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Physicochemical Properties of the Inclusion Complex of Moxifloxacin with Hydroxypropyl-β-Cyclodextrin Synthesized by RESS

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Abstract—A new method for the synthesis of guest–host inclusion complex of moxifloxacin (MF) with 2-hydroxypropyl-β-cyclodextrin (HPCD) by supercritical fluid technology in the rapid expansion of supercritical solutions (RESS) mode was suggested for developing new drug formulations with improved bioaccessibility. The MF–HPCD complex was synthesized by this method as particles of 2–4 μm, which is the optimum size for creating the oral and inhalation MF drug forms. According to the scanning electron microscopy data, the morphology of MF–HPCD particles (irregular polyhedra) obtained by RESS processing differs from that of the starting components and from that of unbound MF and HPCD obtained by lyophilization from aqueous solution. According to X-ray diffraction data, the crystallinity of MF decreased from 95% to 20–30% after the formation of the HPCD complex. The IR spectroscopic and equilibrium dialysis data showed that RESS provides higher efficiency of drug inclusion in the complex with HPCD compared with that of conventional methods such as lyophilization or mixing of solid components.

Keywords: moxifloxacin, 2-hydroxypropyl-β-cyclodextrin (HPCD), guest–host inclusion complex, supercritical fluids (SCFs), rapid expansion of supercritical solutions (RESS), FTIR spectroscopy, drug release kinetics

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INTRODUCTION

Moxifloxacin (MF) is a fourth-generation antibacterial drug from the group of fluoroquinolones (FQs); they inhibit the DNA gyrase of bacterial cells [1, 2] without affecting the DNA topoisomerase of mammal cells. The mechanism of action of FQs differs from that of other antibacterial drugs, which allows them to be effectively used in the therapy of resistant forms of tuberculosis [3–6]. The use of MF, however, is associated with side effects such as gastrointestinal upset, phototoxicity, hemodynamic disorder, and thrombosis [6]. This problem is most acute in the case of serious infections such as tuberculosis when long-term medication using high doses of drug is needed. The side effects of MF can be reduced by decreasing the therapeutic dose of the drug by optimization of its pharmacokinetic characteristics and creating new drug delivery systems.

The pharmacokinetic properties of MF can be optimized by creating inclusion complexes of MF with β-cyclodextrin (CD) and its derivatives. Cyclodextrins are widely used in pharmacy as complexing agents that increase the solubility of hydrophobic drugs in water, increase their bioavailability, and provide controlled release of the drug [7]. Cyclodextrins are absorbed into the gastrointestinal tract (GIT); their macrocyclic ring does not decompose in the stomach and small intestine, but gradually degrades in the large intestine, which makes it possible to create drugs with prolonged therapeutic effect. Currently there are more than 30 CD-containing drugs of various groups, e.g., itraconazole (antifungal agent), hydrocortisone (antiallergic), diclofenac (anti-inflammatory), mitomycin (antitumor antibiotic), etc. [8, 9].

The classic methods for the synthesis of inclusion complexes of FQs with CDs are [8]:

(i) solid-state mixing with grinding;

(ii) lyophilization of an aqueous solution of a mixture of components; and

(iii) evaporation of the solvent from the aqueous solution of the mixture of components.

Previously, we studied in detail the physicochemical properties, structure, and stability of complexes of some fluoroquinolones (levofloxacin, ofloxacin, and moxifloxacin) with the derivatives of β-CD obtained by lyophilization from aqueous solution [10, 11]. The dissociation constant of various FQ complexes is 8×10^{-4}–1×10^{-3} M. Complexation with hydroxypropylcyclodextrin (HPCD) increases the solubility of fluoroquinolones two- to fivefold depending on the drug. This approach is promising for developing new drug

forms of fluoroquinolones with improved pharmacokinetic characteristics.

However, the efficiency of drug inclusion in complexes by lyophilization of an aqueous solution of a mixture of components is generally insufficiently high (20–60%). The obtained drugs contain residual water, which decreases their stability during storage [12] and significantly limits the potential of this approach for creating drug delivery systems.

The new approaches that use supercritical fluid technologies (SCFTs) make it possible to overcome the problems arising when creating new drug forms. SCFTs have a number of undeniable advantages over the conventional technologies used in modern bioengineering: one-stage process and avoiding the use of solvents (including water). The absence of purification and drying stages significantly facilitates the preparation of the desired product [13–19].

This study is aimed at developing a new, highly effective method for the synthesis of inclusion complexes of moxifloxacin and 2-hydroxypropyl-β-cyclodextrin using SCF. For the synthesis of guest–host complexes of FQ with cyclodextrins, the most effective method seems to be rapid expansion of supercritical solutions (RESS): a dry mixture of the components of the system is dissolved in supercritical (SC) fluid, in particular, in SC-CO_2, and is subsequently sprayed via a nozzle of a certain diameter into a vessel in which atmospheric pressure is created. SC-CO_2 is a solvent capable of effectively dissolving various substances [20, 21]. Due to the unique properties of SC fluids (low viscosity, absence of surface tension, high rate of mass exchange) and by optimizing the physicochemical parameters of synthesis (pressure and temperature), it is possible to shift the thermodynamic equilibrium toward complexation and increased efficiency of interaction of the components [20, 22–24].

In the present study, we investigated the possibilities of increasing the efficiency of inclusion of the MF drug in a complex with 2-hydroxypropyl-β-cyclodextrin using the RESS technique. The physicochemical properties of these complexes were studied in comparison with the those of the complexes obtained by lyophilization of an aqueous solution. The results will be used for creating new MF drug delivery systems with improved pharmacokinetic characteristics for treating the resistant forms of tuberculosis.

EXPERIMENTAL

In the study, we used moxifloxacin (Sansh Biotech, India), buffer salts, hydrochloric acid (Galakhim, Russia), hydroxypropyl-β-cyclodextrin (Zibo Industries, China), carbon dioxide (analytical grade); State Standard *GOST 8050-85*; Balashikha Oxygen Plant, Balashikha, Moscow oblast). A sample of the MF–HPCD complex obtained by RESS was kindly supplied by the Institute of Problems of Laser and Information Technologies, Russian Academy of Sciences. The sample was synthesized using SC-CO_2 as a solvent; the working parameters of the process were 80°C, 200 atm.

To obtain a mechanical mixture, the components (MF and HPCD) taken in a molar ratio of 1 : 1 were mixed until a homogeneous powder was obtained; then the mixture was ground in an agate mortar for 15 min.

The synthesis of MF–HPCD complexes by lyophilization was performed in the following way. An MF sample (0.5 g) was dissolved in a 1 mM solution of hydrochloric acid (20 mL, pH 3) and mixed with aqueous HPCD in a molar ratio of MF : HPCD = 1 : 1. The mixture was stored at 30°C for 24 h. The resulting solution of the complex was dried at –60°C for 24 h using a lyophilizer (Thermo Scientific, United States).

The particle morphology and the presence of unbound MF and HPCD in the resulting samples were determined by scanning electron microscopy (SEM) using a SUPRA 40 scanning electron microscope (Carl Zeiss, Germany) by the procedure described in [19]. To achieve the limiting resolution, the pressure in the vacuum chamber in which the samples were placed was brought to less than 5×10^{-6} mbar. The samples were placed on a current-conducting (carbon) Scotch tape and coated with a thin (~15–20 nm) layer of pure gold to remove the electric charge that accumulated during the analysis. Gold was applied by plasma magnetron sputtering in a Z-400 unit (Leybold, Germany). The size analysis of micronized particles was performed using Fiji software. Five micrographs were taken and processed from various regions (center and periphery) of the coating for each sample. Thirty particles on average were processed on one micrograph.

The IR spectra of MF and its solid complexes were recorded on an Impact 410 spectrometer (Nicolet, United States). The spectra of MF powders or its complexes with HPCD mixed with KBr powder (fourfold excess by weight) were recorded. The spectra were measured at a resolution of 2 cm^{-1} (64 scans) and processed with the OMNIC program.

The IR spectra of MF and its complexes with HPCD in aqueous solution (15 mM sodium phosphate buffer, pH 7.5) were recorded on a Tensor 27 IR-Fourier spectrometer (Bruker, Germany) equipped with an MCT detector cooled with liquid nitrogen using the procedure described in [10, 19]. The measurements were performed in a thermostatted frustrated total internal reflection cell (FTIR, BioATR-II, Bruker, Germany) using a ZnSe single reflection crystal at 22°C and constant rate of blowdown of the system with dry air of the Jun-Air apparatus (Germany). A 30-μL sample was deposited on the crystal of the FTIR cell; the spectrum was recorded three times in the range 4000–950 cm^{-1}. The spectrum resolution was 1 cm^{-1}; 70 scans with averaging. The background was recorded in a similar way. The spectra were processed and analyzed with the Bruker Opus 7.0 program

by the following procedure: atmospheric compensation, linear correction of the baseline (three iterations), location of the fundamental absorption bands. Deconvolution of absorption bands was performed by the following procedure: analysis of the second derivative of the spectrum to find the main components of the absorption band, their number and position; Lorentzian curve fitting of peak decomposition with an acceptable RMS error of 0.005.

The rate of dissolution of MF–HPCD complexes was studied by circular dichroism (CD) according to the procedure of [19]. The measurements were performed using a Jasco J-815 CD spectrometer (Japan) at 25°C in a quartz cell with a thickness of 1 mm at an MF concentration of 0.05–0.20 mg/mL in solution.

The MF release was studied by equilibrium dialysis via the Serva membrane, which is penetrable mainly for molecules with a molecular mass of less than 3 kDa. A sample of the solution of the MF–HPCD complex (4 mg/mL for MF) in dilute hydrochloric acid (pH 4) was placed in a dialysis bag and subjected to dialysis with an equal volume of hydrochloric acid solution (pH 4) at 37°C for 3 h while taking samples of 30 μL for recording the CD spectra.

The spectra were studied by XRD analysis on a D2 PHASER diffractometer (Bruker, Germany) using Cu$K\alpha$ radiation. The powders were placed in a cell of organic glass without additional treatment and flattened without adding any components and molding. All the diffraction patterns were recorded at a step of ~0.02 in the range of 2θ from 4 to 50 deg at 15 rpm and storage time of 0.5 s at a point. The components in the samples were analyzed using the EVA program and the PDF-4 Organic database.

RESULTS AND DISCUSSION

Particle Size and Morphology of MF–HPCD Complexes

Figure 1 shows the micrographs of the powders of the starting MF, MF–HPCD mechanical mixture, and complexes obtained by lyophilization and RESS treatment at 80°C and 200 atm. The SEM micrographs of MF after RESS treatment under similar conditions are presented for control.

An analysis of the micrographs shows that the starting MF is represented by prismatic elongated crystals with a length of 8–12 μm (Figs. 1a and 1b). After the RESS treatment, the MF particles are polyhedral thin plates with sizes of 10–20 μm and their aggregates of up to 50 μm (Figs. 1i and 1k). The morphology of MF particles after RESS treatment differs from that of the starting MF sample, which indicates that MF was recrystallized in SC-CO_2. Note that the samples obtained after the RESS treatment of MF at 40°C and 100 atm, on the one hand, and at 80°C and 200 atm, on the other, differ from each other. Thus at 40°C and 100 atm, MF is mainly physically removed from the dissolution chamber; at 80°C and 200 atm, the size and morphology of the resulting particles differ those of the starting MF. This suggests a considerable effect of pressure and temperature on the solubility of MF in SC-CO_2, which is important from the viewpoint of increasing the efficiency of complexation using the RESS method. Similar results were obtained for some other substances [25].

The mechanical mixture of MF and HPCD consists of elongated prismatic parallelepipeds with sizes of 8–12 μm (Figs. 1c and 1d) corresponding to those of MF particles (Figs. 1a and 1b) and of spheres with a porous surface with sizes of 10–15 μm corresponding to those of free HPCD particles. HPCD particles of similar size and morphology were observed in [8]. In addition to MF and HPCD particles, spherical particles with a smooth surface form in the mixture of MF and HPCD with sizes of 3 or 4 μm (Figs. 1c and 1d), most probably corresponding to HPCD with adsorbed MF. The formation of complexes of drug substances with CD during the solid-state mixing of components was reported in [11].

For the MF–HPCD complex obtained by lyophilization, a similar picture was observed, but the contents of free MF and HPCD relative to the particles corresponding to the MF–HPCD complex are smaller than in the mechanical mixture when visualized in the SEM micrographs (Figs. 1e and 1f).

After the RESS treatment, the picture observed for the MF–HPCD mixture differs substantially from that obtained for the mechanical mixture of the components and the lyophilized complex. Thus, after the RESS treatment, the articles corresponding to free MF and HPCD are not observed on micrographs. A new phase forms, which is represented by fragments in the form of irregular polyhedra with sizes of 3 or 4 μm (Figs. 1g and 1h). This phase is evidently the MF–HPCD complex. Importantly, after the RESS treatment, the sample has no free MF particles nor the spherical particles of free HPCD (Figs. 1i and 1k), which retains its morphology after the RESS treatment [8]. The data obtained indicate that the RESS treatment leads to the interaction of MF with HPCD, but not to crystallization of each component of the system. The size and morphology data for the MF–HPCD complexes are given in Table 1.

Structure of Solid Complexes According to IR Spectral Data

To confirm the formation of an MF complex with HPCD at a molecular level and study the structural characteristics of the complexes, we used solid-state IR spectroscopy. This method makes it possible to observe the changes in the microenvironment of the functional groups of MF during its interaction with HPCD directly in microparticles, while the components do not interact with the solvent. Figure 2 presents the spectra of MF, HPCD, a mixture of MF with

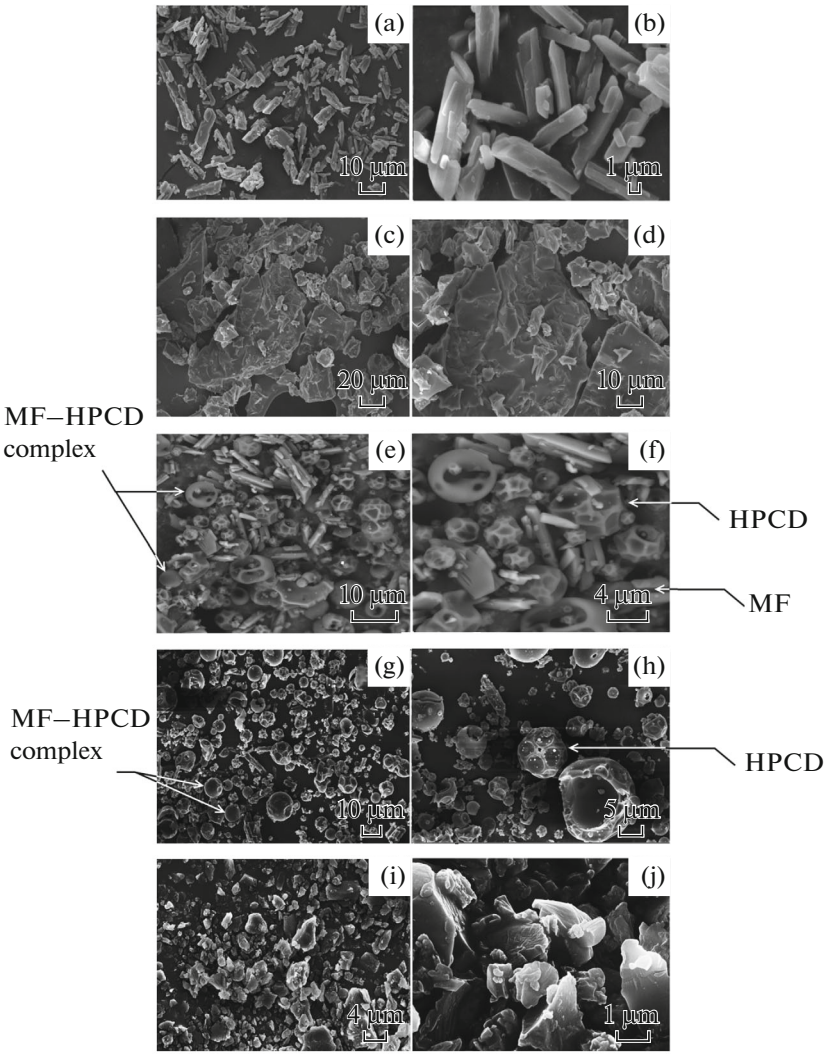

Fig. 1. SEM micrographs of MF–HPCD complexes: (a, b) the starting MF; (c, d) the mixture of MF and HPCD; (e, f) MF–HPCD complex obtained by lyophilization; (g, h) MF–HPCD complex obtained by RESS; and (i, j) MF after RESS treatment.

HPCD, and MF–HPCD complexes obtained by lyophilization and RESS.

The most intense absorption bands in the IR spectrum of MF (curve *1*, Fig. 2) are as follows:

(i) the bands at 1720–1700 cm^{-1} and 1622–1610 cm^{-1}, which correspond to the C=O bond stretching vibrations ($\nu_{C=O}$) in the carboxyl and carbonyl groups of quinolone, respectively;

(ii) the group of bands at 1540–1510 cm^{-1} and 1475–1425 cm^{-1} corresponding to the stretching vibrations of the C=C bond and aromatic structure of MF overlapped by the C–H bond deformation vibrations ($\nu_{C-C}Ar + \delta_{CH} + \delta_{CH_2} + \delta_{CH_3}$) [10, 26, 27].

These bands are most intense and masked by other bands but slightly; they are of greatest interest for analytical purposes.

The main absorption bands of HPCD (curve *2*, Fig. 2) lie in the range 1200–900 cm^{-1}, where the most intense peaks at 1100 and 1172 cm^{-1} correspond to the stretching vibrations of the C–O–C bond and the C–H and C–O–H bonds of the polysaccharide framework, respectively [10]. Table 2 presents the assignment of the characteristic IR absorption bands of MF, HPCD, and MF–HPCD complexes obtained by various procedures.

According to the data of Figs. 2 and 3, the complexation of MF with HPCD led to considerable changes in the absorption range of the functional groups of both MF (1800–1400 cm^{-1}) and HPCD (1200–970 cm^{-1}).

For the mechanical mixture of MF and HPCD, the absorption bands of the carbonyl and carboxyl groups of MF shifted toward high frequencies by 8 and 4 cm^{-1}, respectively (curve *4*, Fig. 3). This may be due to the fact that the intramolecular hydrogen bond between the close-lying –COOH and C=O groups characteristic of fluoroquinolones in the MF molecule decomposes as a result of its interaction with

Table 1. Morphology of FQ and HPCD obtained by various methods

Sample	Particle shape	Particle size, μm	Comments on composition
MF (Figs. 1a and 1b)	Prismatic platelets	9.5 ± 3.5	–
MF–HPCD mixture (Figs. 1c and 1d)	Prismatic platelets (MF) and spheres (HPCD)	9.5 ± 3.5 (MF) 11.2 ± 5 (HPCD) 3.8 ± 1.7 (MF–HPCD)	Mainly MF and HPCD particles, with occasional HPCD–MF particles
MF–HPCD (lyoph.) (Figs. 1e and 1f)	Spheres	8.8 ± 3.1 (MF) 11.2 ± 5 (HPCD) 3.8 ± 1.7 (MF–HPCD)	Mainly MF and HPCD particles; HPCD–MF particles are also observed
MF–HPCD (RESS) (Figs. 1g and 1h)	Polyhedra of irregular shape	3.5 ± 2 (MF–HPCD)	No free MF and HPCD are observed
MF (RESS) (Figs. 1i and 1k)	Thin polyhedral pates	20 ± 10	Aggregates of 40–50 μm are met

HPCD [27] due to hydrogen bonding with the hydroxyl groups of HPCD. This effect was observed earlier for the formation of fluoroquinolone complexes with CD [28–30]. On the other hand, in the IR spectrum of HPCD in the presence of MF, the C–O–H absorption band undergoes low-frequency shifts (Fig. 2 and Table 2); this is consistent with the assumption about the hydroxyl groups of HPCD involved in hydrogen bonding with the carboxyl group of MF. The 1452 cm^{-1} band corresponding to the stretching vibrations of the aromatic C–C bonds shifted by 8 cm^{-1} toward higher frequencies, and the 1516 and 1428 cm^{-1} bands vanished. The decreased intensity of the characteristic absorption bands of MF and their shift toward high frequencies are typical for fluoroquinolones included in complexes with CD derivatives, as reported in [29, 30].

Thus, the IR spectral data suggest that for MF and HPCD, inclusion complexes form already at the stage of formation of a mechanical mixture. This agrees with the SEM data (Figs. 1c and 1d) and the literature data indicating that inclusion complexes of CD with aromatic molecules can form during the mechanical mixing of components, e.g., as reported for ofloxacin and HPCD [1].

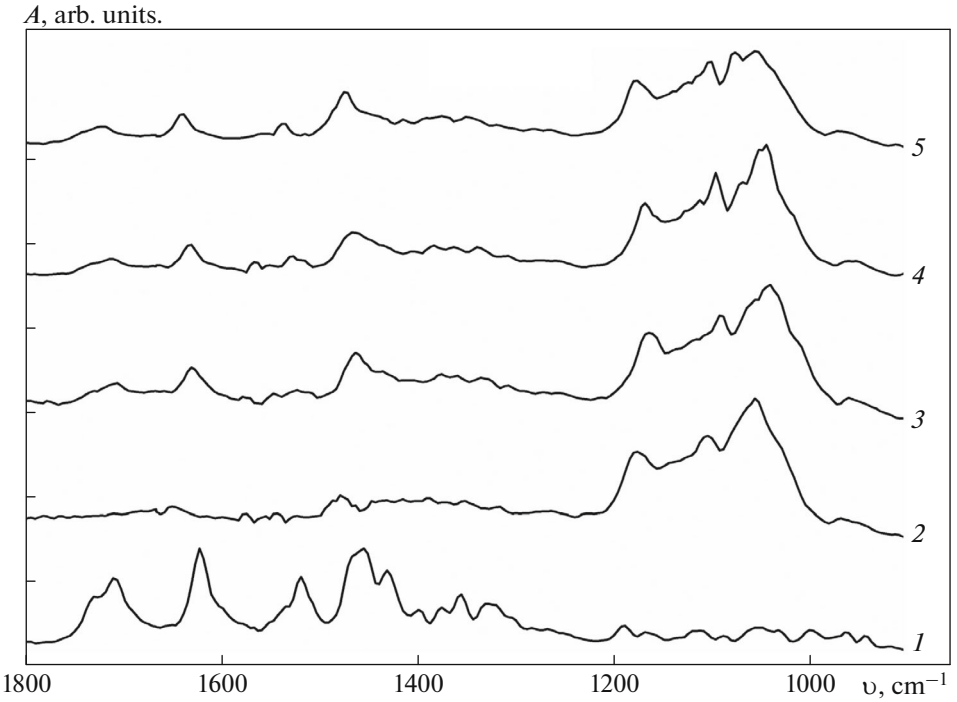

Fig. 2. IR spectra of powders: (*1*) MF, (*2*) HPCD, (*3*) MF–HPCD mixture, (*4*) complex obtained by lyophilization, and (*5*) RESS complex.

Table 2. Main absorption bands in the IR spectra of MF and MF–HPCD complexes

Group/peak position, cm^{-1}	ν_{C-C} (Ar)	$\nu_{C=C}$ (quinolone)	$\nu_{C=O}$	$\nu_{C=O}$ in COOH
MF	1428, 1452	1516	1620	1708
MF–HPCD, mech. mixt.	1460	1518	1628	1712
MF–HPCD, lyophilisate	1464	1526	1628	1706
MF–HPCD, RESS	1444, 1472	1532	1636	1706
Free HPCD	1172	1100	1052	970
HPCD in a mixture	1170	1090	1040	970
HPCD in a lyophilized complex	1168	1088	1036	970
HPCD in a RESS complex	1166	1086	1036	970

When an MF–HPCD complex was formed by lyophilization, in the region of the characteristic absorption bands of MF there were some changes similar to those described above for the mechanical mixture of components, but these changes were more distinct (curve *3*, Fig. 3). Thus, the absorption band of the carbonyl group (1620 cm^{-1}) shifted toward higher frequencies by 8 cm^{-1}; in the region of the ν_{C-C}(Ar) vibrations of the aromatic structure of MF, there is a high-frequency shift (from 1452 to 1464 cm^{-1}). The spectrum of the complex obtained by lyophilization generally differs from the spectrum of the mechanical mixture of MF and HPCD insignificantly. According to the SEM data, the morphology of the complexes obtained by the two methods is also qualitatively the same, which indicates that the MF–HPCD complexes have a similar structure, but the efficiency of MF inclusion in a complex with HPCD increases during lyophilization.

The spectrum of the complex obtained by RESS differs significantly from the spectra of the complex obtained by lyophilization (curve *2*, Fig. 3). For the absorption band of the carbonyl group, the shift toward higher frequencies was more pronounced (by 16 cm^{-1}) than for the lyophilized complex. In the region of absorption of the aromatic structure, we can also observe some characteristic changes: the 1516 cm^{-1} band shifted toward high frequencies by 16 cm^{-1} , and the 1452 and 1428 cm^{-1} bands shifted also toward higher frequencies by 20 and 16 cm^{-1}, respectively

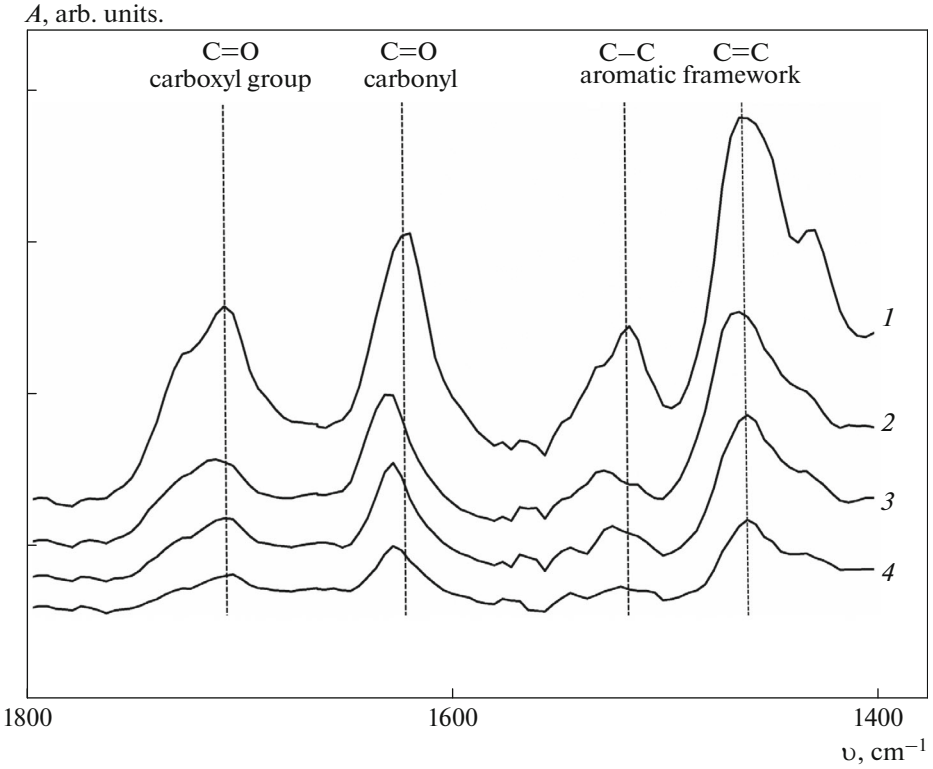

Fig. 3. IR spectra in the range 1800–1400 cm^{-1}: (*1*) MF, (*2*) MF–HPCD RESS complex, (*3*) MF–HPCD complex obtained by lyophilization, and (*4*) a mixture of MF with HPCD.

(Table 2). The observed high-frequency shifts in the IR spectra of MF indicate that the formation of the MF–HPCD complex involves the following functional groups of MF: the aromatic structure of quinolone and the carbonyl and carboxyl groups.

In the IR spectrum of HPCD, during its complexation with MF the absorption bands of the C–O–C, C–H, and C–O–H bonds of the polysaccharide framework undergo low-frequency shifts, indicating that these functional groups of CD are involved in the interaction with MF (Table 2). The observed changes are most pronounced for the MF–HPCD complex obtained by RESS, which suggests higher degree of MF inclusion in the complex.

The observed changes in the spectra of the complexes obtained by different procedures and accordingly the differences in the efficiency of inclusion of MF in the complex are explained by the fact that in the case of lyophilization, the complex forms in aqueous solution, where the motive force of complexation of MF with HPCD are hydrophobic interactions [11, 23]. In the case of RESS, the observed increase in the efficiency of formation of an MF–HPCD inclusion complex during the treatment with SC-CO_2 (80°C, 200 atm) is possibly caused by the molecular factors given below:

(i) the shift of thermodynamic equilibrium toward complexation (the process is performed at a pressure of 200 atm);

(ii) the decrease in the melting and vitrification points of the drug and HPCD, respectively, after the increase in the SC-CO_2 pressure and the transition of the mixture components to a molten state [8, 31, 32], which provides their more effective interaction during the RESS treatment.

In addition, the solubility of some drug substances containing an aromatic fragment and polar carboxyl or carbonyl groups (ibuprofen, acetylsalicylic acid, budesonide, econazole, etc.) considerably increases in SC-CO_2 when its pressure and reaction temperature increase. For various substances, the solubility (mole fraction) in SC-CO_2 at 40°C and 100 atm is $(2–5) \times 10^{-3}–5 \times 10^{-6}$ [33, 34]; when the pressure increases to 250 atm, it can increase 20-fold or more [31, 33–36]. A considerable increase in solubility was also observed when the reaction temperature increased from 40 to 80°C and further to 130°C [36]. This effect is most conspicuous at 200 atm and more, when reasonably high density of SC-CO_2 is achieved [34–36]. This increase in the solubility of MF in SC-CO_2 evidently also promotes the interaction of the components, as in the case of other similar complexes [31, 33].

IR Spectroscopy of MF–HPCD Complexes in Solution

The structural characteristics of the MF–HPCD complexes obtained by RESS and lyophilization were studied in detail by IR spectroscopy in aqueous solution, in which the effect of hydration on the structure of the complex can be taken into account. In addition, this allows evaluation of the stability of the complexes in aqueous solution. Figure 4 presents the IR spectra of the MF–HPCD complexes obtained by RESS and lyophilization in comparison with the data for the starting MF in a phosphate buffer (0.15 M, pH 7.5).

The position of the IR absorption bands of the main functional groups of solid MF and MF in aqueous solution is approximately the same. The relative intensities of these bands differ because of the difference between the physical and hydrated states. In the IR spectrum of MF in aqueous solution, the relative intensity of the band at 1500–1420 cm^{-1} corresponding to the absorption of the ν_{C-C} vibrations of the aromatic structure of quinolone increases relative to that in the solid state. This may be related to the formation of aggregates of MF molecules in aqueous solution due to the hydrophobic interactions [6, 7], in which the aromatic structures of FQ are involved in π-stacking interactions, leading to a change in the molar absorption coefficient of the corresponding functional groups. In contrast, the intensity of the bands of the carbonyl and carboxyl groups decreases (Fig. 4a) due to hydrogen bonding as a result of hydration.

For the MF–HPCD complexes obtained by different procedures, both the intensity and structure of the bands changed in the IR spectra of MF in aqueous solution. This effect is most pronounced for the absorption band of the $\nu_{C=C}$ vibrations of the aromatic structure of MF at 1500–1420 cm^{-1}. To determine the degree of inclusion of MF in the complex with HPCD, quantitative analysis of the relative integrated intensities of the components of this absorption band was performed by deconvolution. Note that the IR absorption bands of aqueous drugs (MF) are often multicomponent, with two to five components that reflect a certain state of the functional group. Thus, the absorption bands of the carbonyl and carboxyl groups are extremely sensitive to changes in the degree of hydration, while the bands of the C–C, C=C, and C–H bonds are mainly sensitive to changes in the polarity of the microenvironment and the packing density of molecules in the case of the formation of supramolecular structures. Studying the reference samples of FQs completely involved in the complex (obtained with a 20-fold mole excess of HPCD) and recording the dependences of the IR spectra of MF on the HPCD concentration, we experimentally determined the ratio of components characteristic of the functional groups in the state bonded with HPCD. Thus, in the IR spectrum of MF, the absorption band at 1500–1420 cm^{-1} is composite: for free MF, there are three main components, namely, 1440, 1452, and 1463 cm^{-1}, their contributions being 59, 35, and 6%, respectively. For the complex with HPCD, the contribution of absorption at 1452 cm^{-1} decreases due to the increased contributions of the other two components. In an excess of HPCD (HPCD : MF = 10 : 1), the

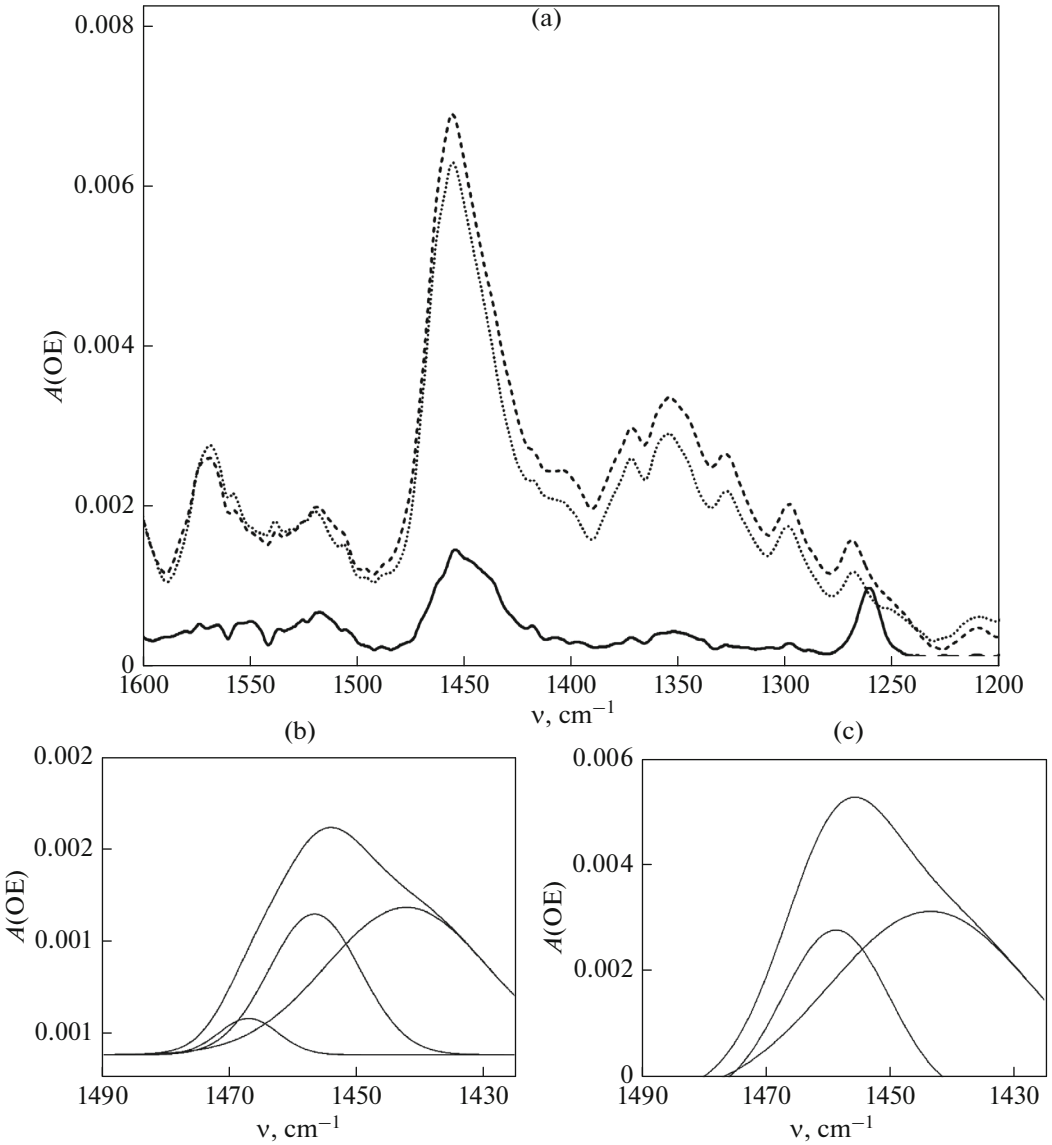

Fig. 4. (a) IR spectra of MF (solid line), MF–HPCD lyophilized complex (dashed line), and MF–HPCD complex (long-dash line) after the RESS treatment; (b) result of deconvolution of the absorption band at 1500–1420 cm^{-1} for the starting MF; and (c) the same for the MF–HPCD complex treated by RESS; conditions: concentration of MF 1.2 mg/mL and of the complex 2.5 mg/mL in 15 mM sodium phosphate buffer, pH 7.5, 25°C.

contribution of the 1452 cm^{-1} absorption decreased almost to zero. It was shown in an independent experiment that the change in the integrated intensity of absorption at 1452 cm^{-1} in the IR spectra of the samples relative to the initial spectrum correlates with the efficiency of inclusion of MF in the HPCD complex (determined by equilibrium dialysis) depending on the synthetic procedure.

For the complex obtained by lyophilization, the contribution of the 1452 cm^{-1} band decreases insignificantly, from 35 (in free MF) to 28%. For the complex obtained by RESS, the intensity of the band decreased to zero due to the increased contributions of the other two components (Fig. 4c). This suggests that in this case, the degree of inclusion of MF in the complex is considerably higher than for the complex obtained by lyophilization.

Kinetics of MF Release from the MF–HPCD Complexes in Aqueous Solution

Figure 5 shows the kinetic curves of MF release from the samples obtained by different methods in aqueous solution (pH 4). When analyzing these curves, it was assumed that two time-spaced processes occurred in the system: MF release into the external solution through the dialysis membrane and MF dissociation from the complex followed by its release into

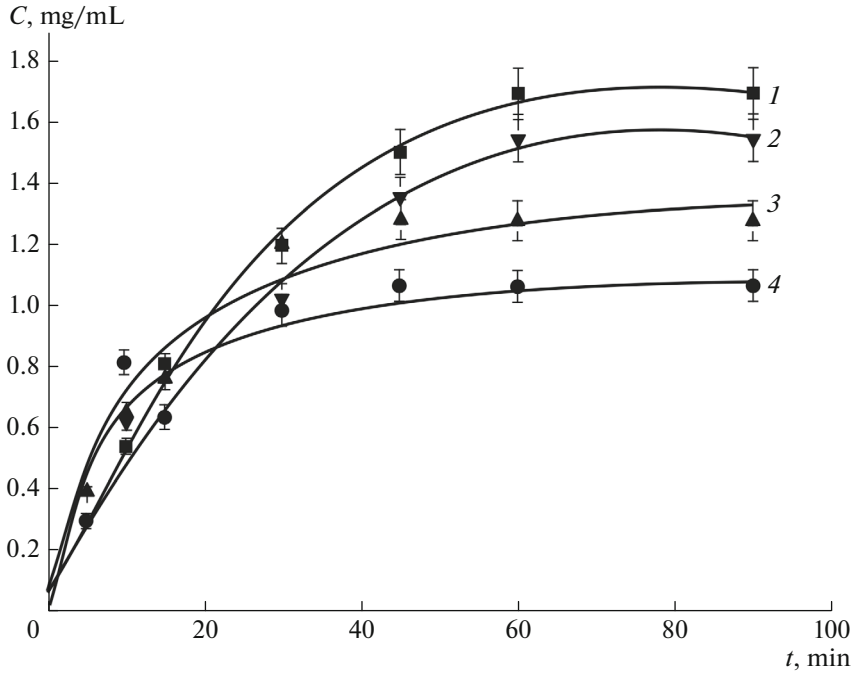

Fig. 5. Kinetic curves of MF accumulation in aqueous solution: (*1*) free MF (control), (*2*) mixture of MF and HPCD, (*3*) complex obtained by lyophilization, and (*4*) complex obtained by RESS. Conditions: 0.1 mM hydrochloric acid solution, pH 4, 37°C, initial MF concentration inside the dialysis membrane 4 mg/mL.

the external solution (which is a slower process). On the initial section of the kinetic curve (at less than 15 min), the MF concentration in the external solution rapidly increases for all the samples, which evidently corresponds to the liberation of MF not included in the complex. The time interval from 20 to 90 min on the kinetic curve shows significant differences in the MF release kinetics depending on the synthetic procedure. For the MF–HPCD mixture, the amount of MF that passed through the dialysis membrane into the external solution within 60–90 min differs from that of free MF by 10–15%, which corresponds to the amount of MF bonded with HPCD.

For the samples obtained by lyophilization and RESS treatment, a considerable amount of MF is retained inside the dialysis membrane due to the formation of the complex, whose dissociation is relatively slow: the retained amount was 25% after 60–90 min storage in solution in the former case and ~40% of MF contained in the system in the latter. This suggests that an MF complex with HPCD formed in both cases, but the degree of MF inclusion in the HPCD complex and the stability of the complex against dissociation in aqueous solution were higher in the case of RESS treatment, which agrees well with the IR spectral data.

Dissolution Rate of MF–HPCD Complexes

In drug design, the drug solubility and dissolution rate are very important characteristics that determine the bioavailability of the drug. The effect of complexation with HPCD on the dissolution rate of MF in model media of intestine and stomach with pH 7.5 and 4, respectively, was studied. The results are presented in Table 3.

The formation of an MF–HPCD complex by lyophilization considerably increases the drug dissolution rate: the amount of dissolved MF increased twofold within 5 min in acid medium and sixfold in alkaline medium. After prolonged (120 min) storage of the complex in weakly alkaline and acid media, the

Table 3. MF dissolution kinetics for MF–HPCD samples obtained by different methods at pH 4 and 7.5

Sample	Amount of MF dissolved at pH 4.0 g/L			Amount of MF dissolved at pH 7.5 g/L		
	5 min	15 min	120 min	5 min	15 min	120 min
MF	13.4 (±1)	16.1 (±1)	25 (±1)	5.8 (±1)	13.4 (±1)	32 (±1)
MF–HPCD obtained by lyophilization	22 (±1)	22 (±1)	35 (±1)	34.4 (±1)	34 (±1)	37 (±1)
MF–HPCD obtained by RESS	13 (±1)	18 (±1)	24 (±1)	6 (±1)	22 (±1)	32 (±1)

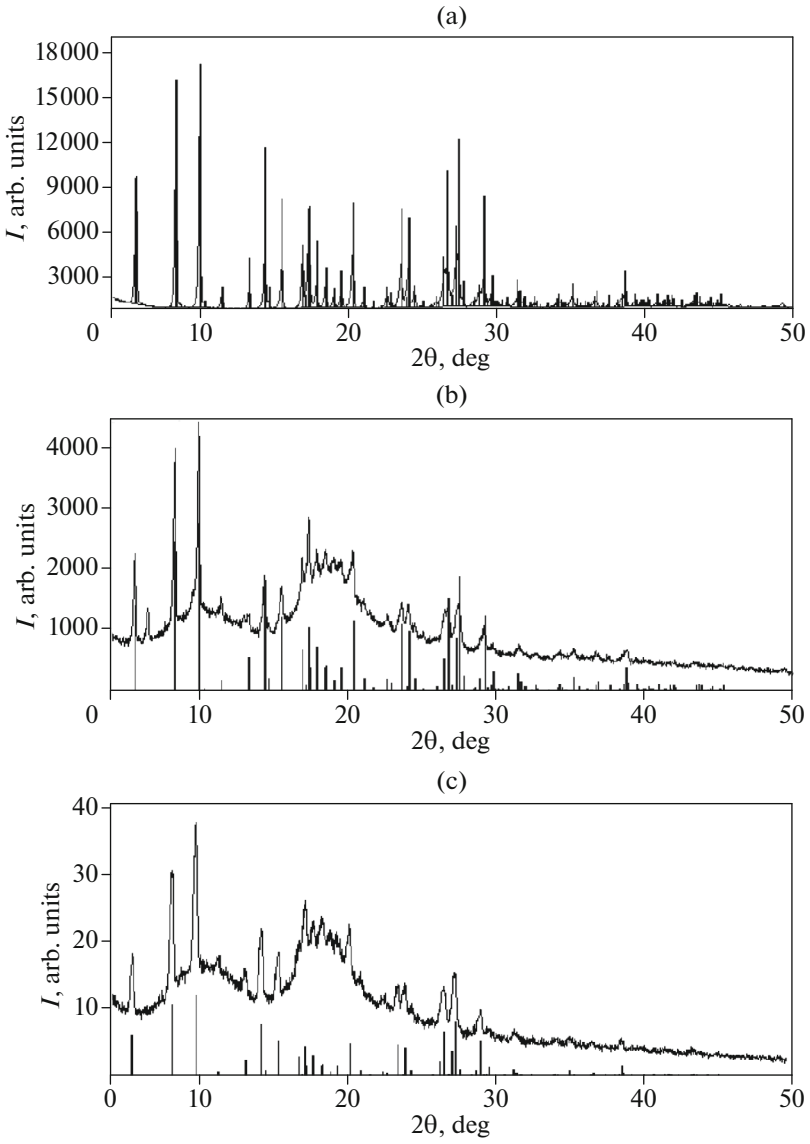

Fig. 6. XRD patterns of (a) the starting MF, (b) MF–HPCD complex obtained by RESS, and (c) MF–HPCD complex obtained by lyophilization.

amount of dissolved MF was higher than for the starting MF by a factor of 1.2 and 1.6, respectively. The dissolution rates of the complex obtained by RESS and the starting MF (Table 3) are comparable over the whole range of storage times in aqueous solutions. This result is slightly unexpected because the size of MF–HPCD particles obtained by RESS (2–4 μm) was considerably smaller than for the starting MF (15–20 μm). The observed differences in the MF dissolution kinetics from the samples obtained by different methods may be explained by differences in the structure of the complexes, particle morphology, or crystallinity.

Crystallinity of MF–HPCD Complexes

The XRD pattern shown in Fig. 6a corresponds to crystalline MF. In the MF–HPCD samples obtained by RESS and lyophilization, the main components are the amorphous phase and crystalline MF (Fig. 6). The crystallinity estimated from the integrated intensities of the amorphous halo and the peaks of the crystalline phases is of the order of 30% for the complex obtained by RESS and 20% for the lyophilized complex.

Thus, when the MF complexes with HPCD are formed by both RESS treatment and lyophilization, the position of the MF peaks in the XRD pattern remains unchanged, indicating that MF is not converted into another polymorphic modification. For MF in the complexes, the fraction of the amorphous phase, which forms due to the interaction of MF with HPCD, increased considerably (to 70 and 80%, respectively). The increased fraction of the amorphous phase is evidently the main reason for the increased rate of dissolution in the case of the MF–HPCD complex compared

with that of the starting MF. An increase in the fraction of the amorphous phase during the formation of the CD complex was also observed for other drugs: taxifolin, ibuprofen, and budesonide, and led, as in the case of MF, to a considerable increase in the dissolution rate of the samples [8, 37].

For MF–HPCD particles obtained by RESS, the rate of MF dissolution changed to a lesser degree compared to that of the starting MF. The differences in the dissolution rate of MF–HPCD particles obtained by different methods seem to be due to the differences in the particle structure and morphology. According to the SEM data, the use of RESS leads to the formation of visually dense particles in the form of irregular polyhedra; the use of lyophilization leads to loose powder with spherical particles.

CONCLUSIONS

An effective method for the preparation of MF–HPCD inclusion complexes by supercritical fluid technology in the RESS mode has been developed. The RESS treatment of a mixture of MF with HPCD leads to the formation of a new phase represented by particles in the form of irregular polyhedra with sizes of 3 or 4 μm. An analysis of the shifts of the characteristic IR bands of MF during the complexation showed that RESS ensures more effective interaction of the components, greater degree of drug inclusion in the HPCD complex compared with lyophilization, and higher stability of the complexes against dissociation in aqueous solution (pH 7.5).

The following mechanism of MF complexation with HPCD in SC-CO_2 can be suggested. As a result of the dissolution of a certain fraction of the drug in SC-CO_2, the MF molecules are included in the pores of CD due to the distribution of the dissolved drug between the CS-CO_2 phase and the inner void of CD accompanied by the formation of many-point intermolecular contacts, hydrogen bonds, and hydrophobic interactions between the MF and CD molecules. Our previous data obtained by molecular simulation of moxifloxacin and levofloxacin complexes with CD derivatives [38] confirm the present conclusions about the involvement of the corresponding functional groups of MF and HPCD in complexation.

The size of the obtained MF–HPCD microparticles (2–4 μm) is suitable for both peroral and inhalation forms of the MF-based drug [39]. The results of this study open up wide prospects for the use of RESS for developing the MF delivery systems of prolonged action for treating tuberculosis.

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