Comparative *in vitro* and *in vivo* taste assessment of liquid praziquantel formulations

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Abstract

The taste of pharmaceuticals strongly affects the compliance of patients. This study investigated the applicability of the electronic tongue and rodent brief-access taste aversion (BATA) model for the bitter compound praziquantel (PZQ) and taste masked liquid formulations for PZQ. In a comparative study maltodextrin (MD) Kleptose® linecaps 17 was selected as an alternative taste masking agent to two cyclodextrins; hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sulfobutyl ether-beta-cyclodextrin (SBE-β-CD). A phase solubility study showed the highest affinity and solubilization capabilities for SBE-β-CD over HP-β-CD and MD, suggesting the highest taste masking ability for SBE-β-CD. No reliable results were achieved for PZQ with the Insent electronic tongue. Thus this system was not used for further evaluation of solutions with MD and CDs to confirm the results of the solubility study. In contrast the BATA model demonstrated conclusive responses for the aversiveness of PZQ. The concentration of PZQ inhibiting 50% of water lick numbers (called IC_{50} value) was 0.06 mg/ml. In contrast to the phase solubility study, the MD enabled an equal taste masking effect *in vivo* in comparison to both CDs. Moreover HP-β-CD showed superior taste masking capabilities for PZQ compared to SBE-β-CD as the SBE-β-CD itself was less acceptable for the rodents than HP-β-CD. In conclusion, the BATA model was identified as a more efficient taste assessment tool for the pure PZQ and liquid formulations in contrast to the electronic tongue and the phase solubility study.

Keywords

Brief-access taste aversion, Electronic tongue, Taste masking, Praziquantel, Maltodextrin, Cyclodextrin

1. Introduction

Taste masking of drugs is of particular importance for the adherence of patients, especially for children or in the veterinary area for picky animals like cats (Walsh et al., 2014). It is reasonable to screen molecules and clinical formulations regarding their taste as early as possible to save costs in the product development of a compound. Due to ethical and toxicological reasons human studies are not possible to perform at an early stage, especially for challenging patient groups like children (Pein et al., 2014). Alternative reported non-human taste assessment tools include *in vivo* methods such as animal preference tests using dogs, cats, rats or mice or even fish or drosophila and electrophysiological methods in primates. Furthermore, *in vitro* methods were developed despite drug release studies which are mostly based on determined taste thresholds in humans. Such *in vitro* methods involve electronic taste-sensing systems (electronic tongues) or cell based systems using calcium imaging (Mohamed-Ahmed et al., 2016; Slack et al., 2010). The most useful non-human taste assessment tools are the electronic tongue and the BATA model (Mohamed-Ahmed et al., 2016).

Electronic tongues are artificial taste assessment tools (Pein et al., 2015). They are analytical sensor array systems characterizing pure substances or formulations in aqueous solutions. The equipped sensors vary in their composition and properties resulting in a selectivity for different substances. The applied measurement principle can be based on potentiometry, voltammetry, amperometry or others (Khan and Kang, 2015). In accordance to the human taste the activity of a compound logarithmically affects the measured signals of the electronic tongue (Woertz et al., 2010, 2011c). Various studies focused on the implementation of these systems for taste assessment in pharmaceutical formulation development for liquid and solid dosage forms and demonstrated correlations to human taste panels (Eckert et al., 2013; Haraguchi et al., 2016; Pimparade et al., 2015; Preis et al., 2012; Rudnitskaya et al., 2013; Wesoły et al., 2017; Woertz et al., 2011a; Woertz et al., 2011b; Woertz et al., 2011c). Electronic tongues are preferable over human taste panels in terms of safety, toxicity and objectivity. They can provide an early screening of new drugs of unknown toxicity and the relative optimization of preclinical formulations (Mohamed-Ahmed et al., 2016). As a prerequisite for the assessment of multicomponent solutions, a calibration for the pure compounds needs to be performed proving a concentration dependent signal. Subsequently mixtures can be compared regarding their taste masking using multivariate data analysis (Lorenz et al., 2009).

Besides the electronic tongue and in terms of readiness, the rodent brief-access taste aversion (BATA) model is the most useful non-human taste assessment tool. Indeed various parameters (necessary time

for data collection, ability to screen pure drugs and formulations, correlation to human *in vivo* data, validation potential and costs) were graded higher comparatively to other tools by Mohamed-Ahmed *et al* (Mohamed-Ahmed et al., 2016). Several studies showed the great potential of rat models designed for the measurement of the palatability of different compounds (Bhat et al., 2005) and the correlation to human taste panels (Clapham et al., 2012; Devantier et al., 2008; Noorjahan et al., 2014; Rudnitskaya et al., 2013; Soto et al., 2016). In the BATA model, samples are presented randomly to rats or mice in several sipper tubes and the number of licks recorded electronically by a lickometer is inversely proportional to the aversiveness of the samples (Soto, 2016). Due to the short period of time of exposure to samples, the intake of each compound is limited, to avoid toxic side effects. New chemical entities with unknown toxicity and well-known pharmaceutical compounds can be screened in early development as pure drugs or in preclinical or clinical formulations (Mohamed-Ahmed et al., 2016).

An example for a compound with an unpleasant taste is praziquantel (PZQ). It is an anthelminthic drug that is used in adults and children against schistosomiasis and worm infections in animals. It is currently used in mass control programs for morbidity control in school-age children and adults at risk (Meyer et al., 2009). A drawback is its intensive bitter and metallic taste, which is accompanied with poor compliance (Passerini et al., 2006).

One approach to improve the taste of aversive compounds is the complexation with cyclodextrins (CDs). They are cyclic oligosaccharides (α-D-glucopyranose) obtained from starch shaped as truncated cones (Szente et al., 2016). Several interactions such as hydrogen bonds, van der Waals', electrostatic, charge-transfer and hydrophobic binding lead to host-guest type inclusion complexes and partly or complete encapsulation of a drug (Loftsson and Brewster, 1996) providing increased drug solubility, bioavailability or stability and decreasing unpleasant taste and smell (Szejtli and Szente, 2005).The β-CD and its derivatives, including hydroxypropyl-β-CD (HP-β-CD) and sulfobutyl ether-β-CD (SBE-β-CD) are the most commonly used CDs in pharmaceutical industry (Jambhekar and Breen, 2016) and were chosen for PZQ on the basis of previous studies (Becket et al., 1999). They are considered as safe as the daily oral dose for HP-β-CD in pharmaceuticals may reach 8 g/day (EMA, European Medicines Agency, 2014). Higher amounts showed an increase in the incidence in soft stools and diarrhea. Further findings upon oral administration where cecal enlargement and renal effects due to systemic absorption (Stella and He, 2008). There is no data available for children below two years (EMA, European Medicines Agency, 2014).

In addition maltodextrins (MDs) were investigated as they can also provide taste masking and solubility enhancement (Preis et al., 2014; Preis et al., 2012). With no limited daily intake (EFSA, European Food Safety Agency, 2013) and their wide use in infant formula and nutritional supply they represent a promising alternative to CDs. They consist of d-glucose units (amylose and amylopectin) connected in chains of variable length with α-1,4-glycosidic and few α-1,6-glycosidic bonds derived from starch. The incorporated amylose builds up a helical structure in aqueous media (Carbinatto et al., 2016). In this way maltodextrins can provide inclusion complexes by hydrophobic and van der Waals' interactions and can encapsulate hydrophobic drugs (Kong and Ziegler, 2014; Luo et al., 2016; Ribeiro et al., 2017). Kleptose® linecaps 17 was chosen as the most promising MD due to its high amylose content and previous promising results regarding taste masking (Preis et al., 2014; Preis et al., 2012).

Former studies reported the complexation mechanisms of PZQ with HP-β-CD and SBE-β-CD improving the solubility and the dissolution of the drug. None of them investigated their taste masking efficiency for PZQ *in vivo* in comparison to MD as a promising alternative to CDs.

Therefore the aim of this study was to investigate alternative taste assessment tools to human taste panels for the bitter compound PZQ. The electronic tongue and the BATA model were chosen as the most promising taste screening tools. Firstly the applicability of both methods was evaluated for the pure compound PZQ. Secondly the efficacy of the BATA model was evaluated by comparing PZQ taste masking capabilities of aforementioned MD and CDs.

2. Materials and methods

2.1. Materials

Racemic Praziquantel was supplied by Merck KGaA (Darmstadt, Germany). Hydroxypropyl-beta-cyclodextrin (HP-β-CD, Kleptose® HPB, 1387.2 g/mol) and maltodextrin (MD, Kleptose® linecaps 17, 12635.0 g/mol) were purchased from Roquette (Lestrem, France). Sulfobutyl ether-beta-cyclodextrin (SBE-β-CD, Captisol®, 2163.0 g/mol) was received from CyDex Pharmaceuticals (Inc., Lawrence, Kansas).

The following substances were used for the reference and washing solutions for the electronic tongue: potassium chloride (Gruessing GmbH, Filsum, Germany), tartaric acid (AppliChem GmbH, Darmstadt, Germany), quinine hydrochloride dihydrate (Buchler GmbH, Braunschweig, Germany), hydrochloric acid (Merck KGaA, Darmstadt, Germany), potassium hydroxide (Gruessing GmbH, Filsum, Germany), absolute ethanol (VWR international, Darmstadt, Germany) and distilled water obtained by in-lab distillation of demineralized water. Deionized water in the BATA-model was prepared by ion exchange. All samples of the phase solubility study were prepared with purified water produced by a Millipore-Milli-Q® integral water purification system (Merck KGaA, Darmstadt, Germany). Acetonitrile and ethanol as LC-MS grade (LiChrosolv®) were provided by Merck KGaA (Darmstadt, Germany).

Self-developed electronic tongue sensors were prepared using Polyvinyl chloride (PVC, Sigma-Aldrich, Steinheim, Gemany) as polymer, isopropylmyristate (IPM, Cognis GmbH, Duesseldorf, Germany) as plasticizer, either tetra-dodecyl ammonium bromide (TB, Sigma-Aldrich), trioctylmethyl ammonium chloride (TC, Alfa Aesar, Karlsruhe, Germany) or bis(2-ethylhexyl) phosphate (BP, Sigma-Aldrich, Steinheim, Germany) as artificial lipids, oleic acid (OA, Fluka Analytical, Steinheim, Germany) and either hydroxypropyl-ß-cyclodextrin (HPßCD, Roquette, Lestrem, France) or a cyclodextrin oligomer (CDO, HHU, Duesseldorf, Germany) as ionophores and tetrahydrofuran (THF, VWR international, Darmstadt, Germany), absolute ethanol (Sigma-Aldrich, Steinheim, Germany) and acetone (VWR international, Darmstadt, Germany) as solvents for the membrane preparation.

2.2. Methods

2.2.1. Phase solubility study with maltodextrin and cyclodextrins

A phase solubility study according to the experimental design of Higuchi and Connors (Higuchi and Connors, 1965) and Loftsson (Loftsson et al., 2007) was conducted to compare the solubility and resulting taste masking capabilities of the maltodextrin (MD) Kleptose® linecaps 17 and the cyclodextrins (CDs) Kleptose® HPB (HP-β-CD) and Captisol® (SBE-β-CD) *in vitro*.

Excess amounts of PZQ (10-fold) were added to aqueous solutions of 2, 5, 10, 15 and 20 mM of the MD or the CDs (n = 3). After stirring for seven days at ambient temperature to reach an equilibrium, the suspensions were filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium). The solubilized drug content was determined via high performance liquid chromatography. The results were plotted against the used concentration of the MD or CDs. The apparent stability constant ($K_{1:1}$) and the complexation efficiency (CE) were calculated as follows:

$$K_{1:1} = \text{slope}/(S_0\,(1-\text{slope})\,) \quad (1)$$

$$CE = \text{slope}/(1-\text{slope}) = [PZQ/CD]/[CD] \quad (2)$$

2.2.2. Analysis of drug content

The solubilized drug content was determined via high performance liquid chromatography (HPLC) using an Agilent 1100 (Agilent Technologies, Santa Clara, USA) at 210 nm. The analysis was performed with a Waters Symmetry® column (Waters Symmetry® Shield RP 18, 150 x 4.6 mm 3.5 µm). Eluents were water and acetonitrile in a validated gradient method with a flow rate of 1.5 ml/min.

2.2.3. *In vitro* taste assessment by electronic tongue

The commercially available electronic tongue TS-5000Z (Insent Inc., Atsugi-chi, Japan) was used for the *in vitro* evaluation of the taste intensity of various PZQ concentrations. The system is composed of a sensor unit with a sample table with two circles of sample positions, two sensor heads with up to eight sensors at a robot arm and a data recording system.

2.2.3.1. Sensors

The commercially available sensors (Insent Inc., Atsugi-chi, Japan) included in this study were SB2AC0, SB2AN0 and SB2BT0 (cationic and neutral bitter compounds), SB2AAE (umami), SB2CT0 (saltiness) and SB2CA0 (sourness). As the detection of non-ionic and slowly water soluble substances like PZQ is limited self-developed sensors were evaluated named as sensor A to G (**Table 1**). The self-developed sensors were prepared according to Immohr *et al.* (Immohr et al., 2016). All sensors were preconditioned in a standard solution (30 mM potassium chloride and 0.3 mM tartaric acid in distilled water) for one day. Prior to each measurement a sensor check was performed.

2.2.3.2. Preparation of reference and sample solutions

A stock solution of 0.5 mM (0.16 mg/ml) PZQ in distilled water was prepared and further diluted to 0.1 (0.032 mg/ml), 0.05 (0.016 mg/ml) and 0.01 mM (0.0032 mg/ml). Using 4 different concentrations a calibration curve was generated to assess reliable drug detection of all sensors. As an external standard quinine hydrochloride with a concentration of 0.5 mM in distilled water was used to monitor the results of each sensor over time and reduce fluctuations in the sensor signals. This is recommended as the sensor response is affected by the environment, e.g. the temperature, and the age of the sensor (Woertz et al., 2011a).

2.2.3.3. Measurement setup

The measurement circle started with three washing steps in a washing solution. For positively charged sensors this was conducted in the standard solution, for negatively charged sensors 100 mM hydrochloric acid and ethanol 30% (w/w) were used. Afterwards a sample was analyzed regarding its taste for 30 s followed by two short washing steps of 3 s and the detection of the aftertaste for 30 s. The aftertaste depicts the change of the membrane potential due to absorption (CPA) of the compound to the lipid membrane of the sensor. This was followed by washing steps ending in the next circle. Each sample was measured 5 times in a randomized order, but always starting with the reference solution to monitor the sensor response.

2.2.3.4. Data analysis

Univariate data analysis was applied for comparison of all sensors and concentrations of PZQ. The results are displayed as a change of the membrane potential in mV. They were calculated in relation to

the reference solution. The first two runs of each sample were discarded as they were considered as preconditioning of the sensors. Based on the last three results of each concentration the mean and standard deviations were calculated for the taste and aftertaste.

2.2.4. *In vivo* taste assessment by Brief-Access Taste Aversion (BATA) model

2.2.4.1. Animals

The taste assessment was carried out with two groups of ten adult male Sprague-Dawley (Charles-River, Kent, UK) in accordance with the UK Animals (Scientific Procedures) Act 1986 (Project License PPL 70/7668). They were housed in pairs in standard cages at 21 ± 2°C and 50 ± 10% humidity with a 12:12 h light/dark cycle. All training and testing occurred during the light phase of the cycle. Animals had free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a water-restriction schedule occurred. Throughout the experiment, daily food and water consumption were monitored. As a safety and welfare measure it was checked that their weight did not drop below 85% of their free feeding weight.

2.2.4.2. Sample preparation

To evaluate the feasibility and the reliable response of the rodents, 6 different concentrations of PZQ in in-lab deionized water were tested. A stock solution of 0.2 mg/ml of PZQ was prepared and diluted with deionized water to 0.005, 0.01, 0.03, 0.05 and 0.10 mg/ml.

After this calibration the taste masking capabilities of the MD in comparison to both CDs was evaluated *in vivo*. Aqueous solutions of each excipient at 20 mM were prepared and excess amounts of PZQ were added as described in 2.2.1 to reach the maximum solubility of PZQ. The resulting suspensions were filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium). The final drug content was analyzed via HPLC. For comparison with the pure PZQ in deionized water, the PZQ concentrations in the mixtures were set to the IC_{50} value of 0.06 mg/ml, the maximum solubility of the pure PZQ of 0.2 mg/ml and the maximum reachable PZQ concentration of 0.27 mg/ml for the MD and 1.3 mg/ml for both CDs. The concentrations were diluted with pure excipient solutions (20 mM) to reach the target concentrations.

2.2.4.3. Experiment procedure

The experimental design consisted of two training and testing days. Each rat was water-deprived for 22 h before each session (training and testing) and was then placed in a lickometer (Davis MS-160, DiLog Instruments, Tallahassee, Florida, USA) for a maximum session-length of 40 min. The initial days of the protocol were dedicated to training with sipper tubes presenting deionized water. During both testing days each sample was presented in a sipper tube randomly up to 4 times to each rat per day. The trial began when the rat took its first lick from the sipper tube, and ended eight seconds later when the shutter closed. The rats received a water rinse between samples for 2 s from a sipper tube to minimize carry over effects. After each testing periods they received tap water for one hour for rehydration. In sum, each sample was tested up to 8 times (4 times per testing day) to 10 rats resulting in a final number of 80 measurements per solution. The taste of each sample was assessed by the number of licks per 8 s recorded by the lickometer. As a reference deionized water or pure excipient solutions were assessed. The experimental procedure was described in detail and optimized in earlier studies (Soto, 2016).

2.2.4.4. Data analysis

All data sets of each concentration of PZQ or the formulations with MD or CDs were statistically analyzed. The IC_{50} value describing the concentration of PZQ inhibiting 50% of the maximum lick numbers compared to the reference (water) was calculated with an E_{max} model as described in earlier studies (Soto et al., 2015). In addition, the percentage of lick inhibition relatively to the reference was determined with the following equation:

$$\%\ \text{inhibition of licks} = \frac{N_0\text{licks}_{\text{reference}} - N_0\text{licks}_{\text{concentration PZQ}}}{N_0\text{licks}_{\text{reference}}}\ \text{x}\ 100 \qquad (3)$$

The results were classified as presented in **Table 2**.

2.2.5. Statistical analysis

The data of the phase solubility study and the electronic tongue were expressed as the arithmetic mean ± standard deviation (S.D.). All BATA results are expressed as the arithmetic mean ± standard error of the mean (S.E.M.). The software SigmaPlot (version 12.5, Systat Software, Erkrath, Germany) was used for all statistical analyses. Statistical comparisons of two groups were performed with the Mann-Whitney test ($p \leq 0.05$). The Kruskal-Wallis test ($p \leq 0.05$) was applied for more than two groups.

3. Results and discussion

3.1. Comparative phase solubility study with maltodextrin and cyclodextrins

The ability of MD to improve the solubility of PZQ and hence a taste masking ability was evaluated by conducting a phase solubility study (Higuchi and Connors, 1965) in comparison to CDs.

Both CDs (HP-β-CD and SBE-β-CD) significantly increased the intrinsic solubility of PZQ in water (0.71 mM) up to 5 to 7-fold at the highest concentration of 20 mM CD ($p < 0.001$) (**Fig. 1**). These findings confirmed previous results of Arrua *et al.* (Arrua et al., 2015) and Maragos *et al.* (Maragos et al., 2009). A linear profile of the solubility increase of CDs indicated a stoichiometry of 1:1, a positive or negative deviation from linearity would be associated with a higher order interaction or a self-association of CDs (Loftsson et al., 2005). Whereas this conclusion is in line with Maragos *et al.* (Maragos et al., 2009), de Jesus *et al.* (de Jesus et al., 2006) and El-Arini *et al.* (El-Arini and Leuenberger, 1996), other studies reported a stoichiometry of 1:2 (Arrua et al., 2015).

The apparent solubility constant $K_{1:1}$ and the complexation efficiency (CE) were calculated from the slope of the plot (**Table 3**). SBE-β-CD showed the highest solubility enhancement and a CE of 0.26 meaning that one out of five SBE-β-CD molecules complexed the drug. For HP-β-CD a lower CE of 0.16 was observed. Less than one out of seven HP-β-CD molecules interacted with the drug. Loftsson *et al.* summarized the reported complexation studies appointing an average CE for HP-β-CD of 0.39 ± 0.47 for 13 different drugs in water (Loftsson et al., 2007). High values for $K_{1:1}$ above $1 \times 10^5\ M^{-1}$ can lead to negative effects on the bioavailability of a compound (Stella and He, 2008). A strong affinity of a CD to a specific compound can result in issues regarding the complete dissociation of the drug from the CD in the gut. As the $K_{1:1}$ values for HP-β-CD and SBE-β-CD in this study are below this critical values no issues were anticipated *in vivo*.

The calculations were not applicable for the MD as there was no remarkable slope of the phase solubility profile. A solubility enhancement as it was shown for loperamide, dextromethorphan or dimenhydrinate leading to a taste masking effect could not be demonstrated for PZQ (Preis et al., 2014; Preis et al., 2012). The incorporated amylose builds up a helical structure in aqueous media (Carbinatto et al., 2016). In this way maltodextrins can provide hydrophobic and van der Waals' interactions to hydrophobic drugs (Kong and Ziegler, 2014; Luo et al., 2016; Ribeiro et al., 2017). Nevertheless these findings could not be confirmed for PZQ in this phase solubility study (**Fig. 1**).

Based on this phase solubility study a higher taste masking ability of both CDs in comparison to the MD was expected.

3.2. Electronic tongue

Pure PZQ was tested with four different concentrations ranging from 0.01 mM to 0.5 mM in distilled water to generate a calibration curve by means of the electronic tongue. The concentration range was comparable to other studies with other compounds (Pein et al., 2015). The detection was evaluated with commercially available sensors (SB2AC0, SB2AN0, SB2AAE, SB2CT0, SB2CA0 and SB2BT0) and self-developed sensors named as sensor A to G (composition described in **Table 1**). The sensor responses were determined as the change of the membrane potential (**Fig. 2**). Depending on the composition of the sensor membrane negative or positive responses were obtained (Kobayashi et al., 2010). None of the sensors supplied a concentration-dependent signal for PZQ in the full concentration range. The commercially available sensors SB2AC0, SB2AN0 and SB2CA0 indicated a log-linear response from 0.01 to 0.1 mM, but not for 0.5 mM limiting the usability. This can be explained by the non-ionic character and the low solubility of PZQ in water resulting in only minor effects on the membrane potential of all tested sensors. The change of membrane potential as the response of the sensor to a sample solution can be generated by different mechanisms. The initial membrane potential of each sensor is formed when the lipid membrane is immersed in the reference solution. An electrical double layer is created at the surface depending on the composition of the incorporated lipids in the membrane. On the one hand, compounds in the sample solution can affect the membrane potential by ions directly altering the double layer. On the other hand, molecules can prevent lipid molecule dissociation which is responsible for the initial membrane potential. Furthermore, bitter compounds adsorb to the hydrophobic parts of the lipid membrane modifying the potential due to a varied charge density (Immohr et al., 2016; Kobayashi et al., 2010). Thus, a limited detection of the sensors has been expected for the non-ionic PZQ providing too little conductivity. Furthermore it could be assumed that the adsorption of PZQ on the lipid membranes of all sensors is too low for reliable interaction and resulting measurement signals. Difficulties regarding the detection of other neutral compounds have been reported before for ibuprofen, caffeine (Woertz et al., 2011a) and acetaminophen (Woertz et al., 2010). A higher concentration of PZQ could have improved the results but could not be provided due to the limited and pH independent solubility of PZQ in water. Modifying the media of the sample solution was not considered as previous studies demonstrated a shift to higher detection limits when evaluating

different pH values and ionic concentrations (Tissen, 2011). As a consequence the detection of PZQ needs to be improved by developing sensors providing a higher sensitivity for the compound (Immohr and Pein-Hackelbusch, re-submitted on 06.03.2017).

The change of membrane potential caused by adsorption or so called aftertaste sensor responses (**Fig. 3**) were smaller for the majority of the sensors due to the washing step in between. Sensor signals before and after washing were significantly ($p < 0.05$) different except for the sourness sensor SB2CA0 ($p > 0.05$). The highest difference between measurements before and after washing were observed for the cationic bitterness sensor SB2AC0. At 0.5 mM CPA values were more than 8 times lower compared to the change of membrane potential before washing ($p < 0.001$). This could indicate an interaction of PZQ with SB2AC0. Nevertheless none of the sensors provided a concentration-dependent response. As similar to the previous experiment (**Fig. 2**) deviations between concentrations for each sensor are below the measured standard deviations and do not provide additional insights regarding the taste intensity of PZQ or the reasonable applicability of one of the sensors in this study.

3.3. BATA model

3.3.1. Evaluation of praziquantel

As an alternative taste assessment tool, the BATA model was used for PZQ and liquid formulations with MD and CDs. A mean value of 44.7 licks ± 1.1 was observed for deionized water as the reference of all 10 rats during both testing days of the pure compound. The water-deprivation of 22 h was suitable to encourage the rats to drink from the various sipper tubes.

The number of licks decreased with increasing concentration of PZQ (**Fig. 4** and **Fig. S1**). PZQ was fully tolerated and did not show a significant decrease in number of licks at the concentration of 0.005 mg/ml (45.3 ± 0.9 licks, 0 ± 1.2% lick inhibition) and 0.01 mg/ml (43.6 ± 1.2 licks, 1.2 ± 2.4% lick inhibition) in comparison to deionized water ($p = 0.896$ and 0.206). For a higher concentration of 0.03 mg/ml a significant decrease in lick number (32.9 ± 1.7 licks) was observed ($p < 0.001$), but was still well tolerated (26.4 ± 3.9% lick inhibition). An increase to 0.05 mg/ml PZQ was tolerated (34.6 ± 4.1%) with 29.2 ± 1.8 licks. The taste of PZQ was perceived aversive at 0.1 and 0.2 mg/ml (19.1 ± 1.5 licks, 57.3 ± 0.2% lick inhibition and 12.5 ± 1.2 licks, 72.0 ± 2.6% lick inhibition).

The IC_{50} value was determined as 0.06 mg/ml (95% CI 0.049-0.082). Thus PZQ showed an extreme aversive taste in the range of quinine hydrochloride (Soto et al., 2015), worse than other bitter compounds such as sildenafil citrate, caffeine citrate, diclofenac or paracetamol (Soto, 2016; Soto et al., 2016). These findings underline the importance of the taste masking for PZQ for the in use palatability of drug products. The individual lick profiles for each rat demonstrate the sensitivity of all rats for PZQ. All rats responded with a decreasing number of licks with an increasing PZQ concentration. This is of high importance as it is well-known that mammalians vary greatly in their taste perception of bitter compounds due to a variability in receptor gene expression in the mouth (Behrens and Meyerhof, 2009; Reed et al., 2006). The variability of both testing sessions (day 1 and day 2) was not significant ($p > 0.1$). Thus the rats did not get used to the aversiveness of PZQ. Furthermore this low variability strengthens the reliability of the results.

3.3.2. Comparative evaluation of maltodextrin and cyclodextrins

Three different concentrations of PZQ were tested with 20 mM MD or CDs. For comparison with pure PZQ in deionized water, the PZQ concentrations in the mixtures were set to the IC_{50} value of 0.06 mg/ml, the maximum solubility of the pure PZQ of 0.2 mg/ml and the maximum reachable PZQ concentration of 0.27 mg/ml in solution with MD and 1.3 mg/ml in solution with CDs.

The taste of pure 20 mM HP-β-CD (55.5 ± 1.2 licks) was similar to water (56.7 ± 1.4 licks) ($p = 0.149$) (**Fig. 5** and **Fig. S2**). However, pure 20 mM SBE-β-CD did significantly decrease the lick number to 43.2 ± 2.4 ($p < 0.001$). Yet, the profiles for formulations at 0.06 and 0.2 mg/ml indicate a taste masking effect for both CDs. The lick numbers measured at 0.06 mg/ml for PZQ with HP-β-CD (53.5 ± 1.8 licks) and SBE-β-CD (44.0 ± 2.2 licks) did not significantly differ from the pure excipient solutions ($p = 0.119$ and 0.616) indicating that these formulations were well tolerated by the rats (**Table 4**). In contrast, the pure PZQ was aversive/ untolerated at 0.06 mg/ml (IC_{50} value). The measured lick numbers significantly decreased for both CD formulations of 0.2 and 1.3 mg/ml ($p < 0.001$), but were better accepted at 0.2 mg/ml than the pure PZQ solution. The complexation of PZQ with CDs improved the solubility of PZQ to 1.3 mg/ml but did not achieve a taste masking. As the complexation of PZQ and CDs is an equilibrium, free drug molecules are in solution (Loftsson et al., 2005). The amount of this free uncomplexed molecules increased with increasing drug concentration which might have lead in turn to a higher taste perception in the rats.

HP-β-CD showed a significant higher numbers of licks ($p < 0.001$) than SBE-β-CD at all concentrations. These findings are contrary to the results of the phase solubility study. SBE-β-CD led to a higher linear increase of the solubility of PZQ resulting in a higher $K_{1:1}$ and CE than HP-β-CD (**Fig. 1**). Thus it was thought that this higher affinity of PZQ to SBE-β-CD would lead to a higher taste masking efficiency. However, the interaction and complexation of PZQ with the CDs is a rapid equilibrium with free drug and CD molecules. Arrua *et al.* (Arrua et al., 2015) described the presence of free PZQ in complexes with CDs. Moreover, CDs can form inclusion and non-inclusion complexes where the compounds interaction is located on the surface of the CD (Loftsson et al., 2007). In this way the compound is not shielded completely for taste perception and the affinity of the drug to the CD might not be solely representative for the taste-masking effect. In addition, the results of the pure excipients outlined an aversiveness of the pure SBE-β-CD in comparison to the pure HP-β-CD. In this study, the phase solubility results were not predictive for the *in vivo* taste assessment in the BATA model.

The MD did not affect the number of licks in comparison to water ($p = 0.810$) (**Fig. 5** and **Fig. S2**). The solution was fully tolerated according to the % lick inhibition (**Table 4**). In contrast to our expectations based on the phase solubility study, the BATA model revealed a taste masking effect of the MD up to 0.06 mg/ml PZQ. The result for this PZQ concentration did not differ significantly from the pure excipient ($p = 0.092$) and was well tolerated. Increasing the concentration of PZQ up to 0.2 and 0.27 mg/ml decreased the number of licks significantly to 39.5 ± 2.9 and 32.2 ± 2.4 licks ($p < 0.001$), but the solutions were still classifiable as tolerated. This can be explained by the helical structure of the incorporated amylose providing a barrier between PZQ and the taste buds in the mouth of the rats resulting in a decreased taste perception (Carbinatto et al., 2016). As previously mentioned MDs can provide inclusion complexes by hydrophobic and van der Waals' interactions and can encapsulate hydrophobic drugs just as CDs (Kong and Ziegler, 2014; Luo et al., 2016; Ribeiro et al., 2017). In the case of PZQ this did not affect the solubility of PZQ but could have resulted in the lower aversiveness of the solutions. Moreover the high amount of MD in the solution could also have led to an increased viscosity and consequently to a taste masking effect drug by decreasing the diffusion of PZQ to the taste buds (Walsh et al., 2014). It should be considered that the molecular weight of the MD is much higher in comparison to the CDs. Solutions with CDs contained 2 – 4% (w/w) in comparison to 25% (w/w) with MDs. Nevertheless, as there is no limited daily intake for MDs the concentration could even be increased to 30 to 40% (w/w) as it is common in syrups.

The taste masking of 0.06 mg/ml PZQ was achieved by using 20 mM of either MD or HP-β-CD. SBE-β-CD was significantly less effective ($p < 0.001$). At higher concentrations (0.2 mg/ml), there was significant reduction of licks for all the formulations regardless of the taste masking agent used. However, the formulation with SBE-β-CD was still significantly less useful compared to the other formulations containing MD or HP-β-CD ($p < 0.001$). Formulations with MD and HP-β-CD were tolerated by the rats with no significant difference between the number of licks ($p = 0.865$).

With regard to the final dosage form MDs would be preferable over CDs due to various reasons such as excipient costs and safety. A liquid formulation development with CDs would not be favorable due to the necessary high volume (> 27 ml/kg bodyweight) of one dose of PZQ. Assuming a single dose of 40 mg/kg bodyweight of PZQ, already a child of 10.3 kg (2 years) would exceed the acceptable daily oral dose for HP-β-CD of 8 g/day. Maltodextrins with no limited daily intake offer a promising alternative in drug product development especially for liquid dosage forms, e.g. in terms of a syrup.

4. Conclusion

The electronic tongue and the BATA model were used as alternative taste assessment tools to human taste panels for the aversive compound PZQ. The evaluated sensors of the electronic tongue in this study were not applicable for the compound PZQ due to the non-ionic characteristic and the low solubility of the drug in water. None of the tested sensors provided conclusive responses for the pure compound and hence for further evaluation of multicomponent systems. In contrast, the rats in the BATA model demonstrated a concentration-dependent sensitivity to the PZQ aversiveness leading to an IC_{50} value of 0.06 mg/ml. The BATA model was shown to be a useful taste assessment tool for PZQ for the comparison of formulations at an early stage in development, avoiding challenging human taste panels. Based on these findings the taste masking efficiency of liquid formulations for PZQ was evaluated and the use of MD was compared to two CDs. Despite the fact that the phase solubility study identified SBE-β-CD as superior to HP-β-CD and the MD, the BATA model revealed the MD as efficient as both CDs to mask the aversive taste of PZQ. Moreover, SBE-β-CD was significantly less useful compared to the other formulations containing MD or HP-β-CD accompanied with a less acceptable taste of the pure excipient solution in the BATA model. This preference for the taste of the rodents needs to be confirmed in further human studies. As MDs are better tolerated excipients than CDs, they could provide a viable alternative in terms of taste-masking of liquids. The mechanisms of this efficient taste masking effect still needs to be further elucidated.

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Declaration of interest

The authors declare that there is no conflict of interest.

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Table 1
Labelling and membrane composition of the applied self-developed sensors.

Sensor labeling	Ionophore	Artificial lipid	Oleic acid
A	ßCD	TB	
B	HPßCD	TC	
C	CDO	TC, BP	x
D	HPßCD	TC	
E	HPßCD	TC, BP	
F		TB	x
G	HPßCD	TB	x

Table 2
Classification of number of licks in the Brief Access Taste Aversion model (Soto, 2016).

Classification	% lick inhibition
Fully tolerated	0
Well tolerated	1 – 30
Tolerated	30 – 50
Aversive/ untolerated	50 – 75
Highly aversive/ highly untolerated	> 75

Table 3
Key figures calculated from the slope of the phase solubility study.

Excipient	Slope	$K_{1:1}$ [M^{-1}]	CE	PZQ: CD	Increase in formulation bulk
HP-β-CD	0.14	228.71	0.16	1: 7.25	32.19
SBE-β-CD	0.21	365.11	0.26	1: 4.85	33.58
Maltodextrin	N/A	N/A	N/A	N/A	N/A

Table 4
Comparative % lick inhibition of pure praziquantel (PZQ) in water and with the addition of 20 mM hydroxypropyl-beta-cyclodextrin (HP-β-CD), 20 mM sulfobutyl ether-beta-cyclodextrin (SBE-β-CD) and 20 mM maltodextrin (MD).

PZQ concentration [mg/ml]	Pure PZQ	PZQ + HP-β-CD	PZQ + SBE-β-CD	PZQ + MD
	% lick inhibition			
0		2.4 ± 2.2	23.9 ± 4.3	-4.3 ± 5.5
0.06	50 (IC_{50})	5.6 ± 3.3	22.4 ± 3.9	9.3 ± 4.6
0.2	72.0 ± 2.6	34.9 ± 3.6	66.3 ± 4.2	32.1 ± 5.0
0.27				44.7 ± 4.2
1.3		80.3 ± 2.3	90.9 ± 0.9	

Figures and captions

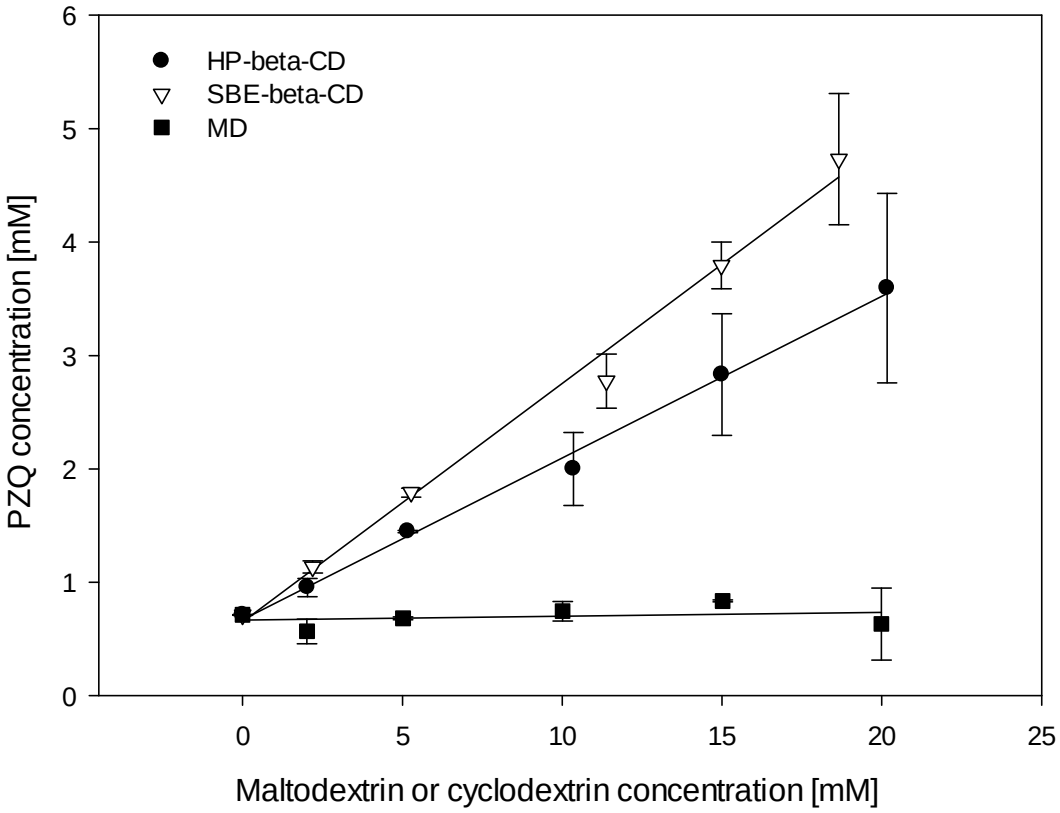

Fig. 1. Phase solubility study. Solubilized concentration of praziquantel (PZQ) plotted against the concentration of maltodextrin (MD, Kleptose® linecaps), hydroxypropyl-beta-cyclodextrin (HP-beta-CD, Kleptose® HPB) and sulfobutyl ether-beta-cyclodextrin (SBE-beta-CD, Captisol®). Arithmetic mean ± S.D. (n = 3).

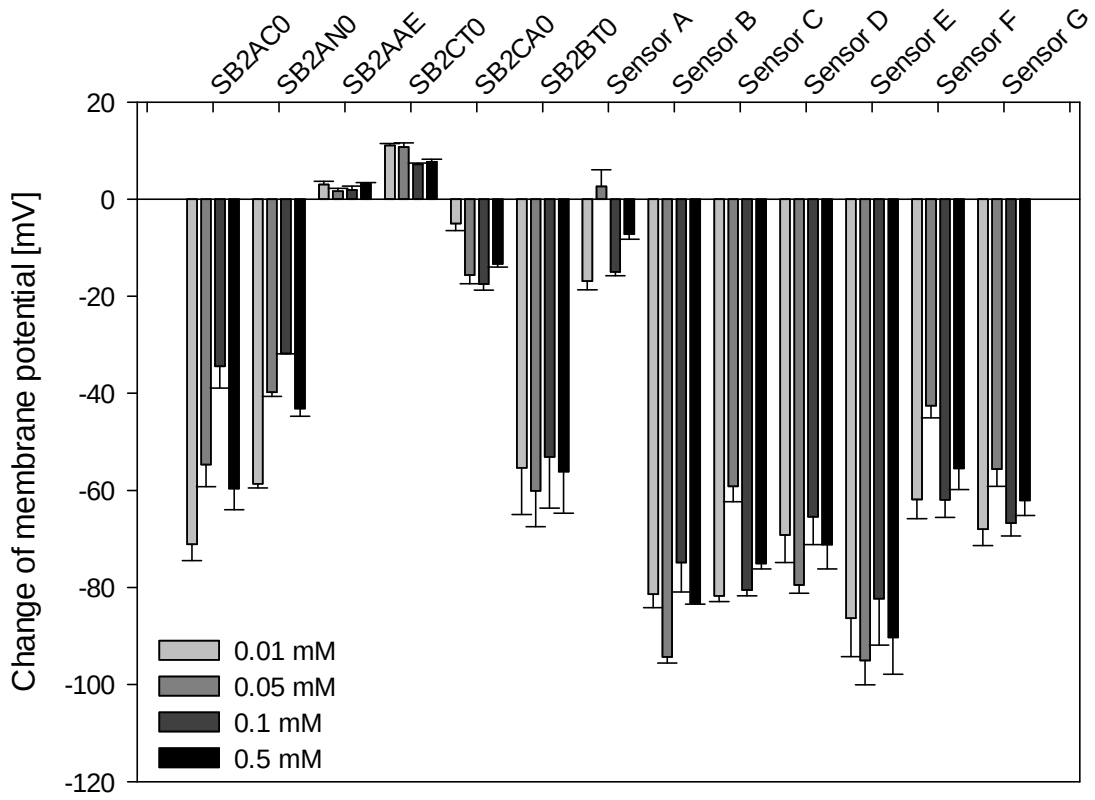

Fig. 2. Calibration for electronic tongue. Sensor signals of commercially available (SB2AC0, SB2AN0, SB2AAE, SB2CT0, SB2CA0, SB2BT0) and self-developed sensors (Sensor A – G) of four different concentrations of praziquantel in distilled water (0.01 – 0.5 mM). Arithmetic mean ± S.D. (n = 3).

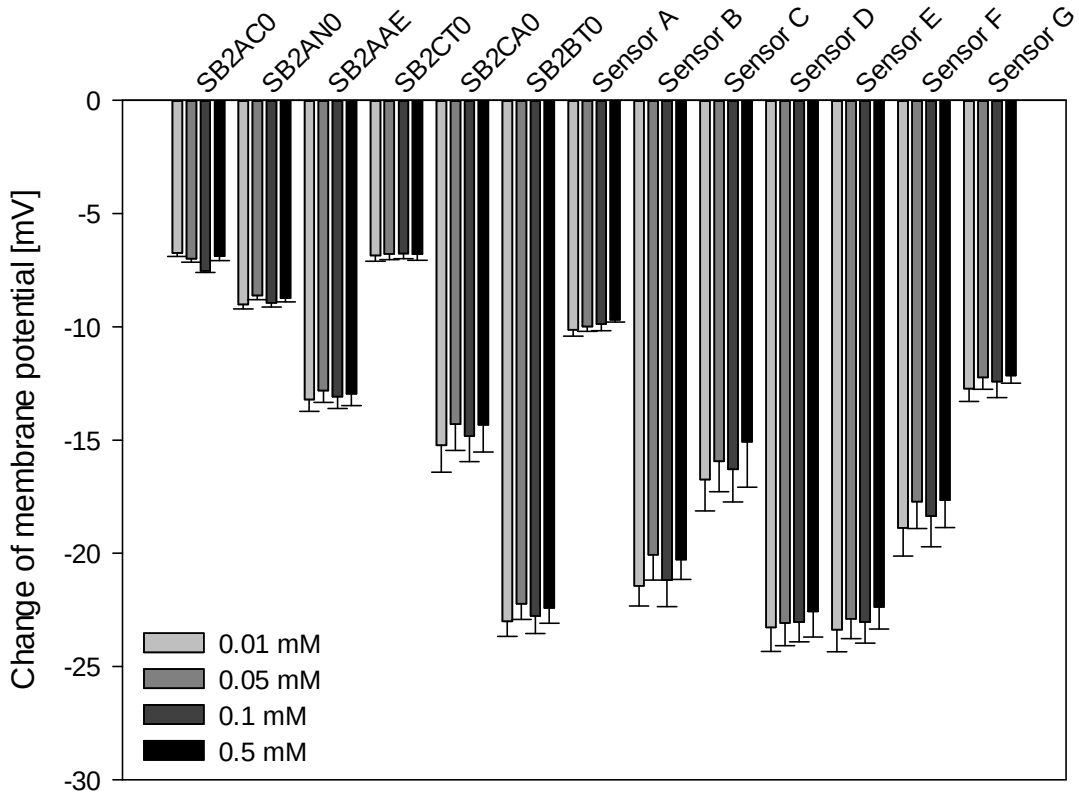

Fig. 3. Measurement of aftertaste of electronic tongue. Change of membrane potential of commercially available (SB2AC0, SB2AN0, SB2AAE, SB2CT0, SB2CA0, SB2BT0) and self-developed sensors (Sensor A – G) caused by adsorption (aftertaste) of four different concentrations of praziquantel in distilled water (0.01 – 0.5 mM). Arithmetic mean ± S.D. (n = 3).

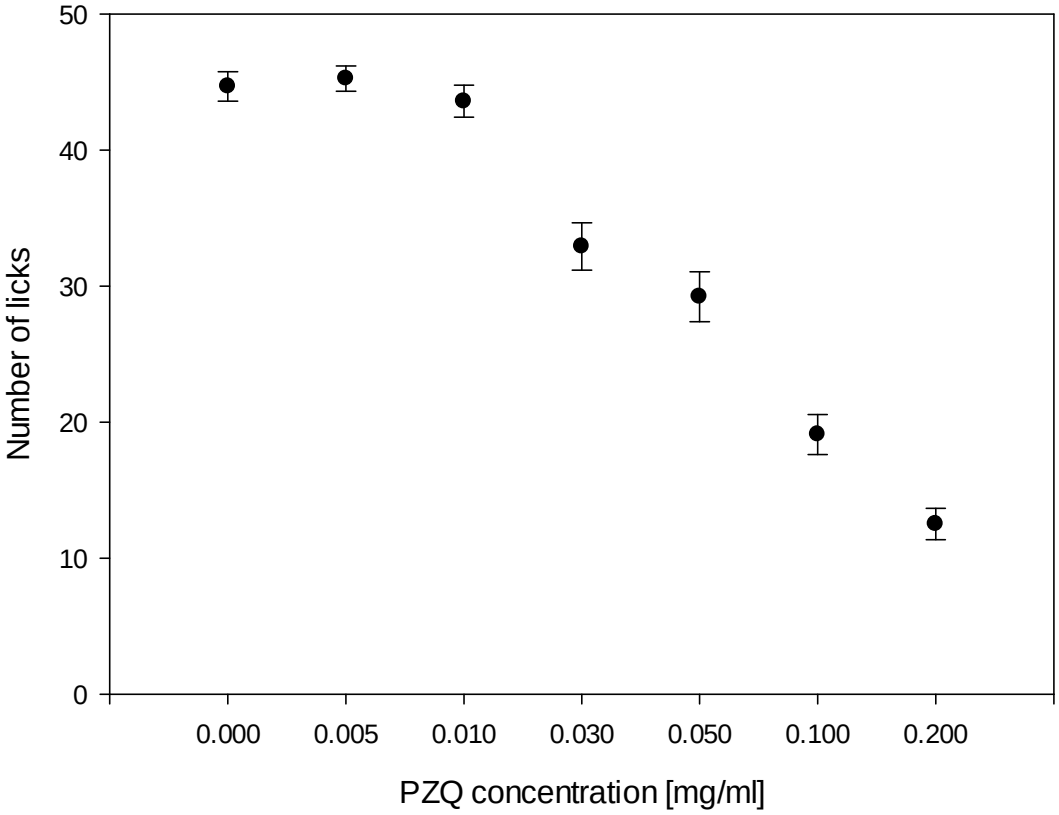

Fig. 4. Calibration for BATA model. Recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration in water. Arithmetic mean (n = 80) ± S.E.M..

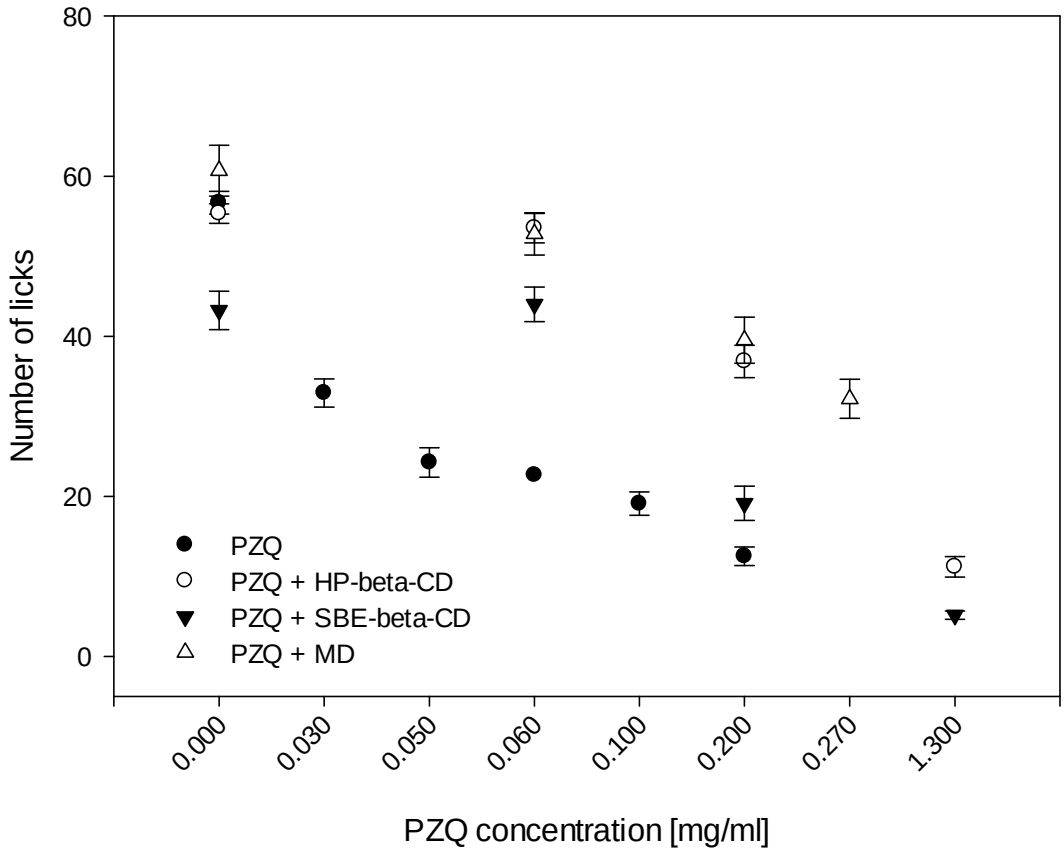

Fig. 5. Comparison of taste masking efficacy in BATA model. Recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration of pure PZQ solution and the solutions with hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and sulfobutyl ether-beta-cyclodextrin (SBE-beta-CD) and maltodextrin (MD). The concentration of 0.000 mg/ml presents pure water in the case of PZQ and pure 20 mM CDs or MD in the case of the formulations. Arithmetic mean (n = 80) ± S.E.M..