ISSN 0036-0244, Russian Journal of Physical Chemistry A, 2018, Vol. 92, No. 12, pp. 2588–2592. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © A.B. Mirgorodskaya, R.A. Kushnazarova, N.A. Zhukova, V.A. Mamedov, L.Ya. Zakharova, O.G. Sinyashin, 2018, published in Zhurnal Fizicheskoi Khimii, 2018, Vol. 92, No. 12, pp. 1983–1987.

BIOPHYSICAL CHEMISTRY

Solubilization of Biologically Active Heterocyclic Compounds by Biocompatible Microemulsions

A. B. Mirgorodskaya[a,*], R. A. Kushnazarova[a], N. A. Zhukova[a], V. A. Mamedov[a,b], L. Ya. Zakharova[a,b], and O. G. Sinyashin[a,b]

[a] *Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, Kazan, 420088 Russia*

[b] *Kazan National Research Technological University, Kazan, 420015 Russia*

**e-mail: mirgoralla@mail.ru, mirgorod@iopc.ru*

Received March 1, 2018

Abstract—Microemulsions based on biocompatible components with high water content are applied to increase the solubility of medicinal drugs of the heterocyclic series indomethacin and 1-[5-(4-chlorophenyl)-3-phenylpyrrole-2-yl]benzimidazole-2(3H)-one (PBI). The solubilizing capacity is characterized quantitatively via spectrophotometry. It is shown that four-component microemulsion water/oleic acid/Tween 80/ethanol increases the limits of indomethacin solubility by more than two orders of magnitude, and that of PBI by more than three orders relative to water.

Keywords: microemulsions, solubilization, solubility, medicinal drugs

DOI: 10.1134/S0036024418120312

INTRODUCTION

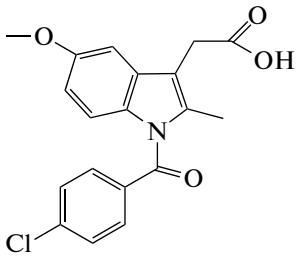
The most important property of systems based on surfactants is their ability to solubilize hydrophobic compounds, thereby increasing their solubility. This property is of utmost practical importance, since it helps to solve problems of reagent compatibility and facilitates their participation in chemical processes, simplifying the administration of biologically active compounds to animals or plants and ensuring the targeted drug delivery [1–4]. The solubilization action of surfactants is associated with formation of supramolecular aggregates in their solutions [5]. One of the simplest examples of such aggregates are micelles composed of self-organized associates of biphilic molecules. These form in solutions of surfactants, eventually reaching what are termed critical micelle concentrations (CMCs) [6]. Another type of practically important supramolecular systems based on surfactants is exemplified by microemulsions. These are macroscopically homogenous, thermodynamically stable microdisperse systems consisting of aqueous and hydrocarbon (oil) phases separated by layers of micelle-forming surfactants that in some cases include such co-surfactants as alcohols C4–C6 [7, 8]. Compared to micellar systems, microemulsions are characterized by slightly larger sizes of aggregates (10–100 nm), high surfactant contents, substantial fractions of disperse phases, and appreciably higher solubilization capacities that ensure the success of their application in various areas of modern chemistry, engineering, and pharmacology.

Features of the use of microemulsions as carriers of medicinal drugs are related to their ability to solubilize both lipophilic and hydrophilic compounds efficiently [9]. Small droplet sizes and extremely extended surface interfaces ensure redistribution of a preparation between the dispersed phase and the dispersion medium of a microemulsion, allowing us to maintain a constant concentration of a drug. The use of microemulsions protects drugs from different types of degradation, increases their bioavailability, reduces the required dosage, and minimizes side effects [10, 11]. Selecting the components of a microemulsion correctly is of great importance: they must consist of biocompatible, nontoxic, and pharmaceutically acceptable compounds [12]. Nonionic surfactants that are compatible with most pharmaceuticals and are not aggressive are used most often as stabilizers [13].

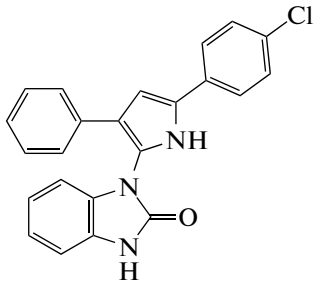
The aim of this work was to create microemulsions based on biocompatible components with high water contents in order to increase the solubility of biologically active compounds of heterocyclic nature. We focused on the familiar nonsteroid anti-inflammatory drug indomethacin, and on the benzimidazole derivative 1-[5-(4-chlorophenyl)-3-phenylpyrrole-2-yl]benzimidazole-2(3H)-one (PBI). The availability of pharmacophore fragments in the molecule of this compound all but guarantees biological activity; preliminary testing

revealed its sugar lowering action in particular [14–16].

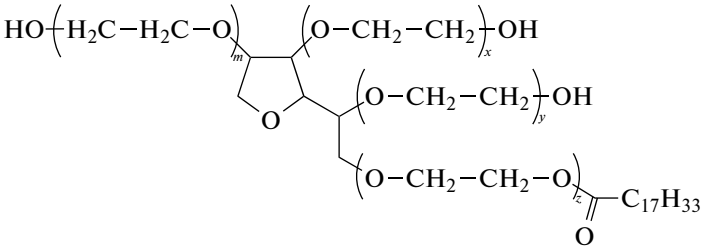
Nonionic surfactants Tween 80 and Tyloxapol were selected as our bases for forming emulsions. The solubilization activity of microemulsions was compared to the action of micellar solutions based on the same surfactants. The formulas of investigated compounds are presented below:



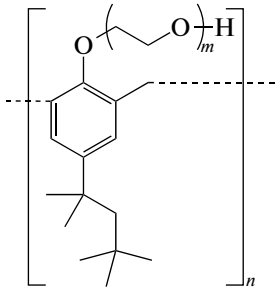
Indomethacin



1-[5-(4-Chlorophenyl)-3-phenylpyrrole-2-yl]benzimidazole-2(3H)-one (PBI)



Tween 80 ($x + y + z = 20$)



Tyloxapol ($n = 5$, $m = 7$)

EXPERIMENTAL

A commercial preparation of indomethacin (SigmaAldrich) containing 99% of the main substance was used in this work. A laboratory sample of benzimidazole derivative (PBI) was produced according to [14], and its characteristics were in agreement with those reported in that work.

Oleic acid, decane, and nonionic surfactants Tween 80 and Tyloxapol were used to prepare microemulsions, and ethyl and butyl alcohols (SigmaAldrich) were used as co-surfactants. Phosphate buffer (pH 6.86) prepared from fixanal and bidistilled water obtained using a Direct-Q 5 UV unit (pH 6.8–7, χ = 2–3 μS cm^{-1}) was used as the aqueous component. Microemulsions were formed by mixing surfactant with the required amounts of oil, to which the co-surfactant and water were then added.

Electronic spectra of the solutions were recorded with a Specord 250 Plus spectrophotometer (Germany). The investigated samples were placed in quartz cuvettes with path length L = 0.1–1 cm and thermostatted at 25°C. Molar extinction coefficients ε were determined from the values of optical density D of each sample at the certain concentration C of solubilizer using the equation $\varepsilon = D/LC$.

The efficiency of micellar solution and microemulsion solubilization towards indomethacin and PBI was determined via spectrophotometry from the content of the compound upon saturation of the system, as in [17, 18]. The content of solubilized compounds in each sample was found from the obtained optical density values with allowance for the extinction coefficients.

The size of aggregates was determined via dynamic and electrophoretic light scattering on a Malvern ZetaSizer Nano photon correlation spectrometer (Malvern Instruments, United Kingdom). A He–Ne gas laser with a power of 10 mW and a wavelength 633 nm was used as the light source. The scattering angle was 173°. The time of pulse accumulation was 5–8 min. Signals were analyzed with a single-plate multichannel correlator connected to computer with a program package for estimating the effective hydrodynamic radius of particles.

The viscosity of microemulsions was determined according to Poiseuille law by measuring the duration of a liquid flow in a calibrated viscometer at an experimental temperature of 25°C.

Distribution oil/water coefficients P were determined by dissolving 10 mL of indomethacin in 10 mL of oleic acid, with the subsequent addition of 10 mL of phosphate buffer. Each mixture was shaken for 5 min

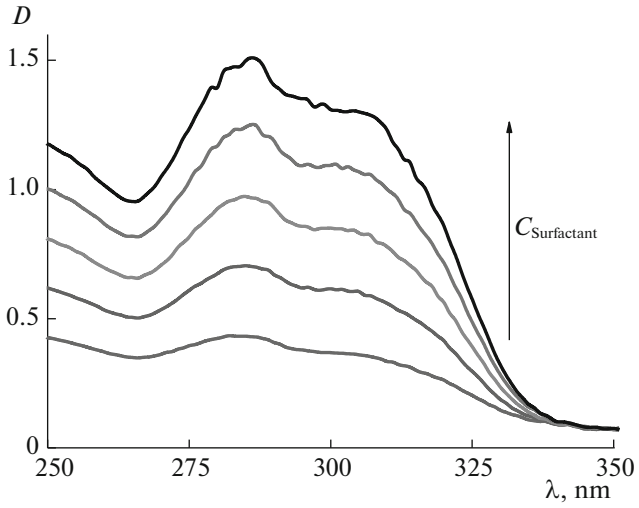


Fig. 1. Absorption spectra of saturated PBI solutions in the presence of Tween 80.

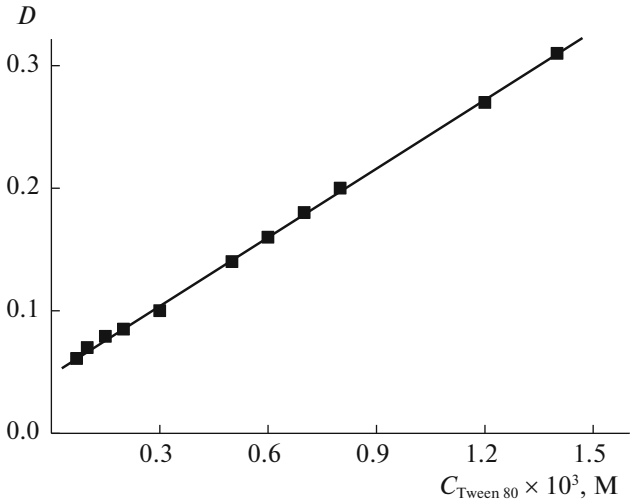


Fig. 2. Dependence of the optical density of a micellar solution saturated with PBI on the concentration of the surfactant (λ = 305 nm; 25°C).

and centrifuged for 1 h for layer separation. The content of indomethacin was then determined spectrophotometrically in the aqueous layer. The effect Tween 80 and ethanol had on the distribution of indomethacin between the water and oil was investigated at a 4% content of them in each system.

RESULTS AND DISCUSSION

A necessary condition for characterizing the solubilization properties of a system is being able to perform the simplest and most reliable analysis of the content of a preparation in it. The molecular structure of compounds investigated in this work allowed us to use electronic spectroscopy for this purpose. We showed in our earlier studies on indomethacin [17, 18] that the content of this compound in a neutral medium can be determined from the absorption maximum at 327 nm (ε = 5800 mol^{-1} L cm^{-1}), which changes only slightly upon moving to micellar solutions of Tween 80. In this work, the electronic spectra of aqueous PBI solutions were recorded for the first time. Two absorption bands are visible in them: the maximum of the first band lies in the region of 285 nm and has a molar extinction coefficient of 24 000 mol^{-1} L cm^{-1}. The second one is at 305 nm (ε = 22000 mol^{-1} L cm^{-1}) (Fig. 1). The second band is less intense, however, and less interference is observed in this region. Because of this, it was selected as an analytical signal. It should be noted that the position and intensity of the band did not change upon moving to alcohol or micellar solutions. Based on the spectral data, it was established that the limit of PBI solubility was no greater than 6×10^{-6} M. The solubility of PBI grew in Tween 80 solutions in the range of concentrations above the CMC upon an increase in the concentration of surfactant.

The dependences presented in Fig. 2 characterize the changes in the optical density of saturated PBI solutions at the maximum of absorption, which were used to determine solubilization capacity *S*. The values of this parameter were calculated using the equation $S = b/\varepsilon$, where *b* is the slope of the linear part of the dependence of adjusted optical density on the concentration of surfactant. The solubilization capacity of Tween 80 with regard to PBI was 0.0085. With regard to indomethacin, it was considerably higher (S = 0.065) [17]. Considering the lower water solubility of PBI relative to indomethacin, however, the efficiency of Tween 80 is higher for this particular preparation.

Moving from micellar systems to microemulsions, we would expect a stronger solubilization effect. To increase the solubility of heterocyclic compounds, we therefore used direct four-component microemulsions with a high water content (79.20 wt %) that were based on Tween 80 [19]. The content of surfactant in each system was 9.43 wt %. *n*-Butyl alcohol was used as the co-surfactant, and decane (1.96 wt %) as the oil. It was shown via dynamic light scattering that particles with hydrodynamic diameters of 23 nm were present in this microemulsion (polydispersity index, 0.30) and were stable for long periods of time. The maximum content attained for indomethacin was 0.004 M (0.15%), and that of PBI was 0.004 M (0.15%). The microemulsions thus had a stronger solubilizing effect than micellar systems. However, the presence of butanol and decane in the investigated microemulsion limits its in vivo applications, requiring further optimization of its composition. Replacing decane with a biocompatible component (oleic acid, squalene, mineral or olive oil) did not allow stable microemulsions to form with the same component ratio described above.

Table 1. Characteristics of four-component microemulsions water/oleic acid/Tween 80/ethanol (c_1 and c_2 are the saturating concentrations of indomethacin and PBI, respectively)

Component	ME I	ME II	ME III	ME IV	ME V	ME VI*
Composition, wt %						
Water (phosphate buffer, pH 6.86)	5	13	17	20.4	23	17
Oleic acid	7	19	25	17.3	17	25
Surfactant	31	24	21	22.6	22	21
Ethanol	57	44	37	39.7	38	37
Characteristic						
Viscosity, St	1.52	3.48	3.81	3.28	3.54	
Diameter, nm	8.7	11.7	28.2	68.1	122.4	
PdI	0.359	0.441	0.245	0.106	0.110	
c_1, mol/L	0.014	0.015	0.040	0.0172	0.0158	0.019
c_2, mol/L	0.0040	0.0044	0.0092	0.0057	0.0050	0.0064

* Tyloxapol was used as the surfactant; ** PdI is polydispersity index.

In the next stage, we used the oil/water microemulsions proposed in [20] for solubilizing rifampicin antibiotic as a basis. Their composition includes Tween 80, oleic acid, water (phosphate buffer pH 6.86), and ethyl alcohol, which acts as both a co-surfactant and a modifier of the aqueous phase in a microdroplet's nucleus. In a number of cases, another nontoxic surfactant—Tyloxapol—was used instead of Tween 80 (Table 1). The investigated compositions with water contents in the range 5–25% were microscopically uniform and stable over time.

Systems saturated with indomethacin and PBI were prepared on the bass of the resulting microemulsions. The solubility limits of these hydrophobic compounds in the investigated microemulsions were determined via spectrophotometry. The obtained results are presented in Table 1. The maximum solubility of indomethacin (0.04 M or 1.5%) was reached in the microemulsion with a water content of 17%. This demonstrates the high solubilization capacity of the tested system, which allowed us to increase the solubility of this preparation by approximately 200 times, relative to water. Similar results were obtained for PBI. The maximum content of that compound in water was 0.006 mM, but in the indicated microemulsion it was 7 mM—an increase in solubility of more than 1000 times. In all of the investigated microemulsions, the solubility of PBI was 3.5–4 times lower than that of indomethacin, allowing us to predict the solubility of one preparation based on the solubility of another determined experimentally. Replacing Tween 80 in the composition with another known solubilizer—Tyloxapol—did not alter the stability of microemulsions, but their solubilization capacity fell slightly (compare ME III and ME V in Table 1).

Investigating the solubility of a hydrophobic preparation in different components of the system and its distribution in binary and ternary compositions allows us to determine its location in the microemulsion. The low solubility of indomethacin not only in water but in oleic acid as well (less than 0.1%) leads us to conclude that the increased solubility in the microemulsion in particular was due primarily to the incorporation of the preparation into the interface layer organized by the surfactant and co-surfactant. This was corroborated by the change in distribution coefficient log P of indomethacin between the aqueous phase (phosphate buffer, pH 6.86) and oleic acid in the presence of Tween 80 and ethyl alcohol: the value of log P in binary system is 1.68. Adding 4% of ethyl alcohol affects the indicated value only slightly (log $P = 1.43$), while introducing the same amount of Tween 80 results in a substantial drop to 0.32, reflecting the lower indomethacin content in the oil phase.

The size of aggregates in the resulting microemulsions with and without indomethacin and PBI additives was determined by means of dynamic light scattering. As can be seen in Fig. 3, the systems were characterized by a monomodal size distribution of particles with hydrodynamic diameters of 3–25 nm (polydispersity index, 0.16–0.4). The size of particles grew along with the content of water in the system. Note that the viscosities of the freshly prepared emulsions we determined with the Stokes–Einstein model (Table 1) were used in analyzing the correlation function. The size of particles remained the same in the presence of hydrophobic preparations, while the polydispersity index grew (0.4–0.65). This leads us to suggest the preparations were distributed in the bulk of the system or in the surface layers of microdroplets. The pattern of the particle size distribution in the investigated microemulsions did not change during prolonged (more than one month) storage.

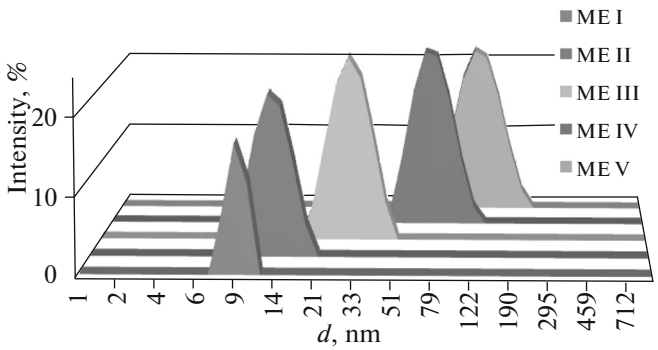


Fig. 3. Dependence of the hydrodynamic diameters *d* of particles in microemulsions on water content.

CONCLUSIONS

Stable microemulsions were prepared on the basis of biocompatible components with high water contents that allowed to increase the limit of indomethacin solubility by more than two orders of magnitude, and that of PBI by more than three orders of magnitude, relative to water. The considerable viscosity and high loading capacity of biologically active compounds, the low toxicity of their components, and the nanometer size range and stability of the microemulsions allow us to recommend them as systems for the transdermal delivery of hydrophobic medicinal drugs of a heterocyclic nature.

ACKNOWLEDGMENTS

This work was supported by the Russian Science Foundation, project no. 14-23-00073.

REFERENCES

1. *Solubilization in Surfactant Aggregates,* Ed. by Sh. D. Christian and J. F. Scamehorn (CRC, New York, 1995).
2. M. N. Khan, *Micellar Catalysis* (CRC, New York, 2007).
3. L. Ya. Zakharova, A. B. Mirgorodskaya, T. P. Zhiltsova, et al., in *Molecular Encapsulation: Organic Reactions in Constrained Systems,* Ed. by U. H. Brinker and J.-L Mieusset (Wiley, New York, 2010), p. 397.
4. K. Gurpreet and S. K. Mehta, Int. J. Pharm. **529**, 134 (2017).
5. L. Ya. Zakharova, R. R. Kashapov, T. N. Pashirova, et al., Mendeleev Commun. **26**, 457 (2016).
6. *Micellization, Solubilization, and Microemulsions,* Ed. by K. L. Mittal (Plenum, New York, 1977), Vols. 1, 2.
7. S. E. Friberg and P. Bothorel, *Microemulsions: Struture and Dynamics* (CRC, Boca Raton, New York, 1987).
8. Sh. P. Callender, J. A. Mathews, K. Kobernyk, et al., Int. J. Pharm. **526**, 425 (2017).
9. D. P. Acharya and P. G. Hartley, Curr. Opin. Colloid Interface Sci. **17**, 274 (2012).
10. M. J. Lawrence and G. D. Rees, Adv. Drug Deliv. Rev. **45**, 89 (2000).
11. A. Azeem, Z. I. Khan, M. Aqil, et al., Drug Dev. Ind. Pharm. **35**, 525 (2009).
12. I. Theochari, M. Goulielmaki, D. Danino, et al., Colloids Surf., B **154**, 350 (2017).
13. K. Shimoni and D. Danino, Langmuir **25**, 2736 (2009).
14. V. A. Mamedov, N. A. Zhukova, T. N. Beschastnova, et al., Org. Chem. **80**, 1375 (2015).
15. V. A. Mamedov, in *Quinoxalines. Synthesis, Reactions, Mechanisms, and Structure* (Springer Int., Switzerland, 2016), p. 397.
16. V. A. Mamedov, RSC Adv. **6**, 42132 (2016).
17. E. I. Yackevich, A. B. Mirgorodskaya, L. Ya. Zakharova, and O. G. Sinyashin, Russ. Chem. Bull. **64**, 2232 (2015).
18. A. B. Mirgorodskaya, L. Ya. Zakharova, E. I. Khairutdinova, et al., Colloids Surf., A **510**, 33 (2016).
19. A. B. Mirgorodskaya, E. I. Yackevich, L. Ya. Zakharova, et al., Chem. Phys. Lett. **567**, 18 (2013).
20. S. K. Mehta, K. Gurpreet, and K. K. Bhasin, Colloids Surf., B **60**, 5 (2007).

Translated by L. Brovko