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Formation of particles in aqueous infusions of the medical plant *Harungana madagascariensis*

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In some aqueous plant extracts the formation of nanoparticles, microparticles and macroparticles has been observed. In the present investigation the particle formation in aqueous infusions of *Harungana madagascariensis* LAM. EX. POIR., a medicinal plant, was investigated using photon correlation spectroscopy (PCS) and scanning electron microscopy (SEM). The results show that nanoparticles with mean diameters of 220 nm are formed in aqueous infusions of the dried leaves of *Harungana*. The particles have an almost spherical shape. In aqueous infusions of the dried stem bark nanoparticles with a mean diameter of 242 nm (PCS) are observed at 25 °C. The particle size distribution has a maximum in the range of 200 nm to 300 nm. Nanoparticles can be detected in infusions of the leaves and the bark in the range of 25 °C to 55 °C. The mean diameter of the nanoparticles in preparations of the bark is temperature dependent: At 55 °C the mean diameter is 144 nm, at 30 °C 197 nm and at 25 °C 242 nm. Lower temperatures result in higher count rates. In infusions of the leaves the mean diameters vary between 220 (25 °C) and 139 nm (55 °C). The particle formation was investigated at pH 2.2, 4.0 and 7.4 at 37 °C. Nanoparticles are detected in infusions of the leaves and the stem bark at each pH. The pH value has an influence on the mean diameter and the count rate.

1. Introduction

In liquid extracts of several plants the formation of nano-, micro- and macroparticles can be observed. In aqueous preparations of black tea nanoparticles are formed. Caffeine takes part in the particle formation. Precipitates are observed in aqueous plant preparations, e.g. in reconstituted extracts of black tea (Roberts 1963, Smith 1968). Caffeine, theaflavins and thearubigins have been identified as components of the precipitate (Roberts 1963).

The formation of precipitates is not only observed in black tea but also in extracts of medicinal plants. In squeezed juices precipitates are formed (Hensel 1999). The formation of precipitates changes the composition of the liquid. A different composition may result in an alternate pharmacological activity of the plant preparations. The formation of precipitates can influence the uniformity of dosing (Hensel and Meier 1999).

Harungana madagascariensis LAM. EX. POIR. is a medicinal plant. Preparations of its leaves and bark are used to treat dyspepsia and exocrine pancreatic insufficiency (Rote Liste® 2003). In Africa aqueous extracts of the herb are taken as medicine for indigestion (Neuwinger 1998). Until now the formation of nano- and microparticles in aqueous extracts of *Harungana madagascariensis* has not been described.

In the present study the formation of nanoparticles in freshly prepared aqueous infusions of the stem and the leaf of *Harungana madagascariensis* was investigated. The influence of the temperature and the pH on the formation of particles was studied.

2. Investigations and results

Aqueous infusions of the leaves and the stem bark of *Harungana* were investigated by photon correlation spectroscopy (PCS). Nanoparticles were photographed under scanning electron microscopy (SEM). The particle formation was investigated at different temperatures. With regard to the oral intake of the infusions PCS measurements were performed at different pH values at 37 °C.

2.1. Particle formation at 25 °C

Aqueous infusions of the leaves of *Harungana* were prepared. Precipitates in the hot liquids were removed by filtration. The infusions were investigated by PCS. Nanoparticles with a mean diameter of 220 nm were detected at a temperature of 25 °C. The particle size distribution has two maxima. An index of polydispersity of 0.28 was calculated. A scanning electron micrograph of the nanoparticles is shown in Fig. 1. The particles have a spherical shape and diameters between about 60 nm and 250 nm, in exceptional cases up to 500 nm.

Infusions of the stem bark were prepared. Precipitates were separated by filtration. Nanoparticles were detected

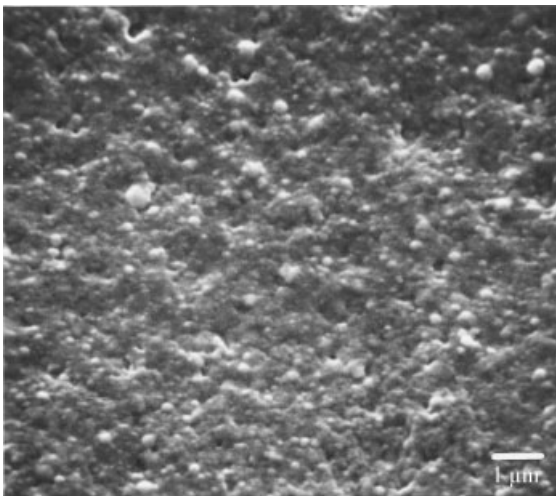

Fig. 1: SEM: Nanoparticles formed in aqueous infusions of the dried leaves of *Harungana madagascariensis* (25 °C)

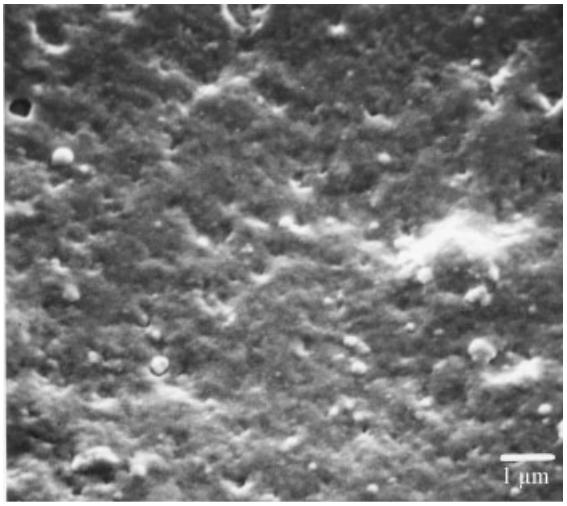

Fig. 2: SEM: Nanoparticles formed in aqueous infusions of the dried stem bark of *Harungana madagascariensis* (25 °C)

by PCS at a temperature of 25 °C. A mean diameter of 242 nm was calculated. The particle size distribution has one maximum in the range of 200 nm to 300 nm. The nanoparticles were collected on a filter and visualized by SEM (Fig. 2).

Compared to Fig. 1 much less nanoparticles can be seen in Fig. 2. Most of the nanoparticles have formed aggregates on the filter surface. The single nanoparticles are located on these aggregates. The particles have diameters between 50 nm and 320 nm, seldom up to 500 nm.

2.2. Influence of temperature and pH

The influence of the temperature on the particle formation was studied. Infusions of the leaves and the bark were prepared. PCS measurements were carried out in a temperature range of 25 °C to 55 °C. Nanoparticles were detected at each temperature. The mean count rates and the mean diameters are presented in Fig 3.

In infusions of the leaves the count rates change slightly between 25 °C and 55 °C. In infusions of the bark differences in the count rate are observed between 25 °C and 35 °C. The mean diameters of the particles are influenced

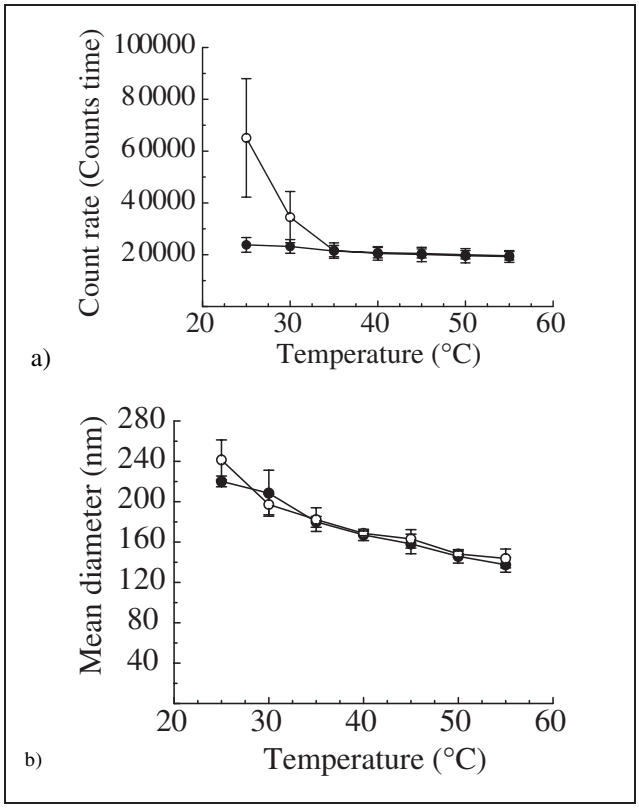

Fig. 3: Mean count rates and mean particle sizes measured by PCS (n = 5; mean ± s) in ● infusions of the leaves of *Harungana madagascariensis,* ○ infusions of the stem bark of *Harungana madagascariensis*

by the temperature in infusions of the leaves and the bark. The mean particle sizes of the nanoparticles in infusions of the leaves and the bark are comparable.

The formation of particles in infusions of the leaves and the bark was investigated at different pH values at a temperature of 37 °C. Nanoparticles are detectable at pH 2.2, 4.0 and 7.4. The mean diameters of nanoparticles in infusions of the leaves and the bark are influenced by the pH in infusions (Fig. 4). In preparations of the bark nanoparticles with a mean diameter of about 200 nm are detected at a pH of 2.2. At pH 4.0 and 7.4 the mean diameter is 179 nm. In infusions of the leaves the mean diameter of the nanoparticles is 54 nm larger at pH 2.2 than at pH 7.4.

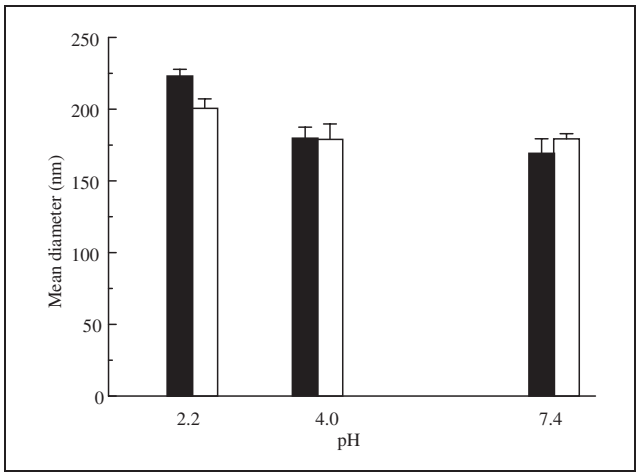

Fig. 4: Mean particle size of nanoparticles in infusions of ■ leaves of *Harungana*, □ bark of *Harungana* at different pH values (n = 5; mean ± s; 37 °C)

The count rate is affected by the change of the pH in infusions of the leaves and the bark. The count rate is higher at pH 2.2 than at pH 4.0 and 7.4. In infusions of the leaves the mean count rate is 23300 ± 3020 counts/s at pH 2.2 and 16900 ± 2210 counts/s at pH 7.4, in infusions of the bark 40300 ± 4920 counts/s at pH 2.2 and 18400 ± 2340 counts/s at pH 7.4.

3. Discussion

Nanoparticles are detected in aqueous infusions of the leaves and the bark of *Harungana madagascariensis* in the range of 25 °C to 55 °C. The size of the nanoparticles is dependent on the temperature. Changes in the solubility of hydrophobic substances may cause changes in the particle size. The solubility of hydrophobic substances in water usually decreases with lower temperature. Substances with low solubility may associate with existing particles. Moreover, the change of solubility could lead to the formation of nanoparticles which would affect the count rate (PCS). The temperature affects the degree of hydration. Therefore, the hydrodynamic radius is dependent on the temperature.

To investigate nanoparticles by SEM a standardised preparation method was used. If infusions of the bark are prepared, most of the particles aggregate on the filter surface. The amount of aggregation is less distinct in case of preparations of the leaves. This difference in properties is a hint for a different composition of the nanoparticles.

Aqueous infusions of *Harungana* are used as peroral preparations. There is no data available about the formation of particles in the gastrointestinal tract and about the effect of particle formation on the absorption of pharmacological active ingredients. The present investigations have shown that nanoparticles exist at 37 °C at pH values which can be found in the gastrointestinal tract. The formation of nanoparticles in plant extracts may influence the absorption of plant substances in the gastrointestinal tract. The stability of the *Harungana* nanoparticles under physiological conditions is still under investigation.

4. Experimental

4.1. Materials

Powders of the dried stem bark and the dried leaves of *Harungana madagascariensis* were obtained from Nigeria. The sodium hydroxide solution (1 mol/l) was purchased from Waldeck (Germany). The hydrochloric acid was purchased from Baker (Deventer; Netherlands). The 3 μm and 0.1 μm cellulose nitrate filters were obtained from Sartorius (Göttingen, Germany).

4.2. Infusions of leaves and bark: Preparation and PCS measurements

Hot distilled water (50 ml) was poured onto 1 g of the powdered leaves or bark ($n = 5$). After 10 min the infusions were filtered through a tea bag and then through a 3 μm membrane filter using a vacuum pump. The filtrates were investigated by single-angle PCS (Malvern AutoSizer 2c; 5 mW Helium Neon laser, wave length 633 nm, beam size small; Multi 8 Correlator with 64 channels) (Malvern, Herrsching, Germany). The diameter of the aperture was 400 μm. 10 measurements by 30 s (delay = 1s) were performed in single-use cuvettes (Sarstedt, Nümbrecht, Germany). The infusions were investigated at 55 °C, 50 °C, 45 °C, 40 °C, 35 °C, 30 °C and 25 °C. Mean diameters, count rates and indices of polydispersity were recorded. Particle size distributions were calculated.

Infusions of the leaves and of the bark were prepared and filtered. Sodium hydroxide solution or hydrochloric acid was added to the filtrate to adjust pH values of 2.4, 4.0 and 7.4 at a temperature of 37 °C ($n = 5$). The infusions were investigated by PCS at 37 °C.

4.3. Scanning electron microscopy (SEM)

Infusions of the leaves and of the bark were prepared and filtered as described above. The filtrates cooled down to 25 °C. 20 ml were filtered through 0.1 μm cellulose nitrate filters. Rectangular pieces of the filters were cut and fixed on aluminium holders (G 301, Plano, Marburg, Germany). The samples were gold sputter coated for 5 min in a SCD040 sputter station (Balzers, FL-Balzers) at 25 mA. A scanning electron microscope Stereoscan S4 (Cambridge Instruments, GB-Cambridge) was used to investigate the particles.

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