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Obtaining Ultradispersed Dioxidine Powder Modified via Cryochemical Synthesis and Determining Its Antibacterial Activity

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Abstract—One way to increase bioavailability and efficiency of drug substances is to decrease their particles up to nanoscale level and to change their crystal structure. A new stable nanoscale form of a polymorphic antibacterial 2,3-bis-(hydroxymethyl)-quinoxaline-*N*,*N*'-dioxyde (dioxidine) modification characterized with a gas chromatography, NMR, XRF, TEM, and thermoanalytic methods (TG, DTG, DSC) was obtained via cryochemical synthesis. The new polymorphic dioxidine modification was proved to be more active in growth inhibition processes of gram-positive *M. cyaneum 98* and gram-negative *E. coli* bacterial strains than officinal modification.

Keywords: nanoparticles, dioxidine, cryochemical modification

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INTRODUCTION

Currently, many drugs have low bioavailability [1]. One way to solve this problem from the point of view of modern pharmacology is to transfer these compounds into nanoscale form. In this case, increase in surface area of a drug leads to increase in its dissolution rate. The bioavailability of low soluble drugs can be improved by obtaining emulsions and suspensions on the basis of nanoparticles of these compounds.

Another way to increase bioavailability is to obtain novel polymorphs of known drugs. Polymorphism is the property of a solid state substance that allows it to exist in different polymorphs, with each having the same chemical composition, but different crystal structures. Different polymorphs of drugs have various physicochemical and biopharmacological properties. Stability, dissolution rate, and efficiency of any drug depend on type of crystal modification. Polymorphism of crystalline drugs has great innovation potential, attracting great attention in the pharmaceutical industry [2, 3].

The most common method to prepare crystalline modifications is recrystallization from solutions [4, 5] and melts [6], including use of solvents under supercritical conditions [7, 8]. New polymorphic substances are also obtained via cryochemical modification, which involves evaporation of a substance in a stream of hot gas carrier followed by vapor condensation on a surface cooled up to the boiling point of liquid nitrogen [9].

The aim of this work is to perform cryochemical synthesis of 2,3-bis-(hydroxymethyl)-quinoxaline-*N*,*N*'-dioxyde (dioxidine) ultradispersed powder and to determine its antibacterial activity. Dioxidine is a synthetic antibacterial agent with bactericidal activity, which is based on a damage of DNA biosynthesis of a microbial cell with a deep injury of a nucleotide structure already upon injection of subinhibitory concentrations. The drug is effective against a broad range of bacteria strains [10, 11].

EXPERIMENTAL

Reagents and Methods

A dioxidine conforming to a pharmacopeial article (PA) 42-2308-97 was used without additional purification. Dioxodine is a yellow-green crystal substance, slightly soluble in water, up to 1% wt under 18–20°C.

Synthesis of a Cryomodified Dioxidine

A dioxidine water solution (1–8 wt %) heated up to 20–100°C was cooled with liquid nitrogen and underwent freeze-drying for 22–27 h.

To confirm identity of a composition and differences in a crystal structure and antibacterial activity of the initial officinal and cryomodified dioxidine, physicochemical and biological measurements were performed.

A chromatographic analysis of the alcohol solutions of initial officinal and cryomodified dioxidine was carried out on a Crystall Lyuks-4000 gas chromatograph (Russia).

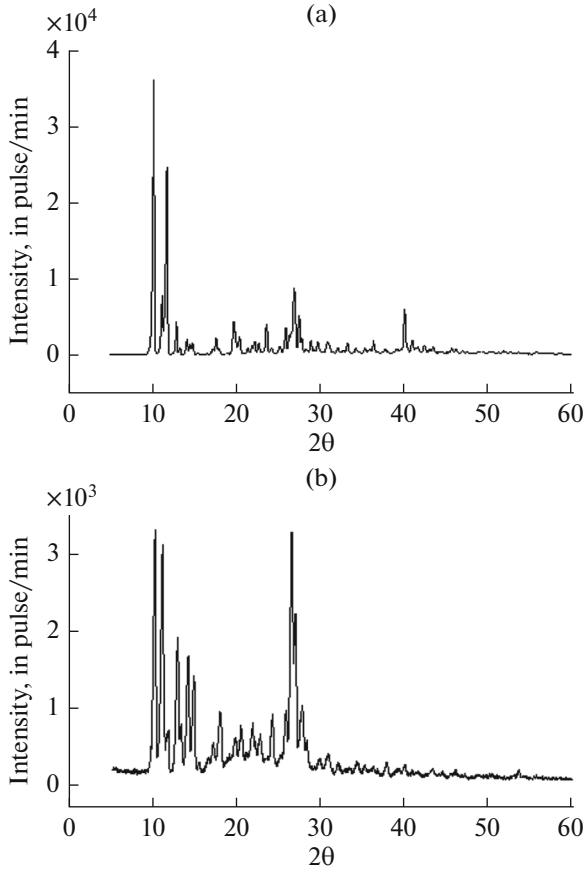

Fig. 1. X-ray diffraction pattern of: (a) initial and (b) cryomodified dioxidine.

Nuclear magnetic resonance (NMR) spectra of initial and obtained compounds were acquired in solutions with deuterated water (D_2O) on a high-resolution Varian VXR-400 NMR spectrometer (UNITED STATES).

X-ray diffraction analysis (XRD) of the samples was performed on a Rigaku D/MAX-2500 diffractometer (Japan) under CuK_α radiation ($\lambda = 1.54056$ Å).

Thermoanalytical studies were carried out on a STA 449 C Jupiter NETZSCH thermal analyzer (Germany) in argon flow upon increase in temperature of 10 degrees per minute. Aluminum cuvettes were utilized as sample holders. Weight of the samples was 4.7–7.8 mg.

Photomicrographs of cryomodified dioxidine were obtained with a transmission electron microscopy (TEM) on a JSM 6380 LA electron microscope with a resolution from 1000 to 20000.

Antibacterial activity of various dioxidine modifications was performed with a disk-diffusion method [12] using compressed tablets of initial and cryomodified dioxidine. Non-spore-forming gram-positive and gram-negative *E. coli 52* and *M. cyaneum 98* bacteria obtained from the Department of Biology of Moscow State University were used as test cultures. The experiments were carried out in Petri dishes with 20 mL of agar dried for one day; thickness of a substrate was 4 mm. Measurements of zones of growth suppression (ZGS) for the test cultures were performed after incubation for 16 and 48 h.

RESULTS AND DISCUSSION

Identity of chemical composition for the initial officinal and cryomodified dioxidine was primarily determined with ^{1}H NMR and gas chromatography.

^{1}H NMR spectrum of initial dioxidine acquired in D_2O contained the signals at δ 4.93–5.21 (m, 4H, $2CH_2$), 7.85–8.05 (m, 2H, H Ar), and 8.38–8.52 (m, 2H, H Ar). That of cryomodified drug involved the resonances at δ 4.95–5.25 (m, 4H, $2CH_2$), 7.86–8.05 (m, 2H, H Ar), and 8.35–8.50 (m, 2H, H Ar); it clearly indicates that these spectra are similar, confirming that the obtained substance is dioxidine.

Chromatographic retention time values (t_{ret}) of the initial substance and those obtained from it are identical and comprise 3.96 min, confirming that their chemical compositions are identical. The chromatograms of both dioxidine modifications contain only dioxidine and solvent peaks, indicating that there are no impurities in the drug composition.

To confirm that the resulting substance is a novel crystalline dioxidine modification, XRD and thermoanalytical measurements were performed.

Comparing X-ray diffraction data of the original dioxidine and that substance obtained from it (Fig. 1) indicates that it is a novel crystalline dioxidine modification, because position of the diffraction peaks and their intensities (I_{rel}, in %) for this substance, 8.740 (100.0%), 8.026 (94.2%), 3.358 (99.3%), and 3.304 (67.6%) are different from those of the original dioxidine observed at 8.638 (100.0%), 7.508 (68, 4%), 3.299 (24.8%), and 2.242 (16.8%).

Thermoanalysis data are shown in Figure 2. DSC curves of the original dioxidine (Fig. 3a) and those of the new crystal dioxidine modification (Fig. 3b) differ in position and size of endothermic melting effects and exothermic effects of thermal decomposition. Melting of the initial dioxidine substance occurs at a temperature of 175.5 ± 0.5°C with a heat effect of –99.8 ± 0.4 J/g, whereas thermolysis proceeds under 199.2 ± 0.5°C with heat effect of 1135 ± 4 J/g. Melting of the new crystal dioxidine modification occurs under 171.7 ± 0.5°C with a heat effect of –38.8 ± 0.4 J/g and thermolysis proceeds under 186.0 ± 0.5°C with heat effect to be 1243 ± 4 J/g. Thus, the thermoanalytical experiments showed that the resulting new crystal dioxidine modification is more active in melting and thermal decomposition processes. Moreover, thermoanalytical studies distinguish the original dioxidine modification substance and the new crystal modification obtained.

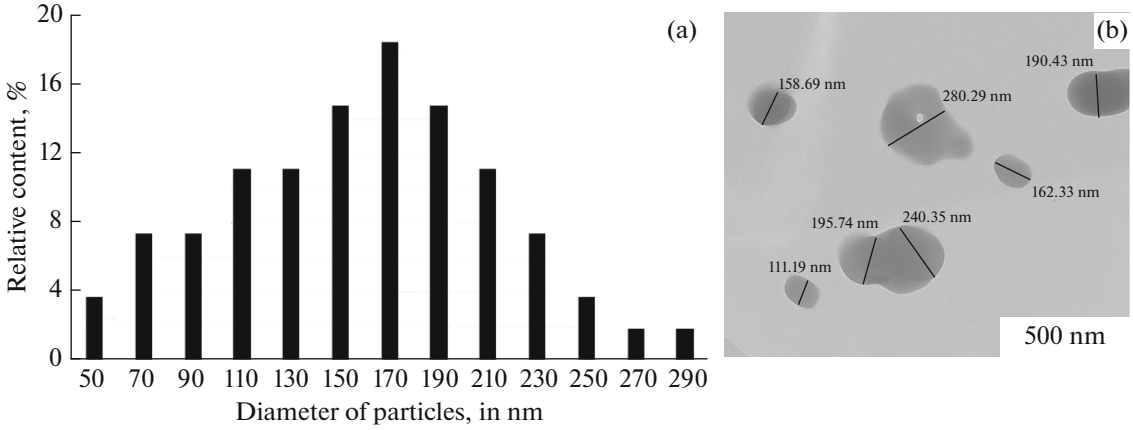

Fig. 2. (a) A particle size distribution for cryomodified dioxidine obtained on the basis of TEM data and (b) a microphotograph of separate units of cryomodified sample.

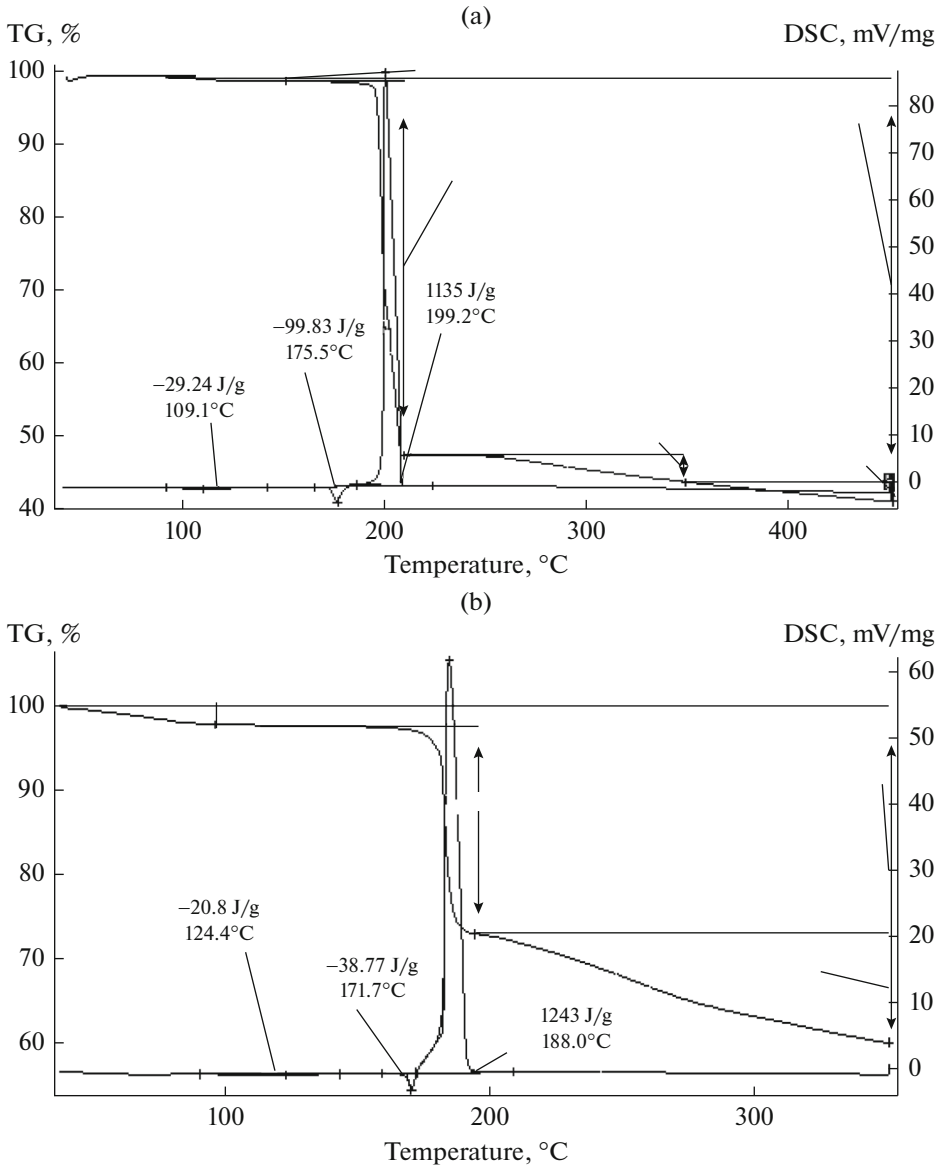

Fig. 3. Thermoanalytical data of: (a) initial and (b) cryomodified dioxidine.

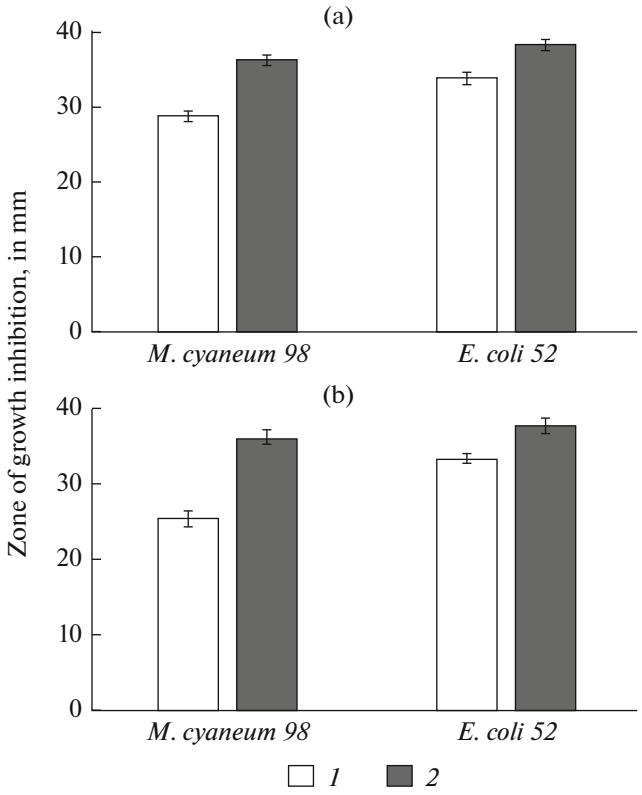

Fig. 4. Zone of growth inhibition for *M. cyaneum 98* and *E. coli 52* around tablets of initial and cryomodified dioxidine after incubation during: (a) 16 and (b) 24 h.

Microphotograph obtained for cryomodified dioxidine with a TEM method indicate that its particle size varies in the range of 50–400 nm. An average particle size obtained from microphotograph of cryomodified dioxidine was 170 nm (Fig. 2a). A microphotograph of the individual units of the cryomodified sample is shown in Figure 2b.

Data on activity of various dioxidine modifications against *E. coli 52* and *M. cyaneum 98* obtained with a disk-diffusion method on compressed tablets are shown in Figure 4.

ZGS diameter (Fig. 4) around the tablets of cryomodified dioxidine obtained for the *E. coli 52* and *M. cyaneum 98* bacterium strains was larger than that of original dioxidine modification after incubation for 16 and 48 h.

In summary, a nanoform of new polymorph dioxidine modification has been obtained via cryochemical synthesis. Disk-diffusion data have shown that the cryomodified drug was more active in growth inhibition processes of *E. coli 52* and *M. cyaneum 98* bacteria than the original pharmacopeia modification.

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