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# A novel approach to the measurement of surfactant parameters in arthropod digestive juices

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ABSTRACT

In arthropods, the determination of two important parameters of digestive juices, i.e. the total surfactant concentration and the critical micelle concentration (CMC), is challenging due to small sample volumes and low surfactant concentrations. In this work, we report a successful implementation of potentiometric titrations using the surfactant ion-selective electrode (SISE) and the pyrene fluorescence method (PFM) for the determination of the total surfactant concentration and CMC in the digestive juice of terrestrial isopod crustaceans *Porcellio scaber*. Pooled digestive juice extracts of four (SISE) or two (PFM) animals were used per measurement run. In both cases, digestive juice extracts in 100 μL of deionized water were sufficient for one measurement run. The total surfactant concentration of *P. scaber* digestive juice was determined to be 9.2 ± 3.5 mM and the CMC was approximately 90 μM. Our work presents an important improvement towards easy CMC determination in small volume samples in comparison with the commonly used stalagmometric technique, where much larger sample volumes are usually needed. To date, the total surfactant concentration was not measured in the digestive juices of arthropods other than *Homarus vulgaris*, *Astacus leptodactylus* and *Cancer pagurus*, for which complex separation and analytical techniques were required. Our results obtained by SISE and PFM therefore present the first successful quantification of surfactants and their CMC in small volumes of arthropod digestive juice without prior separation or purification techniques.

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## 1. Introduction

In invertebrates, digestive surfactants play various roles, many of which are analogous to those of bile salts in vertebrates (Vonk, 1962; Mayer et al., 1997). Digestive surfactants may solubilize food particles, activate or deactivate digestive enzymes, hold hydrophobic compounds in solution beyond their aqueous solubility by incorporating them into micelles, prevent the loss of digestive enzymes due to adsorption onto food, provide lubrication for the transport of food through the digestive system (Mayer et al., 1997), and perhaps play some other roles. In herbivore arthropods, specifically, digestive surfactants may also protect digestive enzymes and dietary proteins from being precipitated by tannin-rich food (Martin and Martin, 1984; Martin et al., 1987; Zimmer, 1997). In insects, digestive surfactants play a crucial role in plant–herbivore interactions, along with the facilitation of nitrogen assimilation from food (Mori and Yoshinaga, 2011). The knowledge of surfactant properties in invertebrate digestive juices is of interest in many fields, such as in crustacean aquaculture with the focus on improving feed digestibility (Sales, 2010; Yue et al., 2013; Perera and Simon, 2014), in plant physiology when studying the defensive reaction of plants to the injuries caused by herbivorous insects (Mori and Yoshinaga, 2011), in marine ecotoxicology with the aim of elucidating the susceptibility of animals to hydrophobic organic pollutants (Voparil et al., 2003), and in nanotoxicology with the goal of explaining the transformations of metal-containing nanoparticles after the consumption by test organisms (Diez-Ortiz et al., 2015; Romih et al., 2015).

The structure of digestive surfactants appears to be very similar throughout the arthropod phylum and, on the other hand, distinctly different from vertebrate surfactants. As opposed to vertebrates, arthropods are in general incapable of endogenous sterol synthesis (Zandee, 1967; Holwerda and Vonk, 1973), therefore the hydrophobic part of the surfactant molecule is represented by a fatty acid chain instead of the cholic acid derivatives (van den Oord et al., 1965; Vonk, 1969; Holwerda and Vonk, 1973; Collatz and Mommsen, 1974; Tumlinson and Lait, 2005; Mori and Yoshinaga, 2011). The structure of the surfactants' polar head group in arthropods varies. It may consist of a sulphate group, such

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as in Chinese mitten crab, *Eriocheir sinensis* (Vonk, 1969), or of different combinations of amino acids or related compounds (such as sarcosine and taurine) in different crustacean, insect and spider species (van den Oord et al., 1965; Holwerda and Vonk, 1973; Collatz and Mommsen, 1974; Tumlinson and Lait, 2005; Mori and Yoshinaga, 2011). The arthropod surfactants may be either anionic or zwitterionic.

Besides structure and charge, two additional parameters of surfactants in the arthropod digestive juices are important: their total concentration and the concentration where micellization occurs, which is termed the critical micelle concentration (CMC), because for many surfactant functions it is important that sufficient amount of micelles is present. However, the information about these two parameters is scarce due to the challenging analysis of digestive juice composition, because only small volumes of juices are present in most arthropod species. To the best of our knowledge, the total concentration and the CMC of arthropod surfactants have so far been quantified only in decapod crustaceans *Cancer pagurus* (Vonk, 1969), *Homarus vulgaris* and *Astacus leptodactylus* (Holwerda and Vonk, 1973). The most likely reason for the lack of data on surfactants in arthropod digestive juices is that for this purpose methods including ion-exchange chromatography, gel filtration, acid hydrolysis, thin-layer chromatography and amino acid analysis were used which demand complex sample preparation and separations before analyses (Vonk, 1969; Holwerda and Vonk, 1973). For insects, such as lepidopteran larvae (*Manduca sexta*, *Lymantria dyspar*) and grasshoppers (*Schistocerca gregaria*), as well as for isopods (*Porcellio scaber*), the exact quantification of the total surfactant concentration has not been attempted and their concentrations were expressed as multiples of (an unidentified value of) CMC (Martin and Martin, 1984; Martin et al., 1987; DeVeau and Schultz, 1992; Zimmer, 1997), which was measured by tensiometry according to the method of Ferguson (1933).

There is a need for methods that would enable the detection of low surfactant concentrations and a simple and rapid analysis, preferably with little or no sample preprocessing. One simple method for the determination of surfactant concentrations as well as of their CMC values, is the titration with surfactant ion-selective electrodes (SISEs), which have been commercially available since approximately 1990. These electrodes enable the determination of a variety of anionic and cationic surfactants (Schmitt, 2001). The titration technique for concentration determination is based on the precipitation reaction between surfactants in the sample and an oppositely charged surfactant in the titrant solution. It is thus not selective for individual surfactants, but determines their total amount in the mixture. Special sample pretreatment is generally not necessary and the analysis of surfactants is possible in various complex matrices, such as wastewaters or commercial cosmetic products (Metrohm Application Bulletin No. 233/3 e). The titration conditions must be optimized for each type of the electrode, as well as for each surfactant, because SISEs from different manufacturers differ in their response time and in the potential difference registered during a titration (Schmitt, 2001).

An alternative method to tensiometry for the measurement of CMC in small-volume samples is the pyrene fluorescence method ([PFM] Kalyanasundaram and Thomas, 1977; Winnik and Regismond, 1996). In this method, pyrene is added to the surfactant solution as a fluorescent probe and the intensity ratio of the first and the third peak ($I_1/I_3$) in the characteristic pyrene fluorescence spectrum is calculated for various surfactant concentrations. The ratio of these two peaks depends on the net hydrophobicity/polarity of the probe's immediate environment. Below the CMC, where no hydrophobic domains are present in aqueous surfactant solutions, the peak ratio value is high, corresponding to the polar environment of water. When surfactant micelles start to form, the ratio decreases and reaches a lower and roughly constant value above the CMC, because pyrene becomes incorporated in the hydrophobic regions of the micelles. The CMC is evaluated at the point where the pyrene polarity ratio as a function of the total surfactant concentration starts to decrease (Winnik and Regismond, 1996). The advantage of PFM over tensiometry for the determination of CMC is the considerably lower amount of solution needed for fluorescence measurements. This makes PFM appealing for the analysis of biological samples where volumes are often extremely limited, especially since fluorimeters have nowadays become common pieces of laboratory equipment.

The aim of the present study is to apply the commercial SISE and PFM to determine the total surfactant concentration and the CMC in the digestive juice of a model arthropod, the terrestrial isopod crustacean *P. scaber*. Terrestrial isopods *P. scaber* are among the most studied organisms in ecotoxicology, ecophysiology (Drobne, 1997), and recently also in nanotoxicology (Romih et al., 2015). In contrast to insects, the main site of digestive processes in isopods is the hindgut, which comprises 80–90% of the total length of the digestive system. The midgut is restricted to the midgut glands (hepatopancreas) that adjunct the alimentary tract ventrally at the junction between the foregut and the hindgut. The digestion of food and microbial cells take place in the anterior hindgut, while the posterior hindgut is the location of water resorption and osmoregulation. The anterior hindgut is dorsally invaginated into a pair of typhlosole channels, which enhance the surface area available for ion exchange with the haemolymph, channel digestive fluids back and forth to ensure mixing of food and digestive enzymes, transport the digestive enzymes from the hepatopancreas to the hindgut and nutrients back from the hindgut to hepatopancreas. The hepatopancreas is the site of the secretion of digestive enzymes and the absorption of nutrients (Zimmer, 2002). Accordingly, the digestive juice was extracted from the *P. scaber* hindgut in our experiments. We discuss the possibility of quantifying surfactant content and their CMC in small volumes of *P. scaber* digestive juice without prior separation or purification techniques. This information is important for the understanding of processes occurring in the arthropod digestive systems.

## 2. Materials and methods

### 2.1. Reagents and solutions

All chemicals were of analytical grade purity. Silver nitrate ($AgNO_3$), *N*-dodecylpyridinium chloride (DPC), sodium dodecyl sulfate (SDS), sodium *N*-lauroylsarcosine (NLS), and pyrene were purchased from Sigma Aldrich (Steinheim, Germany). Water used throughout the work was first deionized and then additionally purified using the Elix 10/Milli-Q Gradient unit (Millipore, Bedford, Massachusetts, USA).

### 2.2. Collection and cultivation of model arthropods

Terrestrial isopods were collected in April 2014 from a compost heap in a non-polluted area near Vrhnika, Slovenia. The animals were kept in a controlled chamber at a constant temperature (20 ± 2 °C) with a light regime (at 16 h light and 8 h darkness cycle) and fed dry common hazel leaves (*Corylus avellana*) for three weeks before the experiments were carried out.

### 2.3. The depuration and dissection of test animals

Immediately prior to the experiments, 50 adult *P. scaber* of both sexes, at the intermoult stage and with the average fresh body mass of 63 ± 19 mg, were collected from the culture and placed individually into plastic Petri dishes (9 cm in diameter) to depurate

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