Marine Chemistry 150 (2013) 19–24

Contents lists available at SciVerse ScienceDirect

Marine Chemistry

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

Purification of meta-cresol purple and cresol red by flash chromatography: Procedures for ensuring accurate spectrophotometric seawater pH measurements

Mark C. Patsavas, Robert H. Byrne *, Xuewu Liu

College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, FL 33701, USA

ARTICLE INFO

Article history:
Received 6 September 2012
Received in revised form 18 January 2013
Accepted 21 January 2013
Available online 9 February 2013

Keywords:
Spectroscopic techniques
Meta-cresol purple
Cresol red
Sulphonephthalein
Seawater
pH
Indicators
Purification
Ocean acidification

ABSTRACT

Impurities in sulphonephthalein indicator salts can result in significant errors in seawater pH determinations. To ensure suitable measurement accuracy and intercomparability on a global basis, impurities must be removed from all indicators used for oceanographic CO_2 system analyses. Previous work has described an effective HPLC (high-performance liquid chromatography) procedure for purification of meta-cresol purple, but the technique is labor-intensive, with each HPLC run producing only a small batch of purified indicator. This work describes the use of flash chromatography to more efficiently produce large batches of purified meta-cresol purple (mCP) and cresol red (CR), the preferred indicators for direct water column determinations of seawater pH.

Several batches of unrefined mCP and CR of independent origin were prepared by flash chromatography. Indicator purity was then assessed in two ways: by (a) HPLC verification and (b) pH measurements of highly buffered solutions. HPLC chromatograms of the various flash-prepared mCPs indicated that the process did not always result in a completely pure product. In terms of performance, however – i.e., pH measurements of highly buffered solutions – no differences were observed between an HPLC-purified reference mCP and the flash-purified mCPs. HPLC examination of the flash-purified CRs indicated that every product was free of detectable impurities. No differences were seen in comparative pH measurements made with the purified CRs. The flash chromatography procedures outlined in this work are suitable for producing bulk quantities of mCP and CR for use in high-precision spectrophotometric pH measurements in seawater.

1. Introduction

High-precision carbon system measurements are required to document the changes in seawater chemistry that accompany the oceanic uptake of anthropogenic atmospheric CO_2 (Clayton et al., 1995; McElligott et al., 1998; Millero, 2007). Prior work has shown that spectrophotometric measurements of pH are simple, fast, and precise (Bellerby et al., 2002; Clayton and Byrne, 1993; Tapp et al., 2000). However, the accuracy of spectrophotometric pH measurements can be adversely affected by impurities in the sulphonephthalein indicator dyes that are used for such measurements (Yao et al., 2007).

The indicator meta-cresol purple (mCP) is currently well suited for water column measurements of seawater pH in most ocean areas (Clayton and Byrne, 1993), but as the upper ocean continues to acidify (Wolf-Gladrow et al., 1999) an indicator with an indicating range slightly lower than that of mCP will be required. In this case, and in regions where the seawater pH is already low (e.g., the Arctic Ocean or Southern Ocean), cresol red (CR), which has a pK lower than that of mCP, is a suitable choice.

The use of unrefined mCP for spectrophotometric measurements can result in systematic errors as large as 0.018 at typical surface ocean pH values (Liu et al., 2011). Errors due to the use of unrefined CR have not been quantified, but offsets of similarly large magnitude could reasonably be expected. Purification is therefore recommended for both indicators.

A method for purifying mCP by high-performance liquid chromatography (HPLC) has been previously established (Liu et al., 2011), but the method lacks large-scale production capability. The present work focuses on developing methods for producing large batches of purified mCP and CR for use in high-precision spectrophotometric seawater pH measurements.

2. Methods

2.1. Reagents

Sodium salts of mCP and CR of independent origin were used for the purification study. The mCP salts were from Acros (Lot# A0182569), Aldrich (Lot# 07005HH), and Ricca (Lot# 4003124). The CR salts were from Acros (Lot# A0255180), Alfa Aesar (Lot# L09754), Biosynth (Lot# 220307/11), MP Bio (Lot# 2045 F), and Ricca (Lot# 2011271). Sodium chloride, TRIS (tris(hydroxymethyl)-aminomethane), EPPS

* Corresponding author. Tel.: +1 727 553 1508; fax: +1 727 553 1189.
E-mail address: rhbyrne@usf.edu (R.H. Byrne).

0304-4203/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.marchem.2013.01.004

(3-[4-(2-hydroxyethyl)-piperazin-1-yl]propane-1-sulfonic acid), MOPS (3-morpholinopropane-1-sulfonic acid), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), trifluoroacetic acid (TFA), and acetonitrile (MeCN, HPLC grade) were obtained from Fisher Scientific and were used without further purification.

A series of highly buffered solutions was prepared by adding 0.08 mol of TRIS, EPPS, MOPS, or HEPES to 0.04 mol of either HCl (TRIS, HEPES) or NaOH (EPPS, MOPS). The solutions were brought to 0.7 mol $(kg{-}H_2O)^{-1}$ ionic strength by the addition of NaCl.

2.2. Determination of the effects of CR impurities on seawater pH measurements

Each of the five unrefined (i.e., off-the-shelf) cresol reds was used to independently measure the pH of the highly buffered solutions described in Section 2.1. For each pH measurement, the buffer solution was weighed (~102.3 g) into a custom-made quartz wide-top 10 cm pathlength spectrophotometric cell (NSG Precision Cells, Inc.). The cell was fitted with a motor-driven stirrer and a lid with a built-in space for a digital thermometer probe. Absorbance measurements were made using a Varian Cary 400 UV–Vis spectrophotometer fitted with a water-jacketed cell holder connected to a recirculating water bath. The solution temperature was maintained at 25.00 ± 0.03 °C and monitored with a VWR digital thermometer (accuracy ± 0.01 °C).

After a blank spectrum was obtained, indicator (25 μL of 10 mM stock solution) was added to the cell. Absorbance was recorded for six replicate scans, which were subsequently averaged. Triplicate measurements were made at each pH point for each indicator batch. Solution pH values were calculated following published protocols for CR (Byrne and Breland, 1989).

2.3. Development of the flash purification procedure

For chromatographic purification, it is generally useful to consult the literature for guidance in selecting a column and a mobile phase appropriate to the molecular weight, solubility, and hydrophobic character of the analyte (Snyder et al., 1997). However, no protocols have been published for flash purification of mCP or CR. Empirical determination of an appropriate procedure was therefore one of the first steps of this work. The solvent system consisted of water, MeCN, and trifluoroacetic acid (TFA). Investigative separations were first conducted using HPLC under isocratic (constant concentration) conditions. The organic phase concentration was determined by trial and error, first starting at 30% MeCN with 0.05% TFA and then increasing by 5% MeCN increments until the organic phase concentration was 80% MeCN. For MeCN concentrations equal to or greater than 80%, no separation of impurities was achieved. The optimal concentration of the TFA mobile phase modifier was determined by incrementally increasing the concentration to attain a general understanding of how the addition of TFA affected the main peak retention time and separation of related impurities.

For the flash chromatographic procedure, a gradient mobile phase profile was used. For mCP, solvent A_{mCP} was water and 0.05% trifluoroacetic acid (TFA), and solvent B_{mCP} was acetonitrile (MeCN) and 0.05% TFA. For CR, solvent A_{CR} was water and 0.2% TFA, and solvent B_{CR} was MeCN and 0.2% TFA. For both indicators, the gradient profile was determined by manually increasing the percentage of solvent B until an impurity began to elute from the top of the column. The percentage of solvent B was then held constant until the impurity was entirely eluted from the column. The process was repeated for all impurities and the pure fraction. After a general gradient was determined, the gradient was optimized to increase efficiency. Table 1 summarizes the gradient steps for mCP and CR. This optimized procedure was used for all subsequent work.

Table 1
Gradient separation procedure for meta-cresol purple and cresol red.

mCP[a]		CR[b]	
Time (min)	% solvent B_{mCP}	Time (min)	% solvent B_{CR}
0–4	5	0–5	5–21
4–5	5–10	5–18	21
5–8	10	18–21	100
8–10	10–30	21–25	5
10–14	30		
14–18	100		
18–21	5		

[a] Solvent A_{mCP} was water and 0.05% TFA, and solvent B_{mCP} was MeCN and 0.05% TFA.
[b] Solvent A_{CR} was water and 0.2% TFA, and solvent B_{CR} was MeCN and 0.2% TFA.

2.4. Flash purification of meta-cresol purple and cresol red

Batches of mCP and CR were purified using a Teledyne ISCO Combiflash Rf-200 UV–vis automated flash chromatography system. This system includes a touch screen controller capable of controlling gradients with up to four solvents, two positive displacement pumps (5–200 mL min^{-1}), an internal fraction collector, a solvent waste management system, and a UV–vis detector.

The flash chromatography column was a 150 g reversed phase Teledyne ISCO Redi*Sep* Gold C18Aq with an average particle size of 20–40 μm. This column prevents C18 chain collapse in highly aqueous conditions and was specifically designed for separation of water-soluble dyes. For storage periods longer than a few hours, a solution of 80% MeCN and 20% water was pumped through the column for 4–6 column volumes; the column was then removed from the system, capped, and stored.

Multiple stock solutions of unrefined mCP and CR were prepared by dissolving the sodium salts in MilliQ water (70 mM). The flash column was removed from the Combiflash system, 25 mL of stock solution was injected into the top of the column from a plastic syringe, and the column was returned to the system. The purification procedure then proceeded according to Table 1. (A detailed description of the results of our method development is given in Section 3.2.) The purified solid (acid forms) of the indicators was obtained by vacuum rotary evaporation of the eluate (indicator in a solution of water, TFA, and MeCN) using a Buchi Rotavapor-R. The evaporation flask was partially submerged in a 40 °C waterbath. One batch of unrefined indicator (MP Bio Lot# 2054F) was excluded from purification because the salt had formed a white precipitate in solution, and when the solution was injected into the flash column, the column became clogged and over-pressurized.

Finally, stock solutions of purified mCP and CR were prepared for use in spectrophotometric pH analyses. The acid form of each indicator batch

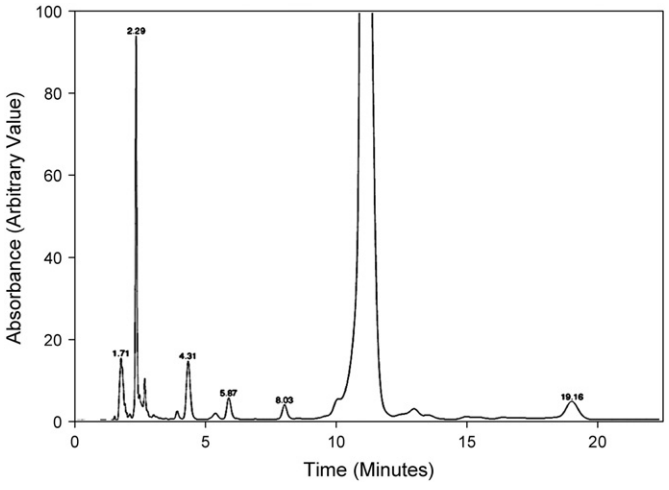

Fig. 1. HPLC chromatogram of unrefined cresol red.