Evaluation of herringbone carbon nanotubes-modified electrodes for the simultaneous determination of ascorbic acid and uric acid

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Abstract
An array of gold interdigitated microelectrodes (Au-IDA) has been modified by casting herringbone carbon nanotubes (hCNTs) producing a new electrochemical sensor suitable for the simultaneous determination of ascorbic acid (AA) and uric acid (UA) in PBS (0.25 M, pH 7.0). The sensor showed good electrocatalytic properties for both analytes with low working potentials, linear ranges between 0 and 600 µM for AA, and between 0 and 420 µM for UA. The empirical limits of detection (LODs) and the limits of quantification (LOQs) for both analytes were 15 µM and 50 µM, respectively. Moreover, UA does not interfere in the measurement of AA and vice versa. Furthermore, the presence of common analytes in physiological fluids as glucose, dopamine and epinephrine do not interfere in the quantification neither AA nor UA.
1. Introduction

Ascorbic acid (AA) and uric acid (UA) are compounds worthy of attention because they play essential antioxidant roles in foods and biological systems [1]. AA is a water-soluble vitamin naturally present in many fruits and vegetables. It is also commonly used as an antioxidant in the food industry in order to stabilize the color and aroma, as well as to increase the shelf life of the product by forming relatively unreactive radicals [2]. On the other hand, AA is of great importance in the human diet due to the antioxidant properties and it performs an important role in bioelectrochemistry, neurochemistry and clinical diagnostics application [3,4]. UA is formed in human body as a result of urine metabolism and represents one of the major circulating low molecular weight antioxidants. Abnormal concentrations of UA indicate symptoms of possible diseases as hyperuricaemia, gout, or Lesch-Nyhan syndrome [1,5]. Both compounds are electrochemically active and they can be determined by electrochemical techniques. The development of a quick, selective and sensitive method for simultaneous determination of AA and UA in real samples is greatly desirable for analytical applications and diagnostic research. In this way, electrochemical techniques are a good alternative and, specially, voltammetric techniques for the detection of AA and UA have attracted great interest due to their rapid response, high sensitivity and miniaturization [6]. However, a main disadvantage is that AA and UA are oxidized at nearly same potential with poor sensitivity at unmodified usual electrodes. The overlapping of the oxidation voltammetric peaks makes the simultaneous determination vastly difficult [7].

Carbon materials due to its properties, such as inertness, low cost, high electrocatalytic activity and wide potential window have been the most used electrocatalytic materials for sensor applications [8,9].

Different carbon nanomaterials, which combine the advantages of carbon properties with those of nanostructures, have been developed and applied as electrocatalysts for different
electrochemical sensors [9–14]. Specifically, carbon nanotubes (CNTs) are excellent electrode materials due to their physical and chemical properties, and they have received enormous attention for the study of electrochemical sensors [15–20]. CNTs have outstanding mechanical, electrical and magnetic properties, different structures and also their surface chemistry can be tailored for specific applications. These properties together with their high specific surface make the CNT a promising material for the development of miniaturized chemical, biological and electrochemical sensors. One of the problems of using CNTs is that their dispersion in aqueous media is really difficult, then, different strategies for its modification have been proposed [21–26]. One of these strategies is the use of carbon pastes electrodes in which the modifier can be mechanically mixed with a binder obtaining a homogeneous paste [27]. In other cases, the CNTs must be pre-treated in concentrated acid and the backbone structure may degrade giving negative effects on their properties. However, few studies have been found in which unmodified CNTs are used as electrodes in sensing of AA and UA applications [28–32]. Also, CNTs have been directly grown on several platforms, like diamond-like carbon thin films [33] or tetrahedral amorphous carbon [34], providing hybrid materials for the determination of neurotransmitters. However, the preparation of the electrodes takes time, involves many reagents and consequently is more expensive than other simple and economic methods. Therefore, the development of novel materials with simple preparation procedures is required. In this context, since defects in CNTs result in perturbations on their electronic structure, they can enhance the reactivity and the electrocatalytic properties. It is well-known that herringbone carbon nanotubes (hCNTs) have many ends sites together with a high surface area, which are responsible for their high chemical and electrochemical reactivity, and they can improve the sensing performance of these carbon materials [16,28,35]
Recently, our group has developed a portable sensor based on functionalized hCNTs [36], which can quantify AA and UA by chronoamperometry. However, the preparation of the electrode involves a previous step of electrochemical functionalization of the hCNTs.

In this work, unmodified commercial hCNTs were explored for the simultaneous determination of AA and UA by cyclic voltammetry (CV). The hCNTs were deposited by drop casting on a gold interdigitated microelectrode arrays (Au-IDA).

2. Experimental

2.1. Reagents and equipment

Herringbone carbon nanotubes (hCNTs) were purchased from TCI Chemicals. Uric acid (≥ 99%, crystalline), L-ascorbic acid (reagent grade, crystalline), glucose, dopamine, epinephrine, glutaraldehyde (50%), and dimethylformamide (DMF) were provided by Sigma-Aldrich. Potassium dihydrogen phosphate (K₂HPO₄) and potassium hydrogen phosphate (K₂HPO₄) were obtained by Emsure® and Sigma-Aldrich, respectively. Sulfuric acid (H₂SO₄, 98%) was provided by AnalR Normapur. Sodium hydroxide (NaOH) was purchased from Merck. Ultrapure water was obtained by a treatment in Purelab Ultra Elga with a resistivity of 18 MΩ cm.

Gold interdigitated microelectrodes array (Au-IDA) was provided by Micrux Technologies.

All electrochemical measurements were carried out in a BIOLOGIC SP-300 potentiostat. A three-electrode configuration has been used (Figure 1), in which, the modified electrode was the working electrode (WE), a Ag/AgCl/Cl⁻(3 M KCl) electrode was the reference electrode (RE) and the counter electrode (CE) was a wire of Pt, which was wrapped around the RE.

Figure 1 shows the electrochemical cell employed in this work, which uses a beaker containing 40 mL of the electrolyte. In order to simulate physiological human fluids all investigations have been carried out in phosphate buffer solution (PBS) (0.25 M, pH 7.0). Stirring was kept always to ensure a good homogenization. All the experiments were carried out in air atmosphere.
Transmission electron microscopic measurements (TEM) were carried out using a JEOL TEM, JEM-2010 model and GATAN acquisition camera.

![Diagram](image1.jpg)

**Figure 1. Schematic illustration of the proposed working configuration (electrochemical cell).**

### 2.2. Preparation of electrochemical sensor

#### 2.2.1. Preparation of modified electrodes

Dispersions of 0.5 mg mL\(^{-1}\) of hCNTs were prepared in dimethylformamide (DMF). The dispersion is stable during the necessary time for preparing the electrodes. To achieve a proper dispersion of the materials, an ultrasound probe was applied for 10 min. To avoid localized heating during sonication, the vessel containing the material suspension was immersed in an ice bath.

The Au-IDA electrode modification by hCNTs was made in several stages. First, the Au-IDA electrode cleaning was performed by CV: ten cycles between -1.0 and 1.3 V vs gold pseudo-reference at 0.1 V s\(^{-1}\) in 0.05 M H\(_2\)SO\(_4\) were applied. Then the Au-IDA was modified by drop casting obtaining the hCNTs/Au-IDA modified electrode with a total mass of hCNTs of 5 µg. The mass was determined from the capacitance (7 F g\(^{-1}\)) that was measured in cyclic voltammogram using a paste electrode with 10 mg. Finally, 1 µL of glutaraldehyde was added to improve the adhesion of the hCNTs on the support. It is important to point out that in these conditions the deposited material is stable under stirring. The current density (j) refers to the deposited hCNTs mass.
Electrochemical behavior of the Au-IDA and hCNTs/Au-IDA electrodes was evaluated towards AA and UA additions by CV. For this purpose, a concentrated AA and UA stock solutions (100 mM) were prepared in PBS, being the pH higher than 8.4 for UA solution. Four different aliquots from the stock solutions were added to the electrochemical cell (0.25 M PBS, pH 7.0) to reach the desired concentrations (50, 120, 420 and 600 µM). According to the pKₐ of the two analytes (pKₐ (AA) = 4.2 [37]; pKₐ (UA) = 5.2 [38]), the quantification of ascorbate monoanion (HA⁻) and urate (HU⁻) was done at pH 7.0. However, these analytes will be referred to as AA and UA throughout the manuscript. Afterwards, CV was applied between -0.35 and 0.4 V, recording the corresponding signal. All measurements were carried out by triplicate with the same electrode, but a new solution was prepared for each calibration curve. The study of mutual interferences has been performed once.

3. Results and discussion

3.1. hCNTs characterization

Firstly, structural characterization of the hCNTs material was performed. Figure 2 shows the TEM images obtained for hCNTs. It can be observed that the hCNTs display a typical herringbone structure, which provides them with a larger number of graphitic edges on their surface [16,35]. The diameter of the CNTs is between 10 and 30 nm, which is included in the common range for CNTs [16].
3.2. Voltammetric behavior towards AA and UA oxidation

Electrochemical behavior of Au-IDA and hCNTs/Au-IDA electrodes towards AA and UA oxidation was evaluated by CV.

3.2.1. AA and UA oxidation on unmodified Au-IDA electrode

Figure 3 presents the voltammetric behavior of the unmodified Au/IDA electrode towards AA and UA oxidation.
Figure 3. A-C) Cyclic voltammogram during 5th cycle for a unmodified Au-IDA electrode in a solution that contains: A) Different concentrations of AA. B) Different concentrations of UA. C) 550 µM AA and 580 µM UA. PBS solution (0.25 M, pH 7.0), v=0.1 V s⁻¹. D-E) Calibration curves of: D) AA. E) UA.
Figure 3A shows that the AA oxidation is produced showing an oxidation peak at around 0.10 V, increasing the peak with the concentration. Figure 3B shows the results obtained for different concentrations of UA. As it can be seen the voltammograms show an oxidation peak at around 0.65 V. Figure 3C displays the voltammogram obtained with the unmodified electrode in a mixture of AA and UA. It is clearly seen that the simultaneous quantification of AA and UA is not possible with this electrode because both oxidation peaks overlap. Figures 3D and 3E present the calibration curves for the individually determination of AA (Figure 3D) and UA (Figure 3E). Both analytes show a clear oxidation peak that corresponds to their irreversible oxidation on gold electrode being the current peak higher in the case of UA in the experimental conditions used. Figures of merit obtained from these calibration curves are presented in Table 1.

Table 1. Analytical figures of merit for the AA and UA voltammetric determination by the Au-IDA unmodified electrode

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AA</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>$1.21 \pm 0.09$</td>
<td>$2.4 \pm 0.3$</td>
</tr>
<tr>
<td>Intercept</td>
<td>$0.02 \pm 0.03$</td>
<td>$0.07 \pm 0.10$</td>
</tr>
<tr>
<td>$r$</td>
<td>0.998</td>
<td>0.994</td>
</tr>
<tr>
<td>$N$</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Linear range</td>
<td>0-550</td>
<td>0-580</td>
</tr>
<tr>
<td>LOD (µM)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>LOQ (µM)</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>$E$ (V)</td>
<td>0.09</td>
<td>0.65</td>
</tr>
</tbody>
</table>

3.2.2. AA and UA oxidation on hCNTs/Au-IDA electrode

Figure 4 shows the voltammograms obtained with the hCNTs/Au-IDA electrode for different concentrations of AA, being the oxidation peak current at 0.015 V related to the oxidation of AA (Figure 4A). As it can be checked, the current difference for each concentration is best
observed in the inset of Figure 4A. As it can be seen, the oxidation peak of AA appears at less positive potential than the unmodified Au-IDA electrode (oxidation peak around 0.1 V, Figure 3A) demonstrating the good electrocatalytic activity of hCNTs for AA electrooxidation. In a similar way, the voltammetric study of the hCNTs/Au-IDA electrode towards different concentrations of UA is shown in Figure 4B. The oxidation peak related to UA oxidation also appears at less positive potentials (0.32 V); however, in this case the process is reversible with the reduction counterpart at 0.24 V. Comparing this oxidation peak for UA with the corresponding obtained in bare Au-IDA electrode (peak about 0.65 V, Figure 3B) the great catalytic affinity offered by the hCNT structure for the UA oxidation is observed, which facilitates the electron transfer reaction.

The ability to use hCNTs/Au-IDA electrodes for the simultaneous quantification of AA and UA was also studied by CV measurements. Figures 4C and 4D show the voltammograms obtained with hCNTs/Au-IDA electrode for the successive additions of AA and UA, with fixed concentrations of UA and AA, respectively. Figure 4C displays the voltammograms for a fixed UA concentration ($C_{UA} = 600 \mu$M) and the successive additions of aliquots of AA stock solution, while Figure 4D shows the behavior of the hCNTs/Au-IDA electrode towards the UA oxidation, with an initial concentration of 600 µM AA and successive concentrations of UA. It can be observed in both figures the presence of two oxidation peaks at around 0 V (AA oxidation) and 0.35 V (UA oxidation) when the two analytes are in the solution.

As it can be checked in Figure 4C and 4D, the CV response shows two well-defined oxidation peaks separated by 0.35 V, which suggests that neither AA nor UA interfere in the voltammetric response of the other analyte. The inset of Figure 4C shows a magnification of the AA voltammetric behavior at the potential at which AA is oxidized. The voltammograms presented in Figure 4C show a slight increase in the anodic current at 0.32 V, belonging to UA oxidation, when several aliquots of AA were added. However, the complete interference study is presented in Figures 4E-F. The opposite behavior was observed in Figure 4D, where it can be
observed that several additions of aliquots of UA in a solution of AA 600 µM just lead to an increase of the current corresponding to UA oxidation, showing no change in the anodic peak at 0 V.

Figure 4. A-D) Cyclic voltammograms during 5th cycle of hCNTs/Au-IDA electrode in a solution that contains: A) Different concentrations of AA (50, 120, 420 and 600 µM). Inset: anodic scans. B) Different concentrations of UA (50, 120, 420 and 600 µM). C) Different concentrations of AA (120, 420 and 600 µM), with a fixed UA concentration (C_{UA} = 600 µM). Inset: anodic scans. D) Different concentrations of UA (120, 420 and 600 µM), with a fixed AA concentration (C_{AA} = 600 µM). E-F) Regression lines of $j_\text{AA}$ vs $j_{\text{AA}+\text{UA}}$: E) $j_\text{AA}$ vs $j_{\text{AA}+\text{UA}}$. F) $j_\text{UA}$ vs $j_{\text{UA}+\text{AA}}$. PBS solution (0.25 M, pH 7.0), $v = 0.1$ V s$^{-1}$. 
Table 2 presents all the figures of merit for each analyte individually and for the simultaneous determination. Regarding the individual analysis, it can be observed in Table 2 that the sensitivity obtained for UA is much higher than for AA, using this hCNTs electrode. This fact could indicate a stronger interaction between UA and the carbon surface than with AA. On the other hand, no significant differences were observed in the simultaneous determination for AA or UA quantification with respect to that observed for the individual analysis. In both cases, the sensitivities are similar to those obtained in the individual analysis.

Table 2. Analytical figures of merit for the AA and UA voltammetric determination by the hCNTs/Au-IDA modified electrode.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AA</th>
<th>AA + UA (C_{UA} = 600 µM)</th>
<th>UA</th>
<th>UA + AA (C_{AA} = 600 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (A g^{-1} mM^{-1})</td>
<td>0.22 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Intercept (A g^{-1})</td>
<td>(2 ± 3) · 10^{-4}</td>
<td>(2 ± 2) · 10^{-3}</td>
<td>0.03 ± 0.12</td>
<td>0.03 ± 0.14</td>
</tr>
<tr>
<td>r</td>
<td>0.999</td>
<td>0.998</td>
<td>0.991</td>
<td>0.984</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Linear range (µM)</td>
<td>0–600</td>
<td>0–600</td>
<td>0–420</td>
<td>0–420</td>
</tr>
<tr>
<td>LOD (µM)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>LOQ (µM)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

The limit of detection (LOD) was the lowest concentration whose signal could be clearly distinguished from the blank, and it was determined empirically, by progressively measuring more diluted concentrations of the analyte. Moreover, the limit of quantification (LOQ) was calculated as 3.3 times the LOD (LOQ = 3.3 LOD).

Comparing Table 1 and 2, it can be concluded that hCNTs modification greatly improves the results obtained. First, the AA and UA oxidations at hCNTs/Au-IDA electrode occur at lower potentials, highlighting the electrocatalytic effect of the material. Even though both electrodes
provide similar sensitivities (taking into account that each modified electrode was prepared with 5 µg of hCNTs), the unmodified electrode is not able to separate the signals belonging to AA and UA, being no feasible the simultaneous quantification of both analytes. This fact justify the necessity of the modification of Au-IDA electrodes for its AA and UA sensor application. Nevertheless, to assess whether AA and UA interfere in the UA and AA measurements, respectively, a regression approach test was applied. It involves plotting the results of the measurement of AA without UA in one of the axis (X axis), and the results obtained with UA in the other axis (Y-axis) (Figure 4E). If the results obtained with and without UA are the same, a straight line with a slope close to one and crossing the origin (intercept zero) will be obtained [39]. The same strategy is applied for the evaluation of AA as a possible interference of UA (Figure 4F). It can be observed that both plots (Figures 4E and 4F) show a good correlation between the results in the individual and simultaneous analysis. The linear equation for AA (Equation 1) shows a correlation coefficient of 0.999, then, it can be concluded that UA does not interfere in the AA determination:

\[ j_{AA+UA}(A\ g^{-1}) = (0.97 \pm 0.08) j_{AA}(A\ g^{-1}) + (0.001 \pm 0.006) \]  

Equation 1

Moreover, the applied regression test allows to assess that AA does not interfere in the UA determination (Figure 4F, Equation 2). The linear equation shows a correlation coefficient of 0.9965.

\[ j_{UA+AA}(A\ g^{-1}) = (0.92 \pm 0.12) j_{UA}(A\ g^{-1}) - (0.00 \pm 0.06) \]  

Equation 2

Then, it can be concluded that neither AA nor UA interfere in the voltammetric quantification of each other.

On the other hand, to know if the presence of other analytes as glucose, dopamine (DP) and epinephrine (EP), usually present in physiological fluids, affects on the oxidation signal of AA and UA, the corresponding study was conducted. Figure 5 shows the CVs obtained for the interference tests carried out with 600 µM of AA and UA while comparing the current changes in the presence of glucose (up to 10 mM), DP (up to 5 mM) and EP (up to 100 µM). Taking into
account that maximum levels of these analytes found in physiological fluids are: 5 mM (glucose) [40], 10 µM (DP) [41] and 0.5 µM (EP) [42] and based on the results presented in Figure 5, it can be concluded that the proposed sensor is able to quantify AA and UA, even in the presence of these analytes.

Finally, Table 3 shows a comparison of some analytical parameters obtained in this work with others results previously reported. As it can be seen, the hCNTs modified electrode exhibits low working potentials, good linear working ranges and acceptable LODs. In addition, a great advantage is that it is relatively simple and easy to prepare and operate, being able to be used in air atmosphere, which represents a step forward for the development of portable sensors. Therefore, it should be considered a suitable and competitive alternative for the simultaneous quantification of AA and UA.

![Figure 5](image-url)

Figure 5. Interference CV study of AA (600 µM) and UA (600 µM) in the presence of: A) different concentrations of glucose. B) Different concentrations of DP. C) Different concentrations of EP. PBS solution (0.25 M, pH 7.0), \( \nu_{\text{scan}} = 0.1 \text{ V s}^{-1} \). 5\textsuperscript{th} cycle.
Table 3. Comparison of analytical parameters for several AA and UA sensors.

<table>
<thead>
<tr>
<th></th>
<th>Working potential (V)</th>
<th>Detection limit (µM)</th>
<th>Linear Range (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>UA</td>
<td>AA</td>
<td>UA</td>
</tr>
<tr>
<td>Chitosan/CPB/GCE</td>
<td>0.200</td>
<td>0.460</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Cysteine sonogel-</td>
<td>0.035</td>
<td>0.330</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>carbon modified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>electrode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNF-CPE</td>
<td>0.125</td>
<td>0.450</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Reduced graphene</td>
<td>-0.035</td>
<td>0.325</td>
<td>300</td>
<td>0.5</td>
</tr>
<tr>
<td>oxide/GCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuRGO/GCE</td>
<td>-0.050</td>
<td>0.260</td>
<td>51</td>
<td>1.8</td>
</tr>
<tr>
<td>PAH-CNTs/GCE</td>
<td>-0.162</td>
<td>0.185</td>
<td>0.92</td>
<td>1.5</td>
</tr>
<tr>
<td>PEDOT/GC</td>
<td>0.215</td>
<td>0.335</td>
<td>45</td>
<td>7</td>
</tr>
<tr>
<td>SWCNT-nanohorn</td>
<td>0.293</td>
<td>0.359</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>hCNTs-4ABA/Au-IDA</td>
<td>0.085</td>
<td>0.320</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>hCNTs/Au-IDA</td>
<td>0.015</td>
<td>0.320</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

CPB = Cetylpyridine bromide; PEDOT = poly(3,4-ethylenedioxythiophene); CNF = Carbon nanofibers; PAH = Poly(allylamine hydrochloride)
Analyzing more carefully Table 3, several comparisons can be done. Even though, some of the sensors presented in Table 3 provide similar or even better results than the hCNTs-modified electrode [3,44,45], the experimental procedure for the preparation of the electrodes is tedious, involving several stages, like electrochemical reduction [46] or electrochemical functionalization [36] taking longer time. The preparation of the carbon material in the hCNTs-modified electrode avoids complex treatment and the hCNTs were immobilized by the simple drop-casting technique. Although Table 3 shows several sensors which preparation was simple and allow to prepare the electrodes in a short time [1,28,43,47], they were prepared by modifying a glassy carbon electrode and subsequently measured in a conventional cell. The developed electrode in this manuscript presents the advantage that it has been prepared on a platform that provides portability. Furthermore, all the experiments were carried out at air atmosphere, presenting a step forward of portable sensors for in situ and field analysis.

4. Conclusions

Unmodified herringbone carbon nanotubes were used for the modification of an array of gold interdigitated microelectrodes (hCNTs/Au-IDA). This modified electrode has shown a good electrocatalytic activities towards the oxidation of AA and UA by decreasing the oxidation potential of both analytes. Based on the CV technique, this electrochemical sensor shows a linear range between 0 and 600 µM for AA and between 0 and 420 µM for UA with a remarkable sensitivity for both analytes. The results show that in PBS (0.25 M, pH 7.0) the sensor can be used for simultaneous determination of AA and UA, with anti-interference ability towards analytes as glucose, dopamine and epinephrine, usually present in physiological fluids. The high surface area and large end sites make this carbon material a promising candidate for electrocatalytic applications.

5. Acknowledgement

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6. Literature


