

Study of Physical and Chemical Properties of Solid Dispersions of Quercetin

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Abstract

Aim: Increasing solubility of medicinal substances is a major problem in the pharmacy, since about 40% of produced pharmaceutical substances are poorly soluble, and of newly synthesized substances up to 60% have low solubility in water and aqueous solutions. For poorly soluble drugs, the limiting stage of the absorption process is usually the rate of its dissolution, and therefore, much attention is paid to the development of ways to increase it. To solve this problem, a number of methods are used which can be conditionally divided into physical and chemical. **Materials and Methods:** Objects of the study were chosen quercetin, quercetin solid dispersions (SDs) with polyvinylpyrrolidone (PVP), polyethylene oxide (PEO)-6000, and β -cyclodextrin (β -CD). The solubility of the samples was studied spectrophotometrically. The particles' shape and size were studied using microscopic analysis. X-ray diffraction analysis was performed on a DRON-3 installation at monochromatic radiation $K\alpha - Cu$ ($\lambda = 1.54 \text{ \AA}$) by recording diffraction mappings on the diffractogram tape in the angular range $2\theta = 5-500$. **Results and Discussions:** In the article, the results of solubility study of quercetin and its SDs based on PVP, PEO, and β -CD obtained by melting with the removal of solvent are presented. The results obtained suggest the possibility of quercetin polymorphic transformations in the composition of SD. Polymer effect on increasing the solubility of quercetin in the composition of the SD has been shown. **Conclusion:** Using physicochemical methods of analysis, change in the crystal structure of quercetin under the influence of high-molecular substances has been proved.

Key words: Microscopic analysis, polyethylene oxide, polyvinylpyrrolidone, quercetin, solid dispersions, solubility, X-ray-phase, β -cyclodextrin

INTRODUCTION

The proper choice of a rational dosage form and technology of its manufacture is a necessary condition for producing high-quality medicines with the necessary therapeutic activity.

Typically, between the rate of dissolution of the active pharmaceutical ingredient (API) in biological fluids and its bioavailability, there is a linear dependence.^[1] For practically water-insoluble substances, therapeutic activity is often determined by their rate of dissolution. Increased solubility will contribute to both release of API from the dosage form, and passage through biological membranes - absorption.^[2] There are various ways to enhance bioavailability, and recently, more focus is on physical modification techniques, creating solid dispersions (SDs). SDs are bi- or multi-component systems, consisting of an API and a carrier representing a highly dispersed solid phase of the API or its solid solutions with the partial formation of variable composition complexes with a carrier material.^[3,4]

Analysis of the literature has shown that as a carrier different polymers (or combinations thereof) can be used including polyethylene oxide (PEO) of varying molecular weight, polyvinylpyrrolidone (PVP), and β -cyclodextrin (β -CD).^[5-7]

Efficacy of drugs depends on the bioavailability of the API. Currently, the World Health Organization has identified groups of APIs that require the study of bioavailability. These primarily include the steroid hormones, cardiac glycosides, hypoglycemic action drugs, anticonvulsants, coumarin anticoagulants, antibiotics, antioxidants, and other chemotherapy drugs.^[8,9]

The aim of our work was the study of macromolecular substances influence on the change of physical and

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chemical properties of quercetin, which has a wide range of pharmacological activity.

MATERIALS AND METHODS

Objects of the study were chosen quercetin,^[9] quercetin SD with PVP, PEO -6000, and β -CD. Dissolution studies were conducted according to the procedure of the State Pharmacopoeia of Ukraine, using a magnetic stirrer with adjustable heater equipped with a device for temperature control (magnetic stirrer RCT BASIC [IKA, Germany]). Test samples were prepared by liquid-phase method by melting with a partial removal of the solvent. Samples of the API and SD to study the dissolution were taken so as to form a saturated solution. Experiment temperature was $37 \pm 1^\circ\text{C}$. Samples were dissolved in 50.0 ml of purified water at stirring (stirrer speed about 200 revs/min), added 30 mL of ethyl alcohol, and brought to the mark (quercetin solution No. 1). 1 ml of No. 1 quercetin solution placed in 100 ml volumetric flask and adjusted to the mark with 0.1 M HCl. As a reference solution used 0.1 M HCl. If necessary, the sample was filtered. For filtering, the selected samples used Minisart injector nozzle having a pore size of 0.45 microns. Adsorption spectra of the test solutions were recorded on evolution 60S spectrometer in cuvettes with 10 mm thickness in the range from 220 to 500 nm.^[10]

X-ray diffraction analysis was performed on a DRON-3 installation at monochromatic radiation $K\alpha$ - Cu ($\lambda = 1.54 \text{ \AA}$) by recording diffraction mappings on the diffractogram tape in the angular range $2\theta = 5\text{--}50$. The phase identification in the test samples was carried out by comparing the diffractograms with standards.^[10]

Microscopic analysis was performed using a scanning electron microscope "SEM-106 I" with the energy-dispersive analysis system and a low vacuum chamber. On the glass-ceramic plate, double-sided adhesive tape was applied. On the adhesive side, stencil was placed with holes-windows for samples. In the windows, using tweezers, samples of solid dispersion were placed. The backing inverted and excess samples powder removed by the air jet. To increase the image contrast copper layer was sprayed. The backing with samples was placed into the object chamber of the microscope. The contact of applied electroconductive film and samples table was provided by clamping electrode. Examination of the samples was carried out in the secondary electrons mode. With a slight increase in field of view, an object was chosen and fixed images at different scales.^[10]

RESULTS AND DISCUSSION

The absorption spectrum of quercetin solution in 0.1 M HCl was recorded on a spectrophotometer evolution 60S in cells with a layer thickness of 10 mm in the area from 220 to 450 nm. The results obtained are shown in Figure 1.

The adsorption spectrum of quercetin test solution in 0.1 M HCl is characterized by a relatively narrow absorption band with a maximum at 254 nm, which is characteristic for the absorption of aromatics and broad, intense long-wavelength absorption band with a maximum at 366 nm characteristic for flavonoids. This band is sufficiently specific, meets all the requirements for analytical absorption bands, and can be used for the quantitative determination of quercetin in a solution, in the absence of the influence of other components of the solution on its total spectrum.

To test the possibility of spectrophotometry using to determine the concentration of quercetin solutions, it was necessary to investigate the influence of excipients comprising the SD on the total spectrum of the quercetin test solution. For this purpose, according to the standard method of solubility, study solutions of placebo tablets with various adjuvants were prepared. The results of research are shown in Figure 2.

All three samples showed a slight sharp peak with λ_{max} at 291 nm. Absorption intensity gradually decreases. In the area of 350–380 nm, absorption of electromagnetic radiation

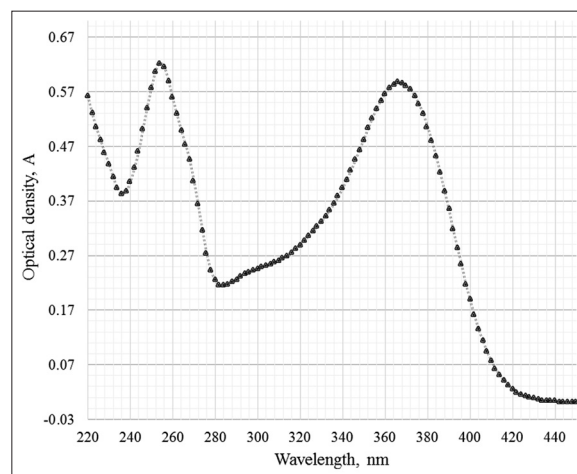


Figure 1: The absorption spectrum of quercetin solution in 0.1 M HCl

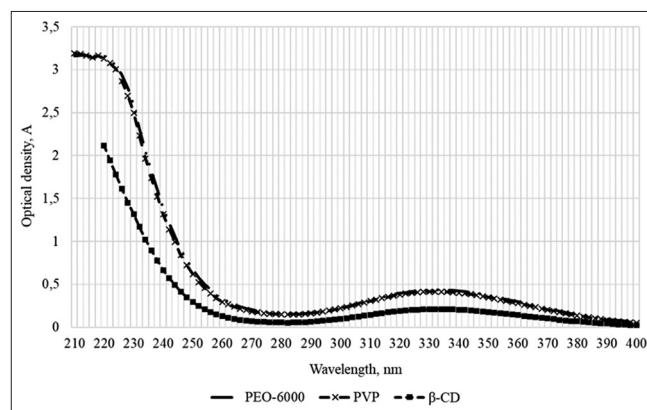


Figure 2: Adsorption spectra of the placebo solutions: (a) Polyvinylpyrrolidone, (b) polyethylene oxide-6000, (c) β -cyclodextrin

is virtually absent, which suggests the absence of effect of excipients on the total spectrum of quercetin test solutions. Thus, the results of studies conducted indicate the possibility of a direct spectrometric determination of quercetin solution in 0.1 M HCl at 366 nm.

One of the main requirements, determining the possibility of spectral methods use to quantify a substance, is its solution light absorption subjection to Bouguer-Lambert-Beer law. Checking subjection to Bouguer-Lambert-Beer law is reduced to constructing a plot of the absorbance A versus the solution concentration. Light absorption of solutions obeys the Lambert-Bouguer-Beer's only in the concentrations in which calibration graph is a straight line. Within this limit, the absorption coefficient χ is calculated as specific absorption index. Specific absorption index of solutions is calculated by the following formula:

$$A_{1\text{Am}}^{1\%} = \frac{A}{cb},$$

Where

A: The absorbance of the test solution,
c: Concentration of the solution g/100 ml,
b: the layer thickness in centimeters.

The calibration curve is shown in Figure 1. $\lambda = 366 \text{ nm}$

Analysis of the data obtained presented in Figure 3 shows that the light absorption of quercetin solution in 0.1 M HCl obeys the Lambert-Bouguer-Beer's law in the range of 0.2–1.8*10⁻³% quercetin in the sample.

Specific absorption rate in the investigated range is = 590 ± 4.4. Investigation of the quercetin solubility showed its solubility - 0.17%.

To determine the degree of high-molecular substances influence on the quercetin solubility, samples of SD obtained by melting with the removal of the solvent in a ratio of 1:1 have been investigated, as comparison model served quercetin substance. The results are shown in Figure 4.

As evidenced by the analysis of the data obtained, the lowest percentage of active substance passed into a solution has pure quercetin (0.17%), and the highest amount of quercetin dissolved is observed in SD with PEO-6000 (5.38%). It is 32 times more than the mass fraction of dissolved pure quercetin. The average value has SD of quercetin with β -CD (4.85%), which is 29 times more than in pure quercetin. Moreover, the lowest value has a SD of quercetin with PVP (1.25%). From the results of the study, it can be concluded that the addition of PEO-6000 has the highest influence on the mass fraction of dissolved quercetin from a SD. It can be assumed that such an effect on the solubility is due to the ability of the PEO to lower melting temperature of a mixture, altering the energy of the crystal lattice of the insoluble API in

the direction of decreasing the energy, and strengthening the crystal lattice at the same temperature in the presence of a source of thermal energy. It is also possible that, after removal of the solvent, the greatest possible contact occurs to form molecular complexes of API-polymer while maintaining a homogeneous polymer structure.

In the formation of SD drug substance in varying degrees can lose its crystallinity. In result of this process partly formed a solid solution or finely dispersed API solid phase in polymer. Proof of the latter can be changed in the radiographs of SD [Figure 5].

Analysis of the obtained quercetin diffractogram showed that the sample has a defined sequence of narrow high diffraction peaks of X-rays that allows suggesting that the material is well crystallized and homogeneous in the lattice parameters.

Diffractograms of quercetin SD show X-rays diffraction on the polymer structure and the reduction or complete disappearance of diffraction on API crystals. Diffractogram of SD with PVP has substantial differences in the nature and intensity of the maxima peaks and absorption, compared with other specimens studied. The results obtained indicate an almost complete amorphization of quercetin in the composition of the sample.^[11]

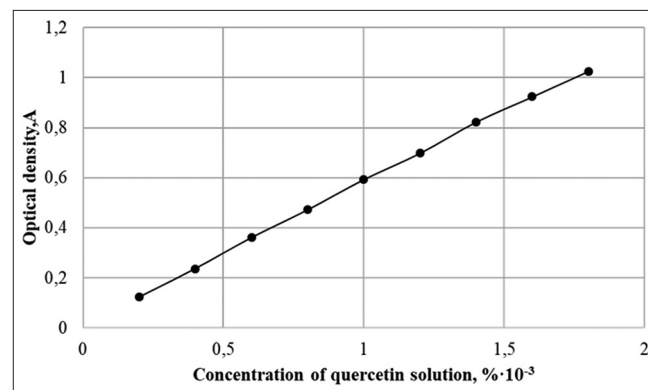


Figure 3: The calibration graph of quercetin solutions absorbance against concentration of quercetin

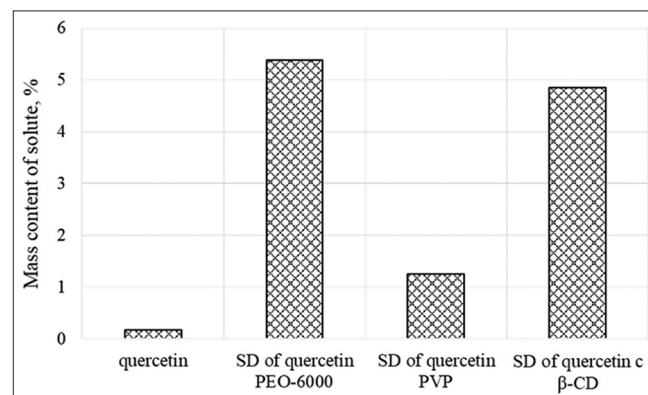


Figure 4: Mass fraction of the dissolved quercetin in test samples

Radiographs of SD with β -CD and PEO represent the sum of the original API and polymer peaks, indicating partial retention of the crystalline structure. Amorphization of quercetin with β -CD is 29%. Radiographs of SD with β -CD show X-ray diffraction on the structure of the polymer and complete disappearance of diffraction on the crystal structure of the API. The intensity of the peaks characteristic of quercetin is reduced by 40%. In the sample with PEO-6000 is formed a new structure, which is confirmed by the presence of peaks atypical for quercetin and polymer. The crystal structure change occurs, presumably, as a result of the failure of the API to return to the original crystal structure at evaporation of the solvent [Figure 5].

Additional insight into the crystalline state of a matter in the SD provides microscopic analysis [Figure 6].

Microcrystalline profiles of quercetin SDs are fundamentally different from the picture of pure API substance. SD after solvent removal is homogeneous, transparent background with different shape inclusions. Quercetin substance is a mixture of yellow needle crystals with a size of at least 20 microns. The appearance of the dispersions obtained indicates visual change in the shape and size of quercetin crystals in the obtained SDs that indicate a possible change in its physical and chemical properties under the influence of the polymers.

For a more detailed study of SDs, their electron microscope images were obtained [Figure 7]. For obtained SDs, characteristic is the formation of solid solutions with inclusions which linear dimension ranges from 1 to 10 microns. In all SD samples is observed changes of how are they look as compared to the original substance, which corresponds to the results of X-ray diffraction analysis.^[12]

For the sample based on PEO, characteristic is the change of the quercetin crystalline structure. SD of quercetin in PEO in terms of microcrystalloscopy presumably represent combined systems, consisting of a solution of the API in the polymer containing amorphous and crystalline forms, that is confirmed by X-ray diffraction data. Crystallization process of PEO-based SD takes place under the influence of the polymer while maintaining a certain degree of quercetin crystallinity; modified API crystals are formed, uncharacteristic for both initial and recrystallized quercetin. Picture of SD with β -CD represents a sum of micronized samples of quercetin and β -CD. Increasing the solubility of quercetin in this case is likely to occur through a combination of factors: Micronization, solubilization, and/or complexing in the dissolution time of the SD with β -CD.

The sample with PVP is characterized by the formation of solid solutions with specific multiple inclusions, and partial

change of the API crystal structure under the action of PVP is observed.

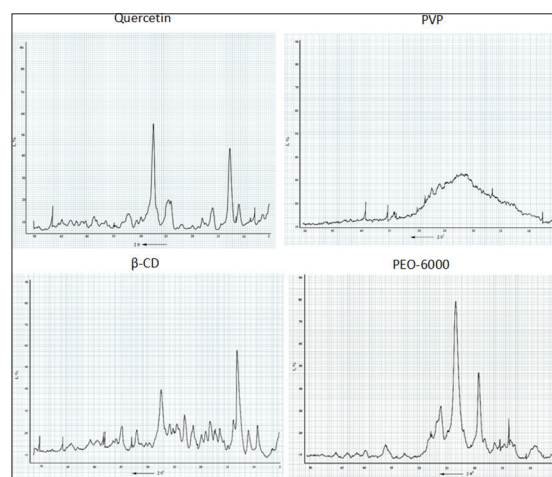


Figure 5: The diffractograms of the studied samples

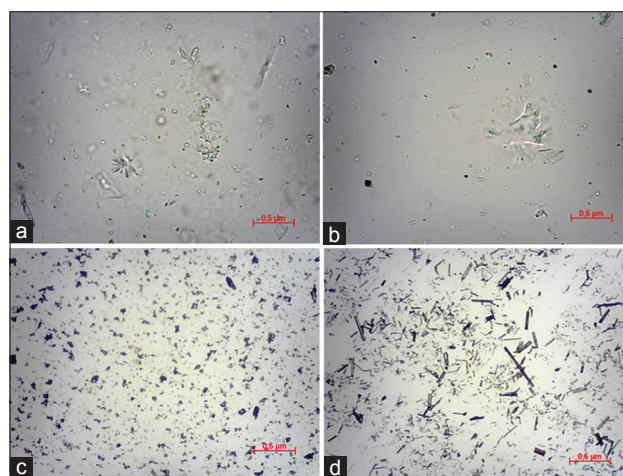


Figure 6: Solid dispersions of quercetin and quercetin substance, (a) PVP, (b) PEO-6000, (c) β -CD, (d) quercetin

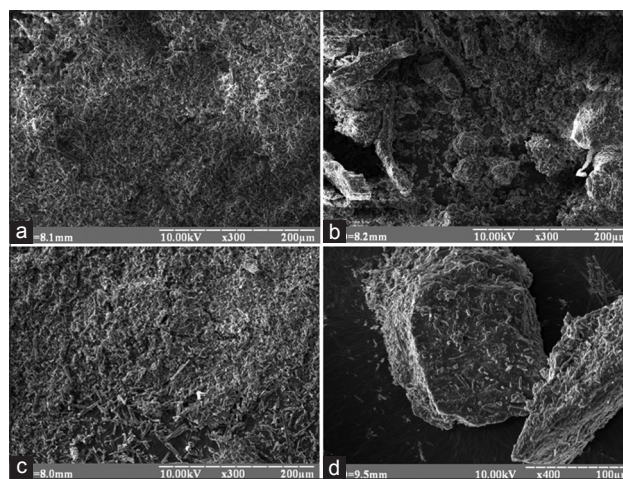


Figure 7: The results of electron microscopic analysis, (a) quercetin, (b) SD with PVP, (c) SD with β -CD, (d) SD with PEO-6000

CONCLUSION

The results obtained indicate that, under the action of polymers on quercetin in SD obtained by the liquid-phase method, polymorphic transformations may occur, i.e., transition of one crystalline structure into another, crystal structure disordering until complete amorphization, and change of quercetin molecular structure.

In comparative aspect, the effect of polymer in the SD composition on increasing the solubility of quercetin has been shown.

Using a complex physicochemical methods of analysis, change in the crystal structure and quercetin amorphization in SD has been proven.

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