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Spectroelectrochemical determination of chlorpromazine hydrochloride by flow-injection analysis

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Abstract

An original, simple and sensitive flow-injection spectroelectroanalytical method for the determination of chlorpromazine in pure form or in pharmaceutical formulations is described. The method is based on the formation of a stable cationic radical by electro-oxidation in sulfuric acid medium (0.1 mol l^{-1}), monitored in situ at $\lambda = 524$ nm. The determination of chlorpromazine hydrochloride in pure form or in pharmaceutical formulations was explored, considering the amperometric and the absorptiometric signal. The association of these two signals enhanced the selectivity of the analysis and proved decisive when other electroactive compounds or excipients like ascorbic acid were present in the formulation. The analytical parameters have been evaluated and the results obtained using standard additions are in agreement with the reference methods.

Keywords: Spectroelectrochemistry; Flow-injection analysis; Chlorpromazine hydrochloride; Gold electrodes; Flow cell; Voltammetry

1. Introduction

The discovery of the antipsychotic agent chlorpromazine hydrochloride in the early 1950s and the advent of even more powerful phenothiazinic psychopharmacological agents represent a landmark in the history of the medical and psychiatric sciences.

Chlorpromazine hydrochloride is the most important compound in the large group of phenothiazine derivatives. It is widely used as a therapeutic agent for treating various mental and personality disorders, in the prevention of vomit spasms and as an intravenous anti-hypertensive. Like other phenothiazines, it easily undergoes oxidation in acid medium under the action of many oxidizing agents leading to the formation of intensely colored oxidation products [1]. The oxidation process involves two subsequent and distinct one-electron steps. The first is reversible and results in the formation of a colored cation-radical. The second, irreversible, giving rise to the colorless sulfoxide [2].

Due their biomedical significance and the continuous introduction of these drugs, the determination of phenothiazines, and in particular of chlorpromazine, has attracted considerable interest and has induced many workers to explore new methods for their determination. The official methods for phenothiazines, listed in the British Pharmacopoeia (BP) and US Pharmacopoeia (USP), consist in the non-aqueous potentiometric titrimetry or spectrophotometry in the ultraviolet region [3,4]. A variety of alternative methods have been reported and the available analytical techniques include: titrimetry with different electrodes or in aqueous phase [5–8], spectrophotometry in the visible region after oxidation of the phenothiazine [9–16], spectrofluorimetry [17,18], chemiluminescence [19,20], high performance liquid chromatography [21–24], differential pulse voltammetry [25], differential pulse polarography [26], differential pulse stripping voltammetry [27–30] and electrophoresis [31–34]. Chromatographic techniques in combination with electrochemistry and mass spectrometry or fluorescence spectroscopy have also been reported [35], exhibiting excellent selectivity, but requiring expensive instrumentation.

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In recent years, flow-injection analysis (FIA) has found wide application as a tool for routine analysis in several fields, including pharmaceuticals [36,37]. The versatility and simplicity of FIA are very convenient to tackle a variety of analytical problems at a relatively low cost. Furthermore, it is particularly attractive for performing automated analysis of large numbers of samples exhibiting similar composition. Some flow-injection methods have been reported for the determination of phenothiazine derivatives [38–45]. In most of them, in-line oxidation of the analyte takes place under the action of a confluent or immobilized oxidant and is followed by spectrophotometric detection of the colored radical. This approach presents two drawbacks: high acid and/or oxidant concentration and the risk of interference of colored excipients. Additionally, some oxidizing reagents used can generate toxic wastes, such as Cr(III) and Cr(VI) from $K_2Cr_2O_7$ and a certain amount of HCN from $Fe(CN)_6^{3-}$ upon reduction followed by decomposition in acid medium. Otherwise, flow-injection analysis methods based on chemiluminescent reactions were proposed for chlorpromazine [20,46,47]. They present high sensitivity but limited selectivity, due to other organic substances capable of generating chemiluninescence or consuming the reagent without light emission or even act as inhibitors of chemiluminescence. For this reason, chemiluminescence methods could not be applied to the determination of phenothiazines in pharmaceutical formulations without prior separation from the excipients or, when applicable, chemometric correction of signal interferences.

The oxidation of the phenothiazines can be conducted electrochemically in a flow cell, and the amperometric signal used for detection. When there is more than one electroactive substance in the sample, depending on their oxidation potential, some interference is likely to occur. However, the combination of amperometric measurements with spectrophotometric detection of the electro-generated products can be beneficial for the determination of phenothiazine derivatives in a range of pharmaceutical formulations, in particular, those containing associated pharmaceutical compounds and dyes.

This paper describes the development and the application of a flow-injection spectroelectroanalytical method for the determination of chlorpromazine hydrochloride in pure form or pharmaceutical preparations using a long-optical-path flow spectroelectrochemical cell, previously developed by the authors [48,49]. Compared to spectrophotometric and amperometric methods used on their own, the proposed spectroelectroanalytical flow method combines a number of desirable properties, including the use aqueous medium, less drastic operating conditions (electroxidation in H_2SO_4: 0.1 mol l^{-1}), similar sensitivity, better selectivity without prior extraction, no need of unstable chemicals and relatively simple and inexpensive instrumentation.

2. Experimental

2.1. Apparatus

The single-line manifold system used for determination of chlorpromazine hydrochloride is shown in Fig. 1. The sample solution is injected through the manual rotary injector into the carrier stream of the sulfuric acid solution (0.1 mol l^{-1}) driven by the peristaltic pump (Ismatec MS-Reglo). The stream passes through the spectroelectrochemical cell, where the chlorpromazine is electro-oxidized and the colored species is formed just before detection. All experiments were conducted at room temperature.

A Hewlett Packard Model 8452A diode array spectrophotometer interfaced to a PC controlled by software from the Hewlett Packard MS-DOS UV/Vis package, was used for spectra acquisition of the colored compound formed in the spectroelectrochemical flow cell that fits directly in the cuvette holder.

All electrochemical measurements were taken using a conventional three-electrode potentiostat built in the authors'

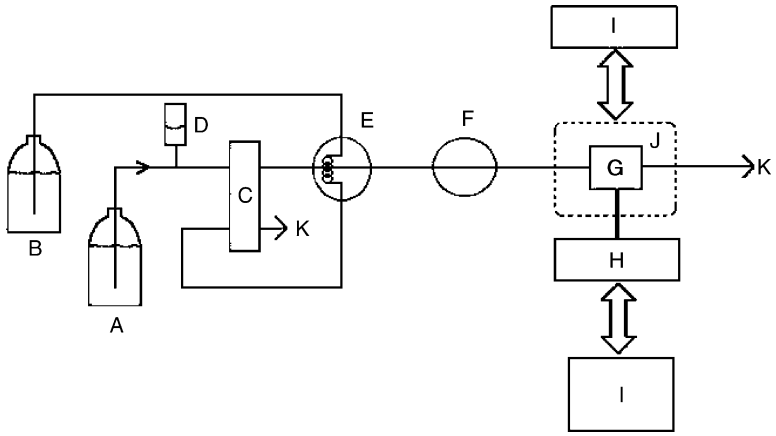

Fig. 1. Schematic diagram of the FIA manifold used for the assay of chlorpromazine hydrochloride. (A) 0.1 mol l^{-1} H_2SO_4 solution (carrier); (B) chlorpromazine hydrochloride solution standards or samples; (C) peristaltic pump; (D) pneumatic damper (T connector with hypodermic syringe); (E) sample injector; (F) debubbler; (G) spectroelectrochemical cell; (H) potentiostat; (I) PC; (J) Hewlett Packard 8452A diode array spectrophotometer; (K) waste.

laboratory and interfaced to a personal computer. The long-optical-path spectroelectrochemical cell and the debubbler used in determination of chlorpromazine hydrochloride are described elsewhere [48]. Two plates of Au-CDtrodes [50], each one with a coplanar working and auxiliary electrode, were assembled face to face in a conventional 1-cm optical-path glass or plastic cuvette. The optical beam passes through the gap between these twin electrodes. A miniaturized Ag/AgCl electrode [51], provided with a salt bridge made with a flexible PTFE tube filled with saturated KCl solution served as reference electrode.

2.2. Reagents

Deionized water and analytical grade reagents were used for all solutions. Appropriate concentration of sulfuric acid solution (0.1 mol l^{-1}) was prepared by dilution from concentrated sulfuric acid (Merck). Chlorpromazine hydrochloride was obtained from Sigma and working solutions were freshly prepared daily by weighting and dissolving appropriate aliquots into 0.1 mol l^{-1} H_2SO_4. The pharmaceutical preparations tested were *Amplictil* tablets (25 mg/tablet) and oral solution (0.04 g/ml) manufactured by Aventis. One tablet of *Amplictil* contain 25 mg of chlorpromazine as active principle and non-reported concentrations of starch, lactose, dextrose, magnesium stearate, titanium dioxide, talc and dye as excipients. Each 1 ml of the *Amplictil* oral solution contain 0.04 g of chlorpromazine as active principle and non-reported concentration of ascorbic acid, glycerin, caramel, ethanol and mint aroma. No sample treatment was required. The *Amplictil* tablets were crushed and dissolved in 0.1 mol l^{-1} H_2SO_4 and the resulting solution filtered. The *Amplictil* oral solution was diluted with 0.1 mol l^{-1} H_2SO_4 as needed, without any treatment.

3. Investigations, results and discussion

Throughout all the experiments, the electrochemical and the spectroscopic signals were monitored simultaneously. Operating conditions, including applied potential, flow rate, injected volume and analyte concentration were carefully examined and the most suitable conditions were selected for the analytical method.

3.1. Spectroeletrochemical investigation

The best working potential was determined from the hydrodynamic voltammogram of 8.6×10^{-4} mol l^{-1} chlorpromazine hydrochloride in 0.1 mol l^{-1} H_2SO_4 (Fig. 2B), which in turn was obtained from a three-dimensional representation of the spectra recorded during of the electro-oxidation (Fig. 2A). The band observed, with a peak at 524 nm, is attributed to the radical cation formed in the first oxidation step upon the removal of one electron from the chlorpromazine molecule [1,29]. Fig. 2B reveals that the potential of 0.800 V

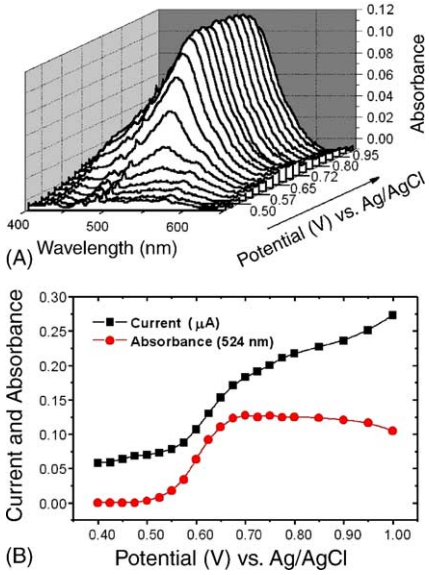

Fig. 2. (A) Three-dimensional representation of the spectra during the electroxidation of the chlorpromazine hydrochloride. (B) Corresponding hydrodynamic voltammogram and voltabsorptiograms of 8.6×10^{-4} mol l^{-1} chlorpromazine hydrochloride solution in 0.1 mol l^{-1} H_2SO_4 at 2.8 ml min^{-1} flow rate.

provides the highest absorbance and current measurements and was therefore adopted for most of the studies described hereafter.

3.2. FIA parameters investigation

The flow rate effect (300 μl injections of 1.0×10^{-3} mol l^{-1} chlorpromazine hydrochloride solution in 0.1 mol l^{-1} H_2SO_4 and 0.800 V constant applied potential) is

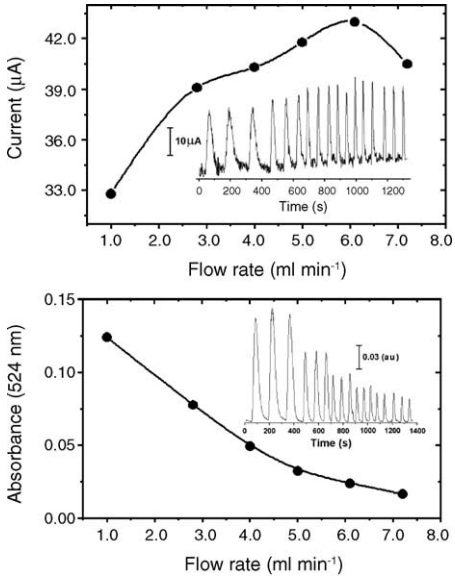

Fig. 3. Flow rate effect on current and absorbance peak heights of 300 μl injections of a 1.0×10^{-3} mol l^{-1} chlorpromazine hydrochloride solution in 0.1 mol l^{-1} H_2SO_4 at 0.800 V applied potential. Inset: recorded signals.

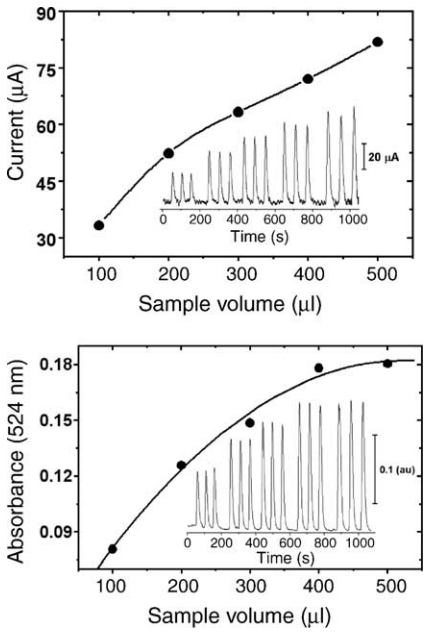

Fig. 4. Effect of sample loop volume on current and absorbance peak heights, at 2.8 ml min^{-1}. Injected-sample volume from 100 to 500 μL of 1.0×10^{-3} mol l^{-1} chlorpromazine hydrochloride solution in 0.1 mol l^{-1} H_2SO_4 at 0.800 V applied potential. Inset: recorded signals.

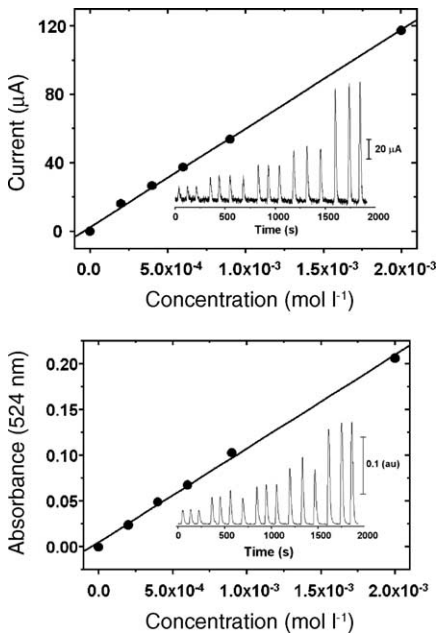

Fig. 5. Analytical curves based on electrochemical and optical peak heights. Injections of 300 μl chlorpromazine hydrochloride solution, 2.0×10^{-4} to 2.0×10^{-3} mol l^{-1} in 0.1 mol l^{-1} H_2SO_4, at 2.8 ml min^{-1} flow rate and 0.800 V applied potential. Inset: recorded signals.

evident from the graph in Fig. 3. This reveals that higher flow rates enhance the current signal because of increased mass transport to the working electrode surface by convection, though in detriment to optical sensitivity. This gain associated with mass transport does not fully compensate the decreased exposure time of the sample to the working electrode.

The influence of the injected-sample volume was studied between 100 and 500 μl for 1.0×10^{-3} mol l^{-1} chlorpromazine hydrochloride solution in 0.1 mol l^{-1} H_2SO_4 at 2.8 mL min^{-1} and 0.800 V constant applied potential. The results (Fig. 4) show that the average current and absorbance values increase with the loop size, as expected. Aiming at compromising between analytical frequency and sensitivity, a sample loop of 300 μl was selected. This and the remaining settings of the flow-injection manifold for the analytical method are gathered in Table 1. With this setup, a series of standard solutions of chlorpromazine hydrochloride was run in triplicate to obtain the analytical curves. The results for the 2.0×10^{-4} to 2.0×10^{-3} mol l^{-1} range are shown in Fig. 5 (average current and absorbance values were used). Excellent linear regression fits were obtained for the electrochemical ($r = 0.999$) and spectrophotometric ($r = 0.998$) response. The limits of detection (3σ) were 7.6×10^{-5} and 4.1×10^{-5} mol l^{-1} for electrochemical and spectrophotometric measurements, respectively, corresponding to limits of quantification (10σ) of 3.3×10^{-4} and 1.6×10^{-4} mol l^{-1}. Table 2 gives the obtained equations and others usual figures of merit, such as the correlation coefficients and the range of linearity.

The system repeatability was examined through 300 μl injections of 1.0×10^{-3} mol l^{-1} chlorpromazine hydrochloride in 0.1 mol l^{-1} H_2SO_4 at 2.8 ml min^{-1} and 0.800 V constant applied potential. The observed relative standard deviations for absorbance and current ($n = 20$) were 3.0 and 2.6%, respectively. The cell washing time accounts for the modest sample throughput, i.e. 52 determinations per hour,

Table 1
Optimized conditions for flow-injection spectroelectrochemical determination of chlorpromazine hydrochloride

Parameters	Studied range	Selected value
Potential (V)	0.200–1.000	0.800
Wavelength (nm)	190–820	524
Flow rate (ml min^{-1})	1.0–7.2	2.8
Sample volume (μl)	100–500	300

Table 2
Analytical characteristic of elaborated method for determination of chlorpromazine

	Amperometric	Spectrophotometric
Quantification range (mol l^{-1})	3.3×10^{-4} to 2.0×10^{-3}	1.6×10^{-4} to 2.0×10^{-3}
Correlation coefficient	0.999	0.998
Equation of calibration curve[a]		
Slope	0.057	102.3
Intercept	2.48×10^{-6}	0.0051
Detection limit (mol l^{-1})	7.6×10^{-5}	4.1×10^{-5}
R.S.D. (%)[b]	0.79	0.77

[a] $y = A + Bx$, where x is the concentration in mol l^{-1}.
[b] Calculated from five determinations.

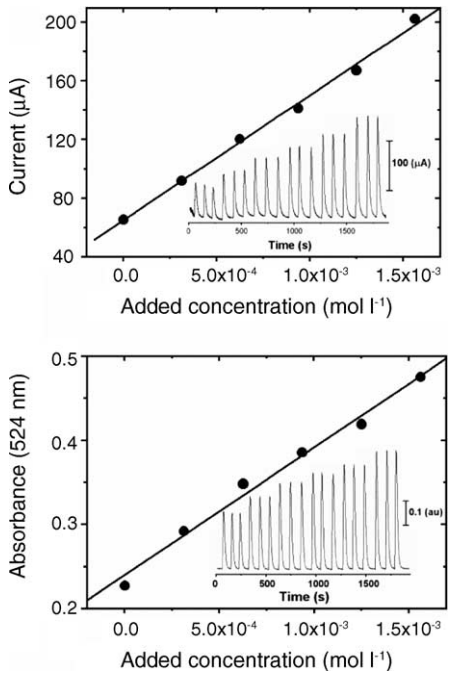

Fig. 6. Spectroelectroanalysis of chlorpromazine hydrochloride in *Amplictil* tablets, method of standard additions. Injections of 300 μl at 2.8 ml min^{-1} and 0.800 V applied potential. Inset: recorded signals.

a value, however, that could be enhanced by operating at higher flow rates, with a moderate reduction in sensitivity of the absorbance peak heights.

3.3. Application

The method was applied for the determination of chlorpromazine hydrochloride in pharmaceutical preparations. The standard addition method was employed for the quantification of the analyte both in the tablets and for oral solutions of *Amplictil*. Typical FIA peaks obtained for quantification in the tablets are shown in Fig. 6.

For the determination of chlorpromazine hydrochloride in the oral solution, the presence of ascorbic acid, acting as antioxidant, must be taken in account. A cyclic voltammogram obtained with an oral solution (Fig. 7) exhibits two

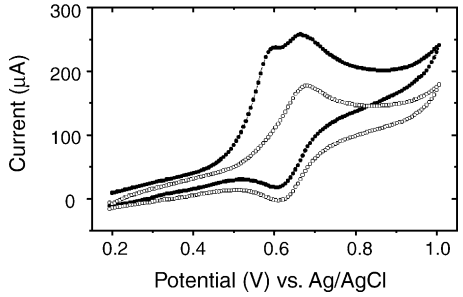

Fig. 7. Cyclic voltammogram of the *Amplictil* oral solution in 0.1 mol l^{-1} H_2SO_4, containing ascorbic acid and 8.6×10^{-4} mol l^{-1} chlorpromazine hydrochloride: (●) first voltammogram; (○) third voltammogram. Spectroelectrochemical cell operated under stopped-flow condition. Scan rate: 11 mV s^{-1}.

Table 3
Results of the determination of chlorpromazine hydrochloride in *Amplictil* tablets and oral solution by the method proposed and reference method

Medicine	Detection	Employed methodology	
		Standard additions	Reference method
Amplictil (25 mg/tablet)	Current	25.1 ± 0.2	–
	Absorbance	25.9 ± 0.2	25.7 ± 0.6[a]
Amplictil (0.04 g/ml)	Absorbance	0.042 ± 0.002	0.0396[b]

Nominal value: 25 mg/tablet and 0.04 g/ml (oral solution). Average of triplicates.
[a] US Pharmacopoeia.
[b] Absorption method used by the manufacturer, Aventis, at production time.

overlapped oxidation waves. The first is originated by the irreversible oxidation of ascorbic acid, while the second wave is associated with chlorpromazine. This not only impedes direct amperometric FIA but interferes with spectrophotometric measurements too, because the cationic radical formed during oxidation of chlorpromazine at the gold electrode and responsible for the absorption band at λ = 524 nm, is reduced back by the ascorbic acid [52]. Therefore, it is necessary to oxidize a large extent of the ascorbic acid at the electrode to allow the colored radical to last until absorbance is measured. Fig. 7 shows that during the third repetition of the cyclic voltammogram of the preparation for oral use, the interference of the ascorbic acid is no longer observed.

In the present work, reducing the distance between the twin working electrodes from 500 to 150 μm and decreasing the flow rate from 2.8 to 2.0 ml min^{-1} overcame the interference problem. A sufficient amount of ascorbic acid is inactivated in the oxidized state to allow the measurement of the colored electro-oxidation product of chlorpromazine with some decrease in the slope of the straight lines defined by standard addition method, that extrapolate to the correct concentration.

The quantification data are summarized in Table 3, where a comparison between the proposed and the reference method is also presented. It is quite clear, from the information in Table 2, the results of spectroelectroanalytical determination for the *Amplictil* tablets are in good agreement with the outcome of the official method (USP). For the *Amplictil* oral solutions, none of the official methods can be satisfactorily applied unless chlorpromazine is previously extracted. Therefore, the results were compared with the value determined by the manufacturer (Aventis, Brazil) for the same batch (L. 205242) during quality control, displaying good agreement.

4. Conclusions

The flow-injection spectroelectroanalytical method proposed for the determination of chlorpromazine hydrochloride in pure form or in pharmaceutical formulations is fast, simple and prone to automation. Compared to other flow-injection methods for phenothiazine drugs, it is one of the

simplest, in terms of instrumentation, and cheapest in terms of cost per assay. Since the oxidation is carried out electrochemically, the generation of a surplus of chemical reagents and the formation of byproducts presenting safety problems and/or having an environmental impact (e.g. chromium salts and/or cyanide) is avoided. In particular, sulfuric acid is used in diluted form, $0.1\,mol\,l^{-1}$, whereas published spectrophotometric methods require, typically, a concentration of $5\,mol\,l^{-1}$ H_2SO_4. Additionally, despite the complexity of the sample matrix, no pretreatment is required, because the combined information (current and absorbance measurements) contributes to a higher degree of selectivity and better discrimination between analyte and interferences signals. In the presence of dyes, the injection of a sample without the application of the electrochemical potential provides a simple and direct procedure to get information on the blank, to be subtracted.

The results obtained in the determination of chlorpromazine hydrochloride in *Amplictil* (Aventis), quantified using the method of standard additions, were satisfactory and sufficiently reliable to recommend the methodology as an alternative to the official methods [3,4]. Furthermore, this work is a remarkable example that with the advent of simplified spectroelectrochemical flow cells [48,49], any laboratory equipped with a spectrophotometer and a potentiostat can introduce spectroelectroanalysis in routine applications and explore its potentialities in research.

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