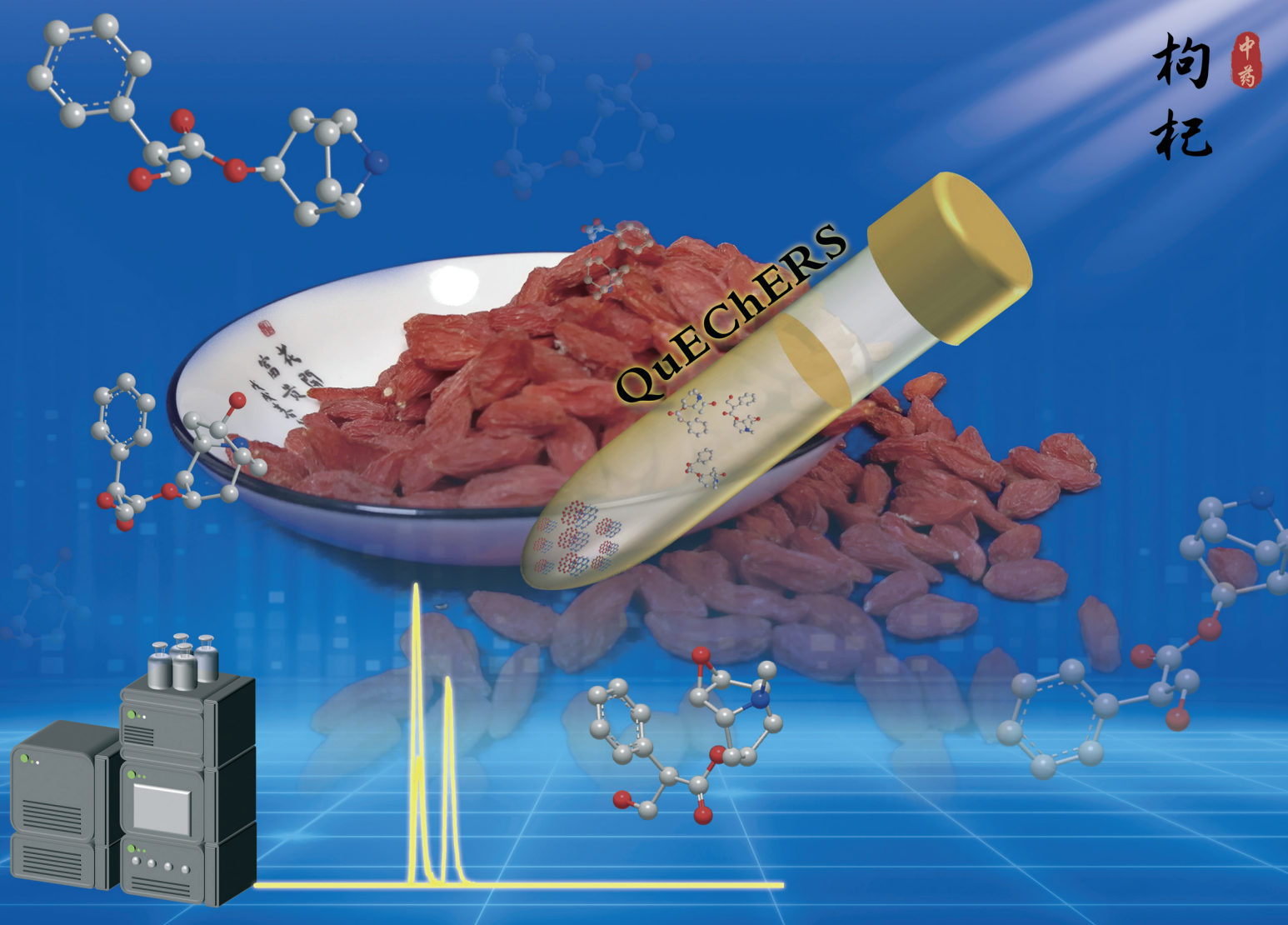


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RESEARCH ARTICLE

Open-tubular capillary electrochromatographic application of a sol-gel matrix with chilli peppers, garlic, or synthetic additives

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This article describes a possible combination of two promising fields of analytical chemistry—the preparation of sol-gel matrices with varying additives and their application in capillary electrochromatography. The inner surfaces of capillaries were coated with the sol-gel solution containing either pure synthetic chemical additive—alliin or capsaicin—or an extract of their natural sources—garlic and chilli pepper, respectively. The modified capillaries were tested for interaction with two neurotransmitters, oligopeptides and nucleotides under conditions of open-tubular capillary electrochromatography. Because both of the natural extracts also contain vitamin C and saccharose, the capillaries with sol-gel modifiers containing each of these substances were also tested. The obtained results from the perspective of changes in the electrochromatograms and the effective mobilities of analytes are discussed with respect to mild conditions both in the preparation process of the sol-gel matrix and during the separations.

KEYWORDS

chilli peppers, garlic, open-tubular capillary electrochromatography, silica sol-gel

1 | INTRODUCTION

Let the following article begin in the kitchen. Chilli peppers and garlic can be counted among very frequently used plants of our daily lives for preparation and seasoning of food. Historically, garlic has been used for medicinal purposes for over 5000 years as an effective drug to treat deafness, leprosy, constipation, and parasitic infec-

tions. Not so long ago, garlic was reported to be an effective agent providing therapeutic effects having bactericidal, antibiotic and fungicidal properties, and its anticancer activity against various tumour types has been reported [1, 2]. A summary of biologically active substances in garlic is as follows: water, fibre, sulfur compounds, adenosine, pectin, fructan, carbohydrates, fatty acids, essential amino acids, nicotinic acid, phospholipids, prostaglandins, lectins, enzymes, vitamins C, E, B₁, B₂, B₆ and minerals P, Zn, Se, K, Fe, Mg, Ca and Na [1, 3, 4]. The most important nonprotein amino acid in garlic is alliin with a content 0.2-2%, which undergoes many chemical reactions during cutting, chewing, crushing, all in conjunction with the pyridoxal phosphate-containing enzyme alliinase, giving other sulfur-containing compounds [3, 5]. A broad spectrum of biologically important compounds found in garlic and their positive effects on human health is nicely and widely summarized in numerous publications [1-4, 6].

Article Related Abbreviations: 5A, Ala-Ala-Ala-Ala-Ala; ACh-Cl, acetylcholine chloride; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; APG, Ala-Pro-Gly; ATP, adenosine 5'-triphosphate; CDP, cytidine 5'-diphosphate; CMP, cytidine 5'-monophosphate; CTP, cytidine 5'-triphosphate; EtOH, ethanol; GDP, guanosine 5'-diphosphate; GGH, Gly-Gly-His; GGTA, Gly-Gly-Tyr-Arg; GMP, guanosine 5'-monophosphate; GTP, guanosine 5'-triphosphate; L(En)₂, Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin); OT-CEC, open-tubular capillary electrochromatography; TEOS, tetraethyl orthosilicate; UMP, uridine 5'-monophosphate; UTP, uridine 5'-triphosphate

Several works have been published in terms of the interaction of garlic with biological samples. Garlic juice was published as a potential agent that disrupted a cell's electrochemical potential and induced apoptosis in yeast [7]. Another application of garlic clove water extract showed it to have antibacterial, antibiofilm, anti-inflammatory, and other activities *in vitro* without cytotoxicity, and thus, opened the promising potential for biomedical applications [8].

The second vegetable fruit, chilli peppers, is well known mainly due to the hot spicy taste (pungency) caused by capsaicin [9]. As well as garlic, chilli peppers are valuable plants (vegetables) because of their efficiency against various degenerative diseases—arthritis, rheumatism, stomach aches, and ulcers [10], cardiovascular health, blood glucose control, thermogenesis, weight management, and intestinal health [11, 12] and because they are rich sources of healthy substances [9, 10, 13, 14]. An extensive, detailed summary of the chemical constituents in chilli peppers can be found in [15], a brief list is phenolics, flavonoids (quercetin, myricetin, luteolin, apigenin, and kaempferol), carotenoids, vitamins A, C, E, B-complex, alkaloids—mainly capsaicin—and several related chemicals containing a series of homologous branched and straight-chain alkyl vanillylamides, collectively called capsaicinoids. Among the mineral constituents, Fe, Ca, K, Mg, P, S, Na, and Se can be found. Capsaicin or the extract of chilli fruits was reported to have antidiabetic, anticancer, antiulcer, anticoagulant, analgesic, antiarthritic activities, as well as immunomodulatory, cardiovascular, pain-relief effects and memory enhancing, respiratory, hepatoprotective, anthelmintic, antifungal and antiviral activity [15–17].

Although most of today's research is focused on the identification and characterization of active specific compounds of plant extracts, many hidden therapeutic molecules or a combination of them should be brought into the limelight [15]. Biological systems usually provide an intelligent combination of many parallel functional processes, which are mutually and dynamically connected to each other in optimized compliance [18]. The extraction of biological compounds from Mediterranean herbal and plant sources, and their placement in a stable carrier while keeping their biological activity is widely summarized in a review [19].

Silicon, the third most common trace element in the human body, plays an important role in the correct functioning of body parts, including especially bones, joints, tendons, hair, nails, skin and blood vessels, securing connective tissue elasticity. It also can eliminate "undesirable" microorganisms, heavy metals, and other waste from a body [20, 21]. Silicon in the form of silicic acid can be predominantly found in food of vegetable origin, like

cereals, legumes, vegetables, and herbs (field horsetail and nettle) [20].

By combining plant extracts with silicates under suitable conditions, we come to a widely evolving methodology of recent years—sol-gel technology. Over the past few decades, sol-gel technology has affected all sectors of industry with an increased interest in applications in science, engineering, and biomaterials [22]. Articles dealing with many aspects of sol-gel preparation and application have been published in large numbers, so it is necessary to visit the library to find out specific purposes of preparation [23–25]. The sol-gel method offers a simple and easy way to immobilize biomolecules in a porous, optically transparent silica matrix while keeping the functional activity of encapsulated biomolecules, especially due to the possibility of tailoring the conditions for specific requirements [26–28]. Bioactive substances in various parts of plants (root, seed, and flower), entrapped in a silica matrix, offer applications in the preparation of new materials, and finally may open new opportunities for optical and biomedical applications [22]. Various biocompatible sol-gel-derived materials have been published and showed significant versatility for the entrapment of a range of biomolecules, especially in the promising form of thin films [29], which was also applied in the open-tubular capillary electrochromatography (OT-CEC) separation technique as well [30, 31]. The latest trends in OT-CEC technology have also been published, including various types of capillary inner surface modifiers, with special attention paid to the separation of peptides and other physiological compounds [32–40]. Finally, the medical benefits of silica sol-gel nanomaterials, describing the whole process of biomimetic synthesis up to its practical application, have recently been published and opened up a promising field of action [41].

With the aforementioned benefits of peppers, garlic, and silicon, we reached the point of our interest. To our knowledge, the anchoring of chilli or garlic plant extracts into a sol-gel matrix and its further application as a stationary phase of a separation technique has not been reported. Thus, this work aimed to combine the following approaches: (a) to simply incorporate extracts from frequent natural plants into the sol-gel structure, (b) to apply these modified sol-gels as the stationary phases for OT-CEC analyses, (c) to observe and quantify changes (if any) in the electrophoretic mobilities of neurotransmitters, oligopeptides, and nucleotides, even though the time of interaction is presumed to be short.

It should first be noted that the results of the present study should be (primarily) understood as an initial study (or insight) of interactions between natural compounds and the basic building blocks of the human body. Apart from the two specific chemical substances characteristic of the two tested plants—capsaicin and alliin, another two

substances found in both of them—L-ascorbic acid (vitamin C) and saccharose—were tested as additives to the sol-gel matrix stationary phase. Because both natural extracts contain at least 200 compounds, these two other additional experimental studies should be considered as a very simple model study.

2 | MATERIALS AND METHODS

2.1 | Electrochromatographic analyses

The electrochromatographic analyses were performed on the Beckmann Coulter P/ACE 5500 (Fullerton, CA, USA) apparatus. Detection with photodiode array (PDA) detector was tuned to the wavelength 254 nm for nucleotides, 214 nm for neurotransmitters and oligopeptides, with bandwidth 10 nm. Alternatively, two cartridges with slightly different width of detection windows were used, that is, $100 \times 200 \mu\text{m}$ or $100 \times 800 \mu\text{m}$. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of total length 27 cm and effective length of 20.5 cm, $75 \mu\text{m}$ id, and $375 \mu\text{m}$ od were used for all separations. The injection was performed with the applied pressure (3.4 kPa). The capillary was cooled with liquid coolant (3 M Fluorinert™ FC-770, Zwijndrecht, Belgium).

2.2 | Chemicals and accessories

Nucleotides were obtained from Lachema (Brno, Czech Republic), Sigma-Aldrich (Munich, Germany) excluding AMP from Merck (Darmstadt, Germany). Tetraethyl orthosilicate (TEOS) was obtained from Sigma-Aldrich as well as neurotransmitters and all oligopeptides except for L-glutathione (Alexis, Lausen, Switzerland). Liquid chemicals, that is, ethanol (EtOH) and chemicals for the preparation of buffer—sodium carbonate and phosphoric acid—were from Lachema. Pure chemicals (+)-L-alliin and capsaicin were obtained from Sigma-Aldrich, saccharose and L-ascorbic acid were from Penta (Chrudim, Czech Republic). Dried chilli peppers (imports from India) and fresh garlic (grown in Czech Republic) were bought in the local market. Parafilm (Bemis, WI, USA) and plastic syringes (Chirana, Stará Turá, Slovakia) were also often needed. Plastic materials, such as the pipette tips (Vertex, CA, USA), Eppendorf tubes (Eppendorf AG, Hamburg, Germany), and nylon syringe filters (13 mm, $0.22 \mu\text{m}$, Labicom, Olomouc, Czech Republic), were used in all experiments. Milli-Q water from a Millipore Direct-Q UV3 Water Purification System (Millipore, Bedford, MA, USA) was used for all experiments.

2.3 | Preparation of modified capillaries for open-tubular capillary electrochromatography

2.3.1 | Preparation of sol-gel additives

The synthetic chemicals, which are capsaicin, alliin, saccharose, vitamin C (L-ascorbic acid), were dissolved in Milli-Q water in concentration 5 mg/mL except for capsaicin, which was dissolved in EtOH to the same concentration. Pieces of sliced garlic and chilli peppers were immersed in EtOH to give a final concentration of 0.3546 and 0.1401 g/mL, respectively.

The whole natural fruits (chilli peppers) or sliced garlic cloves (garlic without peel) were left to leach in pure ethanol for 2 months at room temperature in ground glass beakers covered with aluminium foil. Then, the appropriate liquid part was sucked up (aspirated) by syringe to a clear new vial and finally approximately 2 mL of ethanol extract filtered to the last vial from which the liquid extract was used for the sol-gel-modification procedure. The obtained filtered pure extracts of natural plants were optically clear and had attractive colours, moderate yellow for garlic and moderate orange of chilli peppers.

2.3.2 | Modification of the inner capillary surface

The modification procedure consisted of three steps:

- The fused silica capillary was prewashed with Milli-Q water, 1 mol/L NaOH, Milli-Q water, 1 mol/L HCl, Milli-Q water, ethanol and air flush, each step for 10 min.
- The standard solution of a sol-gel preparation contained the following:

50 μL Milli-Q H_2O , 100 μL EtOH, 50 μL TEOS, 50 μL of additive, 20 μL 0.1 mol/l HCl, all ingredients (components) were mixed, vortexed, and left to react for 20 min. After this part of the sol-gel preparation (a transparent clear solution was obtained), the capillary was washed with this solution using suction in such a way that when one end of the capillary was inserted into home-made tapering plastic tubing connected to an injection syringe, the other end was immersed in the vial with the sol-gel solution. After vacuum aspiration of the solution at the syringe end for 30 min, the ends of a capillary were sealed with parafilm and left at room temperature overnight. For the preparation of a capillary modified with pure sol-gel without any additive, 50 μL of

EtOH was added to the sol-gel solution instead of the additive.

- c. The next day, the rest of the inner wall modifier was sucked out of the capillary with a syringe, then the capillary was blown with air and nitrogen (5 min each, pressure 200 kPa) and left open at room temperature till experiments (usually 4 weeks).

No special condensation agent was added to accelerate the condensation step (3-aminopropyl)triethoxysilane, ammonia solution, or sodium hydroxide solution), to avoid cracking, fast, uncontrolled condensation and the sol-gel becoming opaque, to keep immersed additives in the natural form as much as possible and to avoid shielding of the functional groups of additives by the condensation agent.

2.3.3 | Conditioning (stabilization) of capillaries

After modification and resting at room temperature for 4 weeks, the capillaries were prepared for separation experiments. Before running with the mobile phase, each capillary was washed with water for 10 min and then finally with the mobile phase for 20 min.

All experiments were performed in 0.05 mol/L sodium carbonate solution, pH 7.40, adjusted with 1 mol/L phosphoric acid. The analyses were performed as follows: 2.5 μ L of one analyte was added to 20 μ L of distilled water in a microvial and one analysis was performed. Then, 2.5 μ L of the next analyte was added to the same microvial and further analysis was performed. In this way, the whole given mixture of analytes was tested sequentially to verify the migration time.

2.4 | Analytes

Neurotransmitters: ACh—Cl—acetylcholine chloride, HIAA—hydroxyindole acetic acid.

Oligopeptides: GGTA—Gly—Gly—Tyr—Arg, APG—Ala—Pro—Gly, Angiotensin (fragment 1–7, Asp—Arg—Val—Tyr—Ile—His—Pro), 5A—Ala—Ala—Ala—Ala—Ala, L(En)₂—Tyr—Gly—Gly—Phe—Leu (Leu-enkephalin), GGH—Gly—Gly—His, Thr—Tyr—Ser, glutathione were all dissolved in water to a concentration of 1 mg/mL. Because the absorbance at that concentration was too high, the stock solutions of analytes were diluted 10 times to the final experimental concentration of 0.1 mg/mL.

Nucleotides: Uridine 5'-monophosphate (UMP), adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), cytidine 5'-monophosphate

(CMP), adenosine 5'-diphosphate (ADP), guanosine 5'-diphosphate (GDP), cytidine 5'-diphosphate (CDP), uridine 5'-triphosphate (UTP), adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP). The final concentration of nucleotides dissolved in water and used for experiments was 0.5 mg/mL.

3 | RESULTS AND DISCUSSION

The results obtained by electrochromatography of neurotransmitters and oligopeptides in modified capillaries are shown in Fig. 1. For better clarity, it was necessary to divide the set of all electrochromatograms into two parts, according to the migration times of tested analytes in individual capillaries. Even more, the electrochromatograms were sorted according to their baselines obtained in the analyses because if they were arranged alphabetically by the names of sol-gel modifiers, they would be confusingly mixed. As can be seen in Fig. 1A, the separation of this group of analytes using capillaries with sol-gel modified with alliin (B) and capsaicin (C) had almost the same course except for the slower electroosmotic flow (EOF) in alliin-containing capillary (B). Nevertheless, comparing lines (B) and (C) with line (A) of the pure sol-gel-modified capillary, we can note several changes. Analytes Thr—Tyr—Ser (7) and GGH (8) are baseline—line (B) and almost baseline—line (C) separated, but not at all in line (A). Conversely, glutathione (9) and HIAA (10) are partially divided in line (A), but not at all in (B) and (C). Analyte angiotensin (3) goes separately in lines (B) and (C), not in line (A), compounds APG (4), 5A (5) and L(En)₂ (6) are at least partially separated in line (A), but completely united in lines (B) and (C). The lines (D), (F), and (G) reveal a very similar course of separations differing in duration. An interesting view on the separation of this group of analytes is the line (E) with the sol-gel containing the extract of garlic. First, the analytes GGTA (2) and angiotensin (3) run in one peak, followed with L(En)₂ (6). Analytes APG (4), 5A (5) and Thr—Tyr—Ser (7) form one peak, and then HIAA (10) emerged, which was usually observed as the last one in all other capillaries. Behind it, the remaining GGH (8) and glutathione (9) were detected. The changes in the effective mobilities of analytes calculated for particular capillaries can be seen in Fig. 2 (Top). The biggest changes, i.e. increase of the effective mobilities, were detected using pure alliin (B) and capsaicin (C) as sol-gel modifiers, as compared with the pure sol-gel (A). On the other hand, when extracts of the whole fruits were entrapped in the sol-gel matrix (D, F, G), no dramatic changes in effective mobilities were observed, except that for garlic extract (Fig. 2 (Top), line E). The changes of the relative effective mobilities caused by the various

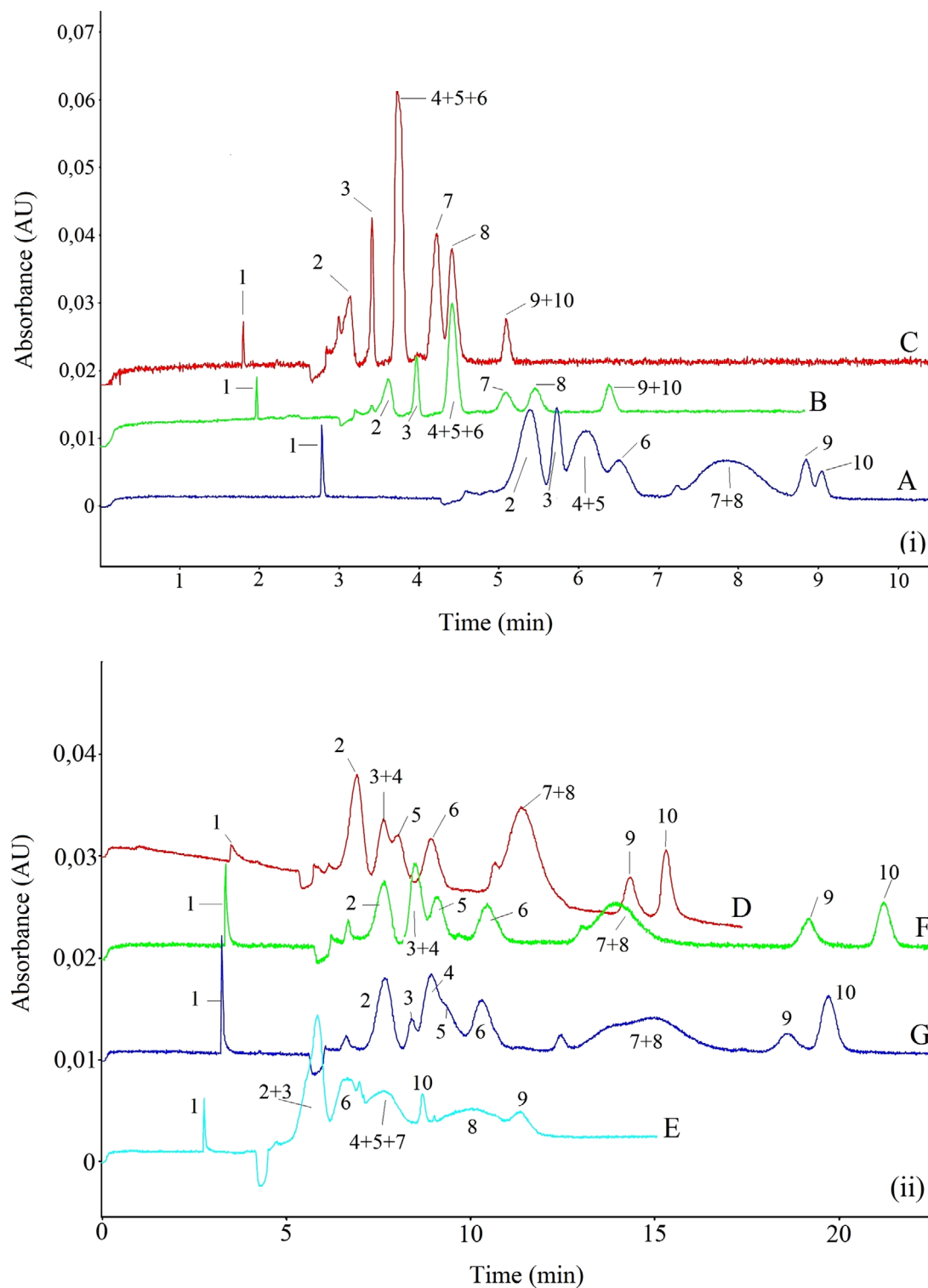


FIGURE 1 Separation of oligopeptides and neurotransmitters, mobile phase 0.05 mol/L $\text{Na}_2\text{CO}_3 + \text{H}_3\text{PO}_4$, pH 7.40, detection 214 nm (bandwidth 10 nm), 5 kV, normal polarity, 20°C, injection 3 s under pressure (3.4 kPa). Electrochromatogram: 1-ACh-Cl, 2-GGTA, 3-Angiotensin, 4-APG, 5-5A, 6-L(En)₂, 7-Thr-Tyr-Ser, 8-GGH, 9-Glutathione, 10-HIAA. (A) Separations in the range to 10 min (lines marked A–C), (B) separation in the range to 25 min (lines marked D–G). Marking of the modified capillaries: A—Pure sol-gel, B—Sol-gel + alliin, C—Sol-gel + capsaicin, D—Sol-gel + chilli extract, E—Sol-gel + garlic extract, F—Sol-gel + vitamin C, G—Sol-gel + saccharose

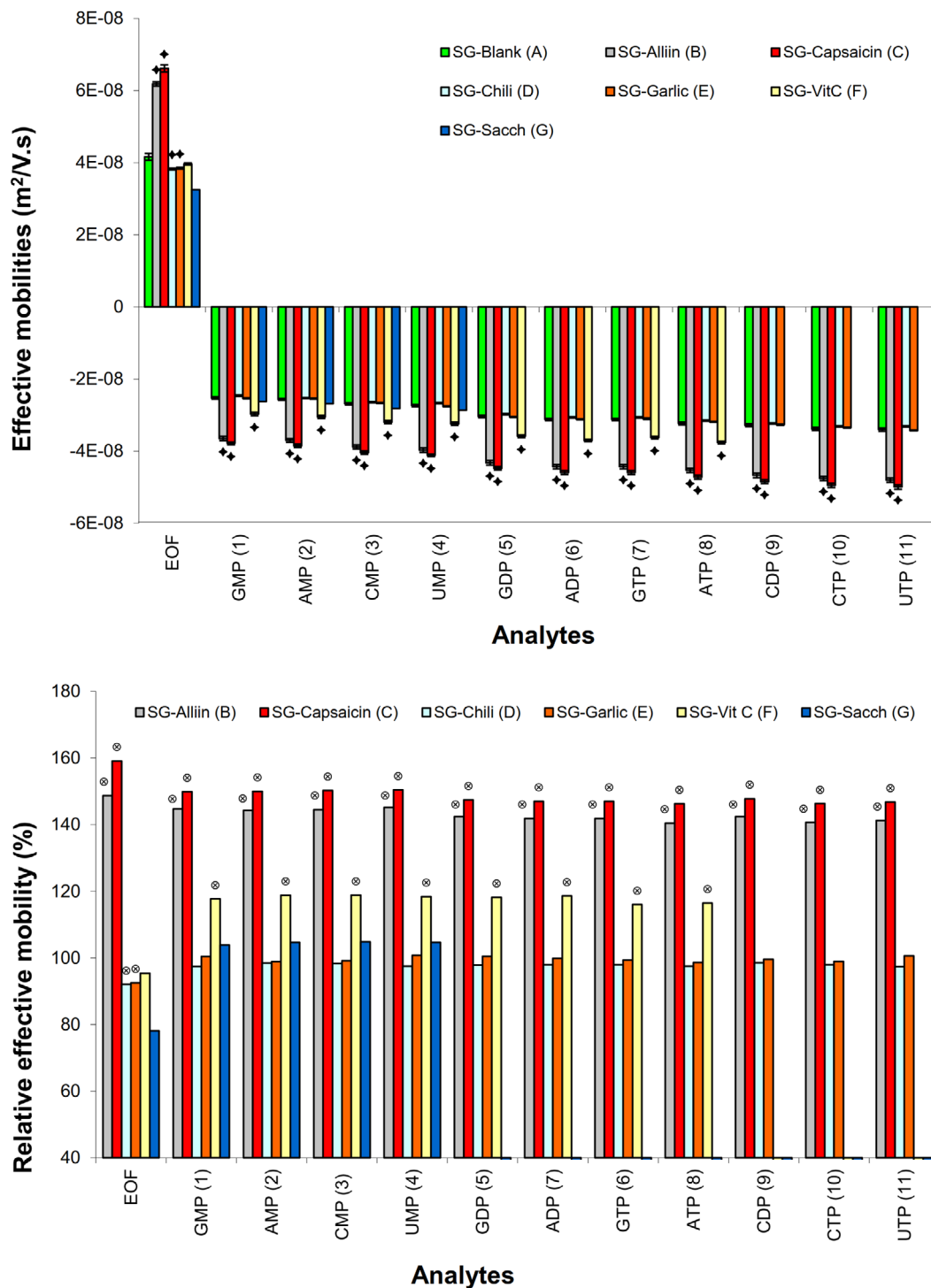


FIGURE 2 Top: Graph of the effective mobilities of neurotransmitters and oligopeptides in modified capillaries: **A**- $n = 3$, **B**- $n = 5$, **C**- $n = 4$, **D**- $n = 5$, **E**- $n = 4$, **F**- $n = 4$, **G**- $n = 4$; n -number of repeated analyses in a particular capillary. The calculation performed according to the t test, ♦ $P < 0.05$ as compared with the pure sol-gel-modified capillary (**A**). Bottom: Comparison of relative effective mobilities of separated neurotransmitters and oligopeptides in additive-modified sol-gel capillaries, related to the mobility in the pure sol-gel capillary (line **A**), which was taken as 100%. The mark ⊗ above the graph bars indicates a greater change in relative effective mobility than 5%, according to the relative standard deviation of the Gaussian normal distribution. The same basic experimental data as in Fig. 2 (Top) were used for the calculation

additives in sol-gel matrices were also calculated, as can be seen from Fig. 2 (Bottom). Looking at this graph, we can see that more significant increase in the relative effective mobilities of analytes can be observed only for modifications of sol-gel with alliin (B), capsaicin (C) and garlic (E), in the last-mentioned only for some analytes. In the remaining capillaries (D, F, G), various tested analytes had a relative effective mobility 25% lower or showed small fluctuations above or below the values of the pure sol-gel capillary, whose relative effective mobility was taken as 100%.

The results obtained during the separations of nucleotides are shown in Fig. 3. The electrochromatograms were divided into two parts again, for the same reason, as in the case of neurotransmitter and oligopeptide separations. The separations of nucleotides in capillaries modified by sol-gel with alliin (B) and capsaicin (C) took half the time of that in pure sol-gel capillary (A) (Fig. 3A), and were even more than three times shorter than in capillaries (D) and (E) (Fig. 3C). In addition, there were two major changes in Fig. 3B: when testing the capillary with immersed vitamin C (line F) in the sol-gel, only eight nucleotides were detected, while CDP, CTP, and UTP did not elute at all. The second change observed using saccharose as the sol-gel modifier (line G) revealed that only monophosphate nucleotides were detected, while di- and triphosphates were not seen at all until 80 min. Further analyses in the last-mentioned capillary brought unexpected difficulty, that is, probable leaking of the saccharose additive because the electric current values began to increase, and di- and triphosphonucleotides began to appear in consecutive electrochromatograms. The reasons for this phenomenon may be first, the insufficient attachment and ageing of the sol-gel inner wall modifier, second, the hydrolysis of saccharose in rather acidic sol-gel preparation solution, leading to easier leaking of the glucose and fructose from the original saccharose molecule and subsequent leaking from the sol-gel matrix. For these reasons, no calculation of the *t* test and repeatability for line G was stated in Fig. 4. The latter figure shows the changes in the effective mobilities of nucleotides in modified capillaries (Fig. 4 (Top)). The increased effective mobilities of nucleotides were achieved with alliin- (line B) and capsaicin-modified capillaries (line C) (as in the case of separations in Fig. 1A). Almost the same values of the effective mobilities were calculated for the nucleotides which have passed the detector when using the sol-gel-modified capillaries with the vegetable extracts and other modifiers, as compared with the pure sol-gel modification (line A) (Fig. 4 (Top)). The relative effective mobilities of nucleotides in individual capillaries, as compared with the pure sol-gel-modified capillary, are shown in Fig. 4 (Bottom). In summary, the capillaries

modified with sol-gel containing either alliin (B) or capsaicin (C) showed increased relative effective mobilities of nucleotides by approximately 40%, a bit lower than 20% for the capillary with incorporated vitamin C in the sol-gel matrix. The rest of the tested matrix-additives showed the same influence on the effective nucleotides' mobilities as in the case of pure sol-gel capillary with slight fluctuations into larger and smaller values.

For the complete evaluation of the effect of the extract presence, we can consider the noncovalent interactions between the analytes and the sol-gel modifiers. They are the following: hydrogen bond, π - π stacking interaction, donor-acceptor coordination bonds of metal ions-electron pairs, ionic bonds and either complete or partial combinations of these interactions. As mentioned in the Introduction section, the extracts of the tested plants provide a wide variety of these interactions, according to their composition.

3.1 | Benefits of the presented work

Following are the benefits of the presented work:

- Described easy preparation of a sol-gel and the procedure of capillary modification with a minimal amount of synthetic chemicals.
- Proven interaction with incorporated extract or pure chemical substance even during a "quick" separation technique.
- Proven different electrochromatographic separations between single pure chemical modifiers and the natural complex extract modifier.
- Sol-gels with extracts remain optically clear and capable of being measured with an ultraviolet detector.
- Even though the time of interaction of an analyte with the modified surface is not very long, there is still proven a non-negligible effect on the separation profile.
- Fully degradable material, not damaging nature.
- Interesting form of application for the modified sol-gel matrix (either containing pure chemically synthesized or naturally occurring mixture of compounds) can work in two tracks: first—interaction with a specific functional group, second—because porous silica sol-gel can work also as a "cleaner," the products of possible interactions can be easily and quickly removed.

3.2 | Limitations and future perspectives

Following are the limitations and future perspective:

- Not all of the individual components of natural extracts were tested, that is, a complete summary of

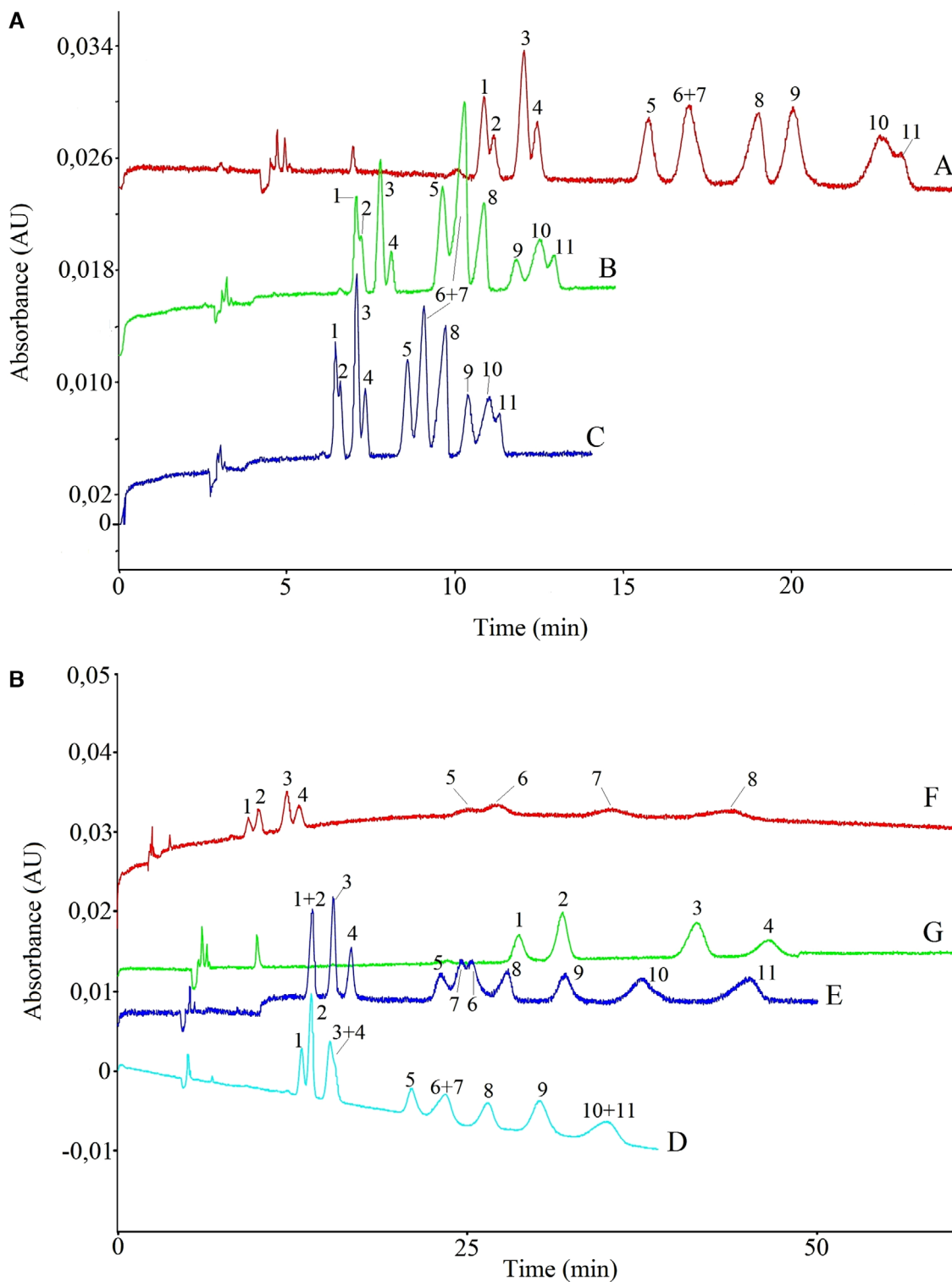


FIGURE 3 Separation of nucleotides, mobile phase 0.05 mol/L $\text{Na}_2\text{CO}_3 + \text{H}_3\text{PO}_4$, pH 7.40, detection 254 nm (bandwidth 10 nm), 5 kV, normal polarity, 20°C, injection 3 s under pressure (3.4 kPa). Electrochromatogram: 1-GMP, 2-AMP, 3-CMP, 4-UMP, 5-GDP, 6-GTP, 7-ADP, 8-ATP, 9-CDP, 10-CTP, 11-UTP. (A) Separations in the range to 25 min (lines marked A–C), (B) Separations in the range to 60 min (lines marked D–G). Marking of the modified capillaries: **A**—Pure sol-gel, **B**—Sol-gel + alliin, **C**—Sol-gel + capsaicin, **D**—Sol-gel + chilli extract, **E**—Sol-gel + garlic extract, **F**—Sol-gel + vitamin C, **G**—Sol-gel + saccharose

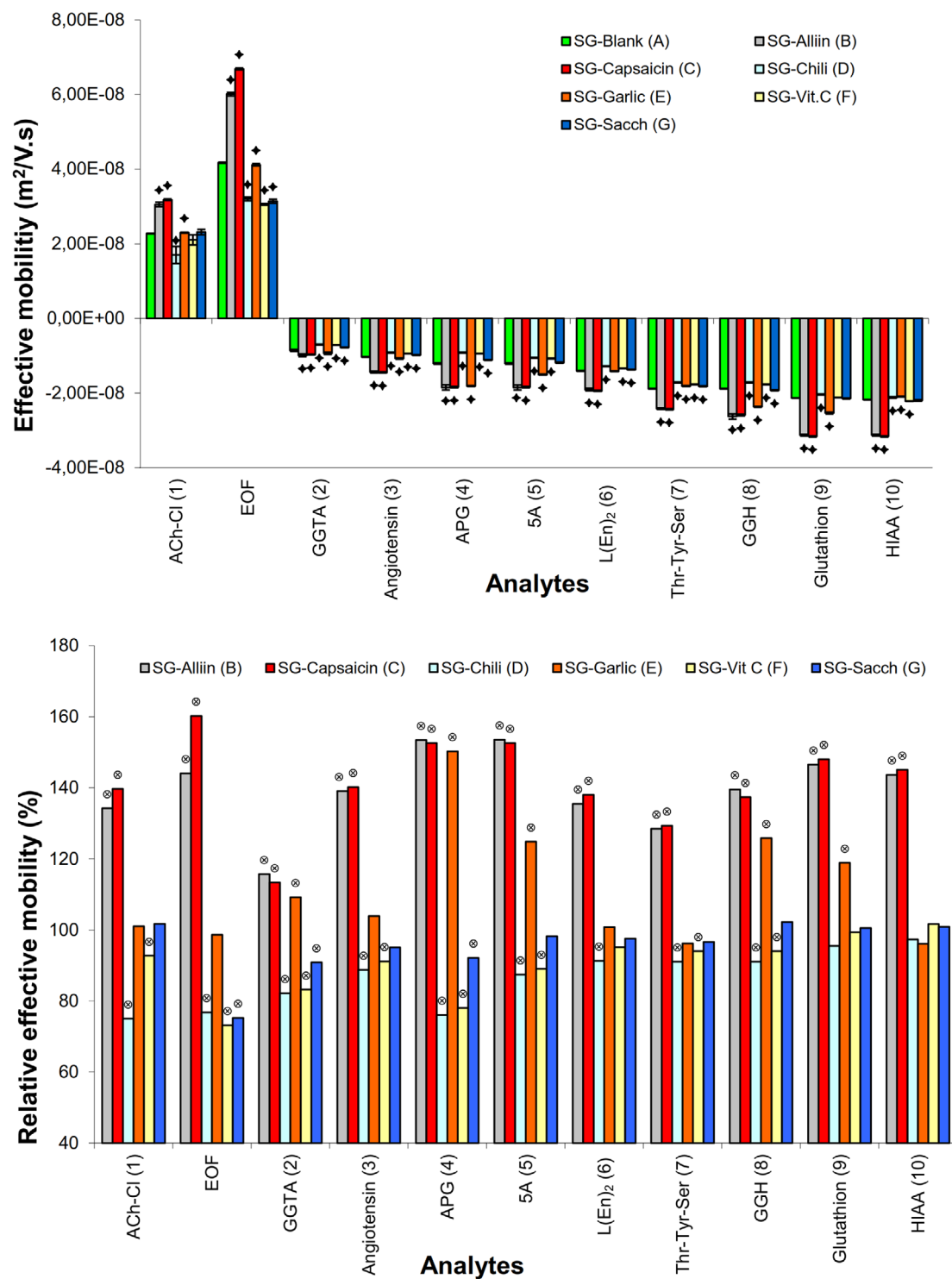


FIGURE 4 Top: Graph of the effective mobilities of nucleotides in modified capillaries: **A**- $n = 3$, **B**- $n = 3$, **C**- $n = 3$, **D**- $n = 3$, **E**- $n = 4$, **F**- $n = 5$, **G**- not calculated, n -number of repeated analyses in a particular capillary. The calculation performed according to the t test, $\blacklozenge P < 0.05$ as compared with the pure sol-gel-modified capillary (**A**). Bottom: Comparison of relative effective mobilities of separated nucleotides in additive-modified sol-gel capillaries, related to the mobility in the pure sol-gel capillary (line **A**), which was taken as 100%. The mark \otimes above the graph bars indicates a greater change in relative effective mobility than 5%, according to the relative standard deviation of the Gaussian normal distribution. The same basic experimental data as in Fig. 4 (Top) were used for the calculation

particular electrochromatograms was not obtained, rather an approximate estimation because it would take a much longer time to test every single chemical substance of the extract (approximately 200) as a sol-gel modifier.

- Although the results of electrochromatography separations do not look ideal—the minimum number of sharp peaks or baseline separations—the individual concentration of pure chemical and the differing concentration in the whole plant extract also has to be considered.
- Different amounts of modifiers were tested (not only measured by weight but also the percentage of an individual compound in the whole plant), a wide range of the involved substances in the case of extract-modified sol-gels.
- We do not know exactly how the extract of a particular plant can be organized in the sol-gel matrix and it is difficult to say if it is possible to find out.
- There is a question if a more concentrated extract of the plants could be entrapped into the sol-gel without changing the PDA detection capability.
- Regarding the electrochromatograms obtained with capillaries modified with sol-gels containing plant extracts, and comparing the effective mobilities of each analyte with those observed in a pure sol-gel matrix, it looks like nothing interesting happened. It leads us to the idea or hypothesis that many of the individual components of the extract can play a specific role, causing either accelerating or slowing down the electromigration of an analyte, finally giving almost no or minimal effect.

4 | CONCLUDING REMARKS

Sol-gel modifications with extracts from different plants offer a wide field of study, for example, their interaction with a wider spectrum of physiologically important compounds, modulation of the sol-gel ratio of basic chemical components and applications to various analytical methods. Another direction of research could be to find out if the possible combination of other plant extracts could bring more benefits. The plant extracts from bark, roots, stems, leaves, flowers and seeds could also be involved in experiments. Because both natural plants and their extracts are so beneficial to health, it offers the combination that could be used in the medicinal field.

This article seems to have raised more questions than answers. Nature offers a near-endless spectrum of chemical compounds waiting to be fully applied in practical life, and where their mutual interactions also play an important, if not the highest role.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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