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The redox behaviour of diazepam (Valium®) using a disposable screen-printed sensor and its determination in drinks using a novel adsorptive stripping voltammetric assay

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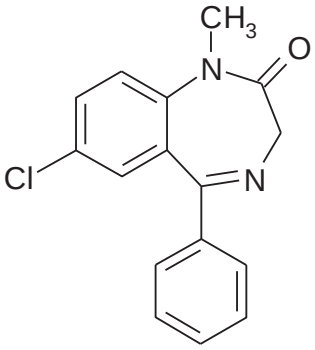
ABSTRACT

In this study we investigated the possibility of applying disposable electrochemical screen-printed carbon sensors for the rapid identification and quantitative determination of diazepam in beverages. This was achieved utilising a previously unreported oxidation peak. The origin of this peak was investigated further by cyclic voltammetry and gas chromatography/mass spectroscopy. At pH 6 the voltammetric behaviour of this oxidation process was found to involve adsorption of the drug allowing for the development of an adsorptive stripping voltammetric assay. Experimental conditions were then optimised for the determination of diazepam in a beverage sample using a medium exchange technique. It was shown that no elaborate extraction procedures were required as the calibration plots obtained in the absence and presence of the beverage were very similar.

1. Introduction

Diazepam is commonly sold under the trade name Valium®, (i) and is one of the most widely prescribed 1,4-benzodiazeapines [1] for the therapeutic treatment of anxiety, insomnia, epilepsy, alcohol withdrawal and muscular spasms [2]. A number of known side effects have been observed including drowsiness, fatigue, ataxia and confusion. The consumption of diazepam along with other depressant substances such as alcohol is known to exacerbate its sedative effects and its increased absorption rate [2–6]. Due to these effects, diazepam has been used in drug-facilitated sexual assault (DFSA) [7–11], often referred to as 'date rape' by the media. Such DFSA cases typically occur in venues where alcohol is served (e.g. clubs and bars) where victims are unaware that their drink may have been 'spiked' with benzodiazepines such as diazepam. Falling prices of diazepam and increased availability of the drug on the black market have also been linked to an increase in these instances [12]. More recently, there have also been reports of diazepam being used in robberies [13,14] via the deliberate adulteration of herbal medicine [15–17]. Concerns have also been raised regarding its occurrence in water and sewage effluents [18,19].



(i)

Due to these factors a range of analytical approaches have been investigated to determine diazepam in beverages and related samples. Commonly, techniques such as high performance liquid chromatography (HPLC) [17,31,33] and GC/MS [20,34] have been employed, having previously gained a great success in medical and pharmaceutical analysis. Table 1 gives an overview of the relative performance characteristics of the techniques used for the determination of diazepam in beverages and related samples. High-performance

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Table 1
Summary of various techniques used for the determination of diazepam in beverage and food samples.

	Technique	Linear range	Detection limit	Sample	Pre-treatment	Reference
Diazepam, flunitrazepam, clonazepam, alprazolam, and ketamine	GC–MS	50–1,000 μg/mL	8.7 μg/mL for diazepam.	Beer and peach juice	Extraction with chloroform: isopropanol 1:1 (v/v)	[20]
Diazepam	High-performance thin-layer chromatography	200–800 ng	30 ng	Cold drinks, Maza and Coke	Liquid-liquid extraction with either chloroform or diethyl ether	[21]
Diazepam, nitrazepam, estazolam, fludiazepam, alprazolam, bromazepam., lorazepam and oxazolam	Direct electrospray probe/mass spectrometry (DEP/MS)	Qualitative rapidly screening method	–	Soda, orange juice, beer and tea	Liquid–liquid extraction (chloroform:isopropanol 1:1 v/v)	[22]
Diazepam	multipumping flow system (MPFS) coupled to a photodegradation fluorimetry		approximately 2.02 μg/mL	Five commercial alcoholic beverages (Eristoff®, Smirnoff®, Bacardi®, Dry Gin® and Brazilian Cachaça 51®).		[23]
Diazepam and chloral hydrate	Differential pulse polarography at a hanging mercury drop electrode.	–	1 μg/ml for diazepam.	Toddy	Dilution in electrolyte	[24]
Diazepam, nitrazepam oxazepam, alprazolam, flunitrazepam, temazepam, 7-aminoflunitrazepam, 7-aminonitrazepam and 7-aminoclonazepam	Capillary electrophoresis-DAD	17.0–90.9 μg/mL for diazepam.	3.0 μg/mL for diazepam.	White wine, Coca-Cola, orange juice, beer, bourbon and Bacardi	Direct injection of sample	[25]
Diazepam	molecularly imprinted polymer modified SPCE conductivity based sensor	0.04–0.62 μg/mL	8 ng/mL	Meat samples.		[26–28]
Diazepam, alprazolam and chloral hydrate	Reversed-phase high-performance liquid chromatography, DAD, UV 210 nm	0.01–1.0 mg/mL	0.8 μg	Toddy (fermented sap or exudate of date, Palmyra, coconut, sago etc.)	Toddy filtered (0.45 μm) and injected to HPLC	[29]
Diazepam and its metabolites. Nitrazepam and lorazepam also determined	Fluorescence polarization immunoassay (FPIA) and liquid chromatography coupled to time-of-flight mass spectrometry (LC-TOF MS)	–	–	Poisoning cases from commenters accepting food or drinks	1 mL of urine sample after enzymatic cleavage of conjugates (*b*-glucuronidase/arylsulfatase) and liquid–liquid extraction at pH 7.0 with 1-chlorobutane/diethyl ether, 1/1, v/v	[30]
Diazepam, oxazolam, nitrazepam, oxazepam, tofisopam, triazolam and clotiazepam	Reversed-phase high-performance liquid chromatography, DAD	2–16 μg/mL for diazepam.	0.5 μg/mL for diazepam.	Dietary supplements	Extraction with methanol	[31]
Diazepam	High-performance thin-layer chromatography with medazepam as internal standard.	200–1000 μg/mL		Cream biscuits	Extraction with ethanol and subsequently by a mixture of methanol: acetonitrile: tetrahydrofuran: water (15:55:4:26 v/v).	[32]
Diazepam	Unmodified SPCE	7.1–285 μg/mL	1.8 μg/mL	Beverage samples; Pepsi Max, Vodka Cherry alcopop	Adsorptive stripping voltammetry with medium exchange	This report

thin-layer chromatography [21] has also been shown successfully for the determination of diazepam in beverages, and the qualitative determination of several benzodiazepines has been reported by direct electrospray probe/mass spectrometry [22]. Recently, Ribeiro et al. [23] have used a multipumping flow system coupled to photodegradation system for the fluorometric determination of diazepam in several different beverages. However, in this instance some samples components were reported to interfere. Husain et al. [24] have reported on the determination of diazepam and chloral hydrate in alcoholic beverages adsorptive stripping polarography. Several nitro substituted 1,4-benzodiazepines have been detected by indirect laser-induced fluorescence using microfluidic device flowing liquid/liquid extraction in several different beverages [35]. Capillary zone electrophoresis has been utilised for the determination of a number of benzodiazepines [36] including diazepam in beverages [25]. Further reports have utilised a molecularly imprinted polymer (MIP) to modify the surface of a screen-printed carbon electrode (SPCE) for the conductometric determination of diazepam [26–28]. A linear range of 0.04 to 0.62 mg/L was obtained with a corresponding detection limit of 8 μg/L. The sensor was shown to be suitable for 'on-the-spot' detection of diazepam in meat samples.

We have recently reported the determination of diazepam, along with flunitrazepam and lorazepam by liquid chromatography with dual electrode detection [37]. A number of other liquid chromatographic approaches utilising electrochemical detection have also been reported utilising boron doped diamond electrodes [38] and hanging mercury drop electrodes [39–42]. However, as far as we are aware, there have been no reports of studies on the voltammetric determination of diazepam at a SPCE for its determination in drinks.

Previous electrochemical studies of diazepam and related 1,4-benzodiazepines at Hg electrodes [43–48] have focused on the $2e^-$, $2H^+$ polarographic reduction of the 4,5-azomethine group at the to give the corresponding dihydro species (Eq. (1)).

$$\text{R-(H)C=N-R}' + 2e^- + 2H^+ \rightarrow \text{R-C(H)-N(H)-R}' \quad (1)$$

This approach has been shown to be highly successful for both medical and pharmaceutical analysis but has been perceived as problematic owning to possible toxicity and disposal problems of Hg. As a result, a number of alternative electrode materials have been investigated such as glassy carbon [37,49–51] and galinstan [52]. In a recent paper we have reported on the electrochemical behaviour of diazepam [37] at an unmodified glassy carbon electrode and suggested a possible electrode reaction.

To our knowledge, the reduction of the azomethine group has been reported by a number of authors as the only electrochemical process occurring at some benzodiazepine drugs such as diazepam. Consequently, in this present study, we have investigated the electrochemical mechanism further using our SPCEs. Cyclic voltammetric studies were first made to investigate and optimize the electrochemical behaviour diazepam. Studies were then made to explore the possibility of using these findings to develop a method for its adsorptive stripping voltammetric (AdSV) determination in beverage samples. Further studies were performed to investigate the possibility of utilising a medium exchange technique; an approach we have shown to be highly successfully in previous studies to improve selectivity and sensitivity for the determination of a several metal ions at these same SPCEs [53,54].

2. Experimental

2.1. Chemicals and reagents

All chemicals were obtained from Fisher (Loughborough, UK), unless otherwise stated. Deionised water was obtained from a Purite RO200—Stillplus HP System, (Purite Oxon., UK). Solutions of disodium, trisodium, sodium *o*-phosphate and *o*-phosphoric acid were made at a concentration of 0.2 M by dissolving the appropriate mass in deionised water. These were then titrated together, to give the desired pH. A stock solution of diazepam (Sigma-Aldrich, Dorset, UK) was prepared by dissolving the required mass in ethanol to give a concentration of 10 mM. Working standards, for initial voltammetric studies, were prepared by dilution of this solution with phosphate buffer to give a final concentration of a 0.1 M phosphate buffer. These were then adjusted with sufficient water to give a 10% ethanol solution. Beverage samples were obtained from local commercial outlets.

2.2. Apparatus and instrumentation

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a Pstat10 potentiostat interfaced to a PC for data acquisition via the General Purpose Electrochemical System Software Package (GPES) version 3.4 (Autolab, Windsor Scientific Limited, Slough, Berkshire, UK). The screen-printed sensor was printed and supplied by Gwent Electronic Materials Ltd. (Pontypool, UK). The working electrode was printed from C10903P14 carbon ink with an Ag/AgCl screen-printed track (2×0.2 cm) printed alongside this serving as the pseudo-reference/counter electrode. The design of this sensor has been shown elsewhere [55]. The screen-printed electrode strip was cut from the PVC support, the connecting strips trimmed to 15 mM and the electrode working area (9 mM^2) isolated with a thin strip of insulating tape (RS Components, Corby, Northamptonshire, UK).

2.3. Cyclic voltammetric studies

Cyclic voltammograms were initially recorded with plain solutions of 0.1 M phosphate buffer, containing 10% ethanol and then in the same solution containing 1 mM diazepam. Degassing was achieved by purging with oxygen free nitrogen (BOC, Guildford, UK) for 5 min to eliminate oxygen reduction waves. A starting and end potential of 0.0 V (vs. Ag/AgCl) was used, with an initial switching potential of −1.7 V (vs. Ag/AgCl), a second switching potential of +1.7 V (vs. Ag/AgCl). A new SPCE was used for each determination. Scan rates were investigated over the range 20 to 200 mV/s.

2.4. Differential pulse adsorptive stripping voltammetry

Accumulation was carried out for 150 s at −2.1 V (vs. Ag/AgCl) at 35 °C. Initially, the stripping voltammogram was recorded using linear sweep voltammetry. Further studies were made by differential pulse voltammetry using a step height of 10 mV, pulse repetition time 0.2 s, pulse amplitude of 70 mV, and pulse duration of 50 ms. The stripping voltammogram was recorded over the potential range −2.0 V to +2.0 V (vs. Ag/AgCl). Calibration standards were prepared by diluting sufficient volumes of the 10 mM diazepam ethanol stock solution to give the desired concentration in a 0.1 M pH 6 phosphate buffer. Beverage samples were prepared in a similar manner to give an overall 0.1 M pH 6 phosphate buffer by dilution with a 0.2 M pH 6 phosphate buffer. A new SPCE was used for each determination.

2.5. Gas chromatography/mass spectroscopy

Extracts for gas chromatography/mass spectroscopy (GC/MS) analysis were obtained by extraction with an equal volume of dichloromethane. Aliquots of this were then examined using a Hewlett-Packard 5890 Series II Plus gas chromatograph coupled to a 5985B quadrupole MS system (Hewlett-Packard, Palo Alto, CA, USA). Manual injections were made using a split–splitless technique

on to an HP-5MS capillary column (15 m × 0.25 mm ID, 0.25 μm film thickness, 5% diphenyl–95% dimethylsiloxane phase) interfaced directly into the ion source. The GC oven temperature was maintained at 70 °C for 3 min and then programmed to 250 °C at 12 °C/min and finally held isothermally for 10 min at this temperature. The injector and transfer lines were at 330 °C. Source and quadrupole temperatures were 200 °C and 100 °C, respectively.

3. Results and discussion

3.1. Cyclic voltammetric investigations of diazepam

Fig. 1A shows the cyclic voltammogram of 1.0 mM diazepam in a 0.1 M phosphate buffer pH 4 containing 10% ethanol at our SPCE. A reduction peak R1 was observed on the first negative going scan similar to that previously reported at carbon [49] and Hg electrodes [43]. Consequently, we believe that R1 seen at our SPCEs results from the same $2e^-$, $2H^+$ reduction of diazepam at the 4,5-azomethine group to give 4,5-dihydro-diazepam. However, unlike that shown in previous reports an oxidation peak O1 is seen on the reverse positive going scan.

Further cyclic voltammetric investigations were carried out on the same solution (Fig. 1B) to explore this phenomenon. However, in this case using a starting and end potential of 0.0 V was used and the scan was initiated in the positive direction first. Under these conditions no oxidation peaks were observable, only the reduction peak R1 was still present on the return negative going scan. We concluded from this that O1 is due to an oxidation process resulting from the reduction product of diazepam generated during peak R1.

This is an interesting finding as earlier reports have indicated that the amide group can be directly oxidised. However, we did not observe a second direct oxidation peak. Further cyclic voltammetric investigations were made on a 0.1 M pH 8.0 phosphate buffer solution containing 10% ethanol, in the presence and absence of 1 mM diazepam. These studies were carried out with both a SPCE and at a glassy carbon electrode (GCE). In the case of the GCE, on the initial going positive scan, without prior reduction, a single oxidation peak with an E_p of +1.7 V was obtained, indicative of the direct oxidation of the amide group. This was found to decrease in magnitude with repeated scans of the same solution. A single reduction peak was seen on the return negative going scan, corresponding to the reduction of the 4,5-azomethine bond, with a further second oxidation peak with the same E_p as O1 occurring on further scans, as previously seen at our SPCEs. The magnitude of all these peaks was found to decrease with repeated scanning.

It should be mentioned that, with our SPCEs this initial oxidation peak was not seen even with repeated scanning, with only the reduction peak R1 and the oxidation peak O1 being recorded as before. However, unlike the behaviour observed with the GCE, the magnitude of both these peaks was found to increase with repeated scanning. This we believe shows that the electrochemical behaviour of diazepam is markedly different at the two different carbon electrode types. The electrochemical processes being diffusion controlled at the GCE and would appear to be adsorption control at our SPCEs. This is an interesting finding as this behaviour suggested the possibility for the development of an adsorptive stripping voltammetry (AdSV) based assay for diazepam.

3.2. Optimisation of conditions for the adsorptive stripping voltammetry of diazepam

3.2.1. Effect of pH

Fig. 2 shows the plots of E_p versus pH for peaks R1 and O1 obtained over the range pH 2–12. Fig. 2A shows that the reduction peak R1 is pH dependent over the whole range investigated. However, two different slopes can be seen (90 mV/pH and 60 mV/pH) with a break point at pH 4.5. We believe that this is a reflection of the p*K*a of the 4,5-azomethine group, which is in good agreement to obtained elsewhere [56]. The changes in slope from 90 mV/pH to 60 mV/pH is in keeping with this proposed mechanism as a $2e^-$, $3H^+$ reduction would be expected below the p*K*a value and a $2H^+$, $2e^-$ above it.

The oxidation peak O1 (Fig. 2B) was found to be pH dependant from pH 4.5 to pH 10 with a slope of 61 mV/pH, indicative of an equal number of protons and electrons involved in the oxidation step. At higher pH values the E_p of this peak was found to be constant. We believe this is again a result of an apparent p*K*a value

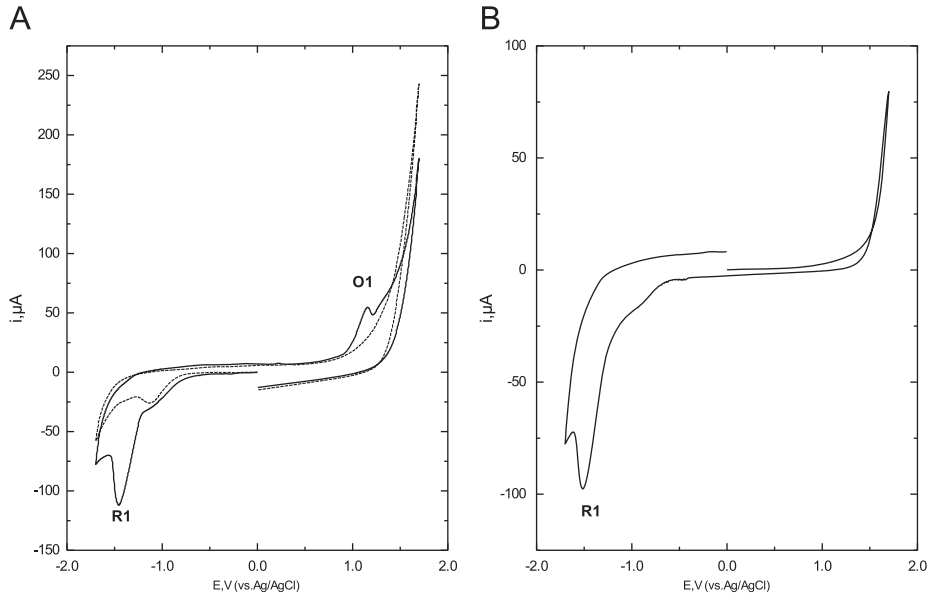


Fig. 1. Cyclic voltammograms, obtained at a scan rate of 50 mV/s, for a 1 mM solution of diazepam in 10% ethanol, buffered with 100 mM phosphate at pH 4. (A) Starting and end potential 0.0 V, initial switching potential -1.7 V, second switching potential +1.7 V. (B) Starting and end potential 0.0 V, initial switching potential +1.7 V, second switching potential -1.7 V. Dashed line in the absence and solid line in the presence of 1 mM diazepam.

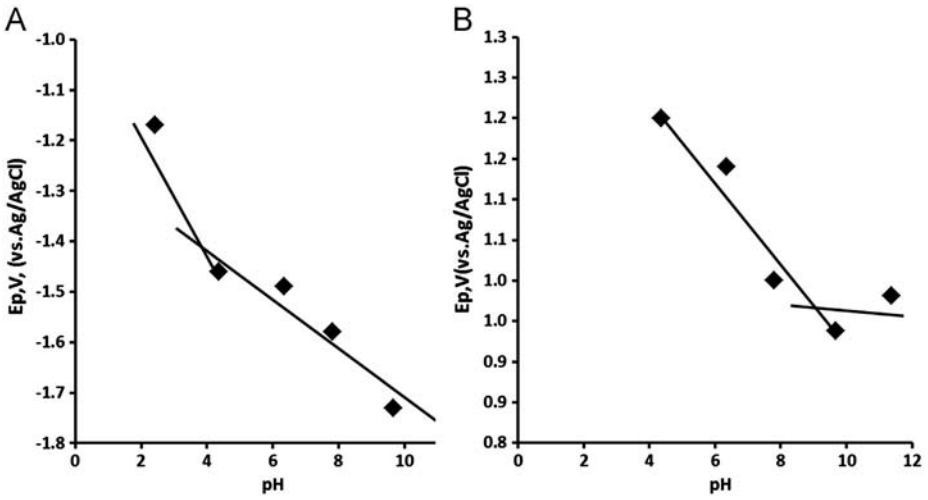


Fig. 2. Plot of E_p vs. pH for diazepam. (A) Peak R1, (B) peak O1. Voltammetric conditions as Fig. 1A.

but this case it is associated with the oxidation product of diazepam.

The peak current (i_p) of both peaks was found to be pH dependent (Fig. 3). Between pH 2 and 8 the peak current of R1 was found to be constant but above pH 8 it was found to increase then plateau, for peak O1, no oxidation current was observed below pH 4; above this pH the i_p increased forming a plateau around pH 8.

3.2.2. Proposed mechanism

Scheme 1 shows our proposed mechanism for the redox behaviour of diazepam at our SPCE. Here diazepam (i) is first reduced via a $2e^-$, $2H^+$ at the 4,5-azomethine group to give 4,5-dihydro-diazepam (ii), resulting in the voltammetric peak R1. The formation of the secondary amine here can then allow for a subsequent opening of the seven-membered ring via the $2e^-$, $2H^+$ oxidation on the return positive going scan to give the corresponding primary amine in a similar manner to that described for the electrochemical oxidation of aliphatic amines previously described [57–59]. Consequently, the proposed mechanism, consists of four steps in which an intermediate ammonium radical (iii) is converted by loss of H^+ to (iv); species (iv) is further oxidised to (v) (a quaternary Schiff base) which is rapidly hydrolysed, opening the seven-membered ring to produce secondary amine and ketone functional groups (vi) (Scheme 1).

To further elucidate the possible electrochemical mechanism, we undertook GC/MS analysis of the product generated from the repeated cyclic voltammetric scans made on at the same SPCE. This showed the presence of a compound with a molecular ion with an *m/z* value of 286, indicative of 4,5-dihydro-diazepam, the reported electrochemical reduction product of diazepam. Species (VI) may be absent from the GC/MS data due to it either being too polar or thermally unstable.

At each pH investigated we explored the effect of scan rate (υ) over the range 20 to 200 mV/s on the cyclic voltammetric behaviour of diazepam. Plots of current function ($i_p/\upsilon^{1/2}C$) versus the square root of scan rate ($\upsilon^{1/2}$) exhibited a positive slope that at pH 6 (Fig. 4) for the oxidation peak O1 which indicates adsorption of species (ii) in order to deduce the possibility of exploiting this adsorption behaviour, we performed further studies using pH 6.

3.2.3. Effect of accumulation potential, time and temperature

Fig. 5 shows the effect of accumulation potential on the result peak current (i_p) magnitude of O1 using an accumulation time of 60 s. Clearly, peak current increased from −1.0 V to a maximum value at −2.0 V (vs. Ag/AgCl) which then plateaus at potentials more negative than this. Consequently, further studies were carried out using an applied potential of -2.0 V (vs. Ag/AgCl).

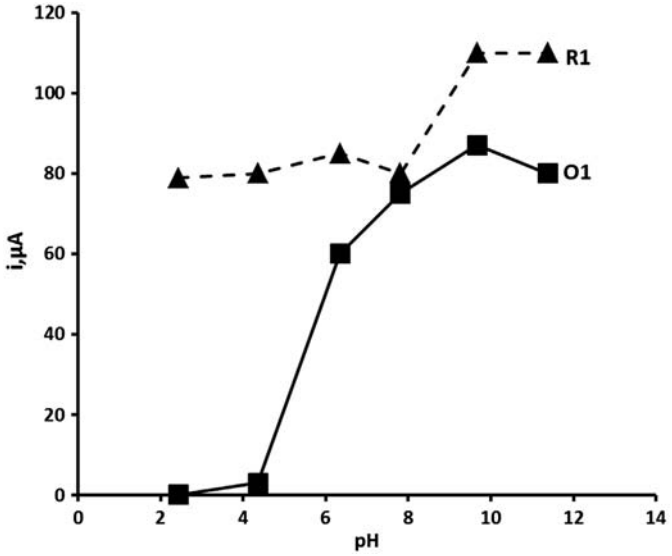


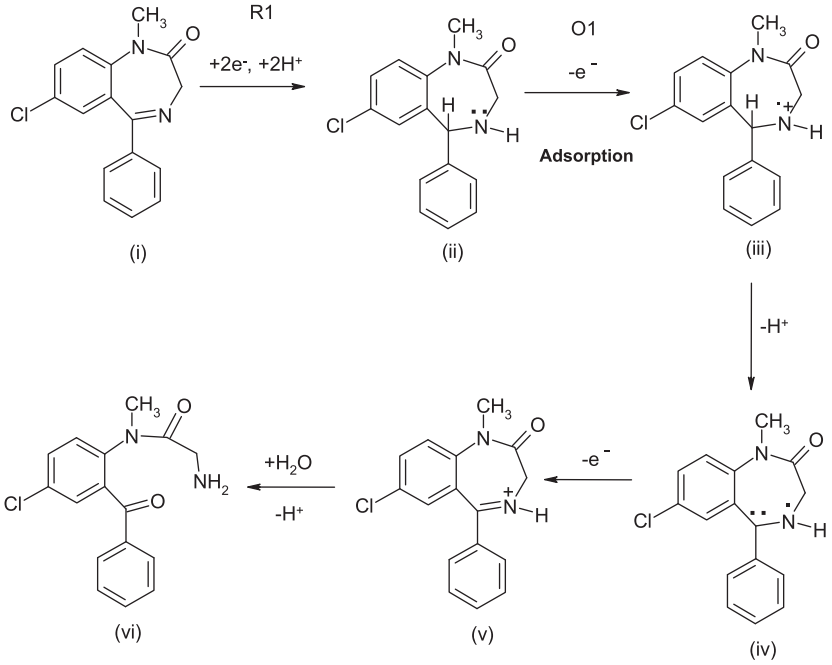
Fig. 3. Plot of i_p vs. pH for peaks R1 and O1. Voltammetric conditions as Fig. 1A.

3.2.4. Effect of accumulation time

Fig. 6 shows the effect of increasing accumulation time at an applied potential of −2.0 V using a 0.08 mM diazepam solution. The resulting oxidation stripping peak was found to increase with time and research a maximum value at 240 s. Therefore, an accumulation time of 240 s was employed in further studies.

3.2.5. Effect of temperature

The effect of temperature (Fig. 7) on the AdSV response of a 0.08 mM was studied over the range 19 °C to 45 °C. The resulting oxidation peak current was found to increase with temperature researching a maximum at 35 °C. Consequently, further studies were made using this temperature, together with an accumulation time of 240 s and accumulation potential of -2.0 V.



Scheme 1 .

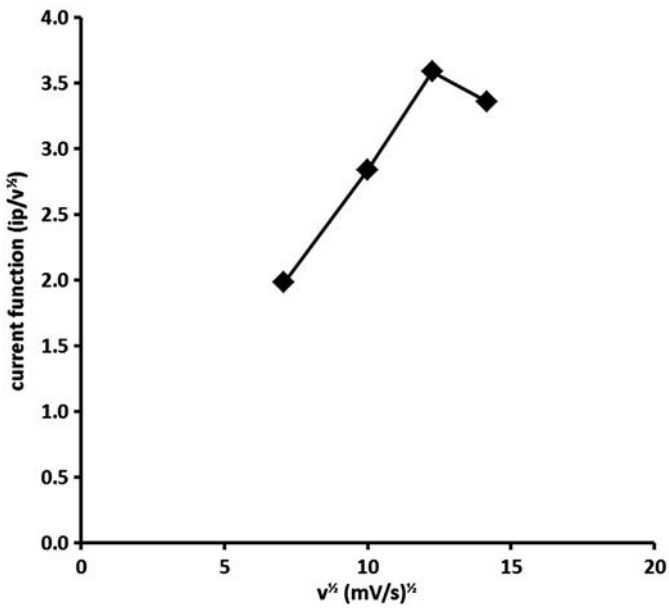


Fig. 4. Plot of current function vs. $v^{1/2}$ for peak O1 in a pH 6 phosphate buffer.

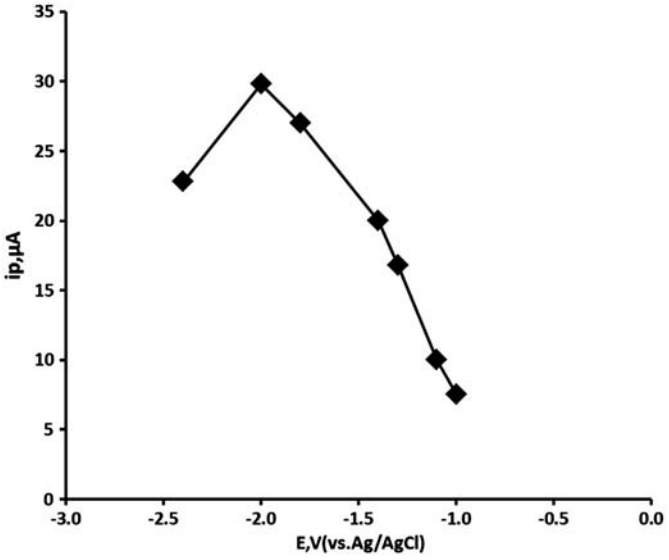


Fig. 5. Effect of accumulation potential on the adsorptive stripping voltammetric response of a 80 µM diazepam. Accumulation time = 60 s.

3.2.6. *Calibration curve and limit of detection*

In order to ascertain whether we could improve on the sensitivity of the assay we decided to investigate the same solutions using DPV. In previous work with these same SPCEs we have shown the superior sensitivity and resolution that can be gained using this waveform [60]. As we were particularly interested in studying forensically relevant concentrations, we calculated that a 10 mg tablet of diazepam in 250 mL of beverage would give a concentration of 40 mg/L; consequently, a concentration range of 7.1 to 285 mg/L diazepam was investigated.

Initial studies were undertaken to study the effect of diazepam concentrations on the magnitude of the adsorptive stripping peak O1 occurring at a potential of +1.0 V. The calibration plot was linear over the range 7.1 to 285 mg/L (R^2 = 0.9969, %CV = 6.4%), with a sensitivity of 26.2 nA/mg L; above this concentration the graph exhibited a plateau. The theoretical detection limit, defined as three times the mean baseline noise was calculated to be 1.8 mg/L. A coefficient of variation of 7.4% was obtained for a 7.1 mg/L solution of diazepam.

3.2.7. *Interference studies*

Utilising the optimised AdSV medium exchange conditions, aspartame (5 mM), ascorbic acid (13 mM), citric acid (25 mM) and paracetamol (15 mM) were found not to interfere with the determination of an 80 µM diazepam solution.

4. Medium exchange approach and analytical application

Fig. 8 shows two representative voltammograms for the DPAdSV determination of diazepam spiked into an alcoholic beverage. Without

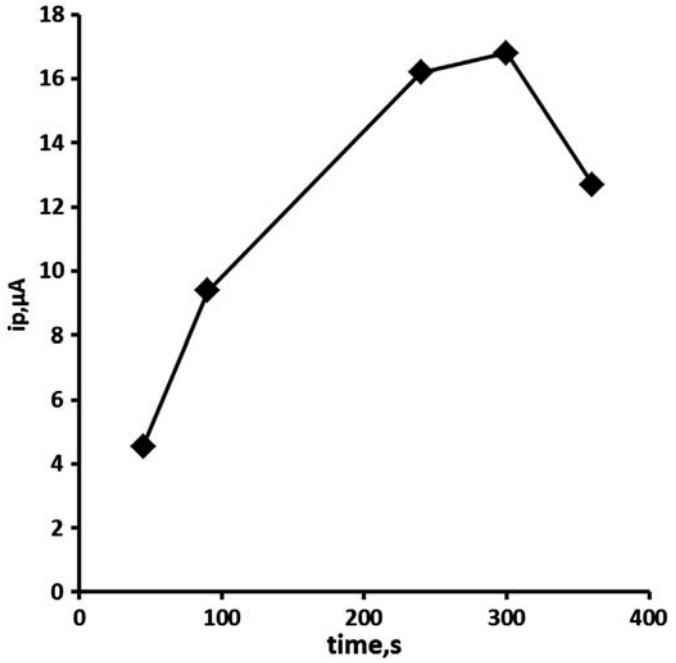


Fig. 6. Effect of accumulation time on the adsorptive stripping voltammetric response of 80 μM diazepam. Accumulation potential = -2.0 V.

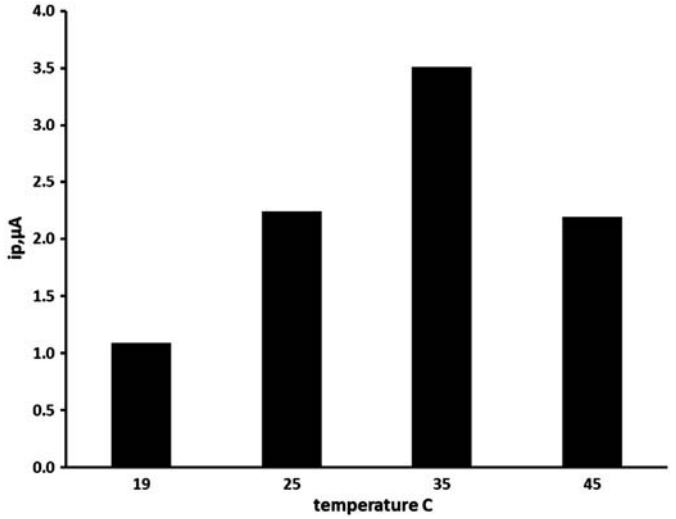


Fig. 7. Effect of temperature on the adsorptive stripping voltammetric response of 80 μM diazepam.

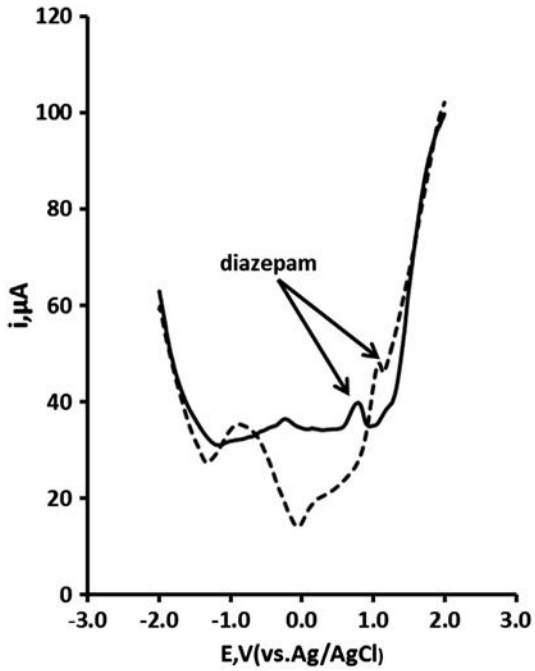


Fig. 8. AdSV determination of diazepam in vodka cherry alcoholic beverage. Solid line with medium exchange, dotted line without medium exchange.

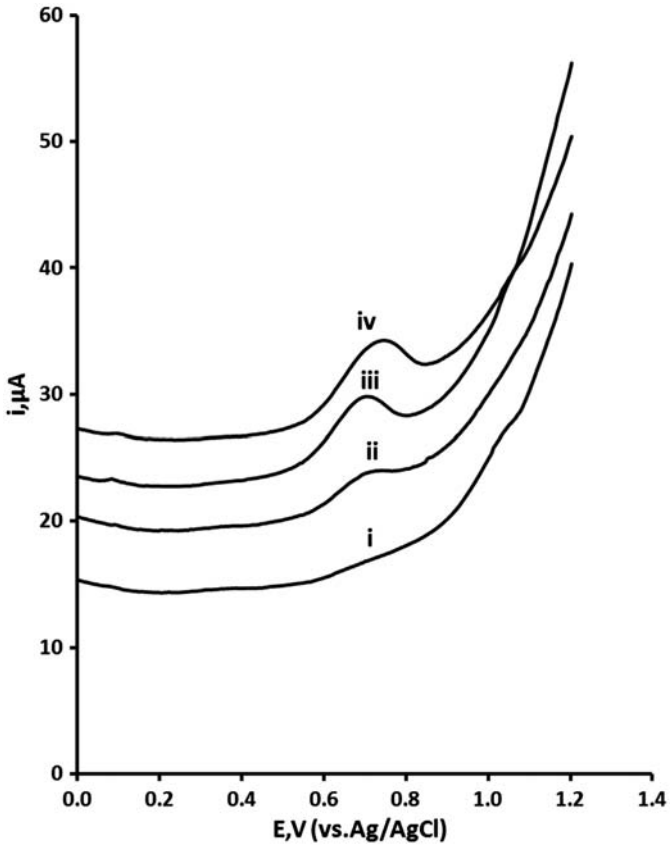


Fig. 9. Adsorptive stripping voltammetry of Pepsi Max fortified with increasing concentrations of diazepam (i) 0 μM, (ii) 42 μM, (iii) 84 μM and (iv) 168 μM. Conditions: -2.0 V for 240 s at 35 °C. Differential pulse voltammetric conditions as described in Section 2.3. A new SPCE was used for each determination.

medium exchange, a large oxidation wave, coupled with a positive shift in E_p makes the determination of diazepam difficult and a consequent decrease in precision is recorded. However, utilising the medium exchange technique, a well-defined oxidation peak for diazepam is readily observed, as it is now completely resolved from the background current. Consequently, we considered that this improvement in selectivity would be beneficial for the analysis of other drinks. Red Russian Ice Cherry, a flavoured vodka based alcohol pop containing 4% v/v ethanol and Pepsi Max (alcohol free) were fortified with diazepam concentrations over the same concentration range and the resulting peak currents recorded. A Student *t*-test showed that the values could be considered to belong to the same population, hence allowing for an external calibration curve to be utilised. Fig. 9 shows the typical adsorptive stripping voltammograms obtained for the Pepsi Max sample (%CV = 12%). Clearly, these results show that the combination of our SPCEs with adsorptive stripping voltammetry utilising the technique of medium exchange offers the possibility of determining diazepam at low levels in beverage samples.

5. Conclusions

The paper demonstrates that diazepam produces two well-defined voltammetric signals at our plain SPCEs via cyclic voltammetry. On the initial going scan a single reduction peak resulting from the $2e^-$, $2H^+$ reduction of the 4,5-azomethine bond to a secondary amine; on the subsequent positive going scan a previously unreported adsorption controlled oxidation signal was found and the voltammetric redox mechanism underlying this was investigated. This was postulated to result from the oxidation, of the secondary amine (which is formed on the negative going scan) via a 2e, $2H^+$ oxidation process; this results in opening of the seven-membered ring diazepine ring to produce a species containing a ketone and a primary amine groups. A simple and convenient assay for diazepam was developed, based on adsorptive stripping voltammetry. The limit of detection, based on a signal to noise ratio of 3:1, was 1.8 μg/mL with a linear response up to 285 μg/mL ($R^2=0.9969$) was achieved using a 120 s accumulation time.

The results indicate that the method gives reliable results using an external calibration method. As far as we are aware this is the first report on the use of the voltammetric adsorptive stripping behaviour of diazepam for a benzodiazepine drug in beverage samples. The oxidation process has not been previously reported. The performance characteristics reported here are comparable to that recently reported for florescence spectroscopy [23].

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