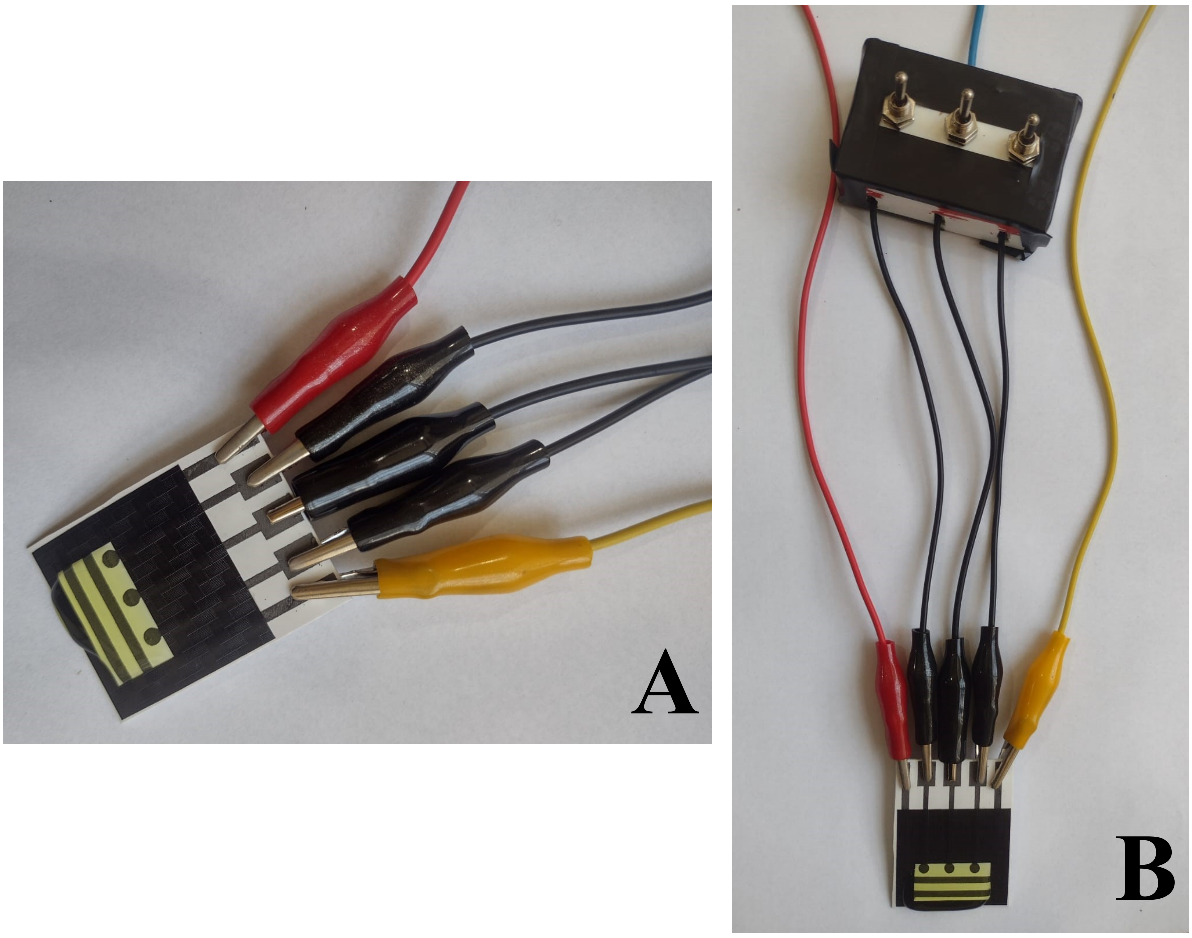
Supplementary Material

Stencil-printed multiplex system for electrochemical determination of Carbendazim, Ascorbic Acid and Glucose in juice samples

Ana Paula Z. Moscardi[a]; *Luís M.C. Ferreira*[a,b]; *Gustavo Martins*[a], *David R.C.D. de Paula*[a], *Anne Raquel Sotiles*[c,d]; *Fernando Wypych*[d]; *Márcio F. Bergamini*[a*], *Luiz Humberto Marcolino-Junior*[a**].

SUMMARY

*Corresponding authors: *bergamini@ufpr.br (MFB); **luiz1berto@ufpr.br (LHM)



S1. Screen-printed multiplex system

Fig. S1. Representative photographs of a screen-printed multiplex system connected to potentiostat cables (A). An expanded image shows the working electrodes integrated into a multichannel analog controller, where each working electrode can be evaluated both separately and in combination (B). In both images, there are 100 µL of a 1 mM ferricyanide solution over the electrodes.

S2. Effect of different treatment of SPE on electrochemical response

Initially, the printed electrodes were subjected to potential sweeps from -1.5 to +1.5 V, using CV, in a 0.1 mol L^{-1} NaOH solution, with a scan rate of 50 mV s^{-1}, for 20 cycles. Then, different electrochemical conditions (voltammetric and amperometric) were evaluated, as illustrated in **Fig. S1**. These treatments aimed to maximize the electrode performance, leading to an improvement in the electrochemical responses.

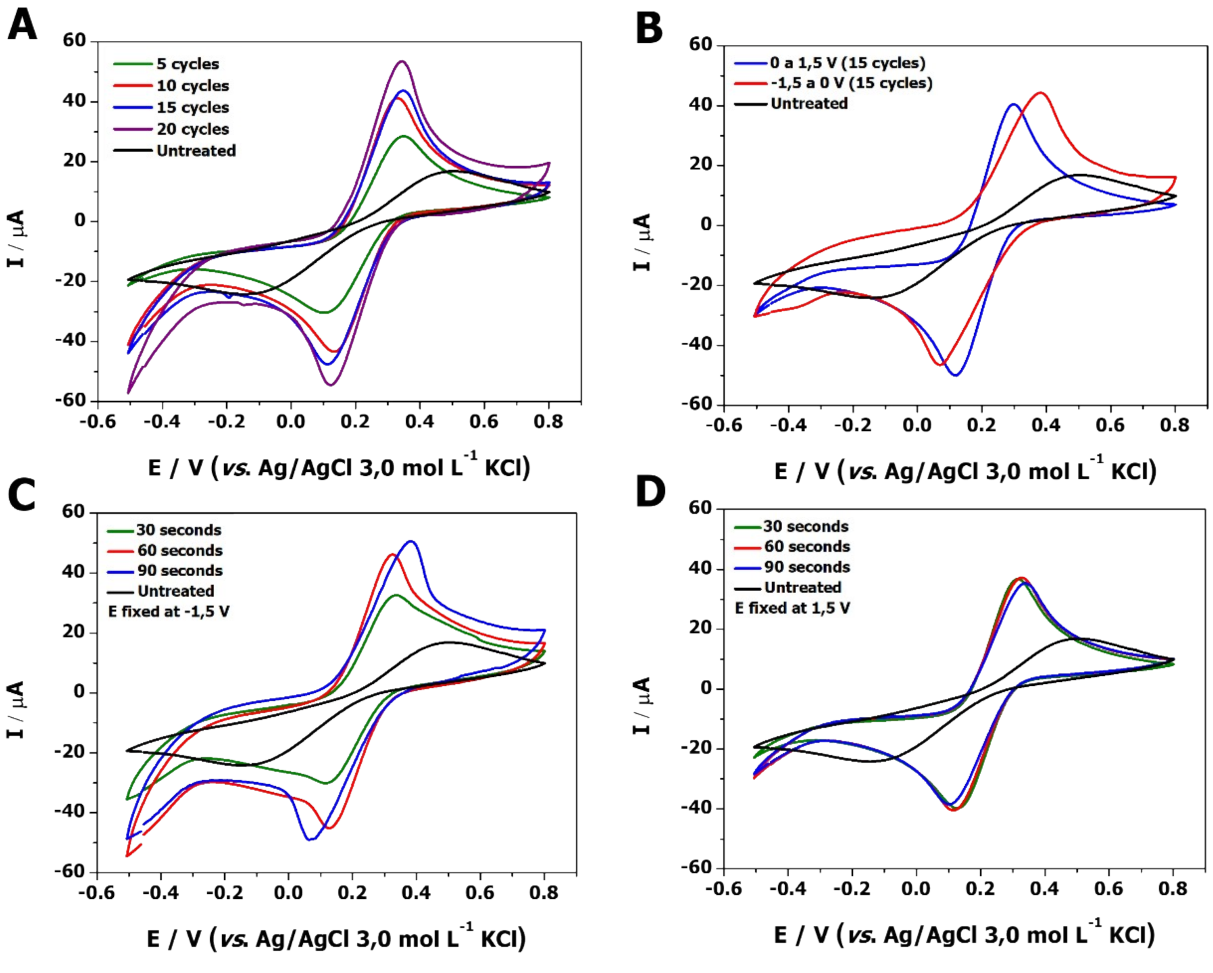


Fig S2. Cyclic voltammograms using $K_3[Fe(CN)_6]$ as the electrochemical probe (5 mmol L^{-1} in 0.1 mol L^{-1} KCl, v = 50.0 mV s^{-1}) after different electrochemical treatment conditions in 0.1 mol L^{-1} NaOH solution. A) Different numbers of voltammetric cycles. B) Different scan potential ranges (positive and negative direction). C) Potential fixed at -1.5 V at different times. D) Potential fixed at +1.5 V at different times.

The response of this treatment was studied using different numbers of cycles as shown in Figure S2 (A). From the analysis of the voltammograms, an increase in peak currents can be observed with an increase in the number of cycles. However, a more capacitive trend is also observed as the number of scans increases. This effect affects the faradaic current, masking the signal, and can lead to problems in electroanalytical assays aiming for low detection limits. Additionally, another unfavorable aspect of this treatment was the execution time, which was about 20 minutes. Thus, due to these limitations, other options for electrochemical treatments were explored to reduce treatment time and improve the electrochemical profile.

The second treatment studied (Figure S2 (B)) involved cycling with a variable scan range, both in the positive direction (0 to 1.5 V) and in the negative direction (0 to -1.5 V). There is no significant difference observed in the peak current values between the different scan directions; however, the cathodic scan is responsible for increasing the capacitive profile of the electrode.

The treatments using constant potential application were evaluated (Figure S2 (C) and (D)). The potentials were fixed at -1.5 V and 1.5 V with intervals ranging from 30 to 90 seconds. In this case, the execution time was considerably reduced compared to CV treatments, which ranged from 15 to 20 minutes. From this evaluation, it was observed that the constant application of -1.5 V potential (Figure S2 (C)) led to an increase in peak currents accompanied by an increase in the capacitive nature of the electrode, also observed in the cathodic voltammetric treatments shown in S1 (B).

Finally, in the treatment with a constant potential of +1.5 V (Figure S2 (D)), applying the potential for intervals longer than 30 s did not significantly affect the electrode response and showed the best electrochemical profile compared to other treatments, featuring well-defined peaks and smaller separation between them (lower ΔE). Additionally, the capacitive effect was minimized, there was an increase in electrical current signal compared to the untreated electrode, and a shorter treatment time (30 seconds). Thus, the electrochemical treatment performed at constant potential (application of fixed potential +1.5 V for 30 seconds) was adopted in subsequent studies. These effects could be attributed to a possible loss of binder on the electrode surface, which could result in an improvement in charge transfer from the electrode due to greater exposure of the conductive material.

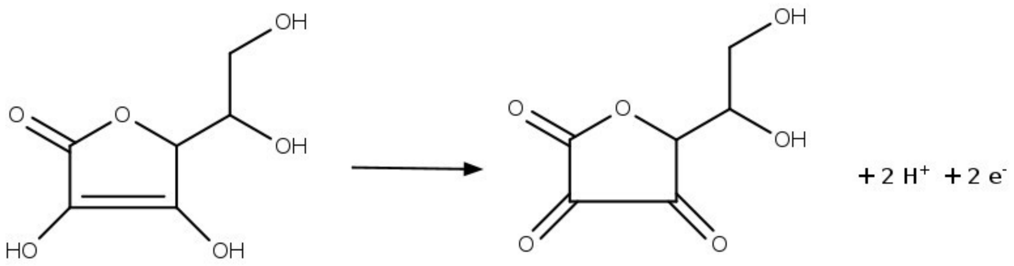
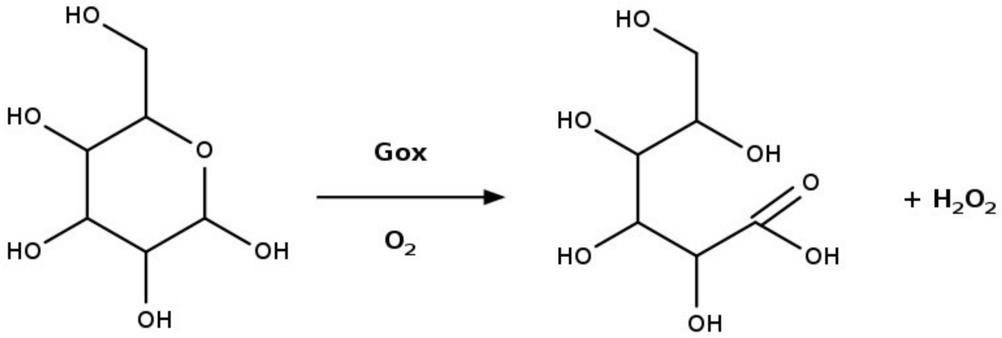


Fig S3. Electrochemical oxidation reaction of ascorbic acid.



$$H_2O_2 + PW \longrightarrow H_2O + PB$$

$$PB + e^- \longrightarrow PW$$

Fig S4. Reactions involved in the process for glucose detection using proposed biosensor using Prussian Blue (PB) and Prussian White (PW) as mediator.

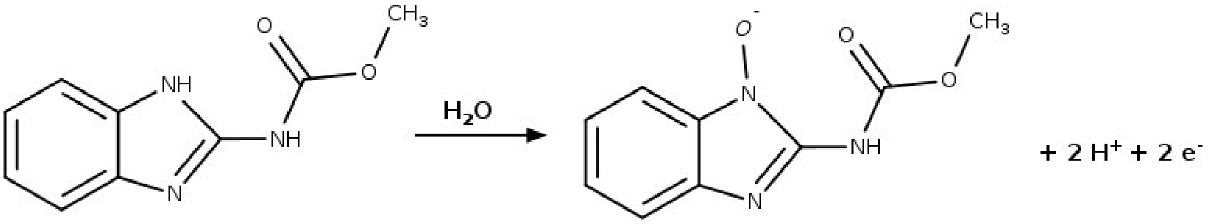


Fig S5. Suggested oxidation reaction of Carbendazim (CBZ).

S3. Influence of experimental parameters (ink composition, pH effects, and accumulation time) on carbendazim response

Layered double hydroxides (LDHs) have adsorptive, cationic and anionic exchange properties, allowing for ex situ pre-concentration of CBZ on the electrode surface, resulting in an increase in the analytical signal. In this context, three different pre-concentration times were evaluated. Due to CBZ exhibiting quasi-reversible behavior under the scan rate condition in CV assays, subsequent studies were conducted using SWV to improve the sensitivity of the methodology. In this study, both unmodified printed electrodes and those modified with LDH at a ratio of 15% (w/w) were examined. Additionally, electrodes modified with different proportions of delaminated LDH (dLDH) were analyzed. The results are shown in Fig. S3.

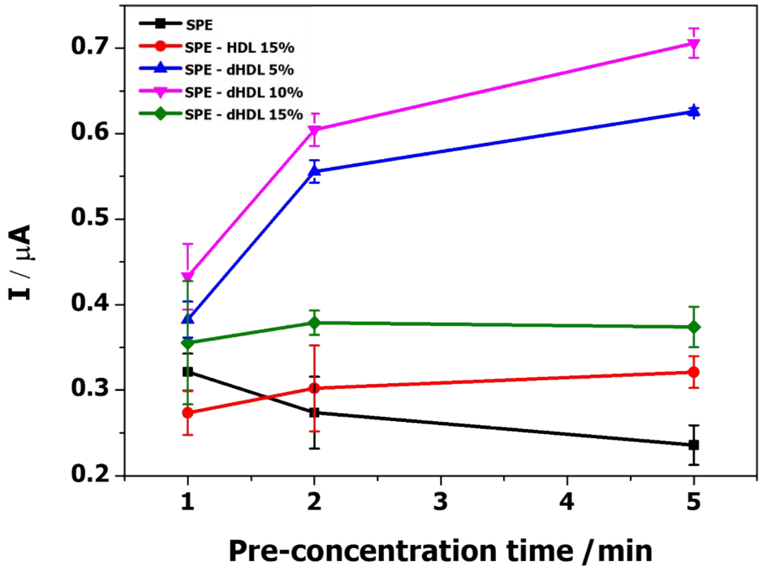


Fig S6. Peak anodic current intensities obtained for CBZ as a function of different pre-concentration times and different proportions of LDH.

The results showed that the presence of the modifier on the proposed electrode leads to an improvement in the response signal compared to the unmodified electrode. For non-delaminated LDH, significant improvements were not achieved compared to the conventional electrode, although there was a signal variation with accumulation time. On the other hand, as the proportion of dLDH was increased, a substantial increase in signal intensity was observed, which may be consistent with an increase in the active surface area capable of adsorbing the analyte. The maximum current value was generated on the printed electrode made with 10% (w/w) dLDH, after which the value starts to decrease. The signal decay may result from the loss of sensor conductivity since the modifier is not an electrical conductor. As graphite (conductor) is replaced by dLDH in higher proportions, the insulating character of the modifier material prevails, leading to a decrease in the recorded current. Therefore, electrodes modified with 10% (w/w) dLDH and a pre-concentration time of 2 minutes were chosen. The increase in electrical signal observed for a pre-concentration time of 5 minutes did not show a significant enough improvement to justify the increased time dedicated to this stage of the process.

A study was conducted to assess the influence of pH on the sensor response. The same supporting electrolyte studied was used in both the pre-concentration and reading stages. Buffer solutions (BR) were prepared for the study in the pH range of 3.0 to 10.0, which were used to pre-concentrate a 10 µmol L^{-1} CBZ solution and also for the electrochemical measurement in buffer. The results obtained are presented in S4.

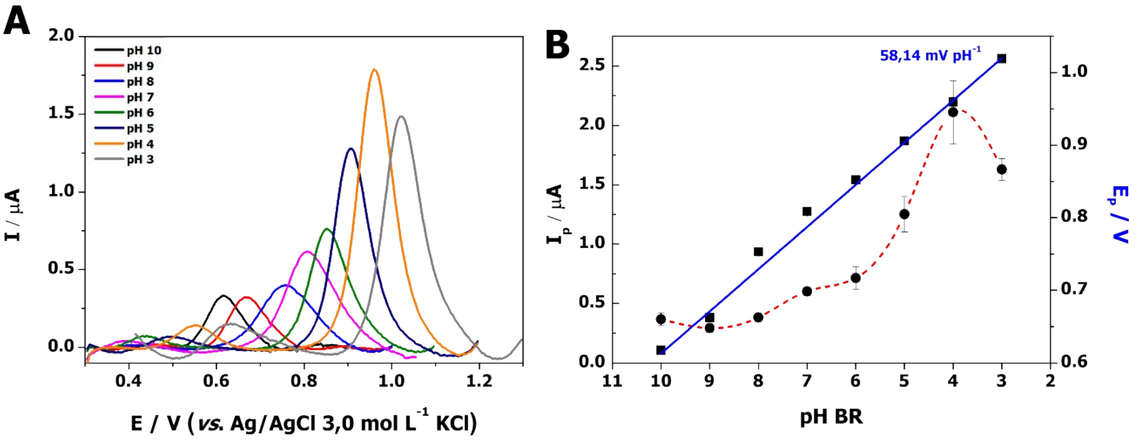


Fig S7. SWV voltammograms (A) and peak anodic current intensities obtained for CBZ as a function of different pre-concentration and reading pH values with the slope value obtained from potential vs. pH (B) (n=3).

The results demonstrate that the analytical signal increases as the pH decreases, reaching its maximum value at pH 4.0. However, a greater dispersion was observed among replicates at this point. This behavior suggests that the interaction between CBZ and dHDL may be of an electrostatic nature, as the analyte's pKa is around 4.53, leading to an increase in the concentration of positively charged (protonated) CBZ in regions near pH 4.0. This chemisorption occurs probably at the edges of the layered particles. On the other hand, the analytical signal decreases at lower pH values, such as pH 3.0, which can be explained by possible dHDL degradation due to an acid-base reaction. In these more acidic conditions, dHDL may not be stable, resulting in a reduction of the analytical signal.

Regarding peak potentials, it was found that the oxidation potential shifts to more positive values as the pH decreases. The slope of the curve revealed a value of 58.14 mV pH^{-1}, indicating that the reaction involves an equal number of electrons and protons. The reaction suggested by Periyasamy et al.[1] follows the observed behavior and is described with two electrons and two protons during CBZ oxidation. Therefore, considering the results, pH 5.0 was defined as suitable for the analyses since it approximates the pH values used to analyze the other target analytes of this device, aiming for multiplex system. Additionally, this pH condition favors the adsorption of the fungicide on the electrode surface without modifier degradation**.**

S4. Effect of matrix on sensitivity of proposed sensors

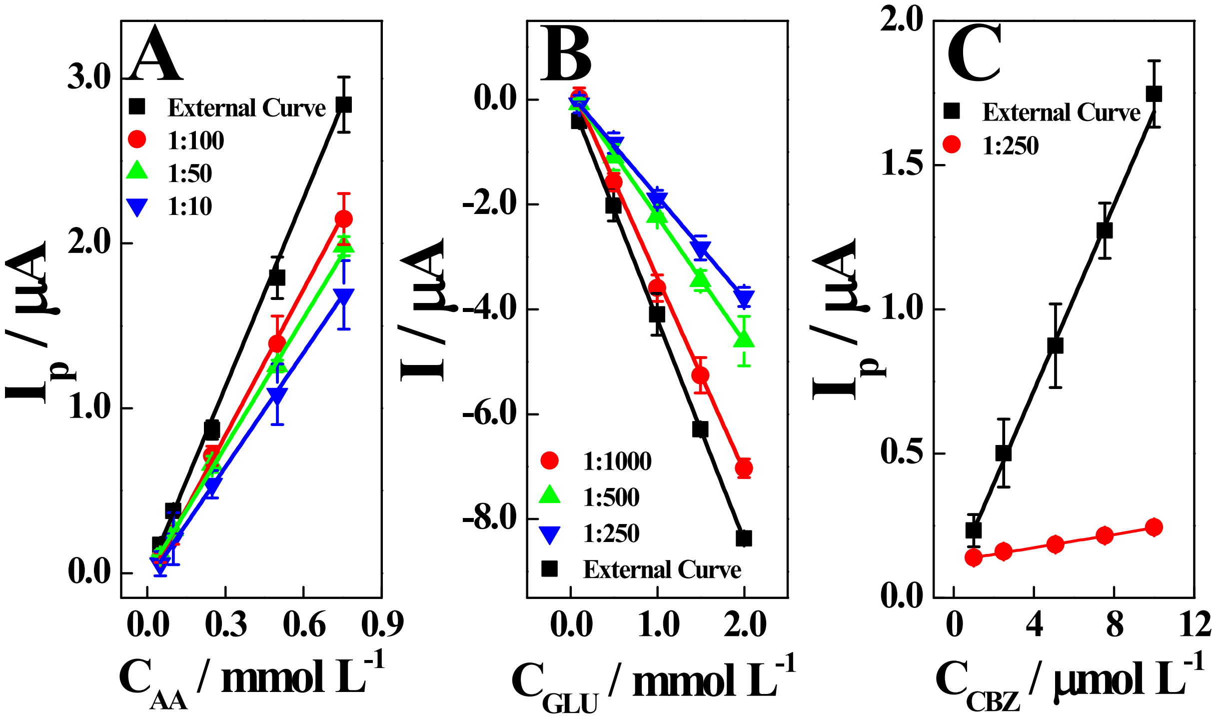


Fig. S8. Comparison of calibration curves constructed in the absence and in the presence of commercial orange juice matrix to evaluate the effect of ascorbic acid matrix at different dilutions (1:10, 1:50, and 1:100) (A). Comparison of calibration curves constructed in the absence and in the presence of commercial orange juice matrix to evaluate the effect of glucose matrix at different dilutions (1:100, 1:250, and 1:500) (B). Comparison of calibration curves constructed in the absence and in the presence of commercial orange juice (1:250) matrix to evaluate the effect of carbendazim matrix (C).

Reference:

1. PERIYASAMY, S.; VINOTH KUMAR, J.; CHEN, S.-M.; ANNAMALAI, Y.; KARTHIK, R.; ERUMAIPATTY RAJAGOUNDER, N. Structural insights on 2D gadolinium tungstate nanoflake: a promising electrocatalyst for sensor and photocatalyst for the degradation of postharvest fungicide (carbendazim). **ACS applied materials & interfaces**, v.11, n.40, p.37172. 2019.