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A study on the alkaline hydrolysis of isatin-β-thiosemicarbazone by capillary electrophoresis with enhanced sample loadability

Pavol Kruk[a], Henrieta Stankovičová[b], Róbert Bodor[a], Anton Gáplovský[b], Marián Masár[a,*]

[a] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina CH2, 842 15 Bratislava, Slovak Republic
[b] Institute of Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina CH2, 842 15 Bratislava, Slovak Republic

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ABSTRACT

An analytical potential of capillary zone electrophoresis (CZE) with enhanced sample loadability (a 200 nL injection volume) in determination of alkaline hydrolysis products of isatin-β-thiosemicarbazone (IBT), a compound with important biological activity, has been studied. The CZE separation conditions for a complete resolution of transformation products, i.e. 2-aminophenylglyoxalate, 2-(2-aminophenyl)-2-semicarbazonoethane, anthranilate and *E-Z* geometric isomers of 2-(2-aminophenyl)-2-thiosemicarbazonoethane, have been optimized. CZE separations with UV detection at 240 nm were performed using glycine running buffer at high pH (9.2) and containing an uncharged β-cyclodextrin as a complexing agent. High sensitivity (with detection limits ranging from 0.1 to 1.2 μM), good repeatability (RSD of migration times less than 0.4% and 0.4–3.4% RSD of peak areas) and linearity over two orders of magnitude were achieved for the compounds studied. The employed CZE method, characterized by simple sample handling (only dilution step needed) and total analysis time of less than 15 min, has been applied successfully to time monitoring of the transformation of IBT in alkaline media. Under optimized CZE conditions, the effect of pH of reaction media, implemented by different concentration of NaOH (0.1–100 mM), on the course of the alkaline hydrolysis of IBT was studied in this respect, as well.

1. Introduction

Isatin-β-thiosemicarbazone (IBT) belongs to the group of substituted indoles which are produced by chemical industry for a variety of applications, i.e. pharmaceuticals, pesticides, disinfectants and dyestuffs. Wide use of these chemicals contributes to the pollution of soil, ground and surface water [1]. Additionally, chemical disinfection of indole-containing waste waters with chlorine disinfectants can result in the formation of carcinogenic chlorinated aromatic products [2]. Various derivatives of IBT have been in interest of pharmaceutical industry since 1-methyl-IBT was found to be active in the treatment of smallpox about 50 years ago [3]. Although biological properties of IBTs such as antibacterial, antifungal, antiviral, anti-HIV, and anti-helminthic activities have been studied extensively over the past few years [4–6], chemical stability of these compounds in alkaline or acidic media is not reported yet.

Currently, typical schemes employed in real-time monitoring of the reaction mechanisms involve mainly non-separative spectrometric techniques, e.g. UV–vis spectrophotometry, MS, NMR and IR spectroscopy [7–10]. Even if routinely used, as simple and rapid analytical tools, these techniques have some restrictions, e.g. for the compounds with weak or slow detection response. These problems can grow with the complexity of the reaction mixture, e.g. resolution of overlapped spectra of the intermediates. Therefore, high performance separation methods, i.e. GC [11–13], HPLC [14,15] and CE [16–22], coupled with already mentioned spectrometric detection techniques have been used in the study of reaction mechanisms and paths of transformation/degradation of many environmentally or biologically important compounds, as well. The coupled techniques combine a high performance separation and quick resolving power of the separation methods with sensitive and identification facilities of spectrometric techniques.

In this work, capillary zone electrophoresis (CZE) with UV detection has been developed for the study of the alkaline hydrolysis of IBT. This, the most widely used mode of CE, is relatively simple and rapid analytical method for determination of ionogenic compounds. The use of CZE with a hydrodynamically closed separation system eliminates negative influences of a varying electroosmotic flow (EOF) on the migration velocities of the separated constituents [23]. Here, a negative impact of EOF on the peak broadening is eliminated, e.g. by its suppression via the use of suitable additives in the running buffer [24]. This CZE approach was already shown

* Corresponding author. Tel.: +421 260296379; fax: +421 260296706.
E-mail address: masar@fns.uniba.sk (M. Masár).

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to provide very reproducible migration velocities of the separated constituents present in complex matrices [23,25].

The narrow bore of the CE capillary (typically 20–75 μm I.D.) puts some limitations on detection techniques. Banks and Paquette [26] showed that UV detector combined with CE works with a path length which is typically at least two orders of magnitude shorter than that for standard spectrometers (1 cm path length). This corresponds to relatively high concentration limit of detection (LOD). The use of the wide bore capillary of 200 μm I.D. in CZE was first described and applied by Mikkers et al. [27]. However, currently such an approach is not used very often probably due to the fact that more attention must be paid to the thermal dispersive effects in contrary to narrow bore capillaries. CE separations in the capillaries of larger I.D.s are usually carried out with hydrodynamically closed separation system to prevent any movement of the solution in which the separation is performed.

In this work, we employed the capillary tube of a 300 μm I.D. to enhance the load capacity and also increase the path length for on-line UV detection. In this way, LOD for the analytes in sub-μM concentrations for the current photometric detection could be achieved [23].

2. Material and methods

2.1. Instrumentation

A 300 MHz Varian Gemini 2000 NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used for identification of synthesized standards of transformation products of IBT. Elemental analysis (C, H and N) was carried out with an Elemental Analyzer 1106 (Carlo Erba, Strumentazione, Italy). UV–vis spectra of synthesized standards were measured on a diode array spectrometer HP Agilent 8453 (Agilent Technologies) with a 1 cm path length quartz cell.

The CZE separations were carried out in the hydrodynamically closed separation system with suppressed EOF. An ItaChrom EA-101 CE analyzer (J&M, Aalen, Germany) with fluorinated ethylenepropylene copolymer capillary of 300 μm I.D. and 300 mm total length (200 mm to the detector) was used. The CE analyzer was operated in a laboratory room with controlled temperature of 24 ± 0.5 °C. The capillary was washed daily with 2% (v/v) aqueous solution of detergent Extran MA 03 (Merck, Darmstadt, Germany) and consequently with demineralized water. Between CE runs, capillary was only re-filled with fresh running buffer. No additional capillary washing steps were needed.

The CZE capillary was coupled to a Spectra 100 photometric detector (Spectra-Physics, Burnsville, USA) operating at 240 nm wavelength. Detection wavelength was optimized in the range from 210 nm through 260 nm with a 5 nm increment. The separation unit of the CE analyzer included an injection valve with a 200 nL sample loop, as well. A CE software WinAces, version 2.4 (J&M) provided a time-programmed control of the CZE runs, attained the detection data and provided their processing. The Savitzky–Golay method was used for data smoothing and evaluating the results.

2.2. Chemicals and reagents

The following standards of analytical grade were synthesized at the Institute of Chemistry, Faculty of Natural Sciences of Comenius University in Bratislava (Slovakia): potassium 2-aminophenylglyoxalate (isatinate; **I**), sodium 2-(2-aminophenyl)-2-semicarbazonoethane (**O**) and sodium 2-(2-aminophenyl)-2-thiosemicarbazonoethane (**S**) comprising two geometric isomers: 71% of *Z*-isomer (**S1**) and 29% of *E*-isomer (**S2**), respectively. Ratio of the isomers **S1** and **S2** was determined from ^{1}H NMR spectra measured in dimethylsulfoxide-d_6 with tetramethylsilane as an internal standard. Elemental analysis values found for carbon, hydrogen and nitrogen were within 0.4% of the calculated values for all synthesized standards. The homogeneity of the standards was determined from ^{1}H NMR and ^{13}C NMR spectra. Antranilic acid (**A**) of analytical grade was purchased from Merck. Stock solutions of these analytes were prepared at 100–200 μM concentrations and stored in refrigerator at +4 °C. Before CZE experiments, model samples were appropriately diluted in a 1 mM aqueous solution of sodium sulfate (Merck, Darmstadt, Germany).

Analytical grade chemicals used for the preparation of running buffers, i.e. 2-aminoacetic acid (glycine) and 1,3-bis(tris(hydroxymethyl)methylamino)propane (bis–tris propane) were bought from Sigma–Aldrich (St. Louis, MO, USA) and Merck. Methylhydroxyethylcellulose 30 000 (MHEC; Serva, Heidelberg, Germany) was used as an EOF suppressor. An aqueous concentrate of this additive prepared at a 1% (w/v) concentration was purified on a mixed-bed ion exchanger Amberlite MB-1 (Merck). It was added to the running buffers at a 0.2% (v/v) concentration and dynamically coated the inner wall of the capillary [24]. No other coating procedure was applied. The concentrations of running buffers constituents employed were 40 mM glycine, 20 mM bis–tris propane, 0.2% MHEC, at pH 9.2 and with addition of 25–100 mM α-cyclodextrin or 8–14 mM β-cyclodextrin.

Water demineralized by a circulation in a Simplicity deionization unit (Millipore, Molsheim, France), was used for the preparation of the electrolyte and sample solutions. The running buffer solutions were prepared from freshly recirculated water and filtered through a 0.8 μm membrane filter (Advantec MFS, California, USA).

2.3. Sample preparations

Preparation of the sample for time monitoring of alkaline hydrolysis of IBT: a solution (170 mL) of IBT with a 0.1 mM concentration in demineralized water and a solution (5 mL) of NaOH with a 35 mM concentration in demineralized water were mixed and the mixture was stirred at laboratory temperature (+20 °C). A 900 μL volume of the reaction mixture was collected into a polypropylene tube at different time after the beginning of the reaction (1 h, 1 day, 1 week, 1 and 2.5 months) and immediately analyzed by CZE after its dilution (9:10) in 1 mM sodium sulfate. No other sample handling or pre-treatment was needed before the analysis.

Preparation of the sample for a study of pH effect on alkaline hydrolysis of IBT: a solution (2.5 mL) of IBT with a 0.2 mM concentration in demineralized water and a solution (2.5 mL) of NaOH in demineralized water with different concentrations (200, 20, 2, 0.6, 0.4, and 0.2 mM) were mixed and the mixture was stirred at laboratory temperature (+20 °C). The mixtures were handled in the same way as previously and analyzed in 5 days from the beginning of the reaction.

3. Results and discussion

Alkaline hydrolysis of IBT seems to be a multistep process. It can comprise two alternative routes for the formation of final product **A**. The mechanistic pathway of the studied reaction is shown in Fig. 1. Isatin and thiosemicarbazide should be formed as products of hydrolysis of IBT. However isatin is unstable in alkaline media, it undergoes indole ring opening to **I** followed by its possible decomposition to **A** [28,29]. An alternative route of alkaline hydrolysis involves IBT indole ring opening resulting in formation of the compound **S**. This intermediate can hydrolyze on C=N bond to form **I** or undergoes nucleophilic exchange of C=S to C=O with subsequent

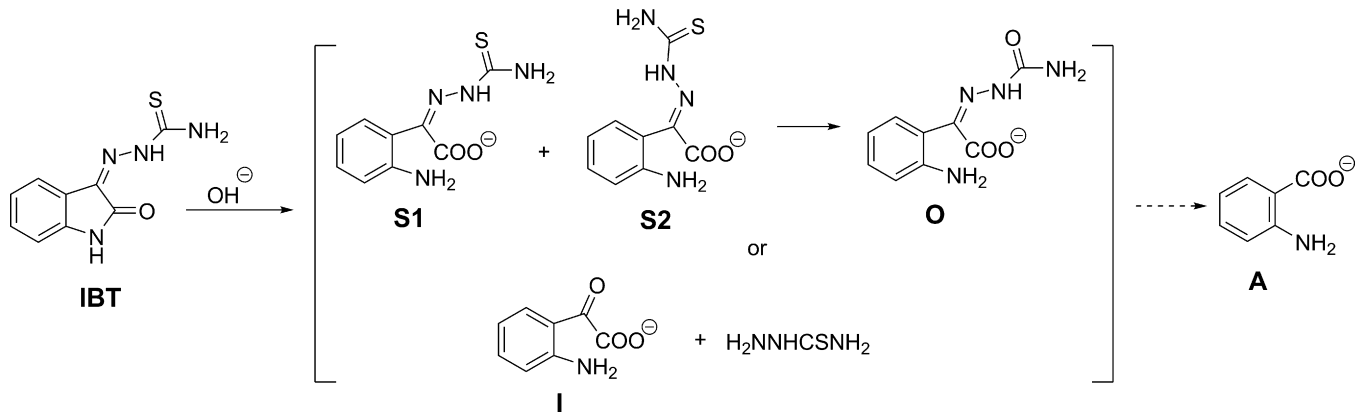

Fig. 1. Proposed pathway of alkaline hydrolysis of IBT. For compound abbreviations, see Section 2.2.

hydrolysis on C=N bond, resulting also in formation of **I**. The aim of this study was to contribute to the clarification of the above process.

UV–vis spectrometry could be suitable for monitoring this transformation process. However, due to the overlapped absorption spectra of the transformation products of alkaline hydrolysis of IBT (see in Fig. 2) it is rather difficult to evaluate the formation of the intermediates including also current chemometrics. Following the scheme proposed in Fig. 1, ionogenic nature of the intermediates and products of alkaline hydrolysis of IBT makes high efficiency CZE potential analytical alternative for their determination. Therefore the CZE separation conditions for the quantitative analysis were optimized in this respect.

3.1. Optimized CZE separation conditions

Considering the relatively high p*K* values of the intermediates **S** and **O**, resulting from their structures with several NH groups, CZE separations were carried out in the anionic mode using a glycine running buffer at high pH (9.2). An electropherogram in Fig. 3a was obtained under such acid–base separating conditions and composition of the running buffer is shown in legend of the figure. The use of high pH of the running buffer led to the baseline resolution of the anions of week acids (**S** and **O**) while anions of stronger acids (**I** and **A**) migrated with identical effective mobilities in one peak (Fig. 3a). A high-molecular water soluble polymer, MHEC, present in the running buffer at a 0.2% (w/v) concentration [24], effectively suppressed EOF. CZE separations were monitored by UV detector and detection wavelength was optimized in the range 210–260 nm with a 5 nm increment and set at 240 nm.

To reach a complete resolution of the compounds studied, two types of native cyclodextrins found to have significant effect on resolution of the aromatic ionogenic compounds with different hydrophobicity and/or spatial arrangement of the atoms in molecule (host–guest complexation), e.g. derivatives of **A** [30] were investigated. Electropherogram in Fig. 3b was obtained from CZE separation of the studied analytes in presence of α-cyclodextrin. Although this complexing agent was not sufficient for resolution of **A** and **I** within the tested concentration range (25–100 mM), it allowed to resolve geometric isomers **S1** and **S2**. A complete resolution of the studied group of analytes was obtained by using an uncharged β-cyclodextrin, having one more glucose unit in the ring than α-cyclodextrin. This complexing agent selectively complexed **A** and **I**, and enabled their baseline resolution when added to the glycine running buffer at a 10 mM concentration or higher. The best resolution of these compounds was found with a 14 mM concentration of β-cyclodextrin, and at the same time, resolution of the geometric isomers **S1** and **S2** was retained (Fig. 3c). A 1 mM sodium sulfate added to the model samples was found to be very effective

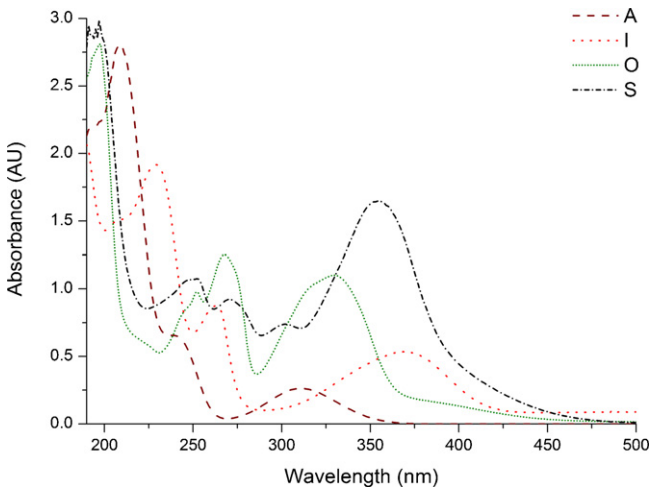

Fig. 2. Absorption spectra of the compounds studied. The model samples contained the analytes, each at a 100 μM concentration. For compound abbreviations, see Section 2.2.

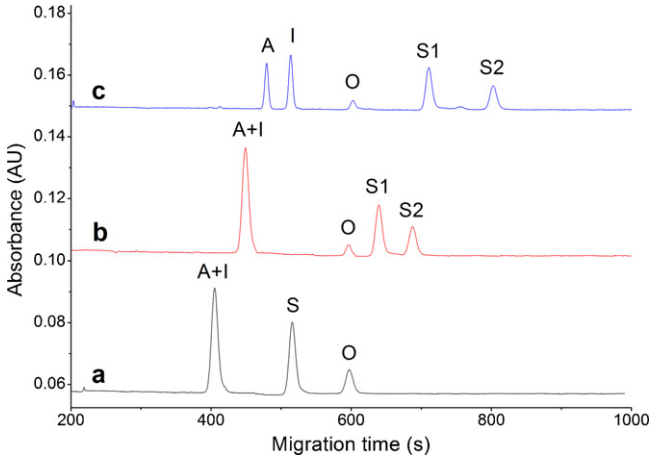

Fig. 3. CZE separations of model compounds under different separation conditions employed CZE separations carried out in the running buffer consisting of (a) 40 mM glycine, 20 mM bis–tris propane, 0.2% MHEC, and with addition of (b) 50 mM α-cyclodextrin and (c) 14 mM β-cyclodextrin (pH 9.2). Driving current stabilized at 70 μA and data acquired at 240 nm. Model sample contained 20 μM **A**, 10 μM **I**, 15 μM **O** and 20 μM **S** (corresponding to 14.2 and 5.8 μM **S1** and **S2**, respectively). The sample loaded in 1 mM sodium sulfate. For compound abbreviations, see Section 2.2.

Table 1
Repeatability data for model compounds.

Parameter	A	I	O	S1	S2
Average migration time (s)	480	515	603	711	805
RSD (%) of migration time	0.3	0.2	0.2	0.2	0.1
Average peak area (mV s)	1882	2362	475	3021	1758
RSD (%) of peak area	0.4	0.6	2.1	2.2	3.4

The intraday repeatabilities evaluated from 6 repeated CZE runs. The concentrations of the compounds in the model sample were as follow: 10 μM **A**, 5 μM **I**, 7.5 μM **O** and 7.1 μM **S1** and 2.9 μM **S2**, respectively. For compound abbreviations, see Section 2.2.

in suppressing the adsorption of the separated constituents on the surface of the CZE capillary [23].

3.2. Method performance parameters

As mentioned above, the CZE separations were carried out in hydrodynamically closed separation system with suppressed EOF. These working conditions minimize the fluctuations in the migration velocities of the separated constituents and, generally, allow achieving high repeatabilities of qualitative and quantitative CZE parameters [23]. The intraday repeatabilities of the migration times for the compounds studied were characterized by RSD of 0.1–0.3% while RSD of the corresponding peak area data ranged from 0.4 to 3.4% (Table 1).

For each of the compounds studied, the LOD has been calculated by using two independent methods [31] based on: (i) 3.3 times of the SD of the detector response (a blank run) and the slope of the calibration line calculated from the peak height and (ii) 3 times of the S/N of the baseline close to the analyte peak. Elaborated CZE method allowed detection of the analytes at trace concentration levels, as summarized in Table 2, using a 200 nL sample loop. In addition, both methods resulted in a good agreement. Linearity over two orders of magnitude of concentration was generally obtained with model samples in a series of CZE calibration measurements. Parameters of the regression equations for the calibration graphs used in the quantitation of the compounds studied are given in Table 2. The obtained values of the correlation coefficients show that the analytes can be reliably quantified in the concentration intervals in which the calibration lines were constructed.

3.3. Determination of transformation products

CZE monitoring of alkaline hydrolysis of IBT was performed in the reaction mixture consisting of 100 μM IBT and 1 mM NaOH at pH 11. For each CZE run, a 900 μL volume of the reaction mixture was taken and the sample collected was diluted 9:10 in 1 mM sodium sulfate and directly analyzed without further pretreatment (see Section 2.2). The electropherograms in Fig. 4 were obtained from the CZE analyses of transformation products of IBT at different time intervals. Corresponding concentration data are summarized

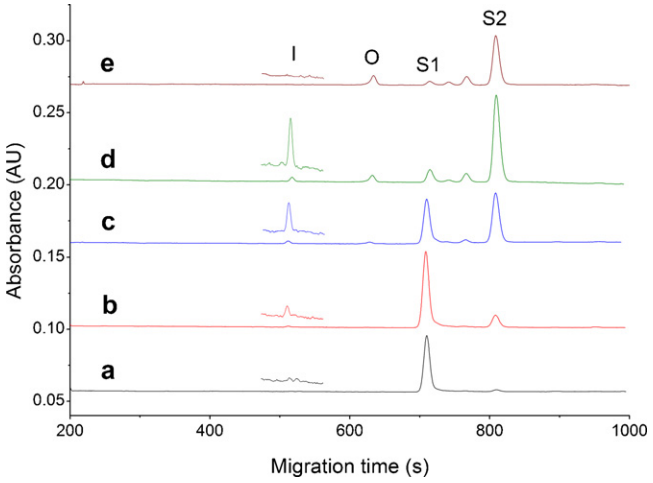

Fig. 4. CZE analyses of reaction mixture consisting of 100 μM IBT and 1 mM NaOH at different reaction times. Separations carried out in (a) 1 h; (b) 1 day; (c) 1 week; (d) 1 month and (e) 2.5 months from the beginning of the reaction in the running buffer as described in the legend of Fig. 3c. Driving current stabilized at 70 μA and data acquired at 240 nm. Each sample diluted 9:10 in 1 mM sodium sulfate. For compound abbreviations, see Section 2.2.

Table 3
Concentrations of transformation products of 100 μM IBT in 1 mM NaOH at different reaction times.

Reaction time	Concentration found (μM)			
	I	**O**	**S1**	**S2**
1 h	0.3	–	43.9	1.2
1 day	0.6	–	57.0	6.8
1 week	1.1	8.0	36.4	26.7
1 month	1.7	26.8	7.5	46.9
2.5 months	–	41.9	1.6	36.0

For compound abbreviations, see Section 2.2.

in Table 3. It can be seen from these data that already within the first hour of the reaction isomer **S1** is formed (Fig. 4a). With increasing reaction time, **S1** is gradually isomerized to **S2** and approximately one week from the beginning of the reaction the concentrations of both isomers become equal (Fig. 4c). At the same time, nucleophilic exchange on C=S bond takes place to form compound **O**. Its concentration is increased with a gradual isomerization of **S1**–**S2** (Fig. 4c–e). Parallel process to the isomerization is alkaline hydrolysis on C=N bond of **S1** resulting in formation of compound **I** (Fig. 4b–d). In about 2.5 months from the beginning of the reaction, the concentration of **S1** was negligible and compound **I** completely disappeared. These facts invoke conclusion that in alkaline media IBT hydrolyses to **S1**, subsequently isomerizes to **S2** and partially hydrolyses to **I**. In parallel, isomer **S2** is decomposed to **O**. Compound **A**, a final product of the transformation reaction, was not observed throughout the study period of 4 months. It must be

Table 2
Calibration parameters and LODs for the compounds studied.

Parameter	A	I	O	S1	S2
Slope (mV s/μM)	195.2	502.2	69.6	414.6	660.3
Intercept (mV s)	−200.9	−98.2	−87.8	664.4	−245.9
Correlation coefficient	0.998	0.999	0.998	0.997	0.999
Calibration range (μM)	1–80	0.3–40	2–60	1.4–57	0.6–23
Number of data points	16	16	16	16	16
LOD[a] (μM)	0.2	0.1	0.7	0.2	0.1
LOD[b] (μM)	0.3	0.1	1.2	0.2	0.2

For compound abbreviations, see Section 2.2.
[a] LOD calculated by method based on the SD of the response and the slope.
[b] LOD calculated by method based on S/N.

Table 4
Repeatability and recovery data for transformation products of 100 μM IBT in 1 mM NaOH.

Parameter	O	S1	S2
Average migration time (s)	623	703	801
RSD (%) of migration time	0.4	0.4	0.4
Average peak area (mV s)	2706	907	12189
RSD (%) of peak area	2.1	2.8	2.3
Concentration found ± SD (μM) in the sample	44.6 ± 0.9	0.7 ± 0.1	20.9 ± 0.5
Recovery (%)	97.5	98.4	92.4

CZE analyses performed in 4 months from the beginning of the reaction. The intraday repeatabilities evaluated from 7 repeated CZE runs. The mean recoveries obtained as the average of 2 repeated CZE analyses of the sample spiked with 20 μM **O** and 10 μM **S** (corresponding to 7.1 and 2.9 μM **S1** and **S2**, respectively). For compound abbreviations, see Section 2.2.

emphasized that other ionogenic compounds appeared in the electropherograms in Fig. 4c–e (peaks in between **S1** and **S2**). However, these constituents have not been identified yet due to the lack of reference standards. These compounds are probably charged intermediates which cannot be isolated or trapped from reaction mixture.

Repeatabilities of the migration times and peak areas for the transformation products of 100 μM IBT in 1 mM NaOH in about 4 months from the beginning of the reaction are given in Table 4. Evaluated data for the compounds **S1**, **S2** and **O** in the reaction mixture are reproduced as well as those obtained for model samples (compare data in Tables 1 and 4). Small differences in their migration times (less than 3%) can be ascribed to the differences in their concentrations in model and reaction mixture samples. The satisfactory recoveries in the range 92–98% (Table 4) as obtained for the compounds studied in the same reaction mixture indicate a good predisposition of the proposed CZE method to provide accurate analytical results.

3.4. Effect of pH

The hydrolysis of isatin and its derivatives show a complex dependence upon pH indicative of subtle changes in mechanisms and rate-limiting step [29]. Therefore, it is possible to assume that the course of the alkaline hydrolysis of IBT also strongly depends on the pH. We used alkaline media with high pH to ensure the studied compound undergoes catalyzed nucleophilic hydrolysis and not solvolysis. Actual concentrations of the transformation products were determined by the employed CZE method in the pH range 10–13. Different pH of the reaction media was implemented by different concentration of NaOH in the reaction mixture. Particular pH values of the reaction mixture (10.0, 10.3, 10.5, 11.0, 12.0 and 12.9) correspond to 0.1, 0.2, 0.3, 1, 10 and 100 mM concentrations of NaOH in the reaction mixture, respectively. The plot in Fig. 5 shows a pH dependence of percentage relative concentration for transformation products calculated as a ratio of intermediate concentration to initial concentration of IBT. The CZE analyses of the reaction mixtures were performed in about 5 days from the beginning of the reaction in order to reach higher concentrations of the compounds produced in the reaction mixture.

As shown in Fig. 5, the relative concentration of the compound **I** in the reaction mixture is very low, although at the highest pH tested (12.9) is approximately 5-times higher than that obtained for lower pH (10.0–10.3). The percentage content and the ratio of isomers **S1** and **S2** increased gradually in the pH range 10–12 of the reaction media and then at the highest pH studied decreased rapidly. On the other hand, the relative concentration of compound **O** in the reaction mixture increased very significantly with increasing pH and over the whole pH interval tested. Especially, at the highest pH the concentration of **O** in the reaction mixture

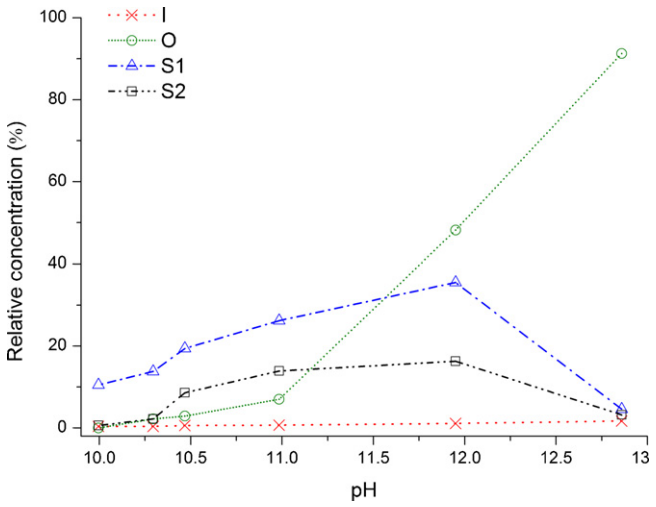

Fig. 5. pH dependence of percentage relative concentration for transformation products of alkaline hydrolysis of IBT. Reaction mixtures consisted of 100 μM IBT and 0.1–100 mM NaOH. The CZE analyses performed in 5 days from the beginning of the reaction. For compound abbreviations, see Section 2.2.

was almost the same as the initial concentration of IBT. Therefore, we can conclude that alkaline hydrolysis of IBT, strongly dependent on the pH, resulted in formation of derivative **O** at the highest pH tested. Formation of proposed final product **A** was not observed.

3.5. Kinetics

A kinetic study on basic hydrolysis of IBT present in the reaction mixture at 100 μM concentration in alkaline media (1 mM NaOH) was performed. Kinetic experiments were measured until the limiting equilibrium concentration was reached (ca. 4 months). As mentioned above, UV–vis spectrometry cannot be used for determining hydrolysis intermediates concentrations with sufficient precision because of overlapped UV–vis spectra of reaction intermediates (Fig. 2). On the other hand, a decrease in absorbance due to decreasing concentration of IBT with time was observed at 285 nm wavelength from UV–vis spectra. In this way, the rate of basic hydrolysis was determined and found to be $1.102 \pm 0.094\ h^{-1}$ with correlation coefficient of 0.998.

As stated above, the basic hydrolysis of IBT is very sensitive to pH (see Fig. 5) due to presence of three electron deficient sites in the molecules (C=S, C=O and C=N) which are suitable for nucleophilic attack. Thus, this transformation is a complex process and proceeds through steps which may be competitive, parallel or consecutive (Figs. 5 and 6). Reaction time dependent changes of ionogenic intermediates concentrations are shown in Fig. 6.

As seen, the change in concentration of individual intermediate with time does not necessarily represent its formation only by one individual reaction process. Therefore, the concentrations of intermediates studied are affected by all processes undergoing the reaction system at the same time. However, the initial rates of formation of each of the intermediates could be determined. They found to be 4.248, 0.302, 0.032 and 0.101 $mM\ h^{-1}$ for compounds **S1**, **S2**, **I** and **O**, respectively. Even if only some kinetic characteristics of alkaline hydrolysis of IBT could be obtained, the employed CZE method provides a new perspective to complex evaluation and clarification of reaction path of IBT transformation. In addition, this approach enables to describe individual reaction steps with improved reliability.

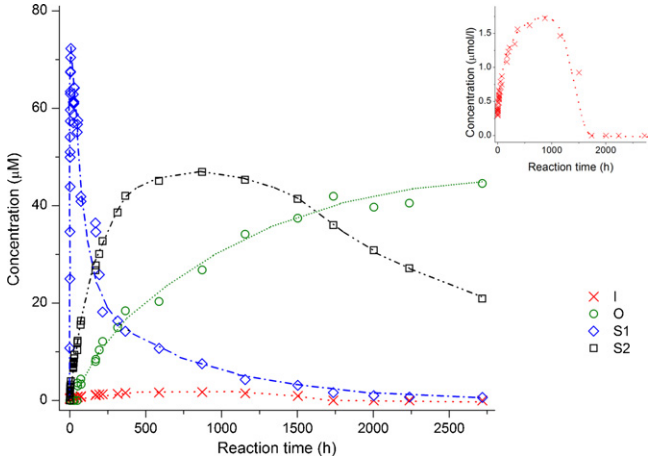

Fig. 6. Reaction time dependent changes of concentrations of ionogenic intermediates in reaction mixture consisting of 100 μM IBT and 1 mM NaOH. For compound abbreviations, see Section 2.2.

4. Conclusion

The present study described a new CZE method with enhanced sample loadability (200 nL sample volume) and UV detection for fast monitoring of the products of alkaline hydrolysis of IBT. The employed CZE method was very useful to confirm the reaction paths of the hydrolysis and determine actual concentrations of the transformation products in the reaction mixture at different time intervals. The use of a 300 μm path length (I.D. of the CZE capillary) of the UV spectrometer working at 240 nm wavelength together with large injection volume applied was very effective in reaching sub-μM detectabilities for the studied compounds. CZE experiments with different concentration of NaOH in the reaction mixture confirmed strong dependence of the course of alkaline hydrolysis of IBT on the pH of the reaction media. Furthermore, considering the overall time of the CZE analyses in about 15 min including also sample pretreatment (only dilution step needed), the proposed CZE method is a good analytical tool for studying the kinetics of this process. Generally, this approach could be used for real-time monitoring of the mechanism and kinetic characteristics of ionogenic intermediate products for different chemical processes.

Acknowledgments

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