analytica chimica acta xxx (2007) xxx–xxx

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/aca

Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms

Kateřina Mervartová[a,*], *Miroslav Polášek*[a], *José Martínez Calatayud*[b]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-500 05 Hradec Králové, Czech Republic*

[b] *Department of Analytical Chemistry, Faculty of Chemistry, University of Valencia, Valencia, Spain*

ARTICLE INFO

Article history:
Received 19 October 2006
Received in revised form
3 January 2007
Accepted 19 January 2007

Keywords:
Indomethacin
Sequential injection analysis
Chemiluminescence
Tris(bipyridyl)ruthenium complex
Pharmaceuticals
Semisolid dosage forms

ABSTRACT

Automated sequential injection (SIA) method for chemiluminescence (CL) determination of nonsteroidal anti-inflammatory drug indomethacin (**I**) was devised. The CL radiation was emitted in the reaction of **I** (dissolved in aqueous 50% v/v ethanol) with intermediate reagent tris(2,2′-bipyridyl)ruthenium(III) ($Ru(bipy)_3^{3+}$) in the presence of acetate. The $Ru(bipy)_3^{3+}$ was generated on-line in the SIA system by the oxidation of 0.5 mM tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bipy)_3^{2+}$) with Ce(IV) ammonium sulphate in diluted sulphuric acid. The optimum sequence, concentrations, and aspirated volumes of reactant zones were: 15 mM Ce(IV) in 50 mM sulphuric acid 41 μL, 0.5 mM $Ru(bipy)_3^{2+}$ 30 μL, 0.4 M Na acetate 16 μL and **I** sample 15 μL; the flow rates were 60 μL s^{-1} for the aspiration into the holding coil and 100 μL s^{-1} for detection. Calibration curve relating the intensity of CL (peak height of the transient CL signal) to concentration of **I** was curvilinear (second order polynomial) for 0.1–50 μM **I** ($r = 0.9997$; $n = 9$) with rectilinear section in the range 0.1–10 μM **I** ($r = 0.9995$; $n = 5$). The limit of detection (3σ) was 0.05 μM **I**. Repeatability of peak heights (R.S.D., $n = 10$) ranged between 2.4% (0.5 μM **I**) and 2.0% (7 μM **I**). Sample throughput was 180 h^{-1}. The method was applied to determination of 1 to 5% of **I** in semisolid dosage forms (gels and ointments). The results compared well with those of UV spectrophotometric method.

1. Introduction

Indomethacin (1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid) is a nonsteroidal anti-inflammatory drug exhibiting analgesic, anti-inflammatory and antipyretic effects. Its chemical structure is depicted in Fig. 1. Indomethacin gels and ointments are broadly used for calming down acute joint and backbone pain and for the treatment of degenerative diseases of joints and ligaments such as rheumatoid arthritis, osteoarthritis and ankylotic spondylitis; on the other hand oral administration of indomethacin may cause severe side effects including irritation of stomach [1,2].

The British Pharmacopoeia [3] uses a titration method for the determination of indomethacin which is time consuming and impractical for routine analyse.

A number of papers have been published concerning determination of indomethacin by various analytical methods in different matrices of pharmaceutical or biomedical

* *Corresponding author*. Tel.: +420 495067391; fax: +420 495067164.
E-mail address: Katerina.Mervartova@faf.cuni.cz (K. Mervartová).

0003-2670/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2007.01.057

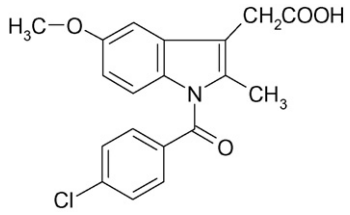

Fig. 1 – Structural formula of indomethacin.

interest. Among them separation methods, namely liquid chromatography [4–14] and capillary zone electrophoresis [15,16] using UV spectrophotometric [3–11,15,16] or mass spectrometric detection [13,14] with limit of quantification (LOQ) of 1.1 nM indomethacin attained the leading position. Recently indomethacin was determined in injections by derivative UV spectrophotometry [17] and by spectrophotometry in visual region with use of molybdate as colour reagent [18]. SIA with fluorescence detection based on alkaline hydrolysis of indomethacin (leading to formation of fluorescing products) was used for its assay in pharmaceutical formulations [19] including automated drug release studies [20]. Adsorptive stripping voltammetry [21–24] with LOD values approaching 0.7 nM of indomethacin and room-temperature phosphorimetry on a poly(vinyl alcohol) substrate [25] are among other methods devised previously for the determination of this drug.

To our best knowledge only one chemiluminescence (CL) method for the determination of indomethacin was published so far [26]. This flow injection – CL method uses non-selective CL reaction of indomethacin with soluble Mn(IV) species and formaldehyde for the assay of >40 ng mL^{-1} (≈0.1 μM) of indomethacin in urine; the interferences were overcome by using selective absorption and pre-concentration of the analyte in a column packed with appropriate molecular imprinted polymer. A single assay (1 injection) took almost 3.5 min.

Several well established CL reaction systems of analytical relevance [27,28] (based, e.g., on luminol, lucigenin, lophine, peroxyoxalate, sulphite, Ce(IV), Mn(VII) and Ru(III) complexes) have been extensively utilized in practice especially in connection with flow methods (FIA, SIA) in the recent decade. The $Ru(bipy)_3^{3+}$ complex can oxidize oxalate or some nitrogenous organic compounds while being reduced to excited $[Ru(bipy)_3^{2+}]^*$ species emanating visible light with maximum emission at 610 nm [29]. The $Ru(bipy)_3^{3+}$ reagent is relatively unstable molecule which needs to be prepared in situ from $Ru(bipy)_3^{2+}$ by chemical, photochemical or electrochemical oxidation. Chemical oxidation of $Ru(bipy)_3^{2+}$ is accomplished for example with Ce(IV) [30–32] or with permanganate [33,34] in acidic medium. Many important drugs contain nitrogen atoms in their molecules; hence the $Ru(bipy)_3^{3+}$ has high potential to become useful reagent for their CL determination. Chemiluminescence of some indole derivatives (indole, indole-3-acetic acid, tryptophan, psilocin and psilocybin) in the reaction with $Ru(bipy)_3^{3+}$ was described previously [35–37].

Automated flow methods such as FIA and especially SIA proved to be powerful tools in solving practical problems occurring in the field of quality assurance of pharmaceuticals [38,39]. One of the advantages of SIA is feasibility of on-line preparation of unstable reagents and at the same time substantial reduction of waste generation as well as minimizing the consumption of sample and reagents.

In our preliminary experiments we used the SIA-CL technique for screening a number of drugs (bulk substances) containing nitrogen atoms in their molecules as potential analytes possibly giving rise to chemiluminescence radiation in the reaction with $Ru(bipy)_3^{3+}$ generated via chemical oxidation of $Ru(bipy)_3^{2+}$ by Ce(IV) directly in the SIA system. Indomethacin showed promising CL response usable for its sensitive SIA-CL determination. The present work is dealing with the development and validation of automated rapid and sensitive SIA-CL method suitable for the assay of indomethacin in semisolid dosage forms (gels and ointments) involving evaluation of its accuracy by comparison with reference UV spectrophotometric method [17].

2. Experimental

2.1. Apparatus

The SIA system was built from CAVRO XL 3000 piston pump (volume 2.5 mL Cavro Scientific Instruments Inc., USA), Vici Valco ten-port selection valve with electrical actuator (Valco Instruments Co., Inc., USA), model FS 970 Schoeffel Instrument Corp. (USA) fluorimetric flow detector (UIV lamp switched off) equipped with a lab-made CL module with spiral geometry; the photomultiplier tube voltage was 320 V. The SIA system involved a holding coil (length 70 cm, i.d. 0.75 mm, PTFE tubing, volume 1.2 mL) and a PTFE tubing (i.d. 0.75 mm). The same tubing was spirally coiled on a 52 mm × 52 mm Perspex plate, which substituted the secondary filter in the fluorimeter; this CL module had a central inlet, peripheral outlet and the diameter of the spiral was 24 mm. The control unit consisted of Pentium 75 MHz PC equipped with additional AT-MIO-16E10 data acquisition and AT-232/4 interface cards (National Instruments Corporation, USA). The whole system was operated by software (FaFSIA, Version 1.2); advanced data calculation and analysis were performed by LPMcalc, Version 1.0 (lab-made programs written in LabVIEW®). The optimization was carried out by our own simplex MS DOS software. Schematic view of the employed SIA manifold is shown in Fig. 2.

The solution stability monitoring and UV spectrophotometry was performed on Agilent UV–vis photodiode array spectrophotometer (Model HP 8453). The CL emission spectra were measured by Shimadzu RF-1501 spectropfluorophotometer.

2.2. Reagents

All solutions were prepared from analytical grade chemicals and a Millipore Milli-Q RG ultra pure water. Standard of indomethacin was obtained from Jiangsu Taicang, China and stored in refrigerator. Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate, acetonitrile, cetyltrimethylammonium bromide (CTAB), sodium dodecylsulphate (SDS), triton X-100 and methanol were purchased from Sigma–Aldrich, Chemie GmbH (Germany). Cerium(IV)-ammonium sulphate was purchased from Fluka (Switzerland). Potassium dihydrogen phosphate and sodium hydrogen phosphate were from Merck (Germany). Acetone, sulphuric acid, potassium permanganate, sodium

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

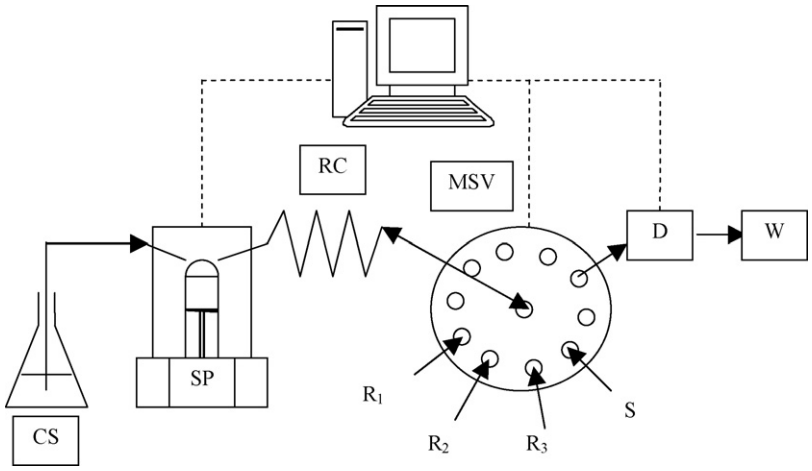

Fig. 2 – Schematic view of SIA manifold: CS, carrier stream; SP, syringe pump; RC, holding coil; MSV, multi-position selection valve; D, detector; W, waste; R_1, Ce(IV) in sulphuric acid; R_2, $Ru(bipy)_3^{2+}$ complex; R_3, buffer; S, indomethacin sample in 50% ethanol.

hydroxide, acetic acid, sodium acetate, orthophosphoric acid, ethanol, hydrochloric acid and boric acid were obtained from Lachema (Czech Republic). Indomethacin was determined in Indobene gel (Merckle GmbH, Germany) and in Vonum cutan ointment (Pharmaceutica GmbH, Germany).

Stock solution of 0.1 mM indomethacin was prepared by dissolving appropriate amount of the substance in ethanol; it was stored at 4 °C. This solution was stable for at least 2 days as found by measuring its UV spectrum. Working solutions were prepared by diluting the stock solution with aqueous 50% (v/v) ethanol.

The buffers used in the experiments were 0.2 M acetate buffer (pH 5–5.6), 0.067 M phosphate buffer (pH 6–8) and Britton–Robinson buffer (pH 2–10).

2.3. SIA procedure

Appropriate volumes of indomethacin test solution or blank, solution of $Ru(bipy)_3^{2+}$, buffer and Ce(IV) in sulphuric acid were automatically aspirated in optimum order into the holding coil, the zones were mixed by flow reversal and the arising CL product was pushed into the detector cell. The transient CL signal was recorded in the form of peaks and processed by our original software written in LabVIEW (involving the peak height calculations in nA). Each measuring cycle was carried out five times at ambient temperature and the mean values of the peak heights and their standard deviations were computed automatically. The net CL signal was calculated by subtracting the mean blank signal from the mean raw signal value where appropriate.

2.4. Optimization

Results of preliminary experiments indicated that the optimum order of the reactant zones aspirated into the holding coil was: (i) Ce(IV) sulphate in sulphuric acid, (ii) aqueous $Ru(bipy)_3^{2+}$, (iii) buffer solution, and (iv) test solution of indomethacin. This order was applied in all subsequent experiments.

2.4.1. Chemical parameters

Initially the effect of the concentration of the individual reactants on the net CL response of 15 μL of 1 μM indomethacin in aqueous 25% (v/v) ethanol was examined by the univariate technique (the concentration of the reactant examined was changed while the other concentrations were fixed). Flow rate of aspirating the zones of the reactants into the holding coil (FRa) was 25 $\mu L\,s^{-1}$ and the flow rate of aspirating the zone of emitting species into the detector (FRd) was 100 $\mu L\,s^{-1}$.

The aspirated volume of the Ce(IV) sulphate in sulphuric acid zone was 70 μL and the concentrations were varied in the range 5–20 mM Ce(IV) and 10–100 mM H_2SO_4. The aspirated volume of the $Ru(bipy)_3^{2+}$ zone was 30 μL and the concentration of $Ru(bipy)_3^{2+}$ changed in the range 0.1 to 5 mmol L^{-1}. The volume of the buffer solution aspirated was 30 μL and the pH varied between 2 and 10 for the Britton–Robinson universal buffer, pH 6–8 for 0.067 M phosphate buffer and pH 5.0–5.6 for 0.2 M acetate buffer; 0.1–0.5 M sodium acetate was also used instead of the acetate buffer.

The effect of the content of 5–15% (v/v) of acetone, 5–15% (v/v) of acetonitrile, 25–100% (v/v) of methanol and 25–75% (v/v) of ethanol in the injected 1 μM indomethacin solution on the net CL intensity was tested at optimized Ce(IV), sulphuric acid, $Ru(bipy)_3^{2+}$ and Na acetate concentrations. In addition possible enhancement of the CL intensity by selected surfactants was examined by treating the injected 1 μM indomethacin solution in 20% (v/v) ethanol with appropriate surfactant to contain 0.02 to 0.21% of Triton X-100, 5 to 80 mM sodium dodecylsulphate or 1 to 10 mM cetyltrimethylammonium bromide.

2.4.2. SIMPLEX optimization of flow parameters

With optimized concentrations of the reactants the volumes of the reagents and the flow rates FRa and FRd were optimized simultaneously by the multi-variate computer-aided SIMPLEX method using a MS DOS software devised in our laboratory. The sample was 15 μL of 1 μM indomethacin in 50% (v/v) ethanol; its volume was maintained constant during the whole SIMPLEX process. The lower and upper limits of FRa and

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

FRd were 25 μL s^{-1} and 150 μL s^{-1} and the aspirated volumes of the reagent zones were optimized within the range 5 μL to 100 μL.

2.5. Calibration curve, LOD, repeatability, robustness

Calibration was performed under the optimum conditions with nine standard solutions of indomethacin covering the concentration range 0.1–50 μmol L^{-1}. For each standard solution five replicate injections were carried out. The calibration data were evaluated by non-linear curve fitting and by linear regression analysis with use of SlideWrite V6 software.

The LOD was calculated by the 3S/N method as the concentration of indomethacin giving rise to CL signal exceeding three times the standard deviation of the blank signal.

The repeatability of the CL peak heights was estimated by performing 10 replicate injections of two standard solutions containing 0.5 μmol L^{-1} and 7 μmol L^{-1} of indomethacin; the relative standard deviation (R.S.D.) of the mean peak height was calculated for either concentration of indomethacin.

The robustness of the method was estimated by examining the effect of varying the concentrations of Ru(bipy)$_3^{2+}$, Ce(IV) and sulphuric acid, and Na acetate and their aspirated volumes as well as FRa and FRd within ±10% around their final optimized values.

2.6. Analysis of pharmaceutical formulations

2.6.1. Procedure for the assay of indomethacin in gel or ointment by the SIA-CL method

Two commercially available semisolid dosage forms were analysed, namely Indobene gel and Vonum cutan ointment. Approximately 0.25 g of a gel or ointment sample was weighed into a 100 mL volumetric flask containing 50 mL of ethanol and the mixture was sonicated for 10 min. Thereafter the volume was adjusted to 100 mL with ethanol and the suspension was filtered through a membrane filter (Nylon syringe filter, pore size 0.45 μm, Teknokroma, Spain). A 1-mL (for gel sample) or 0.5-mL (for ointment) portion of the filtrate was diluted with aqueous 50% (v/v) ethanol to 25 mL in a volumetric flask; this test solution was directly analysed by the proposed SIA-CL method under the optimum conditions with use of calibration curve covering the range 1 to 10 μM indomethacin. Five separately weighed samples of each formulation were analysed and for each test solution five replicate injections were carried out.

2.6.2. Recovery experiments

A 0.25-g sample of the ointment or gel was treated with known volume of a standard solution of indomethacin in ethanol to increase the original content of indomethacin by 40 to 100%. Thereafter the spiked sample was analysed by the SIA-CL method as described above and the recovery of the added amount of indomethacin was calculated. The recovery experiments were run in triplicate for each formulation.

2.6.3. Procedure for the UV spectrophotometric assay of indomethacin [17]

A 2.00-g sample of a gel or 0.40-g sample of an ointment was weighed into a 50-mL volumetric flask containing 20 mL of methanol. Thereafter 20 mL of methanol was added, the mixture was sonicated for 10 min and diluted to 50 mL with methanol. The suspension produced was filtered through the Teknokroma membrane filter (see above), a 2-mL portion of the filtrate was transferred into a 25-mL volumetric flask, 0.5 mL of 1 M HCl was added and the volume was adjusted to 25 mL with methanol. Absorbance of this solution was measured at 318 nm in a 1-cm fused silica cell and corrected for the baseline absorbance measured at 450 nm. The reference standard solution of indomethacin (32 μg mL^{-1}) in methanol contained 20 mM HCl. Five separately weighed samples of each formulation were analysed.

2.6.4. Accuracy

The accuracy of the proposed SIA-CL method was evaluated by the statistical Student's *t*-test [42]. Such a test involved comparison of the results of indomethacin determination in Indobene gel and Vonum cutan ointment as found by the SIA-CL method and by the UV spectrophotometry [17].

3. Results and discussion

3.1. Optimization of chemical parameters

The only CL method for determining indomethacin published to date is a flow-injection assay based on its reaction with soluble Mn(IV) species in the presence of formaldehyde as an enhancer [26]; this method allowed to determine ≥0.1 μM indomethacin at relatively low sample throughput of <20 h^{-1}. Therefore, we attempted to devise another CL method that would be faster and at least as sensitive as the previous one. Initially we found that no CL is emitted if indomethacin is allowed to react directly with acidified solutions of permanganate or Ce(IV) as reagents commonly used in many CL assays. Since the CL of some indole derivatives was observed earlier [35–37] when unstable Ru(bipy)$_3^{3+}$ species was used as oxidant, we decided to employ relatively stable Ru(bipy)$_3^{2+}$ as the reagent in combination with the SIA technique for automated in situ formation of Ru(bipy)$_3^{3+}$ and its on-line CL reaction with indomethacin. We found that the only sequence of reactant zones aspirated into the holding coil leading to CL emission is Ce(IV) in sulphuric acid - Ru(bipy)$_3^{2+}$ - acetate buffer - indomethacin. The orange light emitted showed maximum emission at 590–610 nm; this fact indicates that the emitting species is excited [Ru(bipy)$_3^{2+}$]* [29]. There was no CL signal detected if Ce(IV) was substituted by MnO_4^-.

Sulphuric acid forms complexes with Ce(IV); it has been already pointed out that the reactive species of Ce(IV) are $Ce(SO_4)_2$ and $H[Ce(SO_4)_3]^-$ [40,41] and hence the concentration of sulphuric acid should influence the reactivity of Ce(IV) with Ru(bipy)$_3^{2+}$. On the other hand extremely high acidity of the Ce(IV) reagent could cause decomposition of the Ru(bipy)$_3^{2+}$ complex. It was found that the intensity of CL increases when decreasing concentration of H_2SO_4 down to 0.05 mol L^{-1} but further decrease of its concentration results in the formation of precipitate in the flow system; therefore 0.05 M H_2SO_4 was selected as the optimum parameter. The intensity of CL increased when increasing concentration of Ce(IV) from

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

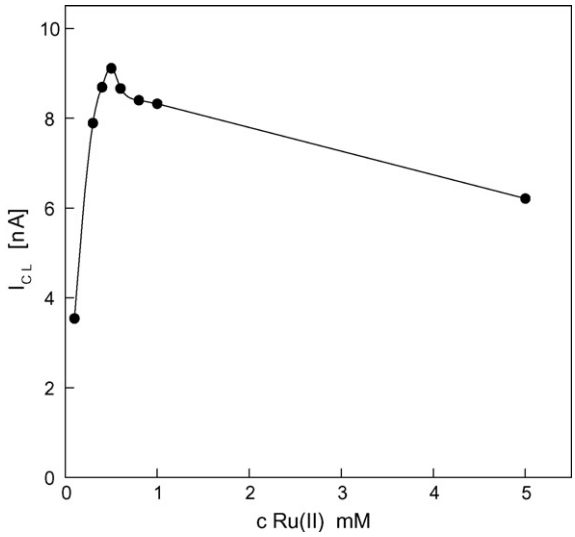

Fig. 3 – Dependence of the intensity of CL on the concentration of Ru(bipy)$_3^{2+}$ complex 15 μL of 1 μM indomethacin in 25% (v/v) ethanol injected; Reagent zones aspirated: 70 μL of 15 mM Ce(IV) in 50 mM H_2SO_4, 30 μL of Ru(bipy)$_3^{2+}$, 30 μL of 0.2 M acetate buffer pH 5.4; FRa = 25 μL s^{-1}; FRd = 100 μL s^{-1}.

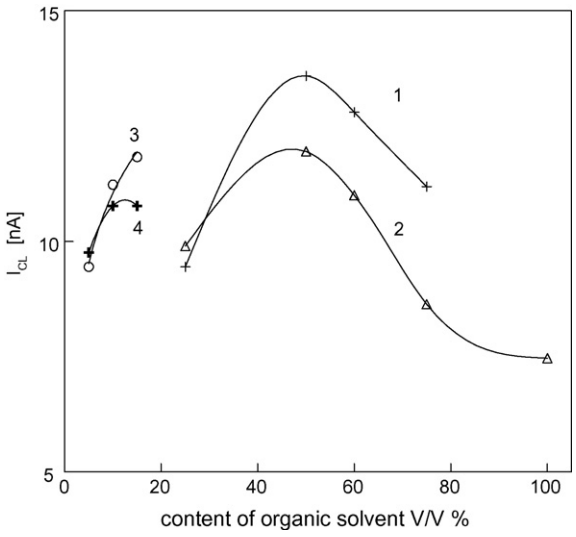

Fig. 4 – Influence of the content of organic solvent in the injected indomethacin solution on the intensity of CL. 15 μL of 1 μM indomethacin in appropriate solvent injected. Reagent zones aspirated: 70 μL of 15 mM Ce(IV) in 50 mM H_2SO_4, 30 μL of 0.5 mM Ru(bipy)$_3^{2+}$, 30 μL of 0.4 M Na acetate; FRa = 25 μL s^{-1}; FRd = 100 μL s^{-1}. Curve 1: ethanol, curve 2: methanol, curve 3: acetone, curve 4: acetonitrile.

5 to 15 mmol L^{-1} and it practically did not change for 20 mM Ce(IV); hence the optimal concentration of Ce(IV) was set to 15 mmol L^{-1}. The effect of the concentration of Ru(bipy)$_3^{2+}$ is depicted in Fig. 3; it can be seen that maximum CL response was attained for 0.5 mM Ru(bipy)$_3^{2+}$.

The CL signal increased with increasing the pH of the 0.2 M acetate buffer from 5 to 5.6. There was no CL emission if acetate buffer was substituted by water, by phosphate buffer of pH 6 to 8 or by Britton–Robinson buffer of pH 2 to 10. Apparently the presence of acetate is essential for the formation of the CL emitting species. Replacement of the acetate buffer by plain sodium acetate solution resulted in further growth (by 126% compared to 0.2 M acetate buffer of pH 5.6) of the CL signal; optimum concentration of the aspirated sodium acetate solution was 0.4 mol L^{-1}.

Indomethacin is sparingly soluble in water but well soluble in common organic solvents such as methanol, ethanol, acetone and acetonitrile. Therefore, its stock solution as well as the working and standard solutions were prepared in organic or mixed aqueous-organic media. The effect of the content of selected organic solvents in the injected test solution of indomethacin on the intensity of CL is shown in Fig. 4. It can be concluded that ethanol exhibits the best sensitizing effect and that its optimum content in the injected sample is 50% (a 40% increase of CL intensity compared to sample containing 25% of ethanol is achieved). Anionic and cationic surfactants examined (see Section 2) did not influence the intensity of CL; insignificant increase of less than 20% of the original value was observed if the injected sample contained about 0.2% of nonionic surfactant Triton X-100; in view of this fact no surfactants were employed in further experiments.

To recapitulate, the optimum concentrations of the reactants in the aspirated zones were 15 mM Ce(IV) in 50 mM H_2SO_4, 0.5 mM Ru(bipy)$_3^{2+}$, 0.4 M Na acetate, and 50% (v/v) of ethanol in the injected test solution of indomethacin.

3.2. *SIMPLEX optimization of flow parameters*

Sixteen SIMPLEX vertexes were run to optimize the volumes of the aspirated zones of the reactants (except of the zone of 1 μM indomethacin with fixed volume of 15 μL) and the flow rates FRa and FRd in terms of achieving maximum intensity of CL. The process was initiated with 70 μL of 15 mM Ce(IV) in 50 mM H_2SO_4, 30 μL of 0.5 mM Ru(bipy)$_3^{2+}$ and 30 μL of 0.4 M Na acetate. The initial flow rates were FRa 25 μL s^{-1} and FRd 100 μL s^{-1}.

After nine vertexes the CL intensity increased by 20% relative to the initial value and it did not improve with further runs. Hence the final optimum conditions found after the 9th vertex were 41 μL of 15 mM Ce(IV) in 50 mM H_2SO_4, 30 μL of 0.5 mM Ru(bipy)$_3^{2+}$, 16 μL of 0.4 M sodium acetate, FRa 60 μL s^{-1} and FRd 100 μL s^{-1}. The optimum time sequence of the SIA control programme is summarized in Table 1.

3.3. *Calibration curve, LOD, repeatability, robustness*

The best curve fit of the calibration data covering the whole concentration range of 0.1–50 μM indomethacin (nine concentrations) examined was a second degree polynomial: $I = -0.156c^2 + 22.193c + 0.767$ ($r = 0.9997$; $n = 9$) where I stands for the mean peak height (CL intensity in nA) and c is concentration of indomethacin (μM). The deviation from rectilinearity in CL methods is a commonly known phenomenon that may be caused for example by absorption of the emitted radiation by the analyte [28]. For 0.1 to 10 μM indomethacin the calibration curve was rectilinear: $I = 19.51c - 4.4$ ($r = 0.9991$;

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

Table 1 – Optimal concentrations of reactants and time sequence of control program for SIA-CL determination of indomethacin

Device	Command	Values	Aspirated solution
SP	Valve position IN		
SP	Set flow rate ($\mu L\,s^{-1}$)	100	
SP	Aspirate (μL)	500	
SP	Valve position OUT		
SP	Set flow rate ($\mu L\,s^{-1}$)	60	
MPV	Set valve position	1	15 mM Ce(IV) in 50 mM H_2SO_4
SP	Aspirate (μL)	41	
MPV	Set valve position	2	0.5 mM $Ru(bipy)_3^{2+}$
SP	Aspirate (μL)	30	
MPV	Set valve position	3	0.4 M Na acetate
SP	Aspirate (μL)	16	
MPV	Set valve position	4	Indomethacin in 50% (v/v) ethanol
SP	Aspirate (μL)	15	
MPV	Set valve position	9	
SP	Set flow rate ($\mu L\,s^{-1}$)	100	
SP	Empty syringe		
D	On		

SP: syringe pump, MPV: multi-position valve, D: detector.

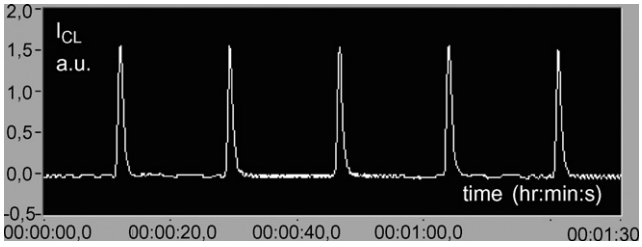

Fig. 5 – SIA-gram of five replicate injections of standard solution of indomethacin 15 μL of 3 μM indomethacin in 50% (v/v) ethanol injected; Reagent zones aspirated: 41 μL of 15 mM Ce(IV) in 50 mM H_2SO_4, 30 μL of 0.5 mM $Ru(bipy)_3^{2+}$ and 16 μL of 0.4 M Na acetate; FRa = 60 $\mu L\,s^{-1}$; FRd = 100 $\mu L\,s^{-1}$.

$n = 5$). The R.S.D. of the slope of this rectilinear calibration curve was 7.1% when repeating the calibration eight times (in eight different days); the correlation coefficient values ranged between 0.9984 and 0.9998 ($n = 5$). The SIA-gram demonstrating five replicate injections of 3 μM indomethacin is shown in Fig. 5.

The LOD estimated as 3S/N ratio was 50 $nmol\,L^{-1}$ of indomethacin.

The R.S.D. values of mean peak heights for 10 replicate injections of 0.5 μM and 7 μM indomethacin were 2.40% and 2.02%, respectively, which is the sign of acceptable repeatability.

Maximum sample throughput was 180 h^{-1}.

As for the robustness of the method, the CL signal was not influenced if the optimized concentrations, volumes and flow rates were changed within ±10% except of decreased concentration of Ce(IV); in this case the CL signal diminished by 7% relative to the optimal value if the concentration of Ce(IV) was decreased by 10%.

3.4. *Analysis of pharmaceutical formulations*

The results of SIA-CL and UV spectrophotometric analysis [17] of Indobene gel and Vonum cutan ointment are summarized in Table 2. Comparison of the results obtained by the proposed and reference methods by statistical Student's t-test [42] revealed that there is no significant difference between the two methods at 95% confidence level. Thus, the proposed SIA-CL method provides accurate results that are not influenced by the presence of excipients quoted by the manufactures such as 2-propanol, diisopropyl adipate, carbomer 980, trometamol and spruce essential oil for Indobene gel and lauromacrogol,

Table 2 – Determination of indomethacin in semisolid formulations and comparison of results with UV spectrophotometric method

Formulation	Nominal content (%)	Content found (% ±S.D.[a])		t-test[b]
		SIA-CL	UV [17]	
Vonum cutan	5	4.80 ± 0.08	4.88 ± 0.08	1.630
Indobene gel	1	1.01 ± 0.04	0.97 ± 0.02	2.003

[a] S.D., standard deviation of the mean value ($n = 5$).
[b] At 95% confidence level; $n_a = n_b = 5$; $t_{critical} = 2.228$ [39].

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

Table 3 – Recoveries of standard additions of indomethacin

Formulation	Indomethacin found by SIA-CL[a] (%)	Indomethacin added (%)	Sum found (%)[b]	Mean recovery (%)	R.S.D.[c] (%)
Indobene gel 1%	1.01	1.00	2.01	99.65	2.50
Vonum cutan 5%	4.80	2.01	6.75	97.31	1.59

[a] In original formulation without analyte addition.
[b] In original formulation with analyte addition.
[c] Relative standard deviation ($n = 3$).

macrogol, glycerol monostearate and cetostearomacrogol for Vonum cutan ointment. This fact is also corroborated by the results of recovery experiments (see Table 3) based on the standard addition technique.

4. Conclusion

Most of available methodologies for indomethacin determination are either time-consuming, need specific and expensive equipment or require a rigorous operator intervention that could affect precision and accuracy. With the sample throughput of 180 injections per hour the devised automated SIA-CL method is by far the fastest method employed for the determination of indomethacin among those published so far. The proposed method is characterised by favourable limit of detection that compares well with that of considerably slower FIA-CL method based on different chemistry [26] which was the only CL one published till now. This was achieved by thorough optimization of the chemical and flow parameters; considerable enhancement of the CL signal was attained by the use of acetate as a novel specific enhancer and by adding ethanol to the analysed indomethacin solutions. Relatively broad calibration range, acceptable reproducibility and selectivity are among main benefits of the method. Since common excipients used in the manufacture of commercial gels and ointments do not interfere with the SIA-CL assay of indomethacin the method could be successfully applied to routine determination of indomethacin in semisolid dosage forms. Its future use in automated studies of the release of indomethacin from pharmaceutical preparations can be envisaged.

Acknowledgements

The authors gratefully appreciate financial support coming from Ministry of Education of Czech Republic, FRVS grant No. 292/2006 and Research Project MSM0021620822 as well as from Charles University Grant Agency GAUK, grant No. 313/2005.

REFERENCES

[1] Czech drug database, AISLP – Automated information system of pharmaceutical preparations.
[2] http://www.healthsquare.com/newrx/ind1209.htm, visited 25/8/2006.
[3] British Pharmacopoeia. The Stationery Office, London, 2004.
[4] M.A. Al Za'abi, G.H. Dehghanzadeh, R.L.G. Norris, B.G. Charles, J. Chromatogr. B 830 (2006) 364–367.
[5] S.-J. Lin, Y.-R. Chen, Y.-H. Su, H.-I. Tseng, S.-H. Chen, J. Chromatogr. B 830 (2006) 306–313.
[6] L. Nováková, L. Matysová, L. Havlíková, P. Solich, J. Pharm. Biomed. Anal. 37 (2005) 899–905.
[7] M.Y. Khuhawar, F.M.A. Rind, A.R. Rajper, Acta Chromatogr. 15 (2005) 269–275.
[8] C. Martínez-Algaba, L. Escuder-Gilabert, S. Zahrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Pharm. Biomed. Anal. 36 (2004) 393–399.
[9] S. Liu, M. Kamijo, T. Takayasu, S. Takayama, J. Chromatogr. B 767 (2002) 53–60.
[10] E. Grippa, L. Santini, G. Castellano, M.T. Gatto, M.G. Leone, L. Saso, J. Chromatogr. 738 (2000) 17–25.
[11] C. Cristòfol, B. Pérez, M. Pons, J.E. Valladares, G. Martí, M. Arboix, J. Chromatogr. B 709 (1998) 310–314.
[12] J. Sato, T. Amizuka, Y. Niida, M. Umetsu, K. Ito, J. Chromatogr. B 692 (1997) 241–244.
[13] D. Löffler, T.A. Ternes, J. Chromatogr. A 1021 (2003) 133–144.
[14] M.E. Abdel-Hamid, L. Novotný, H. Hamza, J. Pharm. Biomed. Anal. 24 (2001) 587–594.
[15] Y.L. Chen, S.M. Wu, Anal. Bioanal. Chem. 381 (2005) 907–912.
[16] K. Makino, T. Yano, T. Maiguma, D. Teshima, T. Sendo, Y. Itoh, R. Oishi, Ther. Drug. Monit. 25 (2003) 574–580.
[17] M. Stolarczyk, J. Krzek, W. Rzeszutko, J. AOAC Int. 87 (2004) 592–595.
[18] T. Aman, F. Naureen, A.A. Kazi, I.U. Ghan, M.A. Kashmiri, Anal. Lett. 35 (2002) 1007–1020.
[19] P.C.A.G. Pinto, M.L.M.F.S. Saraiva, J.L.M. Santos, J.L.F.C. Lima, Anal. Chim. Acta 539 (2005) 173–179.
[20] P. Solich, H. Sklenářová, J. Huclová, D. Šatínský, U.F. Schaefer, Anal. Chim. Acta 499 (2003) 9–16.
[21] C. Reguera, M.J. Arcos, M.C. Ortiz, Talanta 46 (1998) 1493–1505.
[22] A.M.M. Ali, J. Pharm. Biomed. Anal. 18 (1999) 1005–1012.
[23] G.B. El-Hefnawy, I.S. El-Hallag, E.M. Ghoneim, M.M. Ghoneim, Anal. Bioanal. Chem. 376 (2003) 220–225.
[24] C. Reguera, M.C. Ortiz, M.J. Arcos, Electroanal 14 (2002) 1699–1706.
[25] T. Kitade, K. Kitamura, S. Takegami, R. Yoshinage, M. Sasakawa, A. Tokuhira, Anal. Sci. 18 (2002) 835–838.
[26] F. Nie, J. Lu, Y. He, J. Du, Talanta 66 (2005) 728–733.
[27] A.M. García-Campaña, W.R.G. Baeyens, X. Zhang, F. Alés, L. Gámiz, Ars Pharmaceutica 42 (2001) 81–107.
[28] A.M. García-Campaña, W.R.G. Baeyens, Chemiluminescence in Analytical Chemistry, Marcel Dekker, Inc., New York, 2001.
[29] R.D. Gerardi, N.W. Barnett, S.W. Lewis, Anal. Chim. Acta 378 (1999) 1–41.
[30] T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás, J. Fenoll, Anal. Chim. Acta 485 (2003) 63–72.
[31] F.A. Aly, S.A. Al-Tamimi, A.A. Alwarthan, Anal. Sci. 17 (2001) 1257–1262.

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057

[32] F.A. Aly, S.A. Al-Tamimi, A.A. Alwarthan, Talanta 53 (2001) 885–893.
[33] N.A. Al-Arfaj, Talanta 62 (2004) 255–263.
[34] N.A.A. Alarfaj, Anal. Sci. 19 (2003) 1145–1149.
[35] D.R. Skotty, T.A. Nieman, J. Chromatogr. B 665 (1995) 27–36.
[36] K. Uchikura, M. Kirisawa, Chem. Lett. 20 (1991) 1373–1376.
[37] N. Anastos, N.W. Barnett, S.W. Lewis, N. Gathergood, P.J. Scammells, D.N. Sims, Talanta 67 (2005) 354–359.
[38] P. Solich, M. Polášek, J. Klimundová, J. Ruzicka, Trends Anal. Chem. 23 (2004) 116–126.
[39] C.E. Lenehan, N.W. Barnett, S.W. Lewis, Analyst 127 (2002) 997–1020.
[40] J. Xi, B. Shi, X. Ai, Z. He, J. Pharm. Biomed. Anal. 36 (2004) 237–241.
[41] S. Lakshmi, R. Renganathan, In. J. Chem. Kinet. 28 (1996) 713–720.
[42] K. Eckschlager, I. Horsak, Z. Kodejs, Vyhodnocovani analytickych vysledku a metod (Evaluation of Analytical Results and Methods), SNTL, Prague, 1980, p. 34.

Please cite this article in press as: K. Mervartová et al., Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, Anal. Chim. Acta (2007), doi:10.1016/j.aca.2007.01.057