STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS AND PROCESS CONTROL

KINETICS OF DIOXIDINE LIBERATION FROM A FOAMED POLYURETHANE MATRIX IN THE PRESENCE OF TRYPSIN

I. I. Burak,[1] B. M. Sadikov,[1] O. V. Bordzilovskaya,[1] and V. Ya. Bordzilovskii[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 32, No. 3, pp. 50 – 53, March, 1998.

Original article submitted September 5, 1996.

The wide use of proteinase based drugs in medicine is limited because of the instability of most of these enzymatic preparations [1]. The probability of inactivation can be reduced to a certain extent by immobilizing the enzymes on a polymeric matrix [2]. At the same time, the presence of enzymatic components in the known macromolecular therapeutic systems (MTS) can markedly modify their properties.

With a view to this problem and in continuation of the systematic investigation into the synthesis and characterization of polyurethane (PU)-based MTS [3 – 7], we have studied the effect of trypsin (a proteolytic enzyme used in the therapy of various diseases [8]) on the kinetics of liberation of the drug dioxidine immobilized in a foamed PU (FPU) matrix.

Previously we have established that dioxidine introduced, before the synthesis of an FPU based MTS, into a polyol prepolymer (A) evolves at a significant rate from FPU into the liquid phase at temperatures above 293 K. The introduction of trypsin into prepolymer A before the MTS synthesis generally hinders the passage of dioxidine from polymer to the liquid phase [4].

This is illustrated by data presented in Fig 1, showing the kinetic curves for the dioxidine liberation into water at 310 K at various initial trypsin concentrations C_T^0 in the polymer. Figure 1 also shows the dioxidine liberation rate W (calculated from the slopes of initial parts of the kinetic curves constructed in the C versus time coordinates) as a function of the initial trypsin concentrations C_T^0 in the polymer, plotted in the semilogarithmic coordinates.

As is seen from Fig. 1*a*, the kinetic curves of the dioxidine liberation from FPU into water in the presence of trypsin are described by a linear function, which is indicative that the process under consideration has the first order with respect to the drug concentration. In the initial stage, the dioxidine liberation rate linearly decreases with the time. Then the decrease gradually slows down and eventually the plot exhibits a "saturation" region, where the process rate is independent of the trypsin concentration (for $C_T^0 > 0.5 \times 10^{-3}$ M). Taking this fact into account, all the subsequent kinetic investigations were performed for the trypsin concentration in the polymer equal to 1.05×10^{-3} M, ensuring that the effective rate constant of dioxidine liberation would be independent of the enzyme content in the polymer.

The character of variation of the rate of dioxidine liberation depending on the enzyme content (Fig. 1*a*) shows evidence that the dissolution process at small enzyme concentrations (below 1×10^{-4} M) is described by a kinetic equation having the first negative order (– 1) with respect to the initial trypsin concentration in the polymer. This follows from the initial slope of the log W versus log C_T^0 plot, which is close to – 1.

In the range of $C_T^0 > 0.5 \times 10^{-3}$ M, the process has zero order with respect to the trypsin concentration, as confirmed by the fact that the process rate becomes independent of the enzyme content (Fig. 1*a*).

The dependence of the effective rate constant k (sec^{-1}) for the liberation of dioxidine (introduced into prepolymer A prior to the synthesis of FPU-based MTS) on the trypsin concentrations C_T^0 (M) can be described by a correlation equation

$$k = 1.42 \times 10^{-5} - \frac{7.2 \times 10^{-3} C_T^0}{1 + 7.2 \times 10^2 C_T^0}, \qquad (1)$$

having a selected pairwise correlation coefficient of 0.994.

The observed effect of the trypsin concentration on the rate of dioxidine liberation from FPU into water can be explained proceeding from the notion of complex formation be-

[1] Vitebsk Medical Institute, Vitebsk, Belarus.

0091-150X/98/3203-0164$20.00

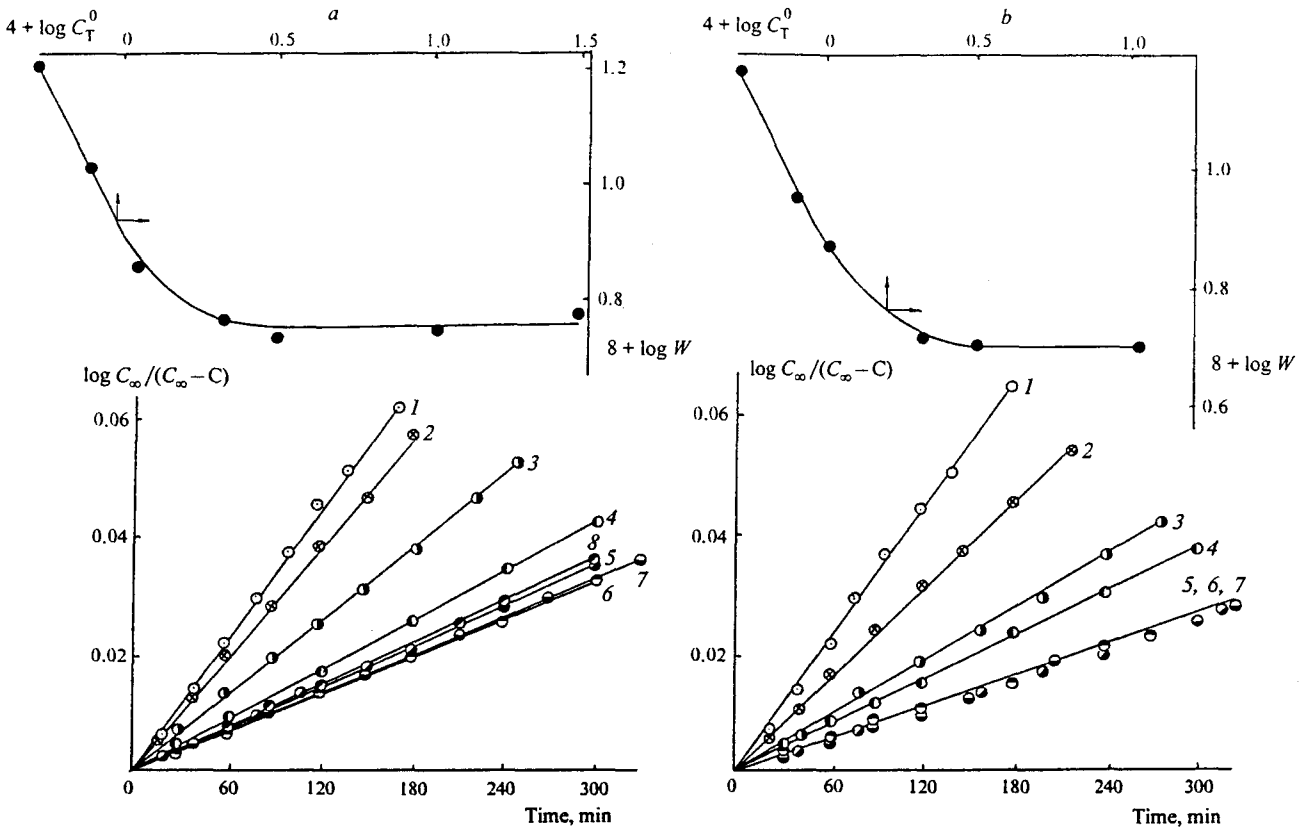

Fig. 1. Effect of the initial trypsin concentration C_T^0 on the yield of dioxidine (D) from an FPU composition (0.4 g) into water (10 ml) at 310 K: *a*) trypsin and dioxidine introduced into prepolymer A; *b*) trypsin introduced into prepolymer B and dioxidine, into prepolymer A; $C_D^0 = 0.34$ M; $C_T^0 = 0$ (*1*), 0.53 (*2*), 0.79 (*3*), 1.05 (*4*), 2.10 (*5*), 3.15 (*6*), 10.50 (*7*), 3.50 M (*8*); *C* is the current dioxidine concentration (M), C_∞ is the value calculated assuming that all dioxidine has passed

tween drug and enzyme. The coordination of trypsin to dioxidine results in the formation of a rather bulky adduct, whose transfer to the polymer – liquid interface is strongly hindered. As a result, the rate of dioxidine passage to the liquid phase markedly decreases. Independence of the drug liberation rate of C_T^0 at sufficiently high enzyme concentrations corresponds to nearly full binding of dioxidine.

The study of the effect of the initial trypsin concentration on the liberation of dioxidine introduced, prior to the synthesis of FPU-based MTS, into an isocyanate prepolymer B showed that the pattern of variation of the process rate with increasing trypsin concentration in the polymer was generally the same as in prepolymer A (Fig. 1*b*): the liberation exhibited an initial linear decrease and then attained a constant level. The experimental data indicate that the process of dioxidine liberation is also initially described by a kinetic equation having the first negative order with respect to the enzyme concentration at small trypsin contents in the polymer, and by a zero-order kinetics at higher trypsin contents. The rate of trypsin liberation under these conditions became independent of the trypsin content at approximately the same initial enzyme concentration as in the case of the polyol prepolymer.

The dependence of the effective rate constant k for the liberation of dioxidine (introduced into prepolymer B prior to the synthesis of FPU-based MTS) on the trypsin concentrations C_T^0 is generally similar to that described by equation (1) for prepolymer A. The corresponding correlation equation is as follows:

$$k = 1.39 \times 10^{-5} - \frac{7.6 \times 10^{-3} C_T^0}{1 + 7.2 \times 10^2 C_T^0} \qquad (2)$$

and has a selected pairwise correlation coefficient of 0.987.

Note that close values of the coefficients of argument in equations (1) and (2) are indicative that the effect of trypsin on the dioxidine liberation from both isocyanate and polyol prepolymers is caused by the same reason, namely, by the formation of dioxidine – enzyme complexes.

A more sharp drop in the effective rate constant k of the drug liberation process with increasing C_T^0 and a lower value of this constant in the region of "saturation" observed in the case of the isocyanate prepolymer are apparently explained by the increasing fraction of dioxidine chemically bound to PU in this matrix. As was established previously [3], such a

binding of the drug to polymer chains generally hinders its passage into the liquid phase.

Introduction of the enzyme into the isocyanate prepolymer B prior to the MTS synthesis did not significantly influence the effective drug liberation rate, irrespective of the type of prepolymer in which the drug was immobilized. This is explained by the fact that trypsin introduced into prepolymer B is capable of chemically interacting with highly reactive isocyanate groups of this prepolymer via mobile atoms of the imidazole group His-57 and hydroxy group Ser-195 of the active enzyme center. As a result, trypsin loses to a significant degree its activity and the ability to form complexes with dioxidine. These notions agree well with the data reported previously [10], according to which the chemical binding of proteolytic enzymes to a PU chain by introducing them into the isocyanate prepolymer leads to almost complete inactivation of the enzymes.

Thus, the results of our experiments show that the introduction of trypsin into the polyol prepolymer prior to the MTS synthesis leads to a decrease in the effective constant of dioxidine liberation from polymer into liquid phase, which may lead to prolongation of the therapeutic action of the drug. This implies that, provided equal dioxidine contents, the liberation of the drug from the matrix in the presence of trypsin will proceed at a lower rate as compared to that from the trypsin-free matrix, ensuring a constant supply of comparatively smaller doses of dioxidine for a longer period of time. The latter mode of the drug supply may lead to the phenomenon of "negative adaptation" of viruses and bacteria in a tissue liquid to dioxidine liberated into this liquid, thus significantly increasing the efficacy of the drug action. This is probably one of the reasons for the increased therapeutic effect observed for MTS of the dioxidine – PU – proteolytic enzyme system used in the treatment of acute purulent infections [11].

Using the above empirical equations, relating the effective rate constant of the drug liberation to the initial trypsin concentration in the polymer, we may readily calculate (without recourse to experiment) the amount of enzyme necessary to provide for the process proceeding at a desired rate and producing the maximum therapeutic action. In other words, once the dioxidine dose ensuring the maximum antibacterial action is known, equations (1) and (2) allows us to estimate a priori the amount of trypsin that has to be introduced into the polymeric composition in order to provide for the dioxidine liberation over a sufficiently long time. Thus, using these relationships in medical practice helps create effective FPU – based MTS with controlled and prolonged action.

The temperature dependence of the rate constant k (determined under the conditions of independence of C_T^0) for dioxidine liberation in the presence of trypsin introduced by different methods into the MTS matrix satisfactorily obeys the Arrhenius equation. The activation parameters calculated using these relationships (together with the kinetic parameters of the drug's liberation in the presence of trypsin) are listed in Table 1.

A comparative analysis of the kinetic and activation parameters presented in Table 1 leads to the following conclusions. The introduction of trypsin into the isocyanate prepolymer prior to the polymer composition synthesis does not significantly affect the activation parameters of the dioxidine liberation in comparison with the process characteristics observed in the absence of trypsin, independently of the type of prepolymer in which the drug was immobilized. This result confirms the earlier conclusion that interaction with the isocyanate groups of prepolymer B leads to inactivation of the enzymatic activity of trypsin and the loss of its ability to coordinate with dioxidine. In this case, the absence of enzyme bound to the PU chain has no significant effect on the characteristics of the drug liberation process.

The introduction of trypsin into the polyol prepolymer leads to an increase in the activation energy and entropy of the dioxidine liberation (Table 1), which is especially pronounced if the dioxidine is also immobilized in prepolymer A (the activation energy is nearly doubled, and the entropy increases by almost 100 entropy units.

This behavior can be explained proceeding from the notion that the presence of dioxidine and trypsin in the polyol prepolymer (chemically inert with respect to both drug and enzyme) creates conditions for almost complete binding of the drug into a complex with the enzyme already in the stage preceding the MTS synthesis. Adding the isocyanate prepolymer, which reacts almost exclusively with the polyol component, does not significantly alter the degree of coordination of dioxidine and trypsin. The transport of dioxidine, bound in the complex with trypsin, to the polymer – liquid interface is hindered, which accounts for the increase in the activation energy for the drug liberation. An increase in the activation en-

TABLE 1. Effect of Trypsin (T) on the Kinetic and Activation Parameters for the Dioxidine (D) Liberation from Foamed Polyurethane into Water (C_D^0 = 0.34 M; $C_T^0 = 1.05 \times 10^{-3}$ M; water volume, 10 ml; polymer weight, 0.4 g; temperature interval, 298 – 315 K)

Components introduced into prepolymer		E, kJ / mole	$\log k_0$	$\Delta H^{\neq}$, kJ / mole	$-\Delta S^{\neq}$, J/(mole · K)	$\Delta G^{\neq}$, kJ / mole
D	T					
A	—	46.2	3.1	43.7	194.4	101.6
A	A	87.7	9.4	85.2	73.4	107.1
A	B	44.6	2.8	42.1	200.4	101.8
B	—	88.1	10.0	85.6	62.0	104.1
B	A	99.7	11.3	97.2	36.6	108.1
B	B	79.4	8.6	76.9	89.3	103.5

Note. The values of $\log k_0$ and E were calculated by the least squares procedure (the rms errors are not exceeding ± 0.3 and ± 6.8 kJ / mole, respectively); the values of enthalpy, entropy, and Gibbs energy for the process activation are reduced to standard conditions.

tropy of the process (that is, a decrease in the "rigidity" of the activated complex) is explained by the geometry of the coordinated dioxidine being closer to the geometry of the transition state before liberation as compared to the case of the uncoordinated drug.

A still higher activation energy for the liberation of dioxidine immobilized in the isocyanate prepolymer is explained by the fact that the drug is partly bound to the PU chain, which additionally hinders its passage to the liquid phase. The liberation of dioxidine from FPU into water in the presence of trypsin is accompanied by the kinetic compensation effect, which is mathematically reflected by a correlation equation

$$\Delta H^{\neq} = 110.3 + 0.35\Delta S^{\neq}, \quad (3)$$

(with a selected pairwise correlation coefficient of 0.986), where $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are reduced to 298 K and expressed in kJ / mole and J/(K · mole), respectively.

The presence of the kinetic compensation effect indicates that the introduction of trypsin into the polymer does not significantly modify the mechanism involved in the limiting stage of dioxidine liberation from FPU into water.

EXPERIMENTAL PART

The polyol prepolymer A was obtained by mixing 94 g (1.9×10^{-2} mole) of laprol 5003-26-10, 31 g (7.8×10^{-3} mole) of laprol 402-2-100, 7.2 g (6.4×10^{-2} mole) of 1,4-diaminobicyclooctane, and 3.0 g (0.167 mole) of water. The content of hydroxy groups in prepolymer A was 1.1 wt.%. The isocyanate prepolymer B according to the State Technological Conditions TU-113-03-29-12 – 83 was obtained by copolymerization of 5 g (2.5×10^{-2} mole) of laprol 202 and 35.2 g (0.133 mole) of 4,4'-diisocyanatodiphenylmethane. The content of isocyanate groups in prepolymer B was 22.7 wt.%.

The PU composition was prepared immediately before experiments in a cylindrical pyrex glass setup specially designed for the study of the dioxidine liberation.

In the case when both dioxidine and trypsin were introduced into polyol prepolymer A, the setup was charged with 0.3 g of prepolymer A, an aliquot (30 mg) of dioxidine, and a definite amount of trypsin. The mixture was thoroughly stirred to a homogeneous mass. To this mass was added 0.1 g of isocyanate prepolymer B, and the mixture was thoroughly stirred for 60 sec.

If both dioxidine and trypsin were introduced into isocyanate prepolymer B, the setup was charged with 0.1 g of prepolymer B and the aliquots of dioxidine and trypsin. The mixture was thoroughly stirred to a homogeneous mass. To this mass was added 0.3 g of isocyanate prepolymer A and the mixture was thoroughly stirred for 60 sec.

If dioxidine and trypsin had to be introduced into different prepolymers, the setup was charged with 0.3 g of prepolymer A and an aliquote of the first component (dioxidine or trypsin). After thorough stirring, to this mixture was added another component (trypsin or dioxidine) in 0.1 g of isocyanate prepolymer B and the mixture was thoroughly stirred for 60 sec.

After completion of the FPU composition formation (12 h after prepolymer blending) 10 ml of water was added and the setup was closed and placed into a thermostat. This moment was considered the onset of the drug liberation process.

The dioxidine concentration in the liquid phase was determined by the spectrophotometric technique with absolute calibration using a KFK-3 instrument measuring the absorption intensity at a wavelength of 409 nm. The reference cell was filled with water.

The experiments were performed with dioxidine satisfying the State Pharmacopeial Issue FS 42-1232 – 79. The drug was additionally purified by multiple recrystallization from absolute ethanol and dried for not less than 6 h at 323 K in a vacuum of 2×10^{-2} Torr to provide for the main substance content of not less than 99.8 % (by IR and chromatographic data).

REFERENCES

1. V. K. Antonov, *The Chemistry of Proteolysis* [in Russian], Nauka, Moscow (1991).
2. A. E. Vasil'ev and A. B. Davydov, *Zh. Vses. Khim. o-va im. D. I. Mendeleeva*, No. 4, 395 – 402 (1985).
3. A. G. Sutulo, B. M. Sadikov, and V. Ya. Bordzilovskii, *Khim.-Farm. Zh.*, **28**(5), 15 – 19 (1994).
4. A. G. Sutulo, B. M. Sadikov, and V. Ya. Bordzilovskii, Dep. VINITI, No. 2404 – B94, Lyubertsy (1994).
5. V. Ya. Bordzilovskii, B. M. Sadikov, and A. G. Sutulo, *Dep. NIITEKhIM*, No. 76-KhP95, Cherkassy (1995).
6. B. M. Sadikov, I. I. Burak, O. V. Bordzilovskaya, et al., *Dep. NIITEKhIM*, No. 178-KhP96, Cherkassy (1996).
7. T. É. Lipatova and Yu. S. Lipatov, *Zh. Vses. Khim. o-va im. D. I. Mendeleeva*, No. 4, 438 – 446 (1985).
8. F. P. Trinus, *Pharmacotherapeutic Handbook* [in Russian], Zdorov'ya, Kiev (1989), pp. 321 – 322.
9. L. N. Yakhontov and V. G. Glushkov, *Synthetic Drug Preparations* [in Russian], Meditsina, Moscow (1983), pp. 201 – 202.
10. L. N. Kirpichenok and V. Ya. Bordzilovskii, *Abstracts of Papers. The All-Union Conf. "Production and Application of Isocyanates"* [in Russian], Dzerzhinsk (1989), pp. 50 – 51.
11. A. N. Kosinets, M. G. Sachek, Yu. I. Moskalev, et al., in: *Mechanisms of Homeostasis Regulation. A Collection of Scientific Works* [in Russian], Vitebsk State Medical Institute, Vitebsk (1994), pp. 123 – 125.