

VOLTAMMETRIC AND AMPEROMETRIC DETERMINATION OF N-NITROSO ANTINEOPLASTIC DRUGS AT MERCURY AND AMALGAM ELECTRODES

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Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th anniversary of the Nobel Prize for polarography.

A hanging mercury drop electrode and a mercury meniscus modified silver solid amalgam electrode were used as electroanalytical sensors for voltammetric determination of anti-neoplastic drugs carmustine, lomustine and streptozotocin containing reducible N-nitroso groups. On the example of carmustine it was shown that its one-step reduction proceeds at substantially more negative potentials at amalgam electrode as compared with mercury electrode. Both electrodes offer satisfactory repeatability of current response (relative standard deviations < 5%) using DC voltammetry and differential pulse voltammetry. The achieved limits of determination lie mostly in the 10^{-7} mol l⁻¹ concentration range. The mentioned voltammetric methods were applied to determination of carmustine and lomustine in pharmaceutical formulations. Further, the mercury meniscus modified silver solid amalgam electrode was employed in a "wall-jet" amperometric detection cell in the determination of carmustine by flow injection analysis. Under optimized conditions (run electrolyte Britton-Robinson buffer of pH 7.0; flow rate 5.5 ml min⁻¹; detection potential -1.5 V; injection volume 0.02 ml) the limit of quantitation 7.1×10^{-6} mol l⁻¹ was achieved.

Keywords: Carmustine; Streptozotocin; Lomustine; Hanging mercury drop electrode; Silver solid amalgam electrode; Nitroso compounds; Voltammetry; Amperometry; Electrochemistry.

Streptozotocin (STZ, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose), carmustine (BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea) and lomustine (CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; structures in Fig. 1) are antineoplastic chemotherapy drugs containing N-nitroso group, classi-

fied as alkylating agents^{1,2}. The alkylation of DNA or RNA of tumor cells causes their inability to divide and leads to their death.

STZ is an glucosamine-nitrosourea compound, originally identified in the late 1950s as an antibiotic³. It is of natural origin, the drug was first isolated from a strain of the soil microbe *Streptomyces achromogenes*⁴. STZ is similar enough to glucose to be transported into the insulin-producing beta cells of the pancreas in mammals by the glucose transport protein GLUT2^{5,6}. Therefore, STZ is used for treatment of certain cancers of pancreatic islets cells. The selective cytotoxicity to pancreatic beta cells inducing insufficient insulin production in mammals suggests the use of the drug as an animal model of diabetes mellitus⁷.

BCNU and CCNU, contrary to STZ, are synthetic drugs prepared by nitrosation of *N,N*-bis(2-chlorethyl)urea and pentachlorophenyl *N*-(2-chlorethyl)-*N*-nitrosocarbamate^{8,9}, respectively. As the other nitrosoureas, unlike most chemotherapeutics, BCNU and CCNU have the unique ability to cross the blood-brain barrier. Therefore, they are useful in treatment of brain tumors. Other cancers treated with BCNU and CCNU include multiple myeloma, Hodgkin's disease, non-Hodgkin's lymphomas, lung cancer and colon cancer^{2,10}.

The extended use of STZ, BCNU and CCNU for medicine purposes initiated further studies on their toxicity: According to the International Agency for Research on Cancer (IARC) classification, BCNU and CCNU are probably carcinogenic to humans (group 2A) and STZ is possibly carcinogenic to humans (group 2B)¹¹.

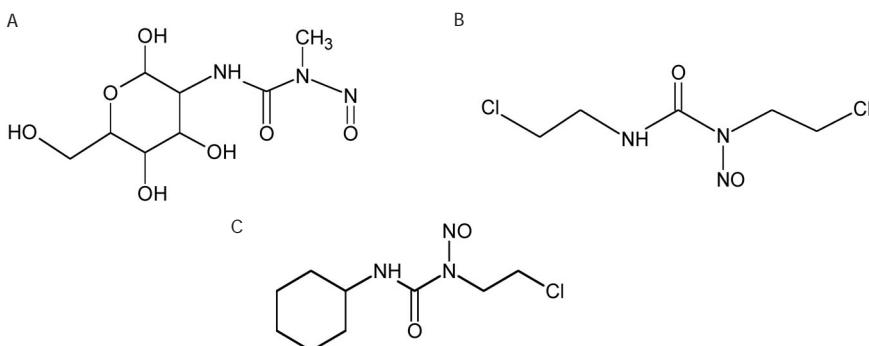


FIG. 1
Structural formulas of STZ (A), BCNU (B) and CCNU (C)

Other efforts are devoted to the development of sensitive analytical methods for determination of trace amounts of nitrosoureas in different biological and environmental matrices and to stability and pharmacological studies. Chromatographic methods including TLC^{12,13}, spectrophotometry¹⁴ and classical polarography¹⁵ were used for investigation of the STZ stability in pharmaceutical preparations. Cojocel et al.¹⁶ used polarography to evaluate the carcinogenic potential of STZ.

Polarographic methods were also used for determination of BCNU and CCNU and structurally related nitrosoureas in pure form, capsules, blood¹⁷, plasma¹⁸, and for pharmacokinetic studies¹⁹. Stability studies of BCNU in aqueous^{13,20}, mixed-solvent and nonaqueous media²¹ were performed by colorimetry after its conversion to an azo compound or by reversed-phase HPLC with UV detection²². This method has been employed also for the determination of BCNU in plasma²³⁻²⁵ or CCNU in a mouse tumor tissue²⁶. Other methods previously used in analysis of plasma or brain tissue containing BCNU or CCNU include gas chromatography with thermoionic N-P detection²⁷, electron capture detection²⁸, or mass spectrometric detection²⁹. Attention was paid also to the analysis of sewage plant effluents, as cytotoxic drugs may appear in clinical waste waters³⁰.

A few previously given examples on the use of polarographic methods show the potential of electroanalysis for sensitive determination of N-nitroso drugs. Besides the mercury electrodes, materials based on solid or paste amalgams are of increasing importance especially for reducible analytes. Solid amalgam electrodes (SAE) were re-introduced in electrochemistry in the year 2000 (refs^{31,32}). They are electrochemically the most similar alternative to mercury electrodes due to a comparable cathodic potential window and relatively high sensitivity. Additional benefits of SAE include generally respected non-toxicity of amalgam materials and mechanical robustness that allows their use in liquid flow systems. Among all the metals forming amalgams, silver amalgams seems to perform best for analytes missing specific interactions (e.g., complexation with metal cations) with metals of the amalgam^{33,34}. Silver solid amalgam electrodes (AgSAE) modified by mercury meniscus (m-AgSAE) were shown to be the most convenient alternative to mercury electrodes regarding sensitivity, limits of determination and repeatability for a number of organic analytes, mostly possessing the reducible nitro group (see reviews^{35,36}). The aim of this study was to test for the first time the suitability of m-AgSAE for organic analytes with reducible N-nitroso group and compare the performance of a m-AgSAE with a hanging mercury drop electrode (HMDE). Thus, in the presented study, the analytical applicability of both electrodes is

demonstrated on batch voltammetric analysis of STZ, BCNU and CCNU using differential pulse voltammetry (DPV) and direct current voltammetry (DCV). The developed voltammetric methods are further modified for quantitation of BCNU and CCNU in pharmaceutical formulations. Moreover, the possibility of combination of flow injection analysis (FIA) with amperometric detection at m-AgSAE in the "wall-jet" arrangement for BCNU determination is presented.

EXPERIMENTAL

Instrumentation

Voltammetric measurements were carried out using a computer-driven EcoTribo polarograph with PolarPro software version 4.0 in combination with a HMDE of the UΜμΕ type (all Eco-Trend Plus, Prague, Czech Republic) or m-AgSAE, a platinum-wire auxiliary electrode and a Ag|AgCl reference electrode (1 M KCl), to which all the potential values are referred. For DPV and DCV at HMDE, the maximum drop size obtained by opening the valve for 100 ms, with a surface of 0.759 mm² and a polarization rate of 20 mV s⁻¹ was used. For voltammetry, the m-AgSAE with the disc diameter 0.55 mm was used. The electrode consisted of a drawn-out glass tube, the bore of which near the tip was filled with a fine silver powder, amalgamated by liquid mercury and connected to electric contact³³. Afterwards, the electrode was immersed into a small volume of liquid mercury and agitated for 15 s. The modulation amplitude in DPV at HMDE and m-AgSAE of -50 mV with pulse duration of 80 ms was used.

For FIA-ED the system consisted of a high-pressure pump L-2130 HTA (Hitachi, Japan) governed by the EZChrom Elite software (Agilent Technologies, USA) working in Windows XP (Microsoft Corporation, USA). Manual injection of samples degassed by passing nitrogen (purity 4.0, Linde, Prague, Czech Republic) using 20 µl Rheodyne (IDEX Health & Science, Rohnert Park, USA) injection valve was used. The run electrolyte was degassed by sonication using PS 02000A ultrasonic bath (Powersonic, USA) followed by passing nitrogen continuously for the whole measurement period. The measurements were carried out at laboratory temperature. Electrochemical detector in "wall-jet" geometry with a three electrode system described earlier³⁵ was used. The working electrode was m-AgSAE with a disc diameter of 2.47 mm. The exact volume of mercury (0.4 µl if not stated otherwise) was applied by Hamilton syringe to form the meniscus of m-AgSAE. Further Ag|AgCl (3 M KCl) reference and platinum-wire auxiliary electrodes (both Monokrystaly, Turnov, Czech Republic) were used. The electrode surface-capillary outlet distance was kept at 0.5 mm if not stated otherwise. The electrode system was driven by ADLC 1 (Laboratorní přístroje, Prague, Czech Republic) potentiostat.

UV/Vis spectra were measured in quartz cuvettes (Hellma, Müllheim, Germany) using a diode array spectrophotometer HP 8453 (Hewlett Packard, Netherlands). pH values refer to those of the buffer measured using a Conductivity & pH meter Jenway 4330 (Jenway, Dunmow, Essex, UK) with a combined glass electrode.

Materials

The stock solutions of STZ and BCNU ($c = 1 \times 10^{-3}$ mol l⁻¹) were prepared by dissolution of the pure substance (Sigma-Aldrich, >98%) in deionized water or ethanol. The stability of these solutions is discussed below. The stock solution of CCNU ($c = 1 \times 10^{-3}$ mol l⁻¹) was prepared by dissolution of the pure substance (Sigma-Aldrich, 99%) in methanol. All stock solutions were stored in the dark. Britton-Robinson (BR) buffers were prepared in a usual way, i.e. by mixing a solution of 0.04 mol l⁻¹ in phosphoric acid, 0.04 mol l⁻¹ in acetic acid and 0.04 mol l⁻¹ in boric acid with an appropriate amount of 0.2 M sodium hydroxide solution (all chemicals were from Lachema, Brno, Czech Republic). Methanol (Lachema, Brno, Czech Republic) and ethanol (Merck, Czech Republic) of analytical grade purity were used. Deionized water was produced by the Milli-Q_{plus} system (Millipore, USA). The analyzed pharmaceutical preparations were lyophilized powder BiCNU® Carmustinum with the declared BCNU content 100 mg and capsules CeeNU® Lomustine with the declared CCNU content 40 mg (both Bristol-Myers Squibb, Sermoneta, Italy). All solutions were kept in glass vessels, because the studied drugs adsorb at plastic materials²².

Procedures

The general procedure to obtain voltammograms was as follows: An appropriate amount of the stock solution of the tested substance in deionized water (STZ and BCNU) or methanol (CCNU) was placed in a 10 ml volumetric flask, and the system was diluted to volume with a buffer of the required pH. Oxygen was removed from the measured solutions by purging with nitrogen for 5 min.

Before starting the work every day, the electrochemical activation of m-AgSAE was carried out in 0.2 M KCl at -2200 mV under stirring for 300 s. The regeneration of m-AgSAE taking ca. 30 s preceded every voltammetric scan; this included the application of 300 polarizing cycles, consisting of switching the working potential from E_1 to E_2 for 50 ms. E_1 was selected ca. 50–100 mV more negative than the potential of the anodic dissolution of the electrode material, E_2 was selected about 50–100 mV more positive than the potential of the hydrogen evolution in the given base electrolyte. The m-AgSAE used in FIA experiments was activated similarly as in voltammetric measurements in 0.2 M KCl once a week or when a new mercury meniscus was formed. No other electrochemical pretreatment was performed prior to injection.

The quantitation of BCNU in 100 mg of lyophilized powder BiCNU® Carmustinum by DPV and DCV at HMDE was performed as follows: ca. 50 mg of the powder was accurately weighed and dissolved in 18.5 ml of ethanol. 1 ml of this solution was further diluted with 9 ml of ethanol. 100 μ l of this solution was transferred into 10 ml volumetric flask and made up to the mark with BR buffer (pH 2.2). The solution was transferred into the voltammetric cell, deoxygenated with nitrogen for 5 min, and DP and DC voltammograms were recorded. The exact concentration of BCNU was determined using two standard additions of 12 μ l of the BCNU standard stock solution ($c = 1 \times 10^{-2}$ mol l⁻¹) in ethanol, and plotting the resulting analytical curve.

For quantitation of CCNU in capsules CeeNU® Lomustine 40 mg by DPV and DCV at m-AgSAE, the content of the capsule was dissolved in 100 ml of methanol. After 3 min of sonication, 100 μ l of this solution was transferred into 10-ml volumetric flask, 900 μ l of methanol was added and the flask was made up to the mark with 0.05 M acetate buffer (pH 4.0). The solution was transferred into the voltammetric cell and deoxygenated with nitro-

gen for 5 min. DP and DC voltammograms were recorded for this sample using regeneration potentials $E_1 = -250$ mV and $E_2 = -1400$ mV prior to every scan. The exact concentration of CCNU was determined using one standard addition of 171 μl of the CCNU standard stock solution ($c = 1 \times 10^{-3}$ mol l⁻¹) in methanol, and plotting the resulting analytical curve. Four parallel determinations were performed for analysis of both pharmaceutical formulations.

All voltammetric and amperometric curves were recorded in triplicate. The heights of the peaks was evaluated from the straight line connecting the minima before and after the peak, if not stated otherwise. The parameters of calibration curves were calculated using statistic software OriginPro 6.0 (OriginLab Corporation, USA). The significance of the intercepts of linear calibration dependences was tested by statistic software ADSTAT³⁷, the non-significant intercepts are omitted in Tables II and IV summarizing the parameters of calibration dependences. The limit of quantitation (L_Q) and the limit of detection (L_D) for voltammetric and amperometric methods were calculated using the standard deviation of the mean of the peak heights obtained by ten consecutive determinations of the lowest measurable concentration (s_c) and the slope b of the analytical curve, related by the equations: $L_Q = 10s_c/b$ and $L_D = 3s_c/b$ (ref.³⁸), respectively. The paired two-sample t-test for equal means was calculated as in ref.³⁹. All statistical data were calculated for the level of significance $\alpha = 0.05$.

RESULTS AND DISCUSSION

Spectrophotometric Studies on STZ, BCNU and CCNU Stability

N-nitroso compounds are not stable in aqueous solutions^{18,22}. The stabilities of the stock solutions of STZ, BCNU ($c = 1 \times 10^{-3}$ mol l⁻¹) and CCNU ($c = 2 \times 10^{-4}$ mol l⁻¹) in water were monitored for 30 days by measuring the absorbance at $\lambda = 230$ nm, where the absorption maximum of the N-nitroso chromophore appears⁴⁰. While CCNU degrades very fast, for STZ the absorbance decreases by ca. 20% during 30 days and the same 20% drop can be observed only after 3 days for BCNU. The instability of aqueous solutions was confirmed earlier by spectrophotometric and polarographic measurements, with a reported maximum stability of the solutions at pH 4.0 for STZ¹⁵ or 2.6–5.0 for CCNU⁴¹. As the pH of the stock solution of STZ itself is 4.5, no other pH regulation of the stock solution was carried out. The stock solution of CCNU in methanol was used instead of that in deionized water. Its stability is characterized by a 10% decrease of absorbance at $\lambda = 230$ nm within 4 days. The reports on stability of BCNU in aqueous solution slightly differ, a highest stability was reported in citrate buffer of pH 3.3–4.8 (ref.²²) or 5.2–5.5 (ref.¹¹). A fresh stock solution of BCNU (pH 5.3) in water was prepared at the beginning of every working day. The stock solution of BCNU in ethanol is stable ($c = 1 \times 10^{-3}$ mol l⁻¹) for at least 30 days judging from constant absorbance at $\lambda = 230$ nm.

Voltammetric Determination of STZ and BCNU at HMDE

The choice of proper pH for analysis of N-nitroso drugs is of high importance due to their instability in aqueous media. The influence of pH on the DC and DP voltammograms of STZ and BCNU was investigated at HMDE in BR buffer in the pH range 2.0–12.0; the obtained curves are depicted in Fig. 2.

For STZ, the voltammograms exhibit one well-developed irreversible peak in the whole investigated pH range in contrast to polarographic studies performed in our group previously, where a second indistinct signal at ca. -1.55 V was recognizable in the pH range 6.0–8.0 (ref.⁴²). Nevertheless, this second peak already appeared in the region of solution instability and it is not suitable for analytical purposes. The main peak presumably corre-

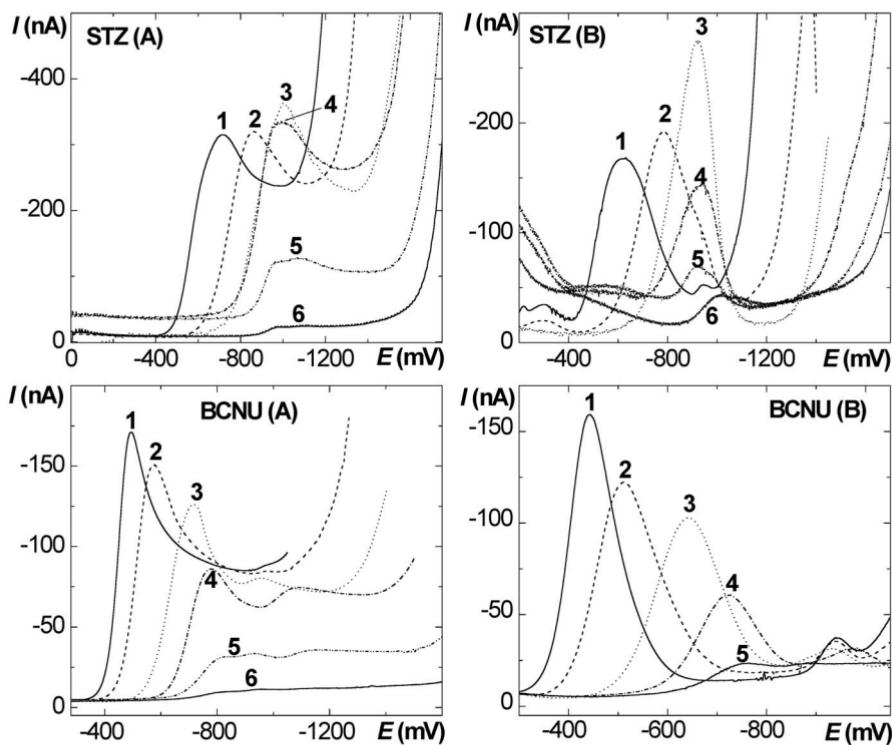


FIG. 2

DC (A) and DP voltammograms (B) measured at HMDE for STZ ($c = 1 \times 10^{-4}$ mol l⁻¹) in BR buffer at pH 2.0 (1), 4.0 (2), 6.0 (3), 7.0 (4), 8.0 (5), 10.0 (6), and for BCNU ($c = 1 \times 10^{-4}$ mol l⁻¹) at pH 2.2 (1), 3.0 (2), 5.0 (3), 7.0 (4), 9.0 (5), 10.0 (6)

sponds to the two-electron reduction of N-nitroso group to corresponding hydroxylamine according to Eq. (1).



Its peak potential (E_p) for DCV varies with pH in the range of 2.0–6.0 according to the relationship E_p (V) = $-0.062 \text{ pH} - 0.607$ ($R = -0.9859$). This shift is in concordance with the fact that the reduction described by Eq. (1) requires proton transfer prior to the rate-determining electron transfer. At higher pH values, the shift of E_p to more negative potentials with increasing pH is less pronounced and the peak height decreases substantially. No signal appears above pH 10.0. This is presumably due to increasing instability of STZ with increasing pH. The present hydroxide anion catalyses the decomposition of STZ to diazomethane, so that only the carboxylyzed saccharide skeleton remains¹⁵.

Similarly, DC and DP voltammograms of BCNU (Fig. 2) at HMDE exhibit one well-developed irreversible peak of decreasing height in the pH range 2.0–10.0 presumably corresponding to reduction described by Eq. (1). Its peak potential (E_p) shifts significantly in the pH range 2.0–4.0 according to the relationship (for DCV) E_p (V) = $-0.088 \text{ pH} - 0.312$ ($R = -0.9968$). At higher pH values (5.0–9.0), the shift is less pronounced, E_p (V) = $-0.030 \text{ pH} - 0.562$ ($R = -0.9992$), and no signal can be observed above pH 10.0. The intersection of these dependences at pH 4.3 could correspond to the pK value of protonation of the hydroxylamino derivative at lower pH values, as presumed for other nitroso compounds⁴³. The second, indistinct peak observable at the voltammograms at higher pH values (Fig. 2) is not suited for analytical purposes, similarly as mentioned for polarographic methods¹⁸.

The optimum conditions for determination of STZ (BR buffer pH 6.0) and BCNU (BR buffer pH 2.2) were estimated based on the height and shape of recorded DC and DP voltammetric peaks. As these pH values lie about one pH unit outside the reported optimum range (see above on STZ and BCNU stability), the repeatability of the peak heights obtained by these methods for the concentrated solutions ($c(\text{STZ or BCNU}) = 1 \times 10^{-4} \text{ mol l}^{-1}$) and solutions of L_Q -close concentrations ($c(\text{STZ}) = 4 \times 10^{-7} \text{ mol l}^{-1}$, $c(\text{BCNU}) = 6 \times 10^{-7} \text{ mol l}^{-1}$) was tested. No statistically significant peak decrease was observed when ten scans were repeated within three hours. The relative standard deviations (s_r) calculated from ten consecutive measurements are summarized in Table I. They are lower than 4.9% even for the L_Q -close concentrations.

Calibration curves measured under optimized conditions are linear over three concentration ranges, i.e. from 1×10^{-4} to ca. 10^{-7} mol l⁻¹, their parameters are summarized in Table II. It follows from that table that slightly lower L_Q s were achieved for DPV than for DCV.

Voltammetric Determination of BCNU and CCNU at m-AgSAE

The pH dependence of the signal of BCNU measured at m-AgSAE revealed one-step reduction exhibiting decreasing peak heights with increasing pH of the supporting electrolyte similarly to HMDE. It follows from comparison of Fig. 2 and Fig. 3 (voltammograms recorded at HMDE and m-AgSAE, respectively) that BCNU is reduced at far more negative potentials at m-AgSAE than at HMDE, i.e., the electron transfer at m-AgSAE requires a higher energy in comparison with the ideally smooth surface of HMDE. This difference – ca. 400 mV – is most pronounced in the pH range 2.0–6.0. In alkaline media the difference decreases to ca. 250 mV, because no E_p shift is observed at m-AgSAE for pH values higher than pH 6.0. For the pH range 2.0–6.0, the E_p shift can be described by the equation (for DCV) E_p (V) = -0.052 pH – 0.819 ($R = -0.9744$). Such potential difference for HMDE and m-AgSAE is sporadic, typical differences for other organic compounds were 0–100 mV (refs^{44–46}). Cyclic voltammograms confirmed irre-

TABLE I

Repeatability of STZ, BCNU and CCNU current response obtained by DCV and DPV at HMDE or m-AgSAE calculated as relative standard deviations (s_r) of peak heights for ten consecutive measurements

Analyte	HMDE				m-AgSAE				
	DCV	DPV	DCV	DPV	DCV	DPV	DCV	DPV	
BCNU	c , $\mu\text{mol l}^{-1}$	100 ^a	100 ^a	0.6 ^a	0.6 ^a	100 ^b	100 ^b	4 ^b	4 ^b
	s_r , %	0.5	0.6	3.3	1.9	2.3	1.9	1.5	1.4
STZ	c , $\mu\text{mol l}^{-1}$	100 ^c	100 ^c	0.4 ^c	0.4 ^c	not investigated			
	s_r , %	1.2	2.8	3.1	4.9				
CCNU	c , $\mu\text{mol l}^{-1}$	not investigated				100 ^d	100 ^d	2 ^d	2 ^d
	s_r , %					2.3	1.1	7.1	6.9

^a BR buffer pH 2.2. ^b BR buffer pH 7.0. ^c BR buffer pH 6.0. ^d 0.05 M acetate buffer pH 4.0–methanol (9:1).

versibility of the electroreduction of BCNU at both electrodes; the observed linear dependences of peak currents on the square root of the scan rate confirm its diffusion control. A more detailed explanation of the differences of electrochemical behaviour of BCNU at HMDE and m-AgSAE would require a more extensive mechanistic study.

CCNU features similar reduction potentials as BCNU at m-AgSAE and peak current maxima at pH 6.0 as follows from DP and DC voltammograms (Fig. 3).

Calibration curves were measured in BR buffer pH 7.0 for BCNU and the mixture of methanol-0.05 M acetate buffer pH 4.0 (1:9) for CCNU. This decision was based on regular shape and easy evaluation of the voltammograms due to a sufficient difference between the peak potential and the onset of the base electrolyte decomposition. The obtained parameters and L_Q s mostly in the 10^{-6} mol l⁻¹ concentration range are summarized in Table II. A lower sensitivity by an order of magnitude and higher L_Q s were obtained for BCNU using DPV at m-AgSAE in comparison with HMDE; this was frequently reported also for other organic compounds⁴⁴⁻⁴⁶. An excellent repeatability of 1.37% (s_r) was obtained for the lowest attainable concentration of BCNU (4×10^{-6} mol l⁻¹), which resulted in L_Q in the 10^{-7} mol l⁻¹ concentration range.

TABLE II
Optimum conditions and parameters of the calibration straight lines for the voltammetric determination of STZ and BCNU at HMDE, and BCNU and CCNU at m-AgSAE

Analyte	Optimum conditions	Method	Linear dynamic range, $\mu\text{mol l}^{-1}$	Slope mA mol ⁻¹ l	L_D^a $\mu\text{mol l}^{-1}$	L_Q^b $\mu\text{mol l}^{-1}$
HMDE						
STZ	BR buffer, pH 6.0	DCV	0.2-100	2.13	0.11	0.38
		DPV	0.2-100	2.85	0.07	0.24
BCNU	BR buffer, pH 2.2	DCV	0.2-100	1.79	0.11	0.36
		DPV	0.2-100	2.36	0.19	0.62
m-AgSAE						
BCNU	BR buffer, pH 7.0	DCV	2-100	0.39	0.25	0.83
		DPV	2-100	0.28	0.21	0.71
CCNU	0.05 M acetate buffer pH 4.0-methanol (9:1)	DCV	2-100	0.24	0.93	3.1
		DPV	2-100	0.30	0.66	2.2

^a Limit of detection. ^b Limit of determination.

It can be concluded that mercury-based electrodes proved satisfactory regarding repeatability, sensitivity and the achieved submicromolar detection limits for batch voltammetric methods.

Analysis of Pharmaceutical Formulations Containing CCNU and BCNU

The developed electroanalytical methodologies were modified for quantitation of BCNU and CCNU in pharmaceutical preparations. While the BiCNU® Carmustinum 100 mg powder contains 100% of BCNU according

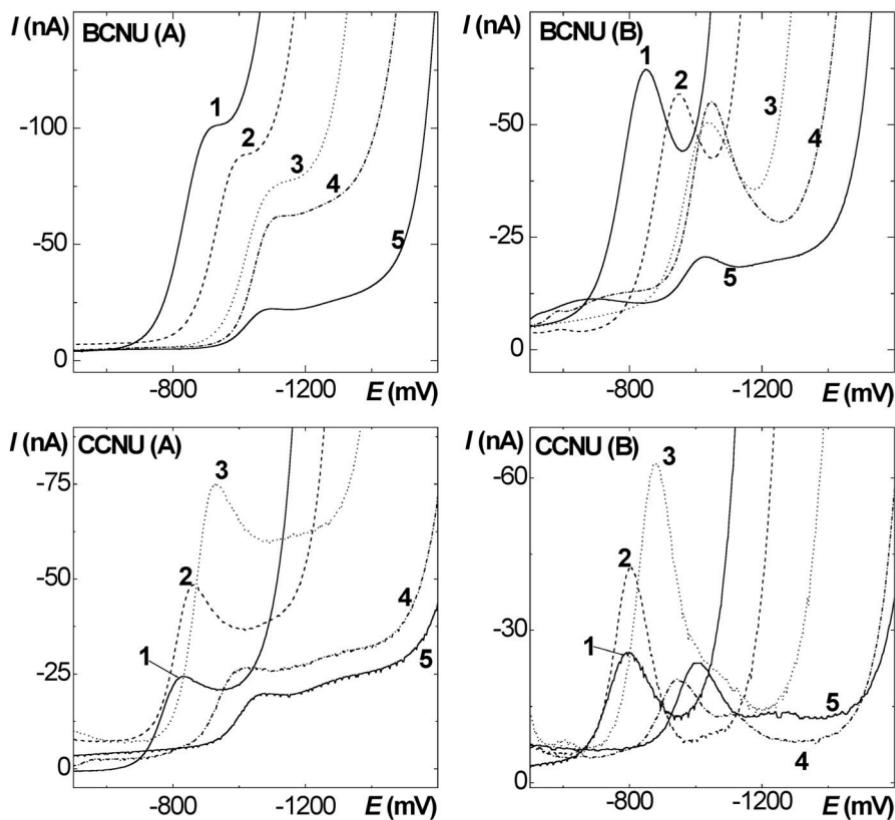


FIG. 3

DC (A) and DP voltammograms (B) of BCNU ($c = 1 \times 10^{-4}$ mol l⁻¹) measured at m-AgSAE in BR buffer at pH 2.2 (1), 3.0 (2), 5.0 (3), 7.0 (4), 9.0 (5), and of CCNU ($c = 1 \times 10^{-4}$ mol l⁻¹) measured at m-AgSAE in a mixture of methanol-BR buffer (1:9) at pH 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4), 10.0 (5)

to the manufacturer and, therefore, the matrix effects are not important, the excipients present in CeeNU® Lomustine 40 mg capsules may complicate analysis, especially when using solid electrodes. To test this, m-AgSAE was chosen for DP and DC voltammetric determination of CCNU in the mentioned capsules. BiCNU® Carmustinum powder was analyzed using the same methods at HMDE. Standard addition methods described in Procedures were developed for these purposes.

The obtained mean values of BCNU and CCNU contents in the analyzed dosage forms and the statistical evaluation are summarized in Table III. The mean value for the percentage content referred to the value declared by manufacturer is in all cases higher than 97%. The accuracy of the DPV methods is higher than that of DCV, especially for m-AgSAE as follows from relative standard deviations s_r . Both methods for a particular analyte afford equal mean values (i.e., percentage content) as confirmed by the paired two-sample t-test for equal means. This test can be applied when the two sets of data are dependent because there is a natural pairing of observations in the samples, such as in our case, when one sample is analyzed using two different methods (e.g., DPV and DCV). No interferences of excipients were observed in the analysis of capsules CeeNU® Lomustine. It can be concluded that the developed voltammetric methods offer a suitable alternative to the methods prescribed in the Czech Pharmacopoeia⁴⁷: UV spectrophotometry at $\lambda = 230$ nm prescribed for BCNU and time-consuming argentometric titration after alkaline hydrolysis for CCNU.

TABLE III

Mean values m_A , standard deviations s , relative standard deviations s_r , limits of confidence $L_{1,2}$ ($\alpha = 0.05$) of percentage content of BCNU in the powder BiCNU® Carmustinum 100 mg and CCNU in the capsules CeeNU® Lomustine 40 mg, analyzed by DPV and DCV at HMDE (BCNU) and m-AgSAE (CCNU)

Analyte/Electrode	Method	m_A , %	s , %	s_r , %	$L_{1,2}$, %
BCNU/HMDE	DCV	98.8	2.3	2.3	3.7
	DPV	100.8	1.8	1.8	2.9
CCNU/m-AgSAE	DCV	97.4	9.8	10	15.5
	DPV	97.2	3.2	3.3	5.4

Amperometric Determination of BCNU Using Detectors Based AgSAE

An amperometric detector with large-area m-AgSAE as an indicator electrode was tested for FIA-ED determination of BCNU. BR buffer pH 7.0 was used as a run electrolyte. It was selected based on hydrodynamic voltammograms (HDVs) measured in BR buffer pH 2.0–10.0 (Fig. 4). The shape of these HDVs respects partly the shapes of DC voltammograms as described above, i.e. the potential of the maximum peak height shifts to more negative potentials with increasing pH. The subsequent decrease of the peak heights after this height maximum is caused by an increase of the background current due to the decomposition of the run buffer. The noise in all of these experiments was comparable and about 100 nA. The maximum signal/noise (*S/N*) ratio was obtained in BR buffer pH 7.0 at the detection potential $E_{\text{det}} = -1.5$ V. This buffer was considered previously as the optimal medium for DP and DC voltammetric determinations. The optimum flow rate F_m was set at 5.5 ml min⁻¹, at lower F_m the peaks were more tailing because of the increased time of the presence of the analyzed zone close to the electrode surface.

The other two optimized parameters impact more the detector design. The electrode surface–capillary outlet distance was varied from 0.5 to 2.5 mm. Both the peak height and area are constant up to the 2 mm distance; at higher values, a steep drop of both was observed. To prevent this, the mini-

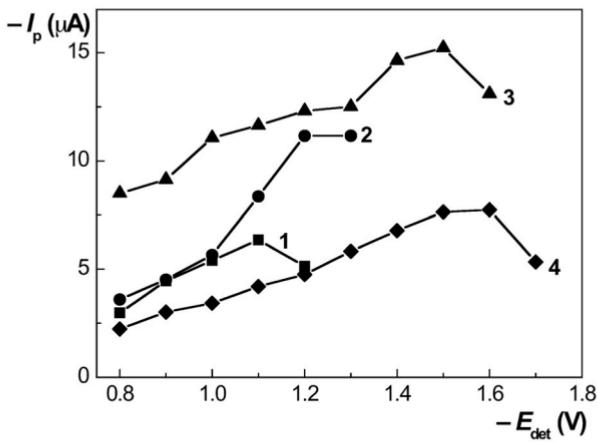


FIG. 4

The dependence of peak heights I_p of BCNU ($c = 1 \times 10^{-4}$ mol l⁻¹) on applied detection potential E_{det} at m-AgSAE in "wall-jet" arrangement in FIA-ED. Run electrolyte BR buffer at pH 2.0 (1), 5.0 (2), 7.0 (3), 10.0 (4). Flow rate 1 ml min⁻¹, injected volume 20 μ l

mum distance 0.5 mm was kept. Further, the m-AgSAE signal is affected by the mercury volume (V_{Hg}) forming the meniscus. Although, the peak height and area increase when increasing V_{Hg} from 0 μl (polished AgSAE) to 2 μl and the meniscus is stable up to F_m of 10 ml min^{-1} , the meniscus formed by higher volumes could be inadvertently diminished or removed during manipulation with the detector or electrode itself. Therefore, 0.4 μl of Hg was used for the meniscus in further measurements.

The repeatability of the measurement was tested using the injection frequency one sample per 30 s. For ten repeated injections of BCNU ($c = 1 \times 10^{-4} \text{ mol l}^{-1}$), no statistically significant changes of the peak heights (s_r 1.86%) and peak area (s_r 2.34%) were observed even without any m-AgSAE pretreatment. Calibration dependences are linear in the range from 6×10^{-6} to $1 \times 10^{-4} \text{ mol l}^{-1}$; their parameters are summarized in Table IV and the corresponding records of FIA-ED signals are depicted in Fig. 5. A lower L_Q of $7.1 \times 10^{-6} \text{ mol l}^{-1}$ was achieved using the evaluation from peak heights instead of peak areas due to better repeatability of the former at the lowest attainable concentration of $6 \times 10^{-6} \text{ mol l}^{-1}$ as obvious from Table IV. This L_Q is comparable with that using DCV and DPV at m-AgSAE.

To conclude, it was presented in this study that HMDE and m-AgSAE prove satisfactory in voltammetric determination of antineoplastic drugs BCNU, CCNU and STZ containing reducible N-nitroso groups. On the example of BCNU it was shown that its one-step reduction proceeds at substantially more negative potentials at amalgam electrode as compared with mercury electrode. Both electrodes offer satisfactory repeatability of current response (relative standard deviations $< 5\%$) using DCV and DPV. The

TABLE IV
Parameters of the calibration straight lines and repeatability for the FIA-ED determination of BCNU using m-AgSAE. For conditions see Fig. 5

Linear dynamic range $\mu\text{mol l}^{-1}$	Slope ^a	Correlation coefficient	L_D $\mu\text{mol l}^{-1}$	L_Q $\mu\text{mol l}^{-1}$	s_r^b %
Evaluated from peak heights					
6–100	6.96×10^4	0.9996	2.2	7.1	9.3
Evaluated from peak areas					
6–100	5.61×10^4	0.9992	3.2	11	15

^a For peak heights (in $\mu\text{A mol}^{-1} \text{l}$), for peak areas (in $\mu\text{A s mol}^{-1} \text{l}$). ^b For $c = 6 \times 10^{-6} \text{ mol l}^{-1}$.

achieved limits of determination lie mostly in the 10^{-7} mol l⁻¹ concentration range, which is sufficient for a number of applications including the in this study suggested voltammetric determination of BCNU and CCNU in pharmaceutical formulations. Further, a m-AgSAE was employed in a "wall-jet" amperometric detection cell in the determination of BCNU by flow injection analysis. Under optimized conditions (run electrolyte BR buffer of pH 7.0, flow rate 5.5 ml min⁻¹, detection potential -1.5 V, injection volume 0.02 ml) the limit of quantitation 7.1×10^{-6} mol l⁻¹ was achieved. The suitability of the m-AgSAE for the determination of tested N-nitroso drugs in biological matrices is under consideration, as the influence of interferences, especially the surface active compounds, on the electrochemical response of the solid surface of m-AgSAE, has to be investigated firstly.

In general, it can be concluded that the amalgam-based electrodes investigated during last ten years as a non-toxic alternative to mercury electrodes strengthen their position for reducible analytes, among others electroanalytical sensors, because, despite all efforts, no other electrode material can offer a potential window and inertness to oxygen adsorption at the surface comparable with mercury. Advantageous is also the robustness and mechanical stability of amalgam electrodes which substantiate their use in

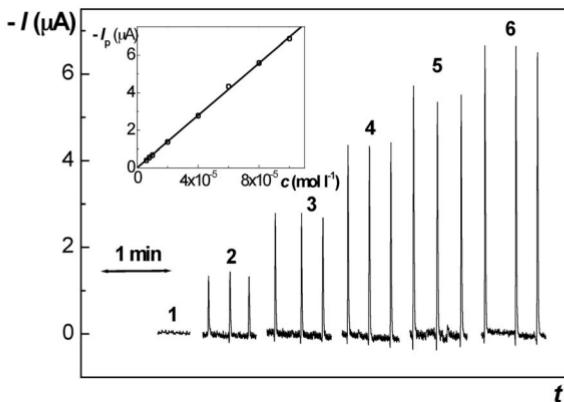


FIG. 5

The record of FIA peaks in the determination of BCNU with amperometric detection at m-AgSAE modified by 0.4 μl of Hg; $c(\text{BCNU})$: 0 (1), 2×10^{-5} (2), 4×10^{-5} (3), 6×10^{-5} (4), 8×10^{-5} (5), and 1×10^{-4} (6) mol l⁻¹. Inset is the calibration dependence evaluated from peak heights I_p . Run electrolyte BR buffer of pH 7.0, detection potential -1.5 V, flow rate 5.5 ml min⁻¹, injected volume 20 μl , capillary outlet-electrode distance 0.5 mm

liquid flow systems. The usefulness of mercury and mercury-based electrodes in analytical chemistry demonstrated also in this study provokes questions and disapproval to the fears of liquid mercury even in minimal amounts inciting to proposals of ban in the European Union, USA and other countries.

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