The aptasensors developed showed reproducibility of the R_{et} value which ranges from 4.5% for single measurements performed with six aptasensors for 10 nM OTA to 7.5% for a series of six measurements with the same aptasensor within a weak operation. Commonly, the metrological characteristics obtained with the same sensor appear better than those of different sensors. In this case, the decrease of reproducibility is related to insufficient recovery of the aptasensor response. Treating the aptasensor with 0.1 M EDTA solution and 0.1 M NaCl did not lead to full recovery on the EIS characteristics. Instead, the response tends to decrease down to 80% recovery in six consecutive measurements. Meanwhile the storage of the aptasensors prepared prior to their application in dry conditions at 4 ℃ for at least two weeks did not alter the signal, while its variation increased during the storage period by 1.5–2.0 fold. For these reasons, the developed aptasensors can be recommended for a single use without any regeneration after their contact with the sample. Taking into account very low amounts of modifiers as well as simple preparation protocol, this does not lead significant increase in the measurement cost. As regards the Au/Boltorn H30® suspension, it can be stored at 4 ℃ for at least one month without significant changes of the characteristics of the aptasensor prepared from it. Moderate improvement of the signal reproducibility can be achieved by sonication of the suspension prior to its use for 5–10 min. The procedure does not lead significant changes in the distribution of the Au nanoparticle size, but increases the uniformity of the sensing layer.

3.4. Selectivity and Real Sample Assay

The selectivity of the developed aptasensor was estimated under similar experimental conditions using an ochratoxin B standard solution. The slope of the calibration curve obtained in the concentration range from 1.0 nM to 100 nM was 25 kΩ/log*c*, or three times lower than that of OTA. The LOD of 1.0 nM makes it possible to detect at least 50-times higher concentration of the target analyte without any intereference and up to 50 nM with less than 10% deviation of the result. The maximal difference in the signals toward OTA and ochratoxin B were achieved for the OTA concentration of 10 nM. This is quite acceptable for direct detection of OTA in foodstuffs.

The application of Au nanoparticles as an aptamer carrier can interfere with some biological compounds, e.g., amino acids or thiols which are frequently present in the samples tested. However, no significant influence of 0.1 mM glycine, alanine, phenylalanine and cysteine added prior to or together with 10 nM OTA on charge transfer resistance was observed. The stability of the aptasensor signal can be referred to a strong interaction of Au nanoparticles with thiolated aptamer which is placed on their surface prior to contact with the sample. Rather dense coverage of the carrier surface with aptamer molecules prevents amino acids and thiols from their reaction with golden nanoparticles.

To confirm the prospects of the aptasensor in real sample assay, it was tested on the spiked samples of light and dark beer ("White Bear" and "Žatecký Gus Černý", respectively). Prior to OTA spiking, the beer samples were boiled for 15 min until foaming stopped and then mixed with distilled water to their initial volume. The signal was measured in the conditions described for standard solutions. The recovery of about 70% for light beer and 78% for dark beer was obtained for six measurements with 5 nM OTA. Some losses of the analyte can be related to the OTA adsorption on solid particles remained in the beer. In HPLC experiments they are removed by filtration prior to OTA addition.

The direct detection of OTA in undiluted beer accelerates testing and makes it possible to detect the OTA quantities below the maximal admissible levels established for foodstuffs [13,14].

4. Conclusions

In this work, an impedimrtric aptasensor has been developed for OTA detection on the base of novel aptamer carrier based on Au nanoparticles suspended in the dendrimeric hydrophilic polymer Boltorn H30®. Measurements of electrochemical properties of the modifier confirmed the high activity of Au nanoparticles in the electron transduction as well as improvement of the aptasensor characteristics in comparison with Boltorn H30® and naked electrode. The use of the polymeric form of Neutral Red and thiolated aptamer against OTA made it possible to develop an easy protocol of aptamer immobilization and ensured the high sensitivity of the response. A LOD of 0.02 nM achieved under optimal conditions of biolayer assembly is lower than that of similar aptasensors with other signal transduction principles.

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Conflicts of Interest

The authors declare no conflict of interest.

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