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Fast and direct determination of butylated hydroxyanisole in biodiesel by batch injection analysis with amperometric detection

Thiago Faria Tormin, Denise Tofanello Gimenes, Eduardo Mathias Richter, Rodrigo Alejandro Abarza Munoz *

Instituto de Química, Universidade Federal de Uberlândia, Av. João Naves de Ávila, 2121, Uberlândia, MG, Brazil

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ABSTRACT

We report here, for the first time, application of batch injection analysis (BIA) with amperometric detection for determination of the phenolic antioxidant butylated hydroxyanisole (BHA) in biodiesel. A sample plug was directly injected onto a boron-doped diamond electrode immersed in 50% v/v hydroethanolic solution with 0.1 mol L^{-1} $HClO_4$ using an electronic micropipette. Importantly, the only preparation step required for biodiesel analysis is dilution in the same hydroethanolic electrolyte solution. Our proposed method has several advantages for routine biodiesel analysis, including: a low relative standard deviation between injections (0.29%, $n = 20$), high analytical frequency (120 h^{-1}), adequate recovery values (93–101%) for spiked samples, satisfactory accuracy (based on comparative determinations by high-performance liquid-chromatography), and a low detection limit (100 ng of BHA per g of biodiesel). Finally, our method can be adapted for the determination of other antioxidants in biodiesel samples.

1. Introduction

Highly efficient analytical methods are required in both routine and research laboratories. Flow injection analysis (FIA) combined with electrochemical detectors provides high-speed, sensitivity, selectivity, accuracy and precision; and thus has been widely employed in the development of analytical methods [1,2]. In FIA, a sample plug is injected at regular intervals, and transported to an electrochemical detector by a continuous liquid carrier stream [1,2]. Batch injection analysis (BIA) is an attractive alternative to FIA for the development of analytical methods for routine purposes [3]. BIA involves injection of a sample plug through a micropipette tip directly onto the working electrode surface (wall-jet configuration), which is immersed in a large-volume blank solution. Disadvantages associated with the pump and valves of the FIA system (especially when non-aqueous solvents are used as the carrier), and disposal of carrier solutions, are eliminated [4]. However, in spite of its simplicity in comparison with FIA, application of BIA with electrochemical detection remains uncommon [1,4]. One possible explanation is the difficulty of introducing a sample preparation step into the BIA system, which is essential for analysis of complex matrix samples [5]. In addition, electrochemical detection is susceptible to interference from surface-active compounds, and a Nafion-coated electrode was proposed to overcome such problems for analysis of ecotoxicological test media [6].

Electrochemical detection of analytes in inaccessible systems, such as petroleum-based products, oil and biodiesel samples, is very challenging because of the high-resistance of the electrochemical matrix; thus, different approaches have been proposed for such samples [7–11]. For example, a protocol based on the formation of an ensemble of microdroplets of an organic sample on the working electrode, which is then immersed in an aqueous electrolyte, has been shown to enable voltammetric detection of methylcyclopentadienyl manganese(I) tricarbonyl in kerosene [7]. In addition, the use of a gold wire microelectrode to perform voltammetry in oil was reported [8]; while stripping chronopotentiometry, which is less susceptible to interference from organic compounds than voltammetry [9], has been used for direct determination of copper in biodiesel samples [10]. Electroanalysis of antioxidants in biodiesel was also accomplished via multiple-pulse amperometric detection using a cleaning potential pulse to remove adsorbed by-products from the working electrode surface eliminating sample matrix effects [11]. Mercury-drop electrodes have also been proposed for voltammetric analysis of biodiesel samples [12], and have the advantage of provide a continually renewable surface minimizing electrode surface passivation. However, widespread use of mercury electrodes has been limited by their high toxicity [13].

Although biodiesels are a very promising substitute for petroleum-based fuels, they display relatively low oxidation sta-

* Corresponding author. Tel.: +55 34 3239 4143xr213; fax: +55 34 3239 4208.
E-mail address: raamunoz@iqufu.ufu.br (R.A.A. Munoz).

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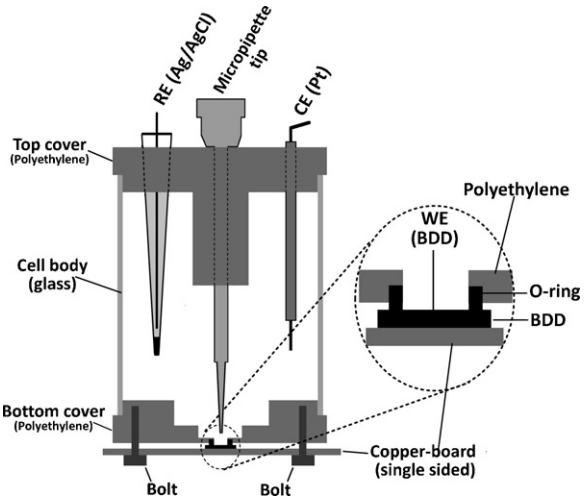

Fig. 1. Schematic diagram of the bath injection cell.

bility. However, this stability can be improved by the addition of synthetic phenolic antioxidants (a common food additive) [14].

In the present study, we report a novel application of BIA with amperometric detection in hydroethanolic medium, for fast and direct determination of the phenolic antioxidant butylated hydroxyanisole (BHA) in biodiesel fuel samples.

2. Material and methods

2.1. Reagents and biodiesel samples

Highly pure deionized water ($R \geq 18\,M\Omega\,cm$) obtained from a Milli Q water purification system (Millipore, Bedford, MA, USA) and was used to prepare all aqueous solutions. Analytical grade perchloric acid (70% m/v), acetic acid (65% m/v), phosphoric acid (85% m/v), sodium acetate and potassium nitrate were obtained from Vetec (Rio de Janeiro, Brazil), and were used without further purification. Butylated hydroxyanisole (BHA) (98.5% m/m) was purchased from Synth (Diadema, Brazil). HPLC grade methanol, ethanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Working standard solutions were prepared immediately before use by appropriate dilution of stock solution. A stock BHA standard solution ($54\,g\,L^{-1}$) was prepared in ethanol. Biodiesel samples were obtained from local factories and were produced from soybean oil and recycled cooking oil. Different amounts of BHA were added to the biodiesel samples just after its acquisition.

2.2. Electrochemical measurements

Electrochemical recordings were conducted using a μ-Autolab Type III potentiostat (EcoChemie, Utrecht, The Netherlands). Injections of standard solutions or samples were conducted using an Eppendorf electronic micropipette (multipette® stream), which permits injections from 10 to 1000 μL (using a 1 mL combitip®) at a programmable dispensing rate (from 28 to 250 $\mu L\,s^{-1}$). A home-made BIA cell was designed (Fig. 1) based on a similar cell [15]. The BIA cell had an internal volume of 180 mL, and was constructed from a glass cylinder (internal diameter = 7 cm) and two polyethylene covers, which were firmly fitted on the top and bottom of the cylinder. The top cover contained 3 holes for the counter (CE in Fig. 1) and reference (RE in Fig. 1) electrodes and micropipette tip (combitip® syringe shape). The micropipette tip (with a regular external diameter = 6 mm) was firmly introduced into the hole (diameter = 6.1 mm) in the center of the cover in such a manner that the injection procedure was highly reproducible. The bottom cover contained a single hole (which was also precisely located at the center of the cover) in which the working BDD electrode (WE in Fig. 1) was inserted. The electrode area was enclosed by an organic solvent resistant O-ring ($0.50\,cm^2$) [16]. The electric contact was made with the copper-board positioned under the BDD electrode. In this arrangement, the micropipette tip was approximately 2 mm from the working electrode surface in a wall-jet configuration.

The reference and auxiliary electrodes were a miniaturized Ag/AgCl (saturated KCl) [17] and a platinum wire, respectively. A thin film (around 1.2 μm) of boron-doped diamond (around 8000 ppm doping level) on a polycrystalline silicon wafer was used as the working electrode (Adamant Technologies SA, La Chaux-de-Fonds, Switzerland). Prior to use, the BDD electrode ($0.50\,cm^2$) was cathodically pretreated in $0.5\,mol\,L^{-1}$ H_2SO_4, by applying −3.0 V for 900 s [18]. This electrode treatment was applied just once during the workday.

Multiple-pulse amperometry was used to measure the hydrodynamic voltammogram of BHA. Ten sequential potential pulses (from +0.7 to +1.6 V, 70 ms each) were applied for injection of a BHA standard solution (in triplicate) through the BIA system.

Amperometry (coupled with the BIA system) at a constant potential (+1.0 V) was used for BHA determinations. BHA standard solutions used to construct analytical curves were prepared in 50% (v/v) hydroethanolic solution containing $0.1\,mol\,L^{-1}$ $HClO_4$ (electrolyte final concentration). Biodiesel samples were diluted in the same electrolyte before injection and 180 mL of the same electrolyte was added to the BIA cell.

All electrochemical measurements were performed at room temperature, in the presence of dissolved oxygen.

2.3. HPLC analysis

HPLC measurements were performed using a Shimadzu LC-10VP HPLC equipped with a UV/VIS detector (SPD-10AV), LC column (Lychrispher 100 Å RP18-C18, 250 mm × 4.6 mm, 5 μm), column oven (CTO-20A), degasser (DGU-20A5), small auto-injector, and pump (LC-10AD-VP). The mobile phase was composed of (75:25 v/v) acetonitrile and water at pH 2.1 (adjusted with phosphoric acid before mixing with acetonitrile), and the flow rate was $1.0\,mL\,min^{-1}$. The UV/VIS detector wavelength was fixed at 280 nm. Biodiesel samples were diluted in mobile phase before injection.

3. Results and discussion

In preliminary experiments, the electrochemical oxidation of BHA was investigated in phosphate buffer, acetate buffer and perchloric acid solutions with 50% (v/v) ethanol, by cyclic voltammetry (voltammograms not shown). The electrolyte solution which provided the highest analytical signal was $0.1\,mol\,L^{-1}$ $HClO_4$ solution with 50% (v/v) ethanol. These results are in agreement with previous studies on the electrochemistry of phenolic antioxidants using different carbonaceous materials (glassy-carbon and BDD electrodes), where acidic media provided the best performance for electrochemical oxidation [19–21]. The electrochemical oxidation involves a two-electron process generating tert-butylquinone [22–24]. This process was investigated at BDD electrodes and it was found that the electrode process is controlled by mass transport [24]. Addition of methanol or ethanol to the electrolyte composition was necessary to dissolve the phenolic antioxidants. Although most studies reported the use of methanol in their electrolyte composition, in this work ethanol was selected due to its lower toxicity.

A hydrodynamic voltammogram, in the potential range covering the electrochemical oxidation of BHA, was obtained by multiple-pulse amperometry (using the BIA system). Ten sequential potential pulses of 70 ms each (0.70; 0.80; 0.90; 1.00; 1.10; 1.20; 1.30; 1.40; 1.50 and 1.60 V) were continuously applied (as

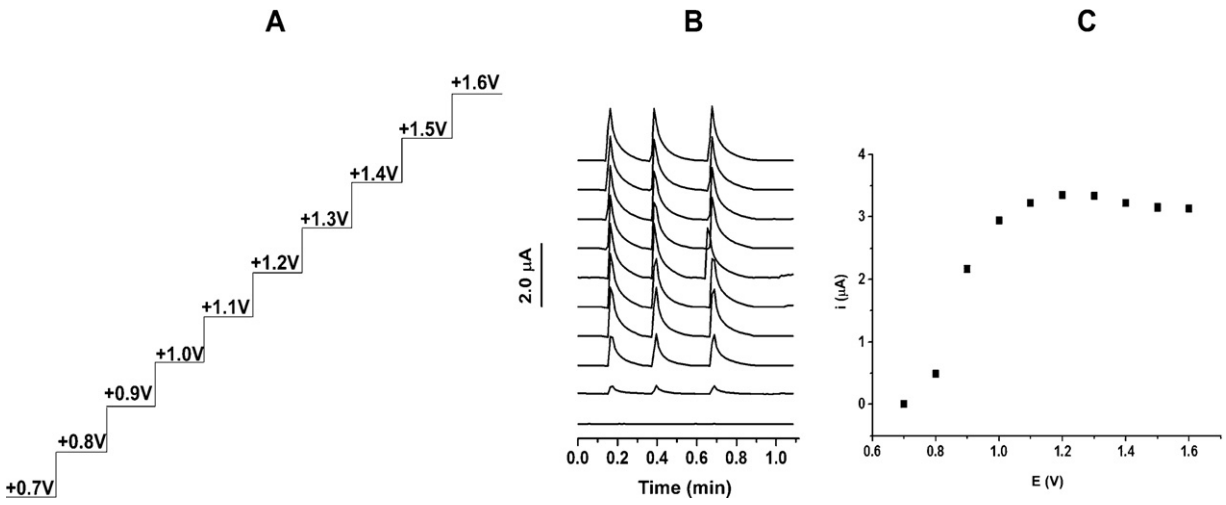

Fig. 2. (A) Multiple-pulse amperometry (MPA) waveform (cyclic form) applied to the BDD working electrode as a function of time; (B) BIA-MPA recordings obtained from 3 successive injections of 75 μM BHA; (C) hydrodynamic voltammogram obtained by plotting the peak current values (average of 3 injections) as function of the corresponding applied potential pulses. Potential pulse time: 70 ms each. Electrolyte: 50% (v/v) ethanol–water with 0.1 mol L^{-1} $HClO_4$.

illustrated in Fig. 2A). The current at each potential pulse was monitored continuously during 3 injections of 75 μmol L^{-1} BHA through the micropipette tip (Fig. 2B). The respective current peak at each potential pulse was measured and used to construct a hydrodynamic voltammogram for the electrochemical oxidation of BHA (Fig. 2C). The working BDD electrode was immersed in a 50% (v/v) ethanol–water solution with 0.1 mol L^{-1} $HClO_4$ (final concentration).

Based on this hydrodynamic voltammogram, a potential of 1.0 V was selected for the electrochemical oxidation of BHA during amperometric recordings.

The initial purpose of this work was to directly inject an aliquot of BHA-doped biodiesel, without prior dilution or electrolyte addition, through the micropipette tip onto the BDD electrode immersed in electrolyte, and measure the respective current at a constant potential (1.0 V). The first injections of BHA-doped biodiesel aliquots through the BIA system revealed that the current baseline was not affected and BHA current peaks were observed. However, the BHA peak height was considerably decreased (by a factor of 30) versus BHA standard solutions. For these experiments, the three-electrode system was immersed in hydroethanolic electrolytes with different ethanol concentrations (30, 50 and 70%, v/v ethanol–water solutions, keeping the final $HClO_4$ concentration constant at 0.1 mol L^{-1}) and BHA standard solutions were prepared in the same electrolyte. Brett et al. [25] suggested the use of BIA with amperometric detection for sample analysis without pre-addition of electrolyte, based on injections of ferrocyanide standard solutions without electrolyte. In contrast, Gunasingham et al. [26] reported a 10-fold decrease in peak height during amperometric detection of estrogens in normal-phase HPLC using a large volume wall-jet cell (analogous to the BIA system), in which no supporting electrolyte was added to the eluent. This result was attributed to the increased ohmic drop between the working and reference electrodes. Gunasingham et al. also verified that the solvent composition (increasing amounts of hexane in a hexane-ethanol eluent) affected the current response more than the electrolyte concentration [26]. Thus, our preliminary results for injections of BHA-doped biodiesel aliquots without addition of electrolyte are in agreement with Gunasingham et al. [26], and biodiesel's lower dielectric constant may also be responsible for the observed decrease in peak height.

Based on the above results, the next step was to test dilution of the biodiesel sample in hydroethanolic electrolyte prior to injection onto the BIA system. Note that typical concentrations of antioxidants added to biodiesel are high enough to require sample dilution before analysis [14]. A BHA-doped biodiesel sample was diluted 10-fold in 3 different electrolyte compositions: 30, 50 and 70% (v/v) ethanol–water solutions keeping the $HClO_4$ concentration constant at 0.1 mol L^{-1}. For all compositions tested, we observed the formation of a single-phase mixture of biodiesel–ethanol–water (micro-emulsion). The three-electrode system was immersed in the same electrolyte in which the sample was diluted. The best results were obtained for the 50% (v/v) ethanol–water solution, with recovery values between 93 and 101% (recovery values were calculated using current responses obtained for BHA standard solutions, prepared in the same respective electrolyte). Lower recovery values (<40%) were obtained for the composition containing a lower ethanol content (30%, v/v). Although amperometric measurements in 70% (v/v) ethanol–water solution provided acceptable recovery values (95–105%), the current baseline was unstable and noisy; the analytical signal was also significantly affected, and a 50% reduction in the BHA amperometric response was observed compared to that obtained using a 50%, v/v ethanol–water solution.

After selecting the composition of the hydroethanolic electrolyte, BIA parameters such as speed of the programmable pipette and injected volume, were evaluated. A dispensing rate of 160 μL s^{-1} provided the highest current response, while low repeatability was observed at higher dispensing rates. The optimal injection volume for the BIA system was 100 μL, which provided the highest analytical signal. The current peak increased significantly with increasing injection volume, from 20 to 80 μL, and reached a maximum value at 100 μL.

In order to evaluate the precision of the proposed BIA method, a repeatability study was conducted. Fig. 3 presents a series of 20 successive injections of 30 μmol L^{-1} BHA, with an analytical frequency of 120 h^{-1}. The average standard deviation was 0.29%. Such low values are typically obtained using BIA [2,4]. Under optimized conditions, the linear dynamic range was from 0.5 to 75 μmol L^{-1} BHA with a correlation coefficient of 0.996.

The proposed BIA method with amperometric detection was applied for BHA determination in biodiesel samples, using the optimized conditions described above. Fig. 4A presents amperometric responses recorded at 1.0 V for injections of 100 μL (in triplicate) of solutions containing increasing and decreasing concentrations of BHA (a–e: 10–50 μmol L^{-1}) and 4 biodiesel samples. The respective calibration curve is also presented (Fig. 4B). This experiment was

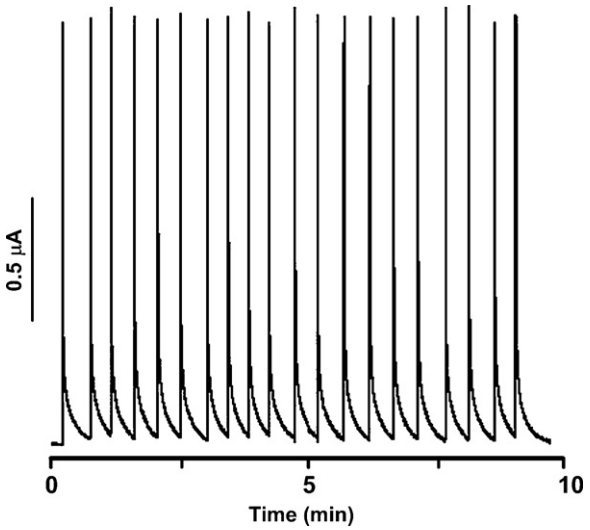

Fig. 3. Repeatability data obtained from successive injections of a solution containing 30 μmol L^{-1} BHA (n = 20). Working potential: +1.0 V; injected volume: 100 μL; dispensing rate: 160 μL s^{-1}; electrolyte: 50% (v/v) ethanol–water with 0.1 mol L^{-1} $HClO_4$.

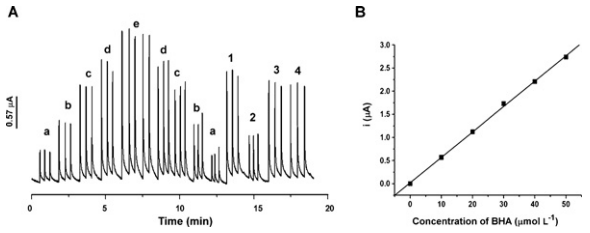

Fig. 4. (A) BIA amperometric responses of the BDD electrode for triplicate injections of (a) 10; (b) 20; (c) 30; (d) 40; and (e) 50 μmol L^{-1} BHA standard solutions and (1–4) four biodiesel samples; (B) corresponding calibration curve (R = 0.999). Experimental conditions as in Fig. 3.

performed in 50% (v/v) ethanol–water solution with 0.1 mol L^{-1} $HClO_4$. Biodiesel samples were diluted 10-fold with the electrolyte prior to injections. For comparison, the biodiesel samples were also analyzed by HPLC. Results are presented in Table 1.

A linear behavior, with a good correlation coefficient (0.999) was observed from 10 to 50 μmol L^{-1} BHA (I = 0.02143 + 0.0550 c). The sensitivity value (slope of the curve) is comparable to the value (0.0559 μA L μmol^{-1}) obtained in a recent work using a BDD electrode and amperometric detection [21]. Electrode fouling was not observed between injections of standard solutions and samples, as evidenced by the fact that current responses were not depleted during amperometric measurements. All results obtained by the proposed BIA method were in agreement with those obtained by HPLC at the 95% confidence level (Table 1).

The detection limit under optimized conditions was estimated to be 50 nmol L^{-1} (with a signal-to-noise ratio of S/N = 3), which corresponds to 100 ng of BHA per g of biodiesel considering the 10-fold dilution (10 μL or 8.70 mg of biodiesel in the 100 μL aliquot injection).

Table 1
Concentrations of BHA obtained by the proposed BIA method and by HPLC (mg g^{-1} of sample) and the respective standard deviation values (n = 3).

Samples	BIA (mg g^{-1})	HPLC (mg g^{-1})
Biodiesel 1	7.4 ± 0.2	7.4 ± 0.2
Biodiesel 2	3.70 ± 0.09	3.7 ± 0.1
Biodiesel 3	6.2 ± 0.2	6.1 ± 0.1
Biodiesel 4	6.3 ± 0.2	6.4 ± 0.1

Both electrochemical oxidation of phenolic compounds [27] and the electrochemistry of inaccessible systems, such as biodiesels, can contribute to electrode passivation during amperometric measurements [10]. Nevertheless, our results indicate that the accuracy and precision of the method proposed here are not affected. The unique properties of BDD electrodes, such as low adsorption and low background currents, coupled with the speed and precision of the BIA technique with amperometric detection, provide a highly sensitive, accurate, precise and fast method for BHA determination in biodiesel. The detection limit of the method (50 nmol L^{-1}) is comparable to the BHA detection limit (30 nmol L^{-1}) reported in a recent work; where a BDD electrode was used to determine the BHA content in mayonnaise samples following sample treatment [21]. This detection limit (30 nmol L^{-1}) was already the lowest reported in the literature [21]. Modified graphite composite electrode with manganese(II) hexacyanoferrate also presented a comparable detection limit (50 nmol L^{-1}) for the amperometric determination of BHA in food samples with the additional advantage of a wide linear dynamic range (from 0.5 μmol L^{-1} to 1.5 mmol L^{-1}) [22]. However, chemically modified electrodes can exhibit gradual decay of signal for long-term experiments [22].

Generally a single antioxidant is added into biodiesel samples. If there are other antioxidants instead BHA such as butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ), the proposed BIA method can be easily adapted for the individual determination of BHT and TBHQ. Considering the presence of mixtures of antioxidants, the amperometric BHA determination would certainly undergo interference from TBHQ, which is electrochemically oxidized at less positive potentials than BHA [20,23,27]. On the other hand, BHT undergoes electrochemical oxidation at more positive potentials than BHA [19,21,24], and then it would not interfere on the BHA determination. BHT did not interfere on the electrochemical BHA detection even with 10-fold excess of BHT using BDD electrodes [21,24]. If mixtures of antioxidants are present, new analytical methods should be developed for biodiesel analysis. Recent reports have been demonstrated the simultaneous determination of antioxidants in food samples [21,24,27]. Similar approaches can be evaluated for biodiesel analysis.

4. Conclusions

We demonstrate, for the first time, the application of BIA with amperometric detection for the determination of BHA in biodiesel. In addition, this is the first reported application of BIA with amperometric detection of analytes in non-aqueous samples such as biodiesel. Samples only required dilution in electrolyte solution prior to analysis. The proposed method is highly precise (RSD = 0.29%, n = 20), accurate (confirmed by comparison with an accepted method and recovery tests), sensitive (LD = 100 ng g^{-1}) and fast (frequency of 120 injections h^{-1}); highlighting the potential of the proposed method for routine biodiesel analysis. Moreover, this method can be applied to assess the content of other antioxidants in biodiesel, and for on-site analysis using commercially available portable potentiostats.

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