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Corresponding Author: Dr. Simona Scarano, Ph.D.

Corresponding Author's Institution: University of Florence

First Author: Simona Scarano, Ph.D.

Order of Authors: Simona Scarano, Ph.D.; Emanuela Pascale; Maria Minunni

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Suggested Reviewers: Dario Compagnone
dcompagnone@unite.it
Similar research fields.

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spotog@unict.it
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Florence, 10th March 2017

Dear Editors,
we would like to submit for publication in *Analytica Chimica Acta* journal the following research article entitled:

'The early nucleation stage of gold nanoparticles formation in solution as powerful tool for the colorimetric determination of reducing agents: the case of xylitol and total polyols in oral fluid'

Authors: S. Scarano, E. Pascale, and M. Minunni*

Herein we report a simple non-enzymatic assay for xylitol and total polyols in water and oral fluid based on the time resolved formation of gold NPs in solution, and their colorimetric detection at fixed wavelength (520 nm). The key novelty of the proposed approach relies on the exploitation of information given by the early nucleation step of NPs formation instead of those related to final products at the end point of AuNPs growth, as generally reported in literature. We demonstrate that the nucleation stage is linearly correlated to the concentration of the reducing agent in solution. On the contrary, the optical reading carried out the end point of the reaction shows non-linear correlation and several undesired features.

As case study, we applied the proposed method to xylitol and polyols determination, first tested in water and spiked oral fluid samples. Afterward, we successfully performed the monitoring of total polyols in oral fluid over time during xylitol-containing gums consumption. The proposed approach is fast, cheap, highly reproducible, and can be extended to other reducing substances of interest for analytical purposes.

We believe that the proposed approach can be extended and effectively contribute to the development of new and attractive (bio)analytical applications.

We feel that this work fits well with the aims of *Analytica Chimica Acta* journal, resulting of interest for the readers.

With my very best regards,

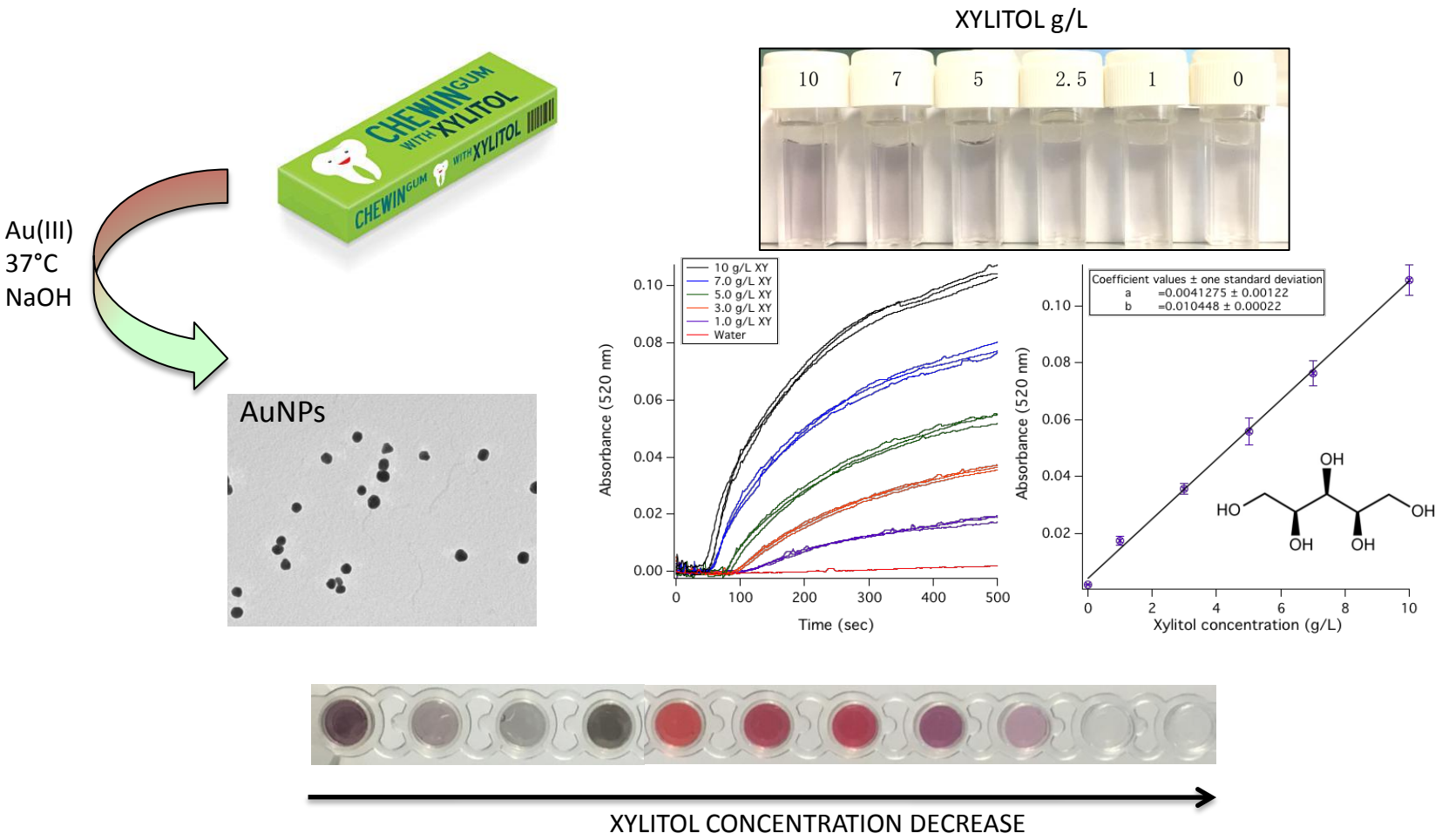
Simona Scarano

Department of Chemistry, University of Florence, via della Lastruccia 3-13, 50019, Sesto Fiorentino, Italy

e-mail: simona.scarano@unifi.it

*Graphical Abstract

Graphical abstract



Highlights

- Xylitol forms highly stable and defined AuNPs under mild conditions in solution
- Xylitol concentration is linearly correlated only during the nucleation step of AuNPs
- A colorimetric assay at fixed wavelength (520 nm) is successfully developed
- Xylitol and total polyols are assayed in spiked oral fluid and during gums consumption
- It is fast, cheap, reproducible, and can be extended to other reducing substances

The early nucleation stage of gold nanoparticles formation in solution as powerful tool for the colorimetric determination of reducing agents: the case of xylitol and total polyols in oral fluid

S. Scarano*, E. Pascale and M. Minunni

Department of Chemistry, University of Florence, via della Lastruccia 3-13, 50019, Sesto Fiorentino, Italy

**e-mail: simona.scarano@unifi.it*

Abstract

Herein we report a simple non-enzymatic assay for xylitol and total polyols in water and oral fluid based on the time resolved formation of gold NPs in solution, and their colorimetric detection at fixed wavelength (520 nm). The key novelty of the proposed approach relies on the exploitation of information given by the early nucleation step of NPs formation instead of those related to final products at the end point of AuNPs growth, as generally reported in literature. We demonstrate that the nucleation stage is linearly correlated to the concentration of the reducing agent in solution. On the contrary, the optical reading carried out the end point of the reaction shows non-linear correlation and several undesired features. As case study, we applied the proposed method to xylitol and polyols determination, first tested in water and spiked oral fluid samples. Afterward, we successfully performed the monitoring of total polyols in oral fluid over time during xylitol-containing gums consumption. The proposed approach is fast, cheap, highly reproducible, and can be extended to other reducing substances of interest for analytical purposes.

Keywords: reducing sugars; gold nanoparticles; colorimetric assay; xylitol; oral fluid; localized surface plasmon resonance.

Introduction

Plasmonic nanomaterials (PNs) display exciting optical properties and tunability, and during last decade their use has been pushed toward innovative and creative applications, combining the design of cheap and smart detection strategies to impressive analytical performances. In literature, different uses of PNs are reported for (bio)analytical purposes through Localized Surface Plasmon Resonance (LSPR) transduction. Most of them exploit the optical features of pre-formed PNs as signal reporters/enhancers by recording wavelength maximum shifts and/or absorbance intensity changes at fixed lambda. Induced spectral changes are thus the direct consequence of either near field perturbation or dispersion of nanoparticles (NPs) in solution (e.g. aggregation and/or plasmon coupling) [1,2]. Differently, a recent and interesting approach relies on the observed correlation existing between the concentration of the reducing agent used to form NPs starting from metallic ions in solution (Au(III) or Ag(I)) and the final optical characteristics of NPs. This strategy has been thus applied for analytical purposes to some class of compounds expressing reducing power. In particular, the quantification of antioxidant activity of natural extracts [3-8] and glucose [9,10] have been proposed by this approach. Therefore, beyond production purposes, the in solution synthesis of NPs upon natural reducing substances has inspired innovative colorimetric methods based on optical properties of metallic NPs formed in solution. Among sugars, to our best knowledge, this is the first report about the application of the above approach to xylitol detection. In this work, a non-enzymatic assay for xylitol (XY) and total polyols in water and oral fluid is reported. Moreover, the monitoring of polyols/xylitol persistence over time in oral fluid during chewing gum consumption is reported.

The method is based on the time resolved formation of gold NPs in solution, and their colorimetric detection at suitable fixed wavelength. The kinetic evolution of absorbance of sugar-stabilized NPs over time shows typical kinetic profiles associated to system parameters, including the reducing/capping agent type and its concentration [11-14]. For XY, a typical sigmoidal evolution of NPs formation is here showed, in which the first early nucleation process give punctual and wide

quantitative information on the reducing/capping agent, xylitol content in this case. The method displays several advantages respect to the available literature on sugars determination by similar approaches; the reproducible and sensitive quantification is obtained under mild conditions, i.e. at low temperature (37°C), without the need long pre-incubation steps at high temperature with surfactants [9] or the vortexing of samples during the reaction [10]. The linear correlation is here widened of more than one order of magnitude with high reproducibility (CV%<7.0%), both in water and oral fluid samples.

The evaluation of polyols content in real matrices is of great interest in many fields, from food to clinical diagnostics. Herein we applied the developed approach to the determination of xylitol in oral fluid. Xylitol (XY) has well-established anticariogenic activity and is widely used as additive in chewing gums, tablets, oral rinses and toothpaste. The quantification of XY (alone or in combination with sorbitol or other polyols) is therefore required both in medical and food control and new, fast, and reproducible methods are constantly required. Presently, assessed non-enzymatic methods for quantification of these sugar alcohols are based on chromatographic techniques, alone or in combination with mass spectrometry [15-17], or on capillary electrophoresis [18-20]. Despite their obvious high analytical performances in terms of speciation of mixed polyols samples and sensitivity, both techniques are time and cost expensive and require bench-top instrumentation available only in equipped laboratory. In this paper, the instrumentation required for optical reading is extremely simple and economic, with optical interrogation at 520 nm obtained by red led on small and commercial spectrometer for food analysis. The method is fast, low-cost and highly reproducible, and can be extended to other reducing (antioxidant) substances of interest.

Materials and Methods

Milli-Q water was used throughout all experiments. Tetra-chloroauric acid trihydrate (TCA),

$HAuCl_4 \cdot 3H_2O$, sodium dodecylsulphate (SDS), NaOH, were from Sigma-Aldrich (Milan, Italy); Xylitol was from Fluka. Polyols and sugars used as calibrators, and all other chemicals used in this study were reagent grade and were obtained commercially.

Instrumentation

Extinction spectra of Xylitol-capped AuNPs were recorded by a Cary 100 spectrophotometer (Varian), in the range 300–800 nm, with 1 nm resolution. For measurements at fixed lambda, the CDR WineLab spectrometer was used in kinetic mode and in color mode (CDR Foodlab s.r.l., Florence, Italy). Extinction values in time (sec) are recorded at chosen wavelength. In case of xylitol-capped gold NPs, 520 nm was the best wavelength for the monitoring of AuNPs growth in solution. Images of formed Xylitol-capped AuNPs are taken by TEM microscopy (STEM CM12, Philips).

Polyols-stabilized AuNPs formation

In a typical synthesis, 250 μL Xylitol aqueous solution (concentrations 0-10 g L^{-1}) is incubated at 37°C for 2 min in a disposable UV-Vis cuvette (CDR Foodlab s.r.l., Florence, Italy) after addition of 5 μL or 7.5 μL NaOH (1 M), directly in the WineLab spectrometer houser. Afterward, 1 mM TCA (750 μL) is added in cuvette and quickly mixed three times with pipette. Immediately after, the zero absorbance reference is taken and then the kinetic recording of the absorbance at 520 nm is started. Samples were recorded up to 2500 sec with 1 sec resolution to investigate the best time window for data analysis. At the end of the recording, AuNPs formed in solution can be stabilized with 0.01 M SDS (10 μL) for further observations. All reported data are averaged on least 5 replicates, both for aqueous and oral fluid samples.

Real samples sampling and processing

Unstimulated oral fluid samples are collected in eppendorf tubes and promptly heated to 95°C (Eppendorf Thermomixer, Hamburg, Germany) for 30 min in order to deactivate α-amylases. This step is mandatory to knock out the natural reducing ability of α-amylases toward gold (III) ions [21] and get negative controls in absence of other reducing agents in solution. After 10 min centrifugation (14000 rpm, Eppendorf centrifuge 5415D, Hamburg, Germany), the supernatant is carefully removed, diluted 10 folds, and then directly assayed or kept frozen (–20 ° C) until use. Controls oral fluid samples are collected after tooth cleaning, followed by thorough rinsing with water. These samples are spiked with known standard xylitol concentrations and used to evaluate the kinetic of NPs formation in real samples.

Polyols-containing chewing gums (Daygum Protex, Perfetti Van Melle S.p.a., Milan, Italy), 1.98 g/piece of total polyols (30% xylitol), are processed as follows: 1 gum is dipped in 1 mL of MilliQ water and lasted for 10 min until the surface layer containing polyols is completely dissolved. After, the supernatant is removed and centrifuged (14000 rpm, Eppendorf centrifuge 5415D, Hamburg, Germany), then diluted 10 folds and directly assayed or kept frozen (–20 °C) until use. For evaluation of persistence of polyols in oral fluid after intake, oral fluid samples are collected at different times, within 60 min, processed as reported above for spiked oral fluid samples, and finally tested.

Results and discussion

The only report about the use of XY to obtain AuNPs in solution is based on the hydrothermal reaction at high temperature (120-180°C) and for long time (24 h) [22]. However, under these strong conditions, the formation of different anisotropic NPs not suitable for optical purposes it has been observed. In particular, triangular and hexagonal nanosheets, which evolve to nanobelts with [XY] increase are obtained. Therefore, first we explored the possibility to obtain xylitol-capped AuNPs under mild conditions and with defined shape and geometry to be exploited by optical

reading.

To investigate the applicability of the method to xylitol determination, we first tested a wide range of [XY], i.e. 0-500 mM (0-76 g L^{-1}), more than enough for our purposes of application. Samples were prepared in water by keeping constant Au(III) and NaOH final concentration. AuNPs features observed at the completion of the reaction, as generally reported in literature, clearly show a great variability of their morphological and optical characteristics. In fact, to obtain the formation of NPs for all the tested samples, a significative temperature increase (25 to 95°C) is gradually necessary with [XY] decrease (Fig. S1). [XY] is therefore at, the same time, the trigger and the limiting factor for AuNPs formation. At high [XY] (500-25 mM, 76-3.8 g L^{-1}) the high XY/Au(III) ratio promotes the instantaneous growth of small (10-20 nm) and interconnected spherical NPs (at room temperature), as displayed in Fig. 1 where colorimetric behavior and TEM images are correlated. Colors observed within this range vary from dark purple to brown and grey, corresponding to complex branched structures giving bichromic effects similar to anisotropic AuNPs (e.g. gold nanostars). By decreasing [XY] (<25 mM), an optimum window between 20 and 1 mM (about 3.0-0.150 g L^{-1}) can be observed, corresponding to pale red NPs of about 20-30 nm. Differently from NPs obtained at high [XY], these are highly stable for months in solution at room temperature without the need of surfactant agents or stabilizers. TEM images display basically round-shaped NPs, well dispersed and with size distribution between 20-30 nm. Below 1 mM [XY], the color of obtained NPs changes to purple red and tends to fade off due to the limiting [XY], which impairs AuNPs formation. To our knowledge, this is the first report of XY-capped spherical AuNPs obtained at atmospheric pressure and moderate temperature within a wide range of xylitol concentration (<100°C).

Optical behavior of these end-point XY-stabilized AuNPs (XY@AuNPs) shows the evolution of both absorption band shape and position (Fig. 2). A broad extinction spectrum at maximum wavelength (λ_{max}) of about 615 nm is obtained for 500 mM XY; then λ_{max} evolves toward sharper

and blue-shifted peaks with [XY] decreasing down to 10 mM (green line, plot B and C). The further decrease of [XY] down to 0.1 mM induces again a red-shift of λ_{max}, in line with the increasing size of NPs already observed by TEM analysis (Fig. 1).

These observations are in line with data available by other authors, which report glucose detection by this approach (end point reading) within a limited concentration range when the colorimetric detection is carried out at fixed lambda [9], or a non-linearity of the maximum wavelength shifts when data are inferred by classic LSPR detection [10]. To overcome these limitations, and having in mind a fast and simple colorimetric assay to be carried out on portable spectrometers, we investigated the feasibility of monitoring the time-resolved change of absorbance at fixed wavelength during the early stage of NPs formation, i.e. nucleation. In fact, we observed that the precise and highly reproducible correlation between absorbance and [XY] is obtainable only at this stage of NPs formation in solution. Contrarily, the correlation is lost at the end point of the reaction, as later discussed.

To reach the goal, the reaction conditions were set up to control the kinetic evolution of NPs formation, to avoid the onset of the growth steps of the reaction which naturally succeed the nucleation step [11-14]. This was achieved by lowering the temperature down to 37°C and the NaOH concentration (from 100 to 50 mM). Under these conditions, we first tested the AuNPs formation in presence of XY within 0-10 g L^{-1} in water, by monitoring the absorbance at 520 nm up to 500 sec. The wavelength was selected as the most sensitive among those available on the instrumentation (269, 366, 420, 520, 578, 620 nm), and confirmed by spectra recorded in wavelength scanning mode (Fig. S2).

After 500 sec, the formation of AuNPs in solution is evident by naked eye down to 1 g L^{-1}, with the intensity of color fading off with [XY] decrease, while keeping the same chromaticity over the explored range (Fig. 3A). Abs_{520} sampled over different time intervals (200 to 500 sec) display well-detectable and reproducible profiles (Fig. S3). Excellent linear correlations for all the time

intervals can be obtained, with slight improvement of the slope moving from 200 to 500 sec. The coefficients of variability averaged on all the tested concentrations (CV%) resulted 3.4% (200 sec), 4.7% (300 sec), and 6.9% (500 sec), respectively. Considering these results, we selected t=500 sec as the best compromise between sensitivity and reproducibility for further measurements. Data collected during this stage of NPs formation (Fig. 3B), plotted versus [XY], give an excellent linear correlation (R^2 =0.998) (Fig. 3C), with an estimated detection limit of 180.0±0.4 mg L^{-1} (3σ=0.006 Abs_{520}). An evident elapse of Abs_{520} increase vs. time is also present with [XY] decrease; this confirms that the nucleation phase of the system is extremely sensitive to the reducing agent concentration. Conversely, if Abs_{520} is monitored for longer time windows, samples at [XY]>5 g L^{-1} evolve toward the second crystallization stage, and pale red to purple red solutions are obtained, indicating different chromaticity (i.e. λ_{max}) of the obtained samples (Fig. 4A). In fact, the relative Abs_{520} values recorded for all the samples at this stage (e.g. 2500 sec) clearly display the loss of the linear correlation between recorded signals and [XY] (Fig. 4B), which is retained until the end-point of the reaction (Abs_{520}=constant), as displayed in Fig. 4C. These findings demonstrate that the proposed method opens a new route in this field, and indicates that the highest analytical performance in terms of correlation with the concentration of the reducing agent (xylitol in this case) can be precisely inferred only at the first stage of AuNPs formation, i.e. during the formation of gold nanocrystallites in solution.

Real samples

Xylitol quantification in oral fluid

Oral fluids containing or not XY at different concentrations were also tested. As required for enzymatic assays, oral fluid samples need a thermal processing (95°C for 30') to deactivate α-amylases naturally present in the matrix. In fact, in our approach α-amylases display the moderate ability to form AuNPs (data not shown), therefore representing an interference leading to false

positive results. After thermal treatment, oral fluid samples were centrifuged and the supernatants recovered and spiked with the same [XY] range tested in water (0-10 g L^{-1}), to compare the efficiency of AuNPs formation in the real matrix. In undiluted oral fluid the formation of XY@AuNPs results inhibited, presumably due to the high protein content of the matrix. Conversely, a 1:10 dilution is enough to allow the triggering of XY@AuNPs formation with a kinetic suitable for a fast assay. However, Abs_{520} values recorded at 500 sec result much lower than the corresponding in water, due to the slower NPs formation in the matrix. To gain the signal, the final pH in solution was thus increased by adding 7.5 µL instead of 5 µL of NaOH (1 M) to each sample before Au(III) addition, as suggested by literature [23]. Moreover, the optical reading was delayed to 1000 sec (instead of 500 sec) to improve both slope and Abs_{520} values. Afterward, the kinetic of the reaction in oral fluid displayed an optimum trend, allowing the reproducible collection of Abs_{520} (Fig. 5A). To be noted that since spiked [XY] is in the range 0-10 g L^{-1} and oral fluid samples are 1:10 diluted, the recorded absorbances are relative to 0-1.0 g L^{-1}. This means that a slight enhancement of pH value under controlled conditions is effective in expanding the detection window of the assay of about one order of magnitude.

Data collected on these samples and used for data processing were inferred from at least five independent measurements for each [XY], conveniently processed as follows: each dilution series of samples are prepared simultaneously, and only one underwent kinetic monitoring in real time (as displayed in Fig. 5A). The replicate series were incubated under the same conditions (i.e. 37 °C for 1000 sec) without monitoring the kinetic. At 1000 sec all the samples were assayed for their Abs_{520}. The linearity of the dose-response resulted highly conserved, with a very good averaged coefficient of variability (6.5%), well comparable to that obtained in water (6.9%) (Fig. 5B). Despite a decrease of slope value can be observed between water and oral fluids samples, it does not affect the optical reading. The estimated detection limit in diluted saliva, calculated with the relative equation, was found to be 44.0±0.7 mg L^{-1}.

As previously observed in water, also oral fluid samples spontaneously evolve toward different final products for t>1000 sec (Fig. 5C), loosing the linear correlation between [XY] and Abs_{520} (Fig. S4). Contrarily, the addition of 10 μL of 0.01 mM SDS to each sample at 1000 sec allows to effectively stabilize the λ_{max} of AuNPs up to days (Fig. 5D). This in turn allows to keep the linearity of the dose-response (Abs_{520} vs [XY]) over time, while XY@AuNPs slowly continue to evolve only in terms of absorbance intensity (Fig. S5). This behavior is induced by the stabilizing/capping action of this anionic surfactant during the nucleation step, and here clearly demonstrates that SDS is crucial when the analytical datum is inferred at the end point of NPs formation instead of during its nucleation phase, as already reported by Palazzo and coworkers for glucose [9]. However, samples treated with SDS (at 1000 sec), at 120 min display a marked enhancement of the intercept (Abs_{520}~0.02) and a certain loss of linearity respect to data sampled at 1000 sec (R^2=0.993 vs 0.998). This means that the negative control (no XY) gives false positive results for long incubation times in oral fluid, probably due to the onset of residual amylases activity and other undesired reactions. The fast reading (1000 sec) is therefore a key parameter to avoid undesired products that should be taken into account for analytical purposes.

Monitoring of polyols in oral fluid during gum consumption

Finally, we tested the feasibility of applying the method on anti-caries agents, chewing gums in this case. Xylitol-containing gums generally contain not only different amounts of xylitol (g/tablet) but also combinations of other sugar substitutes and non-caloric sweeteners (e.g. sorbitol, mannitol, maltitol, and aspartame). As already mentioned, it is known that a variety of sugars and carbohydrates exhibit the ability to reduce Au(III) ions to AuNPs and to stabilize them in solution, included some non-caloric sweeteners such as mannitol [24] and aspartame [25]. However, despite its potential interest, very scarce literature is reported in this sense for food or clinical analyses tests on total polyols. Therefore, we tested the method used for detecting xylitol in oral fluid to monitor

the presence of mixed reducing sugars during mastication of xylitol-containing gums. First we tested only the external coating of gums since, as indicated by the producer, xylitol should be mainly present as crystals in the outer layer instead of the gum tablet.

As reference test for the starting concentration and to exclude the mastication/deglutition effects, gum tablets (n=5, Daygum Protex, 6.9 g of total polyols/ 7 tablets, i.e. 0.985 g/tablet) were dipped in known volumes of oral fluid (processed as described in materials and methods) until complete dissolution of the surface layer. To monitor the persistence of sugars in oral fluid during mastication, oral fluid samples (1 mL) were collected over time (0-60 min), then centrifuged and diluted 10 folds before testing. Kinetic data were collected by applying the reaction protocol suitable for tests in oral fluid.

Considering the declared content of total polyols/tablet (0.985 g), an initial concentration of about 1 g mL^{-1} (0.985 g mL^{-1}) is assumed in sampled oral fluid aliquots. As reported by product information, the xylitol content is 30% of total sugars, i.e. about 0.3 g mL^{-1}.

As showed in Fig. 6, it is very interesting to note the accord between samples obtained by dissolving the sole outer layer of tablets in reference oral fluid (black series) with the first sampling of oral fluid after 2 min from intake (before deglutition and avoiding mastication). Assuming a starting concentration of about 100 g L^{-1}, the percentage persistence of sugars in the collected oral samples displays a fast decay within the first 10 min (to about 20%, i.e. 20 g L^{-1}) and fades off within 60 min. It is also notable that 500 sec (instead of 1000 sec) is the effective time window to avoid the onset of undesired exponential NPs growth (Fig. S6), which could led to the loss of linearity as already demonstrated.

The obtained results are strongly in accord with similar testing performed by commercial enzymatic test for xylitol quantification. Holgerson et al. [26] reported data on the persistence of XY in oral fluid after intake of different xylitol-containing products (chewing gums, sucking tablets, candies, toothpaste, and rinses). It is noteworthy that the reported results display the same trend inferred by

the method here reported, not only in terms of kinetic over time but also in terms of estimated [XY]. In particular, for assays conducted by the authors after chewing gum consumption containing 1 g of XY/tablet, data are strongly correlated. In Fig. 7 the two data sets are directly compared, considering that in our case only the 30% of the overall signal is related to xylitol (as declared by the producer). The overlap of results is strongly indicative of the successful evaluation both of total polyols and the sole contribution of xylitol in the tested samples. It has to be noted that standard deviations reported by Holgerson are referred to clinical tests carried out on 12 children, therefore are statistically higher than those reported for our data (replicates of different oral fluids from a single volunteer).

Conclusions

This paper reports the non-enzymatic colorimetric detection of AuNPs formed in solution upon the reducing ability of xylitol and polyols at fixed wavelength. The method has been first tested in water and oral fluid spiked with xylitol to set up suitable conditions and assess the matrix contribution to the assay. Afterward, the monitoring of total polyols over time during xylitol-containing gums consumption in oral fluid has been performed. The key novelty of the presented assay relies on the effective exploitation of analytical information given by the early nucleation step of NPs formation instead of those related to final products at the end point of AuNPs growth, as generally reported in literature. In fact we show that absorbance at fixed wavelength (520 nm in this case) strongly correlates to the concentration of the reducing agent in solution only during the nucleation stage. On the contrary, the optical reading carried out at the end point of the reaction shows non-linear correlation and several undesired features. Compared to similar papers dealing with quantification of sugars by NPs formation (i.e. glucose), here we demonstrate that most of the relevant limitations previously reported such as scarce linearity, limited concentration range, and low reproducibility can be successfully overcome by the proposed approach.

As proof of concept, we selected xylitol to demonstrate this principle. In fact, the moderate reducing power of xylitol allows a fine control of the nucleation step under mild conditions (37°C without agitation), which can be quantified in a highly reproducible manner at fixed wavelength in less than 10 min. The instrumentation required for optical reading is extremely simple and economic, since the fixed wavelength at 520 nm can be obtained by a led source, as equipped on small and portable spectrometers. In this case we adapted a commercial instrument carrying six different channels, conceived for food analysis. It is fast, low-cost and highly reproducible, even if for quantitative purposes it requires the knowledge and the control of possible competitive reducing agents which may result in false positive responses.

We believe that the proposed approach can be extended to other reducing (antioxidant) substances of interest and that can effectively contribute to the development of new and attractive (bio)analytical applications.

Acknowledgments

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Captions to figures

Fig. 1. Xylitol-capped AuNPs formed in solution by varying [XY] from 500 to 0.1 mM (# = 0 mM). The colorimetric behavior of the samples is here related to TEM images of the obtained NPs.

Fig. 2. (A) Plot of wavelength maxima of the obtained samples against [XY], which evidences the progressive shift of λ_{max} along the series. (B-C) Extinction spectra relative to sample solutions, in which both width and position the peaks can be related to [XY].

Fig. 3. A) Colorimetric result of XY@AuNPs formation after 500 sec at 37°C within 0-10 g L^{-1} concentration (# = 0 g L^{-1}). B) Dynamic trend of XY@AuNPs monitored at 520 nm for 500 sec. Each concentration is reported in triplicate. C) Linear correlation of the calibration of XY in water obtained under the selected conditions.

Fig. 4. A) Colorimetric result of XY@AuNPs formation at the end point of reaction, within 1-10 g L^{-1} XY concentration. B) Dynamic trend of XY@AuNPs monitored at 520 nm for 2500 sec. C) Plot of the dose-response of the system when Abs_{520} is inferred at the end point of AuNPs formation.

Fig. 5. (A) Abs_{520} of XY@AuNPs formation in spiked oral fluid within the range 0-1.0 g L^{-1}. The kinetic was monitored for 1000 sec to improve the detection limit. (B) Direct comparison of the XY calibration in water (purple), and in spiked oral fluid (empty green). (C-D) View of the samples not treated or treated with SDS, after 24 hr at room temperature.

Fig. 6. Monitoring of total polyols persistence in oral fluid during xylitol-containing gums (60 min). (A) the real time formation of polyols-capped AuNPs in solution followed at 520 nm for 500 sec;

(B) the time evolution of polyols content in oral fluid during mastication plotted as function of absorbance values at 520 nm. Green sample is the negative reference (#(neg)) for clean oral fluid; black sample is the positive control obtained in tube by dissolving in oral fluid the external coating of gums without chewing action (#(pos)). C) Visual aspect of samples after AuNPs formation. To better evidence the chromaticity by naked eye, the samples here showed have been stabilized at 500 sec with SDS addition, and the photo taken after 1 h.

Fig. 7. Direct comparison of the estimated xylitol concentration during XY-containing gums consumption obtained in this work and by enzymatic commercial kit [26].

Fig. 1

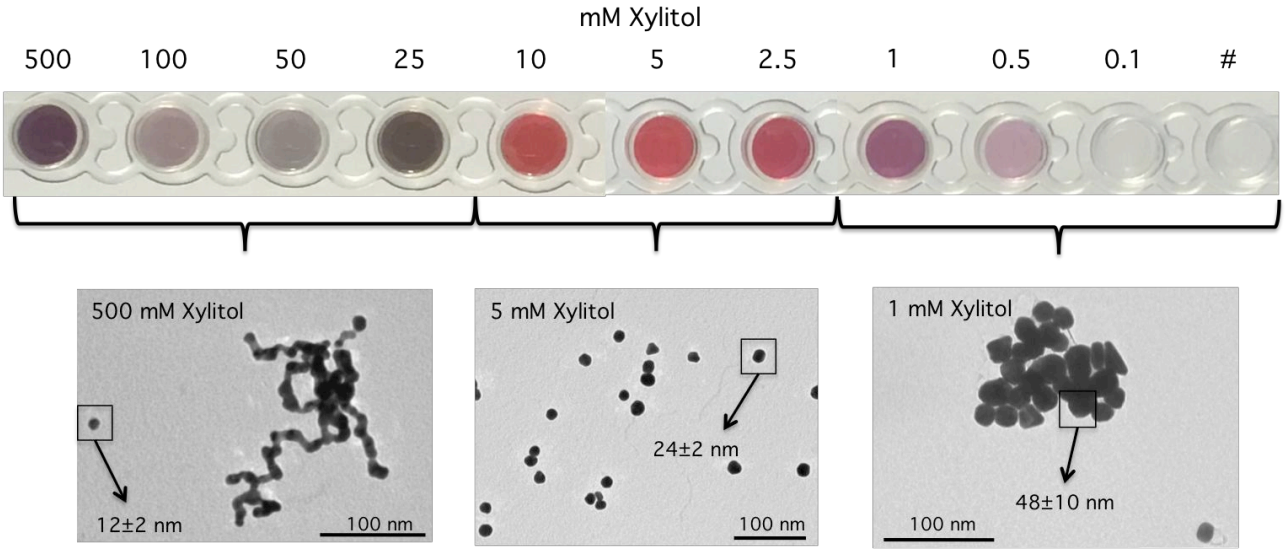

mM Xylitol
500
100
50
25
10
5
2.5
1
0.5
0.1
#
500 mM Xylitol
12±2 nm
100 nm
5 mM Xylitol
24±2 nm
100 nm
1 mM Xylitol
48±10 nm
100 nm

Fig. 2

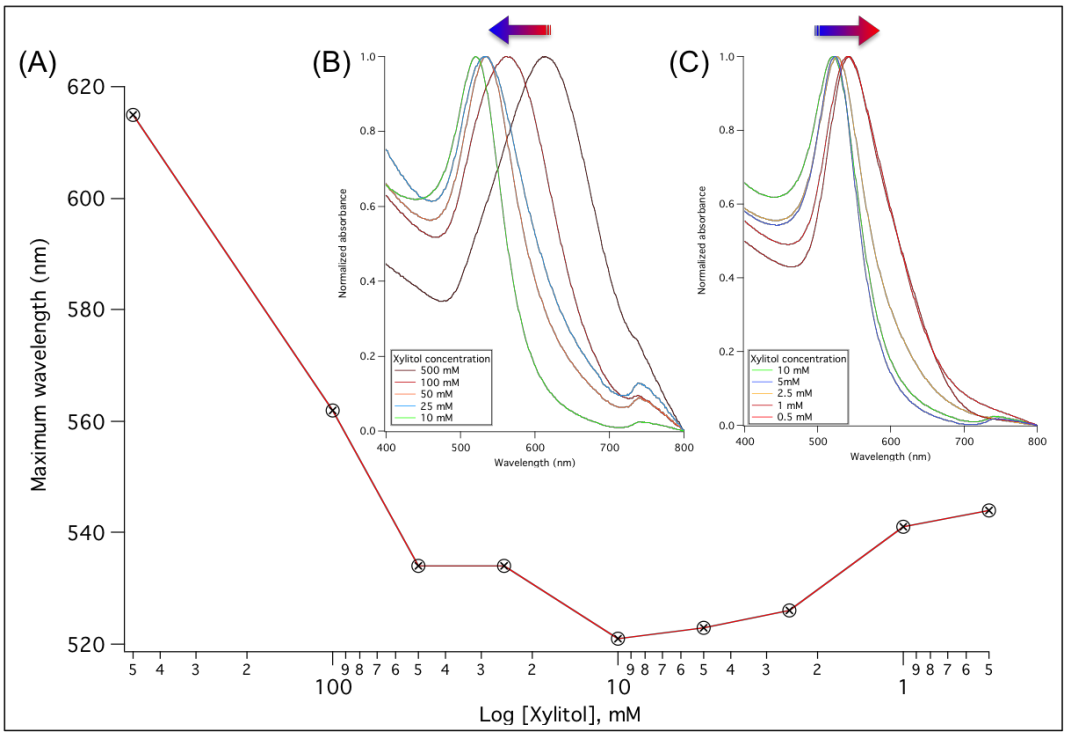

(A)
(B)
(C)
Maximum wavelength (nm)
Log [Xylitol], mM
Normalized absorbance
Wavelength (nm)
Xylitol concentration
500 mM
100 mM
50 mM
25 mM
10 mM
5mM
2.5 mM
1 mM
0.5 mM

Fig. 3

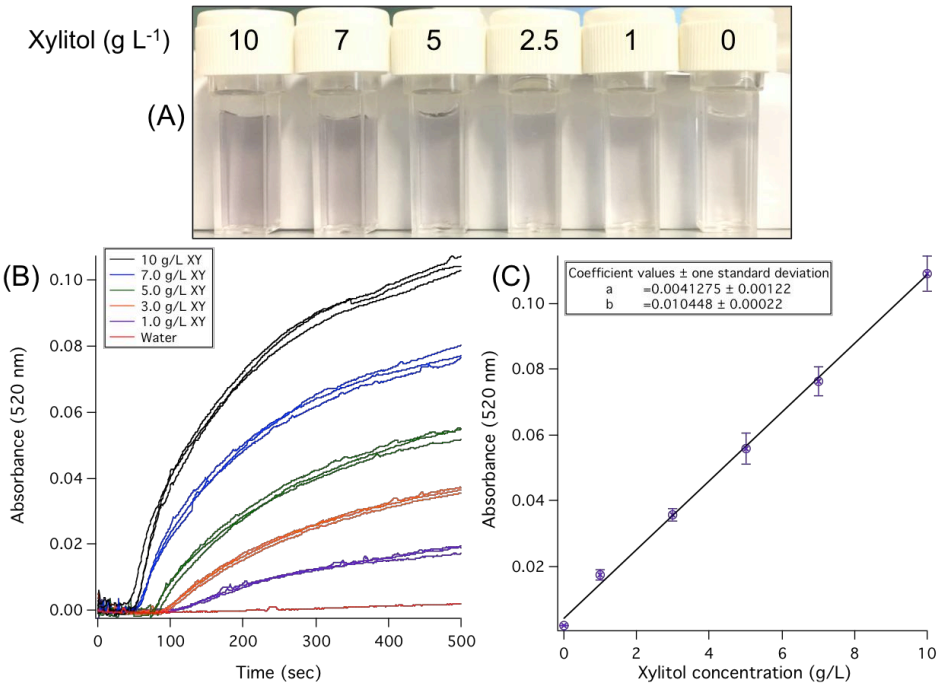

Xylitol (g L-1)
10
7
5
2.5
1
0
(A)
(B)
10 g/L XY
7.0 g/L XY
5.0 g/L XY
3.0 g/L XY
1.0 g/L XY
Water
Absorbance (520 nm)
Time (sec)
(C)
Coefficient values ± one standard deviation
a =0.0041275 ± 0.00122
b =0.010448 ± 0.00022
Absorbance (520 nm)
Xylitol concentration (g/L)

Fig. 4

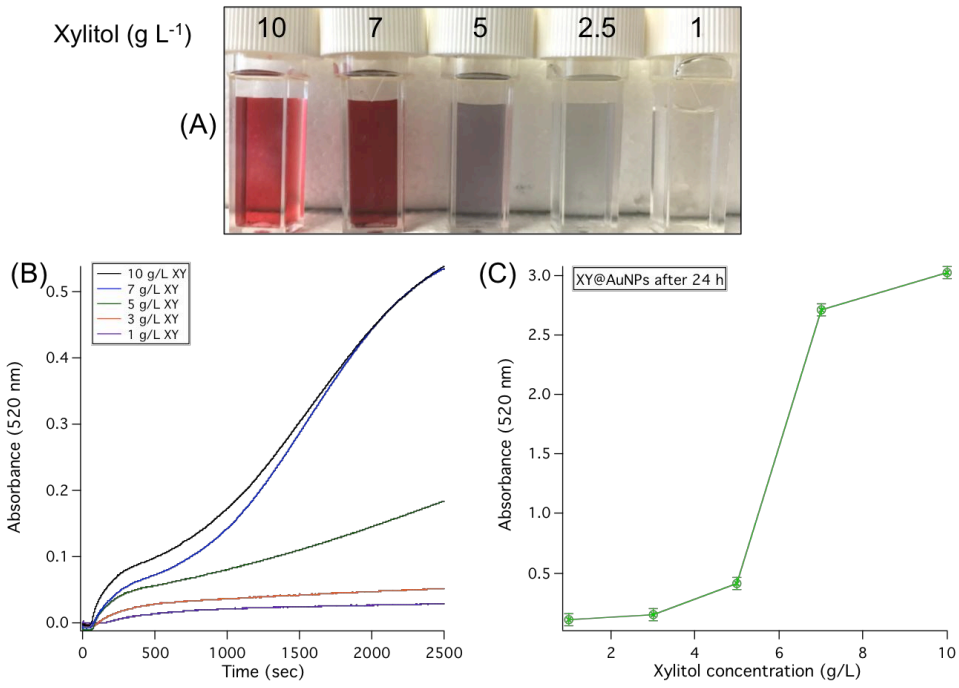

Xylitol (g L-1)
10
7
5
2.5
1
(A)
(B)
10 g/L XY
7 g/L XY
5 g/L XY
3 g/L XY
1 g/L XY
Absorbance (520 nm)
Time (sec)
(C)
XY@AuNPs after 24 h
Absorbance (520 nm)
Xylitol concentration (g/L)

Fig. 5

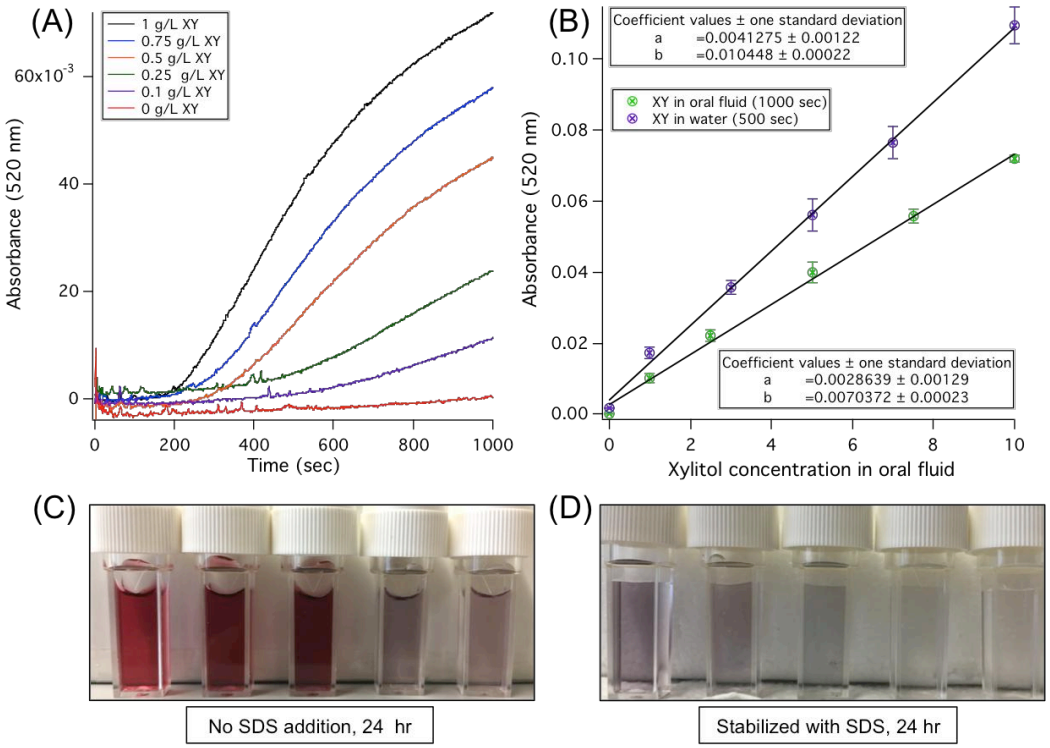

(A)
1 g/L XY
0.75 g/L XY
0.5 g/L XY
0.25 g/L XY
0.1 g/L XY
0 g/L XY
60x10-3
Absorbance (520 nm)
Time (sec)
(B)
Coefficient values ± one standard deviation
a =0.0041275 ± 0.00122
b =0.010448 ± 0.00022
XY in oral fluid (1000 sec)
XY in water (500 sec)
Coefficient values ± one standard deviation
a =0.0028639 ± 0.00129
b =0.0070372 ± 0.00023
Absorbance (520 nm)
Xylitol concentration in oral fluid
(C)
No SDS addition, 24 hr
(D)
Stabilized with SDS, 24 hr

Fig. 6

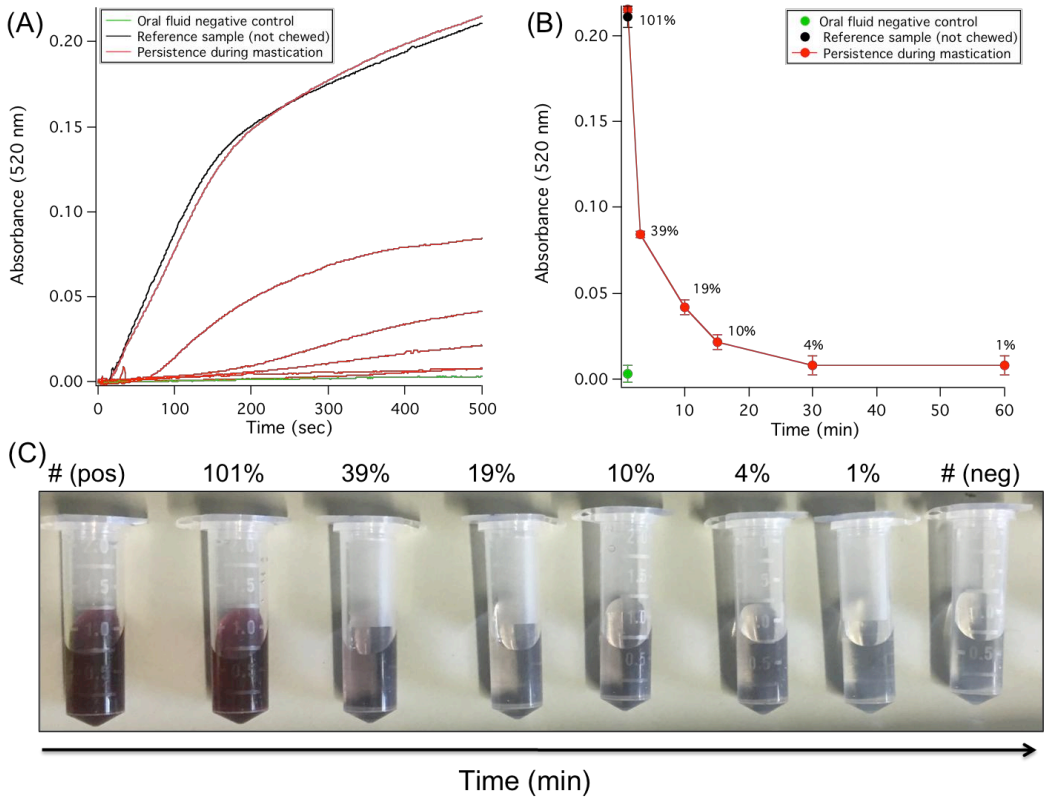
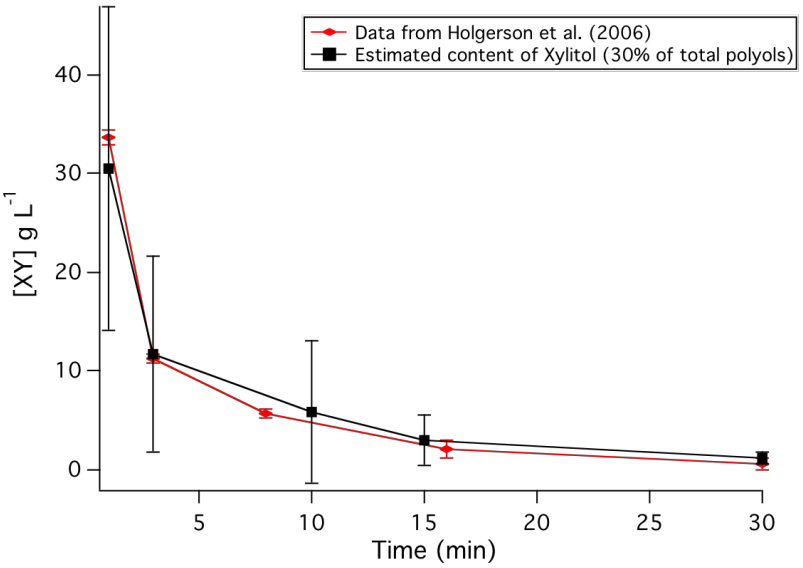

(A)
Oral fluid negative control
Reference sample (not chewed)
Persistence during mastication
Absorbance (520 nm)
Time (sec)
(B)
101%
39%
19%
10%
4%
1%
Time (min)
(C)
(pos)
101%
39%
19%
10%
4%
1%
(neg)
Time (min)

Fig. 7


Data from Holgerson et al. (2006)
Estimated content of Xylitol (30% of total polyols)
[XY] g L-1
Time (min)

Electronic Supplementary Material (online publication only)

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