



## Research paper

Effects of non-ionic surfactants on cytochrome P450-mediated metabolism *in vitro*Anne Christiansen<sup>a,b</sup>, Thomas Backensfeld<sup>a</sup>, Karsten Denner<sup>c</sup>, Werner Weitschies<sup>b,\*</sup><sup>a</sup> Analytical Development, Bayer Schering Pharma AG, Berlin, Germany<sup>b</sup> Department of Biopharmaceutics and Pharmaceutical Technology, Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany<sup>c</sup> Department of Drug Metabolism and Pharmacokinetics, Berlin, Germany

## ARTICLE INFO

## Article history:

Received 7 September 2010

Accepted in revised form 23 December 2010

Available online 8 January 2011

## Keywords:

Non-ionic surfactants

Excipients

Inhibition effect

Cytochrome P450

Intestinal metabolism

Bioavailability

## ABSTRACT

The purpose of the study was to investigate the impact of commonly used non-ionic surfactants on cytochrome P450 (CYP) 3A4-mediated metabolism of testosterone and the CYP2C9-mediated metabolism of diclofenac. Polysorbate 80 (PS 80), D- $\alpha$ -tocopheryl polyethylene glycol (1000) succinate (TPGS), sucrose laurate, Cremophor EL (CR EL), and Cremophor RH 40 (Cr RH 40) were incubated with human liver microsomes at different concentrations to determine the IC<sub>50</sub> of the reduced metabolism of the model substrates. Inhibitory potential in case of all tested compounds could be observed already below their critical micelle concentrations (CMC) and in concentration-dependant manner. The IC<sub>50</sub> of the CYP 3A4-mediated 6 $\beta$ -hydroxylation of testosterone has been determined as 0.40 mM (PS 80), 0.15 mM (TPGS), 0.20 mM (sucrose laurate), 0.60 mM (Cr EL), and 0.80 mM (Cr RH 40). The IC<sub>50</sub> concerning the CYP 2C9-mediated 4-hydroxylation of diclofenac has been calculated to be 0.04 mM (PS 80), 0.30 mM (TPGS), 0.07 mM (sucrose laurate), 0.03 mM (Cr EL), and 0.03 mM (Cr RH 40). The results indicate that these non-ionic surfactants are *in vitro* inhibitors of CYP-mediated metabolism and might have the potential to modify the pharmacokinetics of co-administered drugs, which are substrates of CYP, and thereby enhance their bioavailability.

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## 1. Introduction

Surfactants are historically considered to act as pharmacologically inert excipients in formulations and show no effects on transporters or enzymes. Hence, for a long-time formulation, developers had focused exclusively on their technological functions such as to facilitate the disintegration of solid dosage forms or enhance drug solubilization. This assumption is disproved, because it has been shown *in vitro* and *in vivo* that several non-ionic surfactants interact with intestinal efflux transport systems like P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and the multidrug resistance-associated protein 2 (MRP2), which can lead to an increased absorption rate of drugs that are substrates of these efflux transporters [1–3]. Furthermore, recent studies point to the ability of excipients like surfactants to interfere as well with metabolic enzymes like cytochrome P450 (CYP) which can lead to a diminished degradation and therefore enhanced bioavailability of drugs [4,5]. In case of amphiphilic excipients, there is at present only marginal knowledge about their influence on metabolic enzymes after oral administration. However, it is generally recognized that

inhibition or induction of the CYP-mediated metabolism of an API should be considered to optimize oral bioavailability and to decrease variability at the absorption site [6–8].

Cytochrome P450 enzymes comprise a superfamily of haemoproteins and function as monooxygenases which are mainly present in both intestine and liver, furthermore in lung, brain, and skin. CYP enzymes are localized intracellularly bound to the membrane of the endoplasmic reticulum (ER) where they catalyze various biochemical reactions of the Phase I metabolism, e.g. hydroxylation, N-demethylation, or epoxidation of hydrophobic substrates. The subfamily cytochrome P450 3A (CYP3A) with CYP3A4 as the most abundant isoenzyme in small intestine and liver is responsible for the oxidative metabolism of nearly 50% of all drugs that are currently on the market [9,10]. There is increasing evidence indicating that also CYP2C9 represents an isoenzyme of major importance in human drug metabolism. Amongst others, phenytoin, S-warfarin and numerous nonsteroidal anti-inflammatory drugs (NSAID) like diclofenac and ibuprofen are substrates of CYP2C9 [11,12]. CYP2C9 also is expressed in a number of tissues throughout the human body, including the gastrointestinal tract. Investigators have shown CYP2C9 to be functionally active in intestinal microsomes, as measured by diclofenac 4-hydroxylation [12].

This isoenzyme is also found to a significant percentage of more than 10% of total immunoquantified cytochrome P450 content in the human small intestine [10].

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However, human small intestine epithelial cells (enterocytes) provide the first site for CYP-catalyzed degradation of orally administered drugs. Actually, it has been shown that intestinal cytochrome P450 3A-mediated metabolism plays a significant role in overall first-pass metabolism [13]. Consequently, interactions of excipients like non-ionic surfactants with these metabolic enzymes have to be elucidated, because inhibition can lead to an increased absorption rate or in the worst case overdose of the co-administered drug. To date, very few studies have been conducted on the effects of amphiphilic excipients on cytochrome P450.

Amongst others, non-ionic surfactants are commonly applied to improve the water solubility of active pharmaceutical ingredients (API) and thus possibly enhance their oral bioavailability [14]. It is well known that molecular dispersion in the gastrointestinal tract (GIT) is a precondition for the absorption of drugs across biological membranes, but unfortunately many of the new drug entities (NDE) show poor aqueous solubility, and therefore, limited bioavailability might occur depending on the dose [15]. Considering the fact that the oral route is still the preferred route for drug administration, new formulation strategies were needed to improve the solubility of such APIs [16].

Besides the solubilization of poorly water-soluble drugs, non-ionic surfactants can also alter the composition and character of the gastrointestinal fluid [17]. In recent years, research efforts have focused on the development of lipid-based formulations with particular emphasis on self-emulsifying drug delivery systems (SEDDS), which are isotropic mixtures of oils, surfactants, solvents and co-solvents/surfactants forming a fine oil emulsion under gentle agitation upon dilution with aqueous medium or *in vivo* administration [18]. These lipidic formulations and their digestion products form a range of vesicular and micellar species with endogenous bile salts and phospholipids which leads to an enhanced solubilization capacity for the API in the intestinal lumen [17]. Formulations of Types II–IV according to the Lipid Formulation Classification System (LFCS) contain high amounts of non-ionic surfactants [19].

The compounds tested in this study are widely used non-ionic surfactants, which are polyethoxylated except sucrose laurate. Polysorbate 80 (PS 80) and Cremophor EL (Cr EL) as well as Cremophor RH 40 (Cr RH 40) contain fatty acid esters of polyethylene glycol (PEG). Polysorbate 80 is an oleate ester of sorbitol and its anhydrides copolymerized with 20 moles PEG for each mole of sorbitol and sorbitol anhydrides. Cremophor EL is manufactured by reacting 35 mole of ethylene oxide with castor oil. It contains mainly the tri-ricinoleate ester of ethoxylated glycerol, with smaller amounts of polyethylene glycol ricinoleate and the corresponding free glycols. The composition of Cremophor RH 40 is similar, but it is obtained by the reaction of 40 mole of ethylene oxide with hydrogenated castor oil. D- $\alpha$ -tocopheryl polyethylene glycol (1000) succinate (TPGS) is a mixture formed by the esterification of D- $\alpha$ -tocopheryl acid succinate and polyethylene glycol. The Surfhope<sup>®</sup> sugar ester D-1216 is produced by the esterification of saccharose using the methyl ester of lauric acid obtaining primarily monoesters of sucrose laurate.

The aim of the study was to examine the capability of these non-ionic surfactants to interact with enzymes of the cytochrome P450 superfamily *in vitro*. Human liver microsomes were utilized to examine their inhibitory effects on the isoenzymes cytochrome P450 3A4 (CYP3A4) and 2C9 (CYP2C9), which appear to be the most important enzymes for drug metabolism in liver and intestine. Previous studies have demonstrated that the sequences of intestinal and hepatic cytochrome P450 3A4 cDNAs (complementary deoxyribonucleic acids) are identical [20]. Testosterone and diclofenac are specific substrates of the isoenzymes CYP3A4 (testosterone) and CYP2C9 (diclofenac). The extent of metabolism of these model

compounds was determined after incubation with various concentrations of the surfactant in order to define the IC<sub>50</sub> values.

## 2. Materials and methods

### 2.1. Materials

Polysorbate 80 (HX)<sup>™</sup> was purchased from NOF Corporations (Tokyo, Japan). D- $\alpha$ -tocopherol polyethylene glycol (1000) succinate (TPGS) was provided by Eastman Chemical Company (Kingsport, USA), and Surfhope<sup>®</sup> sugar ester D-1216 (sucrose laurate) was donated by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Cremophor EL and Cremophor RH 40 were contributed by BASF (Ludwigshafen, Germany).

Pooled human liver microsomes were purchased from Cytonet (Weinheim, Germany). Control inhibitors ketoconazole and sulfaphenazole as well as the substrate diclofenac sodium salt were acquired from Sigma–Aldrich (Steinheim, Germany). Testosterone was synthesized by Bayer Schering Pharma AG (Berlin, Germany). The internal standard [<sup>13</sup>C]4-hydroxydiclofenac was synthesized by the Isotope Chemistry Laboratory of Bayer Healthcare AG (Wuppertal, Germany). [<sup>3</sup>H]Testosterone was obtained from GE Healthcare (Munich, Germany). Components of the NADPH-generating system, potassium chloride and magnesium chloride were purchased from Merck (Darmstadt, Germany);  $\beta$ -glucose-6-phosphate and NADP were obtained from Sigma–Aldrich (Steinheim, Germany), and glucose-6-phosphate dehydrogenase was purchased from Roche Diagnostics (Mannheim, Germany).

### 2.2. Inhibition of cytochrome P450-mediated metabolism

The inhibitive potential of non-ionic surfactants on CYP3A4 and CYP2C9 was evaluated as follows. The surfactants were tested in duplicate at various dosing levels. Therefore, they were pre-incubated with human liver microsomes for 20 min at 37 °C together with a control sample containing no surfactant. The incubation mixture (final volume 250  $\mu$ l) consisted of 91.25  $\mu$ l potassium phosphate buffer 100 mM (pH 7.4), 6.25  $\mu$ l NADPH-generating system (38 mM potassium chloride, 8 mM  $\beta$ -glucose-6-phosphate, 1.2 mM NADP, 5 mM magnesium chloride, 1.4 U/ml glucose-6-phosphate dehydrogenase), 100  $\mu$ l protein dilution, and 50  $\mu$ l surfactant solution. The resulting concentration of protein in the incubation mixture was 0.5 mg/ml (CYP3A4 assay) and 0.132 mg/ml (CYP2C9 assay). Subsequently, 2.5  $\mu$ l dilution of the selective substrate of CYP3A4 (mixture of [<sup>3</sup>H]testosterone/testosterone 50  $\mu$ M) and CYP2C9 (diclofenac sodium salt 10  $\mu$ M) was added and incubated for 30 min at 37 °C. For positive control, inhibitors ketoconazole (CYP3A4) and sulfaphenazole (CYP2C9) at different concentrations were tested as well. The reaction was terminated upon the addition of 125  $\mu$ l cold methanol containing an internal standard in case of MS detection of 4-hydroxydiclofenac. After centrifugation for 5 min at 13 000 UpM, the supernatants were collected and transferred into HPLC vials for analysis.

### 2.3. HPLC analysis

The hydroxylation of radio-labelled testosterone into 6 $\beta$ -hydroxytestosterone was quantified via HPLC (HP 1100 series from Agilent Technologies; Waldbronn, Germany) coupled with a flow scintillation analyzer (Radiomatic 525TR from Perkin-Elmer; Waltham, USA). A Hypersil Gold (5  $\mu$ m; 125  $\times$  3.0 mm) C18 column from Ercatech (Bern, Switzerland) was used. Eluents were acetonitrile and water with 0.2% trifluoroacetic acid/acetonitrile 95:5. For gradient analysis, the ratios of the mobile phases were varied to 3:97, 50:50, 95:5 (staying isocratic for 1 min), and

30:70 at 0, 20, 21, and 23 min. Flow was set to 0.5 ml/min at 40 °C. For data analysis, the software Chromeleon 6.5.0 (Dionex; Sunnyvale, USA) was applied.

The metabolite 4-hydroxydiclofenac was determined via HPLC-MS (API3000 from Applied Biosystems; Foster City, USA). A Synergi Polar-RP (4  $\mu$ m; 75  $\times$  2.0 mm) from Phenomenex (Aschaffenburg, Germany) and an Atlantis dC18 (3  $\mu$ m; 2.1  $\times$  10 mm) from Waters (Milford, USA) as pre-column were applied for analysis. Mobile phases were acetonitrile with 0.1% formic acid and water with 0.1% formic acid. For gradient analysis, the ratios of the mobile phases were varied to 3:97, 50:50, 70:30 (remaining isocratic for 0.7 min), and 3:97 at 0, 1.8, 1.9, and 2.9 min. For data analysis, the software Analyst 1.4.1 (Applied Biosystems; Foster City, USA) was utilized.

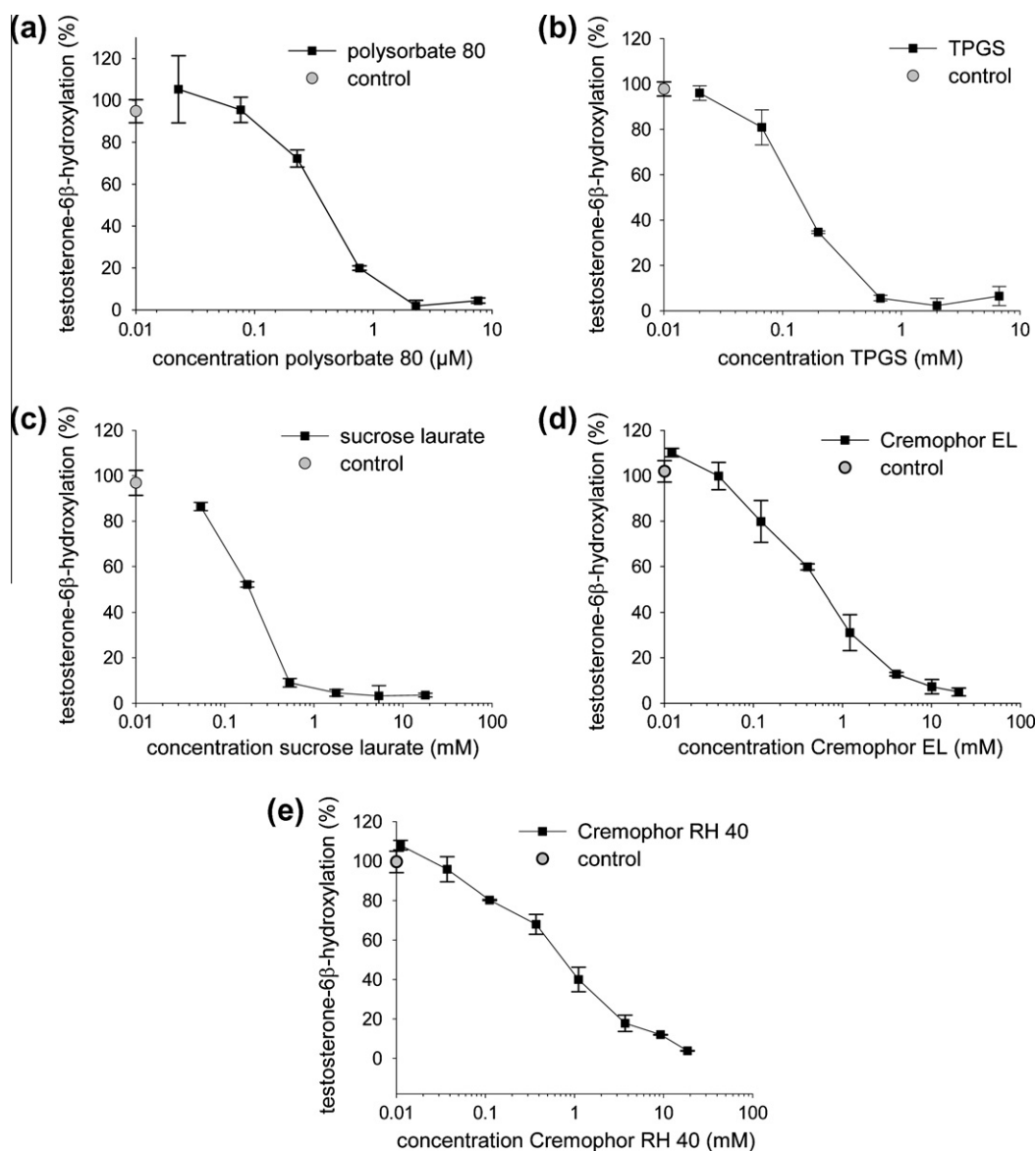
### 3. Results and discussion

The impact of the non-ionic surfactants polysorbate 80, TPGS, sucrose laurate, Cremophor EL, and Cremophor RH 40 on CYP isoenzymes has been studied *in vitro*. It was shown that all tested

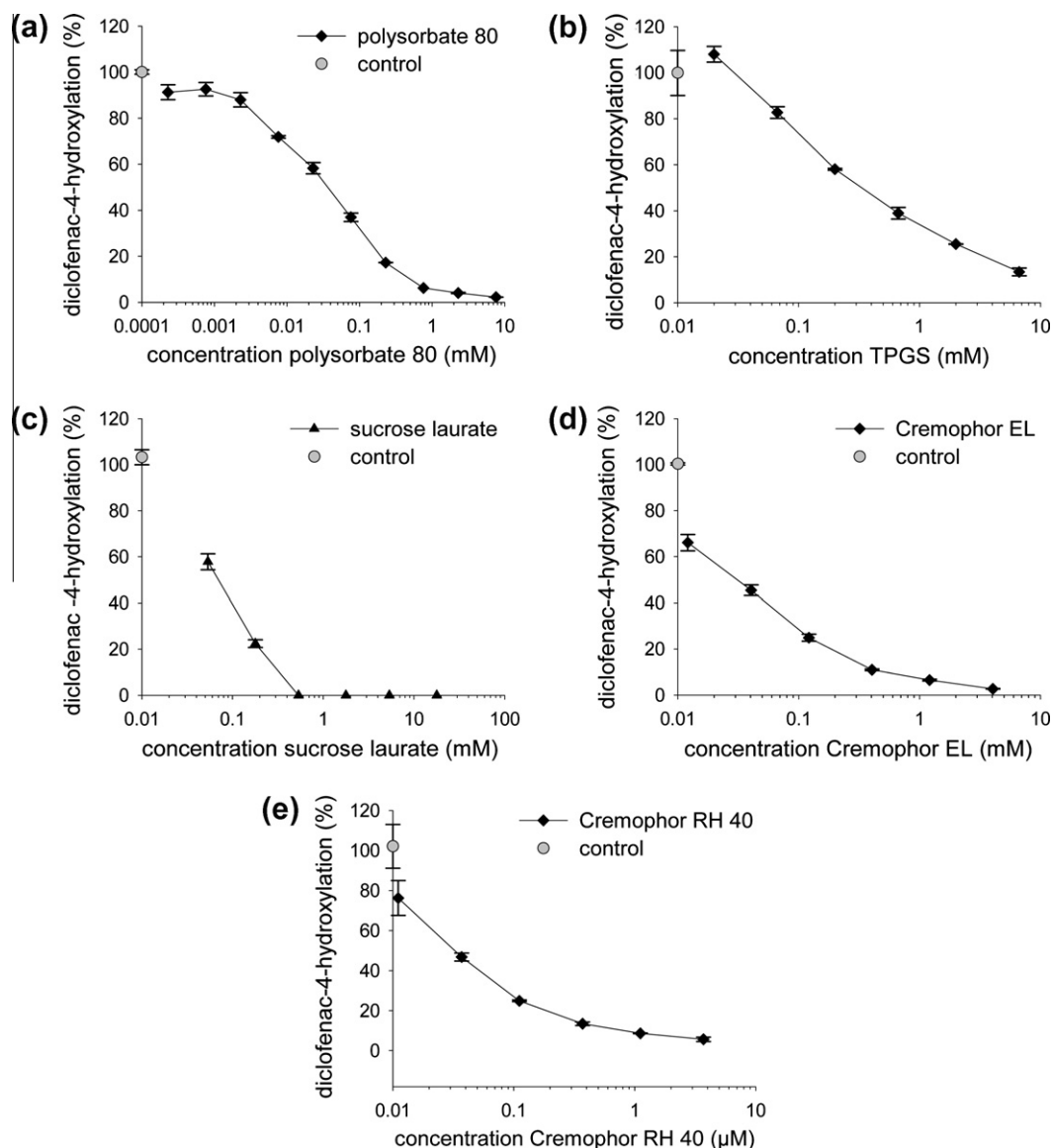
non-ionic surfactants inhibit cytochrome P450 3A4- and 2C9-mediated metabolism of the substrates testosterone (CYP3A4) and diclofenac (CYP2C9) in concentration-dependant manner (Figs. 1 and 2).

The  $IC_{50}$  of the CYP 3A4-mediated metabolism has been determined as 0.40 mM (PS 80), 0.15 mM (TPGS), 0.20 mM (sucrose laurate), 0.60 mM (Cr EL), and 0.80 mM (Cr RH 40). The  $IC_{50}$  of the control inhibitor ketoconazole was 0.09  $\mu$ M in this assay (Table 1). The extent of inhibitory action implying the  $IC_{50}$  is ranked in downward order as follows: TPGS > sucrose laurate > polysorbate 80 > Cremophor EL > Cremophor RH 40.

The  $IC_{50}$  of the CYP 2C9-mediated metabolism has been calculated to be 0.04 mM (PS 80), 0.30 mM (TPGS), 0.07 mM (sucrose laurate), 0.03 mM (Cr EL), and 0.03 mM (Cr RH 40). The  $IC_{50}$  of the control inhibitor sulfaphenazole was 0.32  $\mu$ M in this assay (Table 1). The extent of inhibitory action implying the  $IC_{50}$  is ranked in downward order as follows: Cremophor EL = Cremophor RH 40 > polysorbate 80 > sucrose laurate > TPGS. In case of the control inhibitors, lower  $IC_{50}$  values of two to three orders of magnitude were determined in both assays.



**Fig. 1.** The  $IC_{50}$  determination of non-ionic surfactants concerning the inhibitory effects on CYP3A4-mediated 6 $\beta$ -hydroxylation of testosterone (mean  $\pm$  SD,  $n = 2$ ). Liver microsomes were incubated with 50  $\mu$ M testosterone in the presence of a various concentrations of the surfactants for 30 min at 37 °C. Activities were expressed as a percentage of the 6 $\beta$ -hydroxytestosterone production compared with the control (y-axis). a) Polysorbate 80; b) TPGS; c) Sucrose laurate; d) Cremophor EL; e) Cremophor RH 40.



**Fig. 2.** The  $IC_{50}$  determination of non-ionic surfactants concerning the inhibitory effects on CYP2C0-mediated 4-hydroxylation of diclofenac (mean  $\pm$  SD,  $n = 2$ ). Liver microsomes were incubated with 10  $\mu$ M diclofenac in the presence of a various concentrations of the surfactants for 30 min at 37 °C. Activities were expressed as a percentage of the 4-hydroxydiclofenac production compared with the control (y-axis). a) Polysorbate 80; b) TPGS; c) Sucrose laurate; d) Cremophor EL; e) Cremophor RH 40.

**Table 1**

Values  $IC_{50}$  concerning the inhibition of cytochrome P450.

Surfactant	PS 80 (mM)	TPGS (mM)	Sucrose laurate (mM)	Cr EL (mM)	Cr RH 40 (mM)	Ketoconazole (mM)	Sulfaphenazole (mM)
CYP 3A4	0.40	0.15	0.20	0.60	0.80	0.09	–
CYP 2C9	0.04	0.30	0.07	0.03	0.03	–	0.32

The inhibitory activity of the surfactants appears to be dependent on the type of isoenzyme. TPGS exhibits the strongest effect on CYP3A4, but the lowest on CYP2C9-mediated hydroxylation of the specific substrate. Cremophor EL and Cremophor RH 40 show the strongest effect on CYP2C9, but the lowest on CYP3A4-mediated metabolism. In both assays, polysorbate 80 and sucrose laurate range in the middle of the ranking list. However, the incubation conditions for the two inhibition assays in human liver microsomes were not identical. Substrate concentrations (testosterone and diclofenac) near the apparent Michaelis constant ( $K_M$ ) values were applied. Protein concentrations and incubation times were optimized with regard to linearity and metabolite turnover for the CYP3A4 and CYP2C9 assays, respectively. Therefore, a lower protein concentration was chosen for the CYP2C9 assay.

Measurements of the critical micelle concentrations (CMC) of the surfactants have been taken in water, and reference data from water measurements as well have been used in case of Cremophor EL, Cremophor RH 40, and TPGS, respectively, to compare the obtained  $IC_{50}$  with the CMCs (Table 2). The CMC of a surfactant is dependent on the salt concentration in the medium, and the values from water measurements are lower than the values detected in the test medium. However, due to the different protein and substrate concentrations established in the two assays and the heterogeneous composition of the surfactants themselves, the values would be hardly comparable. Therefore, the CMC measurements were taken in water.

All tested non-ionic surfactants show inhibitory activities towards CYP-mediated metabolism at concentrations below their



**Table 2**  
Critical micelle concentrations (CMC) of the surfactants.

Surfactant	PS 80 (mM)	TPGS (mM)	Sucrose laurate (mM)	Cr EL (mM)	Cr RH 40 (mM)
CMC <sup>a</sup>	0.01	0.13	0.36	0.08	0.10

<sup>a</sup> The critical micelle concentrations (CMC) of TPGS, Cr EL, and Cr RH 40 are specifications from the manufacturers Eastman Chemical Company and BASF. The CMC of PS 80 and sucrose laurate are received from measurements by Analytical Development Physicochemistry, Bayer Schering Pharma AG (data not shown).

CMC (Figs. 1 and 2), while the extent of inhibition appears to be independent from the CMC (Table 2) and varies between the two isoenzymes. Consequently, inhibition is not only caused by incorporation of the co-incubated substrate into micellar structures which also could be a reason for diminished metabolism, but also entailed by interactions of the surfactant monomers with the enzyme. Anyhow, it is worth noting that it is quite challenging to define exact values of the CMCs of polyethoxylated surfactants and that the values reported in literature show remarkable deviations [21–23]. A likely reason for this can be found in their heterogeneous and variable composition.

Furthermore, a direct translation of these concentrations into the *in vivo* situation is critical, and diverse factors like degradation and dilution but also local accumulation of the surfactant have to be considered. The obtained IC<sub>50</sub> values range from 225–2158 mg/l in case of CYP3A4 inhibition, whereas the IC<sub>50</sub> values (37–480 mg/l) are much lower in case of CYP2C9 inhibition. Assuming local accumulation and amounts of non-ionic surfactants in the mg range which are contained in solid and liquid oral dosage forms, these concentrations can be reached in certain cases. However, it has to be considered that inhibition proceeds intracellular, so there is no conclusion possible from the concentration of the surfactant in the intestinal lumen concerning the intracellular inhibitory activity. Moreover, degradation of the excipients might have an influence on their inhibitory activity. Contrary to the *in vitro* situation, after oral administration, the surfactant composition is altered due to the activity of degrading enzymes in the gastrointestinal tract such as pancreatic and gastric lipases or unspecific esterases contained in the pancreatic juice. Previous studies have shown that surfactants, which comprise PEG fatty acid esters, namely polysorbate 80, Cremophor EL, and Cremophor RH 40 in this study, are susceptible to pancreatic enzymes *in vitro* [24,25]. In addition, we recently showed that sucrose laurate [26] and polysorbate 80 (unpublished data) undergo some degradation during incubation in acidic medium (hydrochloric acid solution pH 1.0) at 37 °C simulating the harsh gastric conditions. Accordingly, the extent of decomposition as well as the inhibitory activity of the formed degradation products has to be investigated separately and compared with the original compounds to estimate an effect under physiological *in vivo* conditions.

Free polyethylene glycols (PEG) are already present in the original product as impurities and can also be released by the degradation of polyethoxylated surfactants. Low molecular weight PEGs are orally bioavailable and excreted in the urine [27]. As a consequence, an uptake of this compound into the enterocyte can be assumed, which is a prerequisite for a direct interaction with intracellular membrane-bound cytochrome P450 enzymes. It has been reported that PEG 400 has a significant inhibitory effect on CYP3A-mediated *N*-demethylation of verapamil in isolated rat jejunal mucosa and during *in vitro* microsomal incubation [28,29].

Furthermore, free fatty acids as possible products of the digestion of polysorbate 80, Cremophor EL, and Cremophor RH 40 may interfere with intracellular enzymes. Studies point to the ability of oleic acid and polyunsaturated fatty acids (PUFA) to show inhib-

itory effects on CYP enzymes. Although the saturated fatty acids, palmitic acid and stearic acid, were reported to show no inhibitory effects on the activities of six human CYP isoenzymes at concentrations up to 200 µM, all five examined polyunsaturated fatty acids linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid competitively inhibited CYP-catalyzed metabolic reactions [30]. It has also been described that oleic acid which is a degradation product of polysorbate 80 shows inhibitory action on the CYP3A4-mediated hydroxylation of the model substrate 7-benzyloxy-4-(trifluoromethyl)-coumarin *in vitro* [5]. However, oleic acid is a monounsaturated fatty acid as well as ricinoleic acid, which is the fatty acid component of Cremophor EL. It is worth noting that the non-ionic polyethoxylated surfactant polyoxyethylene (40) stearate which is composed of polyethylene glycol and a saturated fatty acid was also reported to show inhibitory effects on six major CYP isoenzymes during incubation with human liver microsomes [31].

Direct interactions of the non-ionic surfactants with intracellular enzymes would rely on an uptake of the excipient into the enterocyte, which is unlikely respectively possible only to a small extent in case of the most surfactants. Metabolic studies *in vitro* and *in vivo* have shown that sucrose esters of fatty acids are extensively hydrolyzed in the gastrointestinal tract into food constituents prior to absorption and that only small amounts of intact monoesters are absorbed. The incompletely hydrolyzed sucrose esters appear to be excreted in the faeces [32]. In contrast to the sucrose ester, it has been shown that TPGS remains unaffected in the lumen of the small intestine and is taken up by cells as an intact molecule. The decomposition of TPGS is based on intracellular enzymatic hydrolysis by cytoplasmic esterases that liberate free  $\alpha$ -tocopherol to enterocytes [33,34]. Accordingly, an inhibitory effect on intracellular cytochrome P450 enzymes may be rather induced by released free polyethylene glycol or D- $\alpha$ -tocopherol succinate than by the intact molecules of TPGS.

There have been only a few studies on interactions of excipients with CYP-mediated metabolism. Mountfield et al. tested several formulation ingredients, amongst others TPGS, polysorbate 80, and polysorbate 20, concerning their potential inhibitory effects on cytochrome P450 3A4-mediated metabolism of the model substrate 7-benzyloxy-4-(trifluoromethyl)-coumarin. The most dramatic inhibition curves were produced by both polysorbates, in which an IC<sub>50</sub> of 0.0038 mM had been determined in case of polysorbate 20, whereas TPGS showed a considerably lower inhibitory activity towards CYP3A4 [5]. In another study, TPGS, polysorbate 80, and Cremophor EL were shown to moderately reduce verapamil-*N*-demethylase activity, a marker of rat CYP3A4 function [29]. Johnson and co-workers compared the inhibitory effects of TPGS with Pluronic P85 and PEG 400 regarding the *N*-demethylation of verapamil. TPGS was reported to show only marginal effects, while PEG 400 as well as Pluronic P85 showed significant inhibitory effects [28]. Comprehensive *in vitro* investigations into the effects of polysorbate 80 and Cremophor EL on cytochrome P450 3A enzymes were made by Bravo González et al. using rat hepatocytes and microsomes. Their results suggest that both surfactants significantly prevent CYP3A-mediated metabolism of midazolam in concentration-dependant manner at concentrations above 0.03%, in case of polysorbate 80 incubated with liver microsomes already at 0.003% [35]. To date, effects of sucrose laurate and Cremophor RH 40 on cytochrome P450 enzymes have not been described in literature yet. The former results on the non-ionic surfactants polysorbate 80, TPGS, and Cremophor EL using other model compounds and different experimental settings appear to be consistent with our findings.

The mechanisms of inhibition remain unclear. It has been suggested that interactions of solubilizing agents with metabolic enzymes and transporters are largely due to surfactant-induced

membrane fluidization, resulting in perturbation of the local environment and thereby decreasing protein function [36–38]. A reduced P-gp function due to cellular depletion of adenosine triphosphate (ATP) has also been described in case of Pluronic P85 which could also be the cause of a diminished CYP function [39]. González and co-workers additionally tested cytotoxic impacts of the surfactants on the hepatocyte monolayers as well as potential interactions with biological membranes. Neither a cytotoxic effect like alteration in the intracellular ATP content or release of lactate dehydrogenase (LDH) nor a disruption of the membrane integrity could be revealed in case of polysorbate 80 and Cremophor EL [35]. Nevertheless, a direct interaction with the metabolizing enzymes, an alteration in the membrane properties as well as a molecular interaction with the microsomes have to be taken into account when interpreting the observed inhibitory potential of surfactants on CYP-mediated metabolism. Certainly, these effects may be unique to the substrates, and future studies are required to determine whether the metabolism of other substrates will be similarly affected. A quantity of excipients and their degradation products will require further investigations, whereas it has also been elucidated, if the observed effects would exhibit any clinical significance.

Considering that many amphiphilic compounds also have the potential to inhibit P-glycoprotein, addition of surfactants to a formulation for oral delivery in order to effect a systematic inhibition may be an interesting approach for increasing the oral bioavailability of APIs, which are substrates of transporters and cytochrome P450 enzymes [40]. The systematic use of a second API as an inhibitor of CYP3A4 was applied in the pharmaceutical product Kaletra® (protease inhibitor containing lopinavir and low-dose ritonavir), which is approved for the treatment of HIV infections. The extensive first-pass metabolism of lopinavir in the liver is mediated primarily by cytochrome P450 3A4 and 3A5 isoenzymes. The co-administered ritonavir inhibits the activity of CYP3A4 in concentration-dependant manner in human liver microsomes, resulting in an increased plasma concentration of lopinavir [41].

#### 4. Conclusion

Interactions of cytochrome P450 enzymes with common formulation ingredients like non-ionic surfactants have been evaluated. It was found that all tested surfactants act as inhibitors of the CYP-mediated hydroxylation of testosterone and diclofenac, whereas the mechanism by which these compounds exert their influence on metabolizing enzymes appears to be mixed and elucidation requires further investigations. Direct inhibition of the metabolic enzyme, as well as indirect actions on the cellular function such as alterations in membrane fluidity and ATP depletion, has to be taken in consideration.

This study reveals that pharmaceutical industry should act with caution when applying these solubilizing agents in preclinical dosing studies. Such interactions may potentially lead to artificially increased plasma levels of the co-administered drug and diminished plasma levels of CYP-mediated metabolites which entail the risk of assessing incorrect pharmacokinetic parameters for new drug entities. On the other hand, the systematic use of an excipient as an inhibitor of CYP-mediated metabolism might be used in order to increase the oral bioavailability of co-administered drugs that are substrates of cytochrome P450 enzymes.

#### Acknowledgements

This work was supported by a Ph.D. student scholarship from Bayer Schering Pharma AG (Berlin, Germany). The authors gratefully acknowledge A. Reipert from the Department of Pharmacoki-

netics and Drug Metabolism (Bayer Schering Pharma AG) for her active technical assistance in the *in vitro* metabolism experiments.

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