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Nanoparticles in plant extracts – factors which influence the formation of nanoparticles in black tea infusions

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The influence of different factors on the formation of nanoparticles in freshly brewed tea extracts was investigated. A black tea infusion was observed during cooling using photon correlation spectroscopy (PCS). The mean particle size and the number of the nanoparticles increase with decreasing temperature. In the presence of caffeine more particles are formed within the infusion. To study the influence of slight structural differences between methylxanthines, the effect of the addition of caffeine to solutions of freshly prepared decaffeinated tea was compared to that of theophylline and theobromine. In the case of theophylline fewer nanoparticles were formed. Molecular modelling calculations were performed to evaluate the most probable geometries for caffeine-polyphenol complexes. A parallel position and a congruent orientation of the 6-membered ring of caffeine and the aromatic galloyl group is the most probable geometry.

1. Introduction

Tea is one of the most widely consumed beverages in the world. In aqueous infusions of black tea (*Thea nigra*) turbidity can be observed below temperatures of 36 °C. Often a precipitate forms. This phenomenon is well known in the food industry; the precipitate is called tea cream. In previous investigations, we examined the colloidal structure of infusions after cooling down to room temperature using photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) [1]. We detected nanoparticles with a mean particle size of 200–300 nm. Particle formation in redispersed dried tea extracts has already been reported by different authors [2–7]. Tea cream mainly consists of caffeine and tea polyphenols such as thearubigins and theaflavins [8, 9]. Caffeine and polyphenols are able to form complexes. Possible mechanisms and structures are discussed in a review by Spencer et al. [10]. The structure of caffeine-polyphenol complexes has been examined using ^{1}H NMR and X-ray structural analysis [10, 11].

The aim of the present study was to investigate the influence of different factors on the formation of nanoparticles in freshly brewed tea extracts. The mean particle size and number of the nanoparticles at different temperatures were examined. The effect of the addition of caffeine to solutions of freshly prepared decaffeinated tea was compared to the addition of theophylline and theobromine to study the influence of slight structural differences on the formation of the nanoparticles. Molecular modelling calculations were performed to evaluate the most probable methylxanthine-polyphenol complexes.

2. Investigations, results and discussion

2.1. Freshly prepared tea infusions

The formation of colloids in freshly prepared tea extracts on cooling was observed using PCS. The influence of temperature on the particle size and number can be seen in Fig. 1a and 1b. At temperatures of 46 and 40 °C only negligible amounts of particles are detected. Further decrease in temperature (35 °C–23 °C) results in an increase in the amount of particles detected. The mean diameter increases from about 263 nm at 36 °C to 330 nm at 27 °C. Between 27 and 22 °C the mean diameter is almost constant and only a small increase in the count rate is observed. It is important to note that the count rate is a parameter which is dependent on the device used. If another apparatus is used, differences in the absolute values could result.

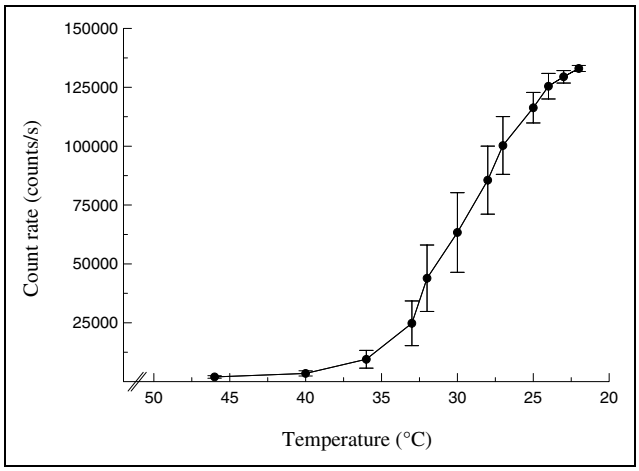

Fig. 1a: Mean count rate of filtered aqueous tea extracts on cooling measured by PCS; n = 5, arithmetic mean ± s

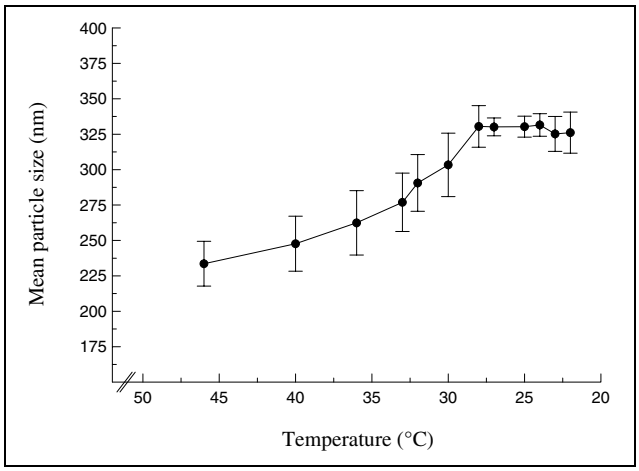

Fig. 1b: Mean particle size of filtered aqueous tea extracts on cooling measured by PCS; n = 5, arithmetic mean ± s

The particle size and number of nanoparticles in aqueous tea extracts depend on the temperature of the extract. At 37 °C, body temperature, particles are already detectable.

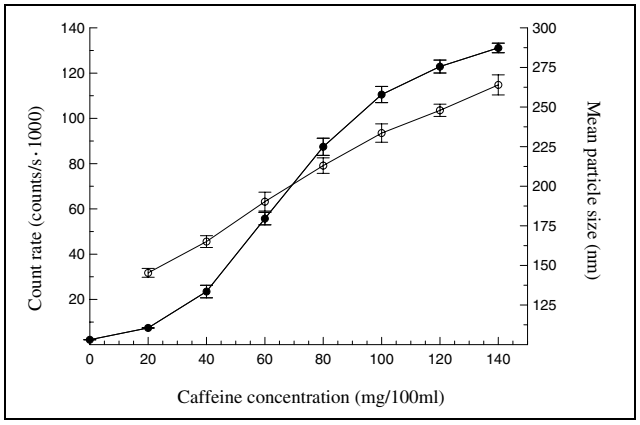

Fig. 2: Count rate and mean particle size of nanoparticles in aqueous infusions of decaffeinated tea after addition of caffeine, measured by PCS; n = 5, arithmetic mean ± s; ● count rate; ○ mean particle size

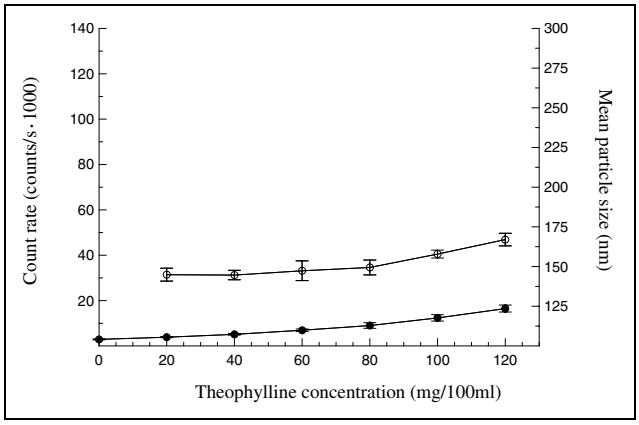

Fig. 3: Count rate and mean particle size of nanoparticles in aqueous infusions of decaffeinated tea after addition of theophylline, measured by PCS; n = 5, arithmetic mean ± s; ● count rate; ○ mean particle size

The count rate is increased by approximately a factor of 10 when the temperature is reduced by 10 °C. Such a temperature dependence has been described for other associates, e.g. in the case of phenothiazines [13–16].

2.2. Addition of methylxanthines to freshly prepared decaffeinated tea infusions

In the following experiments we compared the effect of the three methylxanthines on the formation of nanoparticles in extracts of decaffeinated tea infusions.

In the case of caffeine the formation of nanoparticles is induced by the addition of the drug (Fig. 2). The infusion of decaffeinated tea has only a negligable amount of particles detected by PCS. With increasing addition of caffeine the count rate increases sigmoidally. The mean particle size increases with increasing caffeine concentration.

In the case of theophylline only a few particles are formed (Fig. 3). Even at a concentration of 120 mg/100 ml theophylline the count rate measured was only 16,400 counts per second. At the same concentration of caffeine, more than 130,000 photons per second are measured by the Autosizer IIc. Compared to the tea nanoparticles containing caffeine the mean size of the particles is smaller.

In the case of theobromine the low solubility in water prevented experiments similar to those conducted for caffeine and theophylline. Only 80 mg could be dissolved in hot water, and on cooling crystallisation occurs.

Caffeine seems to be an important factor in the formation of nanoparticles. The addition of caffeine induces the formation of particles in aqueous extracts of decaffeinated black tea. With increasing caffeine concentration the particle sizes and the count rate increase. Our results are comparable to those of experiments reported in the literature [7]. Further molecular modelling calculations were performed to find favourable geometries for the complex of caffeine and tea polyphenols. Theacitrin A, a thearubigin [12], was chosen as a polyphenolic partner.

In Fig. 4 the results of the molecular modelling calculations are shown. On the left side, caffeine is located in a parallel position to the galloyl group of the theacitrin A molecule in a hydrophobic area. On the right side the molecules are presented with caffeine in the foreground. Its 6-membered ring covers the aromatic cycle of theacitrin A almost completely.

It has been reported that caffeine is able to form complexes with some phenolic partners [10, 17, 18]. In the literature, probable structures and models are discussed. Some of these results are summarized in a review by Spencer et al. [10]. A layer-lattice structure was found for different caffeine-polyphenol-complexes in the solid state using X-ray structural analysis. Caffeine is located parallel

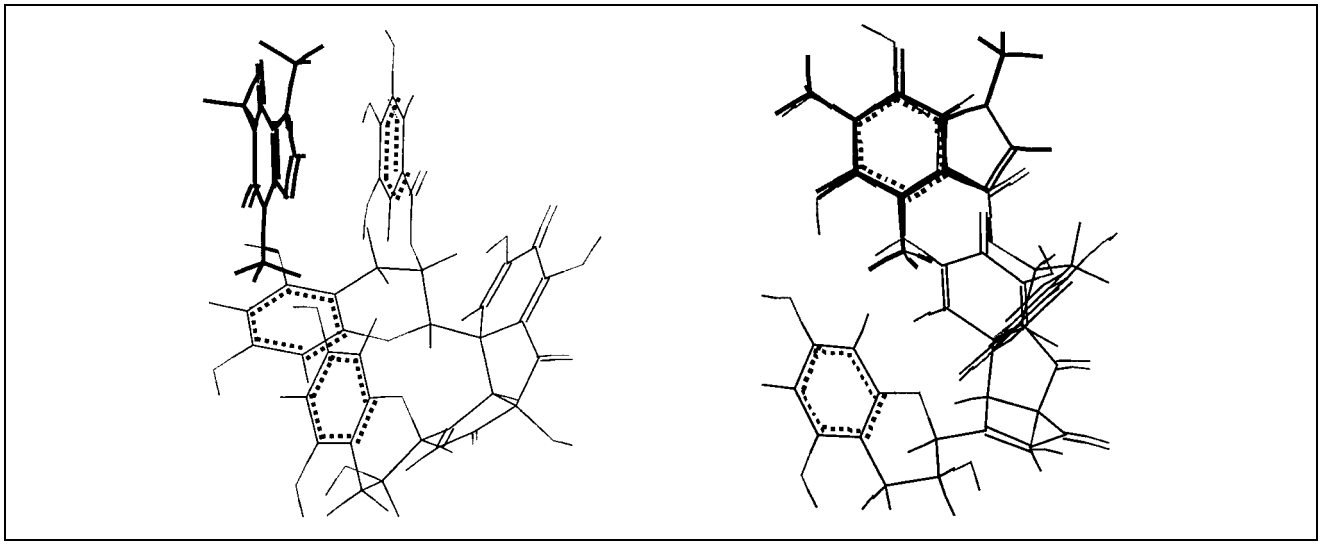

Fig. 4: Calculated structure of a theacitrin A-caffeine-complex, presented from the lateral and front view; aromatic structures are dotted

to aromatic structures. In the case of methyl gallate an in-plane system with hydrogen bonding between the three phenolic hydroxyl groups and the two keto-amide groups and the nitrogen atom in position 9 is described additionally. Interactions between polar groups and a polarisable component may also have an influence on the orientation. The tendency of caffeine and phenolic partners to stack in solution has been demonstrated by ^{1}H NMR spectroscopy.

π-π-interactions are an important factor for the complexation of theacitrin A and caffeine. An influence of temporary polarisation on orientation could not be confirmed in these molecular modelling studies. It is possible that caffeine connects thearubigins and theaflavins by deposition between their polyphenolic structures.

In the case of theophylline fewer nanoparticles are formed than in the case of caffeine. Theophylline and theobromine are able to interact with themselves via hydrogen bonding to form aggregates [18]. Therefore steric hindrance could be a reason why fewer nanoparticles are formed with tea polyphenols.

3. Experimental

3.1. Tea infusions

3.1.1. Preparation of aqueous black tea infusions

Infusions of Assam BOP or Infre BOP were freshly prepared. 3.0 g of the tea was filled in a tea bag and extracted with 150 g of hot purified water. After 10 min, the tea bag was removed. The hot liquid was filtered through a cellulose nitrate filter with a pore size of 3 µm (SM 11302-50-N; Sartorius, D-Göttingen) using a Sartorius filtration unit (SM 16510; Sartorius, D-Göttingen). The filtration rate was increased by a vacuum pump.

3.1.2. Addition of methylxanthines

In case of addition of methylxanthines the drugs were added 30 min after starting the extraction. Concentrations from 20 mg/100 ml to 140 mg/100 ml (steps of 20 mg/100 ml) resulted. The mixture was cooled for 90 min to a temperature of 22 °C. Then the extracts were examined using PCS.

To determine the size of the colloids of the Assam tea extract at different temperatures, the particle size and count rate were measured. PCS measurements were done while the tea was cooling down; the temperature of the spectrometer was equalised with the temperature of the extract. The first measurement was taken at a temperature of 40 °C, the last at 23 °C.

3.2. PCS measurements

To determine the mean diameter and the size distribution of the nanoparticles a Malvern AutoSizer 2c (Malvern, D-Herrsching) with a 5 mW Helium Neon laser (wave length 633 nm) was used with a small-sized beam. The laser illuminates the sample which has been filled in a cuvette (Article no. 67.754; Sarstedt, D-Nümbrecht) and is placed inside a temperature controlled enclosure (temperature: 22 °C). A photomultiplier detects the scattered light at an angle of 90° to the laser beam through an aperture with a diameter of 300 microns. The Autosizer operated with a Multi 8 Correlator with 64 channels. Each experiment lasted for 30 s. The sample distribution width was 1 : 50. A refractive index of 1.334 was determined. The cuvette was filled with a few millilitres of the sample, and five measurements per sample were performed. Between each of these measurements there was a delay of 10 s. In each experiment the mean diameter and the count rate were recorded. The count rate strongly depends on the apparatus used. Histograms of the mass distribution were calculated.

3.3. Molecular modelling

Molecular modelling calculations were performed to examine complexation between caffeine and thearubigins. Theacitrin A [12] was chosen as a representative polyphenolic substance. Molecular structures were calculated in vacuo. Hyperchem (vers 5.1; Hypercube, US-Waterloo) software was used on an AMD personal computer. First the molecular structures were calculated using MM+, a molecular mechanics method. Energy optimisation was performed using the atomic charges obtained by quantum chemistry calculations (AM1). Then the geometry of the complexes was optimised analogously. Changes of the conformation of each molecule were possible just as well as free orientation between the two molecules. The optimisation was finished when the energetic gradient dropped below 0.01 kcal/mol. The calculations were run with different starting geometries to explore the space of force field energies indicating the most probable geometries with the lowest energy values.

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