Research Article

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Rapid chemiluminometric determination of gabapentin in pharmaceutical formulations exploiting pulsed-flow analysis

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ABSTRACT: In this study, a straightforward and automated pulsed flow-based procedure was developed for the chemiluminometric determination of gabapentin [1-(aminomethyl)cyclo-hexaneacetic acid], a new generation antiepileptic drug, in different formulated dosage forms. The software-controlled time-based injection method capitalizes on the decrease of the background chemiluminescence (CL) readout of the luminol–hypochlorite reaction in the presence of gabapentin. In short, gabapentin works as a hypochlorite scavenger. The analytical procedure was implemented in a multi-pumping flow network furnished with a suite of microdispensing solenoid-actuated pumps. The diaphragm-type micropumps might be configured to operate as fluid propellers, commutation units and metering injectors. A dynamic linear working range for gabapentin concentrations in the range 60–350 μmol/L was obtained, with an estimated detection limit of 40 μmol/L. The flow analyser handles about 41 injections/h and yields precise results (RSD < 2%). The miniaturized flow analyser thus has potential to be exploited for in-line monitoring of drug manufacturing within the quality assurance framework of modern pharmaceutical companies. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: gabapentin; chemiluminescence; pulsed-flow analysis; pharmaceutical assays

Introduction

Epilepsy is a polymorphic ailment that is characterized by the periodic occurrence of seizures by abnormal cerebral neuronal discharges, with or without convulsions. It is known that the origin of these epileptic seizures is related to the electrical instability of the cell surface of one or several neurons (1). The reason for the increase of conductivity of the membrane has been attributed to different molecular mechanisms with potential pharmacological amendment. Hence, there exist several groups of effective pharmaceutical preparations for the control of different forms of epilepsy (2).

Traditionally, antiepileptic drug assays in dosage forms and biological specimens relied on column separation methods involving gas chromatography–mass spectrometry (3), liquid chromatography with diode-array, fluorometric or mass spectrometric detection (4–8), capillary electrophoresis (9, 10) or mass spectrometric detection (11, 12). However, current trends in the field are being shifted to the overall automation and miniaturization of the analytical procedures (13, 14) to make them fast and efficient, as demanded in routine quality control tasks for the validation of manufacturing processes, dosage uniformity tests and assessment of the active ingredient concentration in final products (15). To this end, fully automated flow-through analysers based on flow injection and related approaches thereof and utilizing reagent-based assays should be regarded as appealing tools for expeditious ascertainment of the compliance of drug formulations with regulatory authorities (15–18).

As to the chemical assay, there is an increasingly growing interest within the pharmaceutical arena in the exploitation of the scavenging activity of the drug against hypochlorous acid, which is also the basis for *in vitro* explorations of the physiological action of drugs (19–23). Amongst the various detection systems for monitoring the above reaction, chemiluminescence (CL) detection should be regarded as a straightforward, sensitive and cost-effective alternative (22, 23). The analytical application of CL has received a strong impetus by the advent of flow injection and multicommutated flow approaches, as exemplified in several monographs and comprehensive fundamental reviews (24–27). The reproducible mixing of streams and precise timing control inherent to flow-based assemblies are crucial conditions for reliable CL measurements. Flow injection and its sequels should be highlighted as unique strategies to accommodate fast CL reactions, very often involving luminol oxidation in alkaline medium (28–30), in which light emission occurs instantaneously upon merging sample and chemiluminogenic reagent with half-life times of only a few seconds.

In this work, a fully automated flow-based CL analyser capitalizing on pulsed-flow analysis is proposed for fast quality control analysis

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of pharmaceuticals, as demonstrated via the indirect CL determination of gabapentin in various formulated dosage forms, viz. capsules and tablets. The flow network is assembled to measure the decrease of light emission evoked by the luminol–hypochlorite system due to the scavenging activity of the antiepileptic drug. It should be noted that multi-pumping flow systems have potential for large-scale routine analysis as a consequence of the minimal operational maintenance inherent to this novel generation of flow injection (31–34).

Experimental

Reagents and samples

All chemicals were of analytical-reagent grade and deionized water with a conductivity of <0.1 μS/cm was used throughout for solution preparation. A 3 mmol/L analyte stock solution was prepared by dissolution of 26 mg gabapentin (Tecnimede SA, Portugal) in 50 mL deionized water. Working standard solutions were prepared daily by stepwise dilution of the stock with water. The chemiluminogenic reagent consisted of 108 mg 3-aminophthalhydrazide (luminol; Across Organics, NJ, USA) dissolved in 100 mL 0.1 mol/L HPO_4^{2-}/PO_4^{3-} buffer, pH 11.2. A 0.26 mmol/L hypochlorite (Fluka) solution was prepared daily in the above buffer and standarized against thiosulphate, following the *Portuguese Pharmacopeia* recommendations (35).

The pharmaceutical preparations analysed, viz. Bexal 400 mg, Gabamox 300 mg, Ciclum 400 mg, Merck 300 mg, Generis 400 mg and Neurotin 800 mg, were purchased from Portuguese pharmacies. Twenty capsules or tablets of each drug formulation were weighed and homogenized in order to ensure a representative sample. A well-defined portion of the powder to provide a ca. 40 mg/L gabapentin solution was then taken, stirred for ca. 20 min in deionized water and filtered through 0.45 μm cellulose acetate prior to analysis.

Instrumentation and software

The flow network comprised four fixed displacement diaphragm micro-dispensing solenoid pumps (Bio-Chem, Boonton). An 8 μL stroke micropump (090SP, P4) was utilized for sample delivery, while 25 μL stroke micropumps (120SP, P1-P3) were assembled for dispensing carrier and reagents. Further details as to the electronic components for automatic switching of the micropumps have been described previously (36). A schematic illustration of the multipumping flow system is depicted in Fig. 1. The manifold was built from poly(tetrafluoroethylene) (PTFE) tubing of 0.8 mm i.d., using polyvinyl chloride connectors and flow confluences made of poly(methylmethacrylate) (PMMA). The reaction coil (RC) and connecting line between the T-confluence and the detector were 300 and 2 cm long, respectively.

The custom-built CL detector used for collection of the light emission evoked has been described elsewhere (27). In short, it involves a spirally-shaped PMMA flow-through cell with an internal volume of 40 μL and an effective emitting surface of approximately 0.75 cm^2. The CL flow cell was placed over the window of a Hamamatsu HS5784-04 (Ichino-cho, Hamamatsu, Japan) photosensor module (PSM) furnished with a metal-packaged photomultiplier tube, a high-voltage power supply circuit and a low-noise amplifier circuit. The PSM was arranged in a home-made light-tight box. The direct connection between the CL detector and the standard serial port of a PC was accomplished via an Ibercomp RS485–RS232C converter card (Ibercomp, Spain).

The operational procedures for the multipumping-based analyser were computer controlled by the software package AutoAnalysis 5.0 (Sciware, Spain), based on dynamic link libraries (DLLs). Acquisition and processing of the CL data were performed using the same software package. In our particular configuration, the principal protocol of the software was loaded with custom-built DLLs designed for the automatic control of the solenoid pumps and PSM.

Analytical procedure

The pulsed-flow-based analyser capitalizes on the synchronous activation of four solenoid micropumps. These manifold components are accountable for the injection of well-defined plugs of CL reagent (P1), oxidizing reagent (P2), carrier (P3) and sample (P4), respectively. The number of pump strokes and the frequency thereof impose the reagent/sample volume delivered and stream flow rate, respectively. Operational details on the activation (On) of the suite of fluid propellers throughout the assay are pinpointed in Fig. 2.

The analytical run is initiated by the cleansing of the system conduits with hypochlorite and water, followed by the simultaneous loading of the reaction coil with minute slugs of sample and oxidizing reagent (see Fig. 2). The interdispersed zone merges downstream with the chemiluminogenic reagent supplied by P1, the resulting composite segment being directed to the detector for recording of the CL signal. An additional carrier volume is pumped to cleanse the flow-through cell, rendering the system ready to initiate a new analytical sequence.

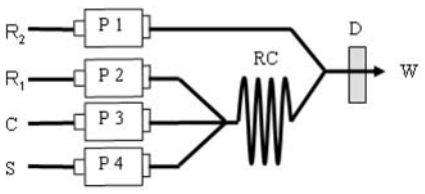

Figure 1. Schematic illustration of the multi-pumping flow manifold for gabapentin determination: P1–P4, solenoid micropumps; RC, reaction coil; D, detector; W, waste; C, carrier; S, sample; R_1, hypochlorite solution; R_2, luminol solution.

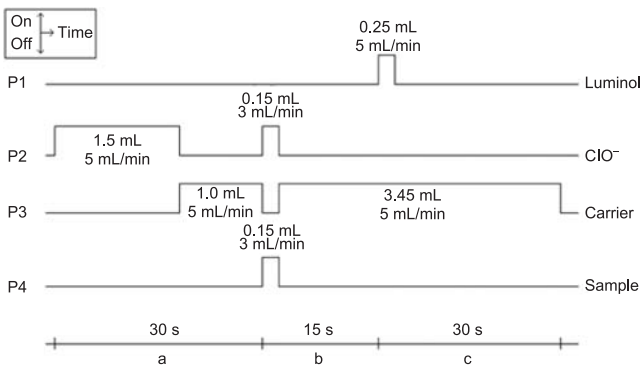

Figure 2. Operational sequence of solenoid micropumps: a, cleansing of flow conduits; b, time-based injection of sample and oxidizing reagent plus development of the redox reaction; c, CL reaction; P1–P4, solenoid micropumps.

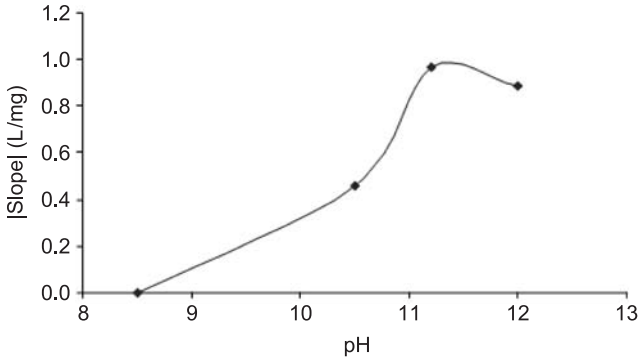

Figure 3. Dependence of slope of calibration graph upon reaction pH. Experimental conditions: sample volume, 150 μL; luminol volume, 250 μL; hypochlorite volume, 150 μL; carrier volume, 1.2 mL; total flow rate, 10 mL/min; hypochlorite concentration, 1.5 mmol/L; luminol concentration, 5 mmol/L; concentrations of gabapentin standard solutions, 10, 20, 30, 40, 50 and 60 mg/L.

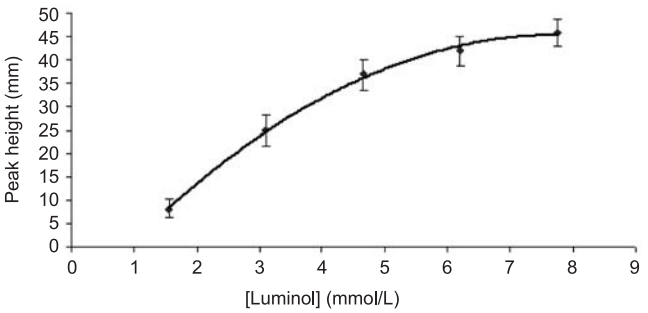

Figure 4. Influence of luminol concentration on the CL signal. Experimental conditions: sample volume, 150 μL; luminol volume, 250 μL; hypochlorite volume, 150 μL; carrier volume, 1.2 mL; total flow rate: 10 mL/min; hypochlorite concentration, 0.3 mmol/L; pH, 11.2.

RESULTS AND DISCUSSION

Selection of chemical variables

A crucial parameter influencing the yield of luminol-based CL reactions is the solution pH. The effect of the pH of chemiluminogenic reagent on the recorded CL intensity was thus examined over the range 8.5–12, using appropriate buffer solutions. The higher the pH, the better was the method's sensitivity up to pH 11.2, whereupon the slope of the calibration graph levelled off, as deduced from the experimental results shown in Fig. 3. A similar trend was reported by Li and Dasgupta (37) when exploiting the luminol–hypochlorite reaction. A 10-fold sensitivity increase was actually encountered when raising the luminol pH from 9 to 11.2, the latter being thus selected for the remainder of the investigations.

The dependence of the CL elicited upon the concentrations of luminol and hypochlorite was assessed using a univariate approach within the ranges 1.5–8.0 mmol/L and 0.05–1.0 mmol/L, respectively, in the absence of antiepileptic drug. The aim of this was to obtain the highest background CL signal that determined both the sensitivity and practical dynamic range for the inhibition reaction.

A second-order polynomial correlation was encountered between the CL signal and the luminol concentration, as shown in Fig. 4. Concentrations >8 mmol/L luminol led to a sharp CL peak drop attributed to the contribution of self-absorption phenomena (38) and gave rise additionally to high blank values. As to the oxidant concentration, a linear correlation was found against the CL emission, yet concentrations >0.3 mmol/L HClO rendered steady CL measurements. Accordingly, the concentrations of luminol and hypochlorite chosen for the multipumping-based analyser were 6 mmol/L and 0.25 mmol/L, respectively.

Selection of physical variables

In order to explore rapid CL reactions based upon luminol oxidation, continuous forward-flow systems are demonstrated to be well suited (24, 25). Hence, the merging of the chemiluminogenic reagent with oxidant should be effected as close as possible to the PSM and at the maximum flow rate available, due to the short lifetime of the light elicited. Various manifold arrangements were examined for implementation of the indirect CL assay of gabapentin. Both reagents (luminol and hypochlorite) and sample might be simultaneously merged at the same confluence or, alternatively, the micropumps might be actuated synchronously to foster on-line mixing of sample with either of the CL reagents prior to further reaction downstream at a second flow confluence. The experimental results revealed that the former configuration gave rise to a 30% CL signal decrease and distorted peaks as compared with flow networks equipped with two merging points. As a consequence of the need for a time for completion of the redox reaction between gabapentin and hypochlorite, the flow network depicted in Fig. 1, in which the oxidizing reagent is scavenged, was utilized for the remainder of the work. The flow manifold was initially mounted with a 25 μL stroke micropump for sample insertion, but replacement by an 8 μL stroke micropump, while fixing the flow rate, yielded improved repeatability and sensitivity. This effect is likely due to the increase of pumping frequency that enhances the chaotic movement of solutions and leads to a more efficient homogenization of the reaction zone, which is in good agreement with earlier observations (34, 39). As to the total sample volume metered and the flow rate of the pulsed stream, it was noticed that the larger the inserted volume, the higher was the CL emission, up to 150 μL, while no significant dependence of the CL readouts upon sample flow rate was encountered over the range 1.0–5.0 mL/min. On the other hand, as expected, the increase of total flow rate from 4 to 12 mL/min gave enhanced collection of evoked light, yet flow rates ≤10 mL/min are recommended for the gabapentin assay to attain repeatabilities of ≤2.0%.

The reaction coil length was found to significantly affect the evoked CL. When increasing the reactor length, the rise of residence time for the gabapentin–hypochlorite reaction was offset by the dispersion increment. Notwithstanding the fact that the peak height deteriorated slightly on increasing the reactor from 100 to 300 cm, the large improvement of coefficients of variation (from 8% to 2%) led us to implement a 300 cm long reactor in the flow network. To minimize the consumption of oxidant and luminol, whose volumes had been set at 150 and 250 μL/assay, respectively, an ancillary carrier stream (see Figs 1, 2) was utilized for delivering the composite sample/reagents zone towards the PSM.

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Table 1. Recovery tests for gabapentin pharmaceutical preparations exploiting automated pulsed-flow analysis

Drug formulation	Gabapentin (mg/L)		Recovery (%)	RSD
	Added	Found		
Bexal 400 mg	0	41.7 ± 0.8	–	1.8
	10.3	52.1 ± 0.7	101	1.3
	20.5	63.0 ± 0.3	104	0.4
Gabamox 300 mg	0	42.3 ± 0.3	–	0.9
	10.3	52.4 ± 0.9	98	1.7
	20.5	63.2 ± 0.6	102	0.9
Ciclum 400 mg	0	43.3 ± 0.5	–	1.1
	10.3	53.4 ± 0.9	99	1.7
	20.5	63.4 ± 0.5	98	0.9
Merck 300 mg	0	42.1 ± 0.5	–	1.9
	10.3	51.8 ± 0.8	95	1.5
	20.5	63.0 ± 0.5	102	0.8
Generis 400 mg	0	41.2 ± 0.5	–	1.2
	10.3	51.0 ± 0.2	95	0.3
	20.5	61.1 ± 0.8	97	1.3
Neurotin 800 mg	0	47.4 ± 0.9	–	1.9
	10.3	57.8 ± 0.6	101	1.0
	20.5	67.5 ± 0.9	98	1.3

The results are expressed as the mean of three assays ± SD.

Table 2. Evaluation of gabapentin concentrations in pharmaceutical preparations obtained by multipumping flow analysis vis-á-vis manufacturer's nominal values

Pharmaceutical preparations	Labelled (mg/unit)	Found (mg/unit)	Error (%)
Bexal	400	411 ± 5	2.6
Gabamox	300	314 ± 3	4.7
Ciclum	400	448 ± 5	11.9
Merck	300	318 ± 6	6.1
Generis	400	420 ± 5	5.0
Neurotin	800	900 ± 12	12.5

The results are expressed as the mean of three replicates ± SD.

Analytical performance and analysis of pharmaceutical preparations

Under the optimized chemical and hydrodynamic variables, a dynamic linear range from 60 to 350 μmol/L gabapentin was found. The experimental data fitted the following equation: $Y = -329\,654C + 132.9$, in which Y is the CL peak height and C is the gabapentin concentration in mol/L, with a correlation coefficient of 0.9992 ($n = 6$). The detection limit, estimated as 40 μmol/L, was calculated as the concentration of gabapentin which provided a signal equal to the background CL minus three times the standard deviation (SD) of the background signal. The coefficients of variation for 10 consecutive injections of a single standard solution were <2% for the overall concentration range. The injection throughput was estimated to be 41/h having accounted for changeover times between samples. As compared with an earlier flow-through spectrophotometric method for gabapentin determination (14), the CL-based multipumping flow system offers improved sampling frequency and a slightly better detection limit.

The interfering effect of several ingredients used as excipients in capsule and tablet formulations was ascertained. To this end, 0.3 mmol/L gabapentin standard solutions containing increasing amounts of excipients, viz., talc, lactose, cornstarch and magnesium stearate, were analysed via the multi-pumping flow set-up. A species was considered interferent when the its concentration affected the CL readings of the standard solution by >3%. No interference effect was detected for any of the assayed matrix components up to a excipient:gabapentin mass ratio of 100.

Inasmuch as there is no reference method for gabapentin assays in the *Pharmacopeia*, the reliability of the proposed flow analyser was assessed by recovery tests. Two different amounts of antiepileptic drug, 0.06 and 0.12 mmol/L, were added to solutions of the various commercially available pharmaceutical formulations, as shown in Table 1. The recovery values were in the range 95–104%, thus denoting the absence of multiplicative matrix interfering effects.

Good agreement was encountered between the experimental data and nominal values reported by the manufacturers for the suite of analysed formulations (see Table 2), with a maximum deviation of 12.5% for Neurotin 800 mg.

Conclusions

The linkage of multipumping flow analysis with CL detection has been exploited for the development of a straightforward and fully automated analyser for the determination of gabapentin in pharmaceutical preparations, regardless of the dosage form. The rapid and reliable determination of the antiepileptic drug makes the flow set-up an appealing tool for ensuring compliance of the formulations to the limits established by pharmacopeias. For example, the *Portuguese Pharmacopoeia* guidelines require that the contents of active ingredients in a single capsule/tablet should be comprised within 75–125% of the labelled value to be commercialized (35). Hence, the pulsed-flow assembly might be advantageously utilized for at-line or on-line monitoring of the manufacturing process, as endorsed by internal and external quality control protocols. Further, the flow analyser is equipped with cost-effective, rugged and versatile components, i.e. solenoid micropumps, and does not require skilful operators.

The applicability of the proposed flow set-up involving the luminol–hypochlorite chemiluminogenic reaction is not merely limited to the determination of gabapentin but can be readily extended to any drug featuring antioxidant capacity with minor modifications of the flow network. As compared with flow injection counterparts relying on streamlined flows, the turbulent flow inherent to pulsed flowing streams leads to a pronounced radial dispersion, thus assuring fast overlapping of sample and reagent zones, with subsequent improvement of method's sensitivity and repeatability. The synchronization of the added solutions within the flow network via the software-controlled actuation of the solenoid micropumps gives rise to a significant reduction of sample and reagent(s) consumption and to a lessening of waste generation with respect to flow injection set-ups. The multipumping CL-based flow analyser should thus be regarded as an attractive green methodology for routine quality control in modern industrial-scale pharmaceutical assays.

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