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Photodegradation of novel oral anticoagulants under sunlight irradiation in aqueous matrices

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HIGHLIGHTS

- Dabigatran undergoes direct and indirect photolysis, $t_{1/2}$ is of 24 h in river water.
- Direct photolysis is predominant for rivaroxaban, $t_{1/2}$ is of 2.2 h in river water.
- Argatroban is mainly photodegraded by photosensitization.
- Structures of main photoproducts have been identified.

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ABSTRACT

Kinetics of photodegradation of novel oral anticoagulants dabigatran, rivaroxaban, and apixaban were studied under simulated solar light irradiation in purified, mineral, and river waters. Dabigatran and rivaroxaban underwent direct photolysis with polychromatic quantum yields of 2.2×10^{-4} and 4.4×10^{-2}, respectively. The direct photodegradation of apixaban was not observed after 19 h of irradiation. Kinetics of degradation of rivaroxaban was not impacted by the nature of the aqueous matrix while photosensitization from nitrate ions was observed for dabigatran and apixaban dissolved in a mineral water. The photosensitized reactions were limited in the tested river water (Isle River, Périgueux, France) certainly due to the hydroxyl radical scavenging effect of the dissolved organic matter. The study of photoproduct structures allowed to identify two compounds for dabigatran. One of them is the 4-aminobenzamidine while the second one is a cyclization product. In the case of rivaroxaban, as studied by very high field NMR, only one photoproduct was observed *i.e.* a photoisomer. Finally, seven photoproducts were clearly identified from the degradation of apixaban under simulated solar light.

1. Introduction

Until recently, oral anticoagulants were synonyms of vitamin K antagonists (VKAs) such as warfarin. These medicines are used for venous thromboembolism, stroke, atrial fibrillation treatment, and most generally in medicinal conditions that require chronic anticoagulation (Hanslik and Prinseau, 2004). VKAs may not be

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appropriate for many patients especially due to the necessity of dietary precautions and frequent laboratory monitoring (El-Helou et al., 2013). In fact, if warfarin concentration is too low the anticoagulation effect does not act efficiently; a too high concentration causes excessive bleeding. In this context, novel oral anticoagulants (NOACs) were developed (Spyropoulos, 2008; Beyer-Westendorf et al., 2014). The U.S. Food and Drug Administration (F.D.A.) recently approved the use of three new active ingredients, namely dabigatran etexilate (2010), rivaroxaban (2011), and apixaban (2012) (Wanat, 2013). Dabigatran etexilate is a prodrug that is converted to dabigatran and acts as a direct thrombin inhibitor (Khoo and Lip, 2010). Rivaroxaban and apixaban are direct factor Xa inhibitors that do not require cofactors for activity (Gulseth et al., 2008; Frost et al., 2013). These three compounds are also increasingly used in Europe. In particular, these NOACs currently represent 30% of ingested anticoagulants in France with an increase in their consumption by a factor of 10 between the second trimester 2012 and the third trimester 2013 (ANSM, 2013). The main limitation for their use was the absence of antidote. In 2015, the F.D.A. approved the idarucizumab as dabigatran antidote while rivaroxaban and apixaban antidote (andexanet alpha) is about to be validated (Pollack et al., 2015; Siegal et al., 2015). Thus, their prescriptions are expected to continue to rise in the near future.

To our knowledge, information reported in the literature about the occurrence of NOACs in the aquatic compartment, their fates, and their toxicity, as well as their degradation products are very scarce (Kasad, 2013; Ramisetti and Kuntamukkala, 2014; Secrétan et al., 2015; Swain et al., 2016; Tantawy et al., 2016; Wingert et al., 2016). This is especially worrying since human metabolism studies have shown that NOACs are eliminated in urines as unchanged forms at levels between 27 and 85% of the delivered dose (eVidal, 2017). In fact, organic pollutants are known to undergo degradation processes in environmental waters which must be taken into consideration. Photolysis can be one of the major ways of degradation for pharmaceutical compounds absorbing solar light as well as for those degraded by photosensitization (Szabo et al., 2011; Zuo et al., 2013; Carlson et al., 2015; Zhang et al., 2016; Chen et al., 2017).

This study has for objective to bring information on the environmental fate of NOACs. In particular, direct and indirect photolysis of NOACs was studied in different aqueous matrices. Results on molar absorption coefficients, rate constants and polychromatic quantum yields of photodegradation are discussed. Rate constants were obtained from simulated solar light irradiation in purified, mineral, and river waters to evaluate the effect of ions and dissolved organic matter naturally present in surface waters. Finally, the elucidation of structures of photoproducts was performed using LC-HRMS, and 700 and 800 MHz NMR spectrometers.

2. Materials and methods

2.1. Chemicals

The novel oral anticoagulants (NOACs) dabigatran (APIChem Technology, CAS 211914-51-1, 99%), rivaroxaban (AKSci, CAS 366789-02-8, 98%), and apixaban (APIChem Technology, CAS 503612-47-3, 99%), as well as 4-aminobenzamidine dihydrochloride (≥99.0%), methanol (CHROMASOLV®, ≥99.9%), and sodium bicarbonate salt (≥99.5%) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Sodium nitrate salt (analytical reagent grade) was provided by Fisher Chemical. All chemicals were used as received. Purified water was produced using a Milli-Q® Direct-Q® 5 system (Millipore, resistivity 18.2 MΩ cm). Mineral water (Volvic) contains 7.2 and 74.0 mg L^{-1} of nitrate and hydrogen carbonate ions, respectively. River water was collected from the Isle River (Périgueux, France). This surface water contained 7.7 and 140–200 mg L^{-1} of nitrate and hydrogen carbonate ions, respectively. Before use, the river water was filtered on a 0.45 μm hydrophilic PVDF Durapore® membrane (Millipore). Stock solutions of NOACs were prepared in purified water at concentrations of 5.0×10^{-5}, 1.0×10^{-5}, and 3.0×10^{-6} mol L^{-1} for dabigatran, rivaroxaban, and apixaban, respectively. During the preparation, volumetric flasks were covered with aluminum foils and solutions were left under magnetic stirring during one week. Then, stock solutions were stored in amber glass bottles at room temperature until use to prevent photodegradation and solubility problems, respectively. The stability of the three compounds was checked.

2.2. UV–visible spectroscopy

UV–Visible absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu, Japan). A 1-nm resolution and a "medium" scan rate were used to record the reference spectrum and spectra in the range 200–400 nm (NOACs do not absorb visible light) using either 1 or 5 cm quartz cells (QS, Hellma Analytics).

Molar absorption coefficients of dabigatran and rivaroxaban in purified water were calculated from the absorbance of five solutions with concentrations included in the range 10^{-6}–10^{-5} mol L^{-1}. Due to the particularly low solubility of apixaban in water, a stock solution was prepared in methanol at a concentration of 9.6×10^{-6} mol L^{-1}. Then, this stock solution was used to prepare five solutions at concentrations between 1.0×10^{-6} and 4.8×10^{-6} mol L^{-1} by adding purified water (*i.e.* the maximal proportion of methanol was of 50%).

The solar light emission spectrum at the Earth surface was recorded using a USB 2000 + radiometer coupled to an optical fiber (Ocean Optics, Florida, USA).

2.3. Irradiation experiments

Solar radiations at the Earth surface were simulated using a SUNTEST CPS apparatus from Atlas Material Testing Solutions (Illinois, USA). The irradiance was set to 250 W m^{-2}. In the case of kinetic studies, purified, mineral, and river waters were spiked with the stock solutions prepared in purified water to obtain final concentrations of 3.5×10^{-7}, 3.5×10^{-7}, and 4.5×10^{-7} mol L^{-1} for dabigatran, rivaroxaban, and apixaban, respectively. For the elucidation of photoproduct structures, non-irradiated and irradiated stock solutions were analyzed without pre-treatment.

Before irradiation, samples were placed into 12 mL transparent glass tubes (optical pathlength: 1.5 cm) and were sealed to avoid water evaporation. NOACs were irradiated in purified water alone and doped with nitrate and bicarbonate ions (same concentrations as in mineral water), in mineral water, and in river water. For each aqueous matrix, two tubes were filled with 10 mL of solution and one of them was wrapped in an aluminum foil for dark control. To obtain kinetic data, aliquots of each sample were collected before irradiation and after scheduled irradiation time. For each kinetics, at least five samplings were performed and the analysis was repeated in triplicate. The maximum irradiation time was of 10 h for dabigatran, 4 h for rivaroxaban, and 19 h for apixaban. To calculate the polychromatic quantum yields of photodegradation, photon fluence rates were measured with a 1 nm interval in the range 290–350 nm using a USB2000 + radiometer coupled to an optical fiber (Ocean Optics, Florida, USA).

2.4. HPLC and LC-QToF-MS analyses

For kinetics studies, HPLC analyses were carried out using an Agilent 1100 series chromatograph (Agilent Technologies) equipped