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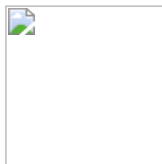


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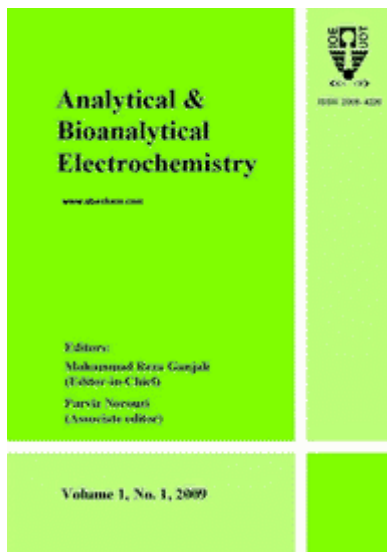


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E-Mails: [a.hajjalizadeh@yahoo.com](mailto:a.hajjalizadeh@yahoo.com); [jahanishohre@gmail.com](mailto:jahanishohre@gmail.com)*Anal. Bioanal. Electrochem., Vol. 10, No. 4, 2018, 404-413; [PDF](#)***Passive and Semiconducting Properties Assessment of Passive Oxide Films Forming on Ti-6Al-4V Titanium Alloy in Ringer's Physiological Solution at 37 °C: Role of Immersion Time and Anodic Passive Potential**

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*Full Paper*

## **Construction and Performance of Creatinine Selective Electrode based on Carbon Paste-Imprinting Zeolite**

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**Abstract-** Carbon paste-modified imprinted zeolite electrodes as a potentiometric sensor for creatinine detection have been constructed. The modified electrodes were fabricated by mixing activated carbon, imprinted zeolite, and paraffin. The electrode that was prepared with a respective mass ratio of 45:15:40 showed the best performance in creatinine detection. In addition, the electrode showed a fast response time (less than 50 s), a wide range of measurement ( $10^{-7}$ – $10^{-5}$  M), and a low limit of detection ( $7.9 \times 10^{-8}$  M). The presence of urea in the solution did not interfere in the detection of creatinine. The proposed creatinine-selective sensor exhibited good reproducibility, accuracy, and a long lifetime. The creatinine-selective electrode based on carbon paste-imprinted zeolite can be potentially used for creatinine detection in the medical field.

**Keywords-** Creatinine selective electrode, Imprinted zeolite, Potentiometry

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### **1. INTRODUCTION**

Creatinine is a product of muscle metabolism, which is excreted through urine. The normal level of creatinine in blood is about 0.6 until 1.2 mg/dL. A lower than normal creatinine concentration may indicate poor nutrition [1], while a high level of creatinine can be a warning for kidney malfunction such as glomerulonephritis, tubular necrosis, and low

filtration of glomerulus [2].

In clinical detection, creatinine concentration is commonly determined using the Jaffe method through reaction between creatinine and picric acid [3]. This method is not specific because not only creatinine, but also ketone, glucose, bilirubin, acetoacetic, and cephalosporin are detected [4]. Chromatography has also been used for creatinine detection; however, this method is time-consuming [5]. Other methods such as flow injection potentiometry [6] and voltammetry using a molecularly imprinted polymer (MIP)-modified hanging mercury drop electrode (HMDE) method [7,8] have been developed for creatinine detection. Detection of creatinine by voltammetry using an electrode based on poly(melamine-co-chloranil)-modified HMD exhibited high selectivity [7]. Unfortunately, this electrode had a short lifetime (can be used only once). The electrode preparation process also depends on polymer solubility. For a low-solubility polymer, electrode fabrication is difficult. A MIP synthesized from aniline as a monomer with ammonium peroxydisulfate as the initiator and creatinine as a template has been used to modify HMDE for voltammetric detection of creatinine. This method yielded high accuracy and sensitivity, but had low precision [9].

Potentiometry is one of the electrochemical methods that determines the difference in cell potential (EMF) between the working and reference electrodes under zero current. Some researchers have developed potentiometric sensors to analyze creatinine [10,11]. However, not all the analytes yielded a high signal response because of the use of low-conductivity electrodes. In this research, a high-response and highly selective electrode is proposed for creatinine detection by potentiometry. A carbon paste electrode was modified with imprinted zeolite to construct the potentiometric electrode. The imprinted zeolite was used to increase the conductivity of the electrode, because of which a high signal response was obtained.

Zeolite is a crystalline  $TO_4$  (T is a tetrahedral unit such as Al, Si, or Ti) with channels and three dimensional framework. The unique properties of zeolite as a porous material have been employed for pore selectivity. The shape and pore size of zeolite are designed to ensure conformity between zeolite pore and the target molecule. Our previous research had produced an imprinted zeolite, whose pores were selective toward uric acid. Titanium silicalite-1 and LTA zeolite have been proposed as selective materials for modification of carbon paste and glassy carbon electrode, which exhibited high selectivity in uric acid detection by potentiometry and voltammetry [12,13].

In this work, we studied the optimum composition of carbon and imprinted zeolite in the preparation of an electrode for creatinine detection by potentiometry. The electrode properties were discussed with respect to response time, Nernst factor, limit of detection, range of measurement, selectivity, precision, and accuracy.

## 2. EXPERIMENTAL

### 2.1. Materials

Creatinine anhydrous (Sigma Aldrich, 98%), SiO<sub>2</sub> (Ludox, 40%), sodium aluminate (Sigma Aldrich, 50%), glacial acetic acid (Merck, 100%), sodium acetate (Merck, 99%), sodium dihydrogen phosphate dihydrate (Merck, 97%), urea (Merck, 98%), sodium hydrogen phosphate dihydrate (Merck, 99%), silver wires with 100% of purity, solid paraffin, carbon powder, and distilled water. All chemicals were used as received without any further purification.

### 2.2. Instruments

The potentiometric was performed on Cyberscan 510 using reference electrode Ag/AgCl. The structure of zeolite was examined using XRD JEOL JDX-3530. The surface area and pore diameter of carbon was measured by N<sub>2</sub> adsorption-desorption using Quantachrome Instruments version 2.0.

### 2.3. Preparation of carbon paste electrode-imprinted zeolite

Firstly, we synthesized zeolite using mole ratio about 4 Na<sub>2</sub>O: 1 Al<sub>2</sub>O<sub>3</sub>: 1.8 SiO<sub>2</sub>: 270 H<sub>2</sub>O [14]. Sodium aluminate as alumina source was mixed with water and SiO<sub>2</sub>. The mixture was stirred and annealed at 100 °C. After 45 h, the obtained mixture was added creatinine with ratio creatinine: Si=0.0306. The mixture was aged for 3 h to trap the creatinine into zeolite pores. Moreover, the mixture was filtered using centrifugation with hot water till neutral. The purpose of this step was creatinine leave prints in the zeolite framework. The residue was dried at 80 °C. The resulted powder was called imprinting zeolite.

Carbon paste electrode-imprinted zeolite was prepared using Ag wires as connector between electrode and potentiometer. A <sup>3</sup>/<sub>4</sub> part of micropipette tube was filled using melted paraffin. After that, we mixed activated carbon-paraffin-imprinted zeolite, and heated at 50 °C to result the paste texture. The formed paste was then filled into <sup>1</sup>/<sub>4</sub> part of micropipette tube.

### 2.4. Optimization of electrode

To obtain the optimum condition of electrode, we investigated the composition of electrode and pH of creatinine sample. The variation of electrode composition was displayed in Table 1. The prepared electrodes were used to measure potential of electrode in the 10<sup>-2</sup>–10<sup>-8</sup> M of creatinine standard solution. The optimum electrode was determined by value Nernst factor, linearity on EMF versus log concentration plot and range of concentration. The pH of creatinine solution was varied to study the influence of acidity/basicity towards electrode performance. The pH was varied of 4, 5, 6, 7, and 8 by adding buffer solution. The

pH that performed constant potential was determined as optimum pH.

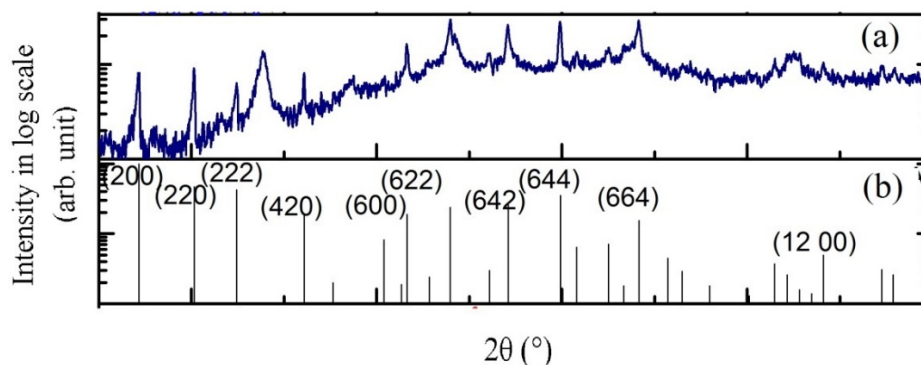
**Table 1.** The composition of activated carbon, imprinting zeolite and solid paraffin in the preparation of carbon paste electrode-imprinted zeolite

Electrode	Composition (%weight)		
	Activated carbon	Imprinted zeolite	Solid Paraffin
E1	60	0	40
E2	55	5	40
E3	50	10	40
E4	45	15	40
E5	40	20	40

### 3. RESULTS AND DISCUSSION

#### 3.1. Influence of electrode composition and pH on potentiometric response of the creatinine-selective electrode

To clarify the structure of zeolite, we characterized the obtained sample by XRD. The diffractogram of the synthesized zeolite is depicted in Figure 1. The XRD profile confirms the structure of zeolite LTA. The diffractogram shows peaks at  $2\theta=7.14, 10.10, 12.40, 16.04, 21.58, 23.90, 27.02, 29.84,$  and  $34.07^\circ$ , among which the peak at  $12.40^\circ$  is associated with the cubic structure of zeolite LTA [15].

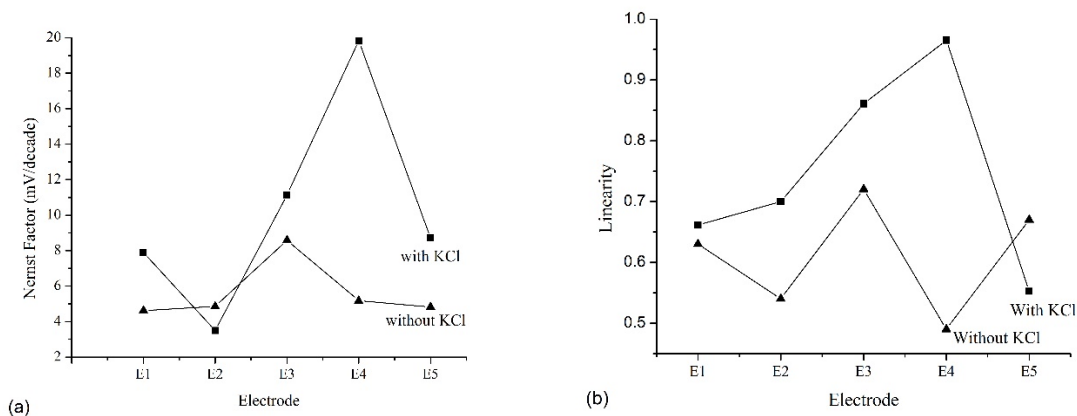


**Fig. 1.** XRD profile of (a) prepared zeolite LTA and (b) JCPDS of zeolite LTA (01-073-2340)

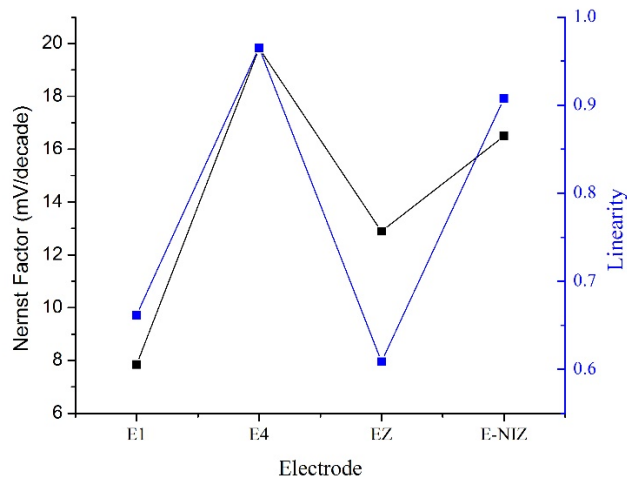
The working electrodes were prepared by mixing activated carbon, imprinted zeolite, and paraffin. Activated carbon was prepared by the activation method using  $10^{-1}$  M  $H_3PO_4$ . It is well known that activated carbon exhibits high chemical stability and conductivity, and has a large surface area and pore diameter of about  $877.463 \text{ m}^2/\text{g}$  and  $3.835 \text{ nm}$ , respectively. Imprinted zeolites were used to increase the selectivity of the electrode because they present selective sites for creatinine molecules. Paraffin was added to bind the composite of carbon and imprinted zeolite



The performance of the electrode was determined by the estimation of linearity, range of measurement, and Nernst factor. The Nernst factors of the resulting electrodes were 4.6–8.5 mV/dec, which were lower than the theoretical value. The obtained linearity was also lower than 1 at about 0.49–0.71. To increase the sensitivity of the method, KCl was added into the solution as it can ionize in water and improve the conductivity of the solution. After KCl addition, the Nernst factor and linearity increased, as can be seen in Figure 2. Among the synthesized electrodes, E4 displayed the highest Nernst factor and linearity. Therefore, for further study, E4 was used as the electrode.



**Fig. 2.** (a) Nernst factor and (b) regression linearity of different electrode compositions



**Fig. 3.** Nernst factor and linearity of E1, E4, EZ, and E-NIZ electrodes

The concentration of imprinted zeolite affected the amount of active sites of the electrode. E4 with a carbon, imprinted zeolite, paraffin mass ratio of 45:15:40 showed the best performance as a sensor in creatinine detection by the potentiometry method, while E5 that contained a higher amount of imprinted zeolite exhibited a low Nernst factor and linearity.

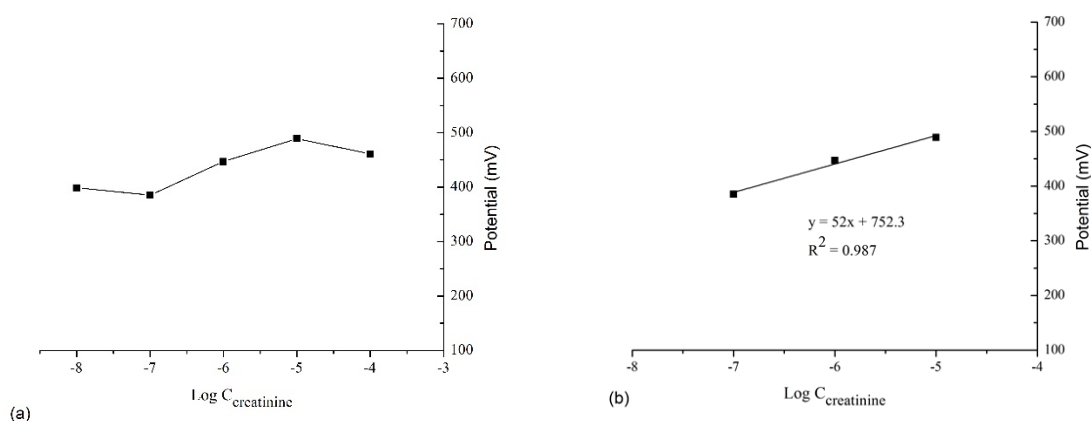
This is probably due to the formation of a rigid membrane, which resulted in the low response of the electrode toward the analyte.

To evaluate the effect of creatinine template on the performance of the electrode, we compared the working performances of E1 (without imprinted zeolite), E4, carbon paste-zeolite (EZ), and carbon paste-non-imprinted zeolite electrodes (E-NIZ). E4 exhibited better linearity and a higher Nernst factor than EZ and non E-NIZ did. This is because EZ did not have a creatinine-selective template, while in E-NIZ, creatinine was trapped in the zeolite framework and could not effectively move from the solution to the electrode. A comparison of the performances of these electrodes is displayed in Figure 3.

The electrode performance in  $H^+$  sensing was also investigated. The performance of the optimal electrode was measured in solutions with varying pH (4, 5, 6, 7, and 8). The pH changed the Nernst factor. At pH 7, the resulting Nernst factor was about 52 mV/dec. Compared to other pH, the electrode showed a constant potential at pH 7. Creatinine has two dissociation constants,  $pK_{a1}=4.8$  and  $pK_{a2}=9.2$ . However, since  $pK_{a2}$  is less than 7, the second protonation of  $H^+$  is difficult. It can be concluded that creatinine was analyzed as a monovalent molecule. Furthermore, creatinine detection at pH 7 is advantageous and effective because it is similar to the blood and urine pH, and thus, does not require pH treatment in the sample preparation.

### 3.2. Calibration graph of creatinine

A standard curve of creatinine was plotted from the electrode potential data of the creatinine measurements and log creatinine concentrations of  $10^{-8}$ – $10^{-4}$  M at pH 7 using the E4 electrode.



**Fig. 4.** (a) Plot of  $\log C_{\text{creatinine}}$  vs. electrode potential and (b) linear curve of creatinine-selective electrode for  $10^{-7}$ – $10^{-5}$  M creatinine solution

From the data, we created the correlation curve of log of creatinine concentration ( $\log C_{\text{creatinine}}$ ) and potential (EMF) (Figure 4a). The  $\log C_{\text{creatinine}}$  from -5 to -7 displayed the regression equation  $y=52x+752.3$ , with  $R^2=0.987$  (Figure 4b). From this equation, the obtained Nernst factor was 52 mV/dec, which is close to the theoretical Nernst factor of monovalent compound. In other words, the linear concentration range of the prepared electrode was  $10^{-7}$ – $10^{-5}$  M.

The limit of detection is derived from intersection of the linear ( $y=52x+752.3$ ) and non-linear ( $y=38x^2+556x+2415$ ) functions in the plot of  $\log C_{\text{creatinine}}$  vs. potential (mV). The limit of detection was calculated as  $7.9 \times 10^{-8}$  M, while the detection limit of the commonly used method for creatinine detection in the medical field (spectrophotometric method) is in the order of  $10^{-5}$  M [16].

### 3.3. Performances of creatinine-selective electrode based on carbon paste and imprinted zeolite

In this study, the response time, reproducibility, accuracy, selectivity, and lifetime of the fabricated electrode were investigated. The response time of an electrode is the time required by the electrode to detect an analyte, and is considered from the time of immersion of the electrode into the solution until the observation of a stable potential [17]. The response time increased with decreasing in creatinine concentration. At a higher concentration, molecules move faster from the solution to the electrode. Table 2 displays the variation in the response time of the electrode with creatinine concentration.

**Table 2.** The response time of electrode for  $10^{-7}$ – $10^{-5}$  M of creatinine

Concentration (M)	Response time (second)
$10^{-7}$	47
$10^{-6}$	38
$10^{-5}$	29

The reproducibility was determined by measuring the electrode performance in three creatinine solutions under the same conditions and concentration. The concentration of creatinine in the solution varied in the range of  $10^{-7}$ – $10^{-5}$  M. The proposed electrode demonstrated high reproducibility with a relative standard deviation less than 2.56%. The prepared electrodes also met the required sensor criteria of the Association of Official Analytical Chemists. It states that the maximum relative standard deviation of a sensor should not exceed 15% for concentrations ranging from  $10^{-7}$  M to  $10^{-5}$  M [18].

In clinical laboratories, spectrophotometry is the commonly used method for creatinine detection. Table 3 shows the accuracies of the proposed potentiometric method using the

carbon paste-imprinted zeolite electrode and the spectrophotometry method in the detection of creatinine solution. The developed electrode shows an accuracy of 80.3–100.4%, while the accuracy of the method that is acceptable for concentrations of the order of  $10^{-7}$ – $10^{-5}$  M is 80–120% [18]. Association of Official Analytical Chemists declares the criteria of analysis method. An analytical method is considered eligible for use in quantitative analysis if it meets certain criteria, including relative standard deviation and accuracy. According to the method accuracy and relative standard deviation, the developed carbon paste-modified imprinted zeolite electrode can be used as an alternative sensor for creatinine detection in the medical field.

**Table 3.** The accuracy of potentiometric method compared with spectrophotometry in the measurement of creatinine solution

Number of sample	Concentration (M)		Accuracy (%)
	Potentiometry	Spectrophotometry <sup>*)</sup>	
1	$3.74 \times 10^{-4}$	$4.66 \times 10^{-4}$	80.3
2	$4.77 \times 10^{-4}$	$5.05 \times 10^{-4}$	94.5
3	$7.07 \times 10^{-4}$	$7.04 \times 10^{-4}$	100.4
4	$3.91 \times 10^{-4}$	$4.55 \times 10^{-4}$	85.9
5	$4.52 \times 10^{-4}$	$4.97 \times 10^{-4}$	90.9

<sup>\*)</sup> data from local clinical laboratory

Selectivity, expressed by the selectivity coefficient,  $K_{ij}$ , is the main parameter for performance evaluation of the prepared electrode. The coefficient of selectivity was investigated to study the selectivity of the electrode in creatinine solution, which also contained urea, which has a similar structure as that of creatinine and is present in high concentrations in blood and urine.

**Table 4.** The selectivity coefficient of carbon paste-imprinting zeolite electrode in  $10^{-5}$  M creatinine

Interference	Concentration (M)	$K_{ij}$
Urea	$10^{-6}$	0.085
	$10^{-5}$	0.126
	$10^{-4}$	0.106

The calculated selectivity coefficient values are presented in Table 4. It can be seen that the carbon paste-imprinted zeolite electrode exhibits high selectivity toward creatinine (i)

than toward urea (j), which is indicated by a  $K_{ij}$  value less than 1. The electrode lifetime was determined to investigate the stability of the electrode after being used several times. The electrodes demonstrated a long lifetime of about 7 weeks (used for 160 times).

#### 4. CONCLUSION

Creatinine-selective electrodes demonstrated high repeatability in the concentration range of  $10^{-7}$ – $10^{-5}$  M. The lifetime of 7 weeks (used 160 times) indicated that the electrodes are suitable for routine detection of medical samples. The detection limit of the developed electrode was much lower than that of the spectrophotometric method, and no interference was observed by the presence of urea. Therefore, the potentiometric method using the developed electrode can be potentially used to determine creatinine levels. Further, the proposed electrode showed an accuracy of 80.3–100.4% (n=5). It can be concluded that the method can be applied for creatinine detection in urine or serum samples.

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

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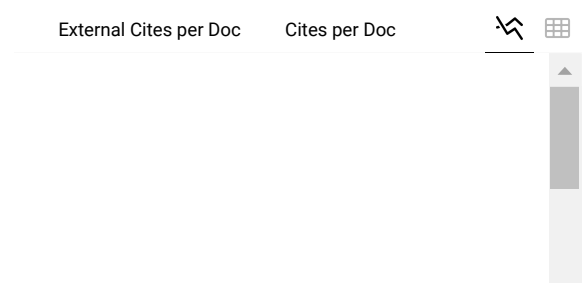
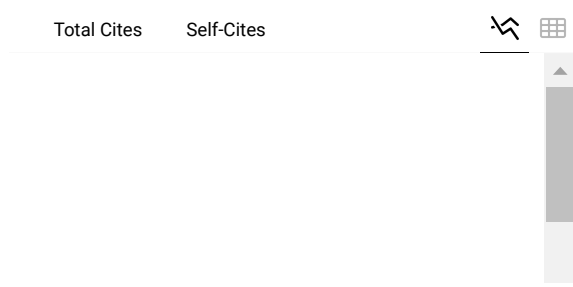
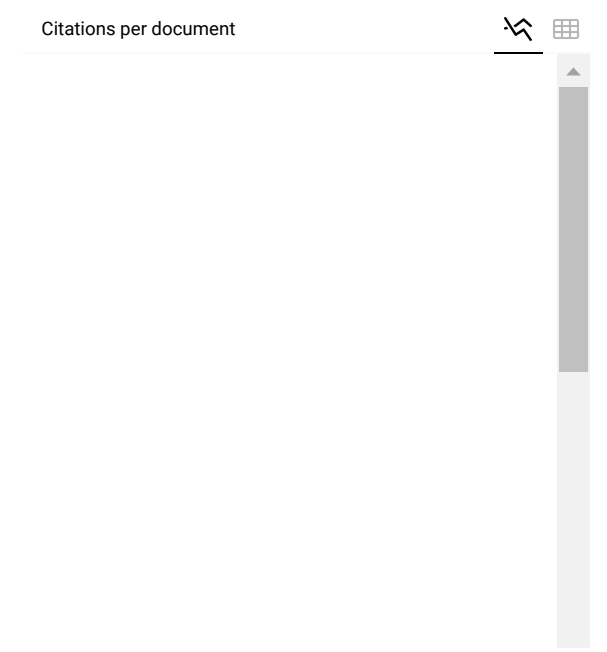
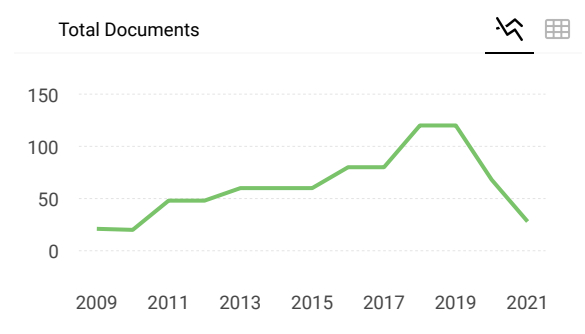
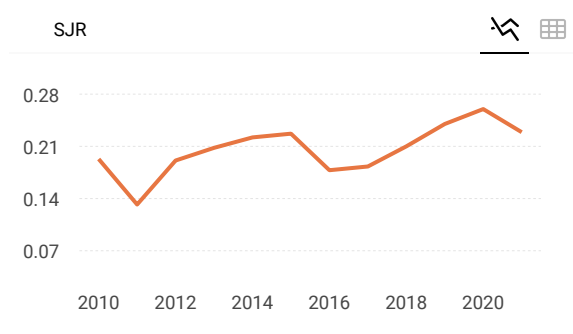
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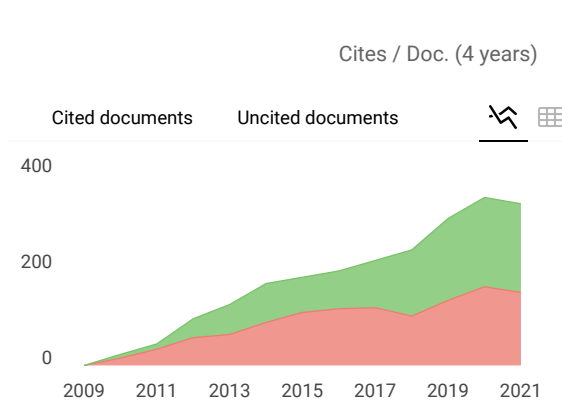
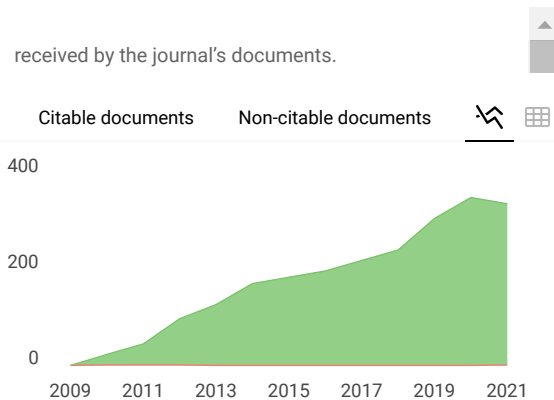
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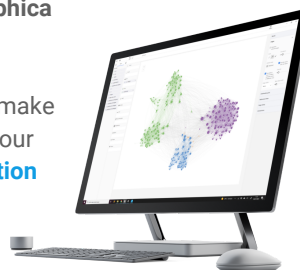
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