SPECTROPHOTOMETRIC AND POLAROGRAPHIC STUDY OF THE EFFICIENCY OF THE CHEMICAL DESTRUCTION OF SOME ANTINEOPLASTIC PHARMACEUTICALS

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The efficiency of the chemical destruction of some antineoplastic pharmaceuticals was studied using visible and ultraviolet spectrophotometry and differential pulse polarography. Doxorubicin, Daunorubicin, Vincristine, Vinblastine, Methotrexate and Dichloromethotrexate are destroyed best by oxidation with potassium permanganate, while some derivatives of N-nitrosourea (Lomustine, Chlorozotocin) are best destroyed by denitrosation with hydrobromic acid. The efficiency of the decomposition can readily be studied by spectrophotometry or differential pulse polarography.

There has recently been a great increase in the laboratories studying the properties and applications of a number of antitumor drugs. The nature of their action makes a number of these substances suspect of chemical carcinogenity. Work with these substances necessarily leads to contamination of the laboratory and the formation of laboratory waste containing these potentially harmful substances. Health safety regulations thus require that efficient methods be developed for destruction of these substances and for decontamination of the laboratory, laboratory equipment and laboratory waste. These methods must be efficient, simple, fast and cheap and must be combined with a suitable analytical method for controlling the chemical efficiency of the decontamination. The development of efficient methods for destruction of various types of cytostatics is required by the appropriate laws and regulations[1] defining the conditions acceptable for work with these toxic substances.

Substances that can be successfully used for treating various types of cancer include Doxorubicin and Daunorubicin, that can be considered as derivatives of 9,10-anthraquinone, Vincristine and Vinblastine, which are alkaloids containing an indole ring, and Methotrexate and Dichloromethotrexate, which are derivatives of L-glutamic acid with a pteridine ring as a substituent. Derivatives of N-nitrosourea, i.e. Lomustine (N-2-(chloroethyl)-N′-cyclohexyl-N-nitrosourea), Carmustine (N,N′-bis(2-chloroethyl)-N-nitrosourea), Semustine (N-(2-chloroethyl)-N′-(4-methylcyclohexyl)-N-nitrosourea), Chlorozotocin (1-(2-chloroethyl-1-nitroso-3-(D-glucoso-2-yl)-urea), and PCNU (N-(2-chloroethyl)-N′-(2,6-dioxo-3-piperidynyl)-N-nitrosourea)

form another large group of cytostatics. The structural formulae, systematic names and a survey of the physico-chemical and biological properties and methods for the determination of all these substances can be found in the monograph[2]. All these substances are strongly mutagenic and most have been shown to be capable of producing carcinogenic growths in various types of organisms; some are suspect of carcinogenic activity in humans. The International Agency for Research on Cancer in Lyon has thus recommended that all these substances be considered as potential chemical carcinogens and that they be included in a systematic study of methods useful for destruction of chemical carcinogens in laboratory waste and for decontamination of laboratories and equipment[2]. Reversed-phase high-performance liquid chromatography with spectrophotometric detection[2] is recommended for control of the efficiency of this chemical destruction. This method has optimal sensitivity and selectivity but requires complex instrumentation and is rather tedious. Thus, study of the efficiency of chemical destruction of these substances by using visible and UV spectrophotometry and differential pulse polarography, which has been found useful for the determination of Doxorubicin[3], Vincristine and Vinblastine[4], Methotrexate[5], Lomustine[6], Carmustine[7] and other N-nitrosoderivatives of urea[8-10] is described in this work.

The actual decomposition was carried out either using oxidation with potassium permanganate in sulfuric acid medium or by reaction with hydrobromic acid in anhydrous acetic acid medium[2].

EXPERIMENTAL

Apparatus

Polarographic measurements were carried out on a PA 3 polarographic analyzer with an XY 4103 XY-plotter (both from Laboratorní přístroje, Prague). A three-electrode system was employed with a platinum auxiliary electrode and saturated calomel reference electrode. The dropping mercury electrode had a drop time of 2·05 s (in 0·1M-KCl at an applied voltage of 0 V vs a saturated calomel electrode at a mercury reservoir height of $h = 36$ cm) and flow rate of 5·77 mg s^{-1} (also at $h = 36$ cm). A polarization rate of 5 mV s^{-1}, pulse amplitude of 50 mV and electronically controlled drop time of 1 s were employed.

Spectrophotometric measurements were carried out on a Pye Unicam PU 8800 UV/VIS spectrophotometer (Philips) in 1 cm quartz cuvettes.

Reagents

The studied cytostatics were provided by the Unit of Environmental Carcinogens of the International Agency for Research on Cancer in Lyon. All the other chemicals (potassium permanganate, sulfuric acid, anhydrous acetic acid, hydrobromic acid, sodium sulfite, powdered zinc, methanol, ethanol, dichloromethane, dimethylformamide and dimethylsulfoxide) were of p.a. purity (Lachema, Brno).

Procedures

Destruction by potassium permanganate in sulfuric acid medium: A modification of the procedure proposed by Castegnaro, Michelon and Brouet in the monograph[2] was employed for destruction of solid substances.

Decomposition of Doxorubicin, Daunorubicin, Vincristine, Vinblastine, Methotrexate and Dichloromethotrexate was carried out by dissolving 10 mg of the substance in 10 ml of 3M-H_2SO_4, to which 1 g of solid potassium permanganate was added. The mixture formed was stirred for two hours on a magnetic stirrer. For spectrophotometric control of the chemical efficiency of the destruction, 2 g of powdered zinc was added, the mixture was stirred for one hour on a magnetic stirrer and the bleached solution was filtered through an S4 frit and its spectrum was measured in the region of the absorption maximum (see Table I). Then 10, 20, 30, 40, and 50 μl of a solution containing 0·1 mg/ml Daunorubicin, 1 mg/ml Doxorubicin, 10 mg/ml Vincristine or 10 mg/ml Vinblastine were added stepwise and the spectrum was recorded after each addition.

In control of the efficiency of the decomposition by differential pulse polarography, the solution obtained after the destruction procedure was decoloured with the minimal amount of sodium sulfite, deaerated by bubbling with nitrogen and the differential pulse polarogram was recorded in the region of the peak of the substance of interest (see Table I). Then 10, 20, 30, 40, and 50 μl of a solution containing 1 mg/ml Methotrexate or Dichloromethotrexate were added stepwise and the differential pulse polarogram was recorded after each addition.

Because of the solubility of the substances determined, the following procedure was employed for Lomustine, Carmustine, Semustine, Chlorozotocin and PCNU: 12 mg of the substance were dissolved in 1 ml of dimethylformamide and 0·5 g of solid potassium permanganate suspended

TABLE I

The efficiency of the destruction of some cytostatics by potassium permanganate

Substance	Efficiency Control[a]	Efficiency %	Mutagenic activity of the residue[b]
Doxorubicin	VIS (490)	99·6	NEG
Daunorubicin	VIS (490)	99·97	NEG
Vincristine	UV (252)	>98	NEG
Vinblastine	UV (264)	>98	NEG
Methotrexate	DPP (−200)	>99·9	NEG
Dichlormethotrexate	DPP (−210)	99·9	NEG
Lomustine	DPP (−610)	99·6	POS
Carmustine	DPP (−460)	99·9	POS
Semustine	DPP (−540)	99·1	POS
Chlorozotocin	DPP (−630)	99·4	POS
PCNU	DPP (−465)	99·9	POS

[a] VIS, UV-spectrophotometry (wavelength in nm in brackets); DPP differential pulse polarography (peak potential in mV vs saturated calomel electrode in brackets); [b] see ref.[14]; POS (NEG) positive (negative) Ames test for mutagenic activity of the solution after destruction.

in 6 ml of 3M-H_2SO_4 were added. The mixture was stirred for 12 h on a magnetic stirrer and then decoloured by addition of the minimal amount of solid sodium sulfite. The solution was bubbled for 15 min with nitrogen and the differential pulse polarogram was recorded in the region of the peak of the substance of interest (see Table I). Then 12, 24, 36, 48, and 60 μl of a solution containing 1 mg Carmustine or PCNU or 2 mg of Lomustine, Semustine or Chlorozotocin per 1 ml of dimethylformamide were added stepwise and the differential pulse polarogram was recorded after each addition.

Expired date Doxorubicin tablets were destroyed in the following manner[11]: A tablet containing 10 mg of Doxorubicin and 50 mg of glucose was dissolved using ultrasonics in 100 ml of 3M-H_2SO_4, 2·5 g of solid potassium permanganate were added and the solution was stirred on a magnetic stirrer for 15 min. Then 5 g of powdered zinc were added, the solution was stirred for one hour and then the decoloured solution was filtered through an S4 frit and its spectrum was measured in the 400—600 nm region. Then 20, 40, 60, and 80 μl of a solution containing 1 mg Doxorubicin per ml were added stepwise to 5 ml of filtrate and the spectrum was rerecorded after each addition.

Expired date Vincristine injections were destroyed in the following manner[11]: To 1 ml of injection solution containing 1 mg of Vincristine, 100 mg of manitol and 1·3 mg of methyl *p*-hydroxybenzoate were added 9 ml of 3M-H_2SO_4 and 0·5 g of solid potassium permanganate and the solution was stirred for 2 h on a magnetic stirrer. Then 1 g of powdered zinc was added and the mixture was stirred for one hour; the decoloured solution was then filtered through an S4 frit and its spectrum was measured in the region 200—400 nm. To 5 ml of filtrate were added stepwise 50, 100, 150, and 200 μl of a solution containing 0·1 mg Vincristine per ml and the spectrum was rerecorded after each addition.

The following procedure was employed to verify the usefulness of this technique for destroying cytostatics in some nonaqueous solvents, using Daunorubicin as a model substance and dimethylsulfoxide as a model solvent[11]: 0·5 mg of solid potassium permanganate suspended in 4 ml of 3M-H_2SO_4 was added to a solution containing 15 mg Daunorubicin in 1 ml of dimethylsulfoxide and the mixture was stirred for 15 min on a magnetic stirrer. Then 1 g of powdered zinc was added, the mixture was stirred for one hour and the decoloured solution was filtered through an S4 frit and its spectrum was measured in the 400—600 nm region. Then 9, 18, 27, 36, and 45 μl of a standard solution containing 0·5 mg of Daunorubicin per ml of dimethylsulfoxide were added stepwise and the spectrum was rerecorded after each addition.

The following procedure was employed using Dichloromethotrexate as a model substance and dimethylsulfoxide as a model solvent[11]: To a solution containing 10 mg Dichloromethotrexate in 1 ml of dimethylsulfoxide were added 1·5 g of solid potassium permanganate suspended in 6 ml 3M-H_2SO_4 and the mixture was stirred for 2 h on a magnetic stirrer. The solution was then decoloured with the minimal amount of sodium sulfite, bubbled with nitrogen and the differential pulse polarogram was recorded. Then 10, 20, 30, 40, and 50 μl of a standard solution containing 1 mg of Dichloromethotrexate per ml of dimethylsulfoxide were added stepwise and the differential pulse polarogram was rerecorded after each addition.

Destruction by hydrobromic acid in anhydrous acetic acid medium: The solid substances were destroyed by the modified method of Castegnaro, Michelon and Brouet[2]: 25 mg of the solid substance were dissolved in 1 ml of dichloromethane dried using Potasit 3A molecular sieve (J. Dimitrov Chemical Works, Bratislava) and 5 ml of 3% hydrobromic acid in anhydrous acetic acid were added. After 15 min, the NOBr formed was removed from the solution by bubbling for thirty minutes with nitrogen, to prevent reformation of the N-nitrosocompound. Then 6 ml of water were added, any bromine formed was removed by addition of about 50 mg of powdered zinc; after dissolving the powdered zinc and bubbling with nitrogen, the differential pulse polaro-

gram was recorded. Then a standard solution containing 2·5 mg of the determined substance per ml of dichloromethane in amounts of 10 to 50 μl for Carmustine, 20 to 100 μl for Lomustine and Chlorozotocin, 40 to 200 ml for Semustine and 100 to 500 μl for PCNU was added stepwise and the differential pulse polarogram was rerecorded after each addition.

The following procedure was employed to destroy a solution of the studied substances in methanol[11]: To a solution containing 25 mg of the substance in 1 ml of methanol were added 17 ml of 4·5% hydrobromic acid in anhydrous acetic acid and the NOBr formed was removed by bubbling after 12 h. Then 18 ml water were added to the solution and the bromine formed was removed by addition of 50 mg of powdered zinc. After dissolving of the powdered zinc, 10 ml of sample were taken and bubbled with nitrogen; the differential pulse polarogram was then recorded. Then, a standard solution containing 1 mg of the determined substance in 1 ml of methanol was added, in amounts of 7 to 35 μl for Carmustine and 14 to 70 μl for Lomustine, Semustine and Chlorozotocin; after each addition, the differential pulse polarogram was again recorded.

RESULTS AND DISCUSSION

Destruction with Potassium Permanganate in Sulphuric Acid Medium

Table I gives the degree of destruction of the substances together with the determination method employed. It can be seen that oxidation of the studied substances with potassium permanganate in sulfuric acid medium is chemically sufficiently efficient. The deep destruction of the molecules and formation of water and carbon dioxide, eventually of inorganic nitrogen compounds and chloride ions can be assumed. From a biological point of view, it should be pointed out that it was found that the solution obtained after destruction of N-nitrosourea derivatives when subjected to the Ames mutagenicity test using *Salmonella typhimurium* TA 1 530, TA 1 535 and TA 100 exhibited weak mutagenic action[14]. This is particularly interesting as oxidation by potassium permanganate in sulfuric acid medium has been found to be useful for the destruction of various types of structurally similar N-nitrosoamines[12] and N-nitrosoamides[13]. It is thus clear that the individual destruction methods cannot be automatically transferred to related types of substances, but that the chemical and biological efficiency of the method used must be verified in each individual case.

Fig. 1 documents the usefulness of spectrophotometry for control of the efficiency of the destruction. The lowest curve corresponds in each case to the solution obtained after the destruction and the subsequent curves correspond to the individual additions of the determined substance, selected so that its concentration was 0·4, 0·8, 1·2, and 1·6% of the initial concentration prior to destruction for Doxorubicin (Fig. 1*a*), 0·03, 0·06, 0·09, 0·12, and 0·15% for Daunorubicin (Fig. 1*b*), 1, 2, 3, and 4% for Vincristine (Fig. 1*c*) and 1, 2, 3, 4, and 5% for Vinblastine (Fig. 1*d*).

Fig. 1 illustrates the usefulness of spectrophotometry for control of the efficiency of destruction of the solid substances (Fig. 1*d*), their solutions in nonaqueous solvents (i.e. the decontamination of a solution of Daunorubicin in dimethylsulfoxide –

see Fig. 1*b*), and expired date pharmaceuticals (i.e. Doxorubicin tablets – Fig. 1*a*, and an injection solution of Vincristine – Fig. 1*c*). The procedures employed in the individual decompositions are given in the experimental part.

Fig. 2 illustrates the usefulness of differential pulse polarography for control of the efficiency of the chemical destruction of the studied substances. It can be seen that differential pulse polarography can be used in the presence of sulfuric acid alone (Fig. 2*a*), of dimethylsulfoxide (Fig. 2*b*) or dimethylformamide (Figs 2*c* to 2*f*). The lowest curve always corresponds to the solution after destruction and the subsequent curves correspond to the individual additions of the studied substances selected so that their concentrations were 0·1, 0·2, 0·3, 0·4, and 0·5% of the initial concentration prior to destruction for Methotrexate, Dichloromethotrexate and PCNU and 0·2, 0·4, 0·6, 0·8, and 1·0% for Lomustine, Semustine, and Chlorozotocin. The procedures are given under Experimental.

Destruction by Hydrobromic Acid in Anhydrous Acetic Acid

Because of the weak mutagenic effect of solutions obtained after destruction of cytostatics derived from N-nitrosourea by the permanganate method, these substances

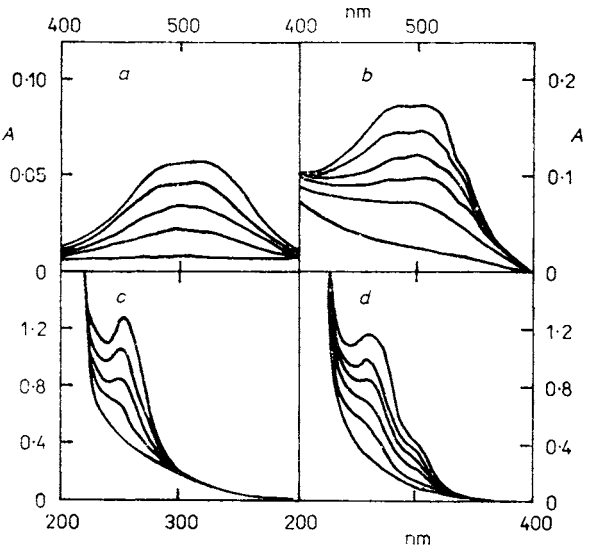
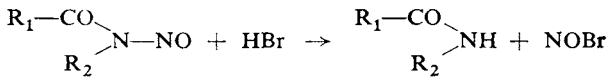


FIG. 1

Spectrophotometric monitoring of the efficiency of the destruction of cytostatics by potassium permanganate. *a* Doxorubicin (tablets); *b* Daunorubicin (solution in dimethylsulfoxide); *c* Vincristine (injection solutions); *d* Vinblastine (solid substance)

were decomposed by denitrosation by hydrobromic acid in anhydrous acetic acid medium. This procedure has been found to be useful for the decomposition of some N-nitrosoamines[12] and N-nitrosoamides[13]. The degree of destruction of the studied solid substances and the method used for their determination are given in Table II. It can be seen that, with the exception of PCNU, the method is sufficiently chemically efficient in all cases. It can be assumed that denitrosation occurs according to the equation



(*A*)

However, The Ames test using the above-mentioned strains indicated slight mutagenic activity of the solutions obtained after destruction of Carmustine and Semustine[14].

The rate of the denitrosation reaction is decreased considerably in the presence of methanol or ethanol, so that 4·5% hydrobromic acid in anhydrous acetic acid must

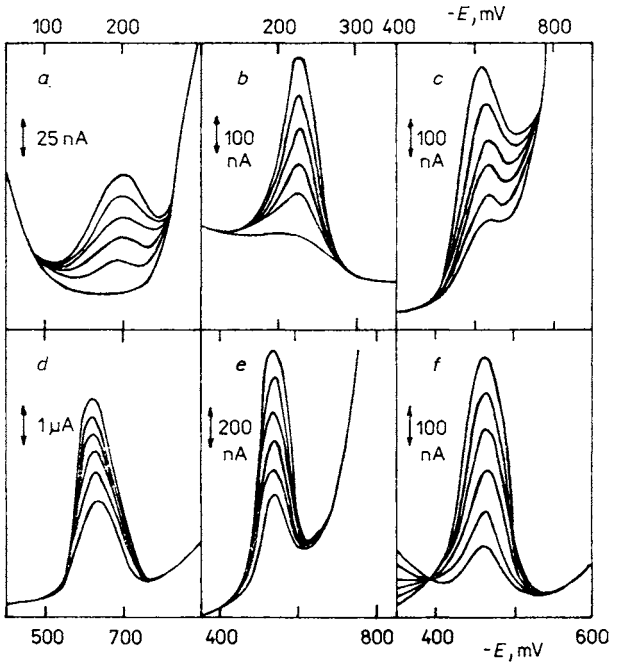


FIG. 2

Differential pulse polarographic monitoring of the efficiency of destruction of cytostatics by potassium permanganate. *a* Methotrexate (solid substance); *b* Dichloromethotrexate (solution in dimethylsulfoxide); *c* Lomustine; *d* Chlorozotocin; *e* Semustine; *f* PCNU; (Figs *c*, *d*, *e*, *f* correspond to destruction of the solid substance after dissolving in dimethylformamide)

be added to the decomposition solution in these solvents in an amount such that the final mixture contained less than 15% alcohol (v/v) and less than 1·5 mg studied substance per 1 ml of solution. In addition, the decomposition time must be increased to at least 12 h (see the procedure under Experimental).

Fig. 3 illustrates the usefulness of differential pulse polarography for controlling the chemical efficiency of the denitrosation. It can be seen that differential pulse

TABLE II

The efficiency of the destruction of some cytostatics by hydrobromic acid

Substance	Efficiency control[a]	Efficiency %	Mutagenic activity of the residue[b]
Lomustine	DPP (−550)	99·9	NEG
Carmustine	DPP (−620)	99·9	POS
Semustine	DPP (−540)	99·9	POS
Chlorozotocin	DPP (−805)	99·5	NEG
PCNU	DPP (−605)	90·0	POS

[a] DPP differential pulse polarography (peak potential in mV vs standard calomel electrode in brackets); [b] see ref.[14]; POS (NEG) positive (negative) Ames test for mutagenic activity of the solution after destruction.

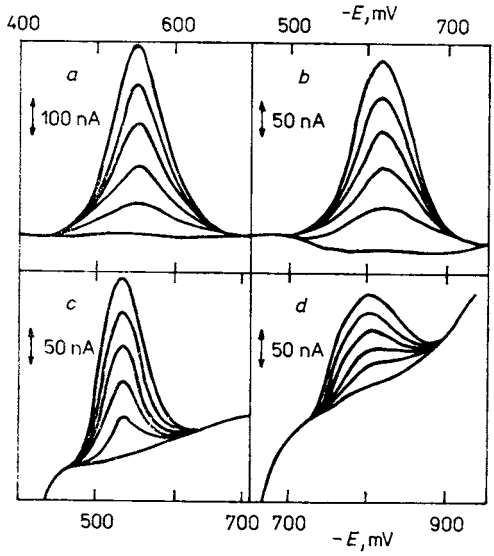


FIG. 3

Differential pulse polarographic monitoring of the efficiency of the destruction of cytostatics by hydrobromic acid. *a* Lomustine; *b* Carmustine; *c* Semustine; *d* Chlorozotocin; (Figs *a*, *b* correspond to destruction of the solid substances after dissolving in dichloromethane, Figs *c*, *d* to destruction of solutions of these substances in methanol)

polarography can be used both for studying the destruction of the solid substances after dissolving in dichloromethane (Figs 3*a* and 3*b*) and for studying their decomposition in methanol solution (Figs 3*c* and 3*d*). The lowest curve always corresponds to the solution after destruction and the subsequent curves to the individual additions of the studied substances, selected so that their concentrations corresponded to 0·1, 0·2, 0·3, 0·4, and 0·5% of the original concentration prior to decomposition for Carmustine and 0·2, 0·4, 0·6, 0·8, and 1·0% for Lomustine, Semustine, and Chlorozotocin.

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