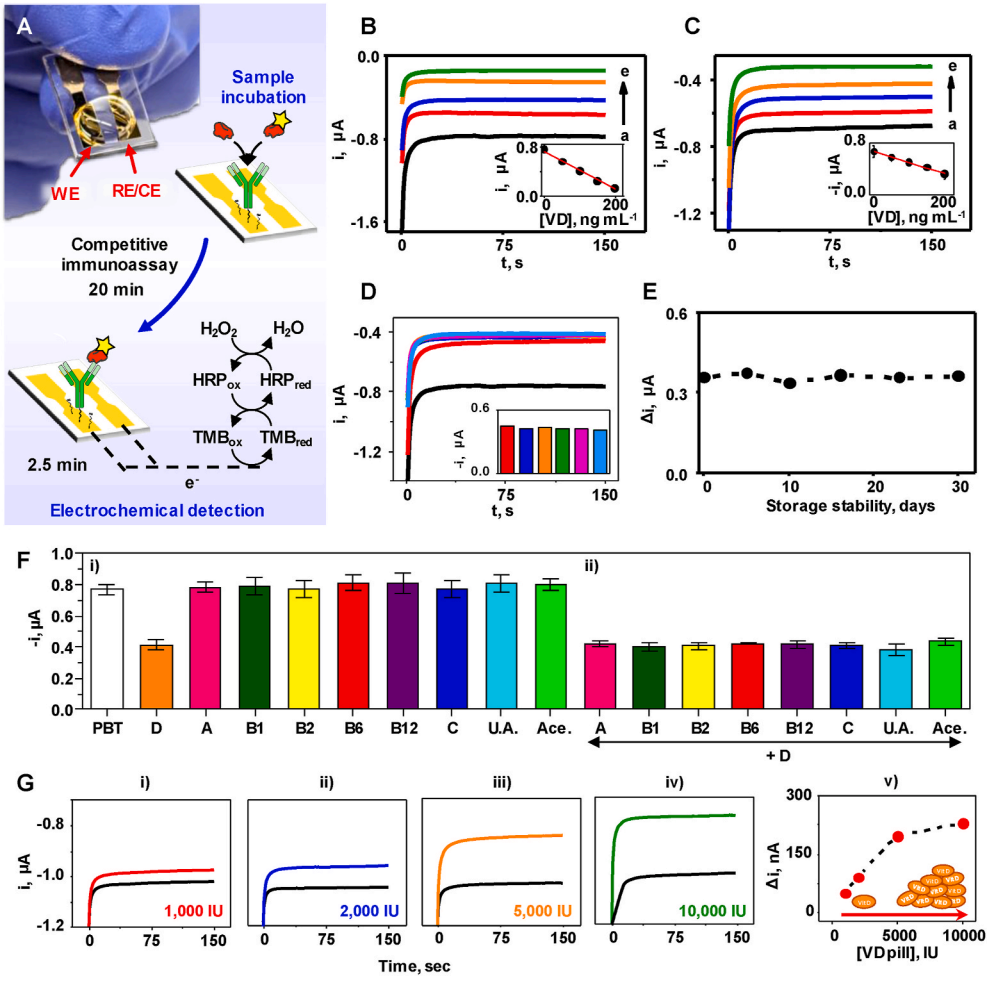


**Fig. 2. VD electrochemical immunostrip characterization. A)** Homemade sputtered sensor platform for single determination consisting of two Au-sputtered electrodes, employed as working (WE) and reference/counter (RE/CE) electrodes, respectively, along with the immunoreaction and transduction processes involved in the VD sensing. Chronoamperometric responses of the immunosensor to increasing VD concentrations from 0 (a) to 200 ng $mL^{-1}$ (e) in 50 ng $mL^{-1}$ increments in buffer **B)** or saliva **C). D)** Reproducibility of the immunoassay for 100 ng $mL^{-1}$ VD standards. **D)** Storage stability at 4 °C of the VD Immunostrip: monitoring of the resulting variation of intensity between the electrochemical responses obtained for 0 and 100 ng $mL^{-1}$ of VD over a 30-day period after its fabrication. **F)** Immunosensor responses in the analysis of PBT (white bar), 100 ng $mL^{-1}$ VD (orange bar), and 500 μM of each interferent in the absence (i) or presence of 100 ng $mL^{-1}$ VD (ii): vitamins A (pink bars), B1 (dark green bars), B2 (yellow bars), B6 (red bars), B12 (purple bars), uric acid (U.A.) (light blue bars) and acetaminophen (Ace.) (light green bars). **G)** Amperometric responses of the VD immunoplatform in the analysis of saliva samples collected before (black lines) and 30 min after taking i) 1000 (red line); ii) 2000 (blue line); iii) 5000 (orange line) or iv) 10,000 IU (green line) of VD pill (as 25-Hydroxyvitamin D3) and v) the corresponding variations of intensity. Dots and bars represent the mean and the error bars represent the standard deviation of the measurements performed with three different chips in B, C and F. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

saliva monitoring (Costantini et al., 2020; Hussein et al., 2020; Sari et al., 2021a; Sari et al., 2021b). The high sensitivity recorded for the saliva matrix reflects the effectivity of the mixed SAM-based surface chemistry used in the immunosensor preparation, which minimizes non-specific adsorption effects and related electrode biofouling in this complex biological fluid (Campuzano et al., 2019; Gooding et al., 2003). It is also worth highlighting that, although several electrochemical immunoplatforms have been described for detecting VD (Carlucci et al., 2013; Chauhan et al., 2018; Chauhan et al., 2019; Kaur et al., 2020; Sarkar et al., 2018), the present study is the first work reporting the direct VD detection in undiluted human saliva samples. The operational parameters of the developed VD immunosensor were evaluated in PBT buffer solutions. The reproducibility of the measurements was assessed by measuring the response to 100 ng $mL^{-1}$ using 6 different immunostrips fabricated using the same protocol (Fig. 2D). The resulting relative standard deviation (RSD) of 4.3% confirms the high reliability of the chip fabrication and corresponding immunoassay protocol. Moreover, the storage stability of the CAb-modified sensors was studied for a 12 electrode-batch fabricated in the same day and stored at 4 °C. As seen in the stability graph in Fig. 2E, the immunostrips tested at 6 days intervals over a 30-day period, display negligible changes in the response for 100 ng $mL^{-1}$ VD, supporting the suitability of the VD biosensor for large scale production. In addition, we evaluated selectivity of the VD immunostrip toward common saliva constituents and commonly used vitamins in dietary supplementation. As illustrated in Fig. 2F, the presence of the vitamins A, B1, B2, B6, B12 and C, uric acid or acetaminophen, resulted in negligible contributions, proving the reliable specific VD detection in the presence of these potential interferents. Such high selectivity of the VD reflects the judicious choice of the specific antibodies and detection conditions.

Following the VD sensor optimization and characterization, we explored the applicability of the developed immunoplatform for short-term monitoring of dynamic VD body levels by direct analysis of saliva samples after consumption of VD-enriched products. Different single doses, ranging from 1000 to 10,000 IU, were tested using the same subject at seven-day intervals in order to maintain similar basal levels for all the trials. Saliva samples were collected from the volunteer before and 30 min after taking the corresponding dose of commercial VD tablets (as 25-Hydroxyvitamin D3). The results, displayed in Fig. 2G, illustrate that the current difference (Δi), before and after the pill intake, was proportional to the corresponding dose, except for 10,000 IU, for which a slight increase of the signal variation was obtained. Such curvature reflects physiological limitations to absorb large amounts of VD (Garland et al., 2011). Additionally, the new sensor was used to evaluate the immediate bioaccumulation of VD by consecutive multiple dosage (results (Fig. S2) and relative discussion are provided in detail in Supporting information). These findings demonstrate the potential of the VD immunostrip to reliably track VD status in saliva, to identify dosage and absorption yield upon analysis of the delta-current signal response. It is important to mention that 25-Hydroxyvitamin D biomolecule in saliva is expected to be unbound of vitamin D binding protein, and therefore the VD status in saliva represents free 25-Hydroxyvitamin D

(Higashi et al., 2008). Additionally, since a moderate and statistically significant correlation between salivary and serum 25-Hydroxyvitamin D has been reported (Sari et al., 2021a), the detection of VD in saliva holds considerable promise as reference for VD screening.

### 3.3. Salivary vitamin D monitoring: toward personalized immune support

Considering that precise VD administration depends upon the unique characteristics of each individual and their nutritional and health status (Griffin et al., 2021; Rastogi et al., 2020; Tarazona-Santabalbina et al., 2021), the use of the VD immunostrip as analytical tool to identify personal needs and guide appropriate vitamin dosage, was evaluated. Accordingly, we studied the VD pharmacokinetic profile of four different subjects by analyzing the stimulated saliva samples collected before and every 20 min after the intake of a single 10,000 IU VD pill; Fig. 3B. In order to estimate the change in the VD signal in saliva (Δi), the difference between the responses obtained before and after the VD pill intake was measured for each subject. As expected, the resulting signal variations were proportional to the dynamically-changing salivary VD levels. Because salivary and serum vitamin D levels have been shown to be correlated (Bahramian et al., 2018), such an approach could be used to estimate its status in the human subject. The corresponding temporal profiles displayed in Fig. 3B v-viii) suggest the distinct VD assimilation and metabolism, achieving maximum VD signals upon 20–40 min after pill intake, followed by decreasing signal with time. We observed highly significant signal change (Fig. 3B vii) or practically steady signal between 40 and 120 (Fig. 3B v), 40 to 60 (Fig. 3B vi) or 20–60 min (Fig. 3B viii), as function of each subject. Therefore, the different profiles obtained for the four volunteers for the same VD3 dose illustrate the potential of the device to guide precise administration according to each subject's absorption kinetics and dietary habits toward enhanced personalized nutrition and care. In order to further evaluate the reliability of the immunosensor measurements, control experiments were carried out by conducting the same temporal profile study protocol i) without VD3 supplementation (Figs. S3A and C) or by ii) using chips non-modified with the CAb for detection after taking 10,000 IU VD3 pill (Figs. S3B and D). The absence of significant current variations in both controls indicates the high specificity of the VD immunostrip and its potential for monitoring salivary VD dynamic changes.

In order to maintain adequate VD body levels for optimal immune system health, frequent VD status measurement is necessary. General recommendations for the administration of VD doses higher than 10,000 IU over short time periods to patients with SARS-COV2 infection to minimize the symptoms, and daily intake of 800–4,000 IU of VD for long time periods for the healthy population have been proposed (Griffin et al., 2021; Jolliffe et al., 2021). However, personalized approaches considering precise dosage and administration period duration while performing close VD monitoring can be essential in critical scenarios such as immunosuppressed and obese patients, or subjects with malabsorption issues (Rastogi et al., 2020; Tarazona-Santabalbina et al., 2021). With this in mind, we proceeded to monitor long-term VD bioaccumulation by intermittent tracking of the vitamin levels every two days (Fig. 3C i) or weekly (Fig. 3C ii). For this purpose, the VD measurements in saliva samples were carried out during daily 2,000 IU VD administration continued for 5 days (Fig. 3C i), or over 4 weeks (Fig. 3C ii), tracking VD status prior to, in the course of (yellow area) and after finishing the VD administration. Note that the saliva samples were collected at least 1 h before the daily intake of VD in order to consider only basal VD level. The change in the VD signal in saliva (Δi) was estimated by measuring the difference between the current signals corresponding to the start day of administration and that obtained on the day of testing. The results obtained show a significant increase of VD levels in saliva over the first days of daily oral administration (Fig. 3C i), compared to basal levels recorded before, and the steady stabilization of the values during the following weeks (Fig. 3C ii). Such behavior is in agreement with previous studies based on serum VD status monitoring

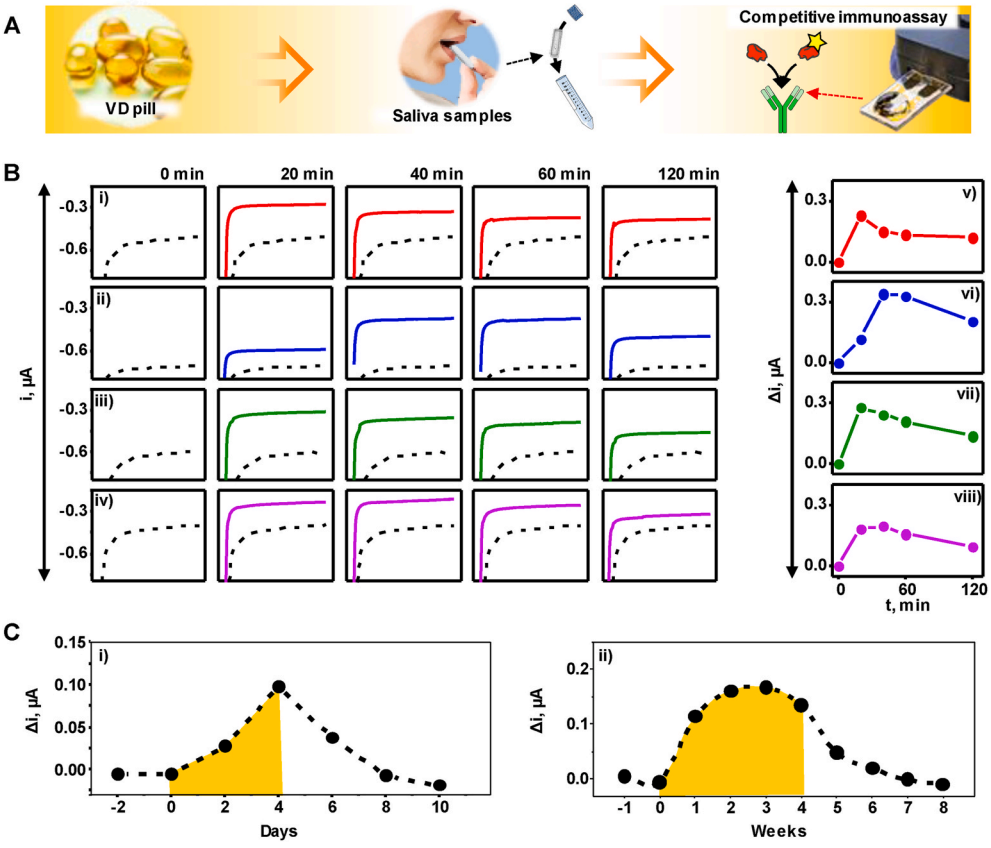


**Fig. 3. VD monitoring in saliva after oral administration of VD pills. A)** Schematic diagram of the followed workflow from the pills intake to collection and analysis of saliva samples. **B)** VD profile in saliva obtained for different subjects: chronoamperometric responses obtained in the detection of VD in stimulated saliva before (black lines) and after (20–120 min) taking 10,000 IU VD pill (as 25-Hydroxyvitamin D3) for four subjects (i–iv) and the corresponding saliva VD temporal profiles of each individual (v–viii). **C)** VD temporal profile in stimulated saliva during daily administration of 2,000 IU VD pill (as 25-Hydroxyvitamin D3) over a period of i) 5 days or ii) 4 weeks. Yellow area denotes the days a) or weeks b) during which VD was administered daily. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Biancuzzo et al., 2010; Tangpricha et al., 2003). Following these series of vitamin D administration, we observed in both cases the progressive decrease of VD levels over time, which was consistent with previous observations of the kinetics of vitamin D metabolism in the literature (Armas et al., 2004; Tangpricha et al., 2003). These results demonstrated the practical applicability of the VD immunosensor for intermittent measurement of salivary VD levels, proving the potential of the developed methodology to guide precise tailoring of vitamin D administration according to the vitamin absorption degree registered with the sensor. Similarly, such attractive performance illustrates the sensor ability to identify optimal dosage and prevent accidental overdosing, which has both safety and economic implications.

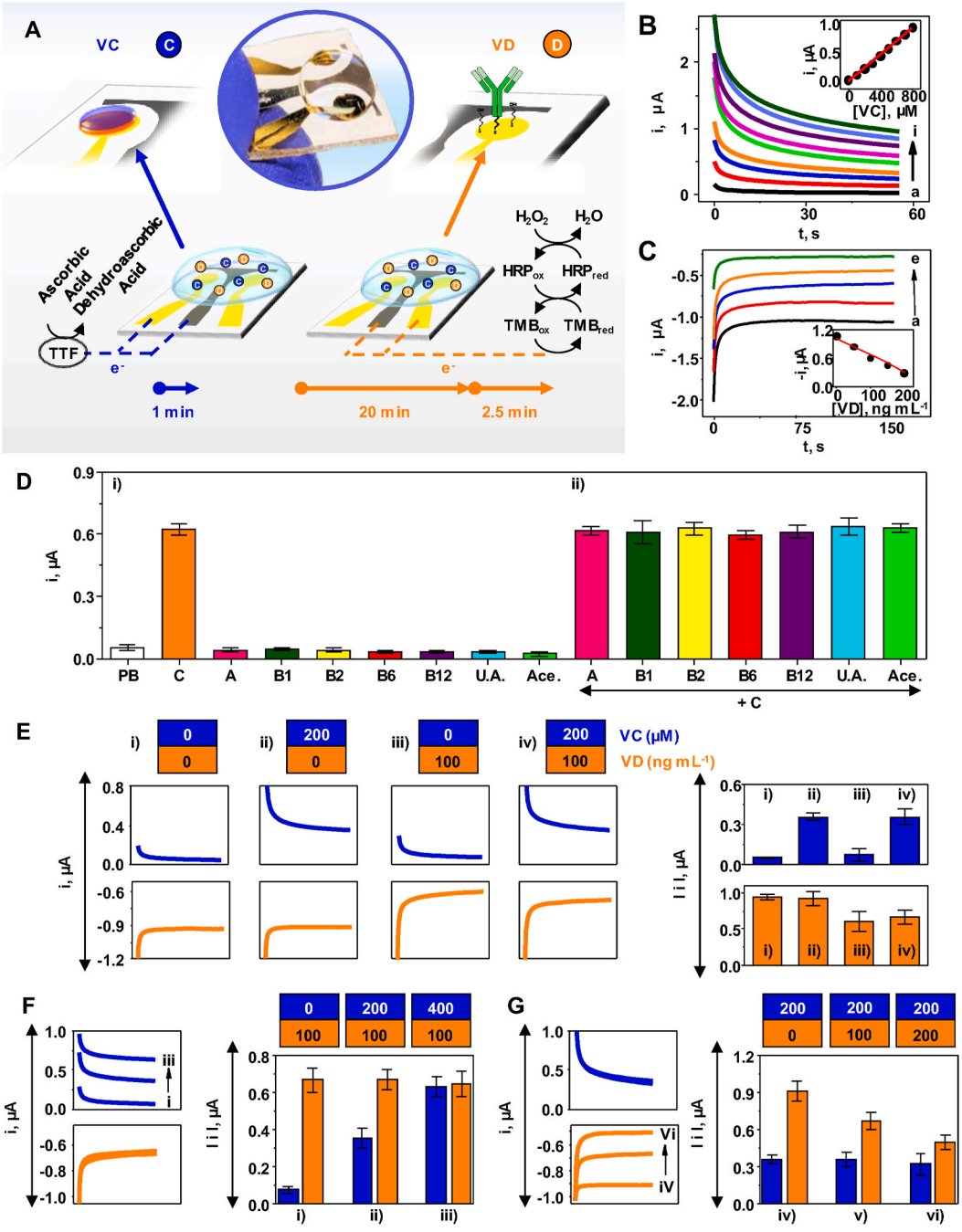


**Fig. 4. VC-D Dual Chip characterization. A)** Homemade sputtered dual sensor chip and schematic diagram of the localized VC and VD measurements on a single chip. Chronoamperometric responses of the dual chip to increasing **B)** VC concentrations from 0 (a) to 800 μM (i) in 100 μM increments or **C)** VD concentrations from 0 (a) to 200 ng $mL^{-1}$ (e) in 50 ng $mL^{-1}$ increments in buffer. **D)** Chronoamperometric responses of the VC sensor in the measurement of PB (white bar), 500 μM VC (blue bar), and 500 μM of each interferent in the absence (i) or presence of 500 μM VC (ii): vitamins A (pink bars), B1 (dark green bars), B2 (yellow bars), B6 (red bars), B12 (purple bars), uric acid (U.A.) (light blue bars) and acetaminophen (Ace.) (light green bars). **E)** Cross-talk evaluation of the dual chips in the analysis of VC-D mixture solutions. Chronoamperometric responses to various combinations of VC-D concentrations: i) absence of both markers; ii) 200 μM VC; iii) 100 ng $mL^{-1}$ VD; and iv) 200 μM VC and 100 ng $mL^{-1}$ VD. Evaluation of the dual chips by measuring **F)** increasing VC concentrations in the presence of a fixed VD level (i–iii) or **G)** increasing VD concentrations in the presence of a fixed VC level (iv–v). Dots or bars represent the mean and the error bars represent the standard deviation of the measurements performed with three different chips. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

*3.3.1. Vitamin C-D dual biosensor chip: integration and cross-talk study*

After successful evaluation of the VD immunostrip, we implemented the VD biosensor along with the VC electrocatalytic sensor in the dual vitamin sensor microchip. The electrode design and sensor modification are illustrated in Fig. 4A. The VD antibody was immobilized on $WE_2$ using the optimized protocol previously discussed, and the VC sensor consisted of the $WE_1$ electrode modified with the TTF mediator, chitosan and glutaraldehyde. VC electrocatalytic sensor optimization on metal-sputtered dual chips and analytical performance are described in detail in Supporting information.

Following the VC-TTF-Au sensor characterization, the sensing performance of the dual VC and VD sensors was interrogated in the individual measurement of 100 μM VC increments (Fig. 4B) or 50 ng $mL^{-1}$ VD increments (Fig. 4C) prepared in PBT buffer solution supplemented with the optimized HRP-VD concentration. The calibration curves showed linear response in the concentration ranges tested and LODs of 28 μM (4.9 ng/9.80x$10^{-5}$ IU) and 25 ng $mL^{-1}$ (250 pg/1.0x$10^{-2}$ IU) for VC and VD, respectively (Table S4), suitable to follow-up VC and VD body levels. Note that such analytical characteristics suggest that the transference of the VD biosensor from the single detection immunostrip to the compact dual chip was successfully realized without negative impact on the immunosensor performance.

Additionally, the selectivity of the VC toward saliva constituents and possible interferent vitamins usually included in multi-vitamin complexes was extensively studied in buffer solution (Fig. 4D and Figs. S5C–D) and saliva samples (Figs. S6C–E). The sensor presented negligible response for vitamins A, B1, B2, B6, B12, uric acid and acetaminophen upon cyclic voltammetry and chronoamperometry analysis

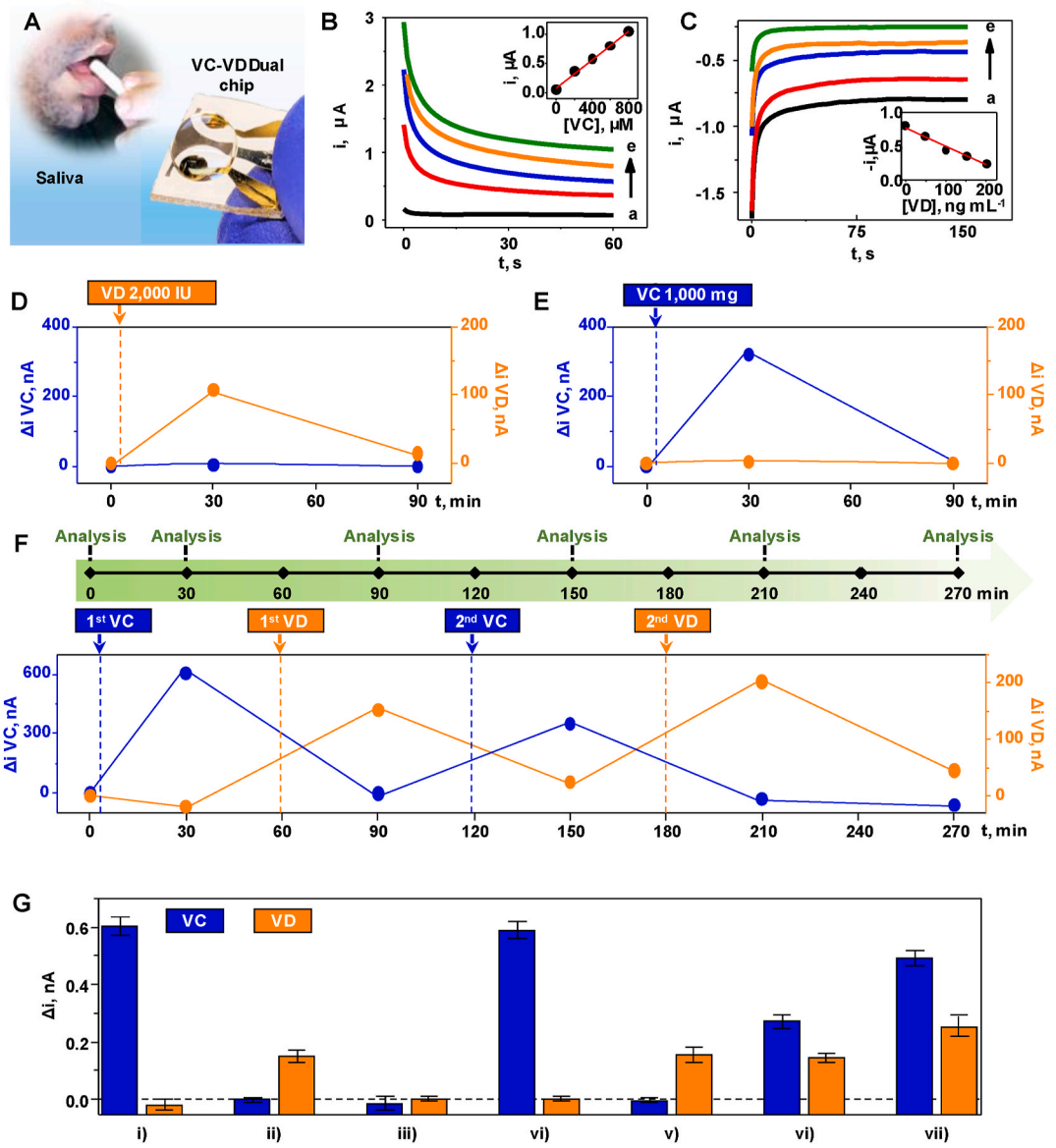


**Fig. 5. Application of the VC-VD Dual Chip in saliva for personalized nutrition. A)** Schematic diagram of the analysis of saliva samples with the VC-VD Dual Chip. Chronoamperometric responses of the dual chip to increasing **B)** VC concentrations from 0 (a) to 800 μM (e) in 200 μM increments or **C)** VD concentrations from 0 (a) to 200 ng $mL^{-1}$ (e) in 50 ng $mL^{-1}$ increments in stimulated saliva. Saliva VC & VD temporal profiles obtained using the VC-VD Dual chip developed after taking 2,000 IU VD (as 25-Hydroxyvitamin D3) (**D**), 1,000 mg VC (as ascorbic acid) (**E**) or both sequentially at 1-h time intervals (**F**). Dots connected by lines represent the Δi obtained for each analysis time. **G)** Variation of electrochemical responses of the VC-VD Dual chip in the analysis of saliva 30 min after intake of: i) 1,000 mg VC pill, ii) 2,000 IU VD pill, iii) vitamin B complex pill, iv) vitamin B complex and 1,000 mg VC pills, v) vitamin B complex and 2,000 IU VD pills, vi) multivitamin pill 1 (500 mg VC and 2,000 IU VD) or vii) multivitamin pill 2 (1,000 mg VC and 5,000 IU VD). Dots or bars represent the mean and the error bars represent the standard deviation of the measurements performed with three different chips in B, C and G.

in both media and proved the reliable VC detection in the presence of these potential interferents, indicating the exquisite selectivity of the VC sensing approach selected.

In order to demonstrate the practical feasibility of dual sensor chip for the joint VC and VD detection in the same 10-μL sample drop, different mixtures solutions of the target vitamins prepared in PBT buffer were analyzed. Fig. 4E–G shows the responses obtained for VC and D using the dual sensor chips in the analysis of various VC-VD mixture concentrations: solutions containing combinatorial levels of 0, 200 μM VC, and 100 ng $mL^{-1}$ VD (Fig. 4E); or increasing VC concentrations (0–400 μM) in the presence of a fixed 100 ng $mL^{-1}$ VD level (Fig. 4F) or increasing VD concentrations (0–200 ng $mL^{-1}$) in the presence of a fixed 200 μM VC level (Fig. 4G). The well-defined amperometric signals obtained in relation to the concentration of each target indicate no apparent cross-talk between the neighboring immune- and catalytic VD and VC sensors on the biochip and using the same 10-μL sample droplet. In addition, the reproducibility of four dual sensor chips in the measurement of a 200 μM VC and 100 ng $mL^{-1}$ VD mixture solution was evaluated (Fig. S8). The RSD values of 3.1 and 1.5% obtained for VC and VD, respectively, confirm the high reproducibility and robustness of the biochip fabrication and operation, as well as of the adapted sequential detection of both vitamins in a single dual sensor chip in less than 25 min.

### 3.4. Probing vitamin C-D salivary changes under vitamins administration

Since the consumption of multi-vitamin complexes contributing different amounts of each vitamin is quite common, the real scope of the developed dual sensor chip was evaluated for the follow-up of the dynamic changes of both vitamins by direct measurement of the saliva after the administration of different commercial dietary supplements. First, with the aim of assessing the sensing performance regarding simultaneous VC and VD nutritional markers monitoring in this biofluid with the dual chip and hence the suitability of the approach to detect relevant physiological levels, the amperometric responses of the dual chip to increasing VC concentrations ranged from 0 to 800 μM in 200 μM increments (Fig. 5B) and VD concentrations from 0 to 200 ng $mL^{-1}$ in 50 ng $mL^{-1}$ increments (Fig. 5C) were recorded, as displayed in Fig. 5. The similar analytical performance obtained, compared to those previously described in PBT buffer solutions (Table S4) and the achieved LODs of 84 μM (14.8 ng/2.96x$10^{-4}$ IU) and 17 ng $mL^{-1}$ (170 pg/6.80x$10^{-3}$ IU) for the VC and VD sensors, respectively, confirmed the suitability of the dual biochip approach for the direct analysis of undiluted raw human saliva samples. Such sensitivity could easily be tailored to move towards other biological specimens or pharmacological requirements through the use of nanomaterials or varying the assay time and volume. In addition, the applicability of the dual sensor biochip for close simultaneous VC and D salivary fluctuant levels tracking after vitamins administration was evaluated. Accordingly, we studied the VC and D pharmacokinetic profiles of three subjects after: single intake of 2,000 IU VD pill (Fig. 5D); single intake of 1,000 mg VC pill (as ascorbic acid) (Fig. 5E); or the intake of the same doses of both vitamins sequentially at 1-h time intervals (Fig. 5F). Note that assays of the saliva samples were realized right after sample collection in order to prevent salivary ascorbic acid degradation (Sharifian and Nezamzadeh-Ejhieh, 2016). Distinct temporal profiles were traced by measuring saliva samples collected at 0, 30, 90 (Fig. 5D and E), 150, 210 and 270 min (Fig. 5F) after intake, as displayed in Fig. 5D–F. The Δi values reflect the corresponding change in the VC or VD levels in saliva, and were estimated as the differences between the responses recorded for both VC and VD markers with the dual chip in a single 10 μL-drop of saliva sample collected before (time 0), used as basal signal reference, and at the indicated times after supplement intake. As can be observed in the resulted variation plots, the individual and concomitant rise and drop of salivary VC and VD levels, reported by the sensor chip responses (Δi) obtained, due to single vitamin or alternate vitamin D and C administrations were satisfactorily recorded.

Similarly, the physiological changes of both vitamins under various scenarios of administrating commercial multi-vitamin complexes were evaluated (Fig. 5G). For this purpose, we measured the response obtained in assays of saliva samples collected from one volunteer before and 30 min after the intake of 1,000 mg VC pill (i), 2,000 IU VD pill (ii), vitamin B complex pill (iii), vitamin B complex and 1,000 mg VC pills (iv), vitamin B complex and 2,000 IU VD pills (v), multivitamin 1 pill (500 mg VC and 2,000 IU VD) (vi), and multivitamin 2 pill (1,000 mg VC and 5,000 IU VD) (vii) in intervals seven days apart in order to keep similar basal levels for all the trials (pills composition listed in Table S5). Fig. 5G shows that similar signal changes for the same doses of vitamins taken individually or along with a vitamin B complex for VC (**i** vs **iv**) and VD (**ii** vs **v**), along with the absence of significant changes for individual vitamin B complex intake (**iii**). This figure shows also the distinct signal changes for the multivitamin supplements (**vi-vii**) according to the VC and/or VD dosage. These results illustrate the ability of the dual VC-VD bioelectronic chip for simultaneous tracking of both vitamins levels during their combined consumption without cross talk or interferences from other vitamins and essential elements. Such attractive performance demonstrates the suitability of the dual vitamin biochip for tracking closely the intake of multivitamins as a function of the dose or commercial trademark employed. Such capabilities of the VC-VD bioelectronic chip are particularly useful for guiding consumers in the establishment of personalized food consumption routines or toward decision-making on certain supplement commercial products and brands based on cost and health benefit.

## 4. Conclusions

Addressing the growing needs for personalized nutrition and boosting the immune system, in wake of the COVID-19 pandemic, this work reports the first example of a decentralized electrochemical VD immunostrip for on-the-spot detection of salivary VD profiles and the first example of a dual bioelectronic chip for tracking simultaneously VC and VD in saliva. The attractive analytical performance and operational requirements have facilitated the reliable application of these bioelectronics chips for successfully monitoring physiological concentration ranges and pharmacological dynamics changes in untreated human saliva samples. Both assays are characterized with high selectivity in the presence of other vitamins, coexisting saliva constituents or dietary supplements. The disposable VD immunostrip offered rapid tracking of salivary VD profiles as a function of the dose ingested, the time course after administration or for periodic follow-up. VC and VD dynamic fluctuations have also been monitored simultaneously under different intake scenarios. The different signals obtained for different subjects using specific dosages along with their distinct temporal profiles, indicate the potential of such a decentralized sensing approach for the accurate and frequent assessment of the subject's individual dose-response relationship towards guiding personalized nutrition.

These advances have been realized by judicious integration on the same biochip of fundamentally different catalytic and immunochemical sensing approaches for multiplexed vitamins tracking. This novel dual sensing strategy combines VC and VD measurements into a single protocol and device to simplify greatly the operation and instrumentation required in the current individual vitamin determination methods. The integration of different recognition and electrochemical detection principles in a single user-friendly sensor chip and the tailored design and fabrication can facilitate the broader scope of this technology for simultaneous assays of these and other relevant vitamins screening. The inherent miniaturization and scalability of the new sensing chip technology facilitate low-cost strip fabrication and reduces the volume of corresponding bioreagents, resulting in a multiplexed vitamin test with an estimated cost of disposable consumables of below 5 US$. The ability to miniaturize and integrate the dual-chip bioassays into microfluidic

systems would further simplify the saliva sampling and analysis workflow or handheld mobile electrochemical devices offers considerable promise for effective at-home self-testing of vitamin status. To advance this technology, future research should focus on the standardization of the developed methodology beyond following temporal profiles towards as quantitative sensing approach, along with critical validation against gold standard clinical reference methods in larger reference populations. Future efforts will broaden the scope of our approach to additional body fluids (particularly serum) and to other relevant molecular forms of VD found in commercial supplements, particularly 25-OH VD2 (considering that the present antibody is specific to both 25-OH VD3 and VD2 forms). The widespread use of such mobile nutrient biosensing technology has potential to provide access to nutrition monitoring at remote settings and can thereby improve nutritional support for low-income individuals prone to weak immune system. By replacing existing lab-based vitamin assays, whose sample-to-answer time can take days and require discomforting invasive sampling such as blood draw or finger-pricking, the new decentralized, affordable, rapid, and non-invasive sensing technology represents a step forward in creating access to timely nutritional status information and will promote personalized nutritional interventions for strengthening the immune system.

## CRediT authorship contribution statement

**Víctor Ruiz-Valdepeñas Montiel:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, and, Visualization. **Juliane R. Sempionatto:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, and, Visualization. **Eva Vargas:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, and, Visualization. **Eileen Bailey:** Conceptualization, Methodology, Writing – review & editing, Supervision, and, Funding acquisition. **Jennifer May:** Conceptualization, Methodology, Writing – review & editing, Supervision, and, Funding acquisition. **Andrea Bulbarello:** Conceptualization, Methodology, Writing – review & editing, Supervision, and, Funding acquisition. **André Düsterloh:** Conceptualization, Methodology, Writing – review & editing, Supervision, and, Funding acquisition. **Nathan Matusheski:** Conceptualization, Methodology, Writing – review & editing, Supervision, and, Funding acquisition. **Joseph Wang:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision, and, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113590.

## References

Abobaker, A., Alzwi, A., Alraied, A.H.A., 2020. Pharmacol. Rep. 72, 1517–1528. https://doi.org/10.1007/s43440-020-00176-1.
Aranow, C., 2011. J. Invest. Med. 59 (6), 881–886.
Armas, L.A.G., Hollis, B.W., Heaney, R.P., 2004. J. Clin. Endocrinol. Metab. 89 (11), 5387–5391.
Arvinte, C., Singh, M., Marik, P.E., 2020. Medicine in Drug Discovery 8, 100064. https://doi.org/10.1016/j.medidd.2020.100064.
Ates, H.C., Yetisen, A.K., Güder, F., Dincer, C., 2021. Nat. Electron. 4, 13–14. https://doi.org/10.1038/s41928-020-00533-1.
Ayseli, Y.I., Aytekin, N., Buyukkayhan, D., Aslan, I., Ayseli, M.T., 2020. Trends Food Sci. Technol. 105, 186–199.
Bae, M., Kim, H., 2020. Molecules 25, 5346. https://doi.org/10.3390/molecules25225346.
Bahramian, A., Falsafi, P., Abbasi, T., Ghanizadeh, M., Abedini, M., Kavoosi, F., Kouhsoltani, M., Noorbakhsh, F., Dabbaghi Tabriz, F., Rajaeih, S., Rezaei, F., 2018. J. Dent. 19 (4), 295–300. PMID: 30680302. PMCID: PMC6338692.
Beard, J.A., Bearden, A., Striker, R., 2011. J. Clin. Virol. 50 (3), 194–200. https://doi.org/10.1016/j.jcv.2010.12.006.
Biancuzzo, R.M., Young, A., Bibuld, D., Cai, M.H., Winter, M.R., Klein, E.K., Ameri, A., Reitz, R., Salameh, W., Chen, T.C., Holick, M.F., 2010. Am. J. Clin. Nutr. 91, 1621–1626.
Bush, C.L., Blumberg, J.B., El-Sohemy, A., Minich, D.M., Ordovás, J.M., Reed, D.G., Yunez Behm, V.A., 2020. J. Am. Coll. Nutr. 39 (1), 5–15. https://doi.org/10.1080/07315724.2019.1685332.
Calder, P.C., Carr, A.C., Gombart, A.F., Eggersdorfer, M., 2020. Nutrients 12, 1181. https://doi.org/10.3390/nu12041181.
Campuzano, S., Pedrero, M., Yáñez-Sedeño, P., Pingarrón, J.M., 2019. Int. J. Mol. Sci. 20, 423. https://doi.org/10.3390/ijms20020423.
Carlucci, L., Favero, G., Tortolini, C., Di Fusco, M., Romagnoli, E., Minisola, S., Mazzei, F., 2013. Biosens. Bioelectron. 40, 350–355.
Carr, A.C., Maggini, S., 2017. Nutrients 9, 1211. https://doi.org/10.3390/nu9111211.
Chauhan, D., Gupta, P.K., Solanki, P.R., 2018. Mater. Sci. Eng. C 93, 145–156.
Chauhan, D., Kumar, R., Panda, A.K., Solanki, P.R., 2019. J. Mater Res Technol. 8, 5490–5503.
Chowdhury, M.A., Hossain, N., Kashem, M.A., Shahid, M.A., Alam, A., 2020. J. Infect. Public Health 13 (11), 1619–1629. https://doi.org/10.1016/j.jiph.2020.07.001. PMID: 32718895. PMCID: PMC7359800.
Costantini, E., Sinjari, B., Piscopo, F., Porreca, A., Reale, M., Caputi, S., Murmura, G., 2020. J Int. J. Mol. Sci. 21 (8), 2669. https://doi.org/10.3390/ijms21082669.
Di Meo, V., Crescitelli, A., Moccia, M., Sandomenico, A., Cusano, A., Portaccio, M., Lepore, M., Galdi, V., Esposito, E., 2020. Nanophotonics 9 (12), 3921–3930. https://doi.org/10.1515/nanoph-2020-0103.
Ebadi, M., Montano-Loza, A.J., 2020. Eur. J. Clin. Nutr. 74, 856–859. https://doi.org/10.1038/s41430-020-0661-0.
Feyaerts, A.F., Luyten, W., 2020. Nutrition 79–80, 110948. https://doi.org/10.1016/j.nut.2020.110948.
Garland, C.F., French, C.B., Baggerly, L.L., Heaney, R.P., 2011. Anticancer Res. 31, 607–612.
Gombart, A.F., Pierre, A., Maggini, S., 2020. Nutrients 12, 236. https://doi.org/10.3390/nu12010236.
Gooding, J., Mearns, F., Yang, W., Liu, J., 2003. Electroanalysis 15, 81–96.
Griffin, G., Hewison, M., Hopkin, J., Kenny, R.A., Quinton, R., Rhodes, J., Subramanian, S., Thickett, D., 2021. Clin. Med. 21 (1), e48–e51. https://doi.org/10.7861/clinmed.2020-0858.
Heureux, N., 2017. Chapter two – vitamin D testing-where are we and what IS ON the horizon?. In: Makowski, Gregory S. (Ed.), Advances in Clinical Chemistry, vol. 78. Elsevier, pp. 59–101. https://doi.org/10.1016/bs.acc.2016.07.002.
Higashi, T., Shibayama, Y., Fuji, M., Shimada, K., 2008. Anal. Bioanal. Chem. 391, 229–238. https://doi.org/10.1007/s00216-007-1780-3.
Hoang, B.X., Shaw, G., Fang, W., Han, B., 2020. J. Glob. Antimicrob. Resist. 23, 256–262.
Hussein, A.S., Almoudi, M.M., Abu-Hassan, M.I., Schroth, R.J., Saripudin, B., Chinna, K., 2020. J. Int. Dent. Med. Res. 13 (4), 1405–1410.
Iddir, M., Brito, A., Dingeo, G., Fernandez Del Campo, S.S., Samouda, H., La Frano, M.R., Bohn, T., 2020. Nutrients 12, 1562. https://doi.org/10.3390/nu12061562.
Im, J.H., Je, Y.S., Baek, J., Chung, M.-H., Kwon, H.Y., Lee, J.-S., 2020. Int. J. Infect. Dis. 100, 390–393. https://doi.org/10.1016/j.ijid.2020.08.018.
Jayawardena, R., Sooriyaarachchi, P., Chourdakis, M., Jeewandara, C., Ranasinghe, P., 2020. Diabetes Metab Syndr 14 (4), 367–382.
Jolliffe, D.A., Camargo, C.A., Sluyter, J.D., Aglipay, M., Aloia, J.F., Ganmaa, D., Bergman, P., Bischof-Ferrari, H.A., Borzutzky, A., Damsgaard, C.T., Dubnov-Raz, G., Esposito, S., Gilham, C., Ginde, A.A., Golan-Tripto, I., Goodall, E.C., Grant, C.C., Griffiths, C.J., Hibbs, A.M., Janssens, W., Khadilkar, A.V., Laaksi, I., Lee, M.T., Loeb, M., Maguire, J.L., Majak, P., Mauger, D.T., Manaseki-Holland, S., Murdoch, D. R., Nakashima, A., Neale, R.E., Pham, H., Rake, C., Rees, J.R., Rosendahl, J., Scragg, R., Shah, D., Shimizu, Y., Simpson-Yap, S., Trilok-Kumar, G., Urashima, M., Martineau, A.R., 2021. Lancet Diabetes Endocrinol 9, 276–292. https://doi.org/10.1016/S2213-8587(21)00051-6.
Kaur, A., Kapoor, S., Bharti, A., Rana, S., Chaudhary, G.R., Prabhakar, N., 2020. J. Electroanal. Chem. 873, 114400 https://doi.org/10.1016/j.jelechem.2020.114400.
Lee, S., Oncescu, V., Mancuso, M., Mehta, S., Erickson, D., 2014. Lab Chip 14, 1437–1442.
Maggini, S., Maldonado, P., Cardim, P., Newball, C.F., Latino, E.R.S., 2017. Vitam. Miner. 6, 3. https://doi.org/10.4172/2376-1318.1000167.
Meltzer, D.O., Best, T.J., Zhang, H., Vokes, T., Arora, V., Solway, J., 2020. JAMA Netw Open 3 (9), e2019722. https://doi.org/10.1001/jamanetworkopen.2020.19722.
Micha, R., Peñalvo, J.L., Cudhea, F., Imamura, F., Rehm, C.D., Mozaffarian, D., 2017. J. Am. Med. Assoc. 317, 912. https://doi.org/10.1001/jama.2017.0947.
Murdaca, G., Pioggia, G., Negrini, S., 2020. Clin. Mol. Allergy 18, 23. https://doi.org/10.1186/s12948-020-00139-0.
Name, J.J., Souza, A.C.R., Vasconcelos, A.R., Prado, P.S., Pereira, C.P.M., 2020. Front. Nutr. 7, 606398. https://doi.org/10.3389/fnut.2020.606398.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J.-H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K., Levine, M., 2003. J. Am. Coll. Nutr. 22 (1), 18–35. https://doi.org/10.1080/07315724.2003.10719272.

Patterson, T., Isales, C.M., Fulzele, S., 2021. Aging and disease 12 (1), 14–26. https://doi.org/10.14336/AD.2020.0918.

Picó, C., Serra, F., Rodríguez, A.M., Keijer, J., Palou, A., 2019. Nutrients 11 (5), 1092. https://doi.org/10.3390/nu11051092.

Prante, M., Schüling, T., Roth, B., Bremer, K., Walter, J., 2019. Biosensors 9, 134. https://doi.org/10.3390/bios9040134.

Rastogi, A., Bhansali, A., Khare, N., Suri, V., Yaddanapudi, N., Sachdeva, N., Puri, G.D., Malhotra, P., 2020. Postgrad. Med. 1–4. https://doi.org/10.1136/postgradmedj-2020-139065, 0.

Rhodes, J.M., Subramanian, S., Laird, E., Griffin, G., Kenny, R.A., 2021. J. Intern. Med. 289, 97–115.

Rondanelli, M., Miccono, A., Lamburghini, S., Avanzato, I., Riva, A., Allegrini, P., Faliva, M.A., Peroni, G., Nichetti, M., Perna, S., 2018. Evid. Based complementary altern. Med. https://doi.org/10.1155/2018/5813095. Article ID 5813095.

Rozga, M., Latulippe, M.E., Steiber, A., 2020. J. Acad. Nutr. Diet. 120, 1074–1085. https://doi.org/10.1016/j.jand.2020.01.020.

Sharifian, S., Nezamzadeh-Ejhieh, A., 2016. Mater. Sci. Eng. C 58, 510–520.

Sari, D.K., Sari, L.M., Laksmi, L.I., Farhat, 2021a. Int. J. Gen. Med. 14, 841–850. https://doi.org/10.2147/IJGM.S302912.

Sari, D.K., Sari, L.M., Laksmi, L.I., Farhat, F., 2021b. Open Access Maced J Med Sci 9 (T3), 40–43. https://oamjms.eu/index.php/mjms/article/view/6329.

Sarkar, T., Bohidar, H.B., Solanki, P.R., 2018. Int. J. Biol. Macromol. 109, 687–697. https://doi.org/10.1016/j.ijbiomac.2017.12.122.

Sempionatto, J.R., Khorshed, A.A., Ahmed, A., De Loyola e Silva, A.N., Barfidokht, A., Yin, L., Goud, K.Y., Mohamed, M.A., Bailey, E., May, J., Aebischer, C., Chatelle, C., Wang, J., 2020. ACS Sens. 5 (6), 1804–1813.

Sempionatto, J.R., Ruiz-Valdepeñas Montiel, V., Vargas, E., Teymourian, H., Wang, J., 2021. ACS Sens. 6 (5), 1745–1760. https://doi.org/10.1021/acssensors.1c00553.

Tangpricha, V., Koutkia, P., Rieke, S.M., Chen, T.C., Perez, A.A., Holick, M.F., 2003. Am. J. Clin. Nutr. 77, 1478–1483.

Tarazona-Santabalbina, F.J., Cuadra, L., Cancio, J.M., Carbonell, F.R., Pérez-Castejón Garrote, J.M., Casas-Herrero, Á., Martínez-Velilla, N., Serra-Rexach, J.A., Formiga, F., 2021. Rev. Esp. Geriatr. Gerontol. 56, 177–182.

Vargas, E., Teymourian, H., Tehrani, F., Eksin, E., Sánchez-Tirado, E., Warren, P., Erdem, A., Dassau, E., Wang, J., 2019. Angew. Chem. Int. Ed. 58, 6376–6379.

Vargas, E., Povedano, E., Krishnan, S., Teymourian, H., Tehrani, F., Campuzano, S., Dassau, E., Wang, J., 2020. Biosens. Bioelectron. 167, 112512 https://doi.org/10.1016/j.bios.2020.112512.

Verma, M., Hontecillas, R., Tubau-Juni, N., Abedi, V., Bassaganya-Riera, J., 2018. Front. Nutr. 5, 117. https://doi.org/10.3389/fnut.2018.00117.

Walter, J.-G., Alwis, L.S.M., Roth, B., Bremer, K., 2020. Sensors 20, 6771. https://doi.org/10.3390/s20236771.

Yu, Y., Nyein, H.Y.Y., Gao, W., Javey, A., 2020. Adv. Mater. 32, 1902083 https://doi.org/10.1002/adma.201902083.

Zhao, J., Nyein, H.Y.Y., Hou, L., Lin, Y., Bariya, M., Ahn, C.H., Ji, W., Fan, Z., Javey, A., 2021. Adv. Mater. 33 (1), 2006444 https://doi.org/10.1002/adma.202006444.