

INDOPOLYCARBOCYANINE DYES AS ANALYTICAL REAGENTS FOR THE DETERMINATION OF NITRITE, IRON(III), AND OXALATE BY FLUORESCENCE SPECTROSCOPY

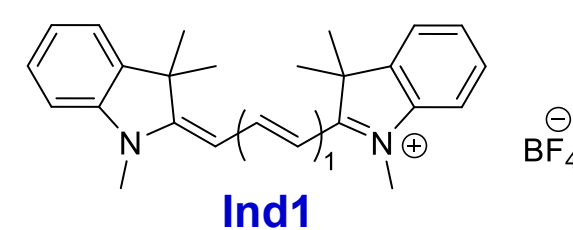
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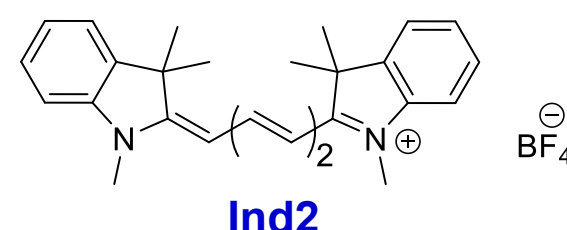
Investigated dyes

$\lambda_{\max}(\text{abs}) = 545 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 1.3 \cdot 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



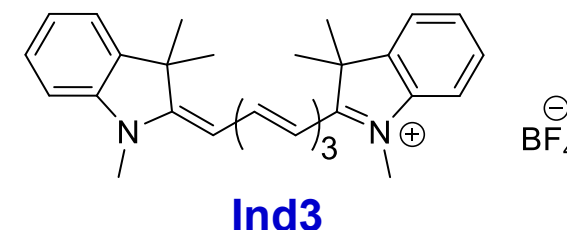
Ind1

$\lambda_{\max}(\text{abs}) = 640 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 2.1 \cdot 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



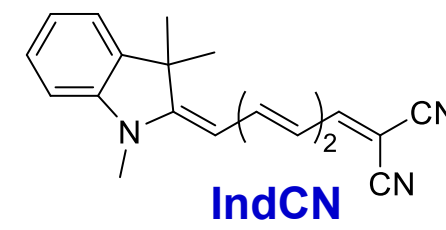
Ind2

$\lambda_{\max}(\text{abs}) = 742 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 2.3 \cdot 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



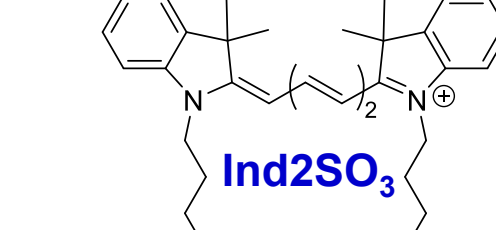
Ind3

$\lambda_{\max}(\text{abs}) = 611 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 8.6 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



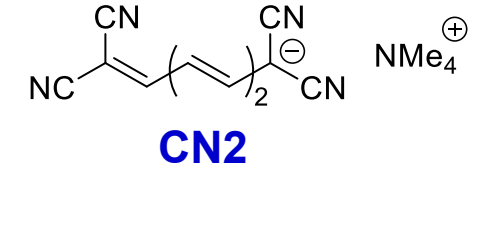
IndCN

$\lambda_{\max}(\text{abs}) = 643 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 2.2 \cdot 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



Ind2SO3

$\lambda_{\max}(\text{abs}) = 542 \text{ nm}$
 $\epsilon_{\max}(\text{EtOH}) = 1.8 \cdot 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$



CN2

The aim of the study was to investigate the relationship between the structure of indopolycarbocyanine dyes and their interactions with nitrite and iron(III) in order to develop highly sensitive, selective, and environmentally friendly methods for the determination of nitrite, iron(III) and oxalate. Among the studied indopolycarbocyanine dyes, the indodicarbocyanine dye (**Ind2**) was found to be the most suitable analytical reagent for the determination of nitrite and iron(III), whereas the indocarbo-cyanine dye showed low reactivity toward both analytes, and the indotricarbocyanine dye exhibited lower stability in acidic media (pH < 2,0) and lower selectivity due to its propensity to react with other oxidants.

Nitrite determination

The fluorescence method for nitrite determination is based on nitration of the polymethine chain of the indodicarbocyanine dye (Ind2) in acidic conditions (pH 1.9 ± 0.05), which leads to decrease in the fluorescence intensity of the Ind2 dye. The use of TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl) as a coreagent was proposed, reducing the detection limit of the method by almost twofold (**2.3 $\mu\text{g/L}$**).

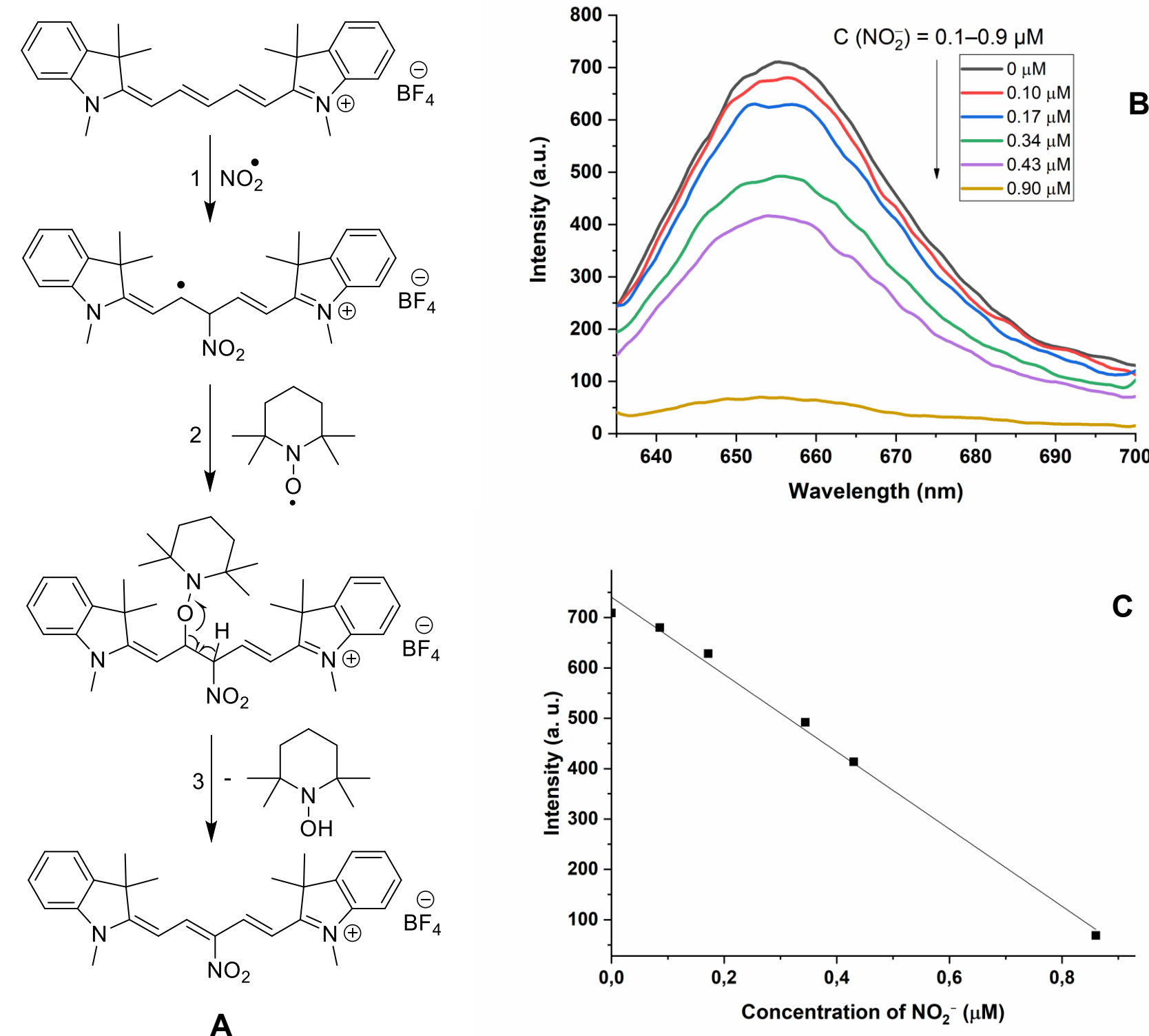


Figure 1 Proposed reaction mechanism of Ind2 with nitrite (**A**), fluorescence spectra of the Ind2 dye in the presence of TEMPO at different nitrite concentrations (0.1 – 0.9 μM) (**B**) and the corresponding calibration curve (**C**).

Iron(III) and oxalate determination

The fluorescence method for iron(III) determination is based on the radical oxidation of indodicarbocyanine by iron(III) in acidic conditions (pH 1.9 ± 0.05) to carbonyl-containing products, resulting in a decrease in fluorescence intensity. The oxalate determination method is based on suppression of Ind2 dye oxidation due to complex formation between iron(III) and oxalate. The limits of detection for iron and oxalate determination are **2.8 and 4.4 $\mu\text{g/L}$** , respectively.

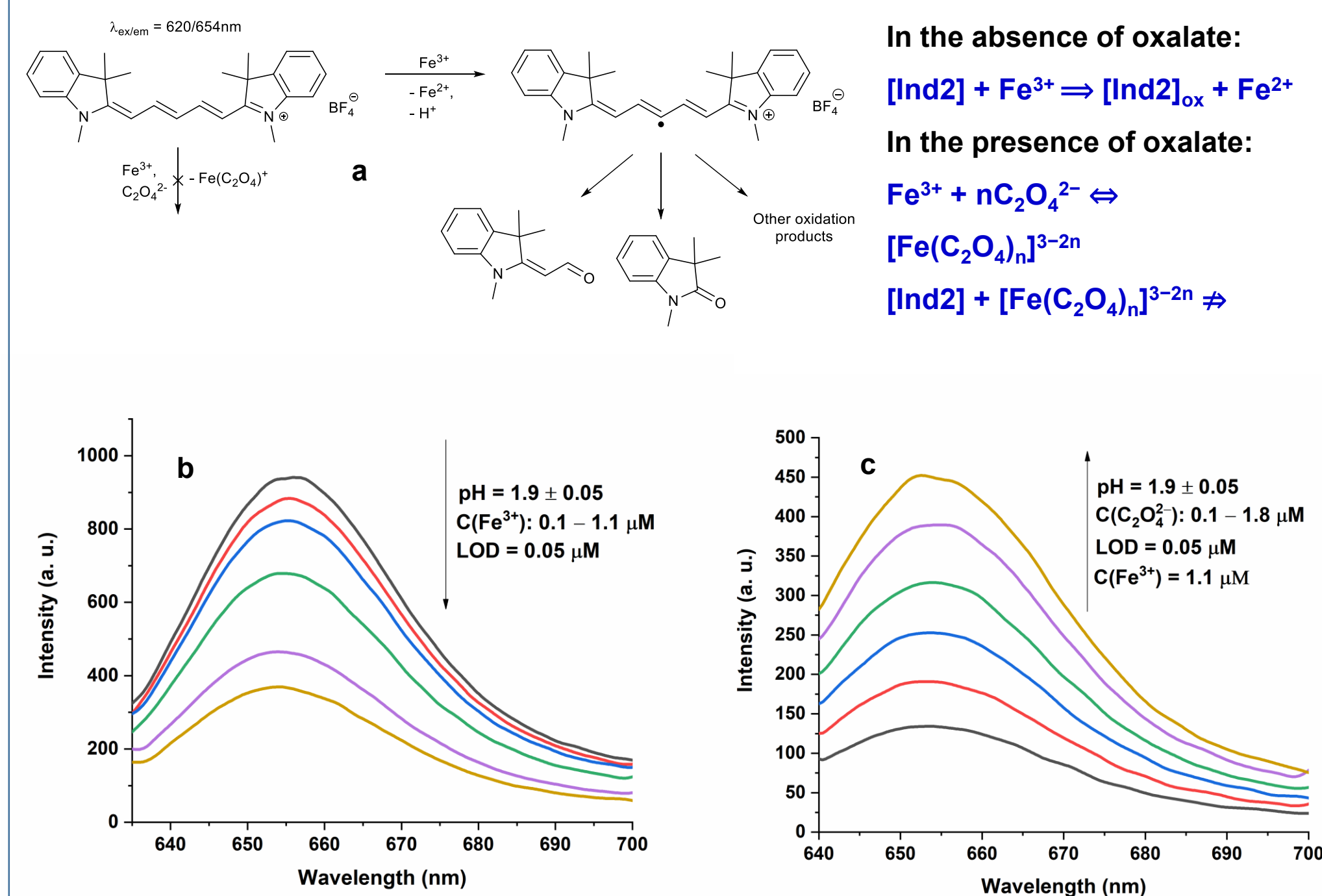


Figure 2 Proposed reaction scheme of Ind2 with iron(III) in the absence and in the presence of oxalate (**a**), fluorescence spectra of the Ind2 dye in the presence of different concentrations of iron(III) (**b**) and oxalate (**c**).

Real sample analysis

The nitrite determination method demonstrates satisfactory selectivity, as the main constituents of drinking water and monosaccharides do not interfere with the determination. The method is successfully applied to bottled water and baby food puree samples, and the results agree with those obtained using the reference Griess method. The iron(III) and oxalate determination methods also exhibit satisfactory selectivity, with interference from anions that form competitive complexes with iron(III) (F^- and H_2PO_4^-) and were validated using sorrel leaf samples and black tea infusion, respectively; the accuracy of the results was confirmed by the spiked-recovery method.

Table 1 Results of nitrite determination in moderately carbonated, still bottled water, and baby puree samples. $P = 0.95$, $n = 3$, $F_{0.05, 3, 3} = 19.00$, $t_{0.05, 4} = 2.78$.

Sample	Added, $\mu\text{M}(\text{water})$, mg/kg (puree)	Found by this method, $\mu\text{M}(\text{water})$, mg/kg (puree)	RSD, %	Recovery, %	Found by this Griess method, $\mu\text{M}(\text{water})$, mg/kg (puree)	F-test	t-test
Moderately carbonated water	0	< MB	-	-	< MB	-	-
	0.43	0.45 ± 0.07	6.5	104.7	0.46 ± 0.11	2.2	0.33
	0.60	0.58 ± 0.07	4.6	96.7	0.62 ± 0.15	8.0	1.08
Still water	0	< MB	-	-	< MB	-	-
	0.43	0.45 ± 0.03	3.0	104.7	0.42 ± 0.09	7.6	1.30
	0.60	0.56 ± 0.03	2.3	93.3	0.55 ± 0.04	1.3	0.88
Baby puree	0	< MB	-	-	< MB	-	-
	3.9	3.7 ± 0.5	5.7	94.9	4.0 ± 0.3	3.2	2.2
	7.8	7.8 ± 0.4	2.3	100.0	7.9 ± 0.6	1.4	0.63

Table 2 Results of the determination of iron(III) in a sorrel sample and soluble oxalate in a tea infusion sample ($P = 0.95$, $n = 3$).

Analyte	Added mg/kg	Found mg/kg	RSD, %	Recovery
Fe^{3+} in sorrel	0	31 ± 2	3.3	-
	43	74 ± 2	1.0	100.2
	76	110 ± 19	6.9	103.4
$\text{C}_2\text{O}_4^{2-}$ in tea infusion	0	9.0 ± 1.3	5.8	-
	2.0	11.1 ± 1.5	5.4	105.0
	5.0	13.7 ± 1.2	3.4	94.0

Conclusions

Among the studied indopolycarbocyanine dyes, the indodicarbocyanine dye (Ind2) was found to be the most suitable analytical reagent for the determination of nitrite and iron(III). The mechanisms of the reactions of cyanine dyes with nitrite and iron(III) were proposed: radical nitration of the dye polymethine chain upon interaction with nitrite in acidic medium, and oxidation of the polymethine chain by iron(III) to carbonyl compounds via formation of a cation radical. The oxalate determination method is based on suppression of Ind2 dye oxidation due to complex formation between iron(III) and oxalate. This approach is promising for developing methods for the determination of other anions that form stable complexes with iron(III) in acidic media, particularly fluoride and phosphate. The Ind2-based optical probe for the determination of nitrite, iron(III), and oxalate exhibits higher sensitivity than most existing methods, with detection limits of 2.3, 2.8, and 4.4 $\mu\text{g/L}$, respectively. The proposed methods do not require toxic organic solvents ($\varphi(\text{EtOH}) < 3 \text{ vol.}\%$), and offer satisfactory selectivity, environmental safety, and operational simplicity. The developed fluorescence method for nitrite determination was successfully applied to the analysis of bottled water and baby food puree. The fluorescence methods for iron(III) and oxalate determination were validated using sorrel leaf samples and black tea infusion, respectively. The proposed approaches for the determination of nitrite, iron(III), and oxalate using indodicarbocyanine dye can be applied to the determination of trace amounts of these analytes and show potential for further integration into automated analytical systems.

[1] Shabelko A., Derevyanko N., Ishchenko A., Tananaiko O., Spectrochim. Acta A, 2024, 321, 124728. doi.org/10.1016/j.saa.2024.124728

[2] Shabelko A., Derevyanko N., Tananaiko O., Microchem. J., 2025, 214, 114068. doi.org/10.1016/j.microc.2025.114068

[3] Shabelko A., Halina S., Lionel Z., Derevyanko N., Tananaiko O., Spectrochim. Acta A, 2026, 358, 127881. doi.org/10.1016/j.saa.2026.127881