Regular paper

A meta-analysis of the influence of the external conditions on the biosensor receptor layer component stability

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The manuscript presented here contains meta-analysis of the influence of the external conditions on the biosensor receptor layer component stability. The novelty of this paper is due to compilation and comparison of studies, based on proposed collective analyses. The presented meta-analysis allows to increase the precision and accuracy of the results by combining and co-analyzing data from five smaller experiments. To understand the significance of presented meta-analysis, the most important conclusions and observations resulting from the conducted five types of research [F1] to [F5] are given. The conducted meta-analysis showed the magnitude of stability differences caused by different external factors. An approach of numerical interpretation of the qualitative stability changes has been offered. The conducted meta-analysis showed that the tested factors influence the stability of the BSA in different ways

INTRODUCTION

Among the parameters influencing biosensor operation, the issue of stability can be distinguished. This problem is important, as the stability of parameters of the device translates into a feasible operational lifetime. Stability and methods of qualitative analysis are often addressed by research groups (Sonawane *et al.*, 2017; Martsenyuk *et al.*, 2016; Penjan *et al.*, 2017; Grabowska *et al.*, 2014; Mosinska *et al.*, 2013). This problem is still open and it will remain open without a doubt in the future. The "biosensor stability" keyword entered in the Web of Science database returns 818 articles for 2019 only, which confirms that the issue is still open. In the biosensor matrix, the active biological material is immobilized. There are several popular methods of immobilization in use. They include entrapment, covalent bonding, adsorption, encapsulation and cross-linking (Kucherenko *et al.*, 2019; Sirisha *et al.*, 2016)

The performed study is related to the cross-linking component of the biosensor receptor layer. This component is BSA (Bovine Serum Albumin). Among the examined methods of immobilization, cross-linking with BSA is superior to other methods in terms of sensitivity, detection threshold, reaction time, as well as operational and thermal stability (Sarika *et al.*, 2015).

The biosensor receptor layer cross-linking component used in the examinations is often used when designing new tests (Żyracka *et al.*, 2005) . It also defines the standard of proteins on glass bed coated with a surfactant, immersed in the indium tin oxide (ITO) electrodes (Lin *et al.*, 2016). Due to the fact that the tested substance is commonly used in pharmacokinetics and pharmacodynamics of new medicaments, its conformational changes under the influence of external factors have been examined before (Zhou *at al.*, 2019; Neuhoff *et al.*, 2006; Torres *et al.*, 2013). However, there is no any information about the type of research we performed, i.e. the influence of the above-mentioned external factors on the cross-linking component of the biosensor receptor layers. The research in this respect was aimed to determine the stability of the biosensor receptor layer cross-linking component in specific time intervals. The aim of the study is to conduct a meta-analysis of the influence of external conditions on biosensor receptor layer stability. The study will allow to increase the precision and accuracy of assessment of the influence of the different factors on the stability of the biosensor receptor layer component. The planned compilation will allow to distinguish factors that improve stability and the destabilizing factors. This study complements the knowledge on the stability of the biosensor receptor layer component.

A diagram of performed tests is illustrated by Fig. 1.

The novelty of this paper is centralizing and compilation of studies based on proposed collective analyses, which are the resultant of source articles. The presented meta-analysis allows to increase the precision and accuracy of the results by combining and co-analyzing data from several smaller experiments. The results come from those five small experiments that were published before separately. In the presented manuscript the results are put together and compared with the use of new metanalysis. The novelty of the paper follows from the comparison of the external factors concerning operational and self-stability. The results demonstrated in this manuscript can be used in the design of biosensors. They help to determine the conditions for storage of that type of devices to prolong their stability. Description of the results leading to conclusions about the stability of the receptor layer cross-linking component used in biosensor matrices. Stability of this component is a very important element, as it forms a kind of a binding element (e.g. for enzymes in the enzyme biosensors) and a change in its conformation may lead to destabilization of the receptor layer structure.

MATERIALS AND METHODS

Bovine Serum Albumin (BSA) as a solid (crystallized and lyophilized powder (purity at least 99%), essential-
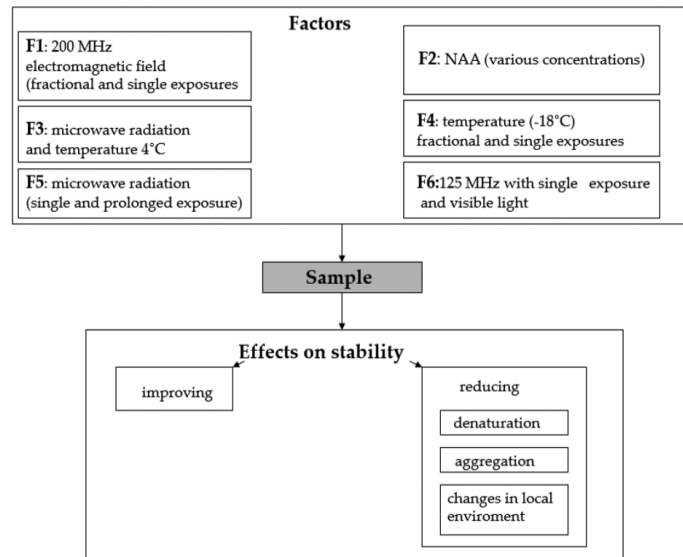
Figure 1. Diagram of applied external factors on the receptor layer component.

ly globulin free BSA (lot: SLBK3063V)) was obtained from Sigma-Aldrich. Bovine Serum Albumin aqueous solution (2 mg/ml) has been investigated. The tests were performed on aqueous solutions, on the basis of scientific reports (Michnik *et al.*, 2005) that such a solution provides a better understanding of the physicochemical properties of the tested substances. To determine the characteristics of the applied electromagnetic field to BSA aqueous solution, a special experimental setup was constructed. It contains a source of electromagnetic field (wave generator), BSA solutions, EMC-Scanner model RSE321, a spectrum analyzer with near field probe and a standard computer. Electromagnetic field scans were collected at 200 MHz. Scans were made in the Y Z plane before the sample (area 2 cm×2 cm) and behind the sample (2 cm×2 cm). The absorption spectra in the range of 250-300 nm were examined with the use of double-beam UV/Vis spectrophotometers (Halo DB-20R) Dynamica.

The track changes in the absorption peak of a protein solution are resulting from the occurrence in the protein of the aromatic residues, such as tryptophan (Trp), tyrosine (Tyr) and Phenyloalanine (Phe) (Hongliang *et al.*, 2013).

In [F1] experiment 200×1 is a description of the sample that was exposed to the 200 MHz only once in 60 min on the first day of the experiment; 200×2 concern sample exposed to 200 MHz two times (60 min in the first day of the experiment and 60 min in the second day of the experiment); 200×3 means exposition to 200 MHz thrice: 60 min in the first day, 60 min in the second day, 60 min in the third day.

For [F2] experiment, brain metabolite NAA (N-Acetyl-L-aspartic acid) (lot: BCBR3519) was obtained from Sigma–Aldrich. An aqueous solution of the BSA and BSA-NAA mixture was investigated.

The study has been performed for two NAA concentrations: for NAA 1mg/ml (1 NAA) and for NAA 3mg/ml (3NAA). In additional, the mixture has been tracked at 4°C and 22°C.

1 NAA means a solution of 2 mg/ml BSA with 1 mg/ml NAA stored at 22°C; 1 NAA 4°C means a solution of 2 mg/ml BSA with 1 mg/ml NAA stored at 4°C; 3NAA means a solution of 2 mg/ml BSA with 3

mg/ml NAA stored at 22°C; 3 NAA 4°C means a solution of 2 mg/ml BSA with 3 mg/ml NAA stored at 4°C

[F3] stands for a study considering the influence of microwave radiation and lowered temperature (4°C).

The solution was divided into groups. One of them was stored at a reduced temperature (4°C), and the next group was exposed to microwave radiation (10 seconds, the power of 750 W), then after 30 min it was also inserted in the refrigerator (4° C) (microwave 10 s and 4°C).

[F4] describes changes occurring in time under the influence of an external factor, which was the reduced temperature (–18°C). A single exposure lasted 60 minutes at (–18°C) (×1) for the first group.

The second group of the sample has been treated with an external agent (–18°C) twice (×2), on the first and second day of the experiment.

The third group of the sample was subjected to three times of exposure (–18°C). On the first, second and third day of the experiment (×3) [F5] concerns microwave radiation applied in fractional and single doses. In the case of single doses, the exposure to microwaves lasted: 10 s, 15 s. No change in temperature of the radiated systems was observed due to the short time of the applied agent.

For fractional doses, radiation was administered in fractions: double (5s×2), the sample was exposed to microwaves twice for 5s each, and a triple (5s×3) sample was exposed to microwaves three times for 5s each; the time intervals for multiple fractional doses were 3 hours, the radiation power was 160 W. The short radiation time did not cause thermal effects.

RESULTS AND DISCUSSION

The most important conclusions and observations resulting from the conducted five types of research [F1] to [F5] one by one

To understand the significance of the presented meta-analysis, the most important conclusions and observations resulting from the conducted five types of research [F1] to [F5] are given.

[F1]: 200 MHz electromagnetic field (fractional and single exposures)

Based on the completed experiments and required analyses (Klos-Witkowska, 2018) it has been shown that:
- there is a cumulative effect of the 200 MHz electromagnetic field impact on the receptor layer cross-linking component,
- exposure to the 200 MHz electromagnetic fields improves the stability of the receptor layer cross-linking component in biosensors,
- multiple exposures of the receptor layer cross-linking component in biosensors to the 200 MHz electromagnetic field improves the component stability.

A very important effect of these tests is a statement that exposure to electromagnetic field (200 MHz) slows down changes occurring in the cross-linking component of the biosensor receptor layer as time passes. This effect is more pronounced for multiple exposures, as compared to single exposure (dosed fractionally).

[F2]: NAA (various concentrations)and temperature 4°C

Tests of stability over time and under variable acidity of the environment and reduced temperature (Klos-Witkowska & Martsenyuk, 2020; Klos-Witkowska et al., 2018) showed the following effects:
- lower stability of biosensor receptor layer components over time,
- changed acidity of the environment by addition of NAA,
- improved stability of BSA-NAA complexes at reduced temperature,
- improved stability of tested biosensor receptor layer cross-linking components as NAA concentration rises and the temperature is reduced.

Completion of the test plan provides evidence that adding NAA to the tested cross-linking

component of the biosensor receptor layer improves its stability. Storage at the temperature of 4°C above zero improves the stability of the cross-linking component of biosensor receptor layers.

[F3]: microwave radiation and lowered temperature (4°C)

Tests presented in publication (Klos-Witkowska et al., 2016) provided evidence for:
- improvement of the stability of the biosensor receptor layer cross-linking component under temperature reduced to 4°C, however, with a concurrent increase in the rate of conformational changes (ageing) under microwave radiation (2.4 GHz, 160 W),
- regardless of the exposure of the biosensor layer cross-linking component to the applied factors (radiation, temperature), progressing conformational changes in time are observed for them.

[F4]: temperature (–18°C) fractional and single exposures

The completed tests provided evidence for:
- reduction of the stability of the cross-linking component of biosensor receptor layers
- caused by the reduction of temperature to the level of –18°C,
- more evident differences between the sample exposed to the factor and control sample, increasing over time.

These tests showed that storage of biosensors at such low temperatures should be avoided, as that large reduction in temperature degrades the stability of the cross-linking component of biosensor receptor layers. This means that biosensors should not be stored in refrigerators. On the other hand, storage in coolers is recommended. Results of these tests were presented in publication (Klos-Witkowska, 2017).

[F5]: microwave radiation (single and prolonged exposure)

The results of completed experiments and analyses were presented in publication (Klos-Witkowska, 2017), in which we provided evidence for:
- the influence of microwave radiation on the stability of the cross-linking component of biosensor receptor layers showing accelerated denaturation processes,
- the impact of radiation in both fractional and single exposure modes,
- higher stability of the samples exposed to fractional radiation,
- more evident differences between the sample exposed to the factor and control sample over time.

To recapitulate, the obtained results of tests covering the impact of selected factors on the stability of the biosensor receptor layer indicate:
- favourable influence of the radiofrequency field ranges of 200 MHz on the stability of the cross-linking component of biosensor receptor layers,
- favourable influence of multiple exposures to the radiofrequency field ranges of 200 MHz on the stability of the cross-linking component of biosensor receptor layers,
- unfavourable influence of light on the stability of the cross-linking component of biosensor receptor layers,
- favourable influence of the increase in NAA concentration and reduction in temperature to +4°C on the stability of the cross-linking component of the biosensor receptor layers,
- unfavourable influence of the microwave radiation (single exposure and fractional exposure to radiation) on the stability of the cross-linking component of biosensor receptor layers,
- unfavourable influence of temperature of –18°C on the stability of the cross-linking component of biosensor receptor layers,
- more evident differences between the sample exposed to the selected factors and control sample over time.

Meta-analysis of [F1] to[F5] experiments. Compilation and comparison of all studies

To compare the influence of F1 to F5 factors on the stability of the receptor layer cross-linking component in biosensors, a meta-analysis has been conducted.

In the first step, we have compiled all of the experiments carried out. The results are listed below in Table 1.

A new type of analysis was carried out to compare the impact of external factors affecting the stability of the receptor layer cross-linking component in biosensors. We have offered an approach of numerical interpretation of the qualitative changes of stability.

Special meta-analysis has been performed to determine which of the applied factors affected the most the stability of the receptor layer cross-linking component in biosensors. For this purpose relative percentage differences were calculated based on a formula

$$K=(A_1/A_0)\cdot 100$$

where: A_1 – absorption under the influence of the factor in the following days of the study; A_0 – absorption of control samples

Then, based on the formula

$$M=100-K$$

Table 1. Comparison of conducted experiments [F1] to [F5] and stability changes examined on appropriate days of the experiments

Days	F1			F2				F3		F4			F5			
	200 MHz			4°C						−18°C			microwave			
	x1	x2	x3	1 NAA	1 NAA 4°C	3 NAA	3 NAA 4°C	4°C	mirowave 10 s and 4°C	x1	x2	x3	x2	x3	10 s	15 s
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+			+	+	+				
3	+	+	+	+	+	+	+			+	+	+				
4				+	+	+	+			+	+	+	+	+	+	+
5				+	+	+	+			+	+	+				
6				+	+	+	+			+	+	+	+	+	+	+
7				+	+	+	+									
8	+	+	+	+	+	+	+	+	+				+	+	+	+
9				+	+	+	+									
10	+	+	+	+	+	+	+									
11				+	+	+	+						+	+	+	+
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The percentages of changes in individual days were calculated $M_1, M_2, M_3, \ldots M_y$.

Based on them, adding up their values, relative ratios were obtained (M_{Dx}), where x=1,2….

$M_1 + M_2 = M_{D1}$,
$M_2 + M_3 = M_{D2}$,
$M_3 + M_4 = M_{D3}$.

Based on the coefficients M_{Dx}, a graph illustrating the change in M_{Dx} value in subsequent days of the experiment was made, and then fitting of functions into a logarithmic trend has been performed.

M_{Dx} matching with a description of logarithmic functions in the form y=aLn (x) $\pm$ b for selected coefficients is presented in Fig. 2.

The chart shows the rising character for y1, y2, y3 and sloping character for y4, y5,y6.

It was interpreted that the parameter "a" in the description of the logarithmic function y=a Ln (x) $\pm$ b is a parameter demonstrating the nature of changes in stability.

When an increase in stability is observed, the parameter "a" takes positive values, whereas when the decrease in stability is noticeable, then this parameter takes a negative value. And so for the y1 coefficients illustrate the F1 experiment (200 MHz×3), y2 illustrates the F1 experiment (200 MHz×2) and y3 illustrates the F3 experiment (4°C) the factors 200 MHz and 4°C are improving stability of receptor layer component in biosensors, whereas y4, y5, y6 coefficients illustrating the F4 (−18°C×3), F4 (−18°C×2), F4 (−18°C×1) reduce the
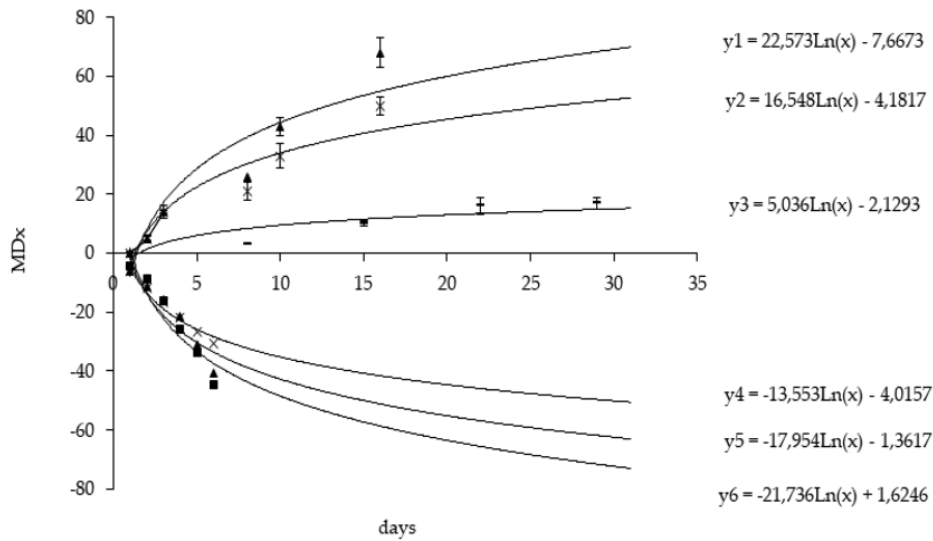
Figure 2. M_{Dx} matching with the description of logarithmic functions for selected coefficients $y1=F_1$: (200 MHzx3), $y2=F_2$ (200 MHzx2), $y3=F_3$: (4°C), $y4=F_4$: (–18x3), $y5=F_4$: (–18x2), $y6=F_4$: (–18x1)

stability of the cross-linking component of biosensor receptor layers.

The same kind of analysis was performed for all measured external factors: F1: 200 MHz×1, F1: 200 MHz×2, F1: 200 MHz×3, F2: 1NAA, F2: 1NAA 4°C, F2: 3NAA, F2: 3NAA 4°C, F3: 4°C, F3: microwave and 4°C, F4: –18°C×1, F4: –18°C×2, F4: –18°C×3, F5: microwave×2, F5: microwavex3, F5: microwave 10 s, F5: microwave 15 s.

The obtained results of this compilation are shown in Fig. 3.

As it is seen (Fig. 3), two types of changes can be distinguished: changes where the "a" parameter is positive and changes for which "a" is negative.

Factors which obtain positive value are interpreted as factors improving stability whereas the factors which obtain negative value are regarded as factors that reduce the stability of the cross-linking component of biosensor receptor layers:

Positive values have been noticed for F1: (200 MHz×1), F1: (200 MHz×2), F13: (200 MHz×3), F2: (1NAA), F2: (1NAA 4°C), F2: (3NAA), F2: (3NAA 4°C), F3: (4°C). Among them, the greatest stability has

been achieved for F2: 3NAA 4°C whereas negative values have been obtain for: F3: (microwave and 4°C), F4: (–18°C×1), F4: (–18°C×2), F4: (–18°C×3), also for microwaves radiation: F5: (microwave×2), F5: (microwave×3), F5: (microwave 10 s), F5: (microwave 15 s).

Based on this analysis it could be said that adding NAA to cross-linking component (BSA) improves its stability the most. NAA is known as a marker of neuronal injury in neurodegenerative disease (Schuff et al., 2006; Tai & Coyle, 1995) For higher concentrations (3 mg/ml) of the added agent, stability was better than for lower concentrations (1 mg/ml NAA). Additionally decreasing the temperature (4°C) compounded the effect.

The performed studies also show a greater destabilizing effect of a significantly reduced temperature (–18°C) than for the microwave radiation.

The presented diagram illustrates factors and its influence on the stability of the biosensor layer cross-linking component.

The destabilization changes in the receptor layer cross-linking component reflect an increase in the amount of helix conformation and a decrease in a beta-sheet structure which leads to loosing of the protein skeleton. It
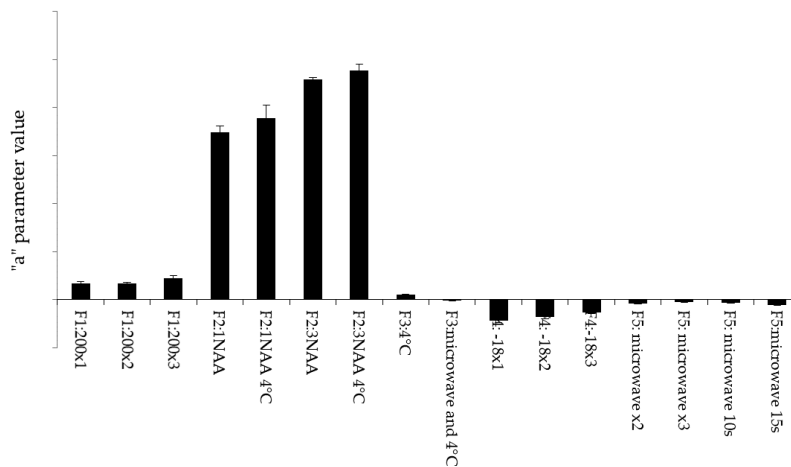

Figure 3. The "a" parameter value interpreted as a factor of improving or reducing the biosensor receptor layer stability

also indicates that fragmentation and aggregation of the studied protein might occur with time. The protein fragmentation in an aqueous solution is affected by the local conformation of a particular amino-acid. Over time, the denaturation of proteins occurs. During this process, the tertiary structure of the protein is disrupted, resulting in loss of biological activity of the protein. Depending on external factors, the tested component of the biosensor matrix can be crushed in whole or only in part (Takeda *et al.*, 1989) have discovered that secondary structural changes occur only after the reduction of disulfide bonds. Then, the helix content drops from 66% to 25%, the structure content increases from 3% to 19%. The receptor layer cross-linking component form may change over time and under the influence of external factors, such as pH and temperature. Further conformational changes were labelled as follows: E — expanded, F — fast-highly changed state, N — normal, 55% α-helix structure, B — basic and A — aged unfolded state. The N–F conformational transition is characterized by a sudden opening of the molecule. The process is very rapid and it means 100 ms and concerns the spreading of the third domain. The F form is characterized by a much lower solubility and decreased helix structure. The E form is a form of stretched protein, which decomposes into a helix I domain that is joined to the helix II domain. Another known conformation is the B conformation from which the next isomerization progresses to the ageing A forms (Michnik *et al.*, 2005; Holiang *et al.*, 2013). The stabilizing effect relates to the slowing down of changes taking place over time.

CONCLUSIONS

This paper presents a meta-analysis of the influence of the external conditions on the biosensor receptor layer component stability. The performed meta-analysis allowed to increase the precision and accuracy of the results by combining and co-analysing data. An approach of numerical interpretation of the qualitative stability changes has been offered. The conducted meta-analysis showed that the tested factors influence the stability of BSA in different ways. Factors improving the stability of the cross-linking component of biosensor receptor layers include radio frequency range (200 MHz) and temperature of 4°C. Higher stability was obtained after adding NAA and at 4°C.

On the other hand, factors that reduce the stability of the cross-linking component of biosensor receptor layers include microwave radiation and reduction of temperatures to –18°C. It is also worth mentioning that differences between the sample exposed to a given factor and the control sample were higher over time.

The conducted meta-analysis shows the magnitude of differences in stability caused by chosen external factors.

The results obtained in the manuscript can be used in the design of biosensors. They help to determine the conditions for storage of that type of devices to prolong their stability.

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