Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Historical Article

Lipids, Chloroform, and Their Intertwined Histories

Carlos A. Ramírez

Department of Chemical Engineering, University of Puerto Rico, Mayagüez, Puerto Rico
Email: carlos.ramirez9@upr.edu

Abstract. Lipids and their fatty acid constituents, in particular, have been the subject of academic and industrial research initiatives since their isolation by Michel-Eugène Chevreul in 1813. Fatty acids can be saturated or unsaturated, their physical properties depending on the aliphatic chain length and degree of saturation. They constitute the building blocks of many lipid groups like triglycerides and phospholipids; are key additives in commercial foods, pharmaceuticals, and cosmetics; and can cross cell membranes. Chloroform was synthesized in 1831 by Samuel Guthrie and has had a tortuous history of interactions with mankind: from an anesthetic in obstetrics, dentistry, and surgery, to being labeled as a potential carcinogen in the 1970s. It has also had important nonmedical applications such as in chemical engineering mass transfer systems designed to estimate binary gas diffusion coefficients. Although chemically dissimilar, lipids and chloroform intertwined their scientific paths through the work of Jordi Folch and associates in the 1940s-1950s, in which many lipid-based brain molecules were isolated and characterized. This article outlines the separate histories of lipids and chloroform, and those research initiatives in which they have acted synergistically. The narrative covers the interplay of chemical compounds with different historical backgrounds, but with physical properties which continue to foster their interaction.

Keywords: lipids, fatty acids, cell membrane structure and function, chloroform synthesis and uses, lipid-based brain tissue components.

OPEN ACCESS

Citation: Ramírez C.A. (2022) Lipids, Chloroform, and Their Intertwined Histories. *Substantia* 6(1): 133-143. doi: 10.36253/Substantia-1498

Received: Nov 11, 2021

Revised: Jan 04, 2022

Just Accepted Online: Jan 10, 2022

Published: Mar 07, 2022

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

I. INTRODUCTION

Modern-day students and young researchers sometimes fail to identify events in one discipline that can help explain similar phenomena in another. They also have difficulty envisioning how materials with different physicochemical properties can lead to common applications. It is precisely this latter problem that serves as the goal of the present article. Lipids and chloroform have long, separate histories. They have also shared scientific pathways for many decades, a fact likely unknown to most readers.

As the initial step of an experimental research project involving a specific class of lipids and chloroform, their individual histories were scrutinized. Their isolation, characterization, and synthesis were thoroughly studied, in many cases going back to the original published sources in the 19th Century.

It is our goal to summarize the historical scientific highlights of lipids (fatty acids in particular) and chloroform, leading to their combined usage to this day. Their individual histories will be addressed separately, while their intertwined pathways will be covered in the last section. The best example of the synergistic use of lipids and chloroform is that their physical and chemical properties served to determine the structure and function of new families of mammalian brain tissue components.

II. LIPIDS AND THEIR FATTY ACID CONSTITUENTS

Biochemistry is the science dealing with the chemistry of life, as well as the title of a timeless textbook written by Professor Albert L. Lehninger of The Johns Hopkins University School of Medicine, Baltimore, MD, USA.[1] A chapter of this venerable reference is entitled "Lipids, Lipoproteins, and Membranes", a subject matter relevant to this work. Lipids are natural substances present in animal and plant tissues. They are mostly insoluble in water, but soluble in many organic solvents. Lipids are actually families of compounds, with similar physical and chemical characteristics, and can be conveniently grouped according to their backbone structure (Table 1 and Figure 1). For example, acylglycerols (triglycerides) are the most abundant lipids in nature. The reader must have heard of them when, during a routine medical checkup, the doctor pulled his/her ear for having a high blood triglyceride level. Solid triglycerides are known as "fats" and their liquid counterparts as "oils". They all have a glycerol (triol) backbone joined through ester linkages to fatty acids. The latter consist of a hydrocarbon (aliphatic) chain of varying length and degree of saturation, indicating the presence or absence of double bonds, with a carboxylic acid terminus. The following discussion will focus on fatty acids, since they are the building blocks of many lipid groups, as well as being part of our current research interests. In a later section, we will return to the broader lipid family as we explore its historical relationship with chloroform.

Fatty acids were first isolated in 1813 from animal fats ("corps gras") by the French scientist Michel-Eugène Chevreul (1786-1889), Professor of Chemistry at the Lycée Charlemagne, Paris.[2] They have been the subject of extensive academic and industrial research ever since. Within the body, fatty acids are found in their esterified form since they are practically insoluble in water. To illustrate with a numerical example, the saturated 6-carbon hexanoic acid has an approximate solubility of 1 g fatty acid per 100 g water at room temperature. This translates to a very small fatty acid mole fraction of order 10^{-3}, with this quantity being relevant in mass transfer processes such as those found typically in the field of chemical engineering. The water solubility decreases considerably as the number of aliphatic carbons in the chain increases. Fatty acids are transported in blood bound to serum albumin, a globular protein with an approximate molecular mass of 68000.[1] They can also cross cell membranes, of which they are key constituents, by diffusion (proportional to a concentration gradient) and protein-facilitated mechanisms.[3-12]

Fatty acids are metabolic precursors of many physiologically-relevant molecules as well as a source of energy for the organism. Free (unbound) fatty acids are either unsaturated (single or multiple double bonds along the aliphatic chain) or saturated (no double bonds). They are named according to the number of carbon atoms in the chain and the location of the double bonds, if any. For example, the 16:0 saturated fatty acid corresponds to the 16-carbon palmitic acid [$CH_3(CH_2)_{14}COOH$] which melts at +63.1°C. On the other hand, the 16:1(D9) monounsaturated fatty acid represents a 16-carbon chain with a double bond between the 9 and 10 carbons (by convention the carboxylic acid is the first carbon). This compound is known as palmitoleic acid

Table 1. Lipid classification according to their backbone structure.[1] The saponifiable lipids can be hydrolyzed to their building blocks which include fatty acids, while the nonsaponifiable lipids cannot be hydrolyzed. Triglycerides are the most numerous in nature. Phospholipids are the main constituents of cell membranes.

	Lipid Type	Molecular Backbone
complex (saponifiable)	acylglycerols or triglycerides	glycerol
	phosphoglycerides or phospholipids	glycerol 3-phosphate
	sphingolipids	sphingosine
	waxes	high-molecular-weight nonpolar alcohols
simple (nonsaponifiable)	terpenes	multiples of the 5-carbon hydrocarbon isoprene
	steroids	perhydrocyclopentanophenanthrene
	prostaglandins	obtained by cyclization of 20-carbon unsaturated fatty acids such as arachidonic acid

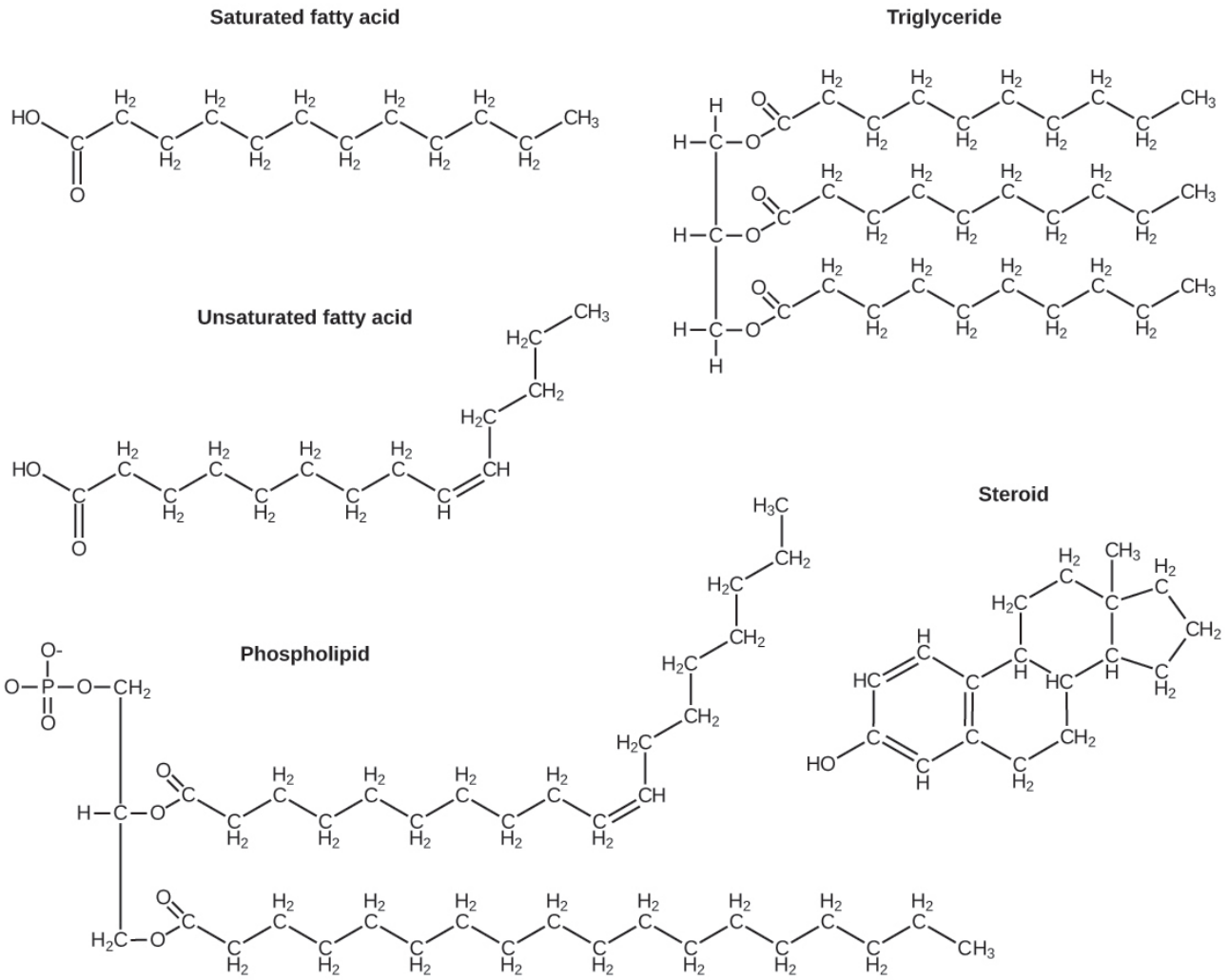

Figure 1. Two-dimensional chemical structures of some of the lipid groups in Table 1. The fatty acids are the building blocks of triglycerides and phospholipids, which are saponifiable through alkali hydrolysis. The simple lipids like steroids are nonsaponifiable. Source: https://cnx.org/resources/00a0b827644d73bfb8695b81a7c7801a.

$[CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH]$ which melts at -0.5°C. Industrial applications of fatty acids include the preparation of soaps, detergents, and lubricants. They are also used as additives in foods, cosmetics, and pharmaceuticals. The book *Fatty Acids and Their Derivatives* by Anderson W. Ralston, Assistant Director of Research at Armour and Company, Chicago, IL, USA, is still the classic starting point for both researchers and enthusiasts of fatty acid biochemistry.[13]

Due to their importance in physiology and food science, fatty acids have been extensively characterized from the standpoint of their physicochemical and dissociation properties. The chronological development of some of these research lines is as follows: a) their very low aqueous dissociation constants were determined as far back as the 1930s, indicating that the vast majority of the fatty acid molecules in solution are nonionic;[14-17] b) the mutual solubility of fatty acids and water has been known since the 1940s, demonstrating the effect of aliphatic chain length and temperature on this property;[18-25] c) solute partitioning studies of fatty acids in organic solvent/aqueous systems, relevant to industrial separation and purification processes, have been undertaken since the 1950s to determine their relative solubility in immiscible media;[26,27] d) chemical reactions at oil/water interfaces involving fatty acid-containing molecules were identified in the 1960s, with applications to the hydrolysis of triglycerides in the intestinal tract and to atmospheric photochemistry;[28,29] and e) Lyman C. Craig and coworkers at the Rockefeller Institute for

Medical Research, New York City, NY, USA, designed in the 1940s-1950s a rotary, multi-stage, liquid-liquid extractor using chemical engineering mass transfer principles to isolate and purify fatty acids from their mixtures, with one benchtop model allowing several thousand quantitative extractions in a few hours of operation![30-34] These landmark historical efforts highlight the importance fatty acids have attained in modern science, particularly their relevance in molecular transport across biological membranes which are discussed below.

Solute exchange across cell membranes is vital to animals and plants. Elegant reviews have addressed the subject of transcellular transport of fatty acids, notwithstanding their limited solubility in aqueous media and the existence of lipid-based membrane resistances.[4-6,12] Debate in the literature lingers on the actual mass transfer mechanisms involved, but it is known that fatty acids are transported with relative ease through triglyceride- and phospholipid-rich cell membranes. To support these findings, the fluid-mosaic model for membrane structure was proposed by Singer and Nicolson in 1972[35] and, even though it has been upgraded conceptually in the last fifty years due to refinements in experimental techniques and instrumentation, remains relevant to this day.[36-48] The original model is sketched in Figure 2 (top) and shows a phospholipid bilayer interspersed with globular (integral) proteins. Membrane phospholipids contain fatty acids attached through ester linkages. Their hydrophilic heads point outward to the aqueous media, while the hydrophobic ends (fatty acids) point inward. The upgraded model depicted in Figure 2 (bottom) indicates that membrane architecture is more complex than originally proposed. Current scientific knowledge describes the membrane as consisting of protein and lipid domains. These are constituted by multiple chemical species that interact dynamically with the cytoskeleton and the extracellular matrix. The most important lines of research related to membrane structure and function since the appearance of the fluid-mosaic model[35] are: a) the isolation and characterization of membrane proteins;[38,47] b) elucidation of membrane molecular signaling and trafficking pathways;[41,42,45-47] c) proof of the existence of membrane protein and lipid domains;[36,37,43-47] d) a description of membrane lateral motion, confinement, and turnover;[38-40,42,43,45-47] e) the roles played by membrane-associated cytoskeletal fences and the extracellular matrix in restricting the lateral diffusion of membrane components;[44,47] and f) characterization of the asymmetry of lipid distribution between the leaflets of the plasma-membrane bilayer.[38,46-48] It is clear from these research thrusts that lipid biochemistry is a continuously evolving discipline, and that it plays a pivotal role in advancing our understanding of cell membrane structure and function.

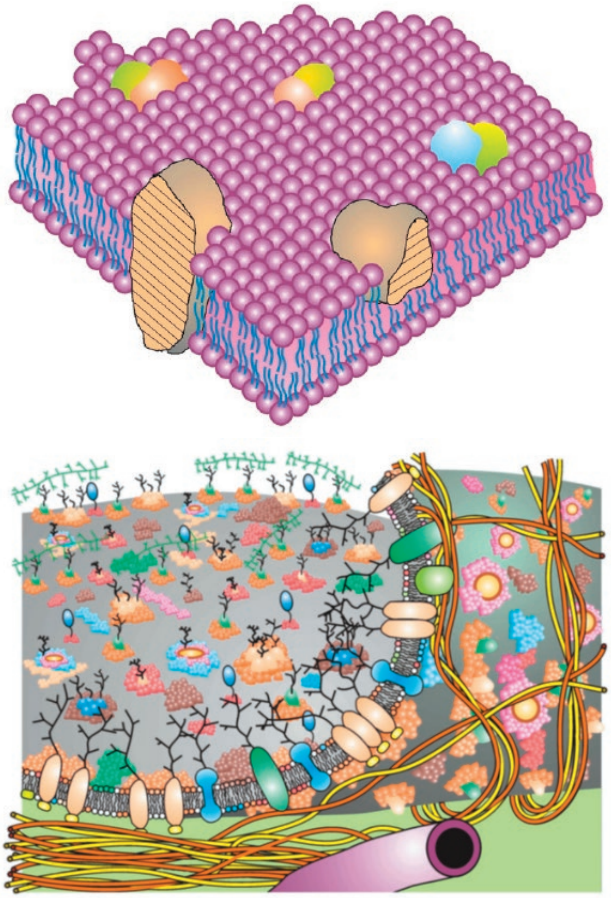

Figure 2. *Top*: Colorized version published by Nicolson (2014)[47] of the Singer and Nicolson (1972)[35] fluid-mosaic membrane model. Originally, it consisted of a phospholipid bilayer with interspersed proteins that could traverse the membrane. The hydrophilic phospholipid heads point to the external and cytoplasmic aqueous media, while the hydrophobic fatty acid tails point inward. *Bottom*: Nicolson (2014)[47] also provided an upgraded membrane model containing what modern-day scientists believe to be more realistic structural and functional features. The basic lipid bilayer architecture is preserved, but in this drawing the membrane has been peeled up (at the right) so that the viewer can appreciate several membrane-associated cytoskeletal (bottom left) and extracellular (top left) interactions. Integral proteins, glycoproteins, lipids, and oligosaccharides are represented by different colors. Protein and lipid domains are evident throughout the membrane. Science moves at a rapid pace in its attempt to unravel the functional attributes of these and many other membrane structures.

We postpone further discussion of lipids to the section following the historical development and uses of chloroform.

III. CHLOROFORM SYNTHESIS AND ITS USES IN MEDICINE AND CHEMICAL ENGINEERING

Chloroform (trichloromethane; $CHCl_3$; CAS Registry Number 67-66-3) was synthesized and purified in 1831 by Samuel Guthrie (1782-1848), who studied medicine under his father's tutelage, and may be considered to be a self-made farmer, chemist, and industrial manufacturer from Sackets Harbor, NY, USA.[49] An interesting historical note is that chloroform's right of discovery was contested for many years, with the French pharmacist Eugène Soubeiran and the German chemist Justus von Liebig claiming priority supported by their independent chemical syntheses. In 1888, a committee appointed by the Chicago Medical Society examined the available scientific evidence and concluded that Guthrie was the rightful discoverer of chloroform, almost sixty years after the events in Sackets Harbor had taken place and forty years after his death.

Guthrie worked in his home/farm laboratory to improve gunpowder preparations (leading to several near-fatal explosions!) as well as carrying out liquor distillations. One of his batch reaction-distillations produced what came to be known as "Guthrie's sweet whiskey", a solution of chloroform in ethanol quite popular among the local alcohol-consuming clientele. The consecutive liquid-phase reactions thought to have taken place in Guthrie's boiler-distiller in his historical synthesis are as follows:[50]

a) Ethanol reacts with calcium hypochlorite to give acetaldehyde, calcium chloride, and water.
$2C_2H_5OH + Ca(OCl)_2 \rightarrow 2CH_3CHO + CaCl_2 + 2H_2O$

b) Acetaldehyde reacts with calcium hypochlorite to give chloral and calcium hydroxide.
$2CH_3CHO + 3Ca(OCl)_2 \rightarrow 2CCl_3CHO + 3Ca(OH)_2$

c) Chloral reacts with calcium hydroxide to give chloroform and calcium formate.
$2CCl_3CHO + Ca(OH)_2 \rightarrow 2\mathbf{CHCl_3} + (H\text{-}COO)_2Ca$

Chloroform has a molecular mass of 119.38 g/mol and is a colorless liquid at room temperature with a sweet, ether-like smell. It has a normal boiling point of 61.2°C, a mass density of 1479 kg/m^3, and a viscosity (a quantity proportional to its resistance to flow) of 5.37 x 10^{-4} Pa×s [kg/(m×s)] at 25°C. These are very important physical properties for describing its behavior in momentum, energy, and mass transport systems such as those typically encountered in chemistry and chemical engineering. For comparison, liquid water has a molecular mass of 18.02 g/mol, a normal boiling point of 100°C, a mass density of ~1000 kg/m^3, and a viscosity of ~1.0 x 10^{-3} Pa×s [kg/(m×s)] at room temperature. Thus, chloroform is denser but less viscous than water at room temperature. Its solubility in water is very low since 1 mL dissolves in about 200 mL water at 25°C. However, as we shall see later, chloroform is an excellent solvent for lipids!

Chloroform was first used as an obstetric anesthetic in 1847 by Sir James Young Simpson (1811-1870) of Edinburgh, Scotland. A very famous patient who received the wonderful chemical on April 7, 1853, was Her Majesty Queen Victoria (1819-1901). After inhaling chloroform for 53 minutes through a folded handkerchief during labor, she delivered her eighth child, Prince Leopold.[50] Illustrations of chloroform's early use as an anesthetic are shown in Figure 3 (top and middle). Following this headline performance, chloroform quickly gained worldwide popularity in related medical specialties such as dentistry and surgery. It has seen action in the treatment of battlefield wounds since the American Civil War [1861-1865; Figure 3 (bottom)]. Readers may also recall its sinister role in well-known fictional crime novels such as Agatha Christie's *The Plymouth Express*[51] and *Why Didn't They Ask Evans?*,[52] in which the assassins subdue their victims temporarily with a chloroform-soaked textile fabric applied to the nose and mouth pending further plans. Due to its potential carcinogenicity, chloroform was banned from human use as an additive in pharmaceuticals and cosmetics by the United States Food and Drug Administration in 1976.[53] However, it is still used in carefully-monitored industrial operations as an intermediate in the production of bactericides, fumigants, insecticides, and fluorinated refrigerants. The synthesis/discovery and medical applications of chloroform have been discussed in excellent books[49,50] and archival references.[54,55]

An important nonmedical use of chloroform, perhaps unknown to the scientific community at large, is its role in mass transfer experiments leading to the estimation of diffusion coefficients (diffusivities) of binary gas pairs, with atmospheric air being the traditional second component. Diffusion coefficients are key parameters in the design and analysis of mass transfer systems typically found in chemical engineering. Examples of the latter include gas absorption, membrane separations, evaporation phenomena, multicomponent distillation, and controlled drug release from diffusion-based systems. The diffusive mass transport rate of a substance in a given medium is directly proportional to its diffusivity; therefore, knowledge of its magnitude is critical to the chemical engineer involved in research or designing an industrial-scale process.[56-59]

Figure 3. *Top*: Inducing chloroform anesthesia by inhalation through a cloth. Source: https://survivalstronghold.com/wp-content/uploads/2016/11/Administering_Chloroform_RAG.png. *Middle*: Chloroform flasks and masks for holding a textile material over the patient's nose and mouth. Source: https://www.riverjunction.com/assets/images/4299/EtherSet.jpg. *Bottom*: Chloroform use for the treatment of a wounded soldier during the American Civil War. Source: https://www.ourgreatamericanheritage.com/wp-content/uploads/2015/08/2363273317_f9cc2da0ea_o.jpg.

The classical method to estimate binary gas diffusivities is the Stefan column developed by Josef Stefan (1835-1893), Professor of Mathematics and Physics at the University of Vienna, during the second half of the 19th Century.[60,61] The original vertical column consisted of a pure liquid phase of volatile species A up to an initial height z_{10}, overlaid by a gas phase containing the evaporated A and a stagnant species B (usually air). All symbols are defined in the Nomenclature. Through the years, the most common operational version of the column, depicted in Figure 4, has been the descending interface modality, which does not require liquid replenishment. In this case, the liquid-gas interface descends to $z_1(t)$ after an elapsed time t following the start of the evaporation-diffusion process, while gas A ascends by diffusion and convection (bulk gas motion) to the top of the column at z_2. A sweeping stream of pure gas B flows steadily and slowly at the top to remove gas A, maintaining its concentration at essentially zero at that location. This boundary condition is critical when developing mathematical models for the transport of gas A within the column. For the experimental situation depicted in Figure 4, the solution of the mass conservation differential equations in the gas and liquid phases is well established in the literature,[58] yielding a simple algebraic expression from which the diffusivity of A in B may be calculated:

$$z_1(t) = z_{10} - (z_2 - z_{10})\left\{\left[1 + \frac{\lambda t}{(z_2 - z_{10})^2}\right]^{1/2} - 1\right\} \quad (1)$$

$$\lambda = 2\frac{c}{c_L} D_{AB} \ln\frac{1 - y_{Az_2}}{1 - y_{Az_1}} \quad (2)$$

In Equation (1), the z-coordinates represent specific vertical locations in the column (m), with z = 0 corresponding to the bottom (refer to Figure 4), and t is time (s). In Equation (2), c is the molar density of an ideal gas at constant temperature and pressure (mol/m^3), c_L is the molar density of pure liquid A (mol/m^3), D_{AB} is the Fickian binary gas diffusivity of A in B (m^2/s), and y_A is the mole fraction of gas A at a specific location within the column. At $z_1(t)$, y_A is calculated by stipulating that vapor-liquid equilibrium conditions prevail at the interface. At z_2, y_A is commonly assumed to be zero due to infinite dilution of gas A in the sweeping stream. For a standard evaporation-diffusion experiment using the Stefan column, D_{AB} can be calculated by regression analysis from Equations (1)-(2) and experimental data of interfacial position versus time, $z_1(t)$.

At this point the alert reader may rightfully ask: How is chloroform related to the Stefan column? Inter-

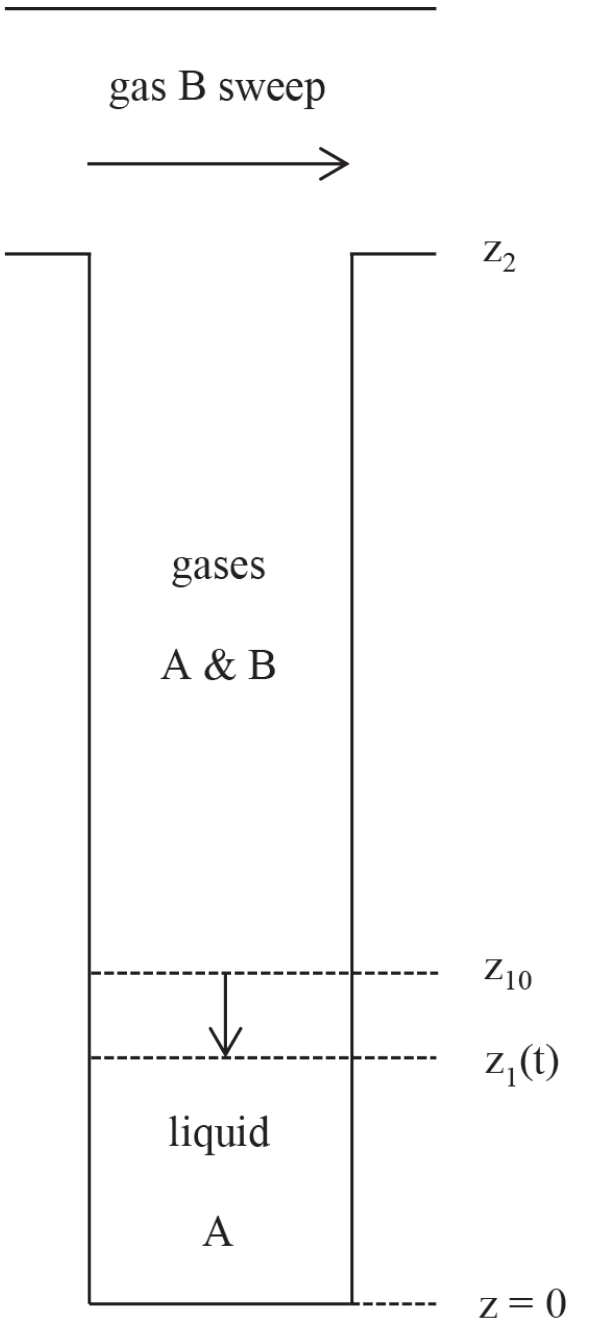

Figure 4. Classic sketch of the Stefan diffusion column showing the liquid and gas compartments.[68,69] The operating modality depicted is that of the descending liquid-gas interface without liquid replacement. Pure volatile liquid A is charged initially to a height z_{10}. Its level falls to $z_1(t)$ after an elapsed time t following the start of the evaporation-diffusion process. Liquid A evaporates at the interface and its vapor ascends through stagnant gas B (air) until it reaches the top of the column at z_2. A steady and slow gas B sweep at the top maintains the concentration of A very close to zero at that location. The binary gas diffusivity of A in B can be calculated from fundamental mass transport principles applied to gas A and experimental interfacial descent versus time data (refer to the text for details). Symbol definitions can be found in the Nomenclature.

estingly, chloroform was one of the first substances tested in the column, when Georg Baumgartner in 1877 determined its diffusion coefficient in air to be about 7.3 x 10^{-6} m^2/s within a temperature range of 17.5-20.3°C.[62] Although this value may seem small at first sight, leading one to the erroneous conclusion that chloroform is a poor diffusant in air, diffusivities of the order 1 x 10^{-5} m^2/s are quite common in binary gaseous systems, as well as being several orders of magnitude *higher* than those in liquids! Therefore, for a given chemical species such as chloroform, its diffusion in gases is very fast relative to its diffusion in liquids. This information is crucial to a chemical engineer when attempting to design a diffusion-based mass transfer system. For the interested reader, well-known compendia are available containing diffusivities obtained by the Stefan column method for many gas pairs, including references to the original publications.[63-65] In addition, a detailed compilation of binary diffusion coefficients for inorganic and organic compounds in air relevant to atmospheric chemistry has been presented recently.[66,67]

In the 1950s researchers first became aware that the Stefan column binary diffusivities may be inaccurate due to "end effects" at the top of the column, where the sweeping stream interacts with the gas phase, and at the liquid-gas interface, where curvature due to surface tension can affect the mass transport area and the diffusion path length for gas A from $z_1(t)$ to z_2. Unfortunately, the early studies could not correlate such end effects to the systems' operational and geometrical settings, limiting their subsequent use. In recent years, we have addressed this gap in scientific knowledge by reporting our efforts to quantify the various Stefan column end effects and their impact on the gas diffusivity estimates. These studies clearly show that factors such as column nonisothermality,[68] sweeping gas stream Reynolds number (dimensionless ratio of fluid inertial to viscous forces) and column aspect ratio (gas phase height to column inside diameter),[69] liquid phase composition,[70,71] and interfacial shape and curvature[72] must be rigorously accounted for in the diffusivity calculations to minimize estimation errors. Our findings to date have hopefully made Stefan column researchers aware of some of the common pitfalls the analyst may find when estimating binary gas diffusivities from experimental interfacial descent versus time data. By continuing this line of research, chloroform and other common volatile solvents may help us attain an even better understanding of the Stefan column mass transport dynamics, leading to more accurate binary diffusivity estimates.

IV. MERGING OF THE LIPID AND CHLOROFORM HISTORIES

Lipids and chloroform intertwined their scientific paths in the 1940s and 1950s through the work of Spanish biochemist/Professor Jordi Folch (1911-1979) and collaborators at the Rockefeller Institute for Medical Research, the McLean Hospital Research Laboratories, and at Harvard Medical School. In 1957, researchers Jordi Folch, M. Lees, and G. H. Sloane Stanley modified their own published procedure for the isolation and purification of total lipids from animal tissues.[73] They developed painstaking analytical methods to extract and purify lipids from animal tissue quantitatively, concentrating on white (axon bundles) and gray (neural cell bodies) brain matter. The team relied on chloroform's dissolving power for organic lipid-based compounds as the key reagent. The tissue was first homogenized with a 2:1 (volume:volume) chloroform-methanol solution followed by filtration. The filtrate, which contained lipid and nonlipid matter, was washed with a five-fold volume of water with minimal lipid losses in the wash. The resulting liquid mixture separated into two phases, the lower chloroform phase containing the total lipid extract. The article qualified the chloroform-based, lipid extraction-purification procedure as: a) operationally simple; b) applicable to any scale of starting biological material; c) capable of decreasing lipid losses incidental to the water washing process; and d) yielding an extract which could be taken to dryness without foaming or splitting of the proteolipids.[73] Besides developing this landmark analytical methodology for extracting total lipids, over the same time period other Folch groups isolated and identified many lipid components of brain tissue, using chloroform both as a solvent and extraction medium.[74-80] Some of the compounds isolated, purified, and characterized from animal brain tissue, with chloroform playing a central role in the extraction-by-dissolution procedures, included: a) α- and β-phosphatidyl serine, phosphatidyl ethanolamine, and diphosphoinositide derived from cephalin (Figure 5); b) strandin consisting of fatty acids and sphingosine; c) proteolipids (lipoproteins) in normal and tumor tissue; and d) extracts of pure lipids. The isolation and characterization of these molecules led the way to elucidating significant aspects of brain tissue biochemistry.

In the last few decades, the lipid-chloroform interaction has continued in applications such as the extraction of lipids from a wide spectrum of animal and plant tissues, as well as in environmental protection and workplace risk minimization by seeking "friendly" alternatives to the solvent's use. Examples of these modern

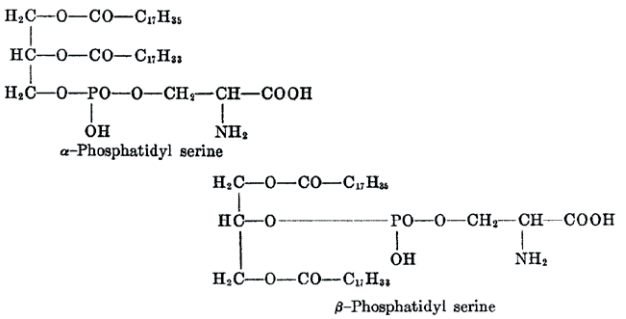

Figure 5. Many lipid derivatives were isolated, purified, and characterized by Professor Jordi Folch and associates from animal brain tissue in the 1940s-1950s using chloroform's dissolving and extraction properties for organic compounds.[73-80] The work of the various Folch teams marked the beginning of the lipid-chloroform scientific interaction, leading to a better understanding of brain tissue biochemistry. It also opened the door to many subsequent research initiatives featuring the synergistic use of both compounds.

research trends involving the synergistic efforts of lipids and chloroform include: a) extraction and purification of unsaturated fish lipids, which require mild treatment to minimize oxidative decomposition and the production of artifacts;[81,82] b) determination of serum triglycerides by extracting the lipids with chloroform, methanol, and diethyl ether, followed by removal of the phospholipids, hydrolysis of the triglycerides, and quantification of their glycerol moieties;[83,84] c) determination of pure lipid solubilities in common laboratory solvents such as chloroform to optimize extraction procedures of diverse biological samples;[85] d) extraction of environmental contaminants such as chlorobiphenyls and other chlorinated pesticides bound and nonbound to fish lipids;[86] and e) reducing the toxicity of chloroform-based tissue lipid extraction methods by substitution with alternate solvents.[87-91] Given their long, intertwined histories in chemistry and biology, it is very likely that lipids and chloroform will continue to find simultaneous use in as yet unforeseen scientific applications.

V. CONCLUSIONS

This article brings together the separate histories of lipids and chloroform. Within the lipid family, fatty acids are highlighted since they are the most abundant in nature and are key constituents of cell membranes. Fatty acids were isolated by Chevreul in 1813[2], and new lipids and lipid-based biomolecules are constantly being identified and characterized due to improved experimental techniques and instrumentation. Not only are lipids of interest to academic researchers, but they find

important industrial use as ingredients in foods, pharmaceuticals, and cosmetics.

Chloroform has gone through its highs and lows along its history. First synthesized by Guthrie in 1831[54], it quickly found worldwide use as an anesthetic in obstetrics, dentistry, and surgery. Military doctors have applied it to injured combatants for wound treatment since the American Civil War. Unfortunately, it is presently banned from inclusion in pharmaceuticals and cosmetic products due to its potential carcinogenicity.[53] Notwithstanding this negative label, chloroform is still used in research laboratories as well as in several carefully-monitored industrial processes.

Lipids and chloroform are chemical species with different physicochemical properties and unique historical backgrounds. The work of Professor Jordi Folch and associates in the 1940s and 1950s intertwined their histories forever.[73-80] The Folch teams spawned many lines of research with definite lipid-chloroform synergism, and these activities continue at an accelerated pace to this day.[81-91] Perhaps the biggest lesson from this story should be addressed to today's young scientists: even though they have dissimilar physical and chemical properties, lipids and chloroform have shared important common ground in the past, and can be used collaboratively and prudently to tackle challenging scientific problems in the future.

ACKNOWLEDGEMENTS

Academic inspiration for this work was provided by University of Wisconsin Professors R. Byron Bird, Warren E. Stewart, and Edwin N. Lightfoot. Their textbook *Transport Phenomena* is a timeless contribution to science, and is highly recommended to young and old readers alike.[58] The Interlibrary Loans Section of the General Library is thanked for procuring most of the archival references. Ana L. Braña provided encouragement throughout the writing of this article.

The manuscript reviewers are gratefully recognized for their insightful comments and suggestions.

REFERENCES

1. A. L. Lehninger, *Biochemistry: The Molecular Basis of Cell Structure and Function*, 2nd ed., Worth Publishers, Inc., New York City, NY, **1978**.
2. M. Chevreul, *Annales de chimie, ou Recueil de mémoires concernant la chimie et les arts qui en dépendent, et spécialement la pharmacie* **1813**, *88*, 225-261. https://gallica.bnf.fr/ark:/12148/bpt6k65741176/f225.item.
3. D. Bassolino-Klimas, H. E. Alper, T. R. Stouch, *Biochemistry* **1993**, *32*, 12624-12637. DOI: 10.1021/bi00210a010.
4. D. Zakim, *Proc. Soc. Exp. Biol. Med.* **1996**, *212*, 5-14. DOI: 10.3181/00379727-212-43986.
5. J. A. Hamilton, *J. Lipid Res.* **1998**, *39*, 467-481. https://www.jlr.org/content/39/3/467.full.pdf+html.
6. J. A. Hamilton, *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1999**, *60*, 291-297. DOI: 10.1016/S0952-3278(99)80002-7.
7. J. A. Hamilton, F. Kamp, *Diabetes* **1999**, *48*, 2255-2269. DOI: 10.2337/diabetes.48.12.2255.
8. E. Barta, S. Sideman, J. B. Bassingthwaighte, *Ann. Biomed. Eng.* **2000**, *28*, 331-345. DOI: 10.1114/1.274.
9. P. K. Fyfe, K. E. McAuley, A. W. Roszak, N. W. Isaacs, R. J. Cogdell, M. R. Jones, *Trends Biochem. Sci.* **2001**, *26*, 106-112. DOI: 10.1016/S0968-0004(00)01746-1.
10. E. Gouaux, S. H. White, *Curr. Opin. Struct. Biol.* **2001**, *11*, 393-396. DOI: 10.1016/s0959-440x(00)00222-0.
11. J. A. Hamilton, R. A. Johnson, B. Corkey, F. Kamp, *J. Mol. Neurosci.* **2001**, *16*, 99-108. DOI: 10.1385/JMN:16:2-3:99.
12. T. Hajri, N. A. Abumrad, *Annu. Rev. Nutr.* **2002**, *22*, 383-415. DOI: 10.1146/annurev.nutr.22.020402.130846.
13. A. W. Ralston, *Fatty Acids and Their Derivatives*, John Wiley & Sons, Inc., New York City, NY, **1948**.
14. H. S. Harned, R. W. Ehlers, *J. Am. Chem. Soc.* **1932**, *54*, 1350-1357. DOI: 10.1021/ja01343a013.
15. H. S. Harned, R. W. Ehlers, *J. Am. Chem. Soc.* **1933**, *55*, 652-656. DOI: 10.1021/ja01329a027.
16. H. S. Harned, R. W. Ehlers, *J. Am. Chem. Soc.* **1933**, *55*, 2379-2383. DOI: 10.1021/ja01333a024.
17. J. F. J. Dippy, *J. Chem. Soc.* **1938**, *paper 231*, 1222-1227. DOI: 10.1039/JR9380001222.
18. C. W. Hoerr, W. O. Pool, A. W. Ralston, *Oil Soap* **1942**, *19*, 126-128. DOI: 10.1007/BF02545490.
19. A. W. Ralston, C. W. Hoerr, *J. Org. Chem.* **1942**, *7*, 546-555. DOI: 10.1021/jo01200a013.
20. C. W. Hoerr, A. W. Ralston, *J. Org. Chem.* **1944**, *9*, 329-337. DOI: 10.1021/jo01186a005.
21. C. W. Hoerr, R. S. Sedgwick, A. W. Ralston, *J. Org. Chem.* **1946**, *11*, 603-609. DOI: 10.1021/jo01175a025.
22. L. M. John, J. W. McBain, *J. Am. Oil Chem. Soc.* **1948**, *25*, 40–41. DOI: 10.1007/BF02593186.
23. D. N. Eggenberger, F. K. Broome, A. W. Ralston, H. J. Harwood, *J. Org. Chem.* **1949**, *14*, 1108-1110. DOI: 10.1021/jo01158a022.
24. M. B. Oliveira, M. J. Pratas, I. M. Marrucho, A. J. Queimada, J. A. P. Coutinho, *AIChE J.* **2009**, *55*, 1604-1613. DOI: 10.1002/aic.11766.

25. C. M. Romero, F. Suárez, *J. Solution Chem.* **2009**, *38*, 315-320. DOI: 10.1007/s10953-009-9375-6.
26. D. S. Goodman, *J. Am. Chem. Soc.* **1958**, *80*, 3887-3892. DOI: 10.1021/ja01548a023.
27. R. B. Simpson, J. D. Ashbrook, E. C. Santos, A. A. Spector, *J. Lipid Res.* **1974**, *15*, 415-422. https://www.jlr.org/content/15/4/415.full.pdf+html.
28. F. H. Mattson, R. A. Volpenhein, *J. Am. Oil Chem. Soc.* **1966**, *43*, 286-289. DOI: 10.1007/BF02609675.
29. S. Rossignol, L. Tinel, A. Bianco, M. Passananti, M. Brigante, D. J. Donaldson, C. George, *Science* **2016**, *353*, 699-702. DOI: 10.1126/science.aaf3617.
30. Y. Sato, G. T. Barry, L. C. Craig, *J. Biol. Chem.* **1947**, *170*, 501-507. https://www.jbc.org/content/170/2/501.citation.
31. B. Williamson, L. C. Craig, *J. Biol. Chem.* **1947**, *168*, 687-697. http://www.jbc.org/content/168/2/687.citation.
32. L. C. Craig, O. Post, *Anal. Chem.* **1949**, *21*, 500-504. DOI: 10.1021/ac60028a013.
33. G. T. Barry, Y. Sato, L. C. Craig, *J. Biol. Chem.* **1951**, *188*, 299-306. https://www.jbc.org/content/188/1/299.citation.
34. E. H. Ahrens Jr., L. C. Craig, *J. Biol. Chem.* **1952**, *195*, 299-310. https://www.jbc.org/content/195/1/299.citation.
35. S. J. Singer, G. L. Nicolson, *Science* **1972**, *175*, 720-731. DOI: 10.1126/science.175.4023.720.
36. J. N. Israelachvili, *Biochim. Biophys. Acta, Biomembr.* **1977**, *469*, 221-225. DOI: 10.1016/0005-2736(77)90185-7.
37. O. G. Mouritsen, M. Bloom, *Biophys. J.* **1984**, *46*, 141-153. DOI: 10.1016/S0006-3495(84)84007-2.
38. S. J. Singer, *J. Membr. Biol.* **1992**, *129*, 3-12. DOI: 10.1007/BF00232051.
39. G. M. Lee, F. Zhang, A. Ishihara, C. L. McNeil, K. A. Jacobson, *J. Cell Biol.* **1993**, *120*, 25-35. DOI: 10.1083/jcb.120.1.25.
40. K. Jacobson, E. D. Sheets, R. Simson, *Science* **1995**, *268*, 1441-1442. DOI: 10.1126/science.7770769.
41. K. Simons, E. Ikonen, *Nature* **1997**, *387*, 569-572. DOI: 10.1038/42408.
42. M. Edidin, *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 414-418. DOI: 10.1038/nrm1102.
43. D. M. Engelman, *Nature* **2005**, *438*, 578-580. DOI: 10.1038/nature04394.
44. K. Ritchie, J. Spector, *Biopolymers* **2007**, *87*, 95-101. DOI: 10.1002/bip.20801.
45. D. Lingwood, K. Simons, *Science* **2010**, *327*, 46-50. DOI: 10.1126/science.1174621.
46. F. M. Goñi, *Biochim. Biophys. Acta, Biomembr.* **2014**, *1838*, 1467-1476. DOI: 10.1016/j.bbamem.2014.01.006.
47. G. L. Nicolson, *Biochim. Biophys. Acta, Biomembr.* **2014**, *1838*, 1451-1466. DOI: 10.1016/j.bbamem.2013.10.019.
48. D. Marquardt, B. Geier, G. Pabst, *Membranes* **2015**, *5*, 180-196. DOI: 10.3390/membranes5020180.
49. J. R. Pawling, *Dr. Samuel Guthrie: Discoverer of Chloroform, Manufacturer of Percussion Pellets, Industrial Chemist (1782-1848)*, Brewster Press, Watertown, NY, **1947**.
50. L. Stratmann, *Chloroform: The Quest for Oblivion*, Sutton Publishing Limited, Stroud, Gloucestershire, England, **2003**.
51. A. Christie, *Hercule Poirot's Casebook*, Dodd, Mead & Company, Inc., New York City, NY, **1984**, pp. 716-727.
52. A. Christie, *Why Didn't They Ask Evans?* Bantam Books, Inc., New York City, NY, **1983**.
53. S. D. Fine, *Fed. Regist.* **1976**, *41*, 26841-26846. https://archive.org/details/sim_federal-register-find_1976-06-29_41_126_3/mode/2up.
54. S. Guthrie, *Am. J. Sci. Arts* **1832**, *21*, 64-65. https://www.biodiversitylibrary.org/item/88794#page/76/mode/1up.
55. O. P. Hubbard, *Trans. N. Y. Acad. Sci.* **1892**, *11*, 149-151. https://www.biodiversitylibrary.org/item/45322#page/183/mode/1up.
56. T. K. Sherwood, R. L. Pigford, C. R. Wilke, *Mass Transfer*, McGraw-Hill, Inc., New York City, NY, **1975**.
57. R. E. Treybal, *Mass-Transfer Operations*, 3rd ed. (McGraw-Hill Classic Textbook Reissue), McGraw-Hill Book Company, Inc., New York City, NY, **1987**.
58. R. B. Bird, W. E. Stewart, E. N. Lightfoot, *Transport Phenomena*, revised 2nd ed., John Wiley & Sons, Inc., New York City, NY, **2007**.
59. E. L. Cussler, Multicomponent Diffusion, Chapter 7 in *Diffusion: Mass Transfer in Fluid Systems*, 3rd ed., Cambridge University Press, Cambridge, England, **2009**, pp. 211-234.
60. J. Stefan, *Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften Wien* **1871**, *63*, 63-124. https://www.zobodat.at/pdf/SBAWW_63_2_0063-0124.pdf.
61. J. Stefan, *Ann. Phys.* **1890**, *277*, 725-747. DOI: 10.1002/andp.18902771206.
62. G. Baumgartner, *Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften Wien* **1877**, *75*, 313-319. http://books.google.com.
63. A. N. Berezhnoi, A. V. Semenov, *Binary Diffusion Coefficients of Liquid Vapors in Gases*, K. Shakhlevich, Russian-to-English translator, Begell House, Inc., New York City, NY, **1997**.

64. T. R. Marrero, E. A. Mason, *J. Phys. Chem. Ref. Data* **1972**, *1*, 3-118. DOI: 10.1063/1.3253094.
65. T. R. Marrero, E. A. Mason, *AIChE J.* **1973**, *19*, 498-503. DOI: 10.1002/aic.690190312.
66. M. J. Tang, R. A. Cox, M. Kalberer, *Atmos. Chem. Phys.* **2014**, *14*, 9233–9247. DOI: 10.5194/acp-14-9233-2014.
67. M. J. Tang, M. Shiraiwa, U. Pöschl, R. A. Cox, M. Kalberer, *Atmos. Chem. Phys.* **2015**, *15*, 5585-5598. DOI: 10.5194/acp-15-5585-2015.
68. J. L. Medina, C. A. Ramírez, *Chem. Eng. Commun.* **2016**, *203*, 1625-1640. DOI: 10.1080/00986445.2016.1223059.
69. M. E. Graniela, C. A. Ramírez, *Chem. Eng. Commun.* **2019**, *206*, 842-860. DOI: 10.1080/00986445.2018.1530992.
70. M. Jaime, S. G. Maisonet, C. A. Ramírez, *Chem. Eng. Commun.* **2020**, *207*, 1658-1684. DOI: 10.1080/00986445.2019.1674815.
71. I. Moreno, M. Moreno, C. A. Ramírez, *Chem. Eng. Commun.* **2022**, *209*, 206-228. DOI: 10.1080/00986445.2020.1852400.
72. M. Moreno, I. Moreno, M. Jaime, S. G. Maisonet, C. A. Ramírez, *Chem. Eng. Commun.* **2021**, *208*, 1219-1237. DOI: 10.1080/00986445.2020.1770233.
73. J. Folch, M. Lees, G. H. Sloane Stanley, *J. Biol. Chem.* **1957**, *226*, 497-509. https://www.jbc.org/content/226/1/497.citation.
74. J. Folch, *J. Biol. Chem.* **1942**, *146*, 35-44. http://www.jbc.org/content/146/1/35.citation.
75. J. Folch, *J. Biol. Chem.* **1948**, *174*, 439-450. http://www.jbc.org/content/174/2/439.citation.
76. J. Folch, *J. Biol. Chem.* **1949**, *177*, 497-504. http://www.jbc.org/content/177/2/497.citation.
77. J. Folch, *J. Biol. Chem.* **1949**, *177*, 505-519. http://www.jbc.org/content/177/2/505.citation.
78. J. Folch, S. Arsove, J. A. Meath, *J. Biol. Chem.* **1951**, *191*, 819-831. http://www.jbc.org/content/191/2/819.citation.
79. J. Folch, I. Ascoli, M. Lees, J. A. Meath, F. N. LeBaron, *J. Biol. Chem.* **1951**, *191*, 833-841. http://www.jbc.org/content/191/2/833.citation.
80. J. Folch, M. Lees, *J. Biol. Chem.* **1951**, *191*, 807-817. https://www.jbc.org/content/191/2/807.citation.
81. E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917. DOI: 10.1139/o59-099.
82. F. Smedes, T. K. Thomasen, *Mar. Pollut. Bull.* **1996**, *32*, 681-688. DOI: 10.1016/0025-326X(96)00079-3.
83. L. A. Carlson, *J. Atheroscler. Res.* **1963**, *3*, 334-336. DOI: 10.1016/S0368-1319(63)80012-5.
84. L. A. Carlson, *Clin. Chim. Acta* **1985**, *149*, 89-93. DOI: 10.1016/0009-8981(85)90277-3.
85. P. Schmid, E. Hunter, *Physiol. Chem. Phys.* **1971**, *3*, 98-102.
86. J. de Boer, *Chemosphere* **1988**, *17*, 1803-1810. DOI: 10.1016/0045-6535(88)90108-7.
87. F. Smedes, *Analyst* **1999**, *124*, 1711-1718. DOI: 10.1039/A905904K.
88. P. Manirakiza, A. Covaci, P. Schepens, *J. Food Compos. Anal.* **2001**, *14*, 93-100. DOI: 10.1006/jfca.2000.0972.
89. K. G. Drouillard, H. Hagen, G. D. Haffner, *Chemosphere* **2004**, *55*, 395-400. DOI: 10.1016/j.chemosphere.2003.11.010.
90. N. El Jaber-Vazdekis, F. Gutiérrez-Nicolás, Á. G. Ravelo, R. Zárate, *Phytochem. Anal.* **2006**, *17*, 107-113. DOI: 10.1002/pca.893.
91. E. Cequier-Sánchez, C. Rodríguez, Á. G. Ravelo, R. Zárate, *J. Agric. Food Chem.* **2008**, *56*, 4297-4303. DOI: 10.1021/jf073471e.

NOMENCLATURE

Latin letters

A species A
B species B
c unsubscripted: molar density of an ideal gaseous mixture; subscripted: molar density of a pure liquid, mol/m^3
D Fickian diffusivity of a species in a binary gaseous mixture (subscripted), m^2/s
t time elapsed after starting a standard Stefan column evaporation-diffusion experiment, s
y mole fraction of a given species at a specific gas-phase location in the column (subscripted), -
z vertical coordinate with origin at the bottom of the column (see Figure 4; subscripted), m

Greek letters

λ constant defined by Equation (2), m^2/s

Subscripts

10 initial location of the liquid-gas interface
1, 2 specific column locations: 1, liquid-gas interface; 2, top
AB A-B gas pair
Az_1 species A at z_1
Az_2 species A at z_2
L liquid