



SIFT-MS quantification of several breath biomarkers of inflammatory bowel disease, IBD: A detailed study of the ion chemistry



Regina Brůhová Michalčíková^{a,b}, Kseniya Dryahina^a, Patrik Španěl^{a,*}

^a J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, 182 23 Prague 8, Czech Republic

^b Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic

ARTICLE INFO

Article history:

Received 26 October 2015

Received in revised form 9 December 2015

Accepted 18 December 2015

Available online 8 January 2016

Keywords:

Selected ion flow tube mass spectrometry

Ion molecule reactions

Three body association

Volatile organic compounds

Chemical ionization

Inflammatory bowel disease

ABSTRACT

Several compounds were previously identified as being present in modified concentrations in the breath of patients with inflammatory bowel diseases, IBD: 2-methylpentane, 1,3,5-triazine, *trans*-2-octene, *cis*-2-nonen-1-ol, 2'-methylacetophenone, 1-heptene and 2-hexanone. A selected ion flow tube, SIFT, study has thus been carried out of the reactions of H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$ with these volatile organic compounds. The rate constants and product ion branching ratios of the primary reactions including three body association rate constants, describing the rate of hydration, were obtained. The results of this study allow accurate SIFT-MS quantification of concentrations of these compounds in exhaled breath of IBD patients with the ultimate aim of establishing their use as non-invasive biomarkers of the disease activity.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Inflammatory bowel diseases (IBD) are a group of chronic relapsing disorders of the gastrointestinal tract causing extensive inflammation and damage to the intestines and rectum. The major two types of IBD are Crohn's disease (CD) [1] and ulcerative colitis (UC) [2]. CD is characterized by chronic discontinuous transmural inflammation that may involve any portion of the gastrointestinal tract, but most commonly the terminal ileum, whilst UC is characterized by inflammation of the colonic mucosa, extending to a variable extent from the rectum to the proximal colon. Several biomarkers including C-reactive protein and faecal calprotectin are currently used for the assessment of activity indices of IBD in addition to colonoscopy. However, the current diagnostic methods are either invasive or have limited sensitivity or specificity. Therefore, identification of new and sensitive non-invasive markers in IBD is highly desirable.

One of the approaches to non-invasive monitoring of IBD activity could be breath analysis of trace amounts of metabolites. Selected ion flow tube mass spectrometry, SIFT-MS, is one of the analytical techniques for real time, on-line quantitative analysis of trace

gases in humid exhaled breath, which is based on chemical ionization in a fast flow tube reactor at a well-defined reaction time [3]. Quantification of different chemical compounds by SIFT-MS is becoming an area of growing interest in several areas of research. In the medical science, volatile organic compounds are analyzed in breath and urine headspace in search for biomarkers that can be used for clinical diagnostics and therapeutic monitoring. Therefore the direct breath testing using SIFT-MS is suggested to have potential for non-invasive monitoring of IBD activity [4].

This suggestion has been validated during a pilot study, where possibility of using SIFT-MS breath analysis as a tool for IBD detection has been tested using exhaled pentane concentration as a biomarker of lipid peroxidation [5]. The results revealed that breath pentane is significantly elevated in the breath of both CD patients and UC patients relative to the healthy controls, being at a somewhat lower mean concentration in UC than CD breath. However, as pentane is a nonspecific inflammatory marker it is important to add other volatile metabolites to the panel of compounds used for breath test of the IBD disease activity.

The group of seven additional biomarker compounds selected for the present SIFT ion chemistry study discussed in this article (methylpentane, 1,3,5-triazine, *trans*-2-octene, *cis*-2-nonen-1-ol, 2'-methylacetophenone, 1-heptene and 2-hexanone) was chosen on the basis of available patent literature [6–9] disclosing lists of potential IBD biomarker compounds as determined by TD/GC/MS

* Corresponding author. Tel.: +420 2 6605 2112; fax: +420 2 8658 2307.
E-mail addresses: spanel@jh-inst.cas.cz, spanel@seznam.cz (P. Španěl).

technique [10] claiming that these volatile organic compounds not only differentiate between IBD and healthy controls, but also between CD and UC and between active diseases or diseases in remission.

The main objective of this study was to study the kinetics of the primary reactions of H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$ reagent ions with these seven compounds. Additionally kinetics of reactions of their primary products with water molecules was also studied, as the ion chemistry used for real time and accurate quantification by SIFT-MS is often influenced by the presence of water vapour and this must be accounted for. Data resulting from this study should allow accurate quantification of these biomarkers in the exhaled breath of IBD patients in real time using SIFT-MS.

2. Experimental methods

The SIFT technique to study the kinetics of ion-molecule reactions at thermal energies and to provide the required rate constants and product ion branching ratios has been described previously [11,12]. SIFT has been used to study numerous ion-molecule reactions in several laboratories around the world and recently to study the reactions of H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$ with many types of organic compounds [11,13–17]. Current small SIFT-MS instruments with a 5 cm long flow tube can be readily used for this purpose, as described previously [18–20] and so only the specific details of the present experimental procedure are outlined below.

2.1. Determination of the branching ratios

The following seven reagents were used: 2-methylpentane, $\geq 99.5\%$, Fluka; 1,3,5-triazine, 97%, Aldrich; *trans*-2-octene, 97%, Aldrich; *cis*-2-nonen-1-ol, $\geq 97\%$, Aldrich; 2'-methylacetophenone (1-(2-methylphenyl)-ethanone), $\geq 99.5\%$, Fluka; 1-heptene, neat, Supelco and 2-hexanone, neat, Supelco. In order to determine the product ions of the reactions of H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$ with molecules of these compounds and their branching ratios, mixtures of dry air and a trace amount (less than 10 parts per million by volume, ppmv) of vapour of a compound were introduced into the SIFT instrument (Profile 3 SIFT-MS manufactured by Instrument Science Limited, Crewe, UK) via a heated calibrated capillary and full scan (60 s integration time) mass spectra were acquired whilst each of the three selected precursor ions were alternately injected into the helium carrier gas. The m/z mass spectral range (typically m/z 10–200) was chosen on the basis of the molecular weights of compounds included in the study, allowing for possible formation of adduct ions. The major ion products were identified by inspection of these mass spectra and their count rates were precisely determined in separate experiments using the multi-ion monitoring (MIM) mode [11] whilst the mixture was introduced into the flow tube by the sample inlet port. The flow rate was measured by a flow meter and varied using a needle valve. The primary product branching ratios of the reactions were then determined by plotting the percentages of the individual product ions on a linear scale as a function of the sample mixture flow rate. By extrapolating to zero flow (i.e., estimating the limit at zero sample concentration) the true primary branching ratios, excluding any secondary reactions were obtained. Only the product ions with resulting branching ratio greater than 5% are reported.

2.2. Determination of the rate constants

It is well established that proton transfer reactions of H_3O^+ proceed at collisional rate when these reactions are exothermic by more than 40 kJ/mol [21]. Thus, the rate constants for the reactions of H_3O^+ were calculated as collisional (k_c) according to parametrised trajectory calculation [22] using the dipole moments,

μ_r , calculated according to the density functional theory (DFT, B3LYP, 6-31G*) using Abalone software (NWChem, version 1.8.88, Agile Molecule) and polarizabilities, α , calculated according to empirical method from the chemical structure of the molecules [23]. The rate constants for the reactions with NO^+ and $\text{O}_2^{+\bullet}$ (k) were determined experimentally in the same way as in the previous studies [10,24] from the experimentally derived decay rates relatively to that for the H_3O^+ reaction by injecting all three precursors simultaneously and allowing them to react with the neat vapours of the compounds introduced at varied concentrations. Uncertainty in the absolute value of the determined rate coefficients is $\pm 20\%$ [10,24].

2.3. Kinetics of the ion chemistry involving water molecules: determination of three-body association rate constants

The three-body association rate constants were determined for protonated molecules formed in the H_3O^+ reactions with the molecules M of potential IBD biomarkers and additional molecules formed in the NO^+ primary reactions.

2.3.1. Reactions of protonated molecules MH^+ with H_2O

It was found out during this study that the three-body association reactions are observed only when the primary reactions of the chosen compounds with H_3O^+ precursor are forming the MH^+ ions. In the presence of water molecules the MH^+ product ions associate with H_2O forming hydrated ions $\text{MH}^+(\text{H}_2\text{O})_{1,2,3}$. The H_3O^+ precursor ions are also partially converted to hydrated hydronium ions $\text{H}_3\text{O}^+(\text{H}_2\text{O})_{1,2,3}$ that can act as additional precursors and contribute to the production of $\text{MH}^+(\text{H}_2\text{O})_{1,2,3}$ via ligand switching reactions. The ratio of the count rates of these ions changes as a function of the concentration of H_2O in the SIFT flow tube than depends on the sample flow rate and the sample humidity. The procedure for determination of the three body rate constants of the primary products of H_3O^+ reactions with H_2O , which is used in this study, has been described previously for a similar study of ion chemistry of esters [19] and carboxylic acids [25]. The relevant equations are also available in Appendix 1 of this article.

2.3.2. Reactions of the primary products of NO^+ reactions with H_2O

When using NO^+ precursor ions to analyze humid samples a fraction of product ions also associate with H_2O molecules forming hydrated ions [26,27]. Ion products of the kind $[\text{M}+\text{NO}]^+$ can be also formed via ligand switching reactions of the $\text{NO}^+(\text{H}_2\text{O})_{1,2}$ ions with the molecules [28]. The primary reactions of NO^+ precursor ions with the seven compounds, M, result in the formation of various types of product ions. In the presence of water molecules some of these product ions associate with H_2O molecules to form the adduct ions. Three-body rate constants for the association reactions of these ions with H_2O molecules in helium, k_{A^+} , describe the rate of hydration of these product ions in the gas phase. The process of formation of these hydrates has been described in previous studies [25,27] and the relevant equations are again available in Appendix 1.

3. Results and discussion

3.1. Rate constants for the primary H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$ reactions

The calculated collisional rate constants, k_c , and the experimentally derived rate constants, k , are summarized in Table 1. H_3O^+ reactions are assumed to proceed at collisional rate for all molecules with exception of 2-methylpentane which does not have sufficiently high proton affinity and which is observed to react at faster rate with both NO^+ and $\text{O}_2^{+\bullet}$ precursor ions. Similar slow reactions

Download English Version:

<https://daneshyari.com/en/article/1192713>

Download Persian Version:

<https://daneshyari.com/article/1192713>

[Daneshyari.com](https://daneshyari.com)