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# Disposable Stochastic Miniplatforms for Simultaneous Recognition and Determination of Vitamins B5, B7, and B9 in Food and Pharmaceutical Compounds

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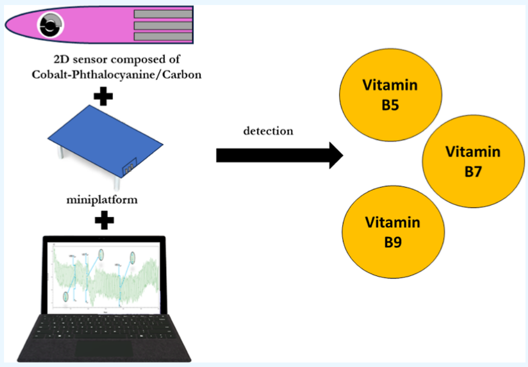
**ABSTRACT:** Vitamins are essential for supporting daily physiological functions and are crucial for metabolic processes, including preventing vascular events and delaying the progression of diabetic nephropathy. The specific assessment of food and pharmaceutical compound quality requires the highly sensitive evaluation of vitamins B5, B7, and B9. Therefore, it is necessary to have a fast screening method for multivitamin tablets, pharmaceutical tablets, water, food, including raw egg yolk, and biological fluids, including urine, in order to ensure the precise identification and measurement of vitamins B5, B7, and B9. This paper introduces a novel disposable stochastic miniplatform that utilizes an incorporated 2D stochastic sensor to be able to simultaneously assay the three vitamins. Cobalt-phthalocyanine was used to modify the carbon film of the 2D sensor. High sensitivities (of $10^2$−$10^8$ $s^{-1}$ $g^{-1}$ mL magnitude order) and low detection limits (of pg $mL^{-1}$ magnitude order) were recorded for the disposable stochastic miniplatforms. The validation procedure involved the utilization of pharmaceutical tablets, supplement tablets, water samples, food samples, including raw egg yolk, and biological samples, specifically urine. The disposable stochastic miniplatforms demonstrate cost-effectiveness and suitability for the fast screening tests, with a time frame of 6 min, across a range of samples, a capacity of over 150 measurements, and an endurance of up to one month. Recovery values higher than 94.00% with RSD (%) values lower than 1.00 were recorded.




## ■ INTRODUCTION

For optimal physiological growth and development, the human body needs a range of essential vitamins, primarily obtained from dietary sources. Several essential vitamins for the human body include vitamin B2, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin D, and vitamin K. Vitamins D and K are classified as fat-soluble, while the vitamins belonging to the B group are classified as water-soluble. Vitamin B5 (VB5), also known as pantothenic acid, is found in natural food sources, incorporated into food products, and can be obtained in the form of dietary supplements. It plays a role in the biosynthesis of acyl carrier protein, a key component involved in the construction of lipid molecules.[1] Vitamin B7 (VB7), commonly termed biotin, is a well-known nutrient. Vitamin B7, a water-soluble B vitamin, is present in certain foods and in the supplement form. Biotin plays a crucial role in facilitating the enzymatic processes involved in the hydrolysis of lipids, carbohydrates, and proteins present in dietary sources. Additionally, it aids in the regulation of cellular signaling and gene expression.[2] Vitamin B9 (VB9), also referred to as folic acid, is a member of the B group of vitamins and is primarily recognized for its significant biological roles, including DNA synthesis and repair, as well as DNA methylation.[3,4] The deficiency of folic acid leads to significant complications that result in malformations of the skull, brain, and spine of the fetus.[5] The significance of three vitamins in the human body's development necessitates the development of methods to ascertain the presence of these vitamins in foods or specific supplements.

Electrochemical approaches are highly advantageous in the advancement of sensors for applications pertaining to health.[6,7] Electrochemical sensors are utilized to quantify the current generated by the working electrode, relying on the electrochemical redox mechanism of an analyte. The sensors are capable of measuring the concentration of a specific analyte, in this case, thiamine, within the electrolyte solution. Comparing

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with chromatographic methods of analysis, they are cost-effective, faster, and highly reliable.

The novelty of this study lies in the development of a compact, disposable stochastic miniplatform that incorporates a 2D sensor composed of cobalt-phthalocyanine/carbon as its active side. Also, this miniplatform enables simultaneous detection of vitamins B5, B7, and B9 through the utilization of a stochastic mode. The miniplatform is cost-effective and highly reliable.

## ■ EXPERIMENTAL SECTION

**Materials and Reagents.** All chemicals were of analytical grade. VB5, VB7, and VB9 were purchased from Sigma-Aldrich. Deionized water, obtained from a Millipore Direct-Q 3 system was used for the preparation of the vitamin solutions, with different concentrations (23.82 $\mu$g mL$^{-1}$ to 6.16 × 10$^{-12}$ g mL$^{-1}$ for VB5 and 2.44 to 6.40 × 10$^{-12}$ $\mu$g mL$^{-1}$ and 4.41 to 5.42 × 10$^{-12}$ $\mu$g mL$^{-1}$ for VB9) using the serial dilution method. All solutions were prepared in a phosphate buffer solution (pH = 7.4). When not in use, the solutions were kept at a temperature of 2−8 °C.

**Apparatus and Methods.** A miniature potentiostat (produced by PalmSens) was used to record all measurements, and laptop/tablet/smartphone were connected to it through wireless. For this, PSTrace software (version 5.9) was used.

**Design of the Miniplatform.** The screen-printed Co-phthalocyanine/carbon electrode, which was integrated on a miniplatform, comprises the stochastic sensor (obtained by modifying a carbon film with a complex between cobalt and phthalocyanine), the auxiliary electrode (carbon film), and a reference electrode (silver film) (Scheme 1).

**Scheme 1. Disposable Stochastic Miniplatform and Its Integration in the Measurement System**

The films were deposited on a ceramic substrate with sizes of L33 × W10 × H0.5 mm. The 2D sensor from the miniplatform was cleaned with deionized water both before and after each measurement. When the 2D sensor from the miniplatform was not in use, it was stored in the dark, at a temperature range of 2−8 °C. The signal produced by the disposable stochastic miniplatform is sent via wireless to laptop/smartphone/tablet and further to specific databases, being able to be continuously monitored.

**Stochastic Modes.** The utilization of the stochastic mode was employed to carry out all of the measurements. The underlying principle of the disposable stochastic miniplatform, which incorporates a stochastic sensor, resides in the measurement of channel conductivity. In the present study, a constant potential of 125 mV was applied, and the resulting current was recorded, as depicted in Figure 1. The diagrams were employed for the purpose of identifying the distinctive signatures ($t_{off}$ values) of vitamins B5, B7, and B9, which served as the recognition parameters for these vitamins. The $t_{on}$ values were employed for all of the quantitative analyses. The disposable stochastic miniplatform underwent a calibration process, wherein a series of solutions with varying concentrations were utilized. These solutions were divided into three sets, each corresponding to VB5, VB7, and VB9, respectively. The calibration equations for the three vitamins were determined using the disposable stochastic miniplatform. The calibration equations were derived through the process of determining the $t_{on}$ values, which were obtained by reading the data between two consecutive $t_{off}$ values. The values of parameters $a$ and $b$ were derived from the calibration equation, $1/t_{on} = a + b \times \text{conc}_{\text{vitamin}}$ using the method of linear regression. The identification of vitamins was accomplished by analyzing water, urine, hen's raw egg yolk, Supradyn, and Optisana and observing their respective signatures or $t_{off}$ values. The $t_{on}$ values were subsequently employed in the calibration equation to measure the concentration of vitamins B5, B7, and B9 in the aforementioned samples.

**Samples.** The proposed disposable stochastic miniplatform was used to determine vitamins B5, B7, and B9 in the following types of samples: two types of multivitamin tablets, Supradyn (vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K, biotin, folic acid, niacin, iodine, calcium, copper, iron, magnesium, manganese, molybdenum, selenium, zinc, and coenzyme Q10), and Optisana (containing vitamin E, vitamin C, thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, and biotin: pantothenic acid, citric acid, and vitamin E), which were purchased from a local pharmacy, water samples collected from various local sources, raw egg yolk (the eggs were purchased from a grocery store), and urine samples, which were collected from healthy volunteers (samples were obtained from the Emergency University Hospital, with the permission to conduct the research by the Ethics Committee of the Hospital, with the number 75/2015).

Supradyn and Optisana multivitamin tablets were dissolved in 10 mL of phosphate buffer solution (pH 7.40). The other samples were analyzed without any pretreatment.

## ■ RESULTS AND DISCUSSION

**Performance Characteristics of the Disposable Stochastic Miniplatform.** As their underlying working principle, stochastic sensors rely on reversible molecular bindings to the walls of the pores and channels of matrices. After a constant 125 mV potential is applied, the conductivity of the pore is measured, and the results are depicted by using the provided diagrams.

The ongoing progress took place in two distinct phases. The first stage, known as the molecular recognition phase, entails the entrance of VB5, VB7, and VB9 into the channels, leading to their subsequent blockage. This phenomenon results in a decrease in the intensity of the present compound to nearly zero for a specific period, which is identified as the characteristic pattern associated with vitamins B5, B7, and B9. The signatures are subsequently denoted as "$t_{off}$" in Figure 1. The value of the signatures is utilized in the qualitative analysis of VB5, VB7, and VB9. The subsequent phase is commonly known as the reaction phase resulting from the interaction between vitamins B5, B7, and B9 and the wall


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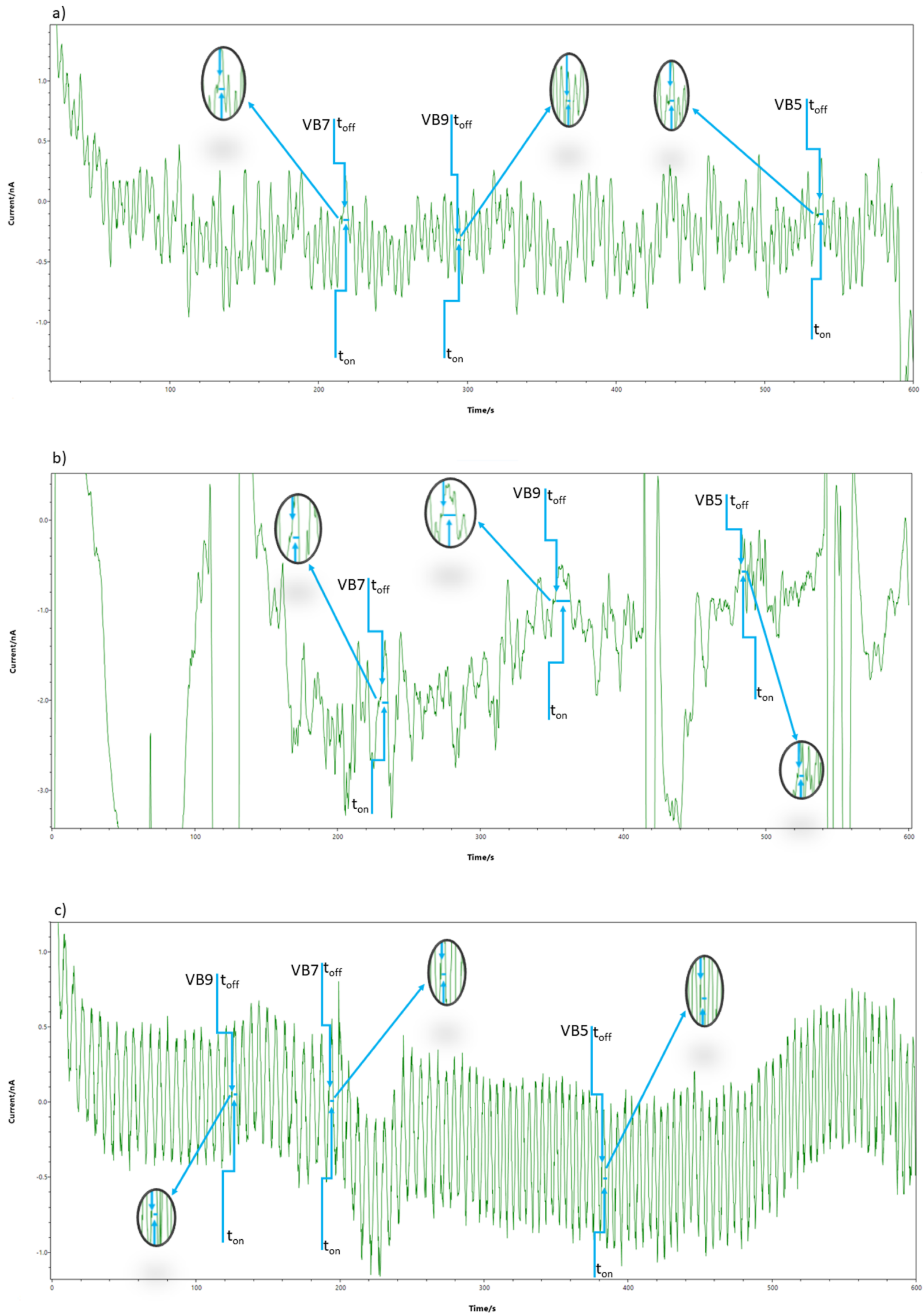


**Figure 1.** Examples of diagrams obtained when screening (a) water, (b) hen's raw egg yolk, (c) Optisana, (d) Supradyn, and (e) urine with the disposable stochastic miniplatform.



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Table 1. Response Characteristics of the Disposable Stochastic Miniplatform Based on a Stochastic Sensor for the Assay of Vitamins B5, B7, and B9[a]

| vitamin | calibration equation and correlation coefficient $(r)$[a] | $t_{off}$ (s) | sensitivity ($s^{-1}$ $g^{-1}$ mL) | linear concentration range (g $mL^{-1}$) | limit of determination g $mL^{-1}$ |
|---|---|---|---|---|---|
| B5 | $1/t_{on} = 0.23 + 3.65 \times 10^5$ C; $r = 0.9998$ | 0.6 | $3.65 \times 10^5$ | $6.16 \times 10^{-12}$–23.82 | $6.16 \times 10^{-12}$ |
| B7 | $1/t_{on} = 0.17 + 1.84 \times 10^8$ C; $r = 0.9995$ | 1.1 | $1.84 \times 10^8$ | $1.60 \times 10^{-10}$–$1.95 \times 10^{-1}$ | $1.60 \times 10^{-10}$ |
| B9 | $1/t_{on} = 0.40 + 1.98 \times 10^2$ C; $r = 0.9999$ | 0.4 | $1.98 \times 10^2$ | $1.35 \times 10^{-10}$–$3.31 \times 10^{-2}$ | $1.35 \times 10^{-10}$ |

[a]$\langle C \rangle = \mu g\ mL^{-1}$; $\langle t_{on} \rangle = s$.

Table 2. Signatures ($t_{off}$ Values, in s) Recorded for Vitamins B5, B7, and B9 and Other Vitamins and Substances Found in the Real Samples

| analyte | vitamin B5 | vitamin B7 | vitamin B9 | vitamin B12 | vitamin D | vitamin C | uric acid |
|---|---|---|---|---|---|---|---|
| signature, s | 0.6 | 1.1 | 0.4 | 3.2 | 2.4 | 1.8 | 1.5 |

channel. This interaction takes place during the equilibrium equation presented below

$$Ch_{(i)} + vitamin_{(i)} \Leftrightarrow Ch\bullet vitamin_{(i)}$$

where Ch denotes the channel, whereas i denotes the interface.[8,9] Furthermore, redox reactions take place. The quantitative parameter, commonly known as $t_{on}$, represents the time needed for the reaction phase to achieve equilibrium. The concentrations of vitamins B5, B7, and B9 are based on the value of this parameter.

The parameters related to the disposable stochastic miniplatform are presented in Table 1. The signatures ($t_{off}$ values in the diagrams) for VB5, VB7, and VB9 exhibit distinct values, thereby demonstrating the miniplatform's capability to simultaneously analyze vitamins B5, B7, and B9.

The disposable stochastic miniplatform demonstrated a most significantly higher sensitivity toward vitamin B7 when compared to that of vitamins B5 and B9. The disposable stochastic miniplatform demonstrated a wide working concentration range for all three vitamins. To the best of our knowledge, there have been no reports of any other electrochemical sensor capable of simultaneously detecting all three vitamins.

Compared with the HPLC method of analysis, which is able to determine 13 vitamin B and related compounds,[10] which is time-consuming and laborious, the proposed method is cost-effective, fast, highly reliable, and presents far lower limits of determinations for the vitamins B5, B7, and B9. Compared with other electrochemical methods of analysis proposed for vitamins B5 (limit of determination 8 ng/mL),[11] B7 (limit of determination 110 nmol/mL),[12] and B9 (limit of determination 100 nmol/mL),[13] the proposed method can simultaneously determine the three vitamins, and the reported limits of determination are lower than those reported earlier.[11−13]

Reproducibility of the design and stability studies were performed. Ten disposable stochastic miniplatforms were designed. For each of these disposable stochastic miniplatforms, the sensitivity for determination of each of the three vitamins was measured; the % RSD values determined were: 0.05% for vitamin B5, 0.08% for vitamin B7, and 0.07% for vitamin B9. To assess the stability over time of the 10 miniplatforms, the sensitivity assay was conducted over a 30 day period of time. The findings of the study revealed that average RSD values of 0.13% were recorded for all three vitamins after the disposable stochastic miniplatforms were used for 30 days, every day for measurements. These results show the high reliability of the design and also high stability over a one month period of time.

The selectivity of the proposed disposable stochastic miniplatform can be determined by analyzing the signatures of vitamins and other substances present in the real samples. If there is a discernible difference in the values of these signatures, this indicates that the disposable stochastic miniplatform exhibits selectivity. The signatures of the analytes were found to be independent of the matrix in which they were detected. However, these signatures were observed to be influenced by factors such as the length and volume of the molecules and their velocity within the channel. Consequently, the analytes within the solution entered into the channel in a specific sequence organized based on the molecules' length and stereochemistry. The method demonstrated selectivity by recording distinct signatures ($t_{off}$ values) for each of the three vitamins, as shown in Table 1. Also, selectivity versus vitamins B12, D, and C and versus uric acid was checked. The signatures of these analytes are shown in Table 2.

Differences in the signatures obtained for vitamins B12, D, and C and versus uric acid showed that the proposed disposable stochastic miniplatform can be used selectively for the simultaneous assay of vitamins B5, B7, and B9.

**Determination of Vitamins B5, B7, and B9 in Food, Pharmaceutical Compounds, Water, and Urine.** The analysis of vitamins B5, B7, and B9 was conducted on various samples including multivitamin tablets, pharmaceutical tablets, water, food (including raw egg yolk), and biological fluids (including urine). In order to determine the concentrations of the vitamins present in the samples, the distinctive signature of each vitamin was identified in the diagrams (Figure 1). Subsequently, the $t_{on}$ value associated with each vitamin was recorded and incorporated into the calibration equation, as outlined in the stochastic method.

The standard addition method was used for the validation of the method using the disposable stochastic miniplatform for the assay of vitamins B5, B7, and B9 in water, urine, and hen's raw egg yolk. The three vitamins were determined in these samples as they were collected from the source and after synthetic mixtures of vitamins B5, B7, and B9 of known concentrations were added following their assay using the disposable stochastic miniplatform. The added amount was compared with the determined amount by using a disposable stochastic miniplatform. Recoveries higher than 98.00% were obtained with % RSD values lower than 1.00% (Table 3).

For the assay of the vitamins B5, B7, and B9 in pharmaceutical formulations Optisana and Supradyn, the


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**Table 3. Recovery Tests of Vitamins B5, B7, and B9 in Various Types of Samples Using the Disposable Stochastic Miniplatform ($N$ = 10)**

| sample type | %, recovery, vitamin | | |
|---|---|---|---|
| | B5 | B7 | B9 |
| water[a] | 98.94 ± 0.45 | 99.99 ± 0.01 | 98.04 ± 0.10 |
| urine[a] | 98.23 ± 0.21 | 99.98 ± 0.02 | 98.26 ± 0.34 |
| hen's egg yolk, raw[a] | 99.12 ± 0.04 | 98.48 ± 0.03 | 99.03 ± 0.04 |
| Optisana[b] | 99.17 ± 0.03 | 96.20 ± 0.02 | 94.02 ± 0.05 |
| Supradyn[b] | 97.55 ± 0.03 | 98.30 ± 0.04 | 98.40 ± 0.02 |

[a]Results reflecting the recovery of the vitamins vs the added amount. [b]Results reflecting the recovery of the vitamins vs the declared amount.

recovered amount was compared with the declared amount: recoveries higher than 94.00% were obtained with % RSD values lower than 0.10% (Table 3).

The recovery rates of vitamins B5, B7, and B9 from various types of samples were found to be significantly high. The data presented in this study demonstrate that the proposed miniplatform exhibits promising capabilities in the detection and quantification of vitamins B5, B7, and B9 in various sample types, including multivitamin tablets, pharmaceutical tablets, water, food (including raw egg yolk), and biological fluids (such as urine). The results obtained from this miniplatform exhibit a high level of accuracy.

## CONCLUSIONS

The disposable stochastic miniplatform was able to reliably analyze simultaneously vitamins B5, B7, and B9 in multivitamins and pharmaceutical tablets, water, food, and biological fluids. Its main feature is the utilization for uniformity content tests of vitamin pharmaceutical formulations, testing of food quality in supermarkets, checking the quality of water regarding the presence of vitamins B5, B7, and B9, and also performing biomedical analysis with the sending of results to mobile devices and further to specialized databases.

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### Notes

The authors declare no competing financial interest.

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