Environmental applications of liquid-waveguide-capillary cells coupled with spectroscopic detection

Laura J. Gimbert, Paul J. Worsfold

Long path-length liquid-waveguide-capillary cells (LWCCs) comprise a capillary with a refractive index (RI) lower than the RI of the liquid core. This enables light introduced into the capillary core to be totally internally reflected down the capillary towards the detector, resulting in an enhancement of sensitivity and improved limits of detection compared with conventional spectroscopic techniques. LWCCs are also versatile and have been used in combination with spectrophotometry, Raman spectroscopy and luminescence detection for quantification of a range of chemical species at nanomolar concentrations.

This article describes the properties and the practical aspects of LWCC instrumentation and applications to environmental matrices. We also present summary tables of applications, including figures of merit, LWCC properties and technical details.

Keywords: Chemiluminescence; Environmental; Fluorescence; Liquid waveguide capillary cells; Spectrophotometry

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1. Introduction

In recent years, liquid-waveguide-capillary cells (LWCCs), also known as liquid-core waveguides (LCWs), have played an important role in the measurement of chemical species at nanomolar concentrations [1]. LWCCs can enhance the sensitivity and improve limits of detection (LODs) of optical instrumentation, by detecting as much of the optical signal as possible while minimising background noise. This is due to the LWCC comprising a capillary that has a refractive index (RI) lower than the RI of the liquid core [2,3]. The light introduced into the liquid core of the capillary is totally internally reflected down the capillary towards the detector.

LWCCs were used in the 1970s with Raman spectroscopy where the capillary consisted of borosilicate with an RI of 1.51 or fused silica with an RI of 1.45. This meant that the liquid core had to have a higher RI for total internal reflection to occur, so it was suitable for use with organic solvents only [4,5]. There was therefore a need for a material with an RI lower than water (<1.33) so that LWCCs could be applied to aqueous samples, and this was achieved in the late 1980s with the introduction of Teflon AF by DuPont. Teflon AF is an amorphous copolymer of tetrafluoroethylene and 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole [4,6] and is available in two grades: Teflon AF 1600 with an RI of 1.31 and Teflon AF 2400 (RI 1.29) [4,6,7]. LWCCs made of Teflon AF tubing are generally classified as Type I waveguides, and those made of Teflon AF-clad silica tubing as Type II. With a Type I waveguide, the light travelling down the capillary is totally internally reflected at the Teflon AF interface, provided that the incident angle, on going from the optically more dense medium (water) to the less dense medium (Teflon AF), exceeds the critical angle. In a Type II waveguide, the light is totally internally reflected at the Teflon AF-silica cladding interface, provided that the incident angle exceeds the critical angle [1]. Type II waveguides are commercially available, in path lengths from 5 cm up to 5 m, from World Precision Instruments (Sarasota, FL, USA), with rigid quartz capillary tubing coated by the amorphous polymer cladding.

LWCCs have been used with a range of different detection techniques, including

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spectrophotometry, fluorescence, chemiluminescence, and Raman spectroscopy [2]. They are therefore versatile, can improve LODs and, although they can be up to 5 m in length, the Teflon AF capillary can be coiled due to its flexibility, thus minimising space.

This article gives an overview of LWCCs and focuses on the important applications of LWCCs to environmental matrices.

2. LWCC properties

Attenuation of the light source within a LWCC cell is minimised because the light is totally internally reflected [1], as shown in Fig. 1 for Types I and II waveguides. For total internal reflection to occur, the incident angle needs to be greater than the critical angle. The critical and incident angles are measured from the normal (perpendicular to the surface of the capillary), and the critical angle is specific to the Teflon AF polymer used, 9.9° and 14.1° for Teflon AF 1600 and 2400, respectively [2]. These values are calculated using the following equation:

$$\theta_c = \cos^{-1}\left[\frac{n_2}{n_1}\right] \quad (1)$$

where θ_c is the critical angle, n_1 is the RI of the fluid core, and n_2 is the RI of the cladding material.

LWCCs can be connected to a light source and a detector (e.g., a spectrophotometer) using fibre-optic cables and can be illuminated either axially or transversely (Fig. 2). In the axial mode, which is generally used for absorption measurements, light is introduced directly into the LWCC via an optical fibre, which

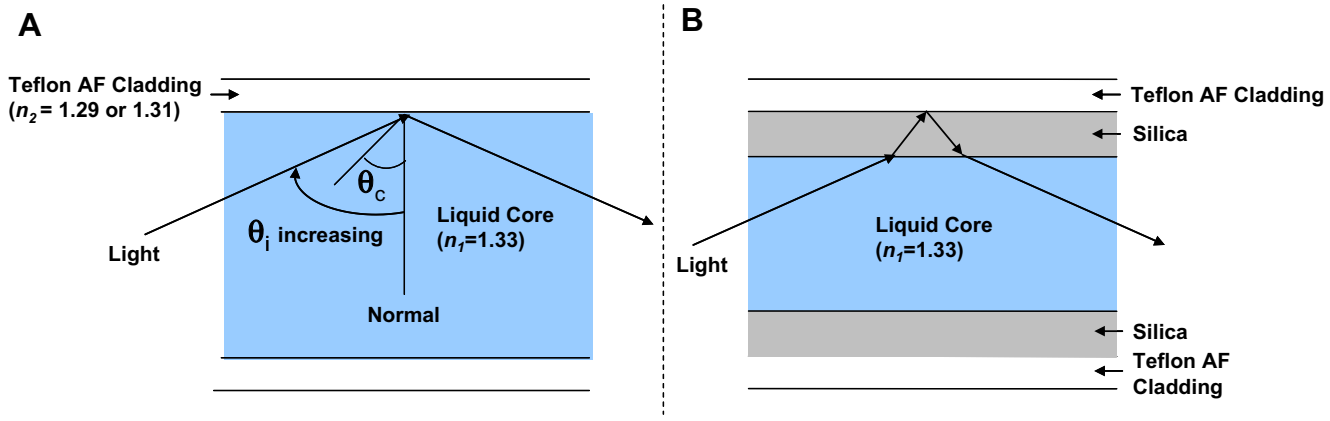

Figure 1. The path of the totally internally reflected light in liquid-waveguide-capillary cells: (A) in a Type I waveguide; and, (B) in a Type II waveguide.

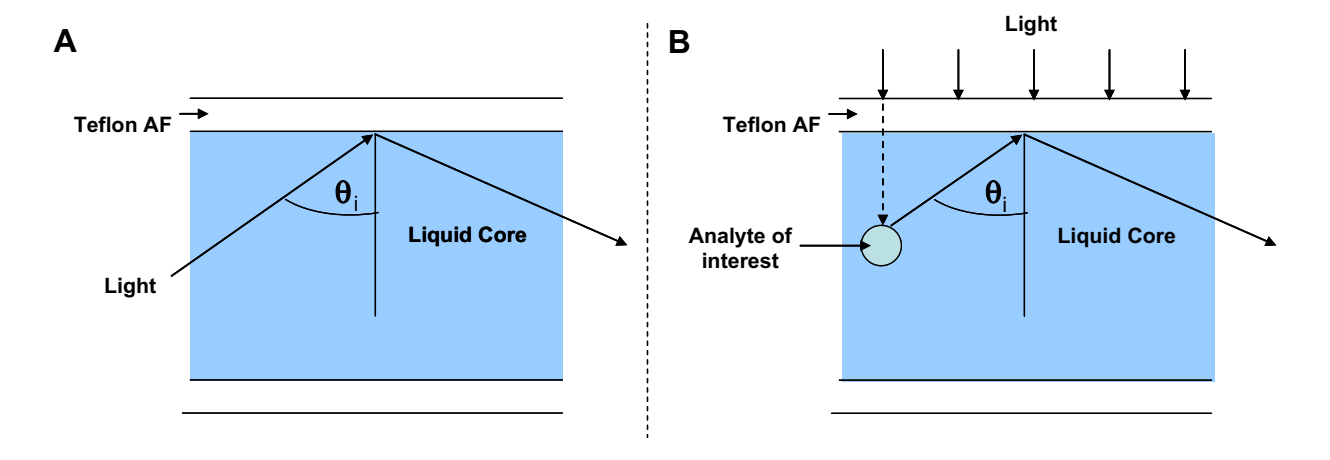

Figure 2. Comparison of axial and transverse illumination in liquid-waveguide-capillary cells (Type I shown for clarity): (A) axial illumination; and, (B) transverse illumination.

ensures that the light signal reaching the detector at the other end of the LWCC is maximised. In the transverse mode, generally used for fluorescence measurements [2], the LWCC is illuminated through the sidewall of the capillary and any light not absorbed by the analyte passes through the capillary [6]. Schematic diagrams of LWCCs in different configurations are shown in Fig. 3.

3. Practical aspects of LWCCs

In addition to an RI lower than water, Teflon AF has other useful beneficial characteristics; it is transparent throughout the 200–2000-nm wavelength range [8] and has a porous structure. However, Teflon AF is prone to adsorption from surface-reactive species in aqueous samples in Type I waveguides, and the hydrophobic surface causes air bubbles to adhere to it, resulting in the light signal being distorted [1,9,10]. The porous structure, however, has been a useful property in the construction of gas sensors, due to its high gas permeability [11–14]. Type II waveguides, with a silica capillary and Teflon AF cladding, have advantages over Type I waveguides as any potential contamination is minimised because the Teflon AF cladding and the analyte are not in direct contact. They are also easier to clean due to the inner silica capillary and the trapping of air bubbles is minimised [1,9]. A 1-m waveguide is easier to work with than a 2-m or 5-m waveguide, as the shorter path length results in formation of fewer bubbles.

Before and after use, the waveguide should be rinsed thoroughly and a manufacturer currently recommends sequentially flushing with acetonitrile, 1 M NaOH, 1 M HCl and then distilled water for 1-2 min each [http://www.wpiinc.com/services/manuals/downloads/LWCC-IM-041901.pdf].

This advice replaced a previous cleaning protocol in which methanol was used instead of acetonitrile. In our experience, the use of organic solvents for cleaning waveguides should be treated with caution, and we have avoided their use by sequentially flushing with ultrapure water followed by 1 M NaOH and 1 M HCl and then again with ultrapure water. This cleaning protocol has kept a waveguide in good working condition for at least two years for the determination of nM concentrations of phosphorus in waters. When the waveguide is not in use, it should be filled with ultrapure water and stored by sealing the ends of the waveguide. This should prevent it from drying out and also prevent microbial growth within it.

4. Environmental applications

Table 1 summarises the application of LWCCs with spectrophotometric detection to a range of analytes in

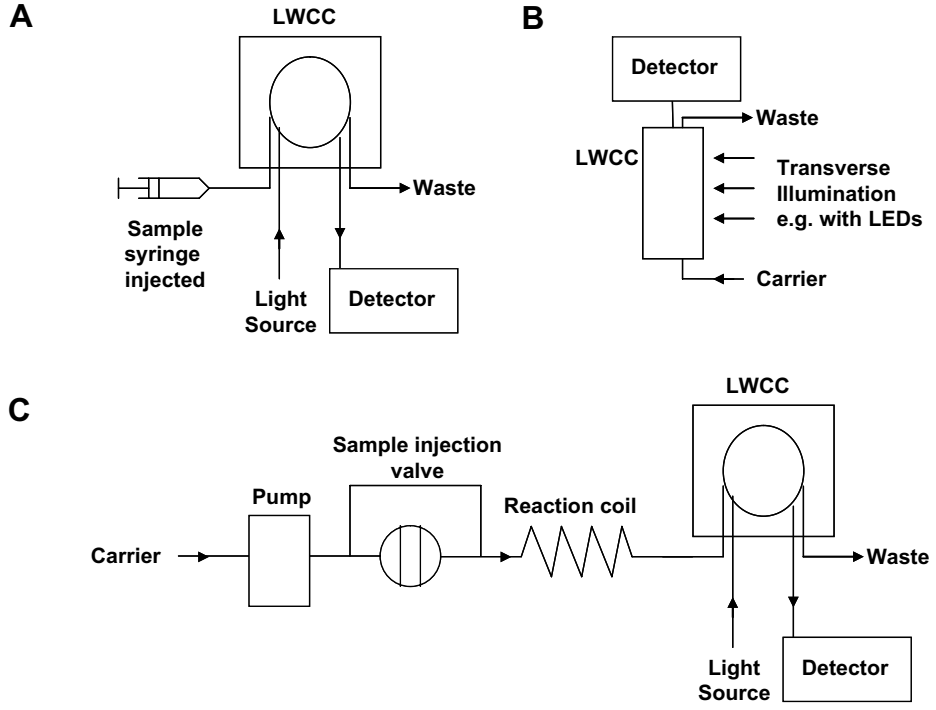

Figure 3. Examples of different manifold configurations using liquid-waveguide-capillary cells (LWCCs): (A) sample is mixed with reagent manually and injected into the LWCC with a syringe (a peristaltic pump can also be used); (B) LWCC transversely illuminated with fluorescence detection; and, (C) LWCC incorporated into a flow system.

standard solutions and environmental matrices. Similarly, Table 2 summarises the application of LWCCs with luminescence detection to the analysis of standard solutions and environmental samples. For each entry in the tables, the LWCC dimensions are given, along with the reported LOD and linear range, interferences and any other important comments (e.g., field deployment). Salient features of these applications are discussed in the following sections.

4.1. Spectrophotometric detection

In absorption spectrophotometry, LWCCs are illuminated axially using fibre-optic cables attached to a light source and a detector, and have been used to measure a wide range of environmental contaminants. The simplest approach to coupling a LWCC with spectrophotometric detection is to mix the sample with reagent manually and then inject the mixture into the LWCC using a syringe (Fig. 2A) [21] or a peristaltic pump [20,23,29]. This method was used for the determination of nitrate and nitrite in seawater samples incorporating the Griess azo dye reaction [29]. LODs of 1.5 nM for nitrate and 0.5 nM for nitrite were achieved, and these were significant improvements over the conventional spectrophotometric method, which can be inaccurate below 50 nM.

4.1.1. Coupling with segmented-flow analysis. An improved but more complicated approach is to use a gas-segmented continuous flow analyser, to give better precision and higher sample throughput. This technique was applied to nitrate and nitrite measurements in seawater and LODs of 2 nM for nitrate and 0.1 nM for nitrite were obtained on board ship during a 1-month cruise [10]. A gas-segmented analyser with a LWCC has also been used to determine, e.g., ammonium in surface seawater [16], iron (using ferrozine) [27] and phosphate (using ammonium molybdate) [1] in natural waters.

The use of gas-segmented continuous flow analysers is not without challenges, due to the gas-segmentation bubbles used [10,16,27]. The presence of these bubbles requires the addition of a suitable surfactant (e.g., Brij-35 [10,16,27] or SDS [1,37]) to the flow stream. The surfactant ensures a smooth flow, with low baseline noise, to establish a regular bubble pattern and minimise bubble breaking.

Other challenges include the effect of RI interferences, if the sample and wash solution have different RIs, as that can cause interference in absorbance measurements [1,10]. Two approaches have been used to compensate for this interference. The first is to apply an RI correction by calibrating the LWCC with deionised water and seawater samples of different salinities [1,10,27]. The second is to match the salinities of the wash solution and sample, hence removing the need for an RI correction [1,37]. When a method with a conventional flow cell is adapted to incorporate a LWCC, the effect of interferences from other species can be amplified, due to the increased sensitivity, so this needs to be systematically reported, but very few LWCC studies to date have done so. However, one good example is the simultaneous measurement of chromium(VI) and molybdenum(VI), in which 5 nM Cr(VI) was determined to have a negligible effect on the determination of Mo(VI) whilst 50 nM Cr(VI) decreased the signal of 5 nM Mo(VI) by ~11%. The interference of Cr(VI) on Mo(VI) measurements was effectively masked with 1% w/v ascorbic acid and 0.05 M EDTA [18].

4.1.2. In situ deployments. LWCCs can be incorporated into in situ analysers. A Type I LWCC used in laboratory studies [18,25,28,29,39] was adapted for use in a compact spectrophotometric elemental analysis system (SEAS) and applied to in situ determination of nitrite in seawater [35]. This was the first application of SEAS in chemical profiling with a relatively rapid sampling frequency of >12 per hour.

This instrumentation was also used with higher temporal resolution measurements to determine the nitrite distribution in the upper 200 m of the water column along a transect in the North Pacific [34]. A Type I LWCC with spectrophotometric detection [23] was also combined with the in situ capabilities of SEAS to determine copper in estuarine waters with an LOD of 3 nM [22]. Before being combined with SEAS, the 4.4-m LWCC was replaced with a 1-m LWCC, thus reducing the sensitivity of the instrument (LOD of 3 nM, down from the original LOD of 0.4 nM). Analysis times were 1–5 min for each sample and copper determinations were made over an estuarine transect with observed concentrations in the range 5–50 nM.

4.1.3. Coupling with flow-injection analysis. LWCCs can also be combined with flow injection (FI), and a portable multi-functional FI instrument has been developed capable of determining aluminium and chromium using spectrophotometry and hydrogen peroxide using fluorescence by switching between the different detection modes [15].

A Type II LWCC, used to study nitrate and nitrite in oligotrophic ocean waters [10,30], has also been incorporated into an FI manifold. This was applied to the measurement of nitrite in aqueous samples using azo-dye chemistry and was tested using three different waveguide lengths (50, 200 and 400 cm) [9]. It was found that the increased path length and small cross-section of the LWCC caused back pressure to build up, resulting in leaks at various joints, so Brij-35 was added to the carrier to reduce the back pressure. It was also found that the reagent needed to be degassed by vacuum suction or sparging with helium, as the air dissolved in the reagent could form micro-bubbles and adhere to the

Table 1. Applications of liquid-waveguide-capillary cells (LWCCs) with spectrophotometric detection

Analyte	Matrix	LWCC properties	Detector	Limit of detection	Linear range	Interferences studied	Comments	Ref.
Aluminium(III), Chromium(VI)	Aqueous solutions	Type I, Teflon AF-2400, 50 cm in length, 0.56 mm i.d., 0.80 mm o.d. (Biogeneral Inc., San Diego, CA)	CCD spectrometer (USB2000, Ocean Optics Inc., Dunedin, FL.). Cr(VI): Absorbance measured at 540 nm with a reference at 680 nm. Al(III): Absorbance measured at 585 nm with a reference at 700 nm	Cr(VI): 0.25 μg/l Al(III): 5 μg/l	Cr(VI): 0–46.2 μg/l Al(III): 0–800 μg/l	None reported	Combined LWCC with spectrophotometry, chemiluminescence and fluorescence to provide an inexpensive portable FI instrument	[15]
Ammonium	Seawater	Type II, 2 m in length, 550 μm i.d., (World Precision Instruments, Sarasota, FL, USA)	Spectra System UV-Vis detector (UV1000). Absorbance measured at 640 nm	5 nM	0–1000 nM	None reported	Automated method using segmented-flow analysis with liquid waveguide	[16]
Carbon dioxide	Seawater	Type I, Teflon AF-2400, 21 cm in length, 550 μm i.d., 625 μm o.d.	Fibre-optic-based spectrophotometer (SpectroPette, World Precision Instruments, Sarasota, FL, USA). Absorbance measured at 434, 620 with a reference at 740 nm		Gas standards 0–2000 μatm prepared. Water standards 300–2000 μatm prepared for field calibrations	None reported	Two wavelengths assessed absorbance peaks of acid and base forms of the indicator, while the third was the reference wavelength. High precision (about ±2–3 μatm in the *p*CO2 range of 200–500 μatm). Used during an underway survey of sea surface *p*CO2	[11]
Carbon dioxide	Natural waters and atmosphere	Type I, Teflon AF-2400, varying lengths in the range 12–21 cm, 550 μm i.d., 625 μm o.d.	Fibre-optic-based spectrophotometer (SpectroPette, World Precision Instruments, Sarasota, FL, USA). Absorbance measured at 560 nm		Gas standards 0–1000 μatm prepared	None reported	The first long path length fibre-optic-based sensor system to measure *p*CO2. High precision (about ±2–3 μatm in the *p*CO2 range of 200–500 μatm) using an 18-cm cell	[12]

Chromium(VI)	Natural waters	Type I, Teflon-AF 3500, 10 mm in length, 0.60 mm i.d. (Ocean Optics)	Photodiode detector (Siemens Model SFH229, silicon PIN-type). Absorbance measured at 574 nm	48.4 µg/l	0.25–1.0 mg/l	Known interferences Mo, V, and Hg to the diphenylcarbazide method were undetected (<1 µg/l) by ICP-MS	Continuous-flow analysis. Results in good agreement with those obtained by ICP-MS	[17]
Chromium(VI), Molybdenum(VI)	Natural and bottled mineral waters	Type I, Teflon AF-2400, 5 m in length, 560 µm i.d., 800 µm o.d. (Biogeneral)	CCD array spectrometer (S2000, Ocean Optics). Cr(VI): Absorbance measured at 546 nm. Mo(VI): Absorbance measured at 630 nm. Both referenced to 700 nm	Cr(VI): 0.2 nM. Mo(VI): 0.6 nM	0–30 nM	Cr(VI): Mo(VI) concentrations on the order of ~0.1 µM produced no interference in the determination of 5 nM Cr(VI). Mo(VI): 5 nM Cr(VI) had a negligible effect on the determination of 5 nM Mo(VI), while 50 nM Cr(VI) decreases the signal of 5 nM Mo(VI) by ~11%	Samples prepared manually and injected into liquid waveguide with peristaltic pump at flow rate of 2 mL/min. Larger reagent blanks observed with Mo(VI) than Cr(VI) as the Mo complex adsorbs to the Teflon tubing. Interference can be masked with 1% (w/v) ascorbic acid and 0.05 M EDTA	[18]
Coloured Dissolved Organic Matter (CDOM)	Coastal waters	Type II, 0.5 m in length, 550 µm i.d. (World Precision Instruments)	Fibre-optic spectrometer (S2000, Ocean Optics, Inc.). Spectral range 350–1000 nm. Due to a difference in salinity between the seawater sample and the freshwater reference, there was a slight offset of the absorption spectra. This was removed by subtracting the average value of absorption between 690 and 700 nm from each absorption value in the spectrum			None reported	A real-time automated system was installed on a research vessel with water that was continuously pumped onboard. Seawater samples filtered (<0.2 µm) before analysis. Results comparable to those obtained using a Shimadzu Model 2501 UV-visible scanning benchtop spectrophotometer with a 10 cm path length cell	[19]

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Table 1 (*continued*)

Analyte	Matrix	LWCC properties	Detector	Limit of detection	Linear range	Interferences studied	Comments	Ref.
Coloured Dissolved Organic Matter (CDOM)	Surface water samples: inland, coastal and ocean waters	Multiple path length waveguide consisting of four optical paths: 2.0, 9.8, 49.3, and 204 cm (Ultrapath, WPI Inc., Sarasota, FL, USA) coupled to a single Teflon AF-2400 Type I cell, 2 mm i.d. (DuPont Fluoroproducts, DE, USA)	16-bit photodiode-array fibre-optic spectrometer (WAH6150, WPI Inc., Sarasota, FL). Absorbance measured using the waveguide between 370 and 725 nm. Absorbance measured using the Lambda-18 spectrophotometer between 300 and 750 nm. Absorbance of phenol red standards measured at 558 nm		Absorption of the phenol red standards measured using multiple path-length waveguide and Perkin Elemer Lambda-18 spectrophotometer was linear ($y = -0.11 + 14.42x$, $r^2 = 0.99$). Standards prepared in the range 0–8 μM.	None reported	Samples injected using a peristaltic pump. Absorption of phenol red standards measured by the waveguide showed linear response with all four path lengths. Results comparable to those obtained using a Perkin Elmer Lambda-18 dual-beam spectrophotometer	[20]
Coloured Dissolved Organic Matter (CDOM)	Seawater	Type II, 0.5 m in length, 550 μm i.d. (World Precision Instruments)	CCD fibre-optics-based spectrometer. Due to a difference in salinity between the seawater sample and the freshwater reference, there was a slight baseline offset of the absorption spectra. This was removed by subtracting the average value of absorption between 700 and 750 nm from each absorption value in the spectrum			None reported	Seawater samples filtered (<0.2 μm) and injected into waveguide using a syringe. Results compared to those obtained using a Shimadzu UV-2401PC double-beam spectrophotometer (spectral range 250–750 nm), a Bausch and Lomb Spectronic 2000 spectrophotometer and a Perkin Elmer Lambda-18 spectrophotometer. Most of the differences between the waveguide and spectrometers were due to differences in baseline corrections	[21]

Copper(II)	Estuarine waters	Type I, Teflon AF-2400, 1 m in length, 813 μm i.d., 1016 μm o.d. (Biogeneral)	CCD-based spectrometer (Ocean Optics). Absorbance measured at 484 nm with a reference at 675 nm	3 nM	0–180 nM	Stated that BDS has a high affinity and specificity for CuI.BDS has been shown to compete effectively with most inorganic and organic ligands including humic and tannic acids, so interferences are not expected to be a significant problem	Spectrophotometric elemental analysis system (SEAS) is a compact, autonomous liquid-waveguide device used for in situ measurements of Cu(II). Method evaluated using a certified copper reference solution (National Research Council of Canada, St. Lawrence, River Sample-4)	[22]
Copper(II)	Seawater, river water and commercial drinking water	Type I, Teflon AF-2400, 4.4 m in length, 560 μm i.d., 800 μm o.d. (Biogeneral)	Spectrophotometer (S2000, Ocean Optics). Absorbance measured at 484 nm with a reference at 660 nm	0.4 nM	0–160 nM	Stated that high affinity CuI ligands that can potentially compete with BDS e.g. CN^-, SCN^-, S_2O8^{2-}, EDTA are not likely to have sufficiently high concentrations in natural waters to interfere with Cu-BDS complexation	Accuracy of method was evaluated using a certified river reference solution (National Research Council of Canada (NRCC, SLRS-4). Sample mixed manually with reagents and injected after a 3.5-min reaction time using a peristaltic pump at a flow rate of 1.5 mL/min	[23]
Hydrogen peroxide	Atmosphere	Type I, Teflon AF-2400 tube, 5 cm in length, 0.74 mm i.d., 1.04 mm o.d. (Biogeneral, San Diego, CA)	Photodiode array spectrometer (Agilent 8453A). Absorbance measured at 450 nm	26 pptv	0–5 ppbv	No interference from MHP, and little interference from SO_2 or ozone	Gaseous hydrogen peroxide collected using a straight inlet Nafion membrane diffusion scrubber. First practical absorbance-based continuously-operating instrument sufficiently sensitive to measure ambient levels of gaseous hydrogen peroxide	[24]

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Table 1 (*continued*)

Analyte	Matrix	LWCC properties	Detector	Limit of detection	Linear range	Interferences studied	Comments	Ref.
Hydrogen sulphide	Aqueous solutions	Type I, Teflon AF-2400, 1.6 m in length, 560 μm i.d., 800 μm o.d. (Biogeneral)	CCD array spectrometer (S2000, Ocean Optics). Absorbance measured at 660 nm with a reference at 710 nm	Of the order of 5 nM	0–50 nM	None reported	Instrument capable of performing fluorimetric and spectrophotometric analyses on aqueous solutions	[25]
Iron(II)	Natural waters	Type II, Teflon AF-2400, 5 m in length (World Precision Instruments, Sarasota, Florida, USA)	Spectrophotometer			None reported	Effect of pH, $NaHCO_3$, temperature and salinity on the oxidation of Fe(II) investigated	[26]
Iron(II)	Natural waters	Type II, Teflon AF-2400, 2 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, Florida, USA)	Photodiode detector. Absorbance measured at 562 nm	0.1 nM	Fe(II): 0–50 nM. Fe(II+III): 0–60 nM	None reported	Gas-segmented continuous-flow method to measure Fe(II) and total dissolved Fe(II+III). Results were in good agreement with those obtained using the luminal-based chemiluminescence method	[27]
Iron(II)	Aqueous solutions	Type I, Teflon AF-2400, 4.47 m in length, 280 μm i.d., 530 μm o.d. (Biogeneral)	CCD array spectrometer (S1000-TR-1, Ocean Optics). Absorbance measured at 562 nm with a reference at 700 nm	0.2 nM	0.5–10 nM	None reported	Samples prepared manually and injected into liquid waveguide using peristaltic pump	[28]
Nitrate, Nitrite	Oligotrophic surface seawater	Type II, Teflon AF-2400, 2 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, Florida, USA)	Photodiode detector. Absorbance measured at 540 nm	During cruise: nitrate 2 nM, nitrite 0.1 nM	Nitrate: 0–>250 nM. Nitrite: 0–50 nM	None reported	Gas-segmented continuous-flow autoanalyser. Method used during a 1-month cruise	[10]

Nitrate, Nitrite	Natural waters	Type I, Teflon AF-2400, 4.5 m in length, 560 μm i.d., 800 μm o.d. (Biogeneral)	CCD array spectrometer (S2000, Ocean Optics). Absorbance measured at 540 nm with a reference at 700 nm	Nitrate: 1.5 nM. Nitrite: 0.5 nM.	0–30 nM	None reported	Samples prepared manually and injected into liquid waveguide with peristaltic pump at flow rate of 2 mL/min	[29]
Nitrate	Oligotrophic surface seawater	Type II, Teflon AF-2400, 2 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, Florida, USA)	Photodiode detector. Absorbance measured at 540 nm	2 nM	0–>250 nM	None reported	Gas-segmented continuous-flow autoanalyser used to study diurnal cycle of nitrate	[30]
Nitrate	Aqueous solutions	Type I, Teflon AF-2400, 203 mm in length	Fibre-optic spectrometer. Different wavelengths measured from 200–220 nm	22 μg/l at 220 nm	6–408 μg/l	None reported	UV sensor	[31]
Nitrate	Natural waters	Type I, Teflon AF-2400, 203 mm in length	Fibre-optic spectrometer. Measured at 220 nm	22 μg/l	13–405 μg/l	None reported	UV sensor. Also measured chlorine at 290 nm, limit of detection 26 μg/l	[32]
Nitrite	River water	Type I, Teflon-AF 1600, 80 cm in length, 0.45 mm i.d., 0.60 mm o.d.	Spectrometer (USB-2000, Ocean Optics Inc., USA)	2.1 nM	0–0.63 μM	None reported	Flow-injection-analysis system	[33]

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Table 1 (*continued*)

Analyte	Matrix	LWCC properties	Detector	Limit of detection	Linear range	Interferences studied	Comments	Ref.
Nitrite	Aqueous solutions	Type II, various lengths 0.5, 2, and 4 m, 550 μm i.d.	Photodiode. Absorbance measured at 520 nm	0.5 m = 6 nM, 2 m = 4 nM, 4 m = 2 nM	0.5 m = 0–4000 nM, 2 m = 0–1000 nM, 4 m = 0–600 nM	None reported	Incorporation of a LWCC into a flow-injection manifold. Three waveguide lengths tested: 0.5, 2 and 4 m. Necessary to use a surfactant to reduce back pressure	[9]
Nitrite	Seawater	Type I, Teflon AF-2400, 97 cm in length (Dupont)	Spectrophotometer (S-2000, Ocean Optics). Absorbance measured at 541 nm with a reference at 700 nm	1 nM	0–150 nM	None reported	Spectrophotometric elemental analysis system (SEAS) is a compact, autonomous device used for in situ measurements of nitrite	[34]
Nitrite	Seawater	Type I, Teflon AF-2400, 1 m in length, 0.81 mm i.d, 1.0 mm o.d. (Dupont)	CCD-based spectrometer. Absorbance measured at 540 nm with a reference at 710 nm	2.5 nM	0–125 nM	None reported	Spectrophotometric elemental analysis system (SEAS) is a compact, autonomous device used for in situ measurements of nitrite	[35]
Phosphate	Freshwater	Type II, Teflon AF, 1 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, FL)	Miniature fibre-optic spectrometer (S2000-FL, Ocean Optics, Dunedin, FL, USA). Absorbance measured at 710 nm with a reference at 447 nm	10 nM	10 nM – 1 μM	Silicate at a concentration of 2 mg/l seriously interfered, so 0.1% w/v tartaric acid added to mask the Si interference effectively	Flow-injection analysis coupled with a LWCC. Limit of detection increased to 11 nM with addition of 0.1% w/v tartaric acid	[36]

Phosphate	Natural waters	Type II, Teflon AF-1600, 2 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, FL)	Photodiode. Absorbance measured at 710 nm	0.5 nM	0–200 nM	Stated that to minimise silicate interference colour was developed at room temperature, no results given	Gas-segmented continuous-flow autoanalysers. Added SDS to ascorbic acid reagent to achieve a smooth flow with low baseline noise	[37]
Phosphate	Natural waters	Type II, Teflon AF-1600, 2 m in length, 550 μm i.d. (World Precision Instruments, Sarasota, FL)	Photodiode. Absorbance measured at 710 nm	0.5 nM	0–200 nM	Stated that, to minimise silicate interference, colour was developed at room temperature; no results given	Gas-segmented continuous-flow autoanalysers. Added SDS to ascorbic acid reagent to achieve a smooth flow with low baseline noise	[1]
Phytoplankton	Estuarine to offshore, oligotrophic waters	Type II, 0.5 m in length (World Precision Instruments)	Fibre-optic spectrometer (SD-2000, Ocean Optics). Resulting absorption spectra normalised to the mean absorption between 400 and 700 nm			None reported		[38]
Total inorganic carbon (TIC)	Riverine samples	Type I, Teflon AF-2400, 10 cm in length, 0.5 cm i.d., 2 cm o.d. (Biogeneral)	Spectrophotometer (Ocean Optics Inc.) and a Cary 400 spectrophotometer. Absorbance measured at 589 and 432 nm with a reference at 730 nm			None reported	Method involves CO_2 equilibration across the permeable wall of the waveguide. Spectrophotometric measurements of natural riverine samples in good agreement with measurements obtained using coulometry	[39]

Table 2. Applications of liquid-waveguide-capillary cells (LWCCs) with luminescence detection

Analyte	Matrix	LWCC properties	Detector	Limit of detection	Linear range	Interferences studied	Comments	Ref.
Ammonium	Aqueous solutions	Type I, Teflon AF-2400, 150 mm in length, 0.84 mm i.d., 1.04 mm o.d. (BioGeneral, San Diego, CA)	Miniature PMT (Hamamatsu H5784)	120–260 nM	0–120 μM at 60 μA, 0–12 μM at 10 μA	None reported	For trace determinations, a current level of 10 uA was chosen	[8]
Ammonium/ Ammonia	Aqueous solutions	Type I, Teflon AF-2400, 115 mm in length, 0.84 mm i.d., 1.04 mm o.d. (BioGeneral, San Diego, CA)	Blue-sensitive integrated photodiode-operational amplifier available in a TO-99 (OPT-301, Burr-Brown, Tucson, AZ). Fluorescence measured at 365 nm	35 nM	0–60 μM	None reported		[40]
Chlorophyll *a*, Quinine sulphate	Aqueous solutions	Type I, Teflon AF-2400, 1.6 m in length, 560 μm i.d., 800 μm o.d. (Biogeneral)	CCD array spectrometer (S2000, Ocean Optics). Chlorophyll *a*: Fluorescence measured at 671 nm, Quinine sulphate: Fluorescence measured at 489 nm	Chlorophyll *a*: 0.03 nM, Quinine sulphate: 0.06 nM	Chlorophyll *a*: 0.056–0.56 nM, Quinine sulphate: 0.31–6.14 nM	None reported	Instrument capable of performing fluorimetric and spectrophotometric analyses on aqueous solutions	[25]
Formaldehyde	Atmosphere	Type I, Teflon AF-2400 tube, 1.25 mm i.d. (Random Technologies, San Francisco, CA)	Miniature PMT (Hamamatsu H5784)	Aqueous formaldehyde: 10 nM. Gaseous formaldehyde: 30 pptv	Aqueous formaldehyde: 100 nM–5 μM. Gaseous formaldehyde: 0–25 ppbv	H_2O_2 interference reduced by adding H_2O_2 to the diffusion scrubber liquid. Response from a 0.21-μM aqueous formaldehyde sample and a 600-pptv gaseous sample was unaffected by addition of up to 50 μM H_2O_2, and up to 100 ppbv H_2O_2, respectively. However, the method raises the background, increasing the LOD	A new robust design of a thermostatted Nafion-membrane diffusion scrubber was developed for sampling atmospheric formaldehyde. Transverse illumination of waveguide. Instrument can be used for aqueous and gaseous samples. Instrument deployed in a field campaign	[41]

Hydrogen peroxide	Aqueous solutions	Type I, Teflon AF-2400, 50 cm in length, 0.56 mm i.d., 0.80 mm o.d. (Biogeneral Inc., San Diego, CA)	Fluorescence: Photodiode array spectrometer (CDI-PDA-512, Control Development Inc., South Bend, IN). Chemiluminescence: Miniature PMT (Hamamatsu H5784)	Fluorescence: 16 nM, Chemiluminescence: 4 nM	Fluorescence: 0–10 μM, Chemiluminescence: 0– 500 nM	None reported	Combined LWCC with spectrophotometry, chemiluminescence and fluorescence to provide an inexpensive portable FI instrument	[15]
Hydrogen peroxide	Atmosphere	Type I, Teflon AF-2400, 150 mm in length, 0.84 mm i.d., 1.04 mm o.d. (BioGeneral, San Diego, CA)	Miniature PMT (Hamamatsu H5784)	25 pptv	0–100 ppbv	No observable interference from NO_2 up to 200 ppbv. Little interference from SO_2 (1.43 pptv H_2O_2 loss per ppbv SO_2). Below 50 ppbv, there was no interference from ozone. Addition of 200 ppbv ozone to 1ppbv H_2O_2 standard increased response to a level equivalent to 2.1 ppbv H_2O_2	Gaseous hydrogen peroxide collected using a straight inlet Nafion-membrane diffusion scrubber	[13]
Hydrogen peroxide	Atmosphere	Type I, Teflon AF 2400, 1.25 mm i.d. (Random Technologies, San Francisco, CA)	Miniature PMT (Hamamatsu H5784)	Aqueous hydrogen peroxide: 11nM. Gaseous hydrogen peroxide: 13.5 pptv	Aqueous hydrogen peroxide: 0–100 μM. Gaseous hydrogen peroxide: 0–1 ppbv	There was a little interference from SO_2 (1.43 pptv H_2O_2 loss per ppbv SO_2) and ozone (1 ppbv ozone = 2.37 pptv H_2O_2).	Gaseous hydrogen peroxide collected using a straight inlet Nafion-membrane diffusion scrubber. Instrument can be used for gaseous and aqueous measurements. The system responds to H_2O_2 and hydroxymethyl hydroperoxide. Instrument deployed in a field campaign	[14]

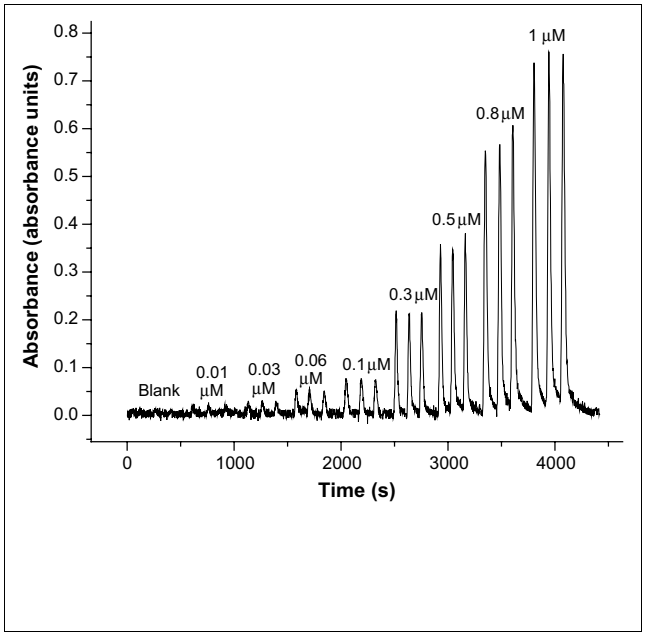
inner surfaces of the LWCC. The result of using a LWCC and on-line vacuum degassing was a stable baseline with an LOD improved by two orders of magnitude compared with a FI manifold incorporating a conventional 1-cm flow-through cell. The LOD achieved with the 4-m LWCC was 2 nM.

Nitrite in river water samples has also been determined using a Type I LWCC of 80-cm path length. The LOD was 2.1 nM, which is the same as the LOD previously obtained with the 4-m Type II LWCC, even though the path length was significantly different. This was probably due to using a Type I cell comprising Teflon AF-1600 that had direct contact with the sample [33].

A FI manifold for the determination of phosphate [42] was also adapted by replacing the 1-cm flow cell with a 1-m LWCC [36]. In order to ensure a stable baseline and a low LOD (10 nM), the manifold was modified by increasing the carrier flow rate and decreasing the reagent flow rates, improving the mixing by increasing the length of a reaction coil and increasing the sample volume (130–500 μL) to ensure that the sample zone completely filled the LWCC and hence eliminated RI effects.

A typical calibration trace for phosphate is shown in Fig. 4. The effect of silicate was systematically investigated and serious interference was observed with 2-mg/L Si at room temperature, but was overcome by masking the Si with 0.1% w/v tartaric acid. In contrast, using a gas-segmented continuous-flow method, Zhang and Chi reported no interference from silicate in the determination of phosphate at room temperature [1].

4.1.4. Gas sensors. The high gas permeability of Teflon AF makes it well suited as a gas sensor because it can be used as both the optical cell and the equilibration membrane. The first long path-length pCo_2 fibre-optic sensor using a gas-permeable Teflon AF LWCC was applicable to gas- and liquid-phase samples, and measurements were carried out at a single wavelength [12]. The same set-up was used for real-time measurement of pCo_2 in surface seawater with multiple wavelengths measured, two at the absorbance maxima of the acid and base forms of the indicator and a third as a reference wavelength to improve stability [11].



Other gases of environmental interest have also been studied using LWCCs combined with spectrophotometric detection. A continuous monitor that was sufficiently sensitive to measure ambient hydrogen peroxide in the atmosphere, with an LOD of 26 pptv, was developed using a 5-cm LWCC coupled with a spectrophotometer [24]. This was comparable with the LOD obtained using chemiluminescence detection with a 15-cm LWCC [13]. The method worked by converting gaseous hydrogen peroxide into the liquid phase using a Nafion diffusion scrubber and reacting the sample with a Ti(IV)-porphyrin complex to form a yellow-coloured solution [24].

4.1.5. Other applications. In addition to the use of LWCCs with spectrophotometric detection in segmented-flow and flow-injection analysers, in situ analysers and gas sensors, there have been a limited number of other applications (e.g., coloured dissolved organic matter (CDOM) absorbance spectra have been obtained using LWCCs [19–21]). A Type II LWCC (0.5 m length, 550 μm i.d.) was used for the measurement of CDOM in seawater [21] where the absorption measurements were referenced to pure freshwater. This method suffered from baseline offsets due to RI differences between the freshwater reference and the seawater sample, so the spectra had to be corrected. This technique was then automated to improve spatial and temporal resolution of sampling for CDOM, with the spectra again requiring correction for baseline offsets [19].

The work involving CDOM measurements led to discussion about the contrasting behaviour of Type I and Type II LWCCs [3,43]. Byrne and Kaltenbacher [3] suggested that the baseline-offset correction required for CDOM spectra would be solved simply by using a Type I LWCC, as light in a Type II LWCC would be trapped in the silica layer (RI 1.46) without being totally internally reflected. However, D'Sa and Steward [43] reported that the light would not be trapped and would be totally internally reflected at the Teflon AF-silica interface in a Type II waveguide. They also stated that Type II LWCCs are easier to clean and have less of a tendency to form bubbles.

LWCCs have also been used to determine iron in aqueous solutions [28], study the oxidation of nanomolar concentrations of Fe(II) with oxygen in natural waters [26], determine nitrite concentrations by direct

absorbance in the UV [31,32], and measure phytoplankton species in seawater [38].

4.2. Chemiluminescence and fluorescence detection

There are fewer reports of chemiluminescence and fluorescence detection combined with LWCC technology. Chemiluminescence has been combined with FI for the determination of ammonium in aqueous solutions [8]. In this study, the LWCC was able to act as both the mixing coil and the light-carrying conduit with LODs of 120–260 nM. Chemiluminescence has also been used for hydrogen peroxide determinations [13]. This utilised the same method as that reported by Li and Dasgupta [8], except that it incorporated a Nafion diffusion scrubber to collect the gaseous hydrogen peroxide. The hydrogen peroxide was then quantified by Co(II)-catalysed oxidation of alkaline luminol.

Hydrogen peroxide has also been determined using a portable, multi-functional FI instrument capable of determining aluminium and chromium by absorbance spectrophotometry and hydrogen peroxide by fluorescence by switching between the different detection modes, but it was not applied to real environmental samples [15]. With fluorescence detection, LWCCs are illuminated transversely. This combination has been used to determine ammonia/ammonium in aqueous solutions [40] and formaldehyde [41] and hydrogen peroxide [14] in atmospheric samples, with samples collected using a temperature-controlled Nafion diffusion scrubber [14].

Fluorescence detection has also been combined with absorbance detection for aqueous solutions by transversely illuminating the LWCC. Hydrogen sulphide was determined by spectrophotometry and chlorophyll *a* and quinine sulphate by fluorescence. The instrument worked by turning on a tungsten-halogen lamp and turning off a UV-excitation lamp in absorbance mode, and then, in fluorescence mode, turning off the halogen lamp with the UV lamp on to excite fluorophores in the liquid core [25].

5. Conclusions and future trends

The emerging potential of LWCC for the determination of chemical species at low concentrations has enhanced our capability to investigate environmental processes and monitor environmental systems (e.g., the determination of macronutrients in oligotrophic waters). This is because conventional spectroscopic techniques do not have the required sensitivity to determine important analytes, such as macronutrients, at environmentally relevant (e.g., nanomolar) concentrations.

LWCCs are versatile and can be used:

- as stand-alone systems with manual injection;
- incorporated into a flow system;
- as gas sensors due to their high gas permeability; and,
- coupled with absorbance, chemiluminescence, and fluorescence detectors.

They can also be used in situ to obtain detailed information with high temporal resolution (<5 min sampling frequency) and map real-time concentrations of transient chemical species in natural waters. Although some work has been done in this area, as shown in Tables 1 and 2, there is still a need for the wider use of such methods to determine nM and sub-nM concentrations of trace elements (e.g. iron) and macronutrients in aquatic environments to enhance our understanding of global processes. Selective detection of specific redox states (e.g., Fe(II)) and individual macronutrient species (e.g., phytase hydrolysable phosphorus) will further enhance the demand for sensitive methods incorporating LWCCs.

There is also a challenge to interface LWCCs with ''front-end'' separation techniques to provide enhanced analytical information for complex environmental matrices. For example, we are currently investigating the feasibility of coupling a LWCC detection system with flow-field-flow fractionation (FlFFF) to determine the phosphorus associated with different sizes of material in complex soil suspensions and agricultural run-off waters. This separation technique greatly dilutes an injected sample [44,45], and combining with LWCC technology could provide a complete physico-chemical profile of the distribution of the element in aquatic colloidal material.

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