Review of Doctoral Thesis

Author: **Zahari Penkov Vinarov**

Title: Enhancing the solubility of hydrophobic drugs by solubilization in micellar surfactant solutions

Thesis is presented to obtain the academic degree "Doctor of philosophy" (Ph.D.) in professional area: 7.3 Pharmacy (Pharmaceutical technology and biopharmaceutics) in Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, Department of Pharmaceutical and Applied Organic Chemistry.

Reviewer: Prof. Dimitar Rachev Rachev, Ph.D.

General description

The structure of thesis conforms to principles and requests to the structure of scientific thesis according to the state low and the University rules for academic degree "Doctor of philosophy".

The thesis consists of four chapters: Introduction, Materials and methods, Progesterone solubilization and Solubilization of Fenofibrate and Danazol. In thesis are included also: Main conclusions from the thesis, Contributions of the thesis and Literature (110 references). It consists of 77 pages, 33 figures and illustrations and 4 tables.

Topicality and aim of the thesis

The thesis research generates significant knowledge in a scientific area of biopharmacy – enhancing the solubility of poor water soluble drugs.

Poor water solubility is characteristic for more than 40% of the new chemical entities that emerge from the modern drug discovery programs. The slow and incomplete dissolution of such drugs in the gastro-intestinal fluids limits their oral bioavailability and is a major problem in drug product development. One of the classical approaches to improve the water solubility of hydrophobic drugs that is still being used in the pharmaceutical industry is to solubilize them in surfactant micelles.

Micellar solubilization is a powerful alternative for dissolving hydrophobic drugs in aqueous environments.

The aim of the thesis is to clarify how the surfactant and drug molecular structures determine the micellar solubilization capacity and to provide mechanistic physicochemical interpretation of the observed effects. For this purpose, is studied systematically the

solubilization of three hydrophobic drugs (progesterone, danazol and fenofibrate) by a wide range of surfactants and to establish the main trends from the obtained data, and to define possible mechanisms and hypotheses to explain the results.

Description and evaluation of the thesis

Introduction

Chapter 1 provides an introduction to the thesis and outlines the goals of the study. The author discusses the problems in the conceptual role of drug solubility and membrane permeability for the oral bioavailability, methods for enhancing drug solubility and in detail solubilization of drugs in micellar surfactant solutions.

Materials and methods

The three drugs are used in the thesis (progesterone, danazol and fenofibrate) that are BCS class II compounds, characterized by low solubility and high permeability Homologue series (from C10 to C18) of surfactants with different charge were studied: nonionic (polysorbates; ethoxylated alcohols), anionic (alkylsulfates) and cationic (trimethylammonium bromides). The materials are described accurately and completely.

The HPLC or gas chromatography (GC) methods are used for determination of drug solubilization in micellar surfactant solutions. Calculation of the drug solubilization capacity of the micelles is done. The shift of the absorption spectrum (UV/VIS) of the solubilized molecules is used to assess the polarity of their surroundings in the micelle. Laser diffraction is used to determination of CMC and micelle's size.

The thesis methodology is described in a way that allows reproducing the experiments.

Progesterone solubilization

The solubilization of Progesterone by 17 surfactants with a variety of hydrophilic head groups and hydrophobic chain lengths (C10-C14) are studied. Charged surfactants show highest solubilization capacity, increasing Progesterone solubility above 3 mg/ml (300 times higher than Progesterone solubility in water), whereas all nonionic surfactants have much smaller effect (0.5 to 1 mg/ml Progesterone solubility).

The high solubilization of Progesterone in charged surfactant micelles the author explains by ion-dipole interactions. The validity of the ion-dipole interaction hypothesis is checked by solubilization experiments with Androstane: a hydrophobic molecule with simple steroid structure, which in contrast to Progesterone does not contain any polar atoms or unsaturated groups.. The results show that the solubilization capacity of the ionic surfactants

for Androstane is much lower than for Progesterone. Therefore, ion-dipole interactions between Progesterone and surfactant head groups are the key for the micellar solubilization capacity. Ion-dipole interactions are confirmed in additional experiments at high ionic strength of 600 mM NaCl.

Increasing drug solubilization is observed with increasing of hydrophobic chain length for all studied surfactants, regardless of the type and charge of the hydrophilic head. Most likely, the improved solubilization is due to increased palisade layer volume.

Based on these results the author concludes that the best candidates to improve oral Progesterone absorption through solubility enhancement are surfactants with long hydrophobic chain and charged hydrophilic head group (e.g. alkylsulfates). Maximal solubilization capacity ($\chi max \approx 250$ mM/M) is obtained by the ionic surfactant - $C_{14}SO_4Na$.

Solubilization of fenofibrate and danazol

The solubilization of two drugs with different structure is studied: fenofibrate, which contains two aromatic rings, and danazol, which has a steroidal structure (similar to progesterone but one order less soluble). Two general trends are observed: (i) ionic surfactants solubilize danazol much more efficiently than fenofibrate and (ii) the nonionic surfactants solubilize fenofibrate better than danazol. Maximal solubilization capacity for fenofibrate ($\chi max \approx 50$ mM/M) is attained by several nonionic surfactants, while danazol is solubilized best by the ionic surfactants - maximal solubilization ($\chi max = 90$-100 mM/M) that is much higher than that of fenofibrate.

To analyze the effect of surfactant structure on solubilization capacity the effect of the hydrophobic chain length and hydrophilic head group are studied.

Linear increase of surfactant solubilization capacity with the increase of hydrophobic chain length is observed for both studied drugs for all surfactant types studied (nonionic, cationic, and anionic). Comparing the magnitude of solubilization capacity increase per CH_2-group, one sees that the effect is greater for danazol than for fenofibrate, for all surfactants studied. The effect is due to the increased volume for solubilization in the micelles.

The effect of the hydrophilic head group on the solubilization capacity of surfactants with C12 hydrophobic chain is compared. The micellar solubilization capacity of the polar fenofibrate and danazol molecules is affected dramatically by the surfactant head group. The results show that danazol is solubilized much more efficiently by ionic surfactants, compared to fenofibrate. This high solubilization of danazol in the ionic surfactant micelles is most likely due to ion–dipole interactions, like in the case of progesterone. This hypothesis is

supported with latter experiments with androstane (a hydrophobic molecule with simple apolar steroid structure, in contrast to danazol). As predicted, the solubilization capacity of the ionic surfactants for androstane is much lower than that for danazol.

The best solubilization of fenofibrate is achieved for the surfactant with sulfate head group. The addition of ethylene oxide groups in between the sulfate group and the alkyl chain decreases very strongly the solubilization capacity.

The studies of location of the fenofibrate solubilized molecule inside the surfactant micelles show that solubilization is in the palisade layer of ionic surfactant micelles and in the hydrophobic core of the nonionic surfactant micelles.

Final evaluation statement

This thesis represents a great deal of work. The influence of surfactant structure on the solubilization of hydrophobic drugs was clarified. The results are well presented and their interpretation is at a high scientific level. Thesis research generates significant new knowledge in a scientific area. The methodology scientifically sounds and described in a way that allows reproducing the experiments. The thesis demonstrates a solid understanding of the state-of-the-problem in the research area and the knowledge of the most important and current literature. The results presented clearly, with the appropriate controls and statistical analysis. The results discussed in relation to the research of others, and demonstrate a good understanding of the implications of the work in a broader scientific context.

It is clear that author is able to organize and realize the significant research work.

Contributions of the thesis

The performed study demonstrates the strong dependence of drug solubilization on the surfactant molecular structure and provides molecular-based insight on the probable mechanisms and interactions that control the observed effects. The role of specific intermolecular interactions on the micellar solubilization of hydrophobic drugs is clarified. The obtained information can be used for rational surfactant selection during the formulation development of poorly water-soluble drugs by micellar solubilization. The identified interactions and mechanisms can be used to develop theoretical models that predict solubilization. The generated database of drug solubilization by surfactants can be used to define drug solubilization in physiologically-based pharmacokinetic models.

Assessment of the criteria for PhD. Degree

The author has published two research papers in connection with the dissertation in: Journal of Drug Delivery Science and Technology (2018) and Drug Development and Industrial Pharmacy (2018). These two papers have received 39 citations (SCOPUS, self-citations removed). He has published other six publications linked to the topic of the thesis with 74 citations. Thus Zahari Vinarov fulfils the National criteria for acquiring the educational and scientific degree of Ph.D..

Conclusion

Based on foregoing, the thesis of Zahari Vinarov "Enhancing the solubility of hydrophobic drugs by solubilization in micellar surfactant solutions" fulfils all the National criteria for gaining the Ph.D. degree in professional area: 7.3 Pharmacy (Pharmaceutical technology and biopharmaceutics). ***I will vote confidently Zahari Penkov Vinarov to get the degree of Ph.D..***

Sofia, February, 12, 2021

Prof. D.Rachev, Ph.D.