Voltammetric Reckoning of Caffeine at Trace Level in Local Available Drinks Formulations and Pharmaceutical Formulations.

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Abstract- Caffeine, available in plant’s kingdom worldwide, has remarkable insecticidal, weedicide, beverage and medicinal values, along with a few fatal actions to humanity if uptake is beyond limit. For healthy cause, it’s reckoning at trace level in local drinks and pharmaceutical of routine use has become vital. An environment friendly chemically modified carbon paste sensor has been fabricated. Square wave voltammetry technique has been deployed to carry the present investigations in procured real samples of routine use medicines and local drinks in Mekelle city. Experimental conditions and square wave parameters have been optimized with prepared sensor for anodic oxidation of caffeine in buffer. The peak signal observed two-fold upswing in presence of this sensor as compared to unmodified sensor. Adequate catalytic activity of anthraquinone and irreversible nature of oxidation process at the interface has been observed. The perceived effect of analyte concentration on both signals i.e. peak current and peak potential exposed mainly the diffusion controlled mechanism at interface, besides some interfering surface processes at high caffeine concentrations. The peak current and caffeine concentration observed linear dependence in low range 0.00 – 100 μM with correlation coefficient ‘R’ = 0.9999 (n = 10) and standard deviation ‘σ’ = 0.0596. The observed regression equation is “Ip(μA) = 34.465 + 1.282C(μM)” with the detection limit of 0.1395 μM. Caffeine quantification conducted in local available drinks and medications by using standard addition/spiked method have yielded very good recovery. An electro-oxidation process of caffeine at the interface received resilient catalytic action of modifier. Excellent recovery of caffeine’s spiked samples of drugs and local drinks represent an inordinate potential of sensor for its prompt quantification in real samples.

Index Terms- anthraquinone; caffeine; local drinks & drugs; spiked method; square wave voltammetry.

I. INTRODUCTION

Recent trends of divergence in technology, most of the researchers have averted the nature of their work in designing and developing instruments of the kind- inexpensive, more stable, quick responsive, highly sensitive, selective nature with high degree of accuracy and exactitude in the outcomes. To achieve proficiency in different fields of their application, much work is being carried out in the development and fabrication of sensors, worldwide. The electrochemists also developed some sensors in the form of environment friendly electrodes which have been successfully exploited for environmental, biomedical, industrial products analysis along with pharmaceutical formulations and food-product preparations. Volta-metric processes actively being used are electro-dynamic techniques for quantitative analysis of electro-active species in medicines and drugs of common use in developing and underdeveloped nations. These methods when coupled with HPLC become versatile tools for the analysis of complex mixtures. They offer the lowest detection limits in pico-molar range. Caffeine is a strong psychoactive substance. In daily human life it is playing an important role in food and drug chemistry. The beverages products such as coffee, tea, cola and drug formulations belong to the category of highly significant economic zone for any nation. Caffeine (1, 3, 7-trimethylxanthine) is N-methyl derivative of xanthine which is available worldwide in plant’s kingdom. It is remarkably rich in its insecticidal values [1, 2], beverage values [3, 4] and medicinal values [5, 6]. Caffeine is a multi-ingredient medications containing drug; its main effect on one’s body is to make one feel more awake and alert for a while, but it can also cause problems. One feels oneself in heaven, by stimulating one’s brain that consequents one in relaxed mood, decreased fatigue, increased focus, faster and clearer of thought if it is consumed within limit, because it increases blood circulation, heartbeat, respiratory rate and metabolic rate for several hours [7-9]. Contrary to this, the passionate use of it may become alarming for humanity [10] by creating several kinds of disorders in the human body.

Several methods like ion mobile spectroscopy [11], capillary electrophoresis [12], FTIR [4], ESI-IMS [13], FT-R [14], HPLC [3] HPTLC [15], UV [9], etc. have been developed for the endurance of caffeine in variety of fluxes. However, most of these are more expensive with time consuming procedure involving pre-concentration or multi solvent extraction beside skilled technicians [14], and the uses of organic solvents create environment pollution. Hence, there is need to develop an accurate, reliable, cheap and easy operable method/process for sensing and estimation of caffeine in food-stuffs/soft-drinks/beverage/ drugs etc. The oxidation of caffeine ensues at more positive potential which in most of the cases overlap with the discharge of background medium [8]. Modified carbon/ carbon-paste electrodes [16-20] belong to the promising electrochemical or bio-electrochemical sensors of wide applicability and fit well in the above mentioned advantageous
criteria, over all other carbon/ metal electrodes [21]. The present study is an extension of our earlier work [22], in this standard addition method have been employed using AMCPE for sensing and reckoning of caffeine at trace level in local available drinks and pharmaceutical formulations applying square wave voltammetry technique.

II. MATERIALS AND METHODS

Apparatus: Electrochemical measurements were accomplished with a BASCV-50W electrochemical analyzer (Bio analytical system Inc., USA) using conventional three electrode cell with unmodified/ anthraquinone modified carbon paste electrode (AMCPE) as working, silver-silver chloride as reference and platinum wire as auxiliary electrode, respectively. All pH values were restrained with bench microprocessor pH meter (HANNA Instruments, Italy) calibrated with standard buffer solutions of pH 4.01 and pH 7.01. The temperature of all investigations persisted to 25°C with an accuracy of ± 0.2°C.

Reagents: Caffeine (anhydrous, 98.9% HPLC grade) was obtained from Addis Pharmaceuticals Factory (APF), Ethiopia. Anthraquinone (LOBA, India), paraffin oil and hydrochloric acid (Nice, India), graphite powder and HClO₄ (BDH, England), Sodium hydroxide (Scharlau, Spain) were used in present examinations. All chemicals were of analytical grade and utilized without further purification. Stock solution of 1.0 mM caffeine was prepared in 0.1 M HClO₄ solution of pH 0.56. Working standard solutions of lower concentration were prepared by dilution method using the supporting electrolyte. Aqueous solutions of 0.1 M NaOH and 0.1 M HCl were utilized for adjusting the pH of the working solution. Aqueous solutions were prepared using double distilled water.

Samples procurement: In order to examine quantitatively the amount of caffeine in real samples, the procured pharmaceutical preparations were Paramol-c (Franklin laboratories, India), Nicip cold and Nocold (Cipla Ltd., India) whereas the local available drinks as tea bags, cola- and meta-beer beverages has been purchased from the Mekelle market.

Preparation of electrode: The unmodified carbon paste electrode (UCPE) and Anthraquinone modified carbon paste electrode (AMCPE) have been prepared as described in our earlier communication [22]. The surface of each fabricated sensor was then polished on a clean paper before being used in the electrochemical cell.

Real sample analysis: The developed electrode was employed for the determination of caffeine in three commercially available caffeine induced tablets and their solutions were prepared as described earlier [22]. Tea-bag weighing 2 gm tea sample, suspended in 100 ml boiling water for three minute, the resulting tea solution formed by infusion. Four ml of tea solution further diluted to 100 ml; thirty percent solution of cola beverage [6] and the same dilution for meta-beer beverage sample was obtained by direct dilution (v/v). The caffeine contents present in these studied samplings were carried out by deploying the standard addition method.

III. RESULTS AND DISCUSSION

Electrochemical behavior of caffeine: Square wave voltammetry (SWV) because of its fast workability, high sensitivity and excellent background separation was used to investigate the electrochemical behavior of caffeine using the present sensor. The influence of modifier has been clearly demonstrated through Figure 1, in which square wave voltammograms are obtained for 1.0 mM solution of caffeine in 0.1 M HClO₄ as supporting electrolyte of pH 0.56 at (a) UCPE and (b) AMCPE. The observed electrochemical performance of unmodified sensor from this figure revealed us that there is one broad but poor signal at about 1.65 V vs. Ag/AgCl.

On the other hand, under the same experimental conditions there is a clear and sharp signal at about 1.50 V when performed through modified sensor. On using prepared electrochemical sensor, there is a drift of peak potential (Eₚ) from 1.65V to 1.51V, accompanied by approximately two fold enhancements in magnitude of peak current (Iₚ) i.e. from 6.0x10⁻⁵A to 1.22x10⁻⁴A. The observed rise of Ip might be due to increase in number of caffeine cations in strong acidic buffer in presence of modifier in AMCPE. The decrease of nearly 0.14V in the over voltage is quite comparable with values reported earlier [20] for electro-catalytic oxidation of caffeine at the surface of modified electrodes. These are unblemished evidences for the catalytic activity of the modified sensor toward the oxidation of caffeine. Non-appearance of signal in the reverse scans of cyclic voltammograms, reflect prevailing irreversible electrochemical oxidation route at the interface [22].

Choice of supporting electrolyte and pH: Supporting electrolyte and its pH play vital role to maintain sensitivity of the method in use. The selection of perchloric acid as proper supporting electrolyte for present investigations, has been made
out of acetate buffer, phosphate buffer, perchloric acid and phosphoric acid because of its best electro-analytical signal i.e. peak current intensity and shape of voltammogram, elsewhere [22]. The current signals observed for caffeine were pH dependent and pH 0.56 chosen as a suitable working medium for further investigation.

Optimization of swv parameters: Striping potentiometric techniques are more sensitive voltammetric technique as compared to that of cyclic voltammetry to examine the dependence of signal current with caffeine concentration. In operating these techniques, procedure involves the proper optimization of the experimental parameters such as pulse amplitude, square wave frequency, and step potential which affect the magnitude of reduction/oxidation peak current along with the shape of voltammogram.

Choice of pulse amplitude: The applied amplitude of the square wave pulse which mainly determines the sensitivity of the technique is an important parameter that has a strong impact on the observed value of peak-current. Figure 2 demonstrate the square wave voltammogram of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at swv frequency 85 Hz; step potential 6 mV and pulse amplitude of 10, 20, 30, 40, 50, 60 and 70 mV. It is evident from this figure that there is sharp rise in magnitude of oxidation peak current at approximately 1.52V of oxidation peak potential corresponding to increase in the pulse amplitude in studied range. Observed rise in I_p values may be due to upswing in activation effect of current carrier’s up to certain limit of applied pulse potential. On and beyond 60 mV, the I_p behavior deviates from its linearity as in figure 3, which may be substantiated to the fact that at higher pulse amplitude the rate of oxidation of caffeine may decreased.

Choice of step potential: The present study deals with the effect of square wave step potential parameter that deals with potential change between the two data points of the experiment, on the shape and observed peak current from the experimental voltammograms. Different magnitudes of step potential in the range of two to twelve were applied to study the oxidation activities of 1.0 mM caffeine solution using the present sensor. Figure 4, represents the effect of step potential on the oxidation peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at frequency 85 Hz; and pulse amplitude of 50 mV.
Figure 4: The effect of step potential on the oxidation peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at frequency of 85Hz and pulse amplitude of 50 mV.

The observed oxidation peak current behavior at two extreme step potential i.e. 2 mV and 12 mV, has shown a slight deviation from the linearity. Hence, the mid value of applied step potential i.e. 6.0 mV was chosen optimal for conducting the subsequent experiment.

Effect of square wave frequency: In this optimization process pulse amplitude and step potential were kept constant and square wave frequency varied between 10 –105 Hz. The resulting voltammograms presented in figure 5A.

It was perceived from this figure that with increase of square wave frequency parameter, the magnitude of both, anodic peak potential and anodic peak current observe positive shift in their values. This observation could expose partial adsorption of caffeine on sensor’s surface where catalytic action of anthraquinone contributes to its anodic oxidation. Similar characteristics of caffeine have been reported elsewhere using differential pulse voltammetry at MWCNT-Nafion/ GCE interface [23]. Using data from figure 5A, a plot presenting the variation of peak potential as a function of logarithmic frequency is presented in Figure 5B. An equation exploited by Jana et. al. [24] for a completely irreversible and fully diffusion controlled process as

$$E_p = 0.5b \log v + \text{constant.} \quad \ldots (I)$$

In this equation ‘b’ represent the tafel slope. The potential gradient from the plot and the slope from equation (I) on comparing, provide $b=114.42$ mV/Hz. This suggests the equal participation of electrons and protons in oxidation of caffeine at interface. This fact has also been identified and cited in literature [21, 22]. Figure 6 represents variation of the oxidation peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at step potential 6.0 mV and pulse amplitude 25.0 mV versus square wave frequency.
Figure 6: Variation of the peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at step potential 6 mV and pulse amplitude 25 mV vs square wave frequency.

It is evident from this figure that there is almost linear rise in response signals up to 85.0 Hz, but beyond it, response is non-reproducible and underway to smooth out. Hence, 85.0 Hz was chosen for subsequent analytical application. The worth of square root of frequency on the oxidation peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56 using AMCPE at step potential 6.0 mV and pulse amplitude 25.0 mV when frequency vary from 10.0 - 105.0 Hz is linear as in figure 7, with a correlation coefficient of 0.9989.

Figure 7: Effects of square root of frequency on the oxidation peak current of 1.0 mM caffeine in 0.1M HClO₄ of pH 0.56, using AMCPE at step potential 6.0 mV and pulse amplitude 25.0 mV when frequency vary from 10.0 - 105.0 Hz.

This, also, is another esteemed evidence to convey that the anodic oxidation process at the interface is diffusion controlled. This fact gained a decent sport from our earlier cyclic voltammetric studies (22) and the work of Goyal et.al [6]. The square wave frequency together with the step potential defines the effective scan rate under given conditions. Hence, rise in either frequency or step potential results an increase in the effective scan rate. The optimized square wave parameters for present studies are given in Table-1.

Table 1: Optimized swv parameters of 1.0 mM caffeine solution in 0.1M HClO₄ of pH 0.56 at AMCPE.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Parameters</th>
<th>Studied range</th>
<th>Optimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulse amplitude</td>
<td>10-70 mV</td>
<td>50 mV</td>
</tr>
<tr>
<td>2</td>
<td>Square wave frequency</td>
<td>10-105 Hz</td>
<td>85 Hz</td>
</tr>
<tr>
<td>3</td>
<td>Step potential</td>
<td>02-12 mV</td>
<td>06 mV</td>
</tr>
</tbody>
</table>

Effect of caffeine concentration: The impact of caffeine concentration on the anodic oxidation behavior at interface of the sensor and analyte has been observed in terms of peak current using square wave voltammograms at individual concentration and the magnitude of their peak-current has been plotted against its concentration (0.00 μM – 1.00 mM) and presented as in figure 8.

Figure 8: Plot of peak current (Inset: peak potential) vs caffeine concentration (0.00 - 1.00 mM), using AMCPE in 0.1 M HClO₄ (pH 0.56) at optimized parameters.

This figure consists of two linear segments of Ip gradients with concentration as 1.282 and 0.097μA/μM. This revealed that there is a sharp increase in the magnitude of peak current at lower caffeine concentration range (0.00-100 μM) but relatively poor upswing in the peak currents at higher concentration range (100 μM- 1.00 mM). This could be due to the problematic interference by developing its more oxidation products at its high concentrations. Similar reports regarding electrochemical behavior of caffeine at MWCNT-nafion modified sensor using DPSV were presented by Zhang et.al [23]. On the other hand, the peak potential magnitude from the observed behavior when plotted as a function of concentration (inset: figure 8); provide some valuable information’s regarding anodic oxidation behavior.

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at the interface in the present study. There is sharp fall in peak potential at faster negative rate which confirm sturdily a pure diffusion controlled anodic oxidation process at the interface in caffeine solutions of lower concentration. On the other side, relatively poor enhancement in magnitude of peak potential observed at higher concentrations. This peculiar observation of decrease in sensitivity (slope) of the second segment of current versus concentration of caffeine, may be due to some kinetic limitations that may arise to some extent through some surface processes which might be expected to encounter during the diffusion controlled process at the interface, resulting slower rate of diffusion of current carriers with increasing concentration of caffeine in cell solution:

i) its tendency to get adsorbed in mono/ multi layers on the electrode surface;
ii) the oxidation products of caffeine or their intermediates may cover some portion of electrode;
iii) catalytic action of the modifier in sensor; and
iv) the development of electrochemical interactions among caffeine, its oxidation products and the current carriers across the interface.

Calibration curve for caffeine: In order to set-up the practical performance for quantitative estimation of caffeine in local available drugs and drinks of day-to-day use for the welfare of humanity, the observed electro-analytical behavior of caffeine has been presented by making a plot between the magnitude of oxidation peak current (μA) and concentration of caffeine (μM) over a range of 0.00 – 100 μM functional concentrations during present investigations at optimized parameters. This provides support to our earlier finding [22] regarding the non-adsorptive present investigations at optimized parameters. This provides over a range of 0.00 – oxidation peak current (μA) and concentration of caffeine (μM) has been presented by making a plot between the magnitude of oxidation peak current (μA) and concentration of caffeine (μM)

At low caffeine concentrations, the linear regression equation obtained is given as

\[ Ip (\mu A) = 34.465 + 1.282C (\mu M) \quad \ldots \text{(II)} \]

The correlation coefficient and the standard deviation obtained for standard calibration plot, are 0.9999 and 0.0596 respectively.

At higher studied concentrations i.e. from 100 μM to 1.00 mM, enormous deviation from calibration curve and slight disparities with its own linearity have been pronounced. A desperate view of these observations further performs another linear responsive behavior with lower magnitude of slope, as evidenced by inset of figure 9, whose linear regression equation with correlation coefficient ‘R’= 0.9856 is

\[ Ip (\mu A) = 163.53 +0.097C (\mu M) \quad \ldots \text{(III)} \]

The slope of regression (II) is nearly 13 times greater than that of regression (III) which is a direct reflectance towards a poorer flow of current in the irreversible oxidation of caffeine with increasing caffeine concentrations in solution across the interface. On application of reported formula in [25], LOD = 3S/m, in which ‘S’ represents standard deviation of the blank and ‘m’ denote the slope of calibration curve from equation (II), the limit of detection of caffeine has been found 1.39×10⁻⁷ M (n = 10).

Quantitative treatment: The results of investigations regarding recovery of caffeine, in some pharmaceutical tablets of routine use by the folk’s, using cyclic voltammetric technique reported earlier [22]. But, for validation of performance of developed sensor and its application in local drinks to see and quantify caffeine at trace level, the recovery experiments were performed by using standard caffeine spiked samples in this study. The presence of matrix in medications and related contents in local drinks remain nearly ineffective interferers. Furthermore, the studies on pharmaceutical formulations has reinvigorated us to perceive and compute the caffeine contents in the samples of beverage being used by humankind in general and the population of Mekelle city in Tigray region of Ethiopia, in particular, using present technique. The results of electrochemical studies on the drugs and drinks are reported in Table-2.

Table 2: Recovery results of caffeine from spiked/ non-spiked solutions of pharmaceutical tablets and local drinks under optimized conditions at AMCPE.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Sample</th>
<th>Sample Conc. (μM)</th>
<th>Perceived Conc. (μM)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 No</td>
<td>i</td>
<td>00.0</td>
<td>04.28 (± 0.23)</td>
<td>--</td>
</tr>
<tr>
<td>ii</td>
<td>10.0</td>
<td>14.02 (± 0.14)</td>
<td>97.40</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>20.0</td>
<td>24.21 (± 0.39)</td>
<td>99.71</td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td>30.0</td>
<td>34.34 (± 0.26)</td>
<td>100.20</td>
<td></td>
</tr>
<tr>
<td>2 Nic</td>
<td>i</td>
<td>00.0</td>
<td>04.84 (± 0.67)</td>
<td>--</td>
</tr>
<tr>
<td>ii</td>
<td>10.0</td>
<td>15.02 (± 0.32)</td>
<td>101.21</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>20.0</td>
<td>24.05 (± 0.21)</td>
<td>96.05</td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td>40.0</td>
<td>64.13 (± 0.32)</td>
<td>98.82</td>
<td></td>
</tr>
<tr>
<td>3 Param</td>
<td>i</td>
<td>00.0</td>
<td>04.91 (± 0.18)</td>
<td>--</td>
</tr>
</tbody>
</table>
The recovery of caffeine pertains within the range 93.80% - 102.20% in the studied samples. This indicates that the developed method is truthful, easy achievable and also unpretentious by the sample matrix present along with caffeine in medications when the sensor is in use. The repeatability and the stability of the fabricated electrode have already been reported elsewhere [22]. Hence, it can be suggest that AMCPE has an inordinate potential for the determination of caffeine in real samples of drugs and drinks.

Comparison with other methods: The comparison of the results of some notable recent research groups to that with present one, regarding voltammetric determination of caffeine has been précised in Table-3 along with their conducted results of some notable recent research groups to that with Comparison with other methods:

<table>
<thead>
<tr>
<th>Electrochemical sensor used</th>
<th>Applied Technique</th>
<th>Studied linear range (mol/L)</th>
<th>LOD (mol/L)</th>
<th>Referenc e</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQMCPE</td>
<td>SWV</td>
<td>0.0–0.5×10⁻³</td>
<td>3.0×10⁻⁷</td>
<td>26</td>
</tr>
<tr>
<td>GPE</td>
<td>SWS V</td>
<td>0.0–2.58×10⁻³</td>
<td>4.74×10⁻⁵</td>
<td>30</td>
</tr>
<tr>
<td>Nafion-ruthenium oxide/ GCE</td>
<td>SWV</td>
<td>5.0×10⁻⁷–2.0×10⁻⁴</td>
<td>2.0×10⁻⁶</td>
<td>29</td>
</tr>
<tr>
<td>Nafion/MWC NT/GCE</td>
<td>DPV</td>
<td>6.0×10⁻⁷–4.0×10⁻⁴</td>
<td>2.3×10⁻⁷</td>
<td>28</td>
</tr>
<tr>
<td>Nafion/GO/GCE</td>
<td>DPV</td>
<td>4.0×10⁻⁷–8.0×10⁻⁴</td>
<td>2.0×10⁻⁷</td>
<td>27</td>
</tr>
<tr>
<td>CF-UME</td>
<td>FSCV</td>
<td>0.1–1.0×10⁻⁴</td>
<td>3.33×10⁻⁶</td>
<td>17</td>
</tr>
<tr>
<td>AQMCPE</td>
<td>SWV</td>
<td>0.00–1.00×10⁻⁶</td>
<td>1.395×10⁻⁷</td>
<td>this work</td>
</tr>
</tbody>
</table>

Table 3: Comparison of the results of present study with earlier reported, for voltammetric determination of caffeine in real samples.

Moreover, the AMCPE offers simple preparation, easy handling and low cost compared with other electrodes. Therefore, the practical suitability of proposed mode for the determination of caffeine in real samples could be effectively functional.

IV. CONCLUSIONS

As compared to the UCPE, the anthraquinone modified sensor exhibits good electro-catalytic activity for the oxidation of caffeine associated with large peak current enhancement and negative shifts of anodic peak potential at lower concentrations rather than at higher concentrations. Nearly thirteen times decrease in sensitivity at high concentration of caffeine arises due to some surface processes involved rather than at lower ones. Under the optimized experimental conditions, the anodic peak current of caffeine was proportional to the concentration in the range 0.00 to 1.0×10⁻⁴M, with a detection limit 1.395×10⁻⁷M and correlation coefficient of 0.9999. The recovered caffeine concentrations in caffeine instated solutions of the tablets along with locally used drinks were in the range 93.80 to 102.20 %. This suggests the potentiality of AMCPE for the determination of caffeine. Moreover, simple preparation, good stability, excellent reproducibility, sensitivity, low cost, and rapid analysis make the developed method suitable for routine determination of caffeine in real samples.

CONFLICT OF INTERESTS

The authors declare regarding the publication of the present research article that there is no conflict of interests among them.

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