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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 231–236
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Physico-chemical interactions between extracts of *Hypericum perforatum* L. and drugs

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Received 10 February 2003; accepted in revised form 15 April 2003

Abstract

The aim of the present study was to investigate physico-chemical interactions between extracts of *Hypericum perforatum* L. and drugs. The formation of nanoparticles, microparticles and precipitates in aqueous drug-free and drug-containing preparations of *H. perforatum* L. was investigated. In aqueous infusions of Hyperici herba only small amounts of nano- and microparticles were observed using photon correlation spectroscopy and scanning electron microscopy. In the presence of promethazine nanoparticles are formed. Between 20.3 and 5.3% of the drug is bound to nanoparticles. Maximally 4.3% of the drug is associated with precipitates. Warfarin has a negligible effect on the particle formation in aqueous infusions of Hyperici herba. In further studies dry extracts of *H. perforatum* were reconstituted in water. Nanoparticles and precipitates are formed in the drug-free preparations. Scanning electron micrographs show almost spherical nanoparticles with a mean diameter between 100 and 300 nm. After addition of promethazine a precipitate is formed. Maximally 12.8% of the drug is associated with the precipitate. In warfarin-containing reconstituted extracts, nano- and microparticles as well as precipitates are observed. The amount of free warfarin in solution is reduced by 36.6% maximally. A loss of the anticoagulant effect which is described in the literature may partly be caused by particle formation.

Keywords: *Hypericum perforatum*; Interaction; Nanoparticle; Particle formation; Photon correlation spectroscopy; Plant extract; Promethazine; Scanning electron microscopy; Warfarin

1. Introduction

Preparations of *Hypericum perforatum* L. are commonly used to treat mild and moderate depressions. Co-medication of digoxin, cyclosporin and indinavir results in decreased plasma concentrations of the drugs [1–3]. A concomitant use of *Hypericum* preparations and warfarin lead to a reduced anticoagulant effect of warfarin [4]. Further drugs which may interact with *Hypericum* preparations are phenprocumon, oral contraceptives, theophylline, amitriptyline, simvastatin, HIV protease inhibitors and HIV non-nucleoside reverse transcriptase inhibitors, the active metabolite of irinotecan and anticonvulsants [4–9]. Possible reasons for the herb–drug interactions are an influence of contents of *Hypericum* on the CYP 450 isoenzymes and an induction of *p*-glycoprotein [1,3,10–15]. It is still unknown whether physico-chemical interactions are partly responsible for the decreased plasma concentrations of the drugs.

Physico-chemical interactions may cause a reduction of the therapeutic effects of neuroleptics and antidepressants when these drugs are combined with black tea [16,17]. The addition of fluphenazine and other neuroleptics to aqueous infusions of black tea leads to the formation of precipitates which do not dissolve completely at a low pH and are stable at a pH of 6 [16,18]. Precipitates are also formed after addition of antidepressants and black tea infusions [17,18]. Maximally 81% of the amount of added drug is bound in the precipitate.

In this study we examined the formation of nano- and microparticles in aqueous *Hypericum* preparations after addition of promethazine and warfarin. We investigated the amount of drug which is bound to particles.

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0939-6411/03/$ - see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/S0939-6411(03)00094-8

2. Materials and methods

2.1. Materials

Hyperici herba (Ph. Eur.), rutin and the dry extract 'extractum Hyperici siccum' (Lot. no. 00155080) were purchased from Caesar and Lorentz GmbH (Hilden, Germany). Hypericin, promethazine hydrochloride and warfarin sodium clathrate were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Methanol (HPLC grade) (No. 1.06018; Merck, Darmstadt, Germany) and acetic acid (No. 100063; Merck) were used for high-performance liquid chromatography (HPLC) assays. The 3-μm and 0.1-μm cellulose nitrate filters were obtained from Sartorius (Göttingen, Germany), the 0.025-μm cellulose nitrate filters from Schleicher and Schuell (Dassel, Germany).

2.2. Infusions of Hyperici herba

One hundred milliliters of boiling distilled water were poured onto 2.0 g of Hyperici herba. After 10 min, the infusions were filtered through a tea bag and allowed to cool down to 40 °C. Fifty milliliters was either poured onto promethazine hydrochloride (12.5, 25, 50 mg) or onto warfarin sodium clathrate (2.5, 5 mg). Twenty-five milligrams of promethazine/100 ml and 5 mg warfarin/100 ml fit to usual single doses taken with half a glass of liquid. The preparations were stored for 70 min at 20 °C and centrifuged for 10 min (4000 rpm). The supernatants were stored at 20 °C for 30–40 min. Then they were investigated by photon correlation spectroscopy (PCS) using a single-angle PCS (Malvern AutoSizer 2c with a 5 mW Helium Neon laser, wave length 633 nm, beam size small; Multi 8 Correlator with 64 channels) (Malvern, Herrsching, Germany). The measurements were performed in cuvettes (No 67.754; Sarstedt, Nümbrecht, Germany) at a temperature of 20 °C. Four × five measurements were performed with each preparation. Usually an aperture with a diameter of 400 μm was chosen; in the case of 25 and 50 mg promethazine, 300 μm. A measurement had a duration of 30 s (delay: 1 s). Mean diameters and count rates were recorded. Histograms with distribution of light intensity were calculated. Each experiment was carried out six times. Drug-free infusions were prepared and investigated analogous to the drug-containing preparations ($n = 6$).

Promethazine-containing infusions of Hyperici herba were prepared as described above. The amount of promethazine in the supernatant was determined by UV spectroscopy after HPLC separation. The difference between the amount of drug added to the infusions and the amount of drug in the supernatant is the amount of promethazine associated with the precipitate. To determine the amount of drug associated with the nanoparticles, 10 ml of the supernatant were filtered through a 0.025-μm cellulose nitrate membrane filter. The nanoparticles were collected on the filter. The difference between the amount of drug in the supernatant and the amount of drug in the filtered liquid is the amount of promethazine associated with nanoparticles. Each experiment was carried out six times. Ten milliliters of aqueous solutions of promethazine hydrochloride (25 mg/100 ml, 50 mg/100 ml, 100 mg/100 ml) ($n = 6$) were filtered through 0.025-μm membrane filters. The mean amounts of drug adsorbing to the filter were included in the calculations of the total amount of promethazine associated with particles.

Drug-containing and drug-free infusions were prepared ($n = 6$). Instead of centrifuging the infusions the precipitates were collected on 3-μm cellulose nitrate filters and dried. The mass was determined (analytical balance: A210P; Sartorius).

2.3. Reconstituted dry extracts of Hyperici herba

One hundred milligrams of a dry extract of Hyperici herba was reconstituted in 50 ml of distilled water (20 °C) ($n = 6$). Then the extracts were centrifuged (4000 rpm, 5 min) or filtered through a 3-μm cellulose nitrate filter. The supernatant of the centrifugations was used for PCS measurements. Four × five measurements per reconstituted extract were performed at a temperature of 20 °C using the large laser beam. Each measurement lasted for 90 s (delay: 1 s). Precipitates were collected on the 3-μm membrane filters and dried. The mass was determined using an analytical balance (A210P; Sartorius).

Extracts with 12.5, 25 and 50 mg promethazine were prepared ($n = 6$) and filtered or centrifuged. The drug concentration in the filtrate was analysed by UV spectroscopy after HPLC separation. The difference between the amount of the drug added to the extracts and the amount in the filtrate is the amount of promethazine associated with the precipitates. The adsorption of promethazine on 3-μm cellulose nitrate filters is negligible.

Fifty milliliters of aqueous solutions of warfarin (25 mg/500 ml and 50 mg/500 ml) was poured onto 100 mg of the dry extracts. The liquids were centrifuged. The amount of warfarin in the supernatant was determined ($n = 6$). PCS-measurements were performed ($n = 6$). Fifteen milliliters of the supernatant was filtered through 0.025-μm filters. The amount of drug associated with nanoparticles was determined as described above.

Aqueous solutions of warfarin were filtered through 0.025-μm membrane filters ($n = 6$). The amount of free drug in the filtrates was 92.5% (5 mg/100 ml) or 96.3% (10 mg/100 ml). The reduction of warfarin was considered in the calculations on the amount of warfarin associated with nano- and microparticles.

2.4. Thin layer chromatography (TLC)

The precipitates of reconstituted dry extracts of Hyperici herba and of the reconstituted dry extracts after addition of

promethazine and warfarin were analyzed by a TLC method described in the Ph. Eur. 4 (Hyperici herba: Identity C). The precipitates were collected on 3-μm cellulose nitrate filters and dissolved in methanol. Solutions of hypericin (1 mg/5 ml methanol) and rutin (5 mg/5 ml methanol) were prepared as references.

2.5. Scanning electron microscopy (SEM)

Promethazine-containing *Hypericum* infusions were prepared as described above. After separating the precipitate by centrifugation, 20 ml of the supernatants (20 °C) were filtered through 0.1-μm cellulose nitrate filters. Rectangular pieces of the filters were fixed on aluminum holders (G 301, Plano, Marburg, Germany). The samples were gold sputter-coated in an SCD040 sputter station (Balzers, Liechtenstein) for 5 min at 25 mA. At the chosen test conditions the filter surface is almost completely covered by nano- or micro-particles. The particles were visualized using a scanning electron microscope (Stereoscan S4, Cambridge Instruments, Cambridge, UK). Representative cut-outs were photographed. To determine the diameter of particles parts of the photos were scaled up and the size of representative nanoparticles was measured.

2.6. HPLC

The amounts of promethazine and warfarin in the preparations were determined by UV spectroscopy after HPLC separation. The HPLC apparatus consisted of a Liquid Chromatograph 655A-11 pump (Merck/Hitachi, Darmstadt, Germany), a L5000 LC controller, a LaChrom Column Oven (L-7360 Merck (VWR International), Darmstadt, Germany) and a UV detector (UV 655A; Merck/Hitachi). A guard column (LiChroCART 4-4) and a LiChroCART-column 125 × 4 mm, both filled with LiChroSpher100 RP 18, 5 μm (Merck) were used. The column temperature was kept constant at 25 °C. The injection volume was 50 μl. The peak area under the curve was calculated after each measurement and related to external calibration standards of promethazine or warfarin. To determine the concentration of promethazine hydrochloride, a method described in Ref. [19] was modified. A mixture of 88% (v/v) methanol, 11% (v/v) distilled water and 1% (v/v) of an aqueous ammonia dilution (10% (v/v)) was chosen as solvent. The flow rate was 1.0 ml/min. The preparation was monitored by a UV detector at a wavelength of 254 nm. The precision was 0.8% at 0.7 mg/100 ml and 1.2% at 10 mg/100 ml.

To determine the concentration of warfarin a detection wavelength of 305 nm was chosen. The solvent contained 700 ml methanol, 300 ml distilled water and 3 ml acetic acid [20]. The flow rate was 1.1 ml/min. The precision was maximally 1.4% (0.1 mg/100 ml) and minimally 0.3% (5 mg/100 ml).

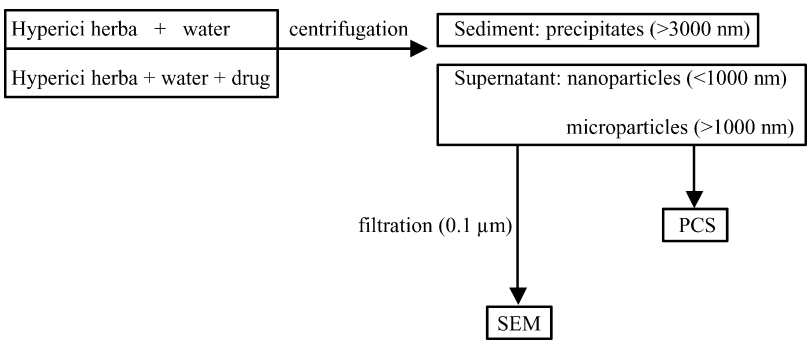
3. Results

3.1. Infusions of Hyperici herba

Aqueous infusions of Hyperici herba (Ph. Eur.) were prepared and cooled down. The formation of particles was examined using PCS. Only small amounts of particles were observed. A count rate of 2400 counts/s was measured (aperture: 400 μm). Promethazine (12.5 mg) was added to freshly prepared aqueous infusions of Hyperici herba. After cooling down, particle formation was observed. To separate particles with a mean diameter greater than 3000 nm the preparations were centrifuged (Scheme 1). The sedimented fraction is named ‘precipitate’. The particles in the supernatant are named ‘nano- and microparticle fraction’. Nanoparticles have a mean diameter between 1 and 1000 nm. Particles in the supernatant with a mean diameter greater than 1000 nm are named ‘microparticles’ in the following text.

In the supernatant nanoparticles with a mean diameter of 289.2 ± 16.2 nm were detected (PCS). The mean index of polydispersity is 0.266. The particle size distribution has one maximum. To characterize the nanoparticles by SEM the infusions were filtered through a membrane filter. The particles were gathered on the surface of the filter. Particles with an approximate spherical shape were detected. The particle diameters are in the colloidal range (Fig. 1).

To quantify the amount of promethazine which participates in the particle formation, HPLC assays were



Scheme 1. Separation of precipitates as well as nano- and microparticles in drug-free and drug-containing aqueous infusions of Heperici herba.

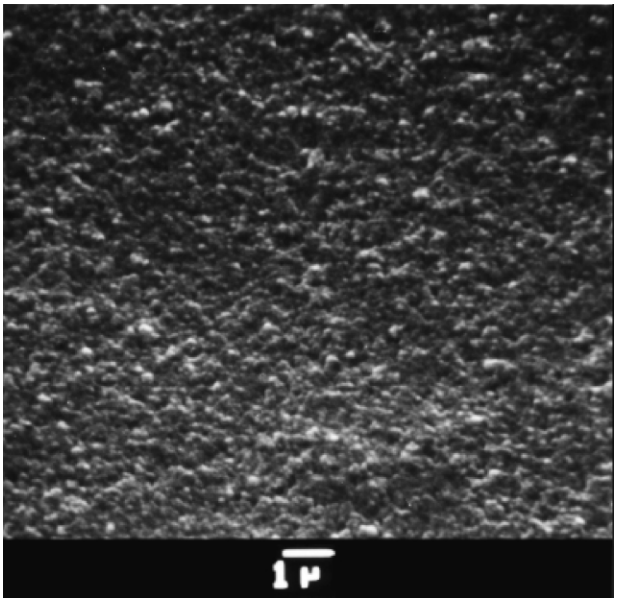


Fig. 1. Scanning electron microscopy. Nanoparticles in aqueous infusions of Hyperici herba after dissolving 12.5 mg promethazine in 50 ml infusion and cooling down to 20 °C.

performed. 20.3% of the drug is associated with the nanoparticles. The results indicate that the amount of promethazine in the precipitate is negligible.

To investigate the influence of the concentration of promethazine on the particle formation, 25 and 50 mg of the drug were added to aqueous infusions of Hyperici herba. Particle formation was observed in both cases. After addition of 25 mg promethazine particles with a mean diameter of 557 nm were detected at a temperature of 20 °C (Table 1). An index of polydispersity of 0.279 was calculated. A count rate of 47800 counts/s was measured using an aperture of 300 μm. 6.3% of the promethazine is bound to nano- and microparticles, 2.7% is associated with the precipitate.

After addition of 50 mg promethazine to aqueous infusions of Hyperici herba a count rate of 8200 counts/s results (aperture 400 μm). 5.3% of the drug is associated with the nano- and microparticles and 4.3% with the precipitate. In Fig. 2 the absolute amounts of promethazine in the infusions after removing the nano- and microparticles as well as the precipitates, and after removing only the precipitates, are shown.

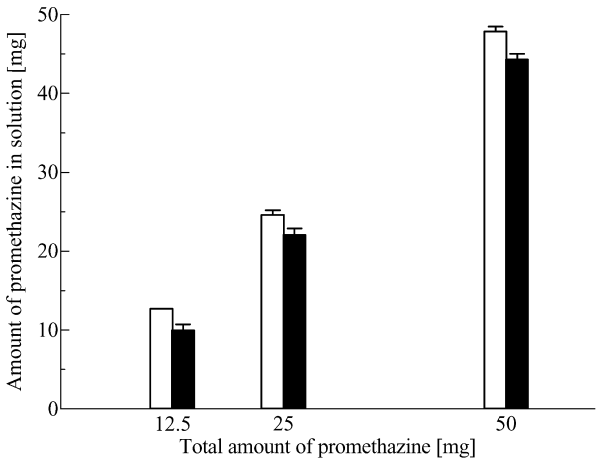


Fig. 2. Absolute amount of promethazine (mg) in 50 ml aqueous infusions of Hyperici herba ($n = 6$). (□) After filtering out the precipitate; (■) after filtering out the nanoparticles and the precipitate.

In the literature a reduction of the pharmacological effect of warfarin is described when the drug and extracts of *H. perforatum* are both taken by patients [4]. It is unknown whether the reduction of the pharmacological effect of warfarin is partly caused by particle formation. To investigate the ability of warfarin to participate in the particle formation, 10 mg warfarin was added to aqueous infusions of Hyperici herba. The count rate was lower than 5000 counts/s (aperture: 400 μm). To quantify the amount of precipitate the infusions were filtered. The precipitates on the filters were dried and weighed. The amount of precipitate (1.6 mg) is comparable to the amount of precipitate in infusions of Hyperici herba (2.4 mg). The effect of warfarin on the particle formation in aqueous infusions of Hyperici herba is negligible. No further investigations were performed.

3.2. Reconstituted dry extracts of Hyperici herba

Dry extracts of *H. perforatum* are used in the treatment of mild and moderate depression [21,22]. To examine physicochemical interactions between contents of a dry extract of Hyperici herba and warfarin or promethazine, the dry extract was reconstituted in water (20 °C). A nano- and microparticle fraction and a precipitate formed in the drug-free aqueous reconstituted preparations. The nano- and

Table 1
PCS measurements of drug-free and drug-loaded infusions of Hyperici herba (20 °C): mean diameter and index of polydispersity (mean ± SD, $n = 6$)

	Infusions of Hyperici herba			
	Without drugs	Addition of promethazine: 12.5 mg/50 ml	Addition of promethazine: 25 mg/50 ml	Addition of promethazine: 50 mg/50 ml
Mean diameter (nm)	[a]	289.2 ± 16.2	556.9 ± 40.9	[a]
Index of polydispersity	[a]	0.266 ± 0.027	0.279 ± 0.039	[a]

[a] Determination is uncertain at low count rates

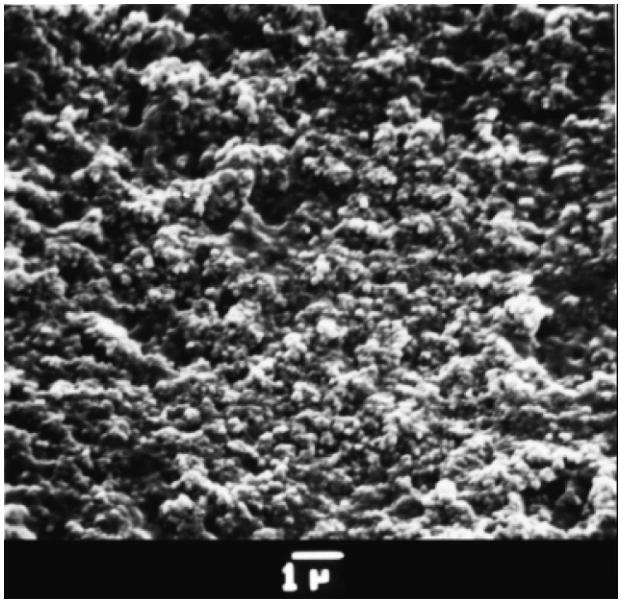


Fig. 3. Scanning electron microscopy: Nanoparticles in aqueous reconstituted extracts of *Hypericum perforatum* (200 mg/100 ml; 20 °C).

microparticle fraction was investigated using PCS. The count rate was 12 600 counts/s (aperture: 400 μm). Particles with more than one preferred size were detected (index of polydispersity: 0.431). We characterized the particles via SEM (Fig. 3). The scanning electron micrograph shows almost spherical nanoparticles with a mean diameter between 100 and 300 nm. The nanoparticles were observed to have aggregated.

We investigated the particle formation in aqueous reconstituted extracts after addition of promethazine. After addition of 12.5 mg promethazine to the aqueous reconstituted extract (50 ml) only negligible amounts of nanoparticles were detected (1300 counts/s). The precipitate was dried and weighed. The amount of precipitate is 137% of the precipitate of the drug-free preparation. In further investigations 25 and 50 mg promethazine were added to aqueous reconstituted preparations of *Hypericum*. Even after addition of 50 mg promethazine the count rate did not exceed 3500 counts/s. The mass of precipitate slightly changed. In Fig. 4 the mass of the precipitates and the amount of promethazine in the precipitates is shown.

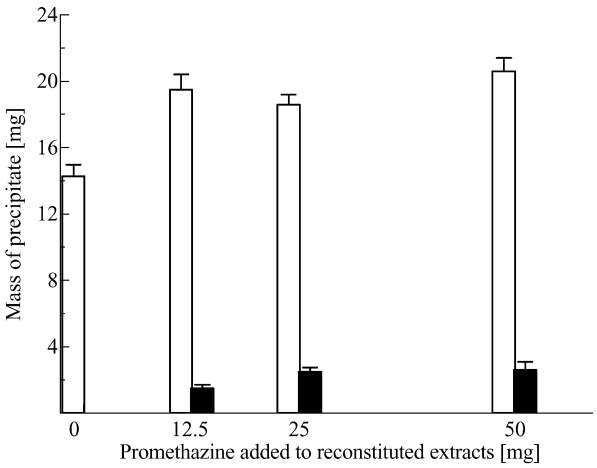


Fig. 4. (□) Total amount of precipitates (mg) in aqueous reconstituted dry extracts of *Hypericum perforatum* after addition of 0, 12.5, 25 or 50 mg promethazine per 50 ml ($n = 6$). (■) Total amount of promethazine (mg) associated with the precipitates in aqueous reconstituted dry extracts of *Hypericum perforatum* after addition of 0, 12.5, 25 or 50 mg promethazine per 50 ml ($n = 6$).

Maximally 12.8% of promethazine is associated with the precipitate. After addition of 12.5 mg promethazine, about 1.5 mg promethazine is associated with the precipitate; after addition of 50 mg promethazine the associated amount is about 2.6 mg.

We investigated the composition of the precipitate by TLC. Hypericin and/or pseudohypericin were identified. Rutin was not found. Hypericin and/or pseudohypericin are also components of the precipitate of the drug-free reconstituted preparations.

To identify the exact composition of the precipitate in drug-free and promethazine-containing reconstituted extracts, further studies are necessary.

After addition of warfarin to aqueous reconstituted extracts of *Hypericum*, nanoparticles and precipitates were detected. The nano- and microparticle fraction was investigated by PCS. A mean count rate of 13 700 counts/s and a mean index of polydispersity of about 0.7 were calculated (2.5 mg warfarin per 50 ml). Five milligrams of warfarin was added to the reconstituted plant extracts. The mean count rate was about 14 000 counts/s. The mean amount of precipitates (15.4 ± 0.5 mg) was comparable to the mean amount of precipitate in aqueous reconstituted dry extracts (14.3 ± 0.6 mg). Hypericin and/or pseudohypericin were identified as components of the precipitate (TLC).

Though the addition of warfarin did not lead to significant changes in the particle formation, we performed HPLC measurements to investigate the amount of drug associated with the particles. 23.2% warfarin is bound in the nano- and microparticle fraction (0.58 of 2.5 mg) and 13.4% is bound to the precipitate. The amount of warfarin in solution is reduced by 36.6%. After addition of 5 mg warfarin to aqueous reconstituted extracts of *Hypericum*, 35.9% of the drug is associated with particles (28.6% with nano- and microparticles, 7.3% with the precipitate).

4. Discussion

Extracts of *H. perforatum* have different compositions, depending on the extraction solvent [23]. The present investigations have shown that particles are only formed in reconstituted *Hypericum* dry extracts. Methanol is the extraction solvent of these dry extracts. If water is used as extraction solvent for Hyperici herba, only small amounts of particles are detected. The differences in the particle formation of *Hypericum* preparations indicate that

the physico-chemical properties of plant extracts are influenced by the extraction solvent. The ratio of plant material to extraction solvent and the temperature also play important roles in the particle formation. In our investigations the production conditions were standardized. The concentration of the *Hypericum* preparations and the amount of drugs were adjusted to the normally used doses.

While more than 90% of promethazine is associated with nanoparticles and precipitates in black tea, about 20% and 12.5%, of the drug is bound in infusions or reconstituted extracts, respectively, of *Hypericum* [24]. Physico-chemical interactions between promethazine and the plant preparations are not expected to be a reason for a reduction of the pharmacological effect of the drug. If warfarin is added to *Hypericum* extracts, the amount of free drug is reduced by about 37%. In this case the reported reduction of bioavailability may be partly caused by physico-chemical interactions.

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