

CHAPTER I

INTRODUCTION

In 1822, Baron Cagniard de la Tour first observed the appearance of a supercritical phase by observing the disappearance of two distinct gas and liquid phases into one visual phase by increasing the temperature of a material in a closed glass container.¹ This early discovery marks the first notation of a supercritical fluid.

A supercritical fluid (SF) is defined as any substance that is above its critical pressure and temperature. The critical pressure is the highest pressure at which a liquid can be converted into a gas by an increase in temperature, while the critical temperature is the highest temperature at which a gas can be converted into a liquid by an increase in pressure. Therefore in the critical region there is only one phase, and this phase possesses both gas and liquid-like properties. The solvating power of the supercritical fluid can be influenced by a change in pressure or temperature. While in the supercritical state, at constant pressure, the solvating power of the fluid decreases with increasing temperature. Likewise, at constant temperature, the solvating power of the fluid increases with increasing pressure. This solvating power can be maximized by changing the density of the SF by manipulating both temperature and pressure. This property allows one to adjust the density so as to solubilize certain types of compounds in a selective fashion. For example, low polarity analytes may be solubilized at low densities and more polar analytes at higher densities.

Supercritical fluids make ideal extraction solvents and chromatographic mobile phases because of their high mass transport properties. As compared to normal liquids, SFs exhibit higher diffusivities, lower viscosities, and near zero surface tension. These gas-like properties allow fast mass transfer into and out of complex matrices. Also when

¹ C. Cagniard de la Tour, *Ann. Chim. Phys.*, **21** (1822) 127, 178.

used as mobile phases, the lowered viscosity, as compared to high performance liquid chromatographic (HPLC) mobile phases, allows the use of higher flow rates, due to a decreased pressure drop along the length of the column. This may allow separations to be achieved in significantly less time than with normal liquids without a large loss in efficiency.

The most commonly used supercritical fluid as an extraction solvent and mobile phase is carbon dioxide (CO₂). It is relatively inert, non-flammable, non-toxic, is readily attainable in high purity, has easily accessible critical parameters ($P_c = 72.9$ atm, $T_c = 31$ °C), and is considered environmentally friendly. Also CO₂ is a gas under ambient conditions. Its use as an extraction solvent and mobile phase may be advantageous. For extraction purposes, it is possible that no solvent concentration following the extraction and prior to analysis will be needed, in direct contrast to most liquid-liquid and liquid-solid extractions. During chromatography, little waste disposal is needed since the gas can be vented directly into the atmosphere.

Carbon dioxide is a non-polar fluid, and its solvating power is comparable to liquid hexane.² For these reasons, CO₂ has been used primarily in the analysis of nonpolar to relatively polar compounds. In order to overcome this limitation, a modifier, consisting of small volumes of organic solvents such as methanol, may be added directly to the fluid or matrix, or a more polar fluid such as ammonia may be used. Ammonia is rarely used due to its toxicity, reactivity, and extreme critical parameters ($P_c = 111$ atm, $T_c = 132$ °C). For this reason, many have used modified CO₂.

Modifiers generally serve two functions: a) increase the solvating power of the SF and b) facilitate the disruption of analyte-matrix interactions. The addition of a modifier to either the SF or to the matrix prior to SFE may not be sufficient for the extraction and separation of multifunctional, highly polar, and ionic/ionizable mixtures such as many pharmaceuticals. A secondary modifier (i.e. additive) may be added to the primary

² C.R. Yonker, R.D. Smith, *Supercritical Fluid Extraction and Chromatography*, B.A. Charpentier, M.R. Sevenants, Eds., ACS Symposium Series, Volume 366, American Chemical Society, Washington, DC (1989) 52.

modifier to achieve successful analyte extraction or separation. The additives typically consist of organic acids, bases, and ion-pairing reagents, and may be added directly to the primary modifier or to the matrix.

Since it is expected that the extraction or the separation of pharmaceutical compounds may be difficult because of low analyte solubility in the supercritical fluid or severe matrix interaction, the extraction or chromatographic conditions may be improved by several means. Several strategies are reported in this dissertation including: ion-exchange, ion-suppression, and ion-pairing. Ion-exchange refers to the displacement of an acidic, a basic, or an ionic species from highly active matrix sites or stationary phase with a “stronger” modifier. Ion-suppression involves charge neutralization by the addition of an appropriate acid or base, while ion-pairing represents the electrostatic interaction of two species of opposite charge with one another to form an ion-pair-analyte complex.

For an extraction method to be deemed successful, both the removal of the analyte from the matrix and the trapping or concentration of the analyte prior to analysis must be optimized. There are generally three-types of off-line trapping systems used today. They include: 1) analyte precipitation onto a cryogenically cooled inert material such as glass beads, 2) analyte precipitation onto a solid-phase sorbent such as octadecyl silica, and 3) analyte collection into a liquid solvent. The main drawback of using liquid collection is that relatively low flow rates of approximately 1 mL/min. (liquid CO₂) or 500 mL/min. (decompressed gas) must be used because of possible solvent evaporation and violent bubbling which may lead to analyte loss. On the other hand with solid-phase trapping, higher flow rates may be used, and the need for preconcentration of the collected extract prior to analysis may be reduced. One of the major drawbacks of solid-phase trapping is the capacity of the solid-phase or the amount of analyte that the solid-phase can retain before analyte breakthrough occurs.

In the following chapters, both the role of trapping and strategies for improving the extraction of neutral and ionic pharmaceutical compounds will be explored. In

Chapter II, the trapping capacities of three solid-phases as a function of analyte polarity and volatility with CO₂ as an extraction fluid will be determined. In Chapter III, the effect of acidic, basic, and neutral secondary modifiers (i.e. additives) in the extraction of lovastatin from MEVACOR® tablets will be investigated. In Chapter IV, the extraction of an anionic compound, triphenylphosphinetrisulfonate, from spiked-sand with CO₂ aided by various ion-pairing additives will be investigated. In Chapter V, the effect of both primary modifiers and additives including ion-pairing reagents, acids, and bases on the extraction of a cationic species, pseudoephedrine hydrochloride, from spiked-sand and Suphedrine tablets will be investigated. In Chapter VI, the separation of neutral and ionic phospholipids via supercritical fluid chromatography as a function of stationary phase, addition of an acidic additive and its concentration, modifier ramp rate, and column outlet pressure will be presented.