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Short communication

Kinetics of photooxidation of papaverine hydrochloride and its major photooxidation products

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Abstract

HPCE methodology was modified to be used in kinetic experiments on photooxidation reactions of papaverine hydrochloride **1** and its oxidation products (papaverinol **2** and papaveraldine hydrochloride **3**) chloroform solutions exposed to UV_{254} light in aerobic conditions. On photooxygenation of the above compounds is formed the final degradation product, a brown compound X **4**, 2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinylim chloride. The rate of **4** formation from the above compounds can be given as **2** > **3** > **1** > **1** HCl. The photooxidation reactions of **1** and **2** proceed with pseudo first-order kinetics and that reaction for **3** follows zero-order kinetics. The most labile compound is **2** whose half-life time is 2.4 times shorter than that one of **1** HCL. The most stable product is **3** whose half-life time is 31-fold longer than of **2**. The specific quantum yields are equal 0.28, 0.30 and 0.10 for **1** HCl, **2** and **3**, respectively which confirm the above stability pattern of the compounds concerned.

Keywords: HPCE quantification; Photooxidation kinetics; Papaverine hydrochloride; Papaverinol; Papaveraldine hydrochloride; Quantum yield; A brown degradation product X; Rate of its formation

1. Introduction

Papaverine **1** salts (hydrochloride and sulfate), used in medical treatments, are susceptible to oxidation in either aqueous (injections) or especially in nonaqueous solutions, in which the solubility of oxygen is greater, as well as in the solid phase. However, **1** base is more stable if compared to its salts. The salts are oxidized to first a secondary alcohol **2** (papaverinol) and second a ketone **3** (papaveraldine) which have been known for a long time [1,2]. The presence of the above **1** oxidation products did not explain the observation that **1** salts solutions had become brown on their storage for an extended period of time when exposed to an either day- or a sunlight conditions, because they are not brown but only yellowish. However, the brown compound—named "the compound X" was first observed in ageing 4% **1** sulfate aqueous injection solutions as a brown spot on a paper chromatogram [3]. The compound X was later on isolated in a yield approx. 40% from a 2 mg/ml **1** hydrochloride chloroform solution stored for over 1 year at the daylight conditions [4]. The formation of the compound X is faster in a nonaqueous solution, e.g. a chloroform one, because the solubility of oxygen in that solvent is much greater than in water [5–7]. The compound X has been lastly identified with a yet unknown chemical **4**—2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinylium salt (e.g., chloride) [8]. Its synthesis procedure from a **2** chloroform solution exposed to a UV light at 254 nm has been published [7]. However, it is not clear, if it is formed from the **1** salts via either the product **3** or the product **2**.

Therefore, the aim of this paper is to follow kinetics and the quantum yield of photochemical degradation reactions of aerobic chloroform **1** chloride, **2** base and **3** hydrochloride solutions exposed to the UV light at 254 nm to find out which of the above compounds favors their degradation to the brown isoquinolinylium chloride **4**.

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2. Materials and methods

2.1. Materials

Loratadine (IS), lot # 7LTL012, Jelfa SA, Jelenia Góra, Poland; papaverine hydrochloride, mp 219–225 °C dec. (lit. 218–223 °C dec. FP VI), Farm-Impex, Gliwice, Poland; papaveraldine hydrochloride $C_{10}H_{19}NO_5HCl{\cdot}2.5H_2O$, synthesized according to Tsatsas [9], mp 211–212 °C (lit. 210 °C [9]); papaverinol $C_{20}H_{21}NO_5$, synthesized according to Gadamer [10], mp 136–137 °C (lit. 137 °C [2]). All other chemicals were of reagent or HPLC grade.

2.2. Apparatus

Melting points were determined on a Boëtius microscope (VEB Wägetechnik Rapido, Germany) and are not corrected. High Performance Capillary Electrophoresis (CE), model 3D 1 apparatus with diode array UV detector (Agilent Technologies, Waldbronn, Germany) was used for quantification of **1**–**3**. A 3D apparatus was equipped with Chemstation used for instrument control, data acquisition and data analysis. The system was controlled by Windows NT software. A low pressure mercury lamp 254 nm Original Hanau TNN 15/32 (Heraeus, Germany) was used in photo-kinetic experiments.

2.3. CE methodology for quantification of photooxidation processes

An Agilent fused silica capillary of 50 μm i.d. and 72 cm total length was used for separation of **1**–**4**. Background electrolyte (BGE) consisted of 50 mmol/l NaH_2PO_4 and 100 mmol/l H_3PO_4 to receive pH 2.5. A new capillary was flushed with NaOH solutions of concentrations 1, 0.1 (mol/l) and subsequently with demineralized water and BGE for 10, 10, 5 and 8 min, respectively. The temperature of the capillary was maintained by a thermostatic system at 25 °C. The samples were automatically injected using hydrodynamic injection at the anode for 2 s (0.5 psi, 3447.38 Pa). The capillary between subsequent injections was washed with 0.1 mol/l NaOH, water and BGE for 5 min each. All experiments were carried out at the voltage 20.0 kV resulting in the current of 21.0 ± 0.3 μA.

2.4. Calibration curve for *1–4*

About 10 mg/l stock solutions each of the above compounds and an internal standard (IS) were prepared in methanol. Then standard solutions each of 0.05, 0.10, 0.20, 0.50, 1.00 and 2.00 mg/l containing each 2.00 mg/l IS were prepared by dilution with methanol. To 16 mm culture tubes were added 200 μl each of chloroform and of a standard solution, and the mixture was vortexed and the solvent was evaporated out at 60 °C under nitrogen. The residue was reconstituted in a mixture (1:1) of methanol and BGE. The solution was injected onto the capillary as mentioned above. The peak area ratio of an analyte to IS was plotted as a function of four analyzed compound concentrations. In order to calculate precision and accuracy different concentrations of the compound considered were determined within-day and between-days. Every single determination was done in triplicate.

2.5. Kinetics of photooxidation reactions

About 2 mg/ml chloroform solutions each of **1**–**3** were exposed to UV light of 254 nm in 15 ml cylindrical silica cells from the distance of 2.5 cm at ambient temperature and in aerobic conditions. At suitable time intervals 200 μl samples withdrawn were diluted with 200 μl IS stock solution and evaporated to dryness. The residue was further treated according to the above specified procedure. The concentrations of **1** and its degradation products (**2**–**4**) were calculated as a function of time from a suitable electropherogram obtained.

2.6. Measurement of the quantum yield

A uranyl oxalate actinometer was applied to measure the quantum yield of the above photolysis experiment. The uranyl oxalate actinometer itself has a known quantum yield ($\varphi = 0.602$) [11]. An aqueous solution (0.01 mol/l) of the actinometer was palced in the above 15 ml cylindrical cell and exposed to UV_{254} light from the distance 2.5 cm for 10, 20 and 30 min.

The actinometer was titrated with manganate(VII) potassium (0.0930 mol/l) prior and after the irradiation. The results of the titrations (Δc) allow us to calculate the intensity of light absorbed by the substrate according to the following formula:

$$I_a = \frac{\Delta c}{\varphi t} \quad (1)$$

where t denotes the time of irradiation.

2.6.1. Quantum yield of photooxidation reaction of a papaverine hydrochloride solution and its degradation products

About 2 mg/ml chloroform solutions each of papaverine hydrochloride, papaverinol and papaveraldine hydrochloride were exposed to UV_{254} light in cylindrical 15 ml silica cells from the distance 2.5 cm in aerobic conditions at the ambient temperature. Their samples were withdrawn at suitable time elapsed and absorbances measured resulting in calculation of the degree of conversion of a substrate. The quantum yields were calculated from Eq. (1) for different degrees of conversion which were extrapolated to the zero degree of conversion which indicates the specific quantum yield of a substrate.

2.7. Calculation of rate constants of photooxidation reactions

Photooxidation reactions of papaverine hydrochloride and papaverinol follow the typical first-order equation:

$$\ln c = \ln c_0 - kt \quad (2)$$

where c and c_0 are concentrations of the substrate at time t and t_0, respectively, and k is an apparent first-order rate constant.

The constant k was calculated as a slope of the above equation by the least-squares regression.

However, the photooxidation reaction of papaveraldine hydrochloride is zero-order according to the equation:

$$c = c_0 - kt \quad (3)$$

where k is an apparent zero-order rate constant.

3. Results

3.1. Validation of HPCE methodology for substrates' quantification

Electropherograms presented in Figs. 1 and 2 indicate all compound of interest which have been separated according to the order: the brown degradation product, papaverinol, papaverine, loratadine (IS) and papaveraldine (Fig. 1). Papaverinol (B)

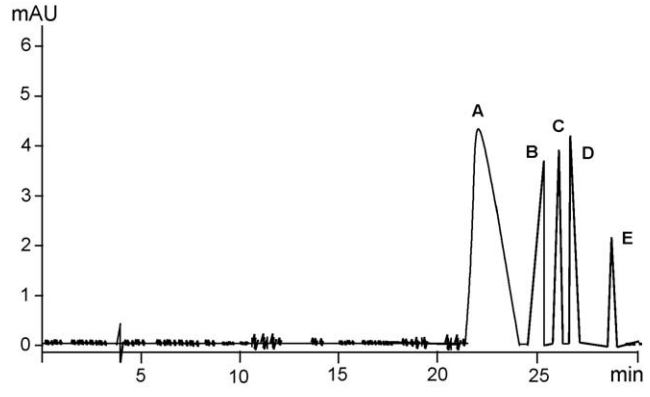


Fig. 1. Electropherogram of a mixture of papaverine (C) and its degradation products: the compound X (A), papaverinol (B) and papaveraldine (E) as well as loratadine, IS (D). Experimental conditions: fused-silica capillary, 50 μm i.d. × 72 cm; temperature, 25 °C; running buffer, 50 mmol l^{-1} NaH_2PO_4 and 100 mmol l^{-1} H_3PO_4 (pH 2.5); separation voltage, 20 kV; injection at the anode for 2 s (0.5 psi, 3447.38 Pa).

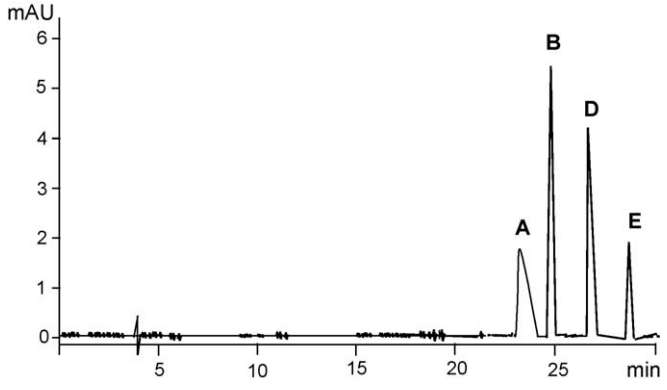


Fig. 2. Electropherogram of a papaverinol chloroform solution exposed to UV_{254} light for 3 h at ambient temperature and in aerobic conditions; (A) the brown photooxidation product of papaverinol X, (B) intact papaverinol, (E) papaveraldine as a photooxidation product of papaverinol, and (D) loratadine (IS). Experimental conditions: fused-silica capillary, 50 μm i.d. × 72 cm; temperature, 25 °C; running buffer, 50 mmol l^{-1} NaH_2PO_4 and 100 mmol l^{-1} H_3PO_4 (pH 2.5); separation voltage, 20 kV; injection at the anode for 2 s (0.5 psi, 3447.38 Pa).

Table 1
Evaluation of calibration curves of papaverine and its photooxidation products by means of least squares regression

Name of analyte	Correlation coefficient, r	Slope $a \pm \Delta a$	Intercept b	Coefficient of variation, CV (%)
Papaverine hydrochloride	0.9998	0.474 ± 0.004	0.0059	0.56
Papaverinol	0.9998	0.393 ± 0.003	0.0333	0.49
Papaveraldine hydrochloride	0.9999	0.622 ± 0.004	0.0118	0.49
Compound X	0.9999	2.25 ± 0.02	−0.0575	0.53

is degraded in its aerobic chloroform solution under influence of UV_{254} light to the brown compound (A) and to papaveraldine (E). Both papaverinol degradation products appear in quite significant and comparable amounts (Fig. 2). Calibration curves of papaverine and its three degradation products were constructed as linear curves for their area under the peak ratios to IS peak area (A/A_{IS}) as a function of their concentration c (mmol/l). Evaluation of linearity of calibrations curves by means of the least squares regression analysis can be learnt from the data provided in Table 1. It indicates pretty good first correlation coefficients (0.9998–0.9999) and second coefficients of variations (0.49–0.56%) for papaverine hydrochloride, papaverinol, papaveraldine hydrochloride and the compound X (a dibenzo[b,g]pyrrocolinium derivative [8]). Precision and accuracy have been also calculated for the data measured within-day assay (WDA) and between-days assays (BDA) for different concentrations of all the analytes. Both parameters for all analytes are low even for the data evaluated for BDA (e.g., 2.2% < CV < 9.8%, Table 2). There is relationship between recovery and the sample concentration (Table 2.) and ranges from 92.25 ± 3.79%, 101.25 ± 1.69% to 106.67 ± 2.71% for the concentrations 0.02, 2.00 and 0.20 mg ml^{-1}, respectively.

Table 2
Precision and accuracy evaluation for BDA of different concentrations of analytes

Name of analyte	Concentration[a] (mg/ml)	Recovery (%)	Correlation coefficient, CV (%)
Papaverine hydrochloride	0.02	94	9.8
	0.20	105	7.2
	2.00	103	4.1
Papaverinol	0.02	91	8.3
	0.20	111	4.3
	2.00	99	3.9
Papaveraldine hydrochloride	0.02	96	6.9
	0.20	110	6.5
	2.00	102	2.6
Compound X	0.02	88	7.4
	0.20	105	4.7
	2.00	101	2.2

[a] Mean value each from six single assay determinations.

Table 3
Kinetic parameters for photooxidation reactions of papaverine hydrochloride and its degradation products in chloroform solutions exposed to UV_{254} light at ambient temperature in aerobic conditions

Parameter	Compound degraded		
	Papaverine hydrochloride	Papaverinol	Papaveraldine hydrochloride
Correlation coefficient	0.9997	0.997	0.9995
Slope, $a \pm S_a$	-0.130 ± 0.001	-0.310 ± 0.005	$(-0.0376 \pm 0.0003) \times 10^{-3}$
Intercept, $b \pm S_b$	1.65 ± 0.02	1.66 ± 0.04	$(5.06 \pm 0.02) \times 10^{-3}$ [a]
$10^5 k$, in s^{-1}	3.62 ± 0.03	8.6 ± 0.1	$(1.036 \pm 0.008) \times 10^{-3}\,l\,mol^{-1}$
$t_{0.5}$ (h)	5.32[b]	2.23[b]	68.77[c]

[a] c_0 in $mol\,l^{-1}$.
[b] Half-life time for a first-order reaction, $t_{0.5} = \ln 2/k$.
[c] Half-life time for a zero-order reaction, $t_{0.5} = c_0/2k$.

Table 4
Specific (extrapolated to zero radiation time) quantum yields of photooxidation reactions of papaverine hydrochloride and its degradation products exposed to UV_{254} light at ambient temperature and aerobic condition

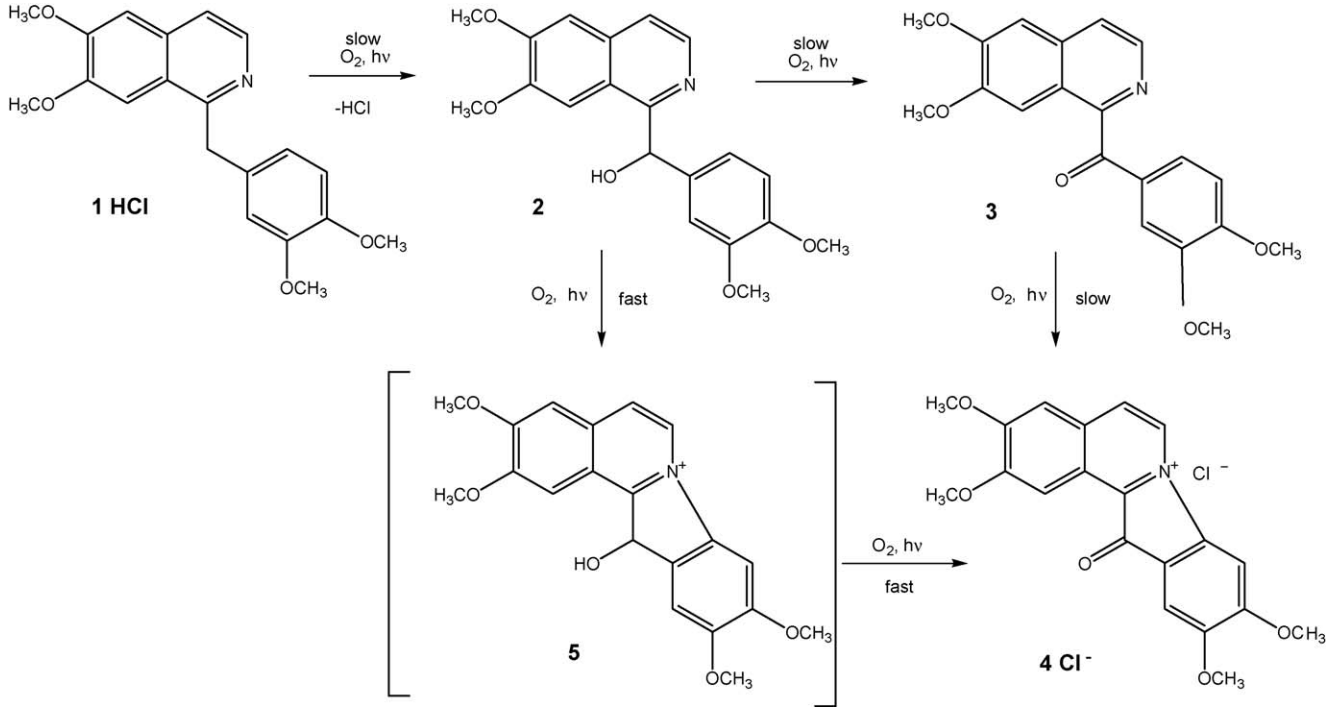
Substrate	Quantum yield, φ	Standard deviation, S.D.
Papaverine hydrochloride	0.28	0.06
Papaverinol	0.30	0.04
Papaveraldine hydrochloride	0.10	0.04

3.2. Kinetics of photooxidation reaction

Typical first-order plots have been generated for photooxidation reactions of papaverine hydrochloride and papaverinol chloroform solutions. However, that reaction for papaveraldine hydrochloride was the slowest of three compounds considered and followed the zero-order kinetics. Since the concentrations of the above analytes as a function of time obey the normal distribution, the least squares linear regression calculations were used to obtain the pseudo-first-order rate constants from Eq. (2) and pseudo zero-order rate constants from Eq. (3) as the absolute values of their negative slopes but taking into consideration sec as a SI unit of time (Table 3).

3.3. Quantum yield measurement

Intensity of light absorbed by the substrate ($I_a = 5.59 \pm 0.04 \times 10^{-4}\,mol\,l^{-1}\,min^{-1}$) was calculated from Eq. (1) taking into consideration the results produced on irradiation the uranyl oxalate actinometer. Quantum yields as a function of a substrate degree of conversion were calculated from Eq. (1) and next they were extrapolated to zero substrate degree of conversion to obtain specific quantum yields (Table 4).



Scheme 1. Pathways of a papaverine hydrochloride (**1** HCl) and its decomposition products (papaverinol **2** and papaveraldine hydrochloride **3**) chloroform solutions degradation on exposition to UV_{254} light in aerobic conditions and at ambient temperature where **4** HCl is the final product (compound X) and **5** is an intermediate postulated.

4. Discussion

An HPCE methodology for determination of aminopyridine derivatives in which papaverine was used as an internal standard [12] was modified and applied in our work for quantification of papaverine and its photooxidation products. The method is selective for papaverine hydrochloride and its either widely known degradation products (papaverinol, papaveraldine hydrochloride) or recently identified (2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinylium salt [8]), previously called a compound X [3]. The presence of any interference to the above analytes was not observed (Figs. 1 and 2). Plotting the sensitivity (response/amount) and correlation coefficients of calibration curves close to unity (0.9998–0.9999, Table 1) give clear indication of the linear range. The linear regression equation applied to the results has an intercept not significantly different from zero (Table 1). Precision of better than 4.7% is achieved for within-day assay and below 10% for between-days assays (Table 2).

The processes of photooxidation of papaverine hydrochloride and papaverinol chloroform solutions follow the first-order reaction kinetics (Eq. (2), Table 3). It confirms a thesis that in pharmaceutical systems, most reported photolysis has been first-order [13]. However, oxidation of papaverine hydrochloride aqueous solutions protected from light is a pseudo-second-order reaction [14]. In general the time frame in photolysis is different from that in usual kinetics [13].

Furthermore, photooxidation reaction of papaveraldine hydrochloride chloroform solutions is pseudo zero-order according to Eq. (3) (Table 3). The photooxidation of chlorpromazine is also an example of a zero-order process [13].

The most labile compound of evaluated ones is papaverinol whose half-life time is 2.4 times lower if compared to papaverine hydrochloride (Table 3). From the other side, papaveraldine hydrochloride is the most stable product whose half-life time is approximately 31 times greater than that one of papaverinol (Table 3). Those differences in photo-stability are approved by the results of partial and specific quantum yields calculated. The greatest specific quantum yield characterizes photooxidation of papaverinol ($\varphi = 0.30$) and the lowest one adheres to papaveraldine hydrochloride ($\varphi = 0.1$).

Our kinetic studies confirm the mechanism of papaverine hydrochloride photooxidation to the compound X proposed via papaverinol [8]. The reactivity of papaverinol to photooxigenation can be explained by the intermediate formation of **5**, arising from oxidation of the nitrogen atom with higher electron density in **2** compared to papaveraldine, but **5** might have only a very short lifetime under the reaction conditions [8] (Scheme 1). We are then entitled to prove previous chromatography and RP-LC/MS experiments [8] that the observed rate of formation of the compound X can be really given as **2** > **3** > **1** > **1** HCl (Scheme 1). Our kinetic experiments substantiate the fact that **2** is a suitable substrate to synthesize **4** chloride with the best yield [7].

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