

Full Paper

Electrochemical Oxidation of Masalazine Drug at Poly(glutamic acid) Modified Glassy Carbon Electrode

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Abstract- A sensitive and quick responsive analytical method was developed for the electrochemical oxidation of masalazine (MSZ) using poly (glutamic acid) modified glassy carbon electrode (MGCE). The modified electrode shows electrocatalytic activity towards the oxidation of MSZ drug in 0.2 M phosphate buffer solution (PBS) of pH 7.4 by cyclic voltammetric (CV) technique. The limit of detection was calculated to be 23.94 nM at modified electrode. The effect of scan rate study reveals an adsorption controlled electrode phenomenon. The modified electrode can be used as an analytical tool for the determination MSZ in pharmaceutical formulations.

Keywords- Masalazine, Modified glassy carbon electrode, Cyclic voltammetry, Electrocatalytic activity

1. INTRODUCTION

The masalazine (mesalmine, 5-Amino-2-hydroxy benzoic acid) is an amino salicylate anti-inflammatory drug widely used for the therapy of bowel disease, ulcerative colitis and inflamed rectum and crohn's disease. It provides protection against the improvement of colorectal cancer in patient suffering from inflammatory bowel disease [1]. In that MSZ

protect cell injury or cell damage in the inflamed mucosa by scavenging reactive oxygen metabolites, thus suppressing their toxicity [2]. In the literature, the abrasive stripping voltammetry was used for determination of MSZ in commercial dosage forms [3,4], but proposed method is not effective for quantitative analysis. The amperometric catalase-peroxidase based biosensor was developed for the measurement of MSZ concentration by indirect method by monitoring the dissolved oxygen level [5]. In the research report, the reduction process and kinetics of differently substituted azosalicylic acid at glassy carbon electrode (GCE) and their voltammetric measurements at the hanging mercury drop electrode was reported [6,7-12]. The effective analysis of the MSZ drug at traditional methods like spectrophotometry, fluorescence, chemiluminescence and ultra-performance liquid chromatography were also been reported [13-18]. In addition to this new method like electrochemical and mass spectrometry identification [19-25] was developed.

Among all the above mentioned methods, the development of new analytical methods for the determination of MSZ drug is of great importance in the modern era. Because of the easy operational procedure, rapid and accurate results with low cost, electroanalytical methods are widely adopted [26-28]. Now a day's electrochemical measurements at solid working electrodes were attracted towards the determination of biologically active molecules. Moreover, polymer modified electrodes (PMEs) have received great importance in this present era. As the polymer film which is deposited as a thin conducting layer onto the surface of the working electrode by electropolymerisation method have good stability, more active sites, homogeneity and strong adherence to the electrode surface. Electropolymerisation is a good approach to immobilize an organic moiety to prepare PMEs [29-31]

The present work describes an electropolymerisation of L-glutamic acid on the surface of glassy carbon electrode by cyclic voltammetric technique. To utilise the electrochemical behavior poly (glutamic acid) modified glassy carbon electrode (MGCE) it was employed for the electro-oxidation of an important drug masalazine. The result obtained at modified electrode shows the stability, sensitivity, reproducibility and anti-fouling property towards the determination of masalazine at physiological pH. Therefore, the fabricated electrode can be used as an effective sensor for the quantification of masalazine drug in pharmaceutical formulations.

2. EXPERIMENTAL SECTION

2.1. Instrumentation

The electrochemical experiments were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation). A conventional three electrode system was employed in an electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum counter electrode and glassy carbon electrode or poly (glutamic acid) modified

glassy carbon electrode as a working electrode. All the voltammograms were recorded at an ambient temperature of 25 ± 0.2 °C. The corresponding oxidation potential of analyte was recorded versus SCE.

2.2. Reagent and chemicals

Masalazine (MSZ) (MW=153.135 g/mol, 99.9%) and L-glutamic acid (MW=143.139, 99.9%) were obtained from Himedia. The stock solutions of 25×10^{-4} M MSZ and 25×10^{-3} M L-glutamic acid were prepared in 0.1 M perchloric acid and double distilled water respectively. Phosphate buffer solution (PBS) of same ionic strength was prepared (0.2 M) by mixing appropriate ratio of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and Na_2HPO_4 . The glassy carbon electrode (GCE) was polished using 0.3 and 0.05 micron $\alpha\text{-Al}_2\text{O}_3$ slurry on a polishing cloth prior to each experiment. After polishing, the electrode was rinsed thoroughly with double distilled water. Until a shiny mirror like appearance was observed.

3. RESULTS AND DISCUSSION

3.1. Preparation of poly (glutamic acid) modified glassy carbon electrode

Cyclic voltammetry (CV) is an effective and convenient technique to coat an amino acid based polymers on the surface of solid working electrode [26].

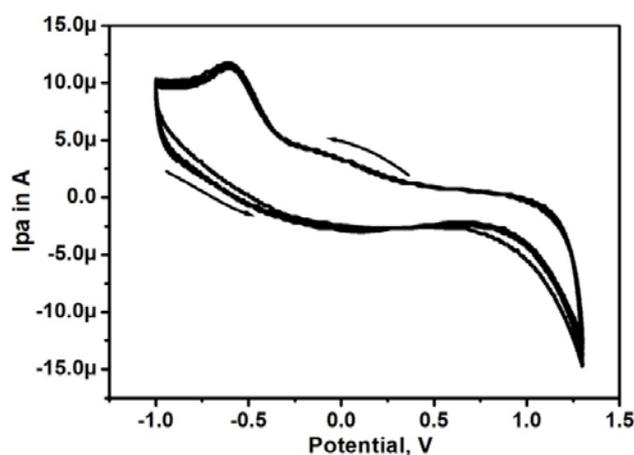
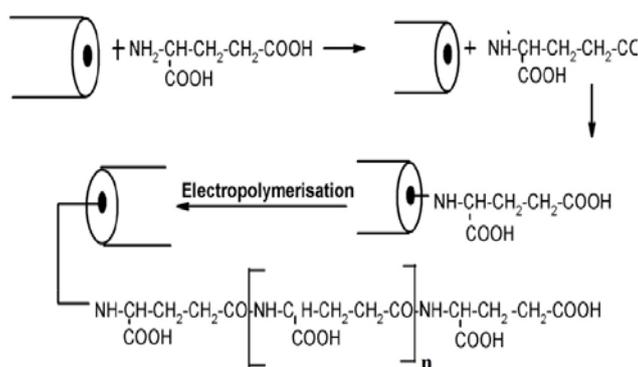


Fig. 1. Cyclic voltammograms of preparation of poly(glutamic acid) MGCE. 1 mM L-glutamic acid solution in 0.2 M PBS of pH 7.4 at 12cycles with scan rate 0.1 Vs^{-1}

The electropolymerisation of glutamic acid on the surface of GCE was achieved by successive sweeping of bare glassy carbon electrode (BGCE) in an electrochemical cell containing 1.0 mM of L-glutamic acid monomer in 0.2 M PBS of pH 7.4 as a supporting electrolyte. For the electropolymerisation process the potential window of -1.0 to +1.3 V was chosen with the scan rate of 0.1 Vs^{-1} for twelve multiple cycles as showed in Figure 1. Higher

the thickness of the modifier leads to decrease in the electrocatalytic activity of the modified electrode. Therefore, twelve multiple cycles were chosen as an optimum [32]. Later, the poly (glutamic acid) modified glassy carbon electrode (MGCE) was rinsed thoroughly with double distilled water several times and used for the determination of MSZ. The probable electropolymerisation mechanism of L-glutamic acid on the surface of GCE was depicted in scheme 1.



Scheme 1. Probable electropolymerisation mechanism of L-glutamic acid on the surface of glassy carbon electrode

3.2. Electrochemical characterization of poly (glutamic acid) MGCE

The freshly prepared stock solution of 1 mM $K_4[Fe(CN)_6]$ in 1 M KCl was placed in an electrochemical cell. The Figure 2 showed the cyclic voltammograms (CVs) recorded for the oxidation of potassium ferrocyanide probe at both BGCE (dashed line) and poly (glutamic acid) MGCE (solid line) with the scan rate 0.1 Vs^{-1} . The low redox peak current response was obtained at BGCE, but in the same identical condition poly (glutamic acid) MGCE exhibited static increment in the redox peak current due to the faster electron transfer kinetics at the electrode surface. An improved voltammetric response was observed at poly (glutamic acid) MGCE. This result suggested the surface property of the modified electrode has been significantly altered and also the results proves that the electrocatalytic activity of the poly (glutamic acid) MGCE. The total available surface area for reaction of species in solution can be estimated by the Randles-Sevcik equation (1) [26,33].

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_0 \nu^{1/2} \quad (1)$$

Where, I_p is the peak current in A. C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electrons exchanged, D is the diffusion-coefficient (cm^2s^{-1}), and ν is the scan rate (Vs^{-1}), A is the electroactive surface area (cm^2). For poly (glutamic acid) MGCE the electroactive surface area is maximum (0.0362 cm^2) as compared with BGCE (0.0292 cm^2).

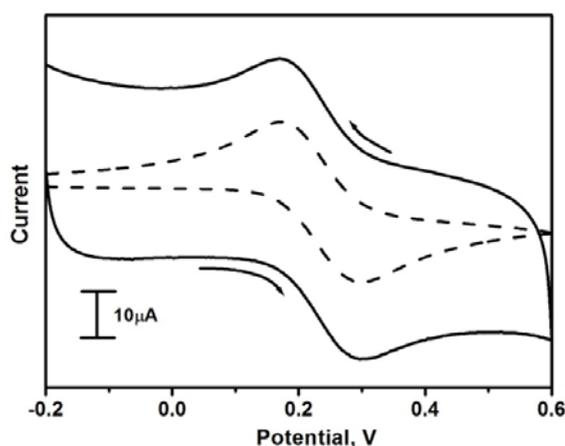


Fig. 2. Cyclic voltammograms of 1 mM potassium ferrocyanide at BGCE (dashed line) and poly(glutamic acid) MGCE (solid line) at scan rate of 0.1 Vs^{-1}

3.3. Electrochemical oxidation of MSZ at poly (glutamic acid) MGCE

Figure 3 showed the CVs recorded for the oxidation of 0.1 mM MSZ at BGCE and poly (glutamic acid) MGCE in 0.2 M PBS of pH 7.4 with the scan rate of 0.05 Vs^{-1} . At BGCE (dashed line) the MSZ showed a broad voltammetric response with low current signals, and was located at 0.276 V (versus SCE).

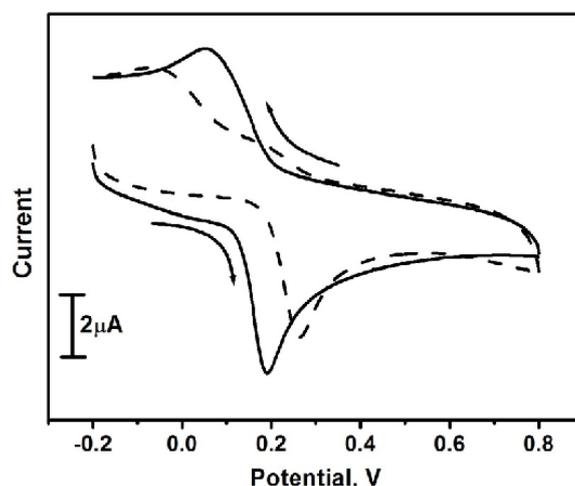
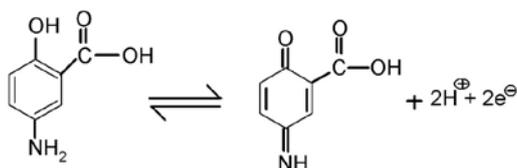


Fig. 3. Cyclic voltammograms of 0.1 mM MSZ in 0.2 M PBS solution of pH 7.4 at BGCE and poly (glutamic acid) MGCE in 0.2 M PBS of pH 7.4 with the scan rate of 0.05 Vs^{-1}

However, on the other hand the oxidation potential of MSZ was observed at 0.192 V at poly (glutamic acid) MGCE (solid line). The peak to peak separation (ΔE_p) between redox potentials at poly (glutamic acid) MGCE was less as compared to BGCE, suggesting the fast electron transfer kinetics at the modified electrode. This minimisation of over oxidation

potential and increment in the current response confirms the electrocatalytic activity of the fabricated electrode towards the feasible oxidation of MSZ. The oxidation mechanism of MSZ was shown in scheme 2.



Scheme 2. Oxidation mechanism of masalazine

3.4. The effect of scan rate on peak current of MSZ

The effect of variation in the applied scan rate for 0.1 mM of MSZ in 0.2 M PBS of pH 7.4 was examined by cyclic voltammetric technique at poly (glutamic acid) MGCE as showed in Figure 4. The experimental results obtained at poly (glutamic acid) MGCE showed increase in the redox peak currents with increase in the applied scan rate in the range 0.02 to 0.2 Vs^{-1} and they are proportional to each other according to Randles-Sevcik equation. The observation showed that there is a slight shifting of anodic peak potential (E_{pa}) towards positive side and cathodic peak potential (E_{pc}) to the negative side. In order to confirm the electrode process, the graph of anodic peak current (I_{pa}) versus scan rate (ν) and I_{pa} versus square root of scan rate ($\nu^{1/2}$) were plotted as shown in Figure 5 and Figure 6 respectively. A good linearity ($r^2=0.9976$) was observed for I_{pa} and ν . Which confirms the adsorption controlled phenomenon at the electrode surface [34,35].

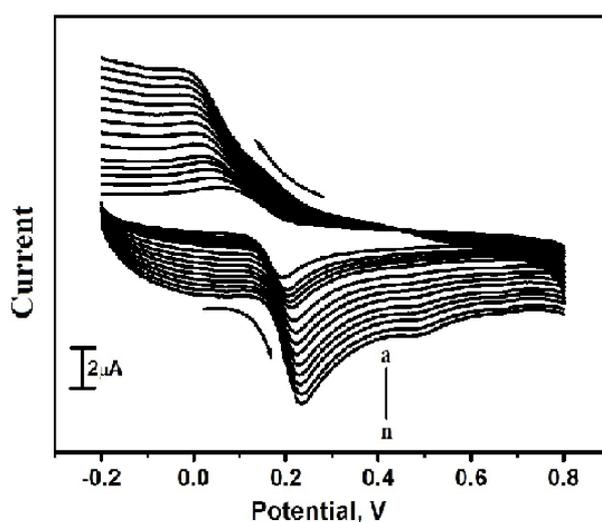


Fig. 4. Cyclic voltammograms of 0.1 mM MSZ in 0.2 M PBS solution of pH 7.4 at poly (glutamic acid) MGCE at different scan rate (a-h; 0.02 Vs^{-1} to 0.2 Vs^{-1})

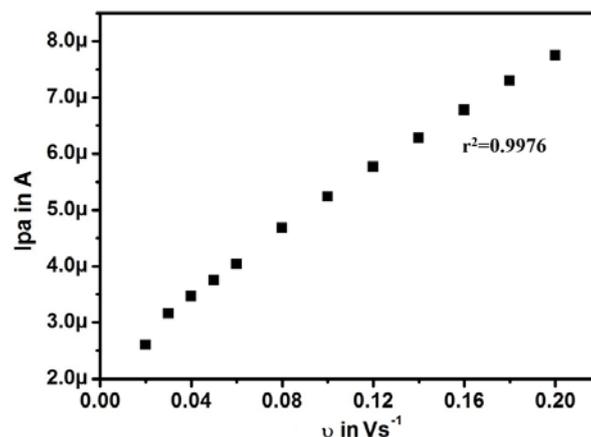


Fig. 5. Graph of anodic peak current versus scan rate

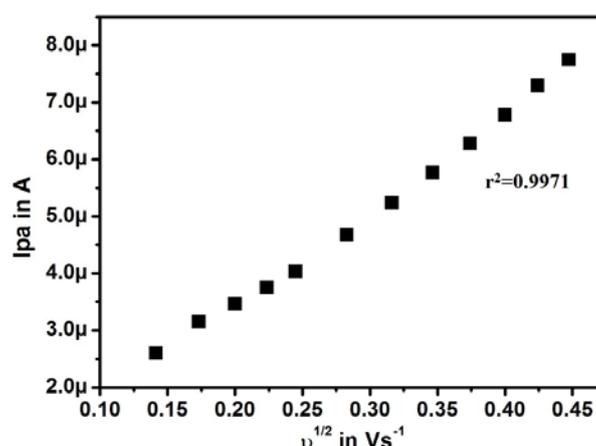


Fig. 6. Graph of anodic peak current versus square root of scan rate

3.5. Effect of MSZ concentration

The electrocatalytic oxidation of MSZ was carried out by varying its concentration at poly (glutamic acid) MGCE. The Figure 7 showed, by increasing the concentration of MSZ from 50 μ M to 0.5 mM the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards less positive and E_{pc} towards least negative side. The graph of I_{pa} vs. concentration of MSZ was plotted as showed in the Figure 8, it gives almost straight line with good linearity. The linear regression equation can be expressed as $I_{pa}(A)=4.9930\times 10^{-6}(C_0 \ 10^{-4} \text{ M/L})+1.8566\times 10^{-6}$, ($r^2=0.9997$). The limit of detection (LOD) was calculated according to the equation (2) [26-28].

$$\text{LOD}=3 \text{ S/M} \quad (2)$$

Where, S is the standard deviation of six blank-solution measurements. M is the slope of the calibration graph [34]. The LOD was calculated in the lower concentration range for MSZ

and was found to be 23.94 nM. The proposed method exhibited lower detection limit than those reported as shown in table 1 [4-5,37-39].

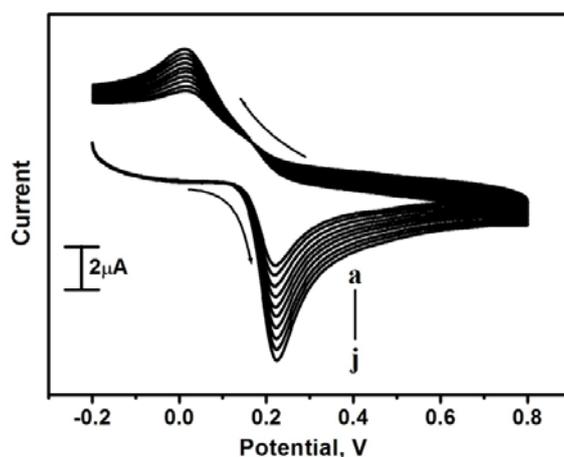


Fig. 7. Cyclic voltammograms of MSZ in 0.2 M PBS solution of pH 7.4 at poly (glutamic acid) MGCE at scan rate of 0.05 Vs^{-1} with different concentration (a–j; $50 \mu\text{M}$ to 0.5 mM)

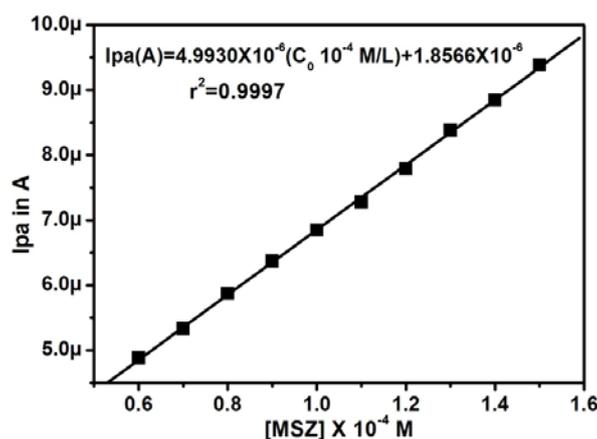


Fig. 8. Graph of anodic peak current versus concentration of MSZ

3.6. Effect of pH value on the determination of MSZ at poly (glutamic acid) MGCE

The pH of the PBS has a significant contribution on the electrocatalytic oxidation of MSZ at the poly (glutamic acid) MGCE by affecting both peak current and peak potential. The effect of PBS pH value on the determination of MSZ at poly (glutamic acid) MGCE was carefully evaluated in a wider pH range of 5.5–8.0. The Figure 9 showed cyclic voltammograms recorded for $0.4 \times 10^{-4} \text{ M}$ of MSZ at poly (glutamic acid) MGCE. The oxidation becomes easy at basic media. The anodic peak potential (E_{pa}) versus pH graph clearly indicated that the E_{pa} depends linearly on the pH value in the range of 5.5–8.0 with a slope of 0.050 V/pH ($r^2=0.9985$) as showed in Figure 10. This suggests there is an

involvement of equal number of protons and electrons in the redox mechanism. This was consistent with the earlier reports in literature [27,36].

Table 1. Comparison of limit of detection with different modified electrodes and poly(glutamic acid) MGCE

Technique	Working Electrode	Concentration range (M)	Limit of detection	Ref.
SWV	Pencil graphite electrodes	9.8×10^{-7} - 7.3×10^{-5}	2.1×10^{-8}	[4]
AdLSV	CNT/PPY doped by 1,5-naphthalenedisulfonic acid	1.0×10^{-8} - 1.0×10^{-6}	3.0×10^{-9}	[5]
Sona LSV	GCE	1.0×10^{-6} - 5.7×10^{-5}	3.0×10^{-7}	[37]
DPV	GCE	2.0×10^{-6} - 1.0×10^{-4}	8.2×10^{-7}	[38]
Amperometry	Catalase-peroxidase based biosensor	2.0×10^{-5} - 6.0×10^{-4}	----	[39]
CV	Poly(glutamic acid) MGCE	50.0×10^{-6} - 0.5×10^{-3}	23.94×10^{-9}	This work

Sona LSV - Sonoelectrochemically enhanced linear sweep voltammetry

DPV - Differential pulse voltammetry

AdLSV - Adsorptive stripping linear sweep voltammetry

SWV- Square wave voltammetry

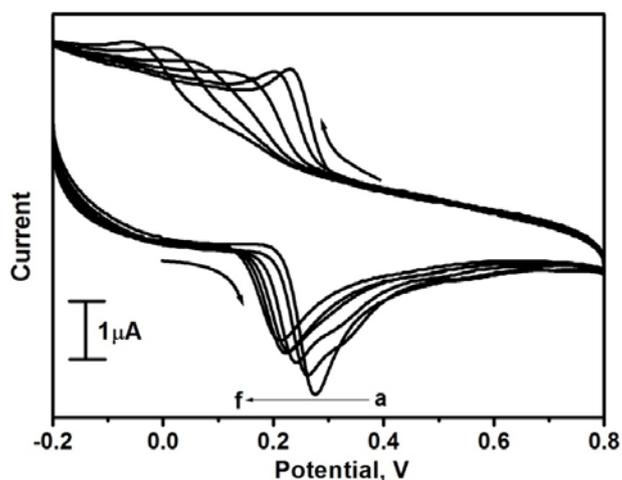


Fig. 9. Cyclic voltammograms of the poly(glutamic acid) MGCE in 0.2 M PBS solution at different pH (a-f: 5.5 to 8.0) at scan rate of 0.05 V s^{-1}

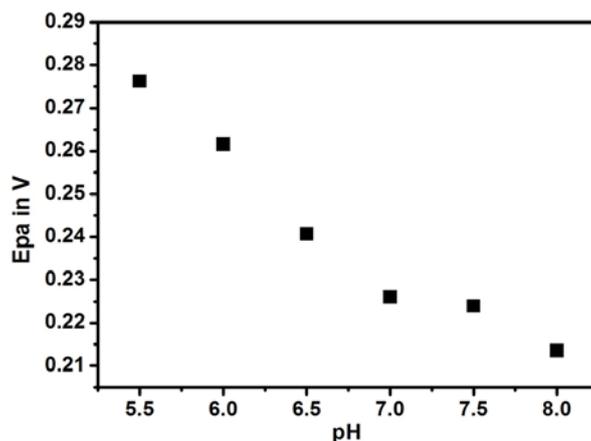


Fig. 10. The effect of peak potential response of 0.4×10^{-4} M MSZ in 0.2 M PBS of different pH

4. CONCLUSION

In order to enhance the performance of bare glassy carbon electrode it was modified by electropolymerising L-glutamic acid monomer by cyclic voltammetric technique. The prepared poly (glutamic acid) MGCE was employed for the electro-oxidation and determination of an important drug MSZ at physiological pH of 7.4 by using CV technique. The detection limit was calculated to be 23.94 nM for MSZ by CV technique. The fabricated electrode showed excellent sensitivity, stability, reproducibility and antifouling property towards the determination of MSZ.

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