

Comparative Study on Voltammetric and Spectrofluorimetric Methods for Fluorescein Detection

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The aim of this research was assessment of suitability of both methods voltammetric and spectrofluorimetric to fluorescein detection. Electrochemical activity of the analyte was examined in the supporting electrolytes of three different pH values due to a strong dependence of fluorescein properties on pH of the sample. Moreover, the electrochemical behavior of fluorescein was studied using different materials of working electrode – graphite paste, graphene paste and glassy carbon. Experiments performed using voltammetric methods indicated that fluorescein is electrochemically active. The influence of pH on its electrochemical activity was confirmed. Fluorescein undergoes one- or two-step irreversible electrochemical oxidation in the considered potential range from 0 to +1.4 V, exhibiting the decrease of peak current with the increase of sample pH. Differential pulse voltammetric measurements enabled successful detection of fluorescein at micromolar concentration levels at graphene and graphite sensors, with the following limit of detection values: for graphene sensors 7.4 μM at pH 4.5, 34.55 μM at pH 7, 23.42 μM at pH 9; for graphite sensors 6.44 μM at pH 4.5, 42.27 μM at pH 7, 95.12 μM at pH 9, whereas results obtained using glassy carbon electrodes were less satisfactory. Comparative study of electrochemical and optical methods for fluorescein detection indicated voltammetry as more suitable for measurements in the acidic solutions, whereas spectrofluorimetry was more advantageous in the case of alkaline media. Thus, we conclude that for quantitative detection of fluorescein in aqueous solutions, both voltammetric and spectrofluorimetric methods are suitable, since complement each other – when associated, they allow to detect fluorescein in wide pH range. On the basis of the obtained results we stated that pH value of the sample affects the utility of each method due to its effect on electrochemical and optical features of fluorescein. Therefore, when choosing the most adequate method – electrochemical or optical, for measurements based on fluorescein detection, pH of the sample should be taken into account, primarily.

Keywords: electrochemical activity; fluorescein; voltammetry; spectrofluorimetry; thick-film amperometric electrodes.

1. INTRODUCTION

Fluorescein (FLSC) is commonly used in variety of applications, especially as a fluorescent label in biochemistry, for detection of e.g. antibiotics or proteins [1-3]. It is known from excellent optical and fluorescent properties such as high quantum yield of fluorescence and good photostability. Functionalization of free acid form with isocyanate or succinimidyl group provide covalent coupling with target molecules such as amino acids, thus contributes in obtaining high sensitivity of determination via various analytical techniques e.g. capillary electrophoresis, fluorescence spectroscopy, high performance liquid chromatography [4-7]. Noteworthy, fluorescein features are strongly depend on pH of the sample [8, 9]. Four basic ionic forms can occur: cation in solutions of pH under 2, neutral form at pH of about 3.3, anionic form in slightly acidic solutions (pH ~ 5.5), and dianions above pH 8. More importantly, these forms coexist in the solution, and their content is pH-dependent. Taking into consideration an absorptive properties of FLSC, the dianionic form is characterized by the highest absorption with the peak at wavelength of 490 nm and wide plateau near 475 nm. In the case of anions, absorption peaks at wavelengths of about 472 and 453 nm appear to be lower compared to dianions. On the other hand, cationic form exhibits relatively small absorption at wavelength of 437 nm, however, the weakest absorptive properties are attributed to neutral form (absorption peak at 434 nm). Similarly, when fluorescence is considered – dianionic form in alkaline media is related with the highest fluorescence, whereas the occurrence of cationic form correspond to the weakest fluorescence. Interestingly, in highly acidic conditions (pH about 1.5-3) under excitation with the wavelength of 430 nm, cationic and anionic forms undergo immediate deprotonation, which results in fluorescence emission mainly associated with the anionic form of FLSC [8].

Vast majority of publications concerning fluorescein are focused on its optical features and applications [10-16], but only a few are related to its electrochemical properties [17-20]. Influence of ultrasounds on electrode processes, using mercury-covered platinum disk electrode, was studied by Eklund and co-workers [19]. The authors proved ability of fluorescein to electroreduction via cyclic voltammetry in two consecutive steps to semi- and leuco- radicals at highly negative potentials without sonication, and immediate reoxidation by means of hydroxyl radicals formed through sonochemical decomposition of aqueous solvent. Another report concerns electrochemiluminescence behavior of fluorescein at polycrystalline gold electrode under conventional cyclic voltammetric conditions [18]. In turn, Queiroz investigated electrochemical oxidation of fluorescein with the use of glassy carbon electrode and proposed mechanism based on a two-step transfer of proton/electron pairs from the positions C3' and C6' of the phenolic group [20]. An approach based on electrochemical sensing of fluorescein acetate was presented by Menon and co-workers, for determination of antibiotic susceptibility of *Escherichia coli* – a gram-negative bacteria [17]. Regarding to the spectrofluorimetric measurements of fluorescein, high fluorescence presented only in alkaline solutions limits its application e.g., excluding the FLSC as a label in the case of acidic samples when fluorimetric spectroscopy is applied. Hence, the main goal of our research was to investigate the electrochemical behavior of FLSC depending on pH and determine its concentration using amperometric electrodes made of different materials using voltammetric methods, as well as by means of spectrofluorimetry in order to elicit the benefits and drawbacks of each technique in quantitative FLSC determination.

Advantages of such approach are, for example, usage of simple and low-cost tools such as amperometric sensors, as well as uncomplicated voltammetric methods. Furthermore, if fluorescent dye would be capable of undergoing electrochemical redox reaction, this advantage opens the possibility of incorporation of two analytical methods into a measurement protocol. The results of our investigations are vital for our further work on methodology for electrochemical sensing of bioanalytes conjugated with fluorescein, such as proteins or DNA.

2. EXPERIMENTAL

2.1. Materials and Chemicals

In the experiments three different materials of working electrodes were voltammetrically examined: graphene paste screen-printed electrodes (GRN) fabricated at Faculty of Mechatronics WUT, graphite paste electrodes (GR) fabricated by direct-printing method in IBBE PAS, and commercially available glassy carbon electrode, GC (mtm anko, Poland). Fluorescein sodium salt, 2-Amino-2-hydroxymethyl-propane-1,3-diol – Tris base and magnesium chloride were purchased from Sigma-Aldrich. Sodium dihydrogen phosphate, sodium hydroxide, potassium chloride, acetate acid, hydrogen chloride and sodium acetate were received from POCH, Poland. For the purpose of pH influence investigation, following buffer solutions were prepared: (1) 0.1 M CH_3COOH in 0.055 M CH_3COONa of pH 4.5, (2) 0.1 M NaH_2PO_4 in 0.01 M KCl of pH 7.0, (3) 0.05 M Tris-HCl in 0.1 M KCl containing 0.1 mM MgCl_2 of pH 9. The deionized water with resistivity of $18 \text{ M}\Omega\cdot\text{cm}$ was used for aqueous solutions, also all reagents utilized were analytical grade. The stock solutions of FLSC were prepared in deionized water and stored in dark at 4°C .

2.2. Methods

A three-electrode system, that operated in batch-mode, consisted of working electrode (GRN, GR or GCE), reference electrode and auxiliary electrode (platinum wire), was implemented to all voltammetric measurements by means of PalmSens potentiostat (Palm Instruments BV, The Netherlands). Calibrations were carried out with the use of a standard addition method in combination with cyclic voltammetry (CV) or differential pulse voltammetry (DPV), according to IUPAC recommendations [21, 22]. The initial volume of buffer solution was 3 mL (blank). The GC electrode was polished with the use of Al_2O_3 powder every time before use to ensure the elimination of possible adsorption of putative reaction products. Additionally, the GC electrode was electrochemically cleaned in 0.5 M H_2SO_4 via CV technique prior to measurements series. To examine the influence of pH on the results of electrochemical measurements, buffer solutions of three pH values (4.5, 7, and 9) were utilized. In order to exclude possibility of interference from reactive oxygen species (ROS) [23, 24], all supporting electrolytes were purged with high-purity gaseous nitrogen for 15 minutes. Experimental parameters of DPV measurements, towards quantitative determination of FLSC, were optimized for each type of working electrode as follows: GRN electrodes – $E_{\text{step}} = 10 \text{ mV}$, scan rate $v = 40 \text{ mV/s}$ at pH 4.5; $E_{\text{step}} = 10 \text{ mV}$, $v = 50 \text{ mV/s}$ at pH 7; $E_{\text{step}} = 10 \text{ mV}$, $v = 50 \text{ mV/s}$ at pH 9; GR electrodes – E_{step}

= 8 mV, $v = 10$ mV/s at pH 4.5; $E_{\text{step}} = 10$ mV, $v = 7$ mV/s at pH 7; $E_{\text{step}} = 10$ mV, $v = 70$ mV/s at pH 9; GC electrode – $E_{\text{step}} = 5$ mV, $v = 10$ mV/s at pH 4.5; $E_{\text{step}} = 5$ mV, $v = 5$ mV/s at pH 7; $E_{\text{step}} = 5$ mV, $v = 5$ mV/s at pH 9. The potential ranges were from 0 V to +1.4 V for all types of electrodes. During the electrochemical measurements, samples were protected from the daylight because of the photosensitivity of the analyte.

The microplate spectrophotometer Synergy HT (BioTek Instruments Inc., USA) was used to perform all optical measurements: fluorescence intensity was recorded at wavelength/bandwidth of emission of 530/25 nm for the samples excited with wavelength/bandwidth of 485/20 nm, whereas the absorbance spectra was collected in the range of 300 – 600 nm. For comparative study of the results obtained in both optical and electrochemical method, the same buffer solutions were used: (1) 0.1 M CH_3COOH in 0.055 M CH_3COONa of pH 4.5, (2) 0.1 M NaH_2PO_4 in 0.01 M KCl of pH 7.0, (3) 0.05 M Tris-HCl in 0.1 M KCl containing 0.1 mM MgCl_2 of pH 9.

In order to eliminate the influence of temperature changes on FLSC properties, all experiments were conducted at 25°C [8].

2.3. Direct-printed electrodes

Graphite amperometric sensors were manufactured with the use of desk-top XYZ-microdosing robot (325 Ultra TT, EFD, USA) and they consisted of three layers: silver paste (5064 DuPont), graphite paste (L-951, ITME, Poland) and insulation layer (7165 DuPont), all deposited onto carrier material (polyester foil Autostat CT7). In order to evaporate the solvents and cure, after deposition, each layer was dried in 120 – 130°C for 15 minutes [25]. After processing and before use, each electrode was vigorously rinsed with MilliQ water.

2.4. Screen-printed electrodes

Screen-printed electrodes were fabricated using graphene paste prepared by mixing graphene nanoplatelets (of surface area of 500-700 m²/g, particles' diameter >2 μm and particles' thickness of 8-15 nm, purchased from Cheap Tube Inc., USA) at the level of 15 wt. % in vehicle solution (PMMA in butyl carbitol acetate). Electrodes were printed with use of a screen-printer Auriel Automation C920 through masks of the designed patterns made on 68T polyester screens. Each sensor consisted of three layers: silver paste layer (L 121), graphene paste layer – both dried in 120°C for 20 minutes, and dielectric paste outer layer (5018A, Dekorglass), which was dried by UV dryer UN60001-0002 Technigraf for 1 hour. The background material of the electrodes was PMMA foil.

The surface areas of working electrodes were estimated to be 5.0 ± 0.1 mm² (\pm standard deviation, SD) in the case of graphite sensors, 9.0 ± 0.1 mm² for graphene screen-printed electrodes, and 3.15 ± 0.01 mm² for commercial glassy carbon sensor.

3. RESULTS AND DISCUSSION

To study the electrochemical behavior of the analyte on GRN, GR and GCE electrodes, cyclic voltammetry was utilized, since this method enables observations of reversed scan of the applied potential [21, 26]. DPV measurements were carried out to determine the linear concentration range of FLSC for the amperometric electrodes.

The results of measurements performed by means of cyclic voltammetry, confirmed the electrochemical activity of FLSC, which undergoes an electrochemical oxidation in the considered potential range from 0 to +1.4 V. There is no visible reduction peaks on bare electrodes, which suggests the irreversible process (Figure 1). However, for various electrode materials, oxidation peak potentials differs significantly, in particular the potential of oxidation peak is about +0.65 V for graphene electrode at pH 7, and about +0.94 V in the case of graphite sensor in the supporting electrolyte with fluorescein at pH 4.5.

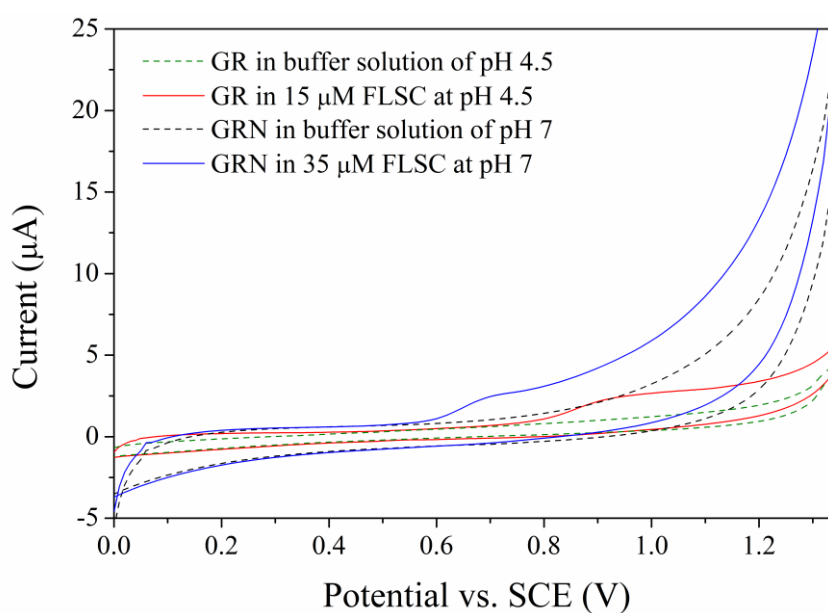


Figure 1. Voltammograms obtained with the use of cyclic voltammetry for 15 μM FLSC and 35 μM FLSC with one oxidation peak (compared to results in buffer solutions), obtained with the use of GR and GRN electrodes at pH 4.5 and 7. Potential range from 0 to +1.4 V, $\nu = 130 \text{ mV/s}$ for both measurements.

Successive scans obtained with the use of cyclic voltammetry by means of graphite sensors are presented in Figure 2 from A to C. Solutions of various pH were examined in order to compare influence of pH on the oxidation potential and the height of anodic peak current. One oxidation peak appeared at the potential of +0.95 V for measurements performed in acidic medium, +0.78 V in solution of pH 7, and +0.76 V in alkaline sample, which is putatively associated with electrooxidation of one phenolic group of FLSC aromatic ring. The compatible results were previously presented, indicating that increase of pH is related to decrease of electrooxidation potential of FLSC [20, 27]. Noteworthy, the difference between potential of anodic peak is much slighter for pH 7 and 9 (20 mV)

than for acidic pH. This leads to the assumption that the anodic potential of FLSC is less influenced by pH after exceeding pK_a value (6.3) of aforementioned phenolic group of FLSC molecule, which undergoes electrooxidation process [20, 28]. The peak height also decreased with the increment of pH of the supporting electrolyte ($0.54 \mu\text{A}$ for pH 4.5, and $0.06 \mu\text{A}$ for pH 7) i.e., the difference between the peak current in the case of pH 4.5 and pH 7 is equal to $0.48 \mu\text{A}$. In turn, the peak current obtained in alkaline media is only by about 22 nA lower than for pH 7. With regard to obtained peak heights, fluorescein ionic form present in acidic media is more electrochemically active comparing with forms prevailing in samples of pH 7 and 9. Considering the consecutive scans (second and third), in the case of measurements performed in pH 4.5, the decrease of peak height is visible, however peaks do not disappear completely. The second peak for pH 7 and 9, decreased significantly, while the third peak practically disappeared, which can be attributed to adsorption of redox reaction products. The GR electrodes utilized for the above described experiments were vigorously rinsed with deionized water and measurements in supporting electrolytes of pH 4.5, 7 and 9 were carried out, afterwards not showing any peaks. Therefore, the hypothesis regarding adsorption of electrochemical oxidation products is justified [20].

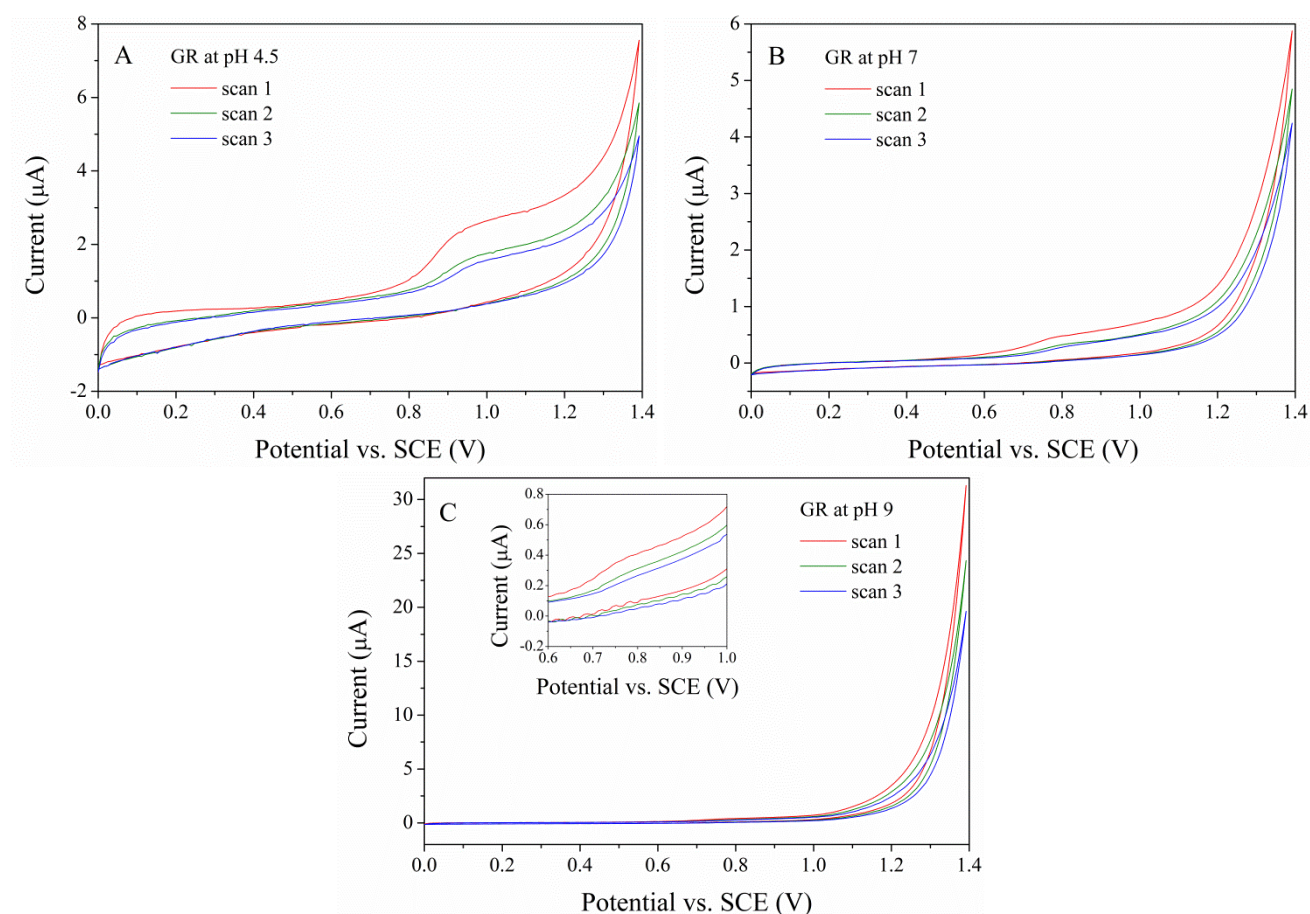


Figure 2. Successive scans obtained with the use of CV method by means of GR electrodes, measurements performed in the solutions of following pH values: **A** – 0.1 M acetate buffer of pH 4.5, **B** – 0.1 M phosphate buffer of pH 7.0, **C** – 0.05 M Tris-HCl buffer of pH 9 (inset illustrates the zoom of the peak region).

As it was previously presented, the absorbance measurements can be used to verify the participation of ionic forms of fluorescein in the samples of investigated pH values [27]. Briefly, at pH 4.5 to absorption peaks at wavelengths 453 and 472 nm were present, indicating on predominant content of anions and neutral form of fluorescein. In the solutions of pH 7, a high content of dianions and anions can be observed, however dianionic form is predominant (absorption peak at 490 nm). Eventually, absorbance spectra obtained in the samples of pH 9 exhibited the prevalence of dianions (Figure 3).

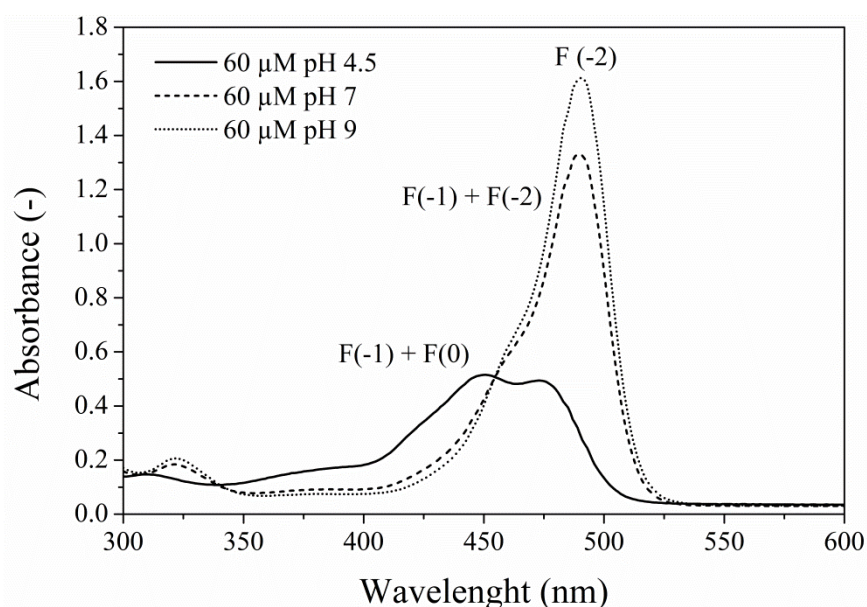


Figure 3. Absorption spectra for 60 μM solution of FLSC of different pH values: 4.5 – purple plot, 7 – orange plot, and 9 – red plot. F(-2) means dianionic form of FLSC, whereas F(-1) and F(0) correspond to anions and neutral forms, according to [27].

Quantitative determination of fluorescein concentration by a standard addition method was performed via differential pulse voltammetry. In contrary to cyclic voltammetry, DPV method allows to eliminate adverse effect of capacitive current, therefore improving sensitivity of the measurements [26].

Figure 4 from A to D shows the exemplary voltammograms recorded with the use of DPV method by means of graphene screen-printed electrodes in the FLSC samples of various pH and corresponding calibration curves. Linear concentration range was relatively wide in the case of measurements performed in solutions of pH 4.5 and 9 – from 0.2 to 3 μM FLSC and from 2 to 10 μM FLSC, respectively. Results obtained for near-physiological pH were characterized with quite poor repeatability visualized via high standard error bars, as well as the lowest linear correlation coefficient $R^2 = 0.96$. Also for measurements using GRN electrodes, the successive DPV voltammograms at pH 4.5 show a slight shift of oxidation potential to lower positive values, which may be associated with the existence of FLSC acid-base equilibrium [20]. Increase of pH revealed to reduce the response of the electrodes, resulting in decrease of mean sensitivity (s – also called slope of calibration curve). The mean sensitivity values obtained for GRN sensors were as follows: 26.19 nA/ μM for pH 4.5, 15.07

nA/ μ M for pH 7 and 8.11 nA/ μ M for pH 9. Similar aforementioned slight negative shift was observed for acidic pH when GRN electrodes were used. On the other hand, potential of electrochemical oxidation was stable during experiments at neutral and alkaline pH. For GRN electrodes, the lowest limit of detection value was obtained for pH 4.5 (7.4 μ M FLSC), which is consistent with the highest sensitivity obtained for acidic solution.

Mechanism of redox reaction that occurred at graphite paste sensors, differed from those received with the use of other types of electrodes – two oxidation peaks were obtained via DPV method at pH 7 and 9, indicating a two consecutive steps (Figures 5 from A to C). Referring to the aforementioned analysis, the potential of oxidation peak appearing at pH 4.5 shifted from +0.85 V to about +0.83 V. Notable, this potential differs of about 0.05 V from the value obtained in the same conditions using GRN sensors. DPV voltammograms obtained for pH 7 and 9 show two oxidation peaks for each pH, the potentials of which are as follows: peak 1a at the potential of about +0.6 V, peak 2a at the potential of +0.71 V – pH 7, and peak 1b at the potential of +0.53 V, peak 2b – at +0.68 V for pH 9.

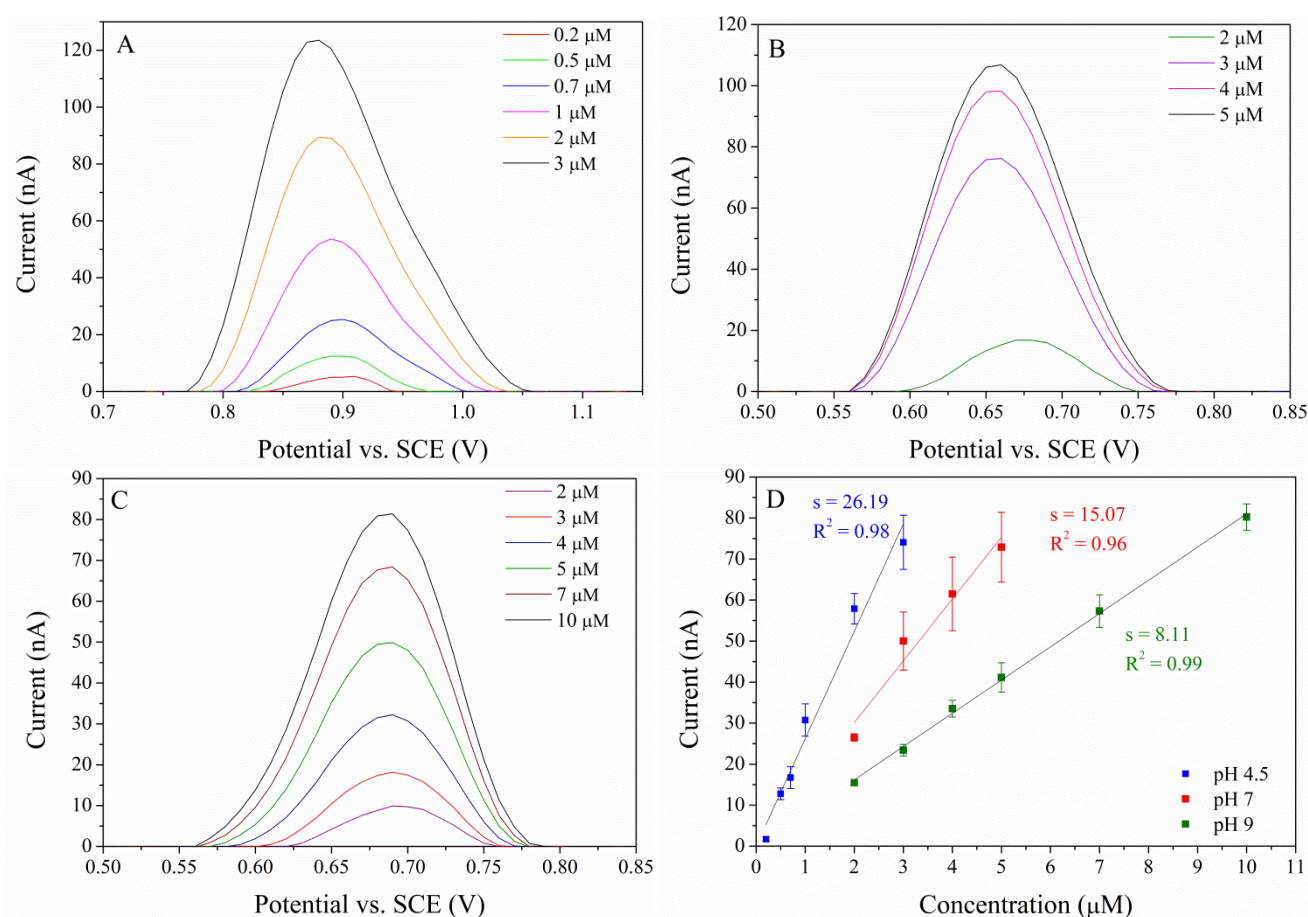


Figure 4. Voltammograms with corrected baseline obtained using GRN electrodes via DPV method at the potential range from 0 to +1.4 V. Results presented for different pH values: **A** – 4.5, **B** – 7, **C** – 9, correspond with calibration curves **D** ($n=4$, \pm standard errors SE, where n is a number of samples for each FLSC concentration).

Sensitivity of GR sensors calculated for samples of pH 4.5 was about 1.5 times lower compared to the value obtained for GRN electrodes, which was 15.2 nA/ μ M (Figure 5 D). Graphite paste electrodes at pH 7 allowed to detect two oxidation peaks, for which mean sensitivity of determination was relatively similar (1.76 nA/ μ M for peak 1a and 1.41 nA/ μ M for peak 2a). However, if the peak 1a is considered then linear range is wider (from 1 to 18 μ M of FLSC) than in the case of the second peak 2a (from 1 to 7 μ M of FLSC). Analyzing the results collected in samples of pH 9, the mean sensitivity indicates more than 4 times higher value for the second peak 2b (4.58 nA/ μ M) in comparison with peak 1b (1.12 nA/ μ M). Moreover, the mean sensitivity for peak 1b was the lowest, for GR electrodes. Interestingly, since the sensitivity of determination increased for the second peak 2b, linear concentration range decreased from the range 3–20 μ M (peak 1b) to 3–10 μ M fluorescein (Figure 5 D).

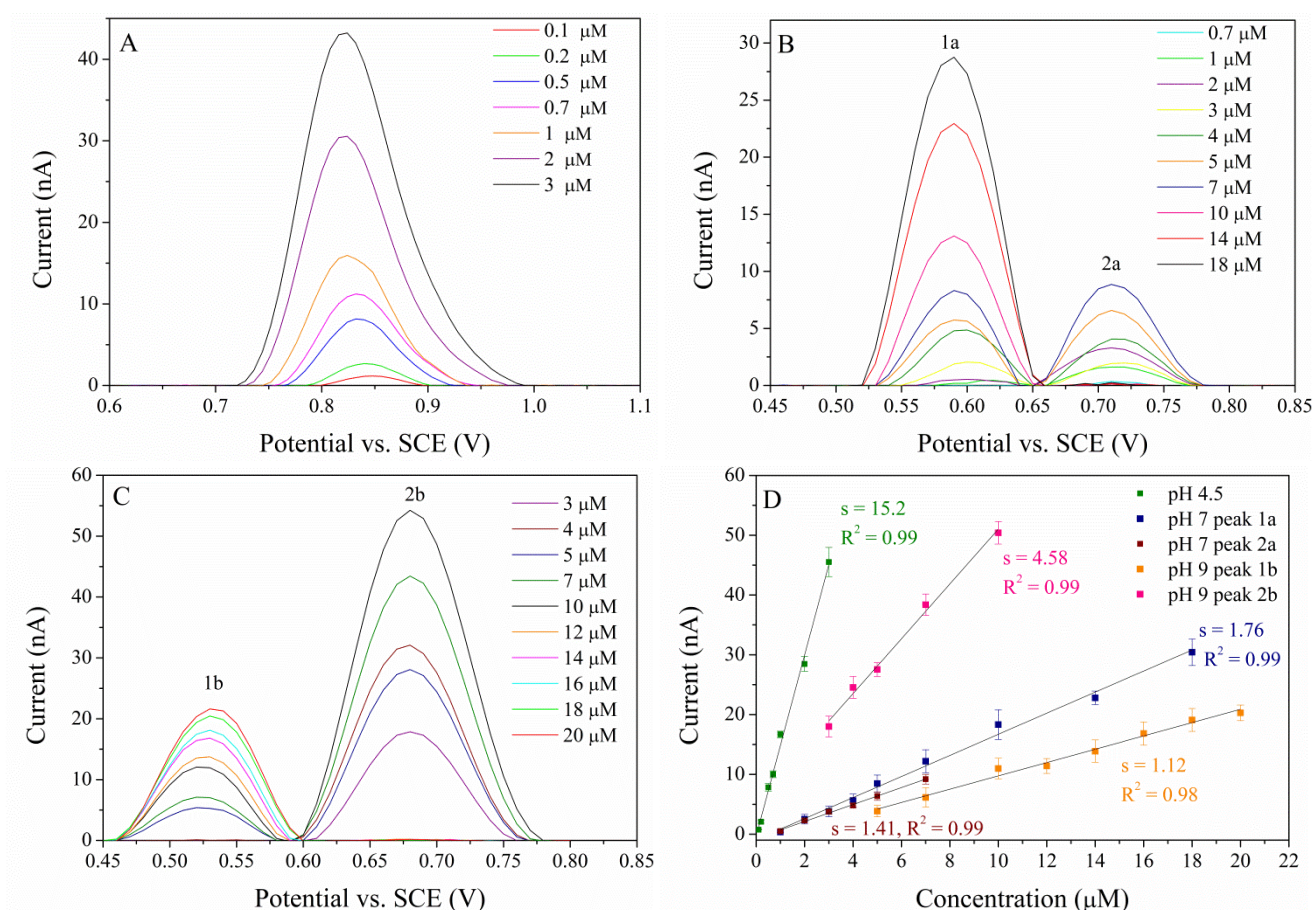


Figure 5. Voltammograms with corrected baseline obtained using GR electrodes via DPV method at the potential range from 0 to +1.4 V. Results presented for different pH values: **A** – 4.5, **B** – 7, **C** – 9, correspond with calibration curves **D** ($n=4$, \pm SE).

Electrochemical oxidation of FLSC was poorly efficient in the case of GCE electrode, where linear correlation between measured peak current height and fluorescein concentration was obtained at pH 4.5 and 7 only (Figure 6). Moreover, the current increment was noticeable when DPV method was applied, whereas cyclic voltammetry did not enable for detection of any peaks. Results received for fluorescein concentration measurements in samples of pH 4.5 as well as pH 7, were characterized with

relatively narrow linear concentration ranges. Comparing the results obtained for all types of sensors measured under the same conditions, it can be concluded that the slope of the calibration curve obtained for GCE at pH 4.5 (6.87 nA/ μ M) was over 3.5 times and about 2 times lower in the case of GRN electrodes and GR sensors, respectively. However, repeatability of GCE, visualized at the calibration curve as standard error bars, was comparable to GR and GRN electrodes.

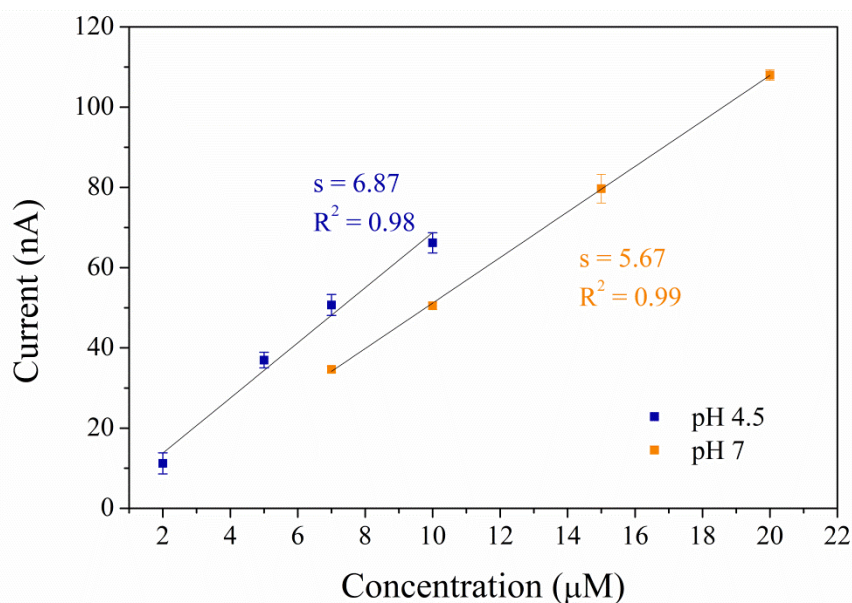


Figure 6. Calibration curves for GCE electrode obtained via DPV method at the potential range from 0 to +1.4 V in buffer solutions of various fluorescein concentration in solutions of pH 4.5 and 7, $n=4$, \pm standard errors. No results obtained in alkaline solution.

In contrary to the results received from electrochemical measurements, the spectrofluorimetric studies showed opposed effect of pH increment onto recorded fluorescence values – the lowest fluorescence appeared in acidic solutions (mean sensitivity was $0.022 \cdot 10^3 \mu\text{M}^{-1}$), whereas much higher values were obtained in samples of pH 7 and 9 (mean sensitivity equaled $1.05 \cdot 10^3 \mu\text{M}^{-1}$ $1.25 \cdot 10^3 \mu\text{M}^{-1}$, respectively). Noteworthy, the difference between mean sensitivity obtained at pH 4.5 is about three orders of magnitude lower comparing with other examined pH values. This confirms the high content of neutral form in acidic sample, since the neutral form of fluorescein does not exhibit fluorescence, itself [13, 28]. These results are also in a good agreement with the previous studies utilizing spectrofluorimetric method of detection [10], which disclosed anionic and dianionic forms of FLSC to be the most fluorescent ones. However, the linear concentration range (from 0.5 to 20 μ M) obtained in samples of both pH values 7 and 9, was much narrower in comparison with the linear region (20 to 200 μ M) received in acidic media.

Referring to the main goal of the study, it can be concluded, that the fluorescein is electrochemically active compound, which can undergo one- or two-step irreversible electrochemical oxidation in the potential range from 0 to +1.4 V depending on the utilized electrodes' material. Differential pulse voltammetric method allowed to quantitatively determine FLSC in aqueous solutions at micromolar concentration levels with good linear correlation coefficients ($R^2 \geq 0.99$). In voltammetric

set-up, the width of obtained linear ranges was comparable with the received in fluorescence measurements in samples of pH 7 and 9. The exception was found in the case of measurements in acidic media, when linear concentration range obtained via optical method was significantly wider. In Table 1, there are values of limit of detection (LOD) calculated on the basis of the data obtained via DPV and spectrofluorimetric method, according to IUPAC guidelines [29], as equal $3 \cdot \sigma/s$ (where σ is a standard deviation and s – a slope of calibration curve). Undeniably, obtained LOD values demonstrate the advantage of voltammetric measurements at pH 4.5 for sensors based on paste of graphene and graphite. However, as it was mentioned before, measurements performed with the use of glassy carbon electrode expose relatively low sensitivity as well as high limit of detection, which indicates a large significance of material used for FLSC detection. Nevertheless, when samples at pH higher than 7 are considered, spectrofluorimetry allows detection at one order of magnitude lower concentration level of FLSC, in comparison with DPV technique.

Table 1. Values of limit of detection for FLSC for two methods: spectrofluorimetric, and differential pulse voltammetric with the use of GRN, GR and GCE sensors.

pH	Spectrofluorimetric method	DPV method		
		GRN	GR	GCE
	LOD [μM]			
4.5	17.56	7.40	6.44	91.14
7	0.42	34.55	42.27	336.69
9	0.35	23.42	95.12	-

As it was mentioned before, thus far only a few authors concerned electrochemical properties of fluorescein. However, only one work describing quantitative voltammetric detection of fluorescein was found [20]. Measurements performed with the use of GCE electrode differed from the presented results by lower detection limit of FLSC obtained at pH 7, which was of $0.63 \mu\text{M}$ – i.e. two orders of magnitude lower compared to results obtained at the same pH using GRN or GR sensors. Nevertheless, in comparison to this study, relatively narrow linear concentration range (from 2.5 to $8 \mu\text{M}$) was presented in [20].

4. CONCLUSIONS

Investigations on electrochemical activity and optical properties of fluorescein with the use of voltammetric and spectrophotometric methods were performed. The electrochemical activity of FLSC in the potential range from 0 to $+1.4 \text{ V}$ was demonstrated using amperometric electrodes: GRN, GR and GCE sensors. Reversed scans, obtained via cyclic voltammetry, revealed irreversible anodic peak, as well as possibility of adsorption of redox reaction products onto electrodes surface (pH 7 and 9). In addition, quantitative determination of FLSC using DPV and spectrofluorimetric techniques was carried out using supporting electrolytes of different pH. Concerning the results of the investigation, it can be assumed that both utilized methods are suitable for FLSC detection, however their utility strongly depends on the pH of the sample, which directly influences on the electrochemical and optical

properties of the analyte – different ionic forms of FLSC. Hence, we assume that the pH of the sample should be taken into account when choosing the analytical method of FLSC detection. Additionally, in the case of voltammetry – material of the electrodes should be considered as well. The comparison of examined methods can be based mainly on the limit of detection values and the estimated ranges of linear relationships between FLSC concentration and the output signals received from both measurement techniques. In detail, LOD calculated for pH 4.5 shows higher suitability of DPV method using GRN and GR electrodes comparing with spectrofluorimetry, since the occurrence of the most electrochemically active – mainly neutral – forms is promoted in the acidic environment. On the other hand, the LOD values calculated for the results of measurements in neutral and alkaline media, indicates spectrofluorimetry to be more beneficial due to predomination of highly fluorescent dianionic form of FLSC, which exhibits poor electrochemical activity at the same time. In the case of linear concentration ranges, they are comparable for pH 7 and 9, whereas in the case of electrochemical method, linear concentration range for pH 4.5 was narrower. Taking into account the analyzed parameters, we state, that voltammetric and spectroscopic methods complement each other, if measurements of fluorescein are concerned. The proposed approach may enable performing measurements in the wide pH range with high sensitivity, which is highly advantageous for example in the case of analysis of environmental samples. Finally, for developing the associated method of indirect detection of biomolecules with the use of compounds acting as tracers, the electrochemical properties of conjugated fluorescent label should be further investigated in respect to the sensitivity of the chosen analyte against changes of pH conditions.

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