DEVELOPMENT OF NEW COST-EFFECTIVE MICROFLUIDICS METHOD FOR L-CARNOSINE DETERMINATION IN DIETARY SUPPLEMENTS

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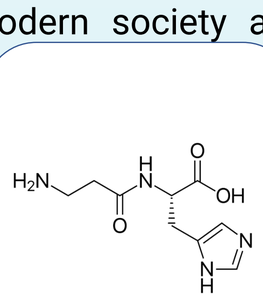
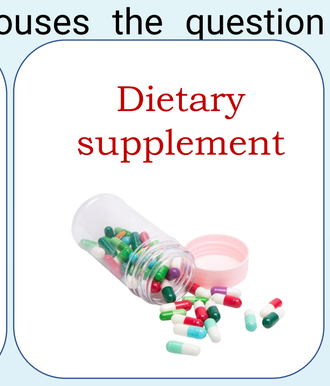
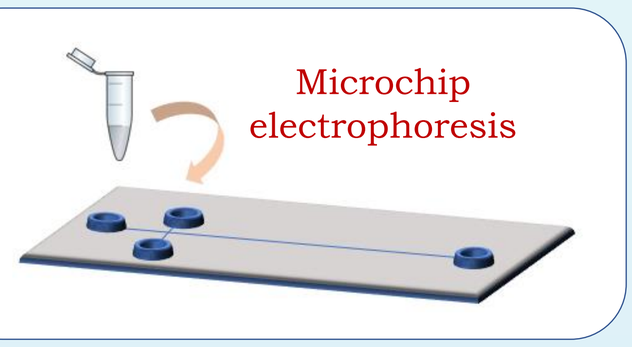
Introduction

Nutritional science emphasizes small peptides as a vital component in the human diet that, besides dietary importance, also have substantial medical significance. Thus they have broad potential therapeutic applications and are used as supplements or functional food ingredients. L-carnosine is a dipeptide used in dietary supplement formulations to improve endurance during intense exercise. However, expanding usage of such products in today's modern society arouses the question regarding their safety. This study aimed to develop new microfluidic methods capable of avoiding the common problems of conventional methods used to analyze small peptides in dietary supplements. Hence, L-carnosine detection method was evolved on microchip electrophoresis (MCE) technology conjugated with capacitively coupled contactless Conductivity detection system (C^4D).







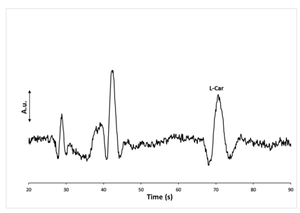
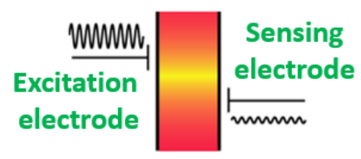


Fig. 1. ME/C^4D electropherogram for L-carnosine dietary supplement (1). MCE conditions: 0.5 M HAc background electrolyte (pH 2.53), electrokinetic injection for 20 s and +2.5 kV separation voltage.



Materials and method

Analysis were performed using MCE devise model ET121 (eDAQ) that incorporated ER430 High Voltage Sequencer (HVS) and ER225 C^4D system. Double-T poly(methyl methacrylate) chip (ChipShop GmbH) with 80 mm microchannel length and 50 x 50 µm cross-section was used for separation. ET121 platform contained four circular points for electrical contacts with C^4D circuity. Two contacts were used as excitation and receiver electrodes, while the other two acted as reference electrodes to minimize the stray capacitance. The chip microchannel was positioned on top of the gold C^4D electrodes electrodes and 40 µL of saple/background electrolyte (BGE) were placed into the chip reservoirs. Detailed optimization of L-carnosine determination method was performed in several stages. The linear response region for L-carnosine was determined using linear regression.

Results

The results indicate the capability of the MCE-C^4D method to quantify L-carnosine in the examined concentration range of 2.5 – 25 µmol/L, achieving adequate accuracy, high linearity, and high resolution. Among various organic acids and their mixtures, the most appropriate background electrolyte (BGE) was 0.5 M acetic acid. The best separation, peak size, and peak symmetry were obtained when an injection voltage of 1 kV was applied for 10 s and a separation voltage of 2.75 kV for 240 s. The proposed parameters were utilized to analyze L-carnosine in two different capsule dietary supplement samples. Obtained electropherogram is shown in Figure 4. The determined concentrations are summarized in Table 2. and show the successful quantification of L-carnosine compared to the data indicated on the label.

Conclusion

A new approach for quantitatively determining L-carnosine was developed using contactless conductivity detection with MCE instrumentation. It was successfully applied in analyzing L-carnosine in two commercial dietary supplement samples. The analytical characteristics of the proposed method showed great potential for further analysis of peptides in these types of samples. This achievement could benefit the nutritional or therapeutic drug industry cause the method is simple, fast, and cost-effective. Furthermore, the MCE-C^4D is environmentally friendly, and there is no requirement for the derivatization process to avoid the matrix effect and interference of additional active ingredients.

Table 1. Analytical characteristics of the ME/C^4D methods.

Characteristics	L-carnosine
Migration time (s)	73
R^2	0.9801
Linear range (mol L^{-1})	2.5×10^{-6} - 2.5×10^{-5}
Slope*	48758
y-intercept*	0.2652
LOD (mol L^{-1})	2.5×10^{-6}
LOQ (mol L^{-1})	2.5×10^{-6}

Output signal: arbitrary units: concentration unit: mol / L.

Table 2. Results of the quantitative determination of L-carnosine in dietary supplements.

Dietary supplement / Active supstance	Determined value (mg/ mg sample)	Declared value (mg/ mg sample)
1 / L-carnosine	$0.142 \pm 2 \times 10^{-3}$	0.142
2 / L-carnosine	$0.807 \pm 2 \times 10^{-3}$	0.833

References

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[2] Smriga M. (2020), International Regulations on Amino Acid Use in Foods and Supplements and Recommendations to Control Their Safety Based on Purity and Quality, *J Nutr* 150, 2602S–2605S.

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