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Short communication

Monitoring benzene formation from benzoate in model systems by proton transfer reaction-mass spectrometry

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ABSTRACT

The presence of benzene in food and in particular in soft drinks has been reported in several studies and should be considered in fundamental investigations about formation of this carcinogen compound as well as in quality control.

Proton transfer reaction-mass spectrometry (PTR-MS) has been used here for rapid, direct quantification of benzene and to monitor its formation in model systems related to the use of benzoate, a common preservative, in presence of ascorbic acid: a widespread situation that yields benzene in, e.g., soft drinks and fruit juices.

Firstly, we demonstrate here that PTR-MS allows a rapid determination of benzene that is in quantitative agreement with independent solid phase micro-extraction/gas chromatography (SPME/GC) analysis. Secondly, as a case study, the effect of different sugars (sucrose, fructose and glucose) on benzene formation is investigated indicating that they inhibit its formation and that this effect is enhanced for reducing sugars. The sugar-induced inhibition of benzene formation depends on several parameters (type and concentration of sugar, temperature, time) but can be more than 80% in situations that can be expected in the storage of commercial soft drinks. This is consistent with the reported observations of higher benzene concentrations in sugar-free soft drinks.

1. Introduction

Benzene is considered to be a human carcinogen [1] and after first reports on the presence of benzene in food [2] (and references therein) several studies investigated the presence of this molecule in different food systems, mostly beverages [3–5]. The concentrations reported are usually below 1 $\mu g\,kg^{-1}$ but, in a few cases, they lie in the range of 10–100 $\mu g\,kg^{-1}$ or above [2,6] and thus higher than the maximum level of 5 $\mu g\,kg^{-1}$ recommended by Food and Drug Administration (FDA) [7]. Concerns about the presence of benzene in food are still widespread even if the expected total daily intake is low [8].

It has been suggested that benzoate, a widespread, otherwise safe preservative, can induce, under certain circumstances, benzene formation in the presence of ascorbic acid [9] added as an antioxidant or being naturally present. Both substances are often present in commercial soft drinks and the worldwide beverage industry is aware of the potential risk of benzene containing products and of the necessity to minimize any potential formation, while still ensuring microbiological standards of the soft drink products [10].

During transport or storage, beverages are often exposed to direct sunlight and, especially during summer time, elevated temperatures can be reached. High temperature and exposure to light have been mentioned as factors enhancing benzene formation [10]. The fact that commercial sugar-free soft drinks contain more benzene than similar sugar-containing products [7,11] suggests a possible inhibition of benzene formation in presence of sugars but, as far as we know, neither direct experimental evidence of this effect has yet been published nor possible differences between different sugars have been considered.

In the studies published, benzene has been usually measured by GC/MS and quantified by comparison with internal standards. In this case a preliminary concentration by adsorption on SPME fibre [11], by purge and trap [12] or by cryofocusing [3] is usually necessary. These techniques are, however, time consuming and not suited for on-line monitoring because they require sample preparation and/or pre-concentration and, in order to increase benzene concentration, a matrix modifier (sodium chloride) is often added in the case of headspace (HS) measurements. It seems thus interesting to introduce fast and direct methods to allow quality control and process/product development and also to support research on

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fundamental aspects related to benzene formation and release in food. These novel methods should also be non-invasive in view of on-line monitoring of benzene.

In this context, we propose proton transfer reaction-mass spectrometry [13] as a possible new tool to address these issues. In the present paper, as a prototypical case study, the formation of benzene in aqueous model solutions containing benzoate and ascorbic acid is discussed as well as the effect of adding different sugars. Results are also compared with GC data.

2. Materials and methods

2.1. Reagents

Benzene standard for GC (≥99.9%) was purchased from Fluka (Milan, Italy) and benzene-D_6 solution (2000 μg/mL in methanol) from Supelco (Milan, Italy). Sodium benzoate (SB), ascorbic acid (AA), D-fructose, D-glucose and sucrose were purchased from Carlo Erba (Italy). SB (1.2 g L^{-1}) and AA (1.2 g L^{-1}) stock solutions were prepared daily.

2.2. Model systems

The model systems used here consisted of aqueous solutions of SB plus AA only or of SB plus AA containing sugars (sucrose, fructose and glucose) at different concentrations (0.1, 2.5 and 0.5 M). The samples (300 mL) with a final concentration of 400 mg L^{-1} of SB and 400 mg L^{-1} of AA were filled into 500 mL Schott Duran® laboratory glass bottles wrapped in aluminium foils to protect the solution from light.

The concentrations of benzoate, ascorbic acid and sugars chosen for the model systems are covering the range expected for commercial products (soft drinks and juices).

2.3. Kinetic experiments: effect of temperature and sugars

The bottles containing solutions of SB plus AA (400 mg L^{-1} of each) were placed in a constant-temperature oven (FED 720 WTB Binder, Labortechnik GmbH, Tuttlingen, Germany) kept at 25 or 45 °C. At intervals corresponding to 5 min, 1 h, 3 h, 6 h, 8 h, 24 h, from the beginning of the experiment, HS volatiles were directly measured by PTR-MS following the procedure described in Section 2.4. During the experiments at 45 °C aliquots of 15 mL were removed from the solutions for GC quantification at the same time intervals listed above (see Section 2.5).

The effect of sugars was studied adding to the model solutions sucrose at three concentration levels (0.1, 0.25 and 0.5 M) and monitoring in each series the benzene formation at 45 °C by PTR-MS over 22 h. From the bottles containing the model solutions with sucrose at 0.5 M three aliquots (15 mL each), at 3.5, 6 and 7 h, respectively, where sampled for GC quantification. To compare the effects of different sugars we repeated the same experiments with fructose at 0.5 M and glucose at 0.1 and 0.5 M.

2.4. Proton transfer reaction-mass spectrometry analysis

All measurements were performed by a high-sensitivity proton transfer reaction-mass spectrometry system (IONICON Analytik GmbH, Innsbruck, Austria) and measured counts per second (cps) were converted to normalized count per seconds (ncps) with respect to 10^6 cps of primary ions and 2.0 mbar of pressure in the drift tube. The PTR-MS system allows an easy, direct, on-line measurement of trace components in gas mixtures [13,14].

For the quantitative determination, the instrument was calibrated using a certified gas mixture (Apel-Riemer Environmental Inc., Denver, CO, USA) containing benzene at the concentration of 1007 ppb_v (±5%), which was dynamically diluted, using a system of two mass flow controllers, down to 1.0 ppb_v in humidified VOC-free air. The accuracy of such measurements is estimated to be ±15% [15].

This calibration procedure allows the absolute quantification of benzene in the HS of the liquid system investigated. To calculate the exact concentration present in the solution it is necessary to know the solution/air partition coefficient for benzene. For this purpose the calibration gas mixtures used to determine the calibration curve was bubbled both through water and one of the model systems prepared with sucrose (0.5 M) with the aim to determine the partition coefficients of benzene between the solution and the HS following the procedure described in Ref. [16] and also applied to hydro-alcoholic solutions in Ref. [17]. Briefly, the calibration gas mixture is bubbled through a stripping cell containing the solution [16] that will gradually be enriched in benzene and at the same time head space is sampled by PTRMS. The benzene concentration in the air leaving the solution at time t (HS(t)), will asymptotically increase as function of time from zero (or background level) to the concentration that enters the stripping cell. The time dependence of the HS concentration is controlled, in the case of perfect mixing, by:

$$\mathrm{HS}(t) = \mathrm{HS}_0 \left(1 - \exp\left(\frac{-t}{\tau}\right)\right) \quad (1)$$

where HS_0 is the concentration in the gas bottle, V_L the liquid volume, V_{HS} the HS volume, φ the flux, He the partition coefficient and:

$$\tau = \frac{V_{HS} + V_L\,\mathrm{He}}{\varphi} \quad (2)$$

Analysis of the time dependence of HS(t) allows the determination of τ and thus, via, Eq. (2), that of He. We prefer to use, for the determination of the partition coefficients, the same bottles (provided with a screw-cap system with Drechsel-head with filter disc, DURAN®, SCHOTT Glass UK Ltd.) and volumes (liquid and HS) used for the actual experiments on benzene formation even if these are not optimized for He measurements (maximum efficiency in obtaining liquid/HS equilibrium).

The actual PTR-MS measurements are carried out by piercing a septum with a stainless steel needle that is connected to the PTR-MS reaction chamber by a Teflon tube held at 70 °C. Even if only the signals at m/z = 79 and m/z = 80 are used in this work, we monitored all masses up to m/z = 240 to have a better control on the possible formation of other compounds. This reduces the response time of the method to one spectrum every 44 s.

2.5. Gas chromatography/mass spectrometry

For GC quantification 10 mL of the solution to be measured was transferred into 20 mL vials and crimp-closed with a Teflon-lined silica cap after the addition of 50 μL of internal standard (benzene-D_6, 2000 μg L^{-1}). The vial was equilibrated in a water bath at 35 °C for 10 min under constant stirring before the 10 min SPME fibre exposure. Benzene was then sampled by means of a manual fibre holder equipped with a SPME fibre coated with a 75-μm film thick layer of carboxen/PDMS (polydimethylsiloxane) purchased from Supelco (Milan, Italy). Thermal desorption of the compounds from the fibre coating took place in the GC injector at 250 °C in splitless mode. A PerkinElmer AutoSystem XL gas chromatograph coupled with a TurboMass Gold (PerkinElmer, Norwalk CT) mass spectrometer was used. Separation was achieved on a HP-InnoWax fused-silica capillary column (30 m, 0.32 mm ID, 0.5 μm film thickness J&W Scientific Agilent Technologies Palo Alto, CA,

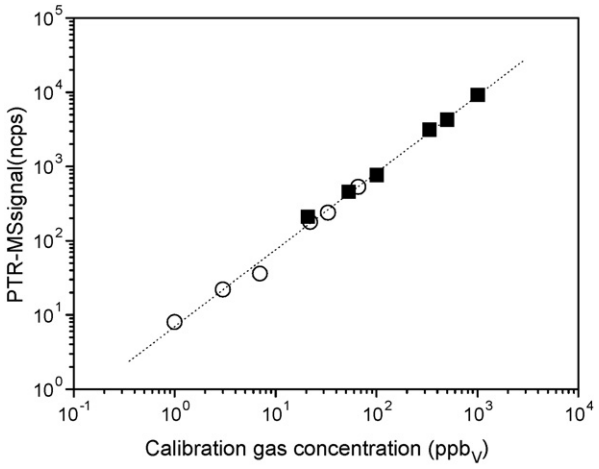

Fig. 1. Calibration curve of benzene in humidified synthetic air. Solid squares (■) refer to protonated benzene at m/z 79, open circles (○) refer to protonated mono-substituted ^{13}C benzene at m/z 80.

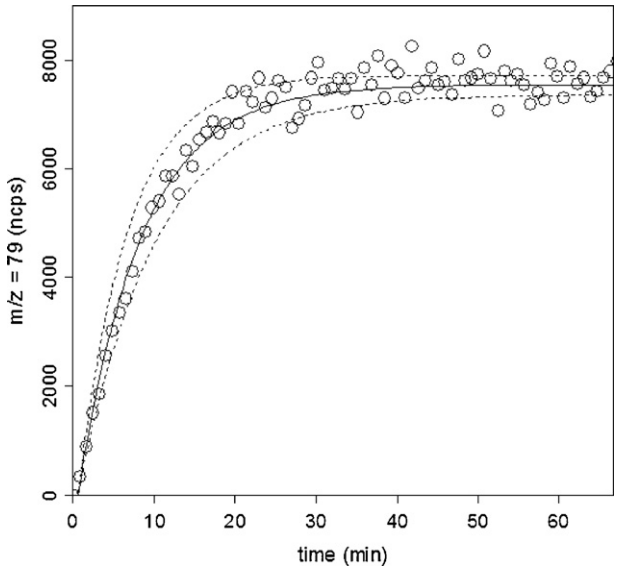

Fig. 2. Example of time evolution of benzene signal in the HS above water while bubbling air containing 1 ppm_v of benzene through the liquid sample. The full line indicates the best fit based on least squares method and the dashed lines indicate the confidence interval (estimated parameters ± 3S.E.).

USA). The oven temperature was programmed as follows: 5 min at 45 °C; 20 °C/min up to 100 °C, hold for 30 s; 10 °C/min up to 220 °C, hold for 5 min.

3. Results and discussion

3.1. PTR-MS calibration

For the PTR-MS quantitative determination of benzene, a calibration curve using the gas standard was prepared. Since it is known that the sensitivity of PTR-MS with respect to benzene is reduced at a higher humidity of the sample air [18], the points for calibration curve were acquired bubbling the calibration gas through water and all further experiments were performed under the same conditions. Fig. 1 shows the linearity of the response of PTR-MS to benzene over 3 orders of magnitude of mixing ratios (from 1 to 1000 ppb_v). Benzene (molecular mass 78) was determined at mass 79, the protonated molecular ion, and also at protonated mass 80 which represents the mono-substituted ^{13}C benzene isotopomer with a natural isotope ratio of 6.6% assuming a ^{13}C abundance of 1.11%.

3.2. Determination of benzene partition coefficients and benzene quantification

Fig. 2 exemplifies the time evolution of the intensity of the signal at m/z = 79 while bubbling air containing 1 ppm_v of benzene through water. Fitting Eq. (1) to the experimental data (full line in Fig. 2) allows the determination of the time constant and thus of the partition coefficient. We prefer to use a least square fitting with 3 free parameters (t_0, HS_0 and τ) but, knowing HS_0 and t_0, it is also possible to linearise Eq. (1). In this case, τ is the negative reciprocal of the slope of the plot of $\log(HS_0 - HS(t))$ versus time.

Within the experimental errors (~20%) we found the same value of He = 0.15 ± 0.02 M atm^{-1} at 25 C for both water and sucrose solutions. Similar results were found for acetate esters in solution containing 20% of sucrose [19]. The Henry law constant found is slightly lower but still compatible (in particular taking into account the bottle size used, see above) with the value reported in Ref. [16] (He = 0.18 ± 0.036 M atm^{-1}). PTR-MS allows the direct measurement of the volatile compounds concentration in the HS but, using the partition coefficients evaluated as above, it is possible to estimate the concentration in the liquid using the following relation:

$$ppb_l = MW \times He \times ppb_v \times 10^{-3} \quad (3)$$

where ppb_l is the concentration of the analyte dissolved in the liquid expressed in $\mu g\,L^{-1}$, MW is the molecular weight of the compound, He is the partition coefficient in M atm^{-1} at a given temperature and ppb_v is the concentration of the analyte in the gas phase in atm 10^{-9}.

It is worth mentioning that both the quantitative determination of the partition coefficients and the determination of the benzene concentration in the HS and in the solution are obtained by PTR-MS analysis without any pre-treatment.

3.3. Comparison between SPME/GC–MS and PTR-MS data

In a previous work we observed significant correlation between SPME/GC–MS and PTR-MS determination for several compounds [20]. Nevertheless PTR-MS results for benzene quantification were compared here with independent SPME/GC–MS measurements. In Fig. 3, PTR-MS data for benzene are plotted against SPME/GC–MS data showing a good agreement between the two determinations. After this check we used only PTR-MS for quantification of benzene in the experiments reported.

3.4. Effect of temperature

In a first experiment, we investigated benzene formation in reaction mixtures of SB plus AA kept at 25 and 45 °C for 72 h. Fig. 4 shows the curves obtained at these two temperatures. The benzene signal is roughly constant (less than 0.1 $\mu g\,L^{-1}$), close to the noise level, over the first 12 h when the reaction solution is kept at 25 °C. Only after 24 h the signal starts to slightly increase reaching a concentration of 0.44 $\mu g\,L^{-1}$ after 70 h. When the reaction solution is kept at 45 °C a strong benzene formation sets in after 3 h reaching a maximum concentration (118.5 $\mu g\,L^{-1}$) after about 24 h and remaining roughly constant (~125 $\mu g\,L^{-1}$) for the next 48 h. Our findings are compatible with those of McNeal et al. [2] who report a concentration of about 300 $\mu g\,L^{-1}$ in a similar solution kept at 45 °C for 20 h.

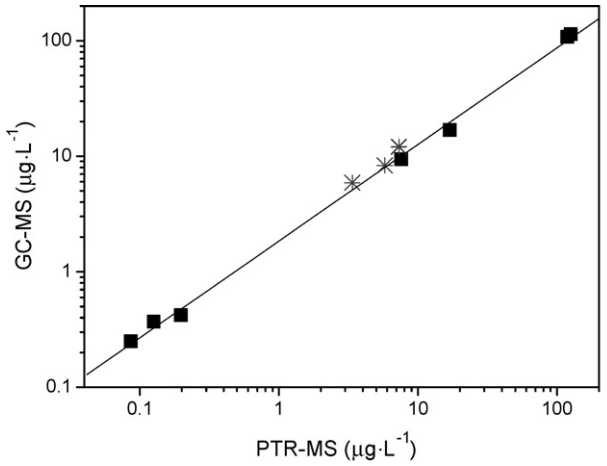

Fig. 3. Intercomparison between measurements of benzene using PTR-MS and SPME/GC–MS on AA plus SB model systems at 45 °C containing sucrose at 0.5 M (✳) or without sugar (■).

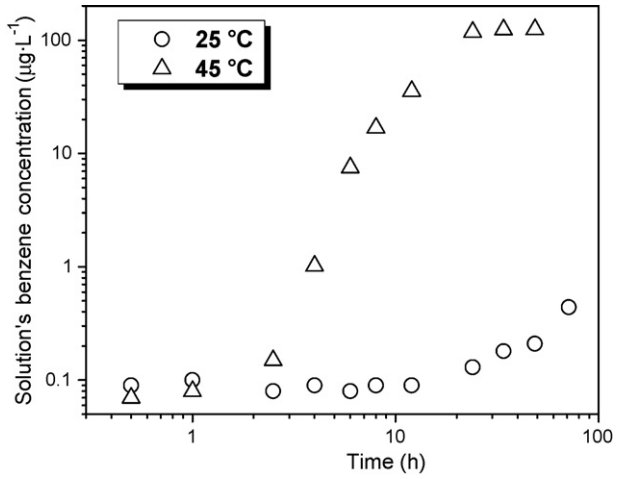

Fig. 4. Example of the time dependence of benzene concentration in aqueous model systems with ascorbic acid and sodium benzoate at different temperatures.

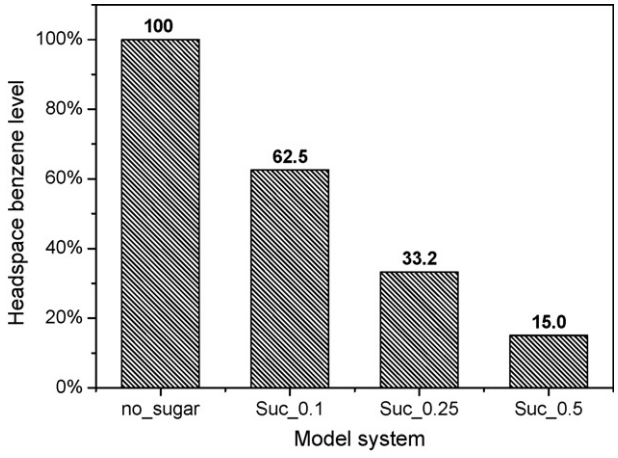

Fig. 5. Effect of sucrose concentration on the benzene formation in an aqueous model system of ascorbic acid and sodium benzoate. Relative concentration (data normalized to the solution with no sugar) of benzene above the solutions after 22 h at 45 °C.

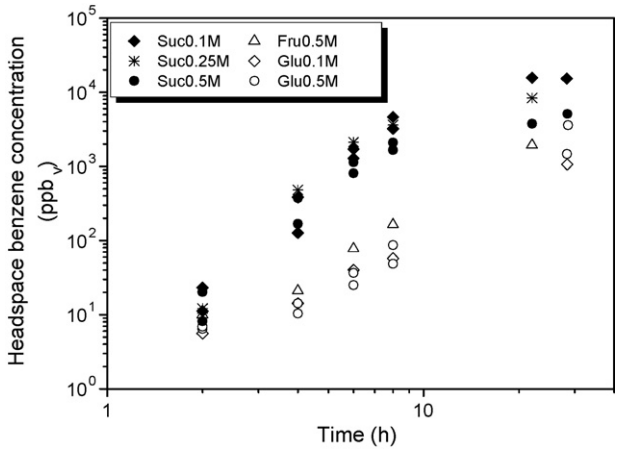

Fig. 6. Effect, at different concentrations, of the reducing (fructose and glucose) and non reducing (sucrose) sugars on the benzene formation in an aqueous model system consisting of ascorbic acid and sodium benzoate at 45 °C. The experiments for the model systems containing 0.1 and 0.5 M of sucrose and 0.5 M of glucose were measured two times.

3.5. Effect of sugars

To evaluate the effect of sugars on benzene formation in model systems of SB plus AA, we added sucrose at three different levels (0.1, 0.25 and 0.5 M). When sucrose is present, benzene formation is reduced and this effect is proportional to the concentration of the sugar as can be observed in Fig. 5 where relative concentrations of benzene formed over 22 h is reported.

In a next experiment we compared the effect of different type of sugars on benzene formation in our model systems, adding also fructose (0.5 M) or glucose (0.1 and 0.5 M). As it can be seen in Fig. 6 not only the concentration of the sugar but also its nature plays a role in the inhibition of benzene formation: the reducing sugars, fructose and glucose, seem to have a higher protection effect than sucrose. Considering the higher sweetening efficiency of fructose (1.7 times than sucrose) and its protection effect on benzene formation its use in soft drink production should be preferred.

4. Conclusions

In this paper we use PTR-MS for direct quantification of benzene in beverages and we have investigated its formation in solutions containing ascorbic acid and benzoate. These systems model the benzene formation in soft drinks that often contain both substances and the present study aims at providing new tools for the research driven by the concerns about the presence of this carcinogenic molecule in beverages. Firstly, we showed that only on the basis of PTR-MS analysis it is possible to determine the concentration of benzene both in the gas and in the liquid phase. In fact PTR-MS provides a direct estimation of HS concentration and a rapid way to evaluate partition coefficients that can be used to calculate the concentration in the liquid. PTR-MS data are in good agreement with independent SPME/GC–MS determination of benzene. We measured a strong dependence on temperature and demonstrated the inhibition effect of sugars on benzene formation in AA plus SB systems also providing indication that reducing sugars are, in this sense, more efficient. The direct application of the proposed method to complex mixtures gives, however, only an upper limit of the concentration of benzene because the absolute quantification must take into account the possibility of interferences at mass 79 due to fragments from larger molecules. Determination of the isotope ratio at mass 80 gives however additional information allowing a more accurate quantification.

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