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Improving the dissolution of lidocaine by dispersing the rapid expansion of supercritical solution

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An experimental study of the grinding of lidocaine by the rapid expansion of supercritical solutions (RESS method) was carried out. The experiments were carried out in the pressure range of 10−35 MPa and temperatures of 308−333 K. The analysis of the morphology and size of particles, as well as the effect of the process parameters on them, was carried out. Qualitative and quantitative spectrophotometric analysis was carried out. The dissolution test was carried out in a phosphate buffered saline medium simulating plasma, which showed that the reduction in particle size gave an increased yield of the drug in a shorter period of time compared to the original drug that was not treated.

Keywords: supercritical fluid, microparticles, spectrophotometric analysis, lidocaine.

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Introduction

The growth in the number of therapeutically active, but relatively insoluble compounds obtained in the development of new drugs, creates serious obstacles in ensuring the required level of therapeutic effect of these substances. Undoubtedly, to achieve it, drugs that are poorly soluble in an aqueous medium must be used in large doses, which, in turn, leads to a large number of undesirable side effects and, in the case of potent drugs, is a serious problem.

The rate and extent to which an active drug ingredient or therapeutic component of a drug is absorbed from a pharmaceutical product and becomes available in the action area can be increased by various methods: production of its salt forms, formation of polymorphs, and the like. However, the most efficient and simple method is the micronization of the pharmaceutical substance. Micronization of hydrophobic drugs will significantly increase the rate of their dissolution in aqueous media, which directly depends on the particle size of the pharmaceutical substance, and, consequently, their bioavailability, reduce weight, thereby reducing the negative effect due to side effects.

The advantages of micronization methods using supercritical fluid media are the possibility of obtaining homogeneous particles with certain physical and chemical properties, sizes and morphology, controlled by changing the values of the regime parameters of the ongoing one-stage process, and environmental safety.

The main goal of this article is to study the effect of lidocaine pharmaceutical substance dispersion by the method of rapid expansion of supercritical solutions (RESS method) at the level of its dissolution. To achieve this goal, the following tasks were solved for the first time: four series of experiments were carried out to study the influence of regime parameters on the lidocaine pharmaceutical substance dispersion by the RESS method, and the size of the obtained particles was analyzed. A qualitative and quantitative analysis of dispersed lidocaine was carried out on a PE-5400UF UV spectrophotometer. The pharmaceutical substance was not subjected to thermal decomposition, molecular degradation, or any other negative effects during processing by the RESS method. The „Dissolution" test for the lidocaine pharmaceutical substance was carried out for the first time in a phosphate buffer solution simulating plasma.

1. Experimental part

1.1. Research procedure and unit

In this article ‚lidocaine is dispersed using a RESS-100 unit (Fig. 1) by Thar Technologies Inc. (USA, official distributor in the territory of the Russian Federation is „SCHAG" CJSC). The technique of the experiment is described in previous articles and dissertations [1,2].

The expansion channel is made of sapphire glass: hole diameter is 150 μm, channel length is 1000 μm.

1.2. Technique for analysis of the particle size and dispersion

Powders obtained as a result of dispersion of substances consist of particles with irregular geometric shapes and different sizes.

A sample of powder under study is placed on a glass slide in the form of a monolayer. To avoid aggregation of particles and their sticking, light mechanical grinding or

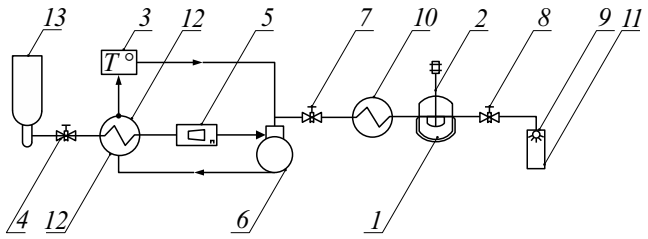

Figure 1. Thar RESS-100 experimental unit scheme: *1* — saturator, *2* — stirrer, *3* — thermostat, *4, 7, 8* — valves, *5* — flow meter, *6* — high pressure pump, *9* — expansion device, *10* — electric heater, *11* — expansion chamber, *12* — cooling heat exchanger, *13* — carbon dioxide cylinder; to cool the cylinders of the high pressure pump *6* and the carbon dioxide in the heat exchanger *12*, the thermostat *3* is turned on.

liquid dispersion using surfactants selected individually are allowed.

In this article, the particles were studied microscopically using a Levenhuk D320L optical microscope. The relative measurement error for 100 measurements and the confidence level of $P = 0.95$ is 1.5%.

1.3. Method for the lidocaine analysis on a UV spectrophotometer

In the State Pharmacopoeia (SPh) of the Russian Federation, physical methods (melting point determination) of analysis and titration are official ones for the lidocaine analysis — there are no articles on the use of any other methods in SPh of the Russian Federation. Thus, based on the analysis of the available literature, a UV spectrophotometric method for establishing the authenticity, purity and quantification of lidocaine was developed. The UV method is the most convenient and easiest for the standard analysis procedure [3,4].

During the analysis, 95% ethanol and a phosphate buffer solution with pH 7 are used as pure solvents. To obtain the spectrum of the sample under study, 2 procedures are performed sequentially:

1) baseline scanning: a blank sample is prepared, which is a pure solvent, in which the test substance will be analyzed later, i.e. null sample containing no analyte. A blank sample is poured into a quartz cell and placed in the path of the light beam into the cell compartment, which is then tightly closed;

2) scanning of the test sample: after performing baseline scanning, the test sample is placed in the working area and the scanning process is started, during which the program window displays a spectrum graph and a data table with the values of wavelengths, absorbency and transmission. To prepare the test sample, 100 mg of test substance is weighed with an error of 0.005 mg of the test substance, dissolved in a pure solvent, the volume is adjusted to 100 ml, and then diluted with the same solvent to concentration of 0.15 mg/ml.

On the spectral curve, the analytical wavelength corresponding to the wavelength of maximum absorption is determined.

1.4. Qualitative analysis Identification procedure

Using UV spectrophotometer and „Scan54" software, the absorption spectra of the original and micronized drug (D) solutions in the same solvent are obtained and compared. The treated D is recognized as identical to the original one in case of observation of the coincidence of the maximum, minimum points, shoulders and inflection points in the spectrum working region.

1.5. „Dissolution"test procedure

The „Dissolution" test is carried out on a heated digital magnetic stirrer, model WiseStir MSH-20D, by DAIHAN Scientific Co. (Republic of Korea).

A certain volume of the dissolution medium (500 ml) is placed in a laboratory vessel and placed on the heating surface of a magnetic stirrer. A phosphate buffer solution simulating plasma with pH 7 is used as a dissolution medium. The temperature of the dissolution medium is controlled throughout the study and is $(37.0 \pm 0.5)^\circ$C.

The exact weighed amount of the drug (0.25 g) is added to the dissolution medium. Sampling is carried out from the zone of the dissolution vessel, located at half the distance between the surface of the dissolution medium and the stirrer blade and at a distance of at least 1 cm from the walls of the dissolution vessel. Sampling is carried out at the initial time, when the analyzed drug was placed in the dissolution medium, and every subsequent 10 min until complete dissolution is achieved.

The samples taken are poured into quartz cells and sequentially placed on the path of the light beam into the cell compartment of the UV spectrophotometer, which is then tightly closed. The analysis is carried out at a previously found analytical wavelength and using saved files with built calibration graphs. The absorbency of the analyzed samples is measured. Next, the concentration value is calculated.

The drug is considered to be completely dissolved when its concentration in the dissolution medium reaches 0.5 mg/ml.

1.6. Materials

In this article, $C_{14}H_{22}N_2O$ lidocaine (white crystalline powder) obtained for research from SIGMA-ALDRICH, with purity over 98%, is used as test substance. Molecular weight is 234.3373 g/mol, melting point is 68.5°C.

According to SPh of the Russian Federation, lidocaine is classified as „moderately soluble" in water, i.e. to dissolve 1 part of lidocaine, 30−100 parts of the solvent will be required.

Carbon dioxide with a purity of 99% (GOST 8050-85) was used as a supercritical solvent. Critical parameters of CO_2: $T_{cr} = 303.9$ K, $P_{cr} = 7.38$ MPa.

Ethyl alcohol 95% (ethanol) (GOST 18300-72) was used as a reference solution for spectrophotometric analysis: a mixture of ethanol and water containing 95% ethanol by volume. It is a solvent for a number of drugs, including lidocaine. As mentioned above, lidocaine is soluble in alcohol, chloroform, benzene and ether. However, all of these solvents, except for ethanol, are highly toxic substances that are not used in pharmaceuticals. This is the reason for the choice of ethyl alcohol as a reference solution.

A phosphate buffer solution with pH 7.0 at temperature of 20°C: a transparent, colorless, odorless mobile liquid (TU 2642 -071-23050963-2009) is used as a reference solution for spectrophotometric analysis and dissolution medium that simulates plasma during the „Dissolution"test. This solvent was used in the analysis of micronized lidocaine.

Table 1. Conditions and results of lidocaine dispersion by the Rapid Expansion of Supercritical Solutions (RESS) method, the line indicates untreated lidocaine, dashed line — lidocaine, treated

№	T_{sat}, K	T_{ed}, K	P_{sat}, Pa	d_i, μm	d_p, μm
Series of experiments № 1					
1	308	338	20	1.4–5.4	2.3
2	313			1.3–3.8	2.2
3	318			1.1–3.9	2.1
4	323			1.2–3.5	1.9
5	328			1.2–3.1	1.9
6	333			1.1–2.5	1.7
Series of experiments№ 2					
1	313	338	10	1.1–2.5	1.6
2			15	1.2–2.8	1.8
3			20	1.3–3.8	2.2
4			25	1.5–4.6	2.2
5			30	1.2–6.6	2.4
6			35	1.4–6.4	2.6
Series of experiments№ 3					
1	308	338	35	1.6–6.5	2.8
2	313			1.4–6.4	2.6
3	318			1.4–4.7	2.1
4	323			1.2–3.2	1.9
5	328			1.1–2.9	1.7
6	333			1.1–2.5	1.6
Series of experiments№ 4					
1	333	338	10	—	—
2			15	1.0–2.9	1.6
3			20	1.1–2.5	1.7
4			25	1.2–3.7	2.9
5			30	1.1–3.8	1.8
6			35	1.1–2.5	1.6

Note. T_{ed} — expansion device heating temperature *9*, (Fig. 1).

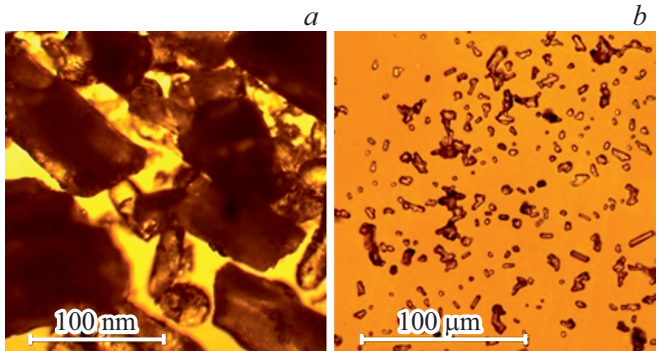

Figure 2. Lidocaine particles: *a* — untreated, average size 108.12 μm (40x magnification); *b* — after RESS grinding, average particle size 1.65 μm (400x magnification).

This buffer system is formed by primary and disubstituted salts of phosphoric acid, where primary salts are weak acids, and disubstituted ones have noticeable alkaline properties.

2. Results and discussion

Dispersion of lidocaine using carbon dioxide as a supercritical solvent was carried out on the RESS-100 experimental unit. In total, four series of experiments were carried out with the following process parameters:

1. Series of experiments № 1. The pressure in the system is 20 MPa, the saturator temperature range is from 308 to 333 K.

2. Series of experiments № 2. The temperature of the saturator is 313 K, the pressure range in the system is from 10 to 35 MPa.

3. Series of experiments № 3. The pressure in the system is 35 MPa, the saturator temperature range is from 308 to 333 K.

4. Series of experiments № 4. The temperature of the saturator is 333 K, the pressure range in the system is from 10 to 35 MPa.

The measurement results are presented in Table 1.

The powder particles obtained in the № 1 and № 4 series of experiments turned out to be so small that the optical power of the microscope used to analyze the sizes and morphology of the studied particles was not enough to determine their average size.

Figure 2 shows lidocaine particles before and after RESS grinding.

The analysis was carried out on a Levenhuk D320L optical microscope with a 400x magnification. A decrease in the average size by more than 60 times was shown.

2.1. Effect of temperature on particle size

As can be seen from Fig. 3, with increasing temperature at a higher pressure, the size of the obtained particles changes more significantly. So, if on the 20 MPa isobar the average particle size at 308 to 333 K decreases from 2.3 to

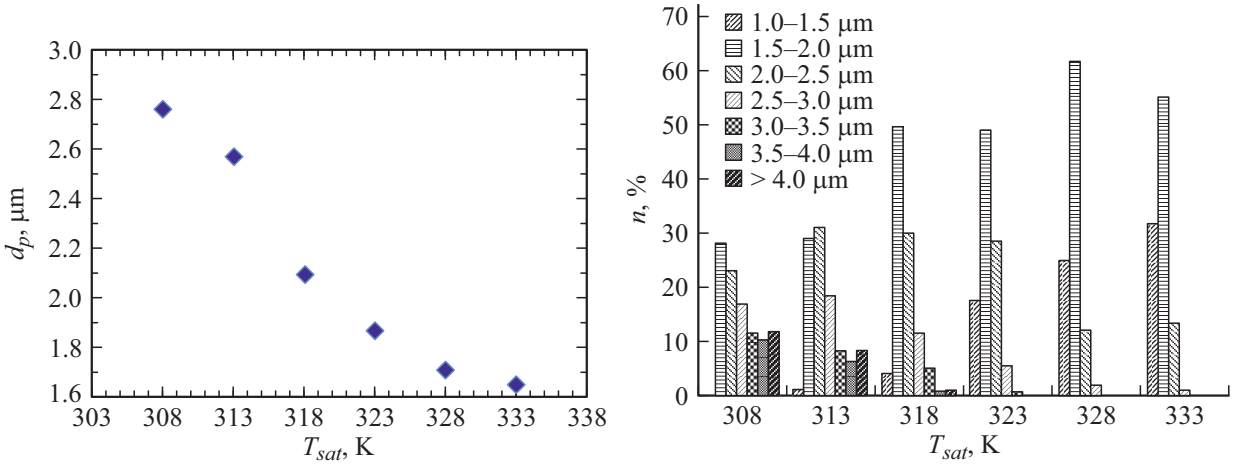

Figure 3. Dependence of the average particle size on the temperature of the saturator and the histogram of the average particle size distribution in a series of experiments № 3 at pressure in the system of 35 MPa.

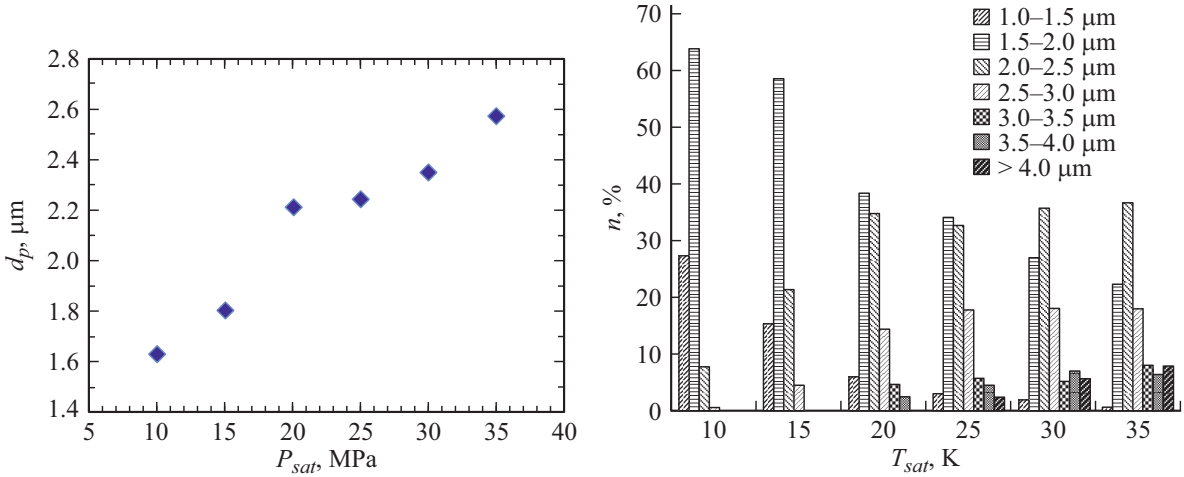

Figure 4. Dependence of the average particle size on the pressure in the system and the histogram of the average particle size distribution in a series of experiments № 2 at saturator temperature of 313 K.

1.7 μm, then at 35 MPa the average particle size decreases already from 2.8 to 1.6 μm.

This can be explained by the fact that higher pressure in the system leads to an increase in the mass flow rate of the solution and causes a decrease in the residence time in the expansion channel. Thus, the time for particle growth in the expansion channel will be reduced. In addition, with an increase in the extraction temperature, according to the article [3], solubility of lidocaine in supercritical carbon dioxide increases, which, in turn, leads to an increase in the degree of supersaturation. In a saturated solution, a greater number of critical nuclei are formed, and this subsequently enables to obtain a greater number of smaller particles, which is clearly reflected in the histograms of the average particle size distribution in these series of experiments: with increasing temperature, the number of particles with sizes of 1–2 μm increases significantly, while at 333 K an insignificant percentage of particles with sizes up to 3 μm and a complete absence of larger particles are observed. Dispersion of the resulting powder increases, the particles acquire the correct spherical shape, and a significant decrease in their agglomerations is observed.

2.2. Effect of pressure on particle size

As can be seen from Fig. 4, the size of the obtained particles on the 313 K isotherm increases with increasing pressure. The solution is saturated at low process parameters, which is clearly reflected in the histogram of the average particle size distribution: in powders obtained at pressures of 10 and 15 MPa, a significant percentage of particles with sizes of 1–2 μm is observed. However, a further increase in pressure leads to an increase in the particle size: the percentage of particles with sizes 1–2 μm decreases significantly, along with this, the number of particles with sizes 2.5–3.0 μm increases, and at 20 MPa, the appearance of particles with sizes from 3 μm and more is observed.

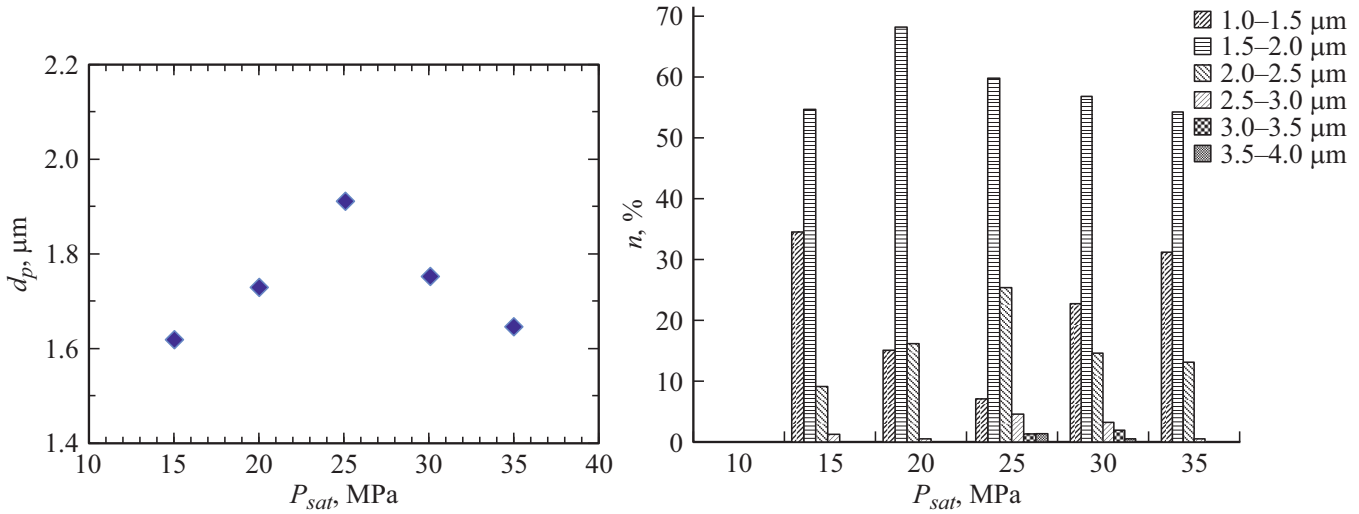

Figure 5. Dependence of the average particle size on the pressure in the system and the histogram of the average particle size distribution in a series of experiments № 4 at saturator temperature of 333 K.

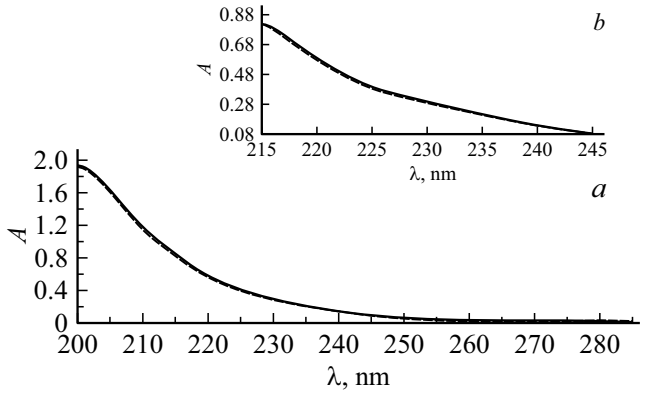

Figure 6. Absorption spectra of ethanol solutions of untreated and RESS-dispersed lidocaine, scanning step 1 nm. Solid line indicates untreated lidocaine, dashed line — lidocaine treated by the RESS method: *a* — wavelength range 200–285 nm; *b* — enlarged region of the UV absorption spectrum of lidocaine, wavelength range 215–275 nm.

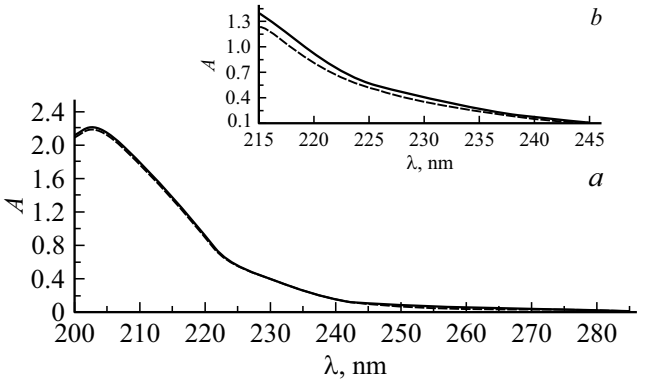

Figure 7. Absorption spectra of untreated and RESS-dispersed lidocaine in a buffer solution, scanning step 1 nm. Solid line indicates untreated lidocaine, dashed line — lidocaine treated by the RESS method: *a* — wavelength range 200–285 nm; *b* — enlarged region of the UV absorption spectrum of lidocaine, wavelength range 215–245 nm.

Figure 5 clearly shows the ambiguous effect of changing the pressure in the system on the size of the obtained pharmaceutical substance particles on the 333 K isotherm.

At 10 MPa, the solubility of lidocaine in supercritical CO_2 was very high, and precisely as a result of a greater degree of supersaturation, particles of such a small size were obtained that they could not be seen in the microscope used in this study. In the extraction pressure range from 15 to 25 MPa, an increase in the particle size is observed. At high pressures (30 and 35 MPa), a tendency to a decrease in particle size was noted: the number of particles with sizes 1–2 μm increases significantly along with a decrease in the number of particles with larger sizes.

2.3. UV spectrophotometric analysis of micronized local anesthetic drug. Samples spectra. Peak definition

Using UV spectrophotometer and Scan54 software in the ultraviolet range with a set scanning step of 1 nm, spectral curves of the dependence of absorbency on the wavelength of untreated and treated lidocaine in 95% ethanol (Fig. 6) and in phosphate buffer solution with pH 7 (Fig. 7) were obtained[4,5].

In each case, the absorption of lidocaine is in the wavelength range 200–285 nm. Solutions of samples in alcohol (Fig. 6) and solutions of samples in a buffer (Fig. 7) in the wavelength range 200–285 nm gave almost identical patterns of the spectrum.

Table 2. „Dissolution"test results

t, min	Untreated lidocaine			Micronized lidocaine		
	α, 1/m	n_k, mg/ml	n, %	α, 1/m	n_k, mg/ml	n, %
0	0.138	0.048	9.6	0.625	0.230	46.0
10	0.354	0.122	24.4	1.045	0.385	77.0
20	0.660	0.228	45.6	1.169	0.430	86.0
30	0.875	0.302	60.4	1.279	0.471	94.2
40	1.005	0.347	69.4	1.342	0.494	98.8
50	1.098	0.379	75.8	1.35	0.497	99.4
60	1.215	0.419	83.8	1.352	0.500	100
70	1.255	0.433	86.6	1.352	0.500	100
80	1.298	0.448	89.6	1.352	0.500	100
90	1.328	0.458	91.6	1.352	0.500	100
100	1.356	0.468	93.6	1.352	0.500	100
110	1.375	0.474	94.8	1.352	0.500	100
120	1.392	0.480	96.0	1.352	0.500	100
130	1.404	0.484	96.8	1.352	0.500	100
140	1.428	0.493	98.6	1.352	0.500	100
150	1.446	0.499	99.8	1.352	0.500	100
160	1.460	0.500	100	1.352	0.500	100
170	1.460	0.500	100	1.352	0.500	100
180	1.460	0.500	100	1.352	0.500	100

The maximum light absorption of each of the studied solutions of untreated and RESS-dispersed lidocaine in 95% ethanol (1.907 and 1.877, respectively) was observed at wavelength of 200 nm (Fig. 6). And at wavelength of 202 nm, a peak was observed for both solutions of untreated and micronized lidocaine — 2.204 and 1.952, respectively (Fig. 7).

Since large light scattering is observed in this wavelength region, it was decided to exclude from consideration the absorbency in the wavelength region below 215 nm; in the absorption spectra in this wavelength region, the components of the buffer solution actively absorb. Since light absorption by all studied solutions of lidocaine is insignificant at wavelength above 245 nm, the selected spectral window was within the wavelength range of 215−245 nm. In this wavelength region, lidocaine solutions show a characteristic shape of the absorption spectrum (Fig. 6, *b* and 7, *b*).

According to the SPh of the Russian Federation XIV [6], the minimum values of the relative error in the measurement of absorbency A are adopted at $A = 0.434$. Therefore, they try to work in the absorbency range of 0.3−0.8, since in this absorbency range the devices are calibrated with the highest accuracy.

Thus, based on these statements, we decided to use $\lambda = 223$ nm as analytical wavelength when working with an ethanol solution, at which $A = 0.447$ for treated lidocaine, and when working with buffer solutions — $\lambda = 228$ nm, at which the optical densities of the untreated and micronized pharmaceutical substance are 0.460 and 0.405, respectively.

A comparative analysis of the spectral curves of the dependence of absorbency on the wavelength of untreated and treated lidocaine in both solvents shows that in the spectra working regions the maximum points (200 nm for ethanol and 202 nm for phosphate buffer solution), shoulders and inflection points coincide. The spectra are almost identical to each other, i.e. the pharmaceutical substance was not subjected to thermal decomposition, molecular degradation, or any other negative effects during processing by the RESS method. Thus, the processed drug is recognized as identical to the original lidocaine.

2.4. „Dissolution"test results

„Dissolution"test was carried out on a heated digital magnetic stirrer, model WiseStir MSH-20D, by DAIHAN Scientific Co. The samples taken were poured into quartz cells and sequentially analyzed using a UV spectrophotometer. The analysis is carried out at a previously found analytical wavelength (when working with buffer solutions $\lambda = 228$ nm) and using saved files with built calibration graphs. The absorbency of the analyzed samples was measured with the subsequent determination of concentrations.

The purpose of this test is to compare the dissolution level of the original pharmaceutical substance and micronized one by rapid expansion of the supercritical solution. The main evaluation criterion is the rate at which the drug will dissolve in a phosphate buffer solution simulating plasma. The results of the „Dissolution" test are listed in Table 2 and clearly shown in Figure 8. One can immediately note the fact that already at the initial time, a larger amount of lidocaine micronized by the RESS method (46%) was dissolved in the phosphate buffer solution compared to its original, non-micronized substance (9.6%).

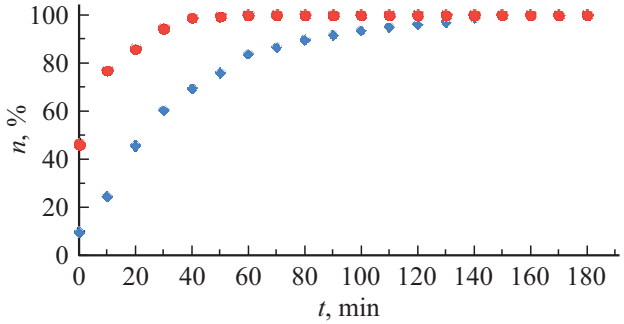

Figure 8. Dissolution dynamics. Diamond — untreated lidocaine, circle — micronized lidocaine.

As stated above, the drug is considered to be completely dissolved when its concentration in the dissolution medium reaches 0.5 mg/ml. Micronized lidocaine reached this 100% indicator after 1 h. Although, as can be seen from Table 2, already after half an hour its concentration in the dissolution medium reached 94.2%, while the original drug was dissolved only by 60%. It reached complete dissolution after 160 min, i.e. it took 3.2 times longer than treated lidocaine. Thus, micronization of lidocaine by the RESS method contributed to an increase in the dissolution rate of pharmaceutical substances. During the first half hour of the „Dissolution" test, the active therapeutic component was released from micronized lidocaine more than 1.5 times compared to the original form of the pharmaceutical substance. This will reduce the consumption of the drug for preparation of solutions for parenteral administration and speed up the technological process of their preparation.

Conclusion

An experimental study of the effect of grinding lidocaine by the RESS method on the activity level was performed. To achieve this goal, the lidocaine pharmaceutical substance was dispersed by the RESS method in the pressure range of 10−35 MPa and temperature range of 308−333 K.

As a result, microparticles of the studied drug were obtained. During the analysis of the particle sizes of the obtained micronized lidocaine powders, it was found that the average particle size significantly depends on the conditions of the experiment (temperature, pressure).

A spectrophotometric analysis of the micronized local anesthetic drug was carried out in the ultraviolet region of the spectrum. The spectra of the test samples were obtained. A qualitative analysis aimed at establishing authenticity showed that during treatment by the RESS method, the pharmaceutical substance was not subjected to thermal decomposition, molecular destruction, or any other negative effects. Calibration graphs are built, suitable for determining unknown concentrations of solutions of the test substance in 95% ethanol and phosphate buffer solution. To prepare the stock solution, 100 mg of lidocaine was accurately weighed, dissolved in ethanol, and the volume was adjusted to 100 ml. Then the initial solution was diluted with ethanol to the required concentrations: 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 mg/ml. To build a calibration graph for the photometric determination of dispersed lidocaine in phosphate buffer solution, the optical densities of ten standard solutions were measured similarly at the same concentrations.

A „Dissolution"test was carried out in a phosphate buffer solution medium simulating plasma, showing that reduction in particle size gave an increased yield in a shorter period of time compared to the untreated original drug.

Thus, micronization of lidocaine by the RESS method contributed to an increase in the volume of the released active therapeutic component of micronized lidocaine at the initial time and an increase in the dissolution rate of the pharmaceutical substance. The performed work and the results obtained in the course of it confirm the efficiency of the use of supercritical fluid technologies for micronization of pharmaceutical substance powders, which are relatively insoluble in water, but are widely used in therapeutic practice.

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Conflict of interest

The authors declare that they have no conflict of interest.

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