



Green chemical processing in the teaching laboratory: a convenient liquid CO₂ extraction of natural products†

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A unique liquid CO₂ extraction laboratory developed for a greener organic teaching lab curriculum provides an effective, inexpensive, and convenient procedure for teaching natural products extraction concepts and techniques using modern green extraction technology. The procedure is appropriate for the teaching lab, does not require any special equipment, and allows the students to see the phase change and extraction as they occur. Students learn extraction and spectroscopic analysis skills, are exposed to a dramatic visual example of phase change, and are introduced to commercially successful green chemical processing with CO₂.

Introduction

Although there have been many advances in green chemistry in the industrial and research fields, integration of these concepts into the teaching environment is still in its infancy. This may be due to the limited availability of educational materials that illustrate the methods, techniques, and principles of green chemistry. To address this problem, we developed a new green organic laboratory curriculum to teach fundamental chemical concepts and techniques along with the tools and strategies of green chemistry.^{2–6} Integration of these goals into the laboratory curriculum required the development of a broad collection of experiments that work well in the laboratory, improve the safety of the laboratory environment, and modernize the curriculum through the introduction of state-of-the-art methods. A green organic chemistry laboratory textbook² and several manuscripts describe the criteria and process for greening experiments.^{3,4,6}

Here we describe a new laboratory exercise that uses liquid CO₂ to extract D-limonene from citrus rind. This safe and convenient procedure successfully addresses the diverse goals of green chemistry experiment development by simultaneously teaching practical techniques, fundamental chemical concepts, and green chemistry applications. In this experiment, the commonly taught concepts of natural product extraction and phase transitions are demonstrated through

a novel procedure employing liquid CO₂ as a green solvent. The experiment introduces modern green chemical approaches through discussions of industrial use of liquid CO₂ as a green replacement solvent (*e.g.*, dry cleaning) and supercritical fluid extraction (SFE) as an example of a successful commercial green process (*e.g.*, decaffeination of coffee). Replacement of traditional natural product extraction experiments through incorporation of this green chemistry research and technology promotes an understanding of the current practice of green chemical methods. This exciting, convenient, and straightforward procedure offers an opportunity to teach core organic chemistry concepts and skills in the context of applicable green chemistry.

In this report, a short review of the background and current industrial uses of CO₂ and the relevance of this technology to the teaching laboratory are described. The new laboratory procedure is summarized in the Experimental section.⁷ In the Results and discussion section, both the chemical and green lessons of this process are discussed and extensions are proposed.

Supercritical and liquid CO₂

At pressures above ambient, carbon dioxide can exist in forms usable as a solvent (*i.e.* as a liquid or supercritical fluid). As shown in the phase diagram in Fig. 1, CO₂ is a liquid under relatively mild temperatures and pressures, in the ranges of –56.6 to 31.0 °C and 5.2 to 73.8 bar. Supercritical carbon dioxide (scCO₂) is produced at temperatures higher than the critical temperature (31.0 °C) and between the critical pressure (73.8 bar) and extremely high pressures (approximately 10⁴ bar). Supercritical fluids have no distinct liquid or vapor phase but retain

properties of each. ScCO₂ is especially beneficial when used as a solvent in selective extraction processes. The gas-like properties, such as very low surface tension and viscosity, allow the solvent to penetrate into the substrate, while the liquid-like properties solubilize compounds and remove them from the substrate. Small changes in pressure or temperature alter the bulk density of the fluid leading to increased or decreased solubility of various compounds. In this way, the use of supercritical fluids can allow for control of separations of materials. Through manipulation of temperature and pressure conditions within accessible ranges, both the phase and properties of CO₂ can be easily controlled.

During the past two decades, technical advances have been made in the industrial use of supercritical and liquid carbon dioxide in place of organic solvents.^{8–10} CO₂ is useful as a green alternative solvent because it provides environmental and safety advantages: it is nonflammable, relatively nontoxic, readily available, and environmentally benign. Processing with CO₂ also poses minimal hazard in the event of unintentional release or residual solvent in the product. Although CO₂ is a greenhouse gas, when used as a solvent it is captured and employed, not generated, resulting in no net environmental harm. Additionally, a closed loop system can be used to compress the gas in order to use the supercritical fluid or liquid in processing, depressurize the solvent for separation of dissolved compounds, and recompress the CO₂ to begin the cycle again. CO₂ extraction processes can also be run at relatively constant pressure when liquid–liquid extraction against water is used for product recovery. These loop systems allow for easy recovery and recycling of the pure solvent.

†Electronic supplementary information (ESI) available: experimental procedure (including instructors' notes, student handout, and demonstration procedure) and movie clip of extraction process. See <http://www.rsc.org/suppdata/gc/b4/b405810k/>

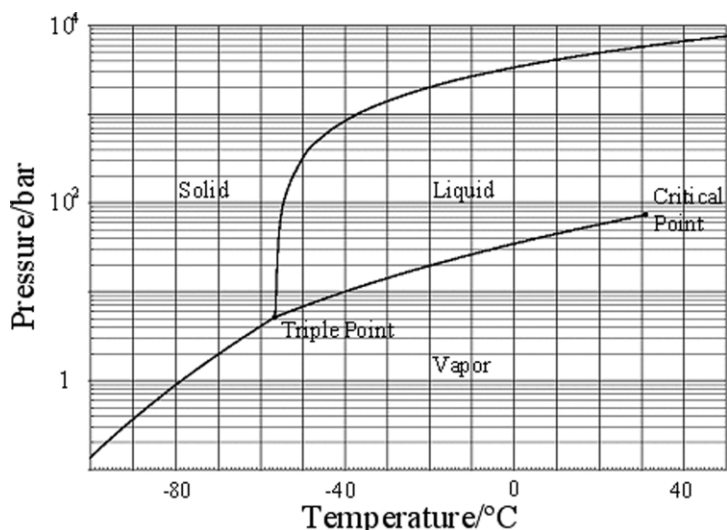


Fig. 1 The temperature–pressure diagram for carbon dioxide clearly indicates the pressures and temperatures for the phase transformations, triple point, and critical point. Adapted and used with permission from ChemicalLogic Corporation.¹

Applications of CO₂ as an alternative solvent

Large-scale CO₂ processing has had commercial success in many separation and extraction processes.^{8,9} The tunable solubility properties, low toxicity, and ease of removal of CO₂ have led to well established scCO₂ technology for the extraction of various food products, including essential oils and hops, and for the decaffeination of coffee and tea. The mild conditions necessary for extraction and absence of residual solvent result in superior products and have motivated an industrial shift to CO₂ from hazardous solvent extractions or steam distillations. The oil products from scCO₂ extraction processes are of higher purity and contain no thermal degradation products. The ability to influence solubility of compounds through variations in temperature and pressure has resulted in enhanced extraction of desired compounds from natural products and in the ability to enrich oils during post-extraction treatment with CO₂.^{11,12} ScCO₂ has also been used in other processes including analytical extractions and chromatography, metal degreasing, and textile dyeing.

A commitment to replacing hazardous solvents and improving environmental footprints has led to many new green methods of materials synthesis and processing with CO₂.^{8,9,13} Carbon dioxide is relatively inert, is resistant to oxidation, cannot serve as a chain transfer agent, and provides for tunable miscibility. These chemical advantages have led to an increasing number of industrial-scale reactions which use CO₂. Carbon dioxide has been employed in the synthesis of polymers such as DuPont™ Teflon® fluoropolymer resins and for commercial-scale hydrogenation and oxidation reactions.⁸ The UNICARB® VOC

Reduction Process uses scCO₂ as the carrier and atomizing agent for spraying paints and coatings.⁹ The variable solubility permits the use of CO₂ in the formation of micron-sized particles, technology which has been employed in the synthesis of inhalable medications.¹⁴ In addition to these demonstrated uses for CO₂, current research is also investigating its applicability in the microelectronics industry, in catalysis reactions, and as a simultaneous reaction medium and raw material.¹³

Although most industrial applications use supercritical CO₂, liquid phase carbon dioxide has also proven effective. The wide range of lower temperatures and pressures offers flexibility in the design of processes using liquid CO₂. The mild conditions prevent degradation of products. As with scCO₂, ideal density, viscosity, and surface tension can be obtained through manipulation of pressure and temperature. Liquid CO₂ has been employed extensively as an industrial solvent for the extraction of essential oils¹⁵ and in new greener methods of dry cleaning.^{8,9}

To date, a convenient liquid CO₂ extraction method using standard laboratory materials has not been reported. The laboratory experience described here brings the liquid CO₂ extraction process into the teaching or research laboratory in an inexpensive, effective, and accessible manner. It offers an opportunity for students to learn extraction techniques, observe striking phase changes, and appreciate the benefits of using greener chemical methods. This carbon dioxide extraction procedure provides a convenient drop-in replacement for currently used natural product steam distillation or solvent extraction laboratories.

Experimental

Dry ice sublimates at atmospheric pressure and temperatures above $-78\text{ }^{\circ}\text{C}$. If the CO₂ is sealed in a vessel during sublimation, the internal pressure in the vessel increases. After the temperature and pressure have increased sufficiently, liquid carbon dioxide forms. Due to this accessible phase change, carbon dioxide can be used for bench top extraction processes. In this experiment (see Fig. 2 and ESI†), approximately two and a half grams of grated orange peel and a wire and filter paper or metal screen solid trap are placed in a 15 mL polypropylene centrifuge tube with plug seal cap (Corning catalog #430052).¹⁶ The centrifuge tube is completely filled with crushed dry ice, capped tightly, and dropped (tapered end down) into a plastic cylinder or polycarbonate bottle which is half-filled with warm (40–50 °C) tap water.

As pressure builds in the tube, gas escapes slowly through the threading of the cap.¹⁷ After approximately fifteen seconds, the solid begins to melt, and liquid CO₂ appears in the tube. Solid, liquid, and gas phases are visible in the tube for a short period of time.¹⁸ The liquid boils, and gas escapes for almost three minutes. During this time, the liquid CO₂ moves through the solid, extracts the oil from the orange rind, and collects in the bottom of the tube. The solid trap successfully prevents the orange rind from moving into the tip of the tube during extraction because the wire coils are supported by the sides of the centrifuge tube at the point where it narrows. After the extraction solvent completely evaporates, isolated product remains in the tip of the tube. Once the liquid has stopped bubbling and gas is no longer escaping, the centrifuge tube is removed from the cylinder with tweezers, and the extraction process is repeated by refilling the tube containing the orange rind and solid trap with dry ice, recapping it, and replacing it in the cylinder. Two or three extraction cycles result in isolation of approximately 0.1 mL of pale yellow oil. Typical yields are comparable to organic solvent extraction or cold pressing (1–2% recovery, based upon initial mass of rind used during extraction).¹⁹ The extracted product is predominantly D-limonene by ¹H NMR and IR analysis and 97% D-limonene as indicated by GC-MS.

Results and discussion

The inspiration for this laboratory came from a desire to develop a reliable, convenient, inexpensive, and safe approach to CO₂ extraction that could be performed easily in any teaching laboratory. A secondary goal was to allow students to visually observe the phase changes of CO₂. Several liquid and supercritical CO₂

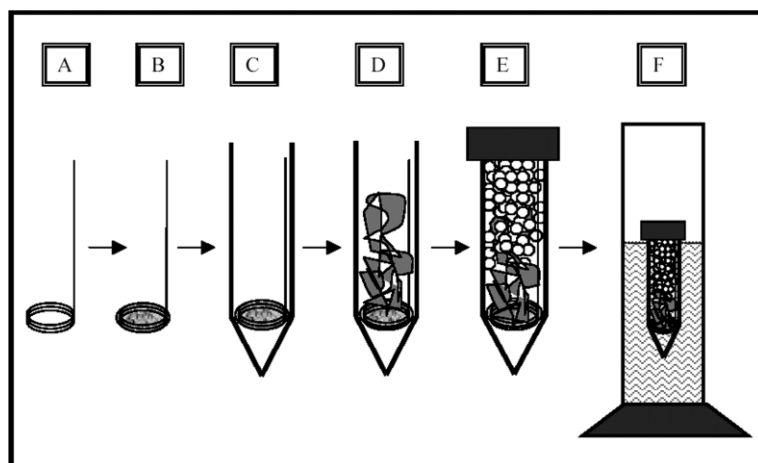
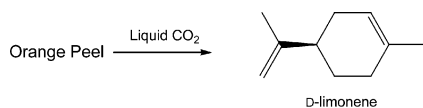


Fig. 2 Illustration of the liquid CO₂ extraction procedure. A solid trap is constructed by (A) bending copper wire into coils and a handle, (B) placing filter paper or metal screen between the wire coils, and (C) placing the solid trap in a centrifuge tube. For extraction, (D) grated orange peel is placed in the tube, and (E) the tube is filled with crushed dry ice and sealed with a cap. (F) The prepared centrifuge tube is placed in the water in the graduated cylinder, and the liquefaction and extraction occur over the following three minutes.

procedures have been developed previously, but these methods show the phase change for a brief time and do not allow for extraction, limit visualization of the phase transitions, or require expensive extraction equipment.^{20–22} We sought to make carbon dioxide extraction of natural products widely accessible and lengthen the observation period of phase changes, while maintaining a high degree of safety. The following sections detail the core organic laboratory concepts and skills as well as the green lessons taught through this new approach. Although the scope of this report is limited to a summary of the laboratory experience, more specific information and detailed instructions are included in the ESI.†

Natural products extraction and spectroscopy

Organic laboratory courses often include a natural product extraction in order to introduce solid/liquid extraction methods and the chemistry of the terpene compounds. The most commonly used methods are those of steam distillation and organic solvent extraction. In the procedure described herein, the techniques of liquid/solid extraction are taught through repeated extractions of orange peel with carbon dioxide (see Scheme 1). The introduction of CO₂ extraction into the curriculum allows students to use a green



Scheme 1 Extraction of D-limonene from grated orange rind using liquid CO₂ as a green extraction solvent. The product is 97% D-limonene and has no solvent residue.

solvent and to compare and contrast separation techniques. The practical and reliable procedure provides reproducible results and isolable yields of pure product which are comparable to those of other extraction methods. The volume of product collected is large enough for analysis by spectroscopic methods, and the use of ¹H NMR and IR spectroscopy provides opportunities for detailed analysis of complex spectra. Gas chromatography-mass spectroscopy can be used to compare the purity of samples generated by different extraction methods or to evaluate the composition of oils from different citrus fruits.

Phase transitions

Changes in physical states of compounds, the effects of temperature and pressure on these states, and their representation with phase diagrams are discussed often in chemistry courses. The opportunity to introduce these concepts in an easy and visual manner is a challenge addressed by few laboratory materials.^{20,21} Students are familiar with the sublimation of CO₂ and with the use of CO₂ in fire extinguishers and fountain drinks. Carbon dioxide provides accessible phase changes requiring low temperatures and relatively low pressures. In particular, the liquid state is accessible through slight increases in pressure. During this laboratory experiment, the solid dry ice melts quickly when appropriate pressure and temperature conditions are reached. For at least one minute, solid, liquid and gas phases are visible. The transparent plastic extraction vessels and containment cylinders used in this procedure allow the students to observe these phase changes safely and easily.

Green messages

In this laboratory, there are opportunities for discussions of many green chemistry principles.²³ Primarily, the focus is on prevention of waste and using safer solvents. Students are encouraged to consider the effects of solvent choice and extraction method on the extraction process and product. Using CO₂ as a solvent presents no risk to human health or the environment, and the extraction is as effective as extraction with other commonly used solvents. Both solvent extraction and steam distillation procedures produce significant amounts of solvent waste for very little product, but there is no solvent waste when carbon dioxide is used. The product of this extraction exhibits higher purity due to the absence of solvent residue. Students also are exposed to a green chemical process which has been widely incorporated into industrial practice. This view of green chemistry as an active and applicable set of principles improves the students' perception of chemistry and provides beneficial preparation for students who may become chemists in industrial or academic settings.

Extensions

Due to the pressure limitations of the simple equipment used in this experiment, extractions with this procedure are limited to those that can be performed in liquid CO₂. Specially-designed equipment is required to extend to supercritical fluid extractions (SFE). Teaching laboratory instructors must balance the need for an inexpensive and convenient procedure with the opportunity to demonstrate the versatility of CO₂ extraction. While lab-scale supercritical fluid extractors are available, they are expensive. If financially feasible, introducing SFE technology into the teaching laboratory through the use of supercritical fluid equipment would provide opportunities for extraction of a wide variety of compounds and improved quantitative analysis.

Extensions of this laboratory exercise include further exploration of the principles and practice of green chemistry. This procedure can be used to help students improve their understanding of the concepts of cleaner processing through the evaluation of yields, purity, waste generated, and energy costs of different extraction methods.² Through reading scientific literature, students can explore the current trends of industrial processing with CO₂. Discussion of the benefits and difficulties of using CO₂ as a green solvent can provide students with an awareness of the challenges involved in solvent replacement.

Although supercritical and liquid CO₂ have been used to extract natural products from coffee, hops, and many fruits,

flowers, and spices,¹⁵ this laboratory procedure has only successfully extracted natural products from citrus rinds. Modification of the extraction conditions to include a larger amount of substrate are required to allow for extraction of materials of lower density, and grinding or other processing may be necessary when introducing large solid matrices.

This lab is readily adapted as a classroom demonstration. Visibility is improved, and safety is enhanced by placing the centrifuge tube in water in a large polycarbonate cylinder. Using this apparatus, students may view the demonstration at closer range. The demonstration also may be projected using a video camera and projector.²⁴

Conclusions

The liquid CO₂ extraction laboratory described herein incorporates modern green chemistry into the organic teaching environment in a visible and exciting manner. Equally important, this procedure fits the constraints of the teaching laboratory, including those of time, safety, effectiveness, affordability, and convenience. The one to two hour procedure, including all preparation, extraction, and analysis, can be performed on its own or conducted concurrently with another experiment. In this laboratory exercise, all chemical hazards of traditional extraction procedures have been removed, and laboratory conditions provide for safe viewing of the phase changes and extraction. All required materials are readily available and inexpensive. Unlike steam distillation or organic solvent extraction procedures, there is no waste disposal cost with CO₂ extraction.²⁵ Comparisons of product recovery and purity, generation of waste, and risk to students and the environment indicate that this procedure provides the greenest natural product extraction laboratory currently available.²⁶ The convenient, rapid liquid CO₂ natural product extraction is based on the foundation of commercially successful green chemical processing. Through the application of principles and strategies of green chemistry and instruction in practical techniques and concepts, this drop-in replacement natural product extraction laboratory exercise easily teaches fundamental chemical

lessons and successfully incorporates green chemistry into the teaching laboratory.

Acknowledgements

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- 16 This procedure has only been successfully tested with centrifuge tubes of this brand and part number. The caps of larger Corning tubes (50 mL) did not withstand the pressure. The tubes must withstand temperatures from –78 to 50 °C and pressures from 1 to at least 6 atm. For safety reasons, the ability to withstand higher pressures is desired.
- 17 Under experimental conditions, carbon dioxide gas leaks from the centrifuge tube at an average rate of 2.5 g min⁻¹.
- 18 The simple apparatus used in this experiment does not allow for accurate determination of temperature and pressure conditions under which the extraction is occurring. Although the system is not at equilibrium, the phase diagram and physical observations can be used to estimate the conditions in the tube. Observation of both the gas/liquid and solid/liquid interfaces indicates that locally the temperature is between the temperature at the triple point (–56.6 °C) and the critical temperature (31.1 °C). Formation of ice on the surface of the tube further brackets the temperature to between –56.6 and 0 °C. The pressure is passively regulated by the leaking from the tube and, due to the liquefaction, is assumed to be above the pressure at the triple point (5.2 bar) but below the pressure that would induce tube rupture. These observations indicate that conditions approach those of the triple point.
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- 24 Detailed demonstration procedures are included in the ESI.
- 25 Cost per student is approximately \$1.00 for laboratory materials including centrifuge tubes, dry ice, and citrus fruit. Plastic containment cylinders can be purchased or constructed for \$2–3 each. One cylinder per student per lab period will be required. Further details are provided in the ESI.
- 26 Product recovery ranges from 1–2% based on initial mass of rind used. GC-MS data of student-performed steam distillation, pentane extraction, and carbon dioxide extraction are available in the ESI.