MODIFICATION AND INSTRUMENTATION
OF A SCREENING UNIT
FOR EXTRACTION OF EGG YOLK LIPIDS WITH
SUPERCRITICAL CARBON DIOXIDE AND ENTRAINERS

By

WILLIAM J. CRERAR

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Agricultural Engineering
University of Manitoba
Winnipeg, Manitoba

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4. RESULTS AND DISCUSSION

4.1 SYSTEM PERFORMANCE

4.1.1 Introduction

The Superpressure SCE screening system was successfully instrumented and modified for the extraction of egg lipids with supercritical carbon dioxide and entrainers. As with any prototype, some debugging and experimentation was necessary to reach the final configuration. However, the system performance has proven generally acceptable during the experimental runs, and in subsequent work. Although there is room for improvement in several areas, the system as modified is a solid research tool for SCE investigations.

4.1.2 Extraction Process Equipment

4.1.2.1 Temperature Control Chamber and Extraction Vessels

Enclosing the extraction vessel in an airbath and adding a temperature equilibration coil to the CO_2 inlet stream has improved the extraction temperature regulation. Extraction temperature (°C) can now be set and maintained to within ±1° of the setpoint, as opposed to ±2° when temperature was controlled by a simple band heater in the original system (Labay, 1991). As well, the original system displayed marked difficulty reaching and maintaining higher temperatures (ie: 75$^+$ °C). The time lag in heating between the extractor surface and the internal thermocouple probe together with uneven heating from the band heater resulted in high temperature overswings on initial heating. A burnt odour was apparent during higher temperature extractions with the original system and the residue removed after extraction had a scorched appearance. With the new airbath system, the extractor is initially heated by transfer from the airbath, with

air temperature regulated by a thermocouple sensor mounted in the oven. The band heater, controlled by the internal thermocouple, is used only to supply supplemental heat during the extraction. Higher extraction temperatures (75-90°C) are now easily attained without evidence of scorching the extracted material. The addition of an air conditioner to the air bath circulation loop also permits lower extraction temperatures for near-critical and liquid CO_2 extraction (down to about 10°C) if necessary. At initial startup, there is still about a 10° temperature rise over the setpoint. This is evidence, perhaps, of temperature gradients forming within the extractor under conditions of low CO_2 flow. The overtemperature dissipates as CO_2 at the extraction setpoint temperature flows into the extractor via the temperature equilibration coil. Well before the first sample vial is changed, the extraction temperature is controlled to the setpoint value.

Mounting the extraction vessels on free-standing bases and connecting them to the extractor process tubing with quick-connect couplings makes vessel filling and cleaning much easier. The only drawback has been the frequent failure of the O-ring seals in the quick-connects. The sliding closure of the quick-connects places shearing stresses on the O-rings and makes them susceptible to tearing. Small tears in the neoprene rubber O-ring allow it to absorb high-pressure CO_2. The absorbed CO_2 expands and causes O-ring rupture when the system is depressurized. However, dusting the O-rings with graphite (for lubrication) and replacing them every 1-2 runs has proven sufficient to ensure reliable operation.

4.1.2.2 Extraction Pressure Control

The original Tescom extraction pressure regulator provides adequate control

of the process pressure (±0.5 MPa). However, there is some tendency for the setpoint to creep upward or downward during the course of an extraction by perhaps 0.5 MPa. The extraction pressure must be monitored and the regulator manually adjusted to compensate for this. Computer control of the regulator setting would overcome this, if suitable servo-mechanical components could be found or constructed to interface the regulator. Also, periodically (eg: about six months) the regulating mechanism will stick and start leaking. At this point the regulator must be disassembled and cleaned, although there is never any visual evidence of contamination. After cleaning and reassembly, the regulator functions well for several months.

4.1.2.3 CO_2 Flow Monitoring and Control

The Sensym pressure transducer which measured CO_2 flowmeter internal pressure was subject to baseline drift despite being warmed up and electronically zeroed at the start of each extraction. During an extraction, it was not uncommon to see the flowmeter pressure creep upward or downward by 2 or 3 kPa when there was no reason to believe that the gage pressure was actually changing by that amount. Due to the fact that CO_2 mass flow is calculated from *absolute* CO_2 pressure, this would lead to a maximum error of only 2 or 3 percent in the calculated mass flow of CO_2. The Sensym pressure sensor was salvaged and may well have been damaged or deteriorated before it was used on this project. However, now that the datalogger/control system has proven viable, replacement of the sensor with a new, reliable unit would be desirable to eliminate this source of experimental error. Ideally, the new pressure sensor would be calibrated to indicate absolute pressure directly. Alternatively, access to an accurate barometer near the experimental site would permit improved incorporation of pressure data into the

calculation of CO_2 mass flowrate.

CO_2 flow control for extractions was somewhat problematic. The extract was in most cases a rather viscous semisolid. The extract tended to spatter out of the collector nozzle, causing constant fluctuation in the flowrate. There did not seem to be a long-term problem with precipitate buildup in the metering valve or nozzle. Material washed out of the extractor lines after extraction was typically only 0.20 to 0.40% of the total amount collected in sampling vials and the mass balance for extraction of freeze-dried yolk with SC CO_2 was generally closed to within 2% of the starting sample mass. However, constant monitoring of the CO_2 flowrate and adjustment of the metering valve was required to keep the indicated time-averaged flowrate from drifting high or low. With reasonable attention, the long-term average flowrate, as measured by CO_2 passage over each sample collection interval was nevertheless highly linear and generally within ±0.25 g CO_2/min of the desired value (Figure 4.1). As was previously mentioned this is entirely adequate for SCE research, given the insensitivity of solubilities in SC CO_2 to flowrate if saturation is ensured (Fattori, 1985). The degree of operator involvement required, however, to prevent the extraction flowrate from either dropping or increasing drastically during the course of a run, was undesirable. This would be an excellent application for an automated valve controller.

Before an automatic control loop could be implemented, electronic flowrate instrumentation would have to be installed. The present method of displaying a running-average flowrate calculated from mass flow and time data is adequate to keep the operator
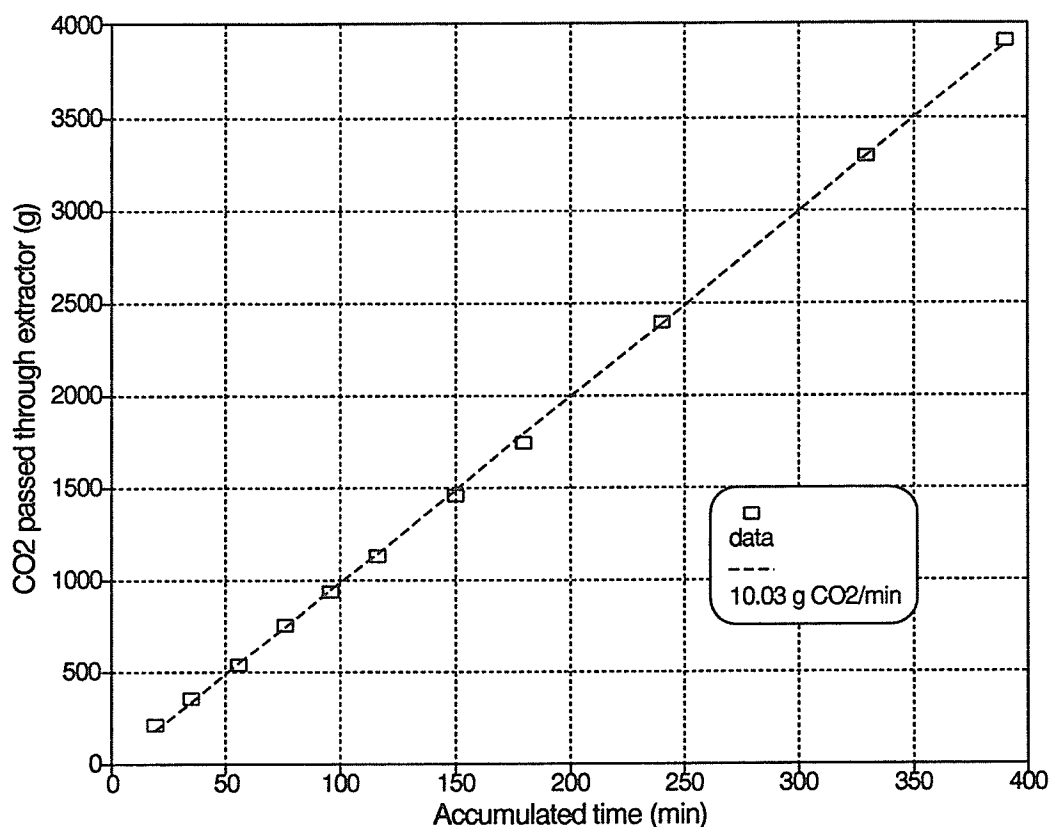
Figure 4.1: Carbon dioxide flow curve for a typical extraction: target flowrate was 10 g CO_2/min, actual flowrate was 10.03 g CO_2/min, $r^2 = 0.9997$.

a rough perception of the flowrate. The operator can use this information to determine the level on the rotameter corresponding to the target flowrate. For the flowrate information required to adjust the metering valve to return the flowrate to the setpoint value, the displayed, running-average flowrate is not adequately responsive. The operator must adjust the metering valve based on the rotameter response while mentally gauging the rotameter readout and correlating it with the displayed value. If the rotameter were replaced with a mass flowmeter calibrated for CO_2 flow, constant operator intervention could be replaced by a computer-controlled setpoint valve control loop.

Another problem related to the CO_2 flow control was erosion of the metering valve needle point. After extended use, it was noticed that the ability of the metering valve to control CO_2 flow at low rates (< 2 g CO_2/min) was deteriorating. The deterioration worsened with use until the minimum controllable flowrate rose to over 5 g CO_2/min. The valve was then removed and torn down (Figure 4.2). Examination of the valve tip revealed pitting and spalling (Figure 4.2a), which was obviously causing problems with the seal between the valve tip and its seat. The valve tip was reground in the Agricultural Engineering Machine Shop using a lathe mounted grinder. As the valve seat was nonremovable, it was reconditioned using a lap made of brass rod ground to the same angle as the valve tip. This operation was necessary three times in as many years. While it might be argued that the tip damage of Figure 4.2a is attributable to overtightening of the valve, the damage pattern sketched in Figure 4.2b is more clearly the result of erosion. The damage, which was observed the first time the valve was reconditioned, consisted of a ragged groove on either side of the tip. It was believed that this damage might have been caused by high-pressure CO_2 entering on one side of the valve and exiting on the other. While problems with seal erosion and extract deposition inside metering valves have been mentioned in the literature (Porter *et al.*, 1992; Sims and Thompson, 1991) little or no mention has been made of erosion of stainless steel components. Still, the author would recommend replacing the existing metering valve with one having a replaceable stem and valve seat to minimize future downtime.

4.1.2.4 Entrainer Removal

The 45°C bath/nitrogen flushing method used to remove entrainer from

a)

SPALLING
AND
ABRASION
OF
VALVE
TIP

b)

VALVE
STEM

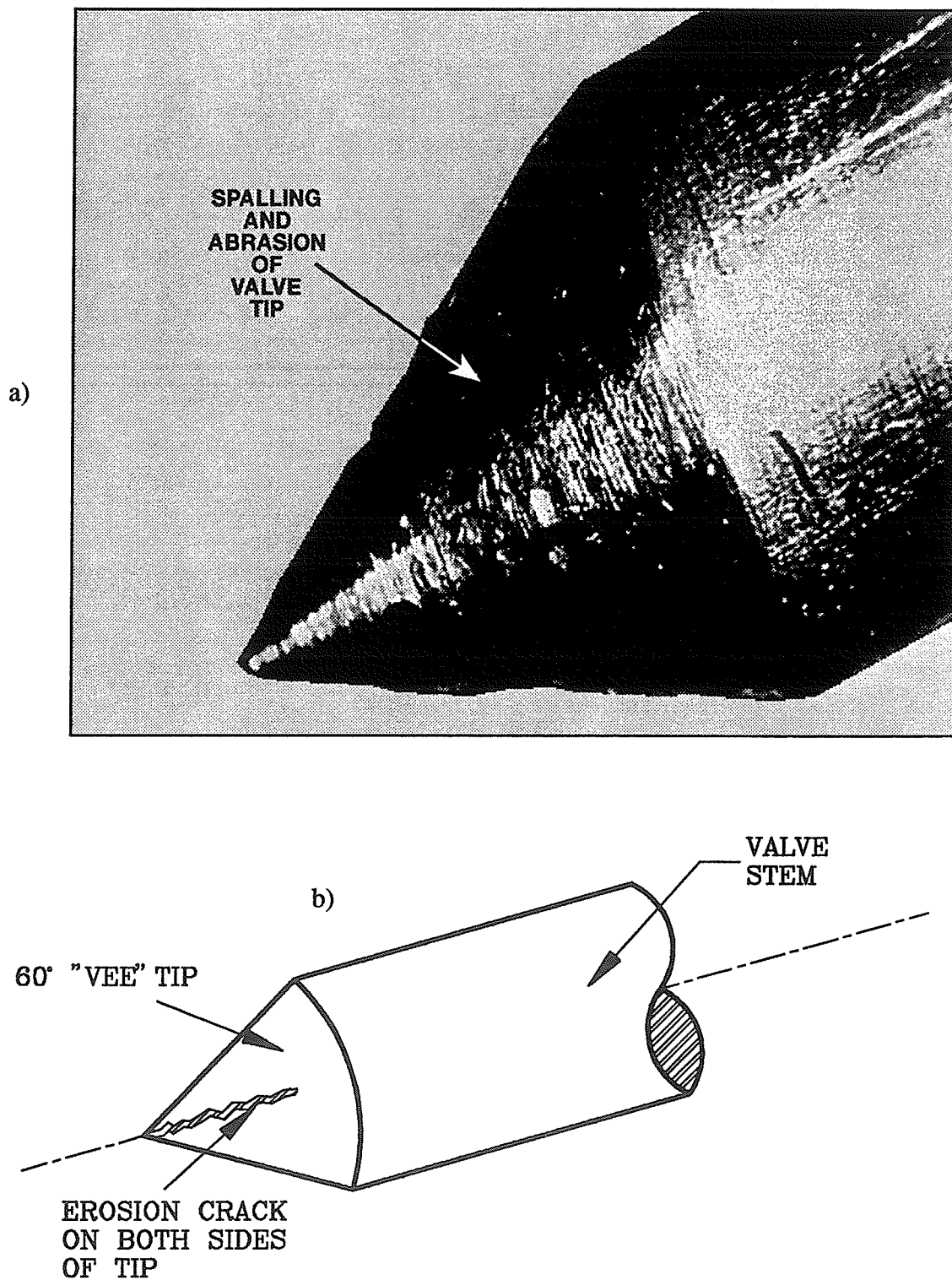60° "VEE" TIP

EROSION CRACK
ON BOTH SIDES
OF TIP

Figure 4.2: Accelerated wear of metering valve: a) photograph showing worn, pitted valve tip before regrinding b) sketch of wear pattern on another occasion, showing flow channel eroded by SC CO_2.

extract samples in this investigation was tested on model mixtures of egg lipids and entrainer (Appendix A). In Figure 4.3, the change in mass is shown for lipid samples which have been mixed with ethanol and methanol (with BHT added) and then dried for 8 h in the 45°C bath. Also shown is the change in mass of blank lipid samples which had no sample added but were placed in the bath for 8 h under nitrogen. Finally, the control samples were simply refrigerated under nitrogen for the duration of the test. As shown in Figure 4.3, the refrigerated control samples and the heated blanks lost a slight amount of mass during the experiment (maximum of 2.5% of the sample mass for the blanks). This was possibly due to evaporation of moisture or volatiles (Christie, 1987). The entrained sample mixtures on the other hand generally gained mass during the experiment, indicating incomplete removal of entrainer. However, the mass change is slight (maximum 2.8%), and not out of line with the general level of experimental accuracy. Comparison of the means of the mass changes (SAS/PC v. 6.04; SAS Institute, Appendix B) in the blanks and entrained lipid samples with the controls using Dunnett's procedure (Montgomery, 1984) found no significant differences at the 95% confidence level (Appendix A).

The mass balances for several extractions of defatted (pre-extracted with SC CO_2 @ 40°C, 36 MPa) egg yolk residue using SC CO_2 mixed with injected ethanol or methanol are given in Table 4.1. The mass balances were closed with either losses of up to 0.6% of the starting sample mass (for the methanol runs) or gains of up to 7.9% (for the ethanol runs). This suggests that there is a greater tendency for samples to retain ethanol than methanol. This might be anticipated by the higher boiling point (78.3 *cf.*
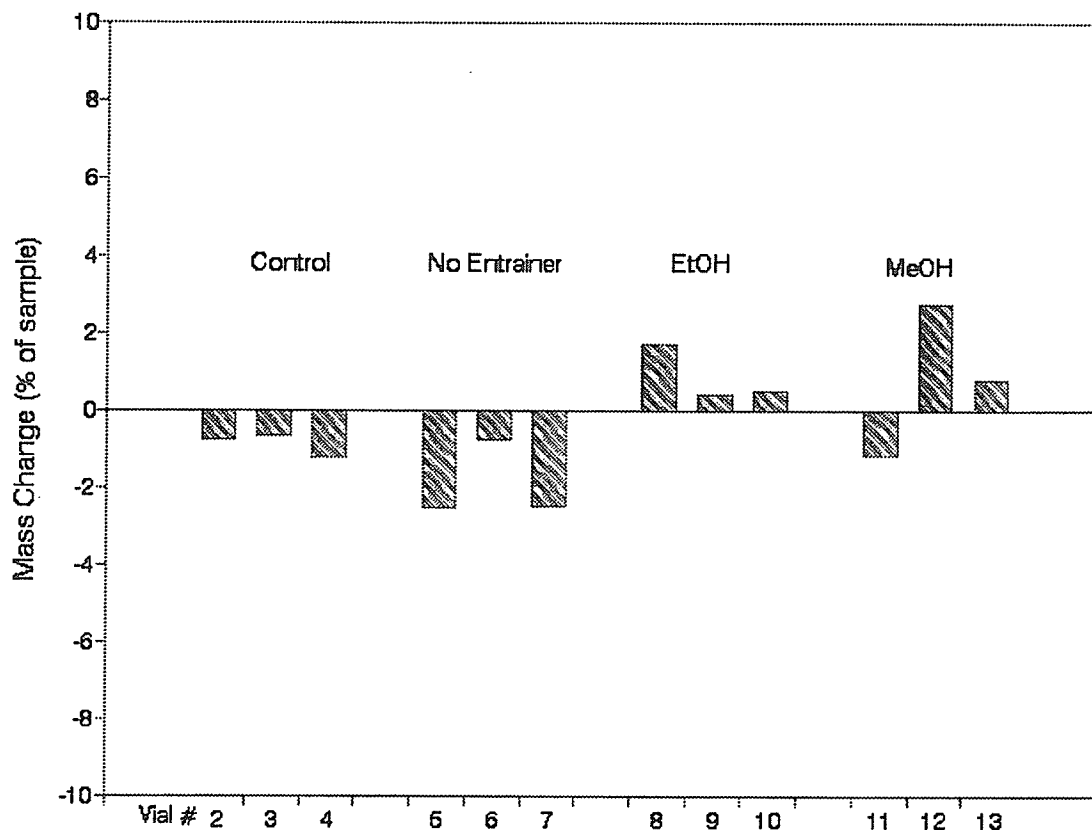
Figure 4.3: Mass changes in control lipid samples and model entrained samples dried under nitrogen in 45°C water bath.

64.7°C) and lower vapour pressure of ethanol as opposed to methanol (Yaws and Hopper,1976).

It must be remembered that the phospholipids are industrially referred to as "gum" (Szuhaj, 1983; Friedrich *et al.*, 1982). When the majority of the solvent has been removed, the egg lipids remaining tend to become viscous and/or form 'skins', hampering the evaporation process. Thus, the complete removal of alcohol entrainers from the egg lipid extracts is very difficult. Nelson (1975) suggested periodic addition of small amounts

Table 4.1: Results of mass balances conducted for several extractions of defatted egg yolk residue using SC CO_2 with injected ethanol or methanol.

Entrainer Type	Concentration (% w/w)	ΔMass Between Starting and Recovered Material (% of starting mass)[§]
Methanol (MeOH)	3	0.58
		0.13
Ethanol (EtOH)	3	-1.05
		-2.95
	5	-7.88
		-2.70
	10	-4.42
		-6.81

[§]Mass balance results are expressed in terms of loss ie: a positive value indicates that total apparent recovery was less than the sample starting mass. Masses of recovered material were measured after 'drying' to remove entrainer.

of chloroform during evaporation to aid in the removal of higher-boiling solvents such as ethanol and water. Several authors (Christie, 1987; Naito and David, 1984; Nelson, 1975) recommended that lipid samples not be stored solvent-free. Rather, a non-aprotic (ie: *not* alcohol) solvent such as chloroform should be added to the samples before longterm storage under nitrogen to prevent glass surface catalysis of lipid breakdown (Nelson, 1975). In retrospect addition of chloroform during evaporation to dryness, and before dry sample storage should probably be incorporated into the entrained extract drying process. However, the entrainer removal procedure as outlined in the Methods section did succeed in controlling the amount of residual entrainer remaining in the lipid

extract samples. The mass balances for entrained CO_2 extractions in Table 4.1 compare

favourably with the apparent mass increases of 10 to 30% for some extractions in the data

of Labay (1990).

4.1.2.5 Entrainer Injection Pump

The (converted HPLC) entrainer injection pump performed adequately.

However, some debugging was necessary before its operation could be considered

reliable. One extraction run was cut short when the filter frits on the HPLC plugged with

a clear gelatinous deposit. The deposit, which is believed to have resulted from initial

contamination of the solvent reservoir/pickup system (eg: the inlet screen) was removed

and not observed again. On several occasions, despite the supplementary check valve

placed in line between the screening system and the injector pump, backflow of SC CO_2

into the injector pump caused it to 'lose its prime' and stop pumping entrainer. To

restore normal operation the selector valve had to be switched to 'BYPASS', after which

the pump was primed and the flow system flushed with entrainer. The situation was

further complicated by the actions of the HPLC on-board microprocessor, which would

shut down the pump if it perceived an error condition despite the fact that the pumphead

was now under PC control. After the completion of the entrainer-injected extractions

reported here, the author discovered a poor connection of the RS-232 serial link ground

wire to the HPLC motherboard had been causing increasingly erratic operation of the

HPLC electronics. Also, a corroded connector for the HPLC pressure/flow transducer was

repaired, eliminating a frequent cause of microprocessor-perceived transducer failures.

The author notes that in subsequent work with the entrainer pump check valves replaced

(Shan, 1993), no major problems with entrainer pump operation were encountered.

Entrainer addition rate was as previously described monitored by the rate of mass depletion from the entrainer reservoir. To evaluate the impact of entrainer evaporation on this measurement the rate of methanol evaporation from the reservoir under typical laboratory conditions was evaluated (Appendix A). It was observed that capping the reservoir with a free-fitting aluminum disk reduced methanol evaporation by 40% (from 0.25 to 0.15 g MeOH/h). The rate of methanol evaporation from the capped reservoir was noted to be lass than 1% of the addition rate when injecting 3% methanol into a CO_2 flow of 10 g CO_2/min.

Once the injector pump had been roughly calibrated for mass delivery rate, the injector system was used in a series of extractions. The entrainer addition rate data was recorded, and later compared (Appendix A) with the addition rates calculated from the nominal entrainer addition rates and CO_2 flowrates. As shown in Figure 4.4, the use of the rough calibration resulted in delivery rates which were approximately 1.8% higher than setpoint. However, the actual delivery rate was extremely linear with the setpoint rate for both ethanol and methanol over an addition rate range corresponding to 3 to 10% w/w. The data were used to calculate a correction equation which accounted for the observed delivery rates with not more than ±0.13% error (Figure 4.4). The correction was applied to the controller program. Based on these results, the controller/entrainer injection system can now be relied upon to accurately deliver ethanol or methanol entrainer within the 3 to 10% w/w concentration range.
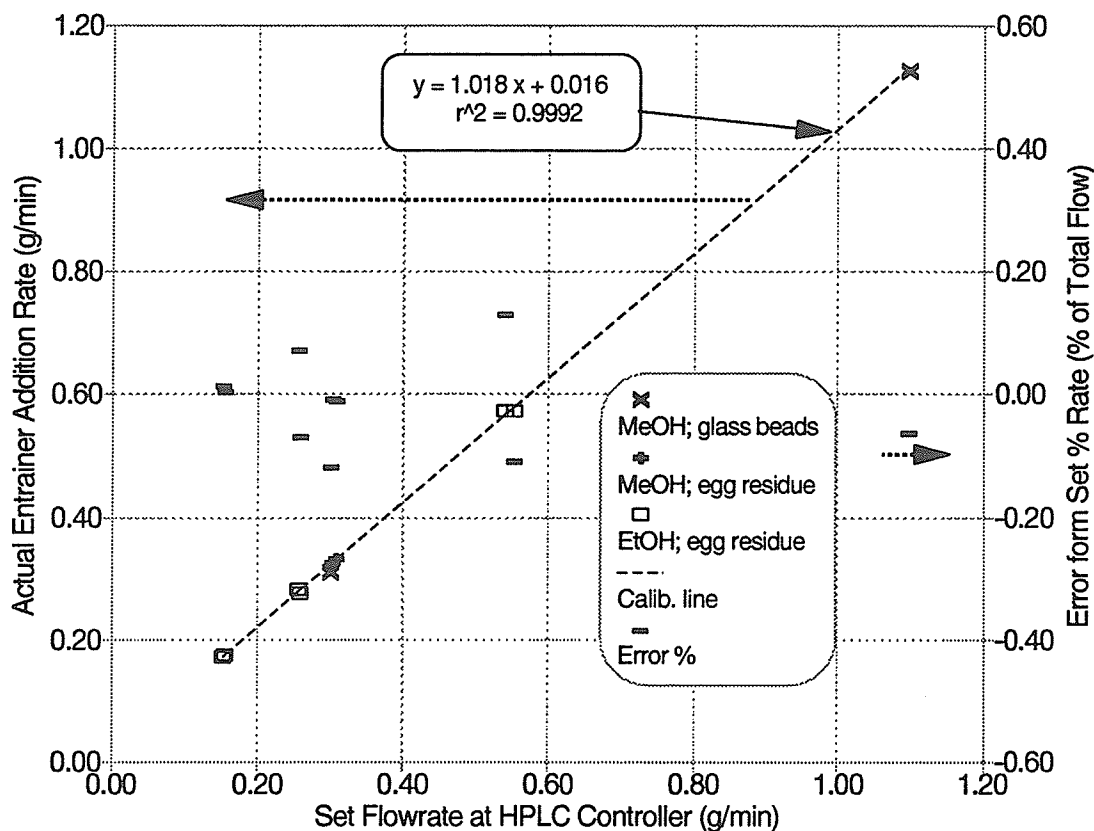
Figure 4.4: Entrainer addition rate calibration curve for entrainer injection system, based on mass addition data obtained during experimental runs.

4.1.3 Compressor Lubrication

Since the continuous-oiling pressure lubrication system was added to the pump main drive in the spring of 1990, no further problems with bearing failure have been experienced. During the initial wear-in period (about 50 h of operation), small flecks of brass were found to accumulate in the oil at the bottom of the slide. This was attributed to final removal of high spots on the mating surfaces and was not observed after the initial period. The pump drive bearings were twice torn down for inspection, most recently in the spring of 1993. The bearing surfaces showed light wear (on the brass

wearfaces of the slide block), but appeared to be perfectly serviceable for the foreseeable future.

Some leakage of oil from the catch pan at the pump drive base is apparent. Frequent replenishment of the lubricating oil is therefore required. In retrospect, a more fully enclosed design would have been desirable, both to prevent oil splashing over the sides and for increased safety. However, such a design would have prevented casual inspection of the drive slide wear surface - vital to monitor the state of the drive bearing and forestall another catastrophic bearing failure. In any case, replacing the present catch pan would require demounting the pump motor and crank drive, which would require another lengthy realignment and lapping-in procedure for the slide bearing. Any such action would wisely be postponed until made necessary (eg: when the brass wearfaces of the slide block become so worn as to require replacement).

4.1.3 Datalogger/Controller and Report Generator

The development of an interactive, PC-based process controller/datalogger system is deceptively simple in concept. Once the human operator becomes part of the control equation, the software is forced to anticipate a wide variety of potential operator requests and/or interventions which must be dealt with in orderly fashion without interfering unnecessarily with the controlling/data gathering process. Most of all, neither unforeseen process events nor operator actions should be permitted to 'crash' the governing programs. The controller/datalogger/analysis software must be effective and user-friendly while remaining robust and reliable for laboratory research use. Developing and

debugging the programs (Appendices E, F, and G) have been the most challenging and time-consuming aspects of this project. Yet the operator must be presented with a consistent and logical interface with which the process can be controlled and data can be stored and analyzed and communicated in meaningful form. Such an interface is key if supercritical extraction is to take its rightful place as a research tool like HPLC or NMR (Nuclear Magnetic Resonance) rather than an arcane art.

Initially, the technology was unproven. The datalogger and controller programs while under development frequently crashed upon encountering untested process situations or incompletely debugged program updates. Therefore, data was manually recorded while being simultaneously datalogged by the monitor program. Manual recording provided a check on the computer-logged results and also provided a backup experimental log in case of program failure.

The first item of interest was the reliability of the dry test flow meter pulse encoder and CO_2 mass flow totalizing software. For manual recording of CO_2 flow, the procedure of Labay (1991) was used. The totalized flow (L of gas) was read from the meter dial at each sample change. The volume of gas for each sampling interval was determined by subtraction. The mean temperature and pressure inside the flowmeter were then used to calculate a representative value of CO_2 density. This density was then used with the volumetric data to calculate mass flow of CO_2. It was found that solubility results recorded and calculated by the datalogger always agreed quite well with the manually recorded data (Figure 4.5). Any small discrepancies between the manually-
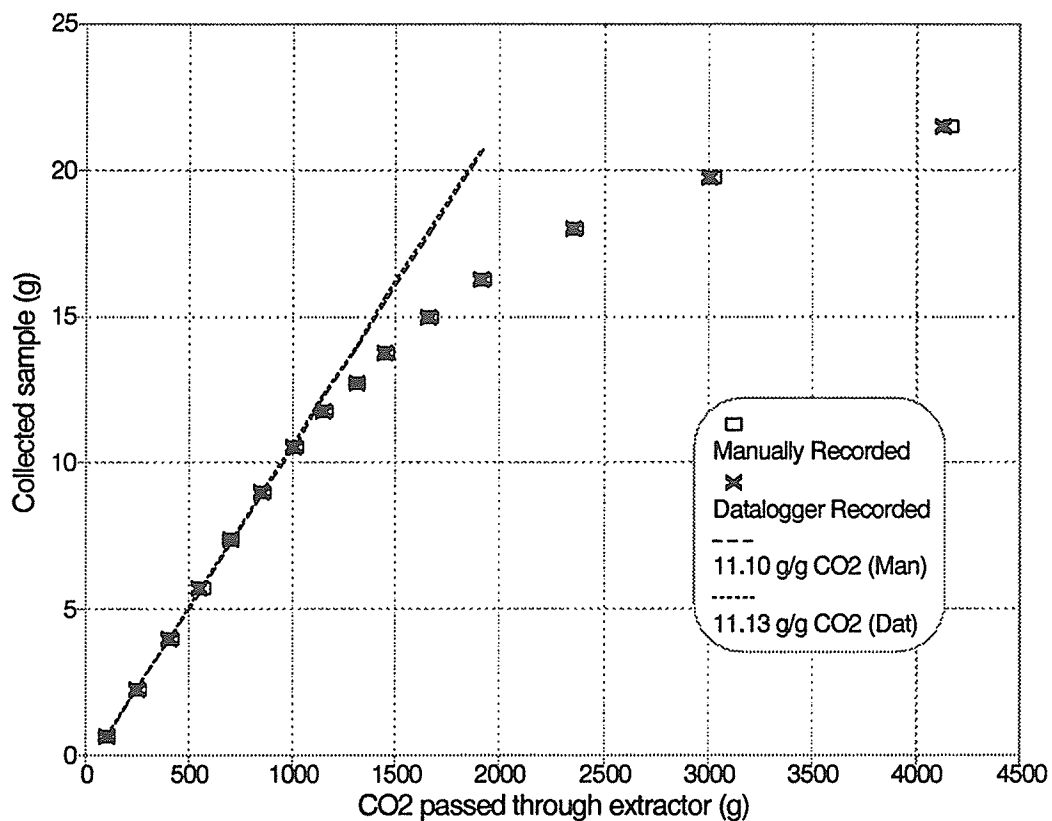
Figure 4.5: Comparison of sample recovery data recorded manually (with CO_2 flow calculated from dry test meter readout) with data recorded by datalogger program (with CO_2 computed from flowmeter pulse encoder, temperature, and pressure data) for a typical SC CO_2 extraction of freeze-dried egg yolk.

recorded and datalogged results are attributable to the fact that the datalogger program uses the flowmeter internal temperature and pressure data to calculate CO_2 density on each data reading cycle (about every 2 seconds) rather than using averaged values for the entire testing period, as in the manual method. The machine-gathered data was therefore inherently more accurate.

Similarly, when the macro data analyzer (Appendix G) was first developed, it and

the datalogger were tested by performing 'dummy' runs. In these runs, the process variables were set to constant values in the datalogger program, and the CO_2 flow was set to increment automatically. The mock extraction was then performed as for a real run, except that the sample mass recovery data entered was copied from previous extractions which had been manually recorded. The results of the dummy run were then saved to disk and run through the analysis macro program. The comparison of the results of the dummy runs with their real counterparts (recorded and calculated in spreadsheets manually) assisted with the debugging and proving of the datalogger/analyzer data handling routines. Further checking of the datalogged results against manually recorded data throughout the courses of the extractions showed that the datalogger and analysis programs performed reliably with the process data.

The controller PC and controller program CNTROLR1.EXE (Appendix F) have proven to be a reliable method of integrated process control. Future modifications would probably involve bringing the other process variables (valve heating, extraction pressure control, CO_2 flow control) under centralized, automatic control. The controller program and hardware have been designed in modular fashion to permit this. However, the controller software, like the datalogger, is presently limited by the system hardware. The use of Taurus KS102 data acquisition cards limits the processing power since the Taurus cards are not compatible with any PC beyond an XT (8086 processor) running at 8 MHz. The controller program running on the Corona PC at 4.77 MHz currently has a process variable update cycle time of about 4 s when controlling entrainer injection. This can be a long time between process control decisions when process variables are changing

rapidly (eg: at startup). If more channels are to be controlled, the controller computer should probably be upgraded to a PC XT compatible running at 8 MHz. At the same time this will not markedly increase controller performance, since the control loop time is also limited by communication with the HPLC pump at its maximum communication rate of 1200 BAUD (Bits AUDio per second). Automation of complex control processes such as CO_2 flowrate may well require the addition of distributed controllers (eg: programmable logic controllers, PLCs) to the system. The function of the controller PC would then be shifted to process variable setting and display via slave controllers rather than direct control as at present.

The datalogger program LOGR.EXE (Appendix E) is well-matched to the capabilities of its host PC XT compatible computer. The display is updated with a mean loop time of 1-2 s, appropriate for monitoring a relatively stable process such as SCE. The present compiled size of the monitor program is about 317 kByte. This leaves some room for future expansion given the 578 kB of base memory available for program use from the PC XT (with 640 kB installed base memory) under the DOS 5.0 operating system. While the monitor function could be improved by colour graphics and realtime display of sample recovery curves for example, this would involve extensive and costly upgrading of the monitor computer/data acquisition system and considerably complicate an already complex program. The current system functions adequately with the available hardware.

The extraction data analyzer macro M1.WQ1 (Appendix G) currently operates as

a one-pass system to format and analyze extract recovery data and print out results in spreadsheet and graph form. Because the macro runs as an 'add-on' to the spreadsheet program QuattroPRO, the analyses can execute on any PC which has the spreadsheet program installed. M1 therefore takes full advantage of the graphical capability and speed of more modern DOS-compatible PCs if available. Because analysis is intended to be performed in one session, M1 has no facility for retrieving spreadsheets from previous extractions which have already been processed. Also, there is no provision for automated incorporation and reporting of extract analysis results (eg: triglyceride, cholesterol, phospholipid contents and extraction profiles). To standardize and automate a procedure for dealing with extract analysis data, let alone deal with all the permutations of missing and available data, is beyond the scope of this project. Indeed there has been considerable effort to keep the software for the controller/datalogger/data analysis system sufficiently general that it might be adapted for future extraction studies with minimal changes. The data analyzer macro has been written in modular form, and relevant parameters and key data locations have been tabulated and stored in each spreadsheet (see Appendix G, "FLAG AND VARIABLE STATUS CELLS", and "BLOCK NAMES AND LOCATIONS") to facilitate future development of more advanced automated data analyses.

As of this writing, the software has been debugged to a useable level. Any fatal program errors situations which surfaced in this research or in subsequent use up to and including the summer of 1993 have been addressed. Some cosmetic defects remain in the user interface. Obscure program branches will sometimes pop up a misspelled or off-

centre menu, for example. Also parentheses or comments will sometimes be overwritten as a selection menu is scrolled.

The data analysis macro benefits greatly from being able to utilize the user interface and graphics capability of QuattroPRO. For the rest of the programming, many viable alternatives exist at the present time to the QuickBASIC coding which was used. Several user-interface "toolboxes" are now available. These, together with a graphical interface like Microsoft Windows would enable a programmer to quickly facilitate the development of new, more user-friendly controller/datalogger/data analysis software, if the hardware investment necessary to run this software were made. If the data acquisition equipment were upgraded, interface drivers for more advanced programming languages such as "C" could be obtained. The existing software tasks could then be performed more efficiently and faster. However, this would require a substantial time investment to recode and debug the existing software into C language, and the readability of the QuickBASIC code would be sacrificed. The advantage of the present system is that it works adequately with the available hardware. It is further hoped that the SCE screening system as modified and documented presents a base for future in-house modification and maintenance of the system as required.

4.1.4 Entrainer Addition and Recovery Studies

4.1.5.1 Introduction

The ability to control or at least monitor the entrainer concentration in the solvent SC CO_2 stream is essential in SCE research. The determination of the effect of

entrainer concentration on component solubility is often a major part of SCE investigations. One obvious method of monitoring entrainer concentration during extraction is by measuring post-extraction entrainer recovery. Labay (1991) performed a series of supercritical fluid extractions of freeze-dried egg yolk and defatted yolk residue using carbon dioxide premixed with ethanol or methanol as the SC solvent. Sample and entrainer were recovered in a chilled vial and U-tube arrangement as was used in the present study. Entrainer concentration was monitored by measuring the mass loss from the sampling vial when the lipid sample was dried under nitrogen in an ice bath. Labay (1991) noted some variation in the amounts of entrainer recovered from the expected values. She hypothesized that the concentration of ethanol or methanol entrainer in the CO_2 was changing as the cylinder became depleted. More recently Schweighardt and Mathias (1993) have cited several researchers including themselves as having noted inconsistent results and unpredictable instrument behaviour associated with premixed CO_2-entrainer mixtures. There appears to be a growing concern that entrainer premixtures may not give adequate control of entrainer concentration. Therefore the present investigation included a study of post-extraction entrainer recovery with the view of monitoring entrainer concentration rates.

This section includes a review of the results of entrainer trapping during the extraction experiments of Labay (1991), and experiments by this author, both using entrainer premixtures. Experiments were also performed in the present study trapping entrainer from entrainer-injected extractions. Finally, the significance of these experimental results is explored with regard to SCE experimental entrainer addition and

capture.

4.1.5.2 Entrainer Addition by Premixture

Entrainer recovery data from the experimental notes of Labay (1990) were used to plot the recovery curves of Figure 4.6. In these investigations (Labay, 1991) freeze-dried egg yolk was extracted using SC CO_2 and entrainer at 40°C and 36 MPa. The ethanol or methanol entrainers had been obtained premixed with liquid CO_2 from Matheson Gas Products Ltd. (Appendix B). The plots in Figure 4.6 show the entrainer recovery as measured by evaporative mass loss from the sampling vials plotted as a function of carbon dioxide usage. In Figure 4.6a, where freeze-dried egg yolk was being extracted, the recovery rates appear at first glance to be comparable to the nominal rates of mixed entrainer used. A closer inspection of curves a (3% MeOH) and d (3% EtOH) in Figure 4.6a shows that the concentration of entrainer drops quite noticeably during the course of the extraction, although the initial slope of recovery curve d (E_{q_0} = 2.96 % EtOH w/w in CO_2) is quite close to the nominal value of 3%. Curves a and b show entrainer concentrations considerably less than the nominal values (E_{q_0} = 2.04 % w/w MeOH $cf.$ 3% and E_{q_0} = 2.62 % w/w MeOH $cf.$ 3%, respectively) while curves c and e show entrainer concentrations *higher* than nominal (E_{q_0} = 3.16 % w/w MeOH $cf.$ 3% and E_{q_0} = 6.90 % w/w MeOH $cf.$ 5%, respectively). Figure 4.6b shows entrainer recovery curves for data recorded by Labay (1990) during re-extraction of defatted freeze-dried egg yolk (pre-extracted with SC CO_2, removing most of the cholesterol and triglyceride - Labay, 1991) with CO_2 and entrainer premixtures. Here the concentrations calculated from the recovery slopes are all much less than the nominal entrainer concentrations. Curve a in Figure 4.6b shows a low initial concentration and a changing slope throughout
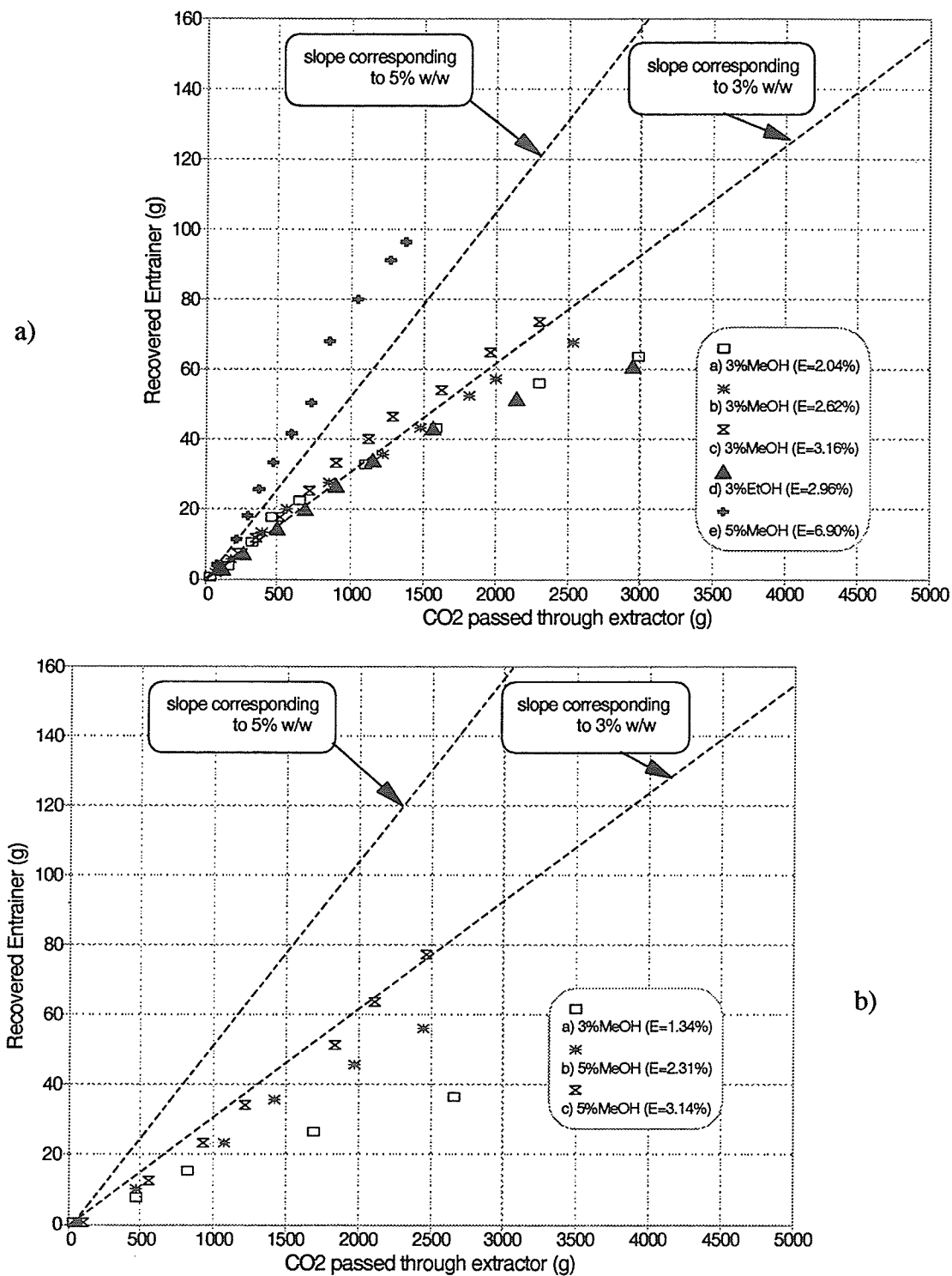
Figure 4.6: Entrainer recovered and evaporated from sample vials after extraction with CO_2-entrainer premixtures: a) extraction of freeze-dried egg yolk b) re-extraction of defatted residue (plotted from unpublished data of Labay, 1990). Legend values refer to nominal entrainer addition rate.

the extraction.

The lower-than-expected recovery rates might be attributed to incomplete recoveries. Generally higher recoveries were observed for the extractions in Figure 4.6a, where whole freeze-dried egg yolk was being extracted, than in Figure 4.6b, where defatted yolk residue was extracted. The higher amount of material extracted from whole freeze-dried yolk would tend to have more surface area and sorption sites for the alcohol entrainer. Increased surface area and sorptive capacity in a chilled trap should theoretically lead to increased trapping of volatiles (Mulcahey and Taylor, 1992a). Comparison of Figures 4.6a and 4.6b supports this hypothesis. As well, Labay (1991) reported that evaporation of entrainer from the entrained lipid samples was sometimes incomplete. This led to artificially high solubility values (and lowered entrainer recovery values). However this does not explain the higher-than-expected entrainer recoveries represented by curves c and e (Figure 4.6a), nor the several curves which had non-constant slopes during the extractions. If entrainer trapping was inefficient and sample drying was incomplete, entrainer recovery values calculated under these conditions should be artificially low rather than high. While it is conceivable that water could be extracted from the extraction sample, condense with the entrainer and inflate the calculated entrainer recovery value, this seems unlikely. The moisture content of the freeze-dried egg yolk was quite low (4.4 % w/w of dry sample - Labay, 1991). The solubility of water in supercritical CO_2 is also quite low, about 0.4% w/w in CO_2 (Schaeffer et al., 1989; Wiebe and Geddy, 1941 - see Figure 4.7). Finally, it is doubtful that water trapped with the sample would have evaporated from Labay's (1991) ice-bath drying
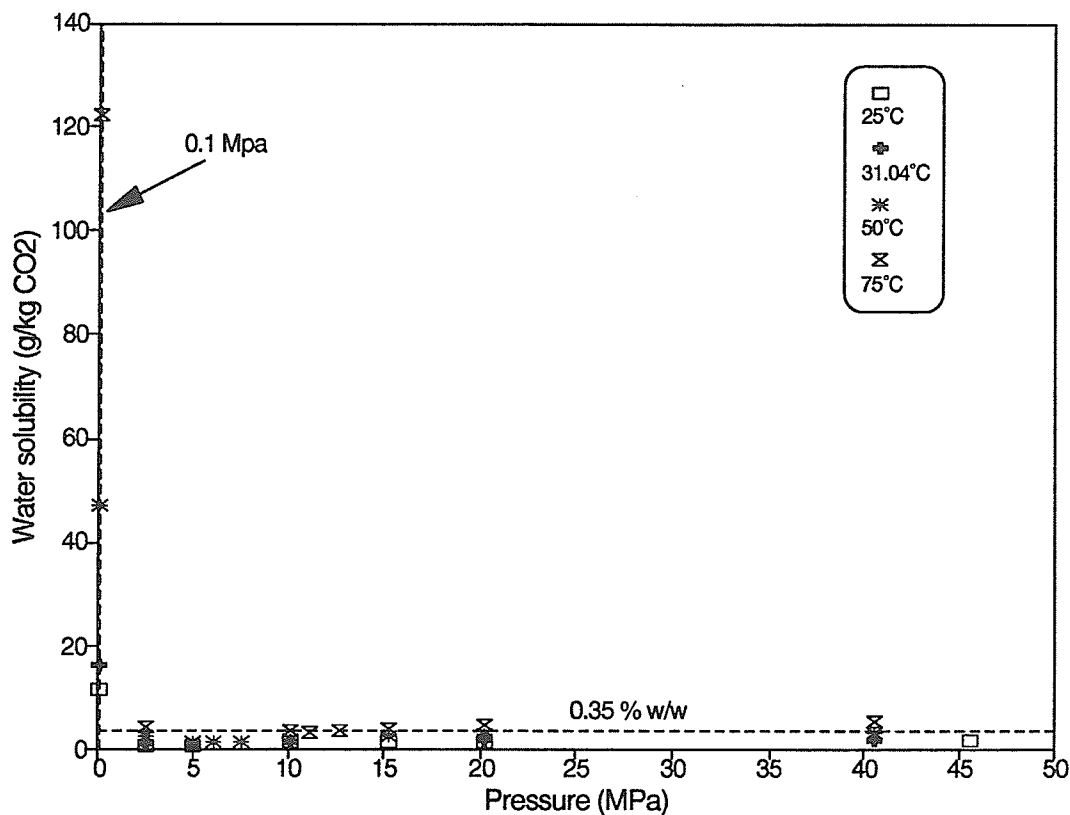
Figure 4.7: Water solubilities in low-pressure, near-critical and supercritical carbon dioxide, plotted as a function of temperature and pressure. (Calculated from the data of Wiebe and Geddy, 1941.)

technique. Rather, any water would have remained with the sample rather than evaporating and being counted as entrainer mass. On the one hand therefore, the higher-than nominal concentration values cannot be dismissed. On the other hand, lower-than nominal trapped entrainer values are associated with extractions in which low solute loadings may have reduced entrainer trapping efficiency. The actual entrainer concentrations may consequently have been higher than the values calculated by solvent trapping. Thus the solubility values reported by Labay (1991) for egg yolk extraction with 3% ethanol w/w or 5 % w/w ethanol or methanol in carbon dioxide might really

have been based on extraction with say 3.5% and 7% w/w entrainer concentrations, respectively.

To further investigate the entrainer concentration/trapping phenomena, a series of new extractions were performed using premixed ethanol or methanol entrainers at 3% and 5% w/w in CO_2. A straight tube (Liebig type) condenser was added to the sampling system downline from the U-tube trap and cooled by the same circulating bath as the methanol chilling baths. This condenser trap was meant to remove all traces of entrainer from the expanding CO_2 and ensure efficient entrainer recovery. The heated bath method was employed for entrained sample drying to promote efficient separation of the sample and entrainer. The total amount of entrainer trapped in the sample vial, the U-tube trap, and the condenser trap was reported as entrainer recovery. Figure 4.8 shows the plots of recovered entrainer from these extractions, which were performed at 40°C, 36 MPa.

The extraction indicated by curve *b* in Figure 4.8 was obtained during an extraction of whole freeze-dried egg yolk. The initial recovery concentration for this curve ($E_\%$ = 3.40% MeOH in CO_2) is higher the nominal 3% mix concentration. The recovery curve slope also decreases markedly during the latter part of the extraction. Recovery curves *a, c, d, e* and *f* were obtained for extractions of defatted freeze-dried egg yolk. The recovery entrainer concentrations for these extractions are all less than the nominal mixture concentrations. Recovery curve *a*, corresponding to an extraction with mixed methanol-CO_2 solvent drawn from a previously-used cylinder, shows a very low initial recovery ($E_\%$ = 0.25% w/w MeOH *cf.* 3 % nominal). The slope of concentration
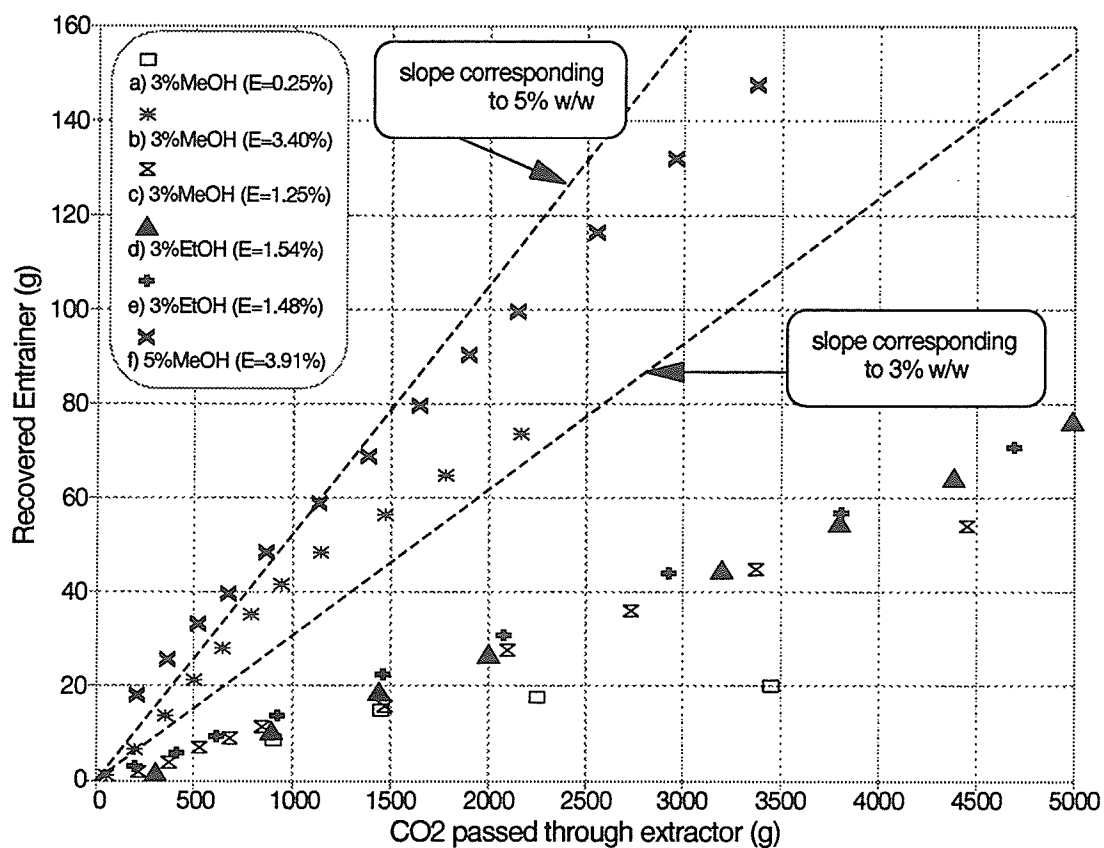
Figure 4.8: Entrainer recovered after extraction of freeze-dried egg yolk and defatted egg yolk residue with CO_2-entrainer premixtures in present study. Values indicate total entrainer recovered from sample vials, U-tube trap, and condenser trap. Legend values refer to nominal entrainer addition rate.

curve *a* also changes during the extraction, becoming flat near the end of the run.

In short, the results shown in Figure 4.8 support the findings from the work of Labay (1991, 1990). The addition of a condenser to improve entrainer trapping efficiency and the improvement of the separation of sample and entrainer still do not appear to have produced quantitative recovery of entrainer under low solute loading conditions. There is some evidence that the entrainer concentration during extraction is higher than the

nominal concentration from the cylinder mixing ratio. Also, there seems to be a common tendency for the entrainer concentration to change during extractions, as indicated by the rate of entrainer recovery. Without an independent means of monitoring entrainer concentration in the extraction system, it is difficult to know whether it is the calculation of trapped entrainer or the assumption of constant entrainer delivery which is inaccurate.

4.1.5.3 Entrainer Addition by Injection

The entrainer injection system was used to provide known concentrations of entrainer in CO_2 for further entrainer trapping trials. It had been established (see section on entrainer injection pump) that the program-controlled pump could maintain injection flowrates at preset rates (although final calibration was still being completed). Also, the entrainer addition rate could be independently monitored by measuring the depletion in mass of the entrainer reservoir. As part of the entrainer mass balance, entrainer accumulations in the extractor vessel and the mixing chamber were measured by the method of evaporative loss measurement at the end of each extraction. These measurements showed entrainer accumulation in the system never exceeded 5% of the entrainer mass added during a session, including entrainer which was absorbed by the sample matrix and entrainer precipitated from the CO_2 during system depressurization. As well, the excellent mass balance results obtained in earlier SC CO_2 extractions of egg yolk indicated minimum leakage from the system. Since the entrainer was being added at a controlled and monitored rate, was not accumulating in the system to any extent, and was not leaking out from the system between the injector and the sampling head, it followed that the concentration of entrainer in CO_2 could be calculated from the addition rate.

During these entrainer trapping trials, the system was modified to maximize entrainer trapping efficiency. The straight-tube condenser downstream from the sampling vial and U-tube trap was replaced by two Friedrichs-type condensers mounted in series, to increase the cold-surface exposure of the CO_2 and entrainer vapours. A third Friedrichs condenser was mounted downstream. This guard condenser was cooled by a separate circulating bath to -15°C. The guard condenser was to monitor the trapping efficiency of the upstream collection systems by removing any remaining entrainer from the low-pressure CO_2 stream.

The entrainer trapping trials were conducted for extractions of defatted freeze-dried egg yolk residue. The extractions were performed at 40°C, 36 MPa. Ethanol or methanol entrainer was added by injection at nominal concentration rates of 3% w/w (methanol) and 3, 5, and 10% w/w (ethanol) in CO_2. Entrainer addition and recovery curves for a typical extraction are shown in Figure 4.9. The addition curve data was calculated by mass depletion of the entrainer reservoir. The recovery curve represents the total of entrainer masses trapped in the sampling vial, the U-tube trap, the series Friedrichs condensers trap, and the guard Friedrichs condenser trap. Both the addition and recovery curves are highly linear. The slope of the recovery curve is lower than that of the addition curve. This indicates that even with all the extra condensers added, the entrainer was still not being efficiently trapped.

Figure 4.10 shows the entrainer recovery data for the same extraction as Figure 4.10, broken down according to trapping method. In the figure, 'in vial' denotes entrainer
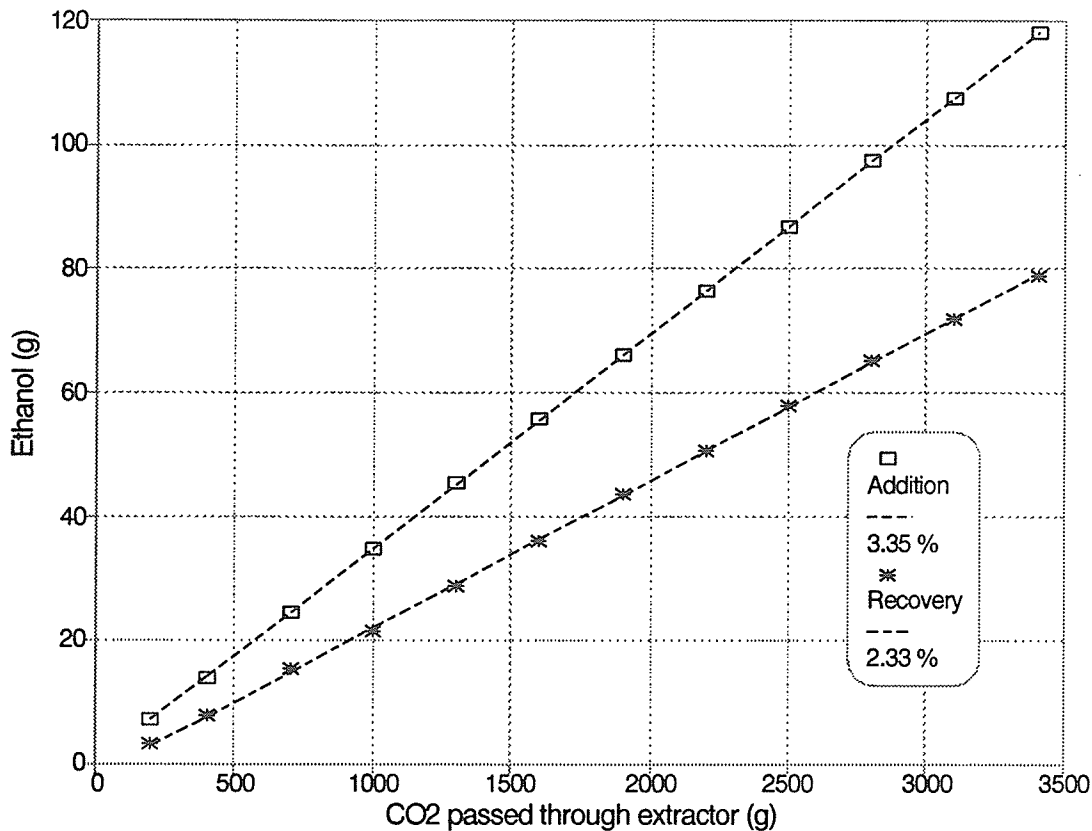
Figure 4.9: Typical curves for entrainer addition and recovery vs. CO_2 passed through extractor vessel for extraction of defatted egg yolk residue with CO_2/injected entrainer mixtures. The CO_2 flowrate was 5 g/min, and the nominal ethanol injection rate was 3% w/w.

recovery calculated by drying the entrained lipid caught in the sampling vials. The entrainer collected from the U-tube trap and the series Friedrichs condensers trap is denoted '1st trap'. The entrainer collected in the guard Friedrichs condenser trap is denoted '2nd trap'. The amounts of entrainer collected in each case have been expressed as percentages of the mass of entrainer added in each sampling interval. Figure 4.10a is the trapping distribution profile for a run with nominal 3% w/w ethanol injected in CO_2, while Figure 4.10b is the trapping profile for an extraction with 10% w/w ethanol.
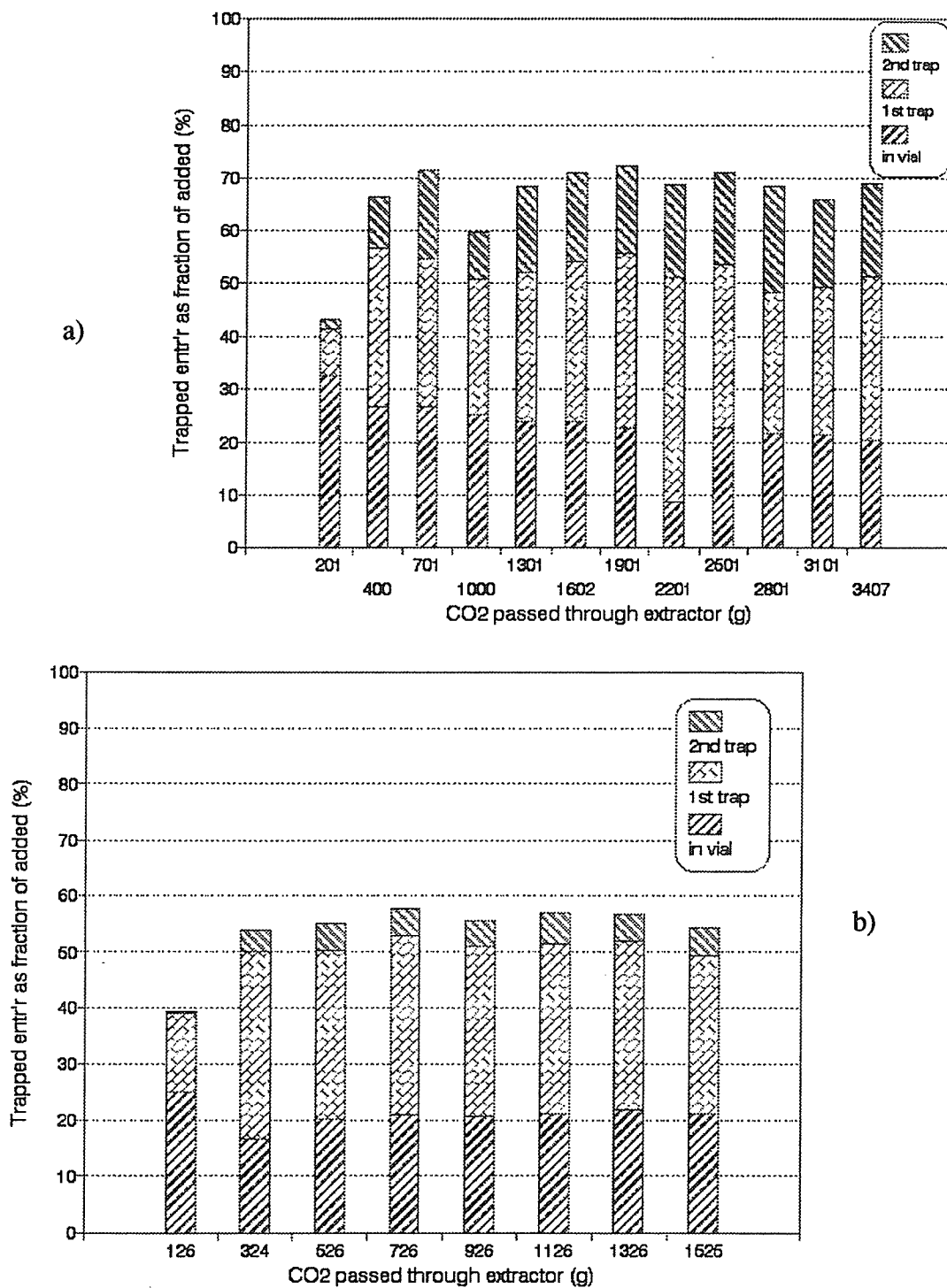
Figure 4.10: Typical entrainer recovery trap distribution profiles. The percentage of added entrainer recaptured in the sampler vial, in the U-tube and first condenser trap, and in the second condenser trap are shown over the course of an extraction. The CO_2 flowrate was 5 g/min, and the nominal ethanol injection rates were: a) 3% w/w b) 10% w/w in CO_2.

In the profiles, it is apparent that the amount of entrainer trapped by each section is relatively constant during the run once a steady state has been established. Constant entrainer addition rates have resulted in relatively constant recovery rates. It is also obvious that each level of trapping has resulted in a substantial gain in recovery. Additional levels of trapping would be required to maximize trapping efficiency. Quantitative recovery of added entrainer is not possible with the existing setup.

In Figure 4.11, the entrainer recovery curves for all of the entrainer-injected extraction runs are plotted as functions of the CO_2 flow. As with the premixture recovery curves (Figures 4.6b and 4.8) the entrainer concentrations calculated from the recovery data are considerably less than the nominal entrainer concentrations, for extraction of defatted egg yolk. However, in Figure 4.8 the recovery curves are all linear, suggesting that entrainer concentrations are constant for any given run. There is considerable variation in recovery rate between replicates (Figure 4.8). The 3% methanol extractions (curves $a1$ and $a2$) have low recovery rates compared with the 3% ethanol extractions (curves $b1$ and $b2$). This is due in part to the higher volatility of methanol, and partly to the fact that the methanol extractions were performed at CO_2 flowrates of 10 g/min, while the remaining runs were performed with flowrates of 5 g CO_2/min. The higher flowrates decreased the entrainer trapping efficiency. The variation in tapping efficiency between replicate extractions may be due to differences in the degree of defatting of the egg yolk residue samples. Variation in the amounts of triglyceride and cholesterol remaining in the samples before extraction would affect the composition and amount of material extracted. This could in turn affect trapping efficiency, as discussed in the
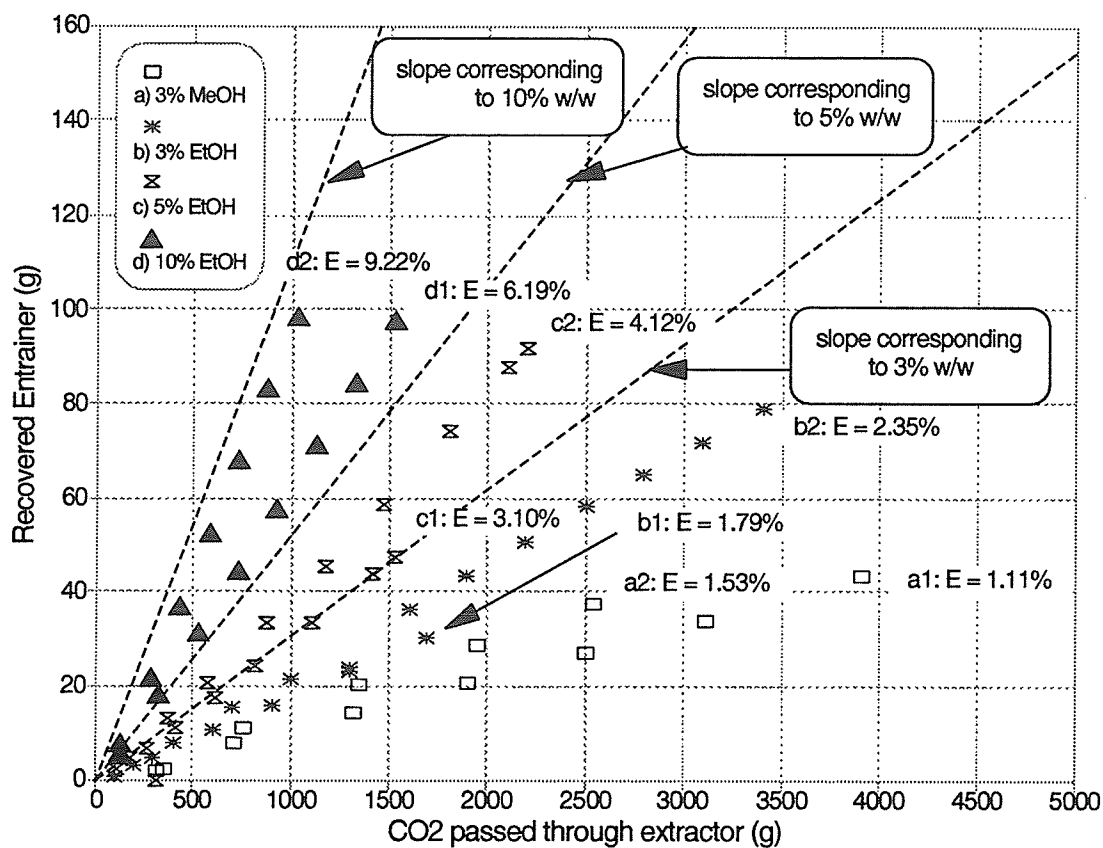
Figure 4.11: Entrainer recovered after extraction of defatted egg yolk residue with CO_2/injected entrainer mixtures. Values indicate total entrainer recovered from sample vials, U-tube trap, and condenser traps. Legend values refer to nominal entrainer addition rate.

section on extraction with premixed entrainers.

4.1.5.4 Significance of Addition/Recovery Results

The entrainer-trapping results in this section have shown that reliably monitoring entrainer concentration in CO_2 by condenser trapping is not a simple procedure. The efficiency of trapping depends on the CO_2 flowrate and to a great degree, on the level and type of solute loading. If solute loading is light, as it was for re-

extraction of defatted egg yolk, extremely efficient condenser systems will be required to obtain quantitative recovery of volatile entrainers such as methanol and ethanol. In the experimental work reported here, quantitative recovery could not be accomplished. Use of glass beads or sorbent materials might be attempted to increase the contact area and improve trapping efficiency. However, traps of this kind would be more susceptible to fouling and plugging. Also, an absorbent trap would have to be installed in the form of a replaceable cartridge to enable periodic measurement of trapped entrainer during an extraction. In the absence of quantitative recovery, entrainer trapping cannot be depended upon for entrainer concentration monitoring.

It has been shown that alcohol entrainers delivered from premixed cylinders may easily vary in concentration (in carbon dioxide) during the course of an extraction. Also, the concentration delivered may well exceed the nominal mixing ratio of the entrainer, as suggested by Schweighardt and Mathias (1993). The entrainer concentrations actually used by Labay (1991), and in the present work with premixed entrainers, are probably at least 1 or 2% higher than their nominal concentrations. In some runs, where the entrainer/CO_2 cylinder had been depleted by previous extractions, the entrainer concentration may have dropped by several percent during the extraction. However, given the unreliability and varied results of the entrainer trapping procedure, it is impossible to determine what the actual entrainer concentrations really were during extractions with premixed entrainers.

Extraction trials with injection of alcohol entrainers into the CO_2 flowstream, on

the other hand, have shown good results. The entrainer addition rate was reliably monitored by the depletion rate from the reservoir. The computer-controlled injector pump was able to reliably control the entrainer addition rate, over a nominal concentration range of 3% to 10% w/w in CO_2, ethanol or methanol. The recovery curves from entrainer-injected extractions, though not quantitative, were highly linear, indicating a constant entrainer concentration in the extraction system during the runs.

On the basis of these results, SC CO_2 extraction using premixed entrainers is not recommended, since the entrainer concentration delivered is extremely difficult to control or monitor. Addition of entrainer by injection will give more reliable results and can be more easily monitored. The reliability of an entrainer injection system will more than compensate for the extra expense of equipment and instrumentation.

4.2 LIPID SOLUBILITY RESULTS

4.2.1 Gross Lipid Solubility in SC CO_2

4.2.1.1 Introduction

Supercritical carbon dioxide extraction of freeze-dried egg at 40°C and 36 MPa resulted in a mean lipid solubility of 11.5 mg/g CO_2 with a standard deviation of 0.73 over 14 extraction trials. This is comparable to the value of 10.46 mg/g reported by Labay (1991). The solubility of egg yolk lipids at 40°C, 36 MPa was used as a benchmark throughout the study. Obtaining a solubility measurement in the range of 10.5 to 12 mg/g CO_2 under these extraction conditions confirmed that the extraction and recording apparatus was functioning properly.

The results discussed in this section refer to the gross solubility of egg lipids extracted from freeze-dried egg yolk with supercritical carbon dioxide. Later sections will cover the extraction fate of specific lipid components (eg: triglycerides, cholesterol, phospholipids) and the effects of lipid extraction with CO_2 mixed with alcohol entrainers.

4.2.1.2 Effect of CO_2 Flowrate

Lipid solubility values from extractions performed at 40°C, 36 MPa with CO_2 flowrates ranging from 9.6 to 10.6 g CO_2/min in the present study were compared with values obtained by Labay (1990). The selected runs from the data of Labay (1990) had also been obtained for extractions of freeze-dried egg yolk (0.85 - 2 mm particle size distribution) performed at 40°C, 36 MPa but at lower flowrates (4.1 to 5.2 g CO_2). The mean lipid solubility measured for the lower flowrates (10.7 mg/g CO_2; standard deviation 0.91 for 3 trials) was not significantly different from the mean value measured for the

higher flowrates (11.6 mg/g CO_2; standard deviation 0.74 for 13 trials) using Duncan's procedure at the 95% confidence level (Appendix A). The fact that measured solubility was not significantly higher at the lower flowrates is consistent with the assumption that the solubilities measured in the present study represent equilibrium solubility values for egg lipids extracted with SC CO_2 (See the discussion of dynamic solubility measurement in the Literature Review).

4.2.1.3 Effects of Extraction Temperature

Figure 4.12 is an extraction profile plot for four typical SC CO_2 extractions of freeze-dried egg yolk. The extractions were performed respectively at process temperatures of 40, 55, 65, and 75°C at a constant pressure of 36.0 MPa. Visually, it is clear that the initial slopes (solubilities) of the four curves are similar. Also, the extraction curves appear to converge asymptotically at a recovery of about 45% of the original sample mass. The composition of egg yolk solids is about 40-45% w/w triglycerides and 2.5-3.5% w/w cholesterol (See Literature Review). Therefore, Figure 4.12 suggests that extraction to exhaustion with SC CO_2 will extract almost all of neutral lipids from the freeze-dried egg yolk independent of extraction temperature in the range 40 to 75°C. This is consistent with the findings of Labay (1991).

The mean lipid solubilities measured for the four extraction temperatures are listed in Table 4.2. Also listed for comparison are the corresponding egg lipid solubility values determined by Labay (1991). The results of the present study show that lipid solubility was not significantly affected by extraction temperature over the range of 40 to 65°C. Lipid solubility dropped significantly (to 10.8 mg/g CO_2) when the results for extraction
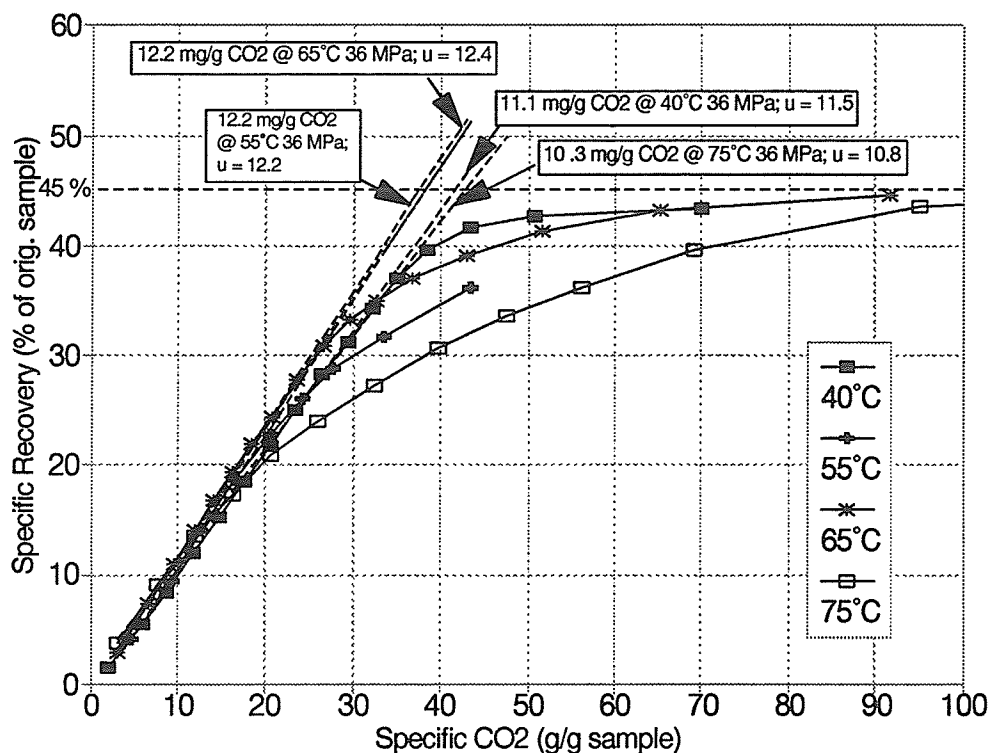
Figure 4.12: Normalized extraction profiles for egg yolk lipids extracted by SC CO_2 as a function of extraction temperature (40, 55, 65 and 75°C) at constant extraction pressure 36 MPa. The profiles and solubility slopes represent individual typical extractions. The mean solubility values $u_1...u_4$ represent 14-replicate determinations for 40°C and duplicate determinations for the other temperatures.

temperature 75°C was compared to those for 65°C (See Appendix A for details of statistical analysis). Labay (1991) also reported no significant effect of extraction temperature on lipid solubility in the range of 40 to 55°C. Labay further noted a significant drop in lipid solubility between 55 and 75°C. However, the values obtained from the present study are slightly higher than those obtained by Labay (1991), and the solubility drop at 75°C is not as severe.

Table 4.2: Egg lipid solubilities in SC CO_2 as a function of extraction temperature at constant extraction pressure 36.0 MPa.

Extraction Temperature (°C)	Gross Lipid Solubility, mg/g CO_2	
	Present Study (mean ± st. dev.)	Labay (1991) (mean ± st. dev.)
40	11.5 ± 0.7[ab]	10.5 ± 1.1[a]
55	12.2 ± 0.1[ab]	9.0 ± 1.0[a]
65	12.4 ± 0.2[a]	
75	10.8 ± 0.8[b]	6.2 ± 0.5[b]

means with different superscript letters in the same column are significantly different ($\alpha = 0.05$)

Examination of the extraction profiles in Figure 4.12 at higher extraction temperatures (especially 65 and 75°C) reveals a tendency to flatten out relatively early in the extractions. After the completion of several of the higher temperature extractions, the extractor bed contents were found to have fused together into one or more lumpy masses. Spaces between the coalesced residue masses and the extractor walls indicated that the SC CO_2 flow was bypassing the sample matrix rather than percolating through it. This tendency to lump formation by the sample matrix with attendant loss of extraction efficiency seemed to be most prevalent when more than a week or two had passed between the freeze-drying and the SC CO_2 extraction of the yolk material. This suggests that the problem may have been partly due to moisture absorption. The coalescing tendency was especially aggravated by higher extraction temperatures. Little tendency to lump formation during extraction was noted when the samples were extracted at 40°C. The extraction curves recorded by Labay (1991) show a similar correlation between high

between high temperature extractions and early flattening of the extraction curves. Supercritical extraction of freeze-dried egg yolk at 75°C has also been associated with denaturation of egg yolk proteins (Arntfield *et al.*, 1992). It is therefore possible that reduced extraction efficiency caused by loss of extraction bed porosity might cause solubility values measured from extraction curve slopes to be artificially low. This could explain the apparent drop in solubility values measured for the extraction temperature of 75°C both in this study and that of Labay (1991). In any case, it appears from these results that lipid solubility does not significantly increase with increasing extraction temperatures in the range of 40 to 75°C. The problems of loss of extraction bed porosity and/or residue scorching do increase with higher extraction temperatures, however. Therefore a maximum extraction temperature of 40°C for SC CO_2 extraction of egg lipids at 36 MPa is suggested.

4.2.1.4 Effects of Extraction Bed Structure

The tendency of extraction beds prepared with freeze-dried egg yolk particles to coalesce during extractions at higher temperatures and to lose their porous structure was noted in the previous subsection. Following these observations, a brief study was made of the effect of extraction bed structure on the progress of supercritical extractions and lipid recovery. Increasing particle size and imposing artificial structure by glass bead addition were considered as possible methods to improve the maintenance of bed structural integrity during extraction.

Extraction profiles from several SC CO_2 extractions of egg yolk at 75°C, 36 MPa are shown in Figure 2.13. Curves *a* and *b* represent extractions from medium (0.85-2.0
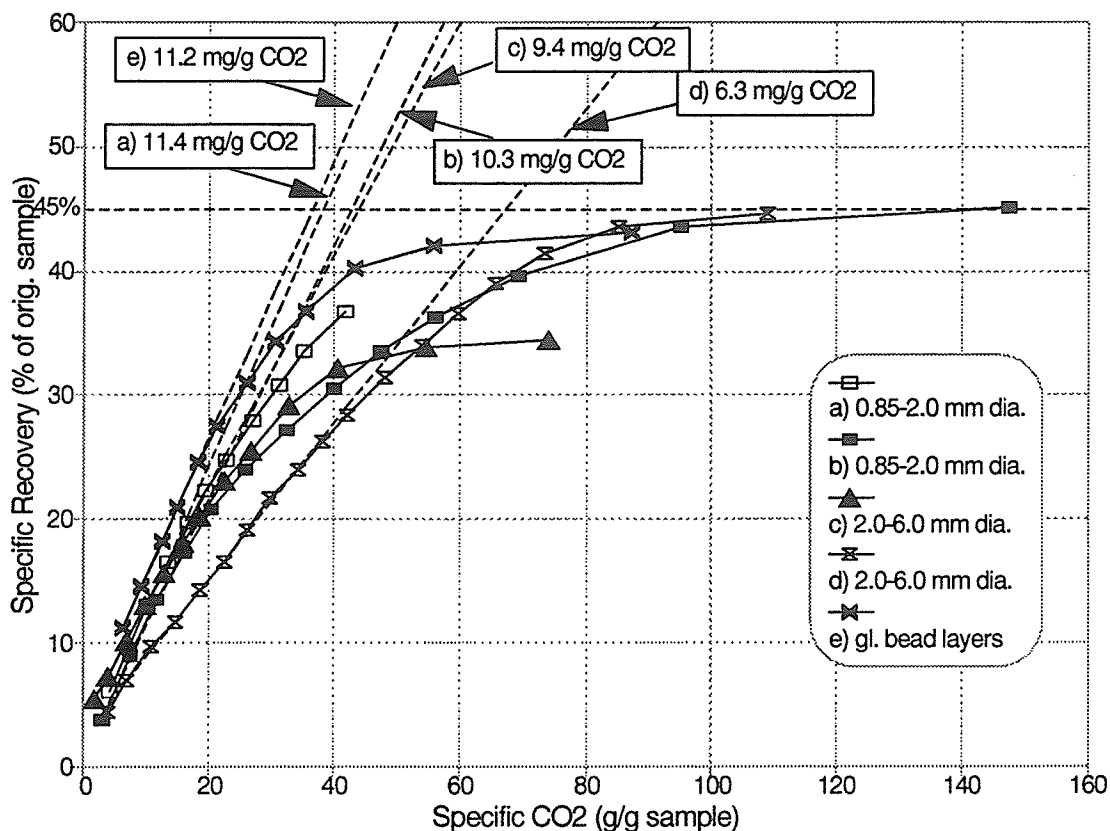
Figure 4.13: Normalized extraction profiles for SC CO_2 extraction of freeze-dried egg yolk at 75°C, 36 MPa. Profiles of runs extracting medium (0.85-2.0 mm ϕ) and coarse (2.0-6.0 mm ϕ) particles, and from particles layered on glass beads are shown. Solubilities indicated on the graph were calculated from the corresponding "Lipid recovery vs. CO_2 passed through extractor" curves.

mm ϕ) egg yolk particles, as were used in most extractions in this study. Curves *a* and *b* exhibit the flattening early in the run which was earlier noted as typical of extraction bed coalescence. Curves *c* and *d* in Figure 4.13 were obtained from extractions of particles which had been sieved to select larger diameter particles (2.0-6.0 mm ϕ). As can be seen from the curves, extraction of larger particles produced varying results. Curve *c* displays almost as much flattening as curve *b*. Also, the recovery from curve *c* is less than that observed for the other curves. However, it should be noted that in this particular run

10.8% of the starting mass could not be accounted for in the mass balance. In contrast the other extractions had more typical mass discrepancies of 1-2% of the starting sample mass. It is therefore possible that the incomplete recovery for curve c was the result of an experimental blunder rather than a genuine phenomenon. Curve d represents an extraction of coarse egg yolk particles (2.0-6.0 mm ϕ) under the same conditions (75°C, 36 MPa) as used in the extraction which produced curve c. Curve d displays good initial linearity, although some flattening is observable in the later stages of the extraction. Ultimate recovery for the curve d appears to be close to the theoretical limit of about 45%, as for the other extractions. However, the lipid solubility calculated for curve d (6.3 mg/g CO_2) is much less than the solubilities determined for the other extractions performed at 75°C and 36 MPa. It appears that the initial sample placement in the extractor vessel must have created voids sufficiently large to permit a portion of the extracting CO_2 flow to bypass the egg yolk particles without intimate contact. The CO_2 exiting the extraction vessel was therefore not completely saturated with dissolved lipids. Construction of extraction beds from fewer, larger particles would be more prone to such sample particle misarrangement and variable results than would the use of smaller particle sizes as were used in the rest of this investigation.

In the final phase of this phase of investigation artificial extraction bed structure was added in the form of 6-mm ϕ glass beads. The normal-sized (0.85-2.0 mm ϕ) freeze-dried egg yolk sample was added to the 300-mL extractor vessel in twelve thin layers, alternating with layers of glass beads. The beads helped to stabilize the extraction bed and also to disperse the CO_2 flow across the extractor vessel, aiding the percolating action

and ensuring intimate contact between the SC CO_2 and the sample matrix. As shown by curve e (Figure 4.13) the resulting extraction curve has a linear initial section and final lipid recovery close to the theoretical limit. There is less evidence of extraction bed coalescence than in the other extractions performed at 75°C, 36 MPa (Figure 4.13). The solubility determined from the extraction represented by curve e is similar to that determined for the other 75°C, 36 MPa extractions (except for the anomalous results represented by curve d). Adding glass beads to the extraction bed required extra effort in bed loading and post-extraction separation of the beads from the egg residue. However, it appeared to be the most successful strategy for counteracting temperature-induced loss of extraction bed structure.

4.2.2 Composition of Lipid Extracts and Estimation of Component Solubility

4.2.2.1 Composition of Egg Yolk and Extraction Residue

For freeze-dried egg yolk samples, proximate analysis indicated 66.7 to 66.8% w/w total lipids. Protein (32±3% w/w) and moisture content (4.4±0.2% w/w) were analyzed in a separate study and reported elsewhere (Arntfield *et al.*, 1992). These values are comparable to reported values for egg yolk composition (see Table 2.10 in Literature Review). Proximate analysis of extractor residue samples after extraction of freeze-dried yolk with SC CO_2 indicated residual lipid contents of 37 to 42% (depending on extraction conditions and duration of extraction).

Selected samples of freeze-dried yolk, SC CO_2 lipid extracts, and extraction

residue were analyzed for triglyceride, cholesterol, and phospholipid (phosphatidylcholine, PC; and phosphatidylethanolamine, PE) content. For each sample, two or more analyses were performed (sample quantity permitting). The results for each sample were then reported as a mean value (% w/w of sample mass) and a standard deviation of analytical results. The composition ranges for freeze-dried egg yolk samples were 43.1 to 45.7% w/w (±8%) triglycerides, 3.5 to 4.0% w/w (±0.6%) cholesterol, 13.6 to 17.8% w/w (±5%) PC, and 3.5 to 3.7% w/w (±0.7%) PE. Once again, the mean composition values are comparable to those in the literature. However, the typical variation in results (bracketed values) for a given sample is quite large, possibly due to non-homogeneous sampling. Therefore, although the analysis results as a body seem reasonable, individual measurements should be viewed with caution.

Analysis of extraction residue (raffinate) samples after SC CO_2 extraction (extraction temperatures ranging from 40 to 75°C, extraction pressure 36 MPa) indicated substantial depletion of neutral lipids. Typical triglyceride levels in the raffinate ranged from 1.6 to 5% w/w, while residual cholesterol levels ranged from 0.5 to 0.7% w/w. Phospholipids were found at concentrated levels in the raffinate. Typical PC and PE levels in the raffinate were in the ranges of 25 to 34% and 3.5 to 4.0% w/w, respectively. Similar lipid concentrations in extractor raffinate after SC CO_2 extraction were reported by Froning et al. (1990), although direct comparisons are impractical due to differences in extraction conditions and run durations (see Table 4.3). Froning and co-workers (1990) proposed substitution of the cholesterol- and triglyceride-reduced raffinate for spray-dried egg yolk in commercial food-processing applications. They reported that emulsion

Table 4.3: Lipid composition of residue samples reported by Froning *et al.* (1990) after SC CO_2 extraction of spray-dried egg yolk with SC CO_2 at various temperatures and pressures using 25±1 g CO_2/g of sample.

Treatment	Total Lipids (% w/w)	Cholesterol (% w/w)	Phosphatidyl-choline (% w/w)	Phosphatidyl-ethanolamine (% w/w)
control (spray-dried egg yolk)	61.12	1.85	18.23	2.78
40°C, 16.5 MPa	59.51	1.55	20.44	3.08
45°C, 24.1 MPa	53.54	1.33	23.89	3.52
45°C, 31.0 MPa	40.43	0.64	32.64	4.64
55°C, 37.9 MPa	38.96	0.63	32.81	4.41

stability was not significantly impaired and sponge cake volumes were significantly increased, relative to the use of ordinary egg yolk powder, for all but the most extreme extraction conditions shown in Table 4.3.

4.2.2.2 Lipid Extract Composition

Analysis of lipid extracts produced by SC CO_2 extraction of freeze-dried egg yolk showed no detectable concentrations of either phosphatidylcholine or phosphatidylethanolamine. The relative insolubility of phospholipids has been extensively noted in the literature (Bulley and Labay, 1991; Froning *et al.*, 1990; Hardardottir and Kinsella, 1988; Fattori *et al.*, 1985; Friedrich *et al.*, 1982). The selective extraction of nonpolar lipids causes a concentration of PC and PE (and protein) in the extraction residue, as reported in the previous section. This led Labay (1990) to propose that extraction with SC CO_2 be used as a concentration step in the isolation of egg phospholipids.

Labay (1990) used fatty acid methyl ester/gas chromatography (FAME-GC) analysis to indirectly quantify triglycerides in SC CO_2 extracts of freeze-dried egg yolk. Labay (1990) reported that the fatty acid distribution of the lipid extracts was similar to that of the lipid fraction of the original freeze-dried egg yolk, and that relative fatty acid concentrations in the extract did not change appreciably during the course of an extraction. Likewise, fatty acid distribution in the extract was not substantially affected by extraction pressure or temperature (temperature range 40 to 75°C @ 36 MPa; pressure range 15 to 36 MPa @ 40°C) or the presence of ethanol or methanol entrainers. Labay (1990) reported triglyceride solubility on the basis of equivalent fatty acids contents determined by FAME-GC. Since FAME did not discriminate the source of the fatty acid moieties, no attempt was made to separately quantify the mono- and di-glycerides, free fatty acids, pigments, sterol esters and vitamin alcohols which together with the triglycerides and cholesterol comprised the neutral lipid fraction of the extracts.

In the present study, HPLC analysis revealed extra peaks corresponding to substantial quantities of mono- and diglycerides and free fatty acids in the lipid extracts. Triglyceride and cholesterol contents together typically represented only about 85 to 95% of the extracts. In Figures 4.14 and 4.15, the triglyceride and cholesterol contents of selected extract samples have been plotted against specific lipid recovery, as a normalized measure of extraction progress. (Recall that a specific recovery of 45% of the original freeze-dried egg yolk mass corresponds to essentially complete extraction of the neutral lipid components.) Although experimental errors undoubtedly contributed to data spread in the results, the results in Figures 4.14 and 4.15 clearly show substantial variation in the
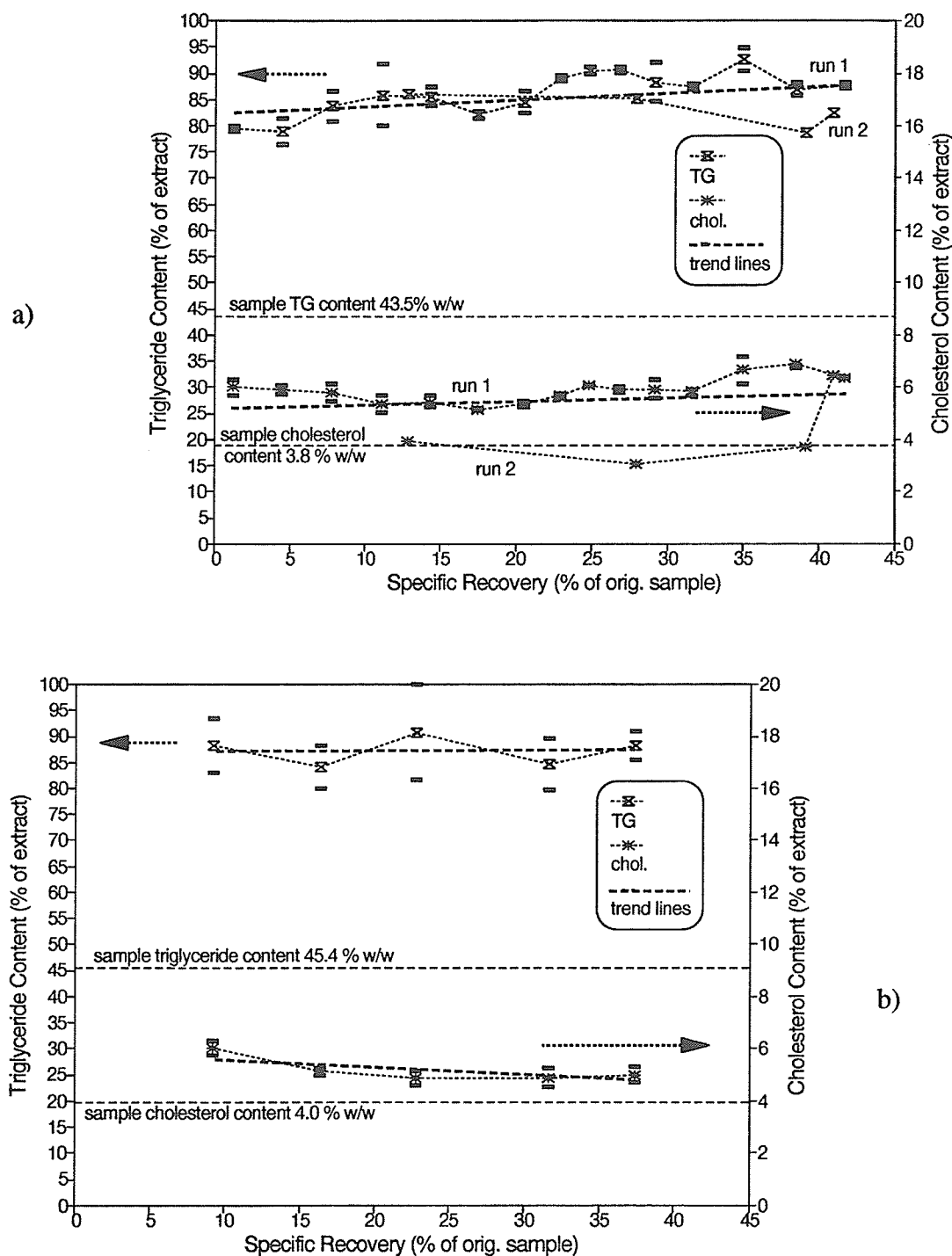
Figure 4.14: SC CO_2 extract composition profiles for lower extraction temperatures: a) 40°C, 36 MPa b) 55°C, 36 MPa. Triglyceride and cholesterol contents of extracts are plotted as functions of extraction progress as measured by percent of extraction sample recovered.
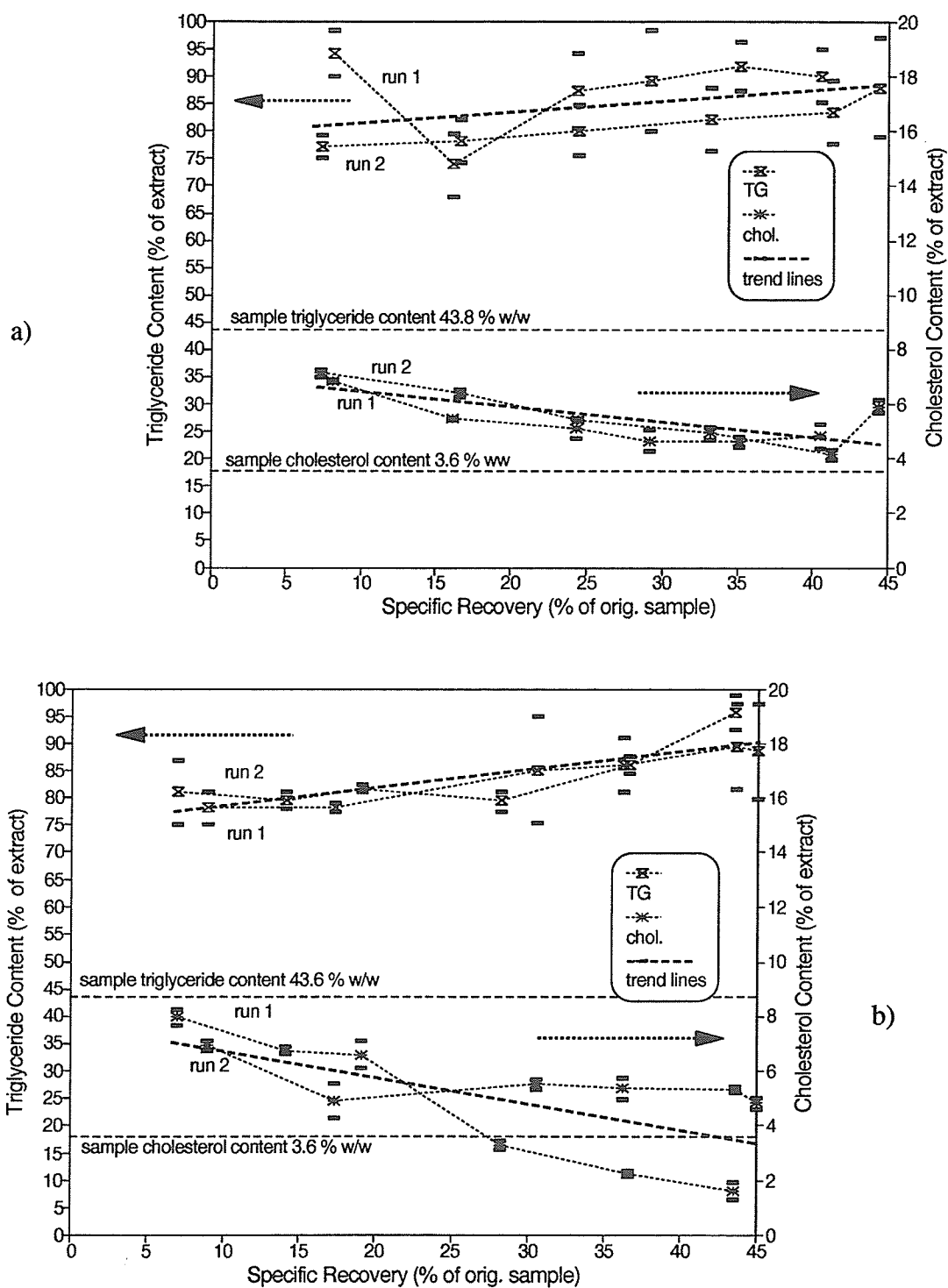
Figure 4.15: SC CO_2 extract composition profiles for higher extraction temperatures: a) 65°C, 36 MPa b) 75°C, 36 MPa. Triglyceride and cholesterol contents of extracts are plotted as functions of extraction progress as measured by percent of extraction sample recovered.

Table 4.4: Effect of extraction temperature on cholesterol:triglyceride ratio in extracts. Average ratios representing the linear section of extractions at 40, 55, 65, and 75°C and 36 MPa are compared, using the cholesterol:triglyceride ratios in the original freeze-dried yolk samples as controls.

Treatment	Cholesterol:Triglyceride Mass Ratio in Extracts (mean ± standard deviation)
Control (original freeze-dried egg yolk)	0.074 ± 0.009
SC CO_2 @ 40°C, 36 MPa	0.057 ± 0.013[*]
SC CO_2 @ 55°C, 36 MPa	0.057 ± 0.006
SC CO_2 @ 65°C, 36 MPa	0.072 ± 0.008
SC CO_2 @ 75°C, 36 MPa	0.077 ± 0.011

[*]differs significantly from control values (Dunnet's procedure, $\alpha = 0.05$)

triglyceride and cholesterol contents, both during the extractions, and between runs conducted at different temperatures. The sample mean values for cholesterol and triglyceride content were used to calculate trend lines (Figures 4.14 and 4.15) by linear regression on specific recovery values. Statistical tests of the trend line slopes (Appendix A) revealed that the triglyceride content of the extracts increased significantly during extraction at 75°C (Figure 4.15b), while cholesterol content of the extracts significantly decreased during extraction at 65°C (Figure 4.15a) and at 75°C (Figure 4.15b). While Labay (1990) reported that the ratio of triglycerides to cholesterol in extracts was constant during extractions, Figures 4.14 and 4.15 seem to indicate some selective extraction of cholesterol at higher extraction temperatures. On the other hand, comparison of cholesterol:triglyceride mass ratios in extracts to the ratios in the original egg yolk samples (Table 4.4) shows little difference in the relative concentrations of cholesterol and

triglycerides between the extracts (for extraction temperatures from 40 to 75°C at 36 MPa) and the original freeze-dried egg yolk. The values in the table were obtained by pooling analytical results obtained from extracts during the linear (CO_2 saturated with lipids) phase of the extractions. The extracts collected at 65 and 75°C appear to have cholesterol:triglyceride ratios comparable to those found in the original; egg yolk (about 0.74 g chol./g TG). Extracts collected at lower extraction temperatures (40 and 55°C) appear to have slightly lower cholesterol:TG ratios (about 0.57). However, only the ratios for 40°C were found to be significantly different from the original egg yolk (see Appendix A), and this appears to be the result of the very low cholesterol contents determined for one replicate (Figure 4.14a).

Labay (1990) reported that cholesterol content during the linear (early) portion of the extraction increased with extraction temperature from 2.9% w/w (@40°C, 36 MPa) to 6.9% (@75°C, 36 MPa). These results are not inconsistent with the cholesterol contents values at the left of Figures 4.14 and 4.15. Additional extraction experiments will be necessary to clearly establish the effects of extraction temperature and extraction progress upon the cholesterol and triglyceride contents of egg lipid extracts. It is suggested that future investigations attempt to quantify and compare the amounts of mono- and diglycerides and free fatty acids present in the extracts as well.

4.2.2.3 Estimation of cholesterol and triglyceride solubilities

Cholesterol solubilities in SC CO_2 for a given set of extraction were estimated by multiplying the sample cholesterol concentrations by the total lipid solubility calculated from the extraction curve. Results for samples representing the straight-line

Table 4.5: Comparison of triglyceride and cholesterol solubilities in SC CO_2 determined in the present study to the values determined by Labay (1990).

| Extraction Conditions | | Estimated Solubility in mg/g CO_2 (mean ± standard deviation) | | | |
| Temperature (°C) | Pressure (MPa) | Present Study | | Labay (1990) | |
		Triglycerides	Cholesterol	Triglycerides	Cholesterol
40	36	9.2 ± 0.3^{ad}	0.56 ± 0.12^{ac}	9.3 ± 0.7^{a}	0.42 ± 0.01^{a}
55	36	10.6 ± 0.4^{b}	0.64 ± 0.07^{bc}	8.0 ± 0.2^{a}	0.49 ± 0.02^{b}
65	36	10.0 ± 1.1^{bd}	0.77 ± 0.10^{b}		
75	36	8.1 ± 0.01^{c}	0.61 ± 0.14^{bc}	5.5 ± 0.7^{b}	0.39 ± 0.001^{a}

means with different superscripts in the same column are significantly different ($\alpha = 0.05$)

portion of the extraction curves were pooled and averaged for each set of extraction conditions. Triglyceride solubilities were estimated similarly. In Table 4.5, the calculated triglyceride and cholesterol solubilities from the present study are listed and compared with the results of Labay (1990). The solubilities estimated for egg lipid extraction at 40°C, 36 MPa were 9.2 mg/g CO_2 (triglycerides) and 0.56 mg/g CO_2 (cholesterol). These are comparable to the values reported by Labay (1990). At extraction temperatures from 55 to 75°C, however, the cholesterol and triglyceride solubilities measured in the present study are significantly higher than those reported by Labay. This reflects the generally higher gross lipid solubilities measured in the present study (see Table 4.2). The trends in triglyceride and cholesterol solubility behaviour with extraction generally followed the pattern observed for gross lipid solubility. Triglyceride solubility increased significantly (to 10.6 mg/g CO_2) when extraction temperature increased from 40 to 55°C, did not change significantly when extraction temperature increased to 65°C, and dropped significantly (to 8.1 mg/g CO_2) when extraction was performed at 75°C. Cholesterol

solubility increased with increasing extraction temperature in the range of 40 to 65°C, then dropped slightly for extraction at 75°C.

4.2.3 Effect of Entrainers on Extract Recovery and Composition

4.2.3.1 Preliminary Results - Entrainer Premixtures

Several supercritical fluid re-extractions of defatted freeze-dried egg yolk (extracted previously with SC CO_2 @ 40°C, 36 MPa) were performed using ethanol or methanol premixed at nominal concentrations of 3% and 5% w/w in CO_2. As previously described in the entrainer recovery study, extractions of residue with premixed entrainers gave highly variable results. The actual entrainer concentration during a given extraction with premixed entrainer could not be confirmed, and there was evidence that entrainer concentration could change during the course of an extraction. Therefore, the results obtained in these preliminary investigations could not be used to quantitatively study the effects of entrainer type and concentration on egg lipid solubility. Qualitatively, however, some interesting observations about egg lipid component solubilities in SC CO_2 with alcohol entrainers can be made.

Figure 4.16 shows extract composition and solubility dynamics during the first stages of a re-extraction of defatted egg yolk residue with nominal 3% w/w methanol entrainer premixed in CO_2. The initial composition of the residue before re-extraction was about 3.1% w/w triglycerides, 0.3% cholesterol, 25.8% PC and 5.9% PE. Triglycerides and cholesterol have equilibrium solubilities in the range of 10 to 40 mg/g CO_2 and 0.6 to 1.5 mg/g CO_2, respectively, when extracted with SC CO_2 with ethanol or

a)

b)
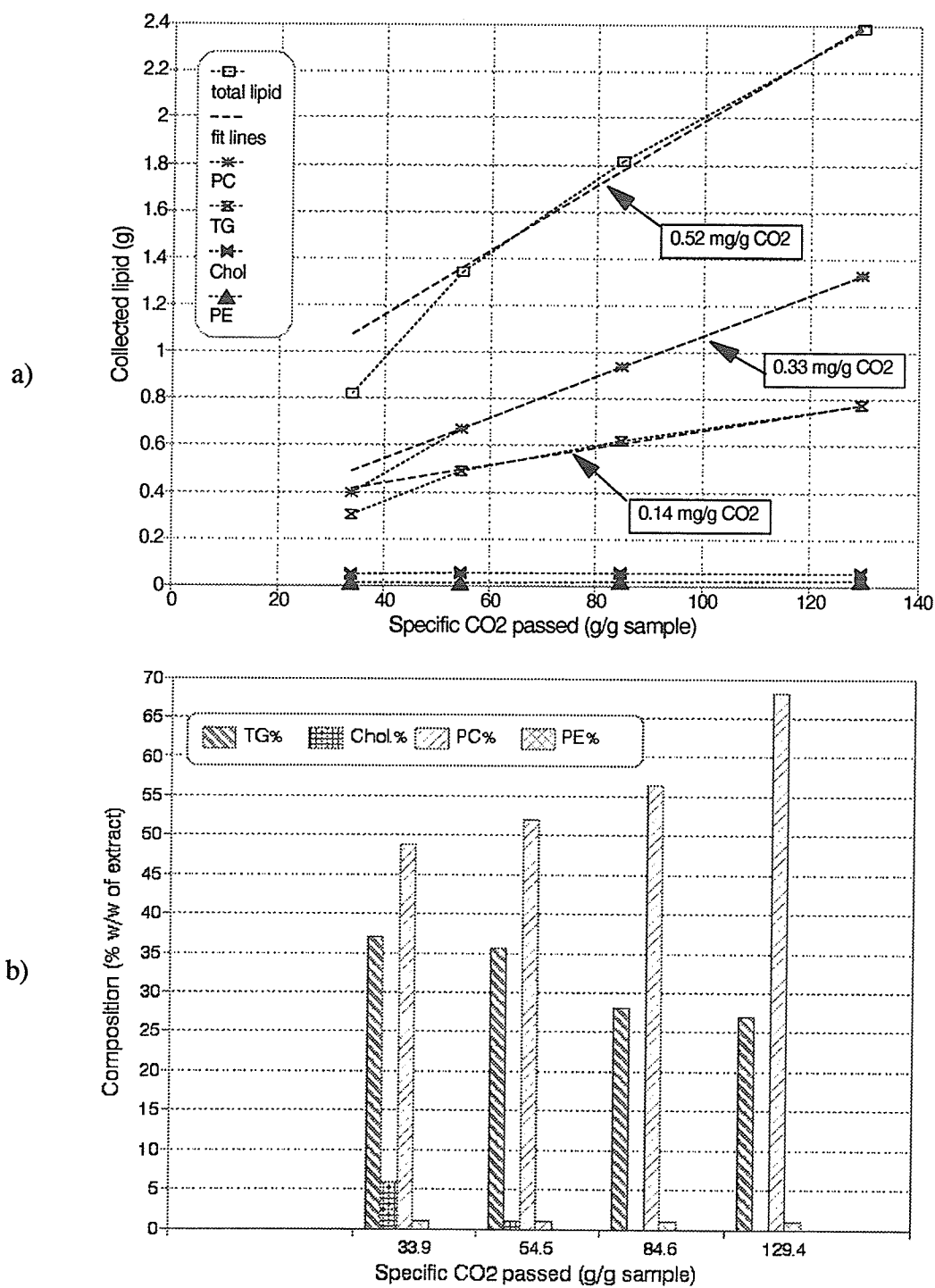
Figure 4.16: a) Extraction profile for SC CO_2 extraction of defatted freeze-dried egg yolk residue with premixed methanol entrainer (nominal 3% w/w in CO_2) at 40°C, 36 MPa, including individual lipid constituent recovery curves. b) Extract compositions at sampling points.

methanol entrainers (Labay, 1991). Consequently triglycerides and cholesterol were prominent constituents in the early fractions collected from methanol-entrained SC CO_2 re-extraction of defatted egg yolk residue (Figure 4.16b). As the residual neutral lipids were depleted, however, their concentration in the extract (particularly the cholesterol content) decreased. As a result, the phospholipid constituents (particularly PC) formed an increasingly large percentage of the extracts with the progress of the extraction.

The changing composition of the extracts during the course of the extraction offers a possible explanation for the 'two-sloped' extraction profiles which were commonly observed during the investigations reported in this subsection (Figure 4.17b). The SC CO_2-egg yolk lipid extraction profiles described previously featured straight-line initial sections and flattened out asymptotically during the later stages of the extraction (Figure 4.17a). In contrast, extraction profiles for re-extraction of defatted residue displayed constantly-changing extraction-curve slopes (solubilities typically 0.5-1.0 mg/g CO_2) during the initial stages of extraction (Figure 4.17b). While the gross lipid solubility continuously decreased during the initial phase of the extraction, at a certain point the extraction profile would assume a constant slope (corresponding to a relatively low solubility in the range of 0.1 mg/g CO_2) for an extended period (Figure 4.17b). These test extractions with premixed entrainer were performed to obtain preliminary data. As previously mentioned, the actual entrainer concentrations during extraction could not be reliably monitored. Consequently, extensive extract analysis was not performed. However, the partial analysis results available seem to indicate that the relatively high initial rates of extraction corresponded to strong extraction and depletion of residual
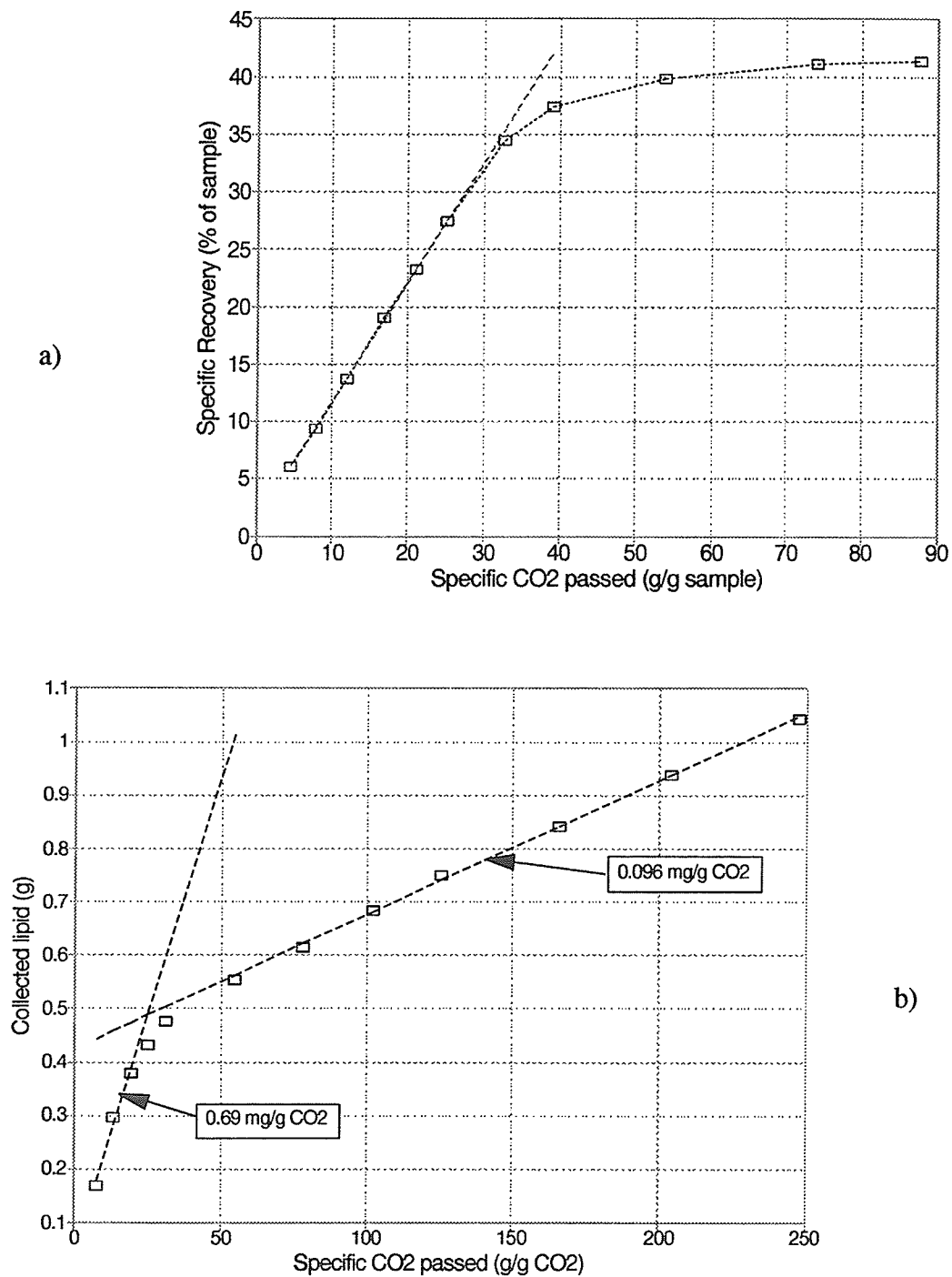
Figure 4.17: a) Typical profile for extraction of one major component from a fixed bed eg: SC CO_2 extraction of egg lipids b) typical 'two-sloped' profile for SC CO_2 and entrainer re-extraction of defatted freeze-dried egg yolk residue eg: extraction with SC CO_2 premixed with methanol entrainer (nominal 3% w/w in CO_2) at 40°C, 36 MPa.

triglycerides and cholesterol. The relatively constant extraction curve slopes and lower solubilities observed later in the extractions were associated with increasingly elevated concentrations of phospholipids in the extracts. The results from one extraction of defatted egg yolk material with nominal 3% ethanol premixed in CO_2 indicated that a PC concentration in the extracts of about 85-100% in the constant-slope region corresponded to an extract solubility of about 0.078 mg/g CO_2.

The results of trial extraction were therefore promising with respect to producing high-purity phospholipid fractions by re-extraction of freeze-dried egg yolk what had been previously defatted by SC CO_2 extraction. Several questions however, were suggested by these preliminary results. For instance, the presence in the extracts of elevated quantities of triglycerides and cholesterol during the initial stages of re-extractions did not appear to be selective extraction *per se*. The slope of the PC collected vs specific CO_2 usage in Figure 4.16a did not increase as the extraction progressed. Rather, it appeared the last traces of the highly soluble neutral lipids were simply being rapidly removed at the start of the extraction. Phospholipids were extracted at all stages, and naturally formed the bulk of the extracts once the neutral lipids were depleted. However, the apparent solubility of the PC fraction of the (50 to 70% PC) lipid extracts in Figure 4.16 was about 0.33 mg/g CO_2. This was considerably higher than the solubilities of about 0.1 mg/g CO_2 associated with higher-purity PC extracts at advanced stages of re-extractions. One possible explanation was that the phospholipids were still bound by the protein matrix. The extraction preparation might have freed a limited quantity of phospholipids, which would be extracted during the initial phase of the re-extraction. If this were so,

the approximately 0.1 mg/g solubilities observed in the later re-extraction stages represented not true solubilities, but rather a slow rate of release/diffusion of bound phospholipids. On the other hand, the apparently higher solubilities of phospholipids in extracts containing more than say 50% neutral lipids might have been attributable to some sort of secondary solubility enhancement due to the presence of the relatively more soluble cholesterol and triglyceride lipids. The pure-component solubility of PC in alcohol-entrained SC CO_2 has not been reported in the literature, and was not determined in this study. It was hoped, however that performing more re-extractions with better control of entrainer addition (by injecting instead of premixing) and extensive analysis of the extracts would confirm the preliminary findings and give additional information on the solubility behaviour of phospholipids during re-extraction of defatted yolk.

4.2.3.2 Extraction with SC CO_2 and Entrainer Injection

Several samples of freeze-dried egg yolk were defatted by extraction with SC CO_2 at 40°C and 36 MPa. The extractions were continued in each case until about 65-70 g of CO_2 had been used per gram of initial sample which resulted in the extraction and recovery of about 42-44% of the starting sample mass. The residue from the extractions was then re-extracted with SC CO_2 and alcohol entrainers which had been added to the CO_2 using the injection apparatus. The extracts and residue samples were analyzed for triglyceride, cholesterol, and phospholipid contents. Extraction/re-extractions were performed in duplicate for re-extraction entrainer concentrations of 3% w/w methanol, and 3, 5, and 10% w/w ethanol in CO_2.

The lipid recovery profiles for CO_2/entrainer re-extractions of defatted residue
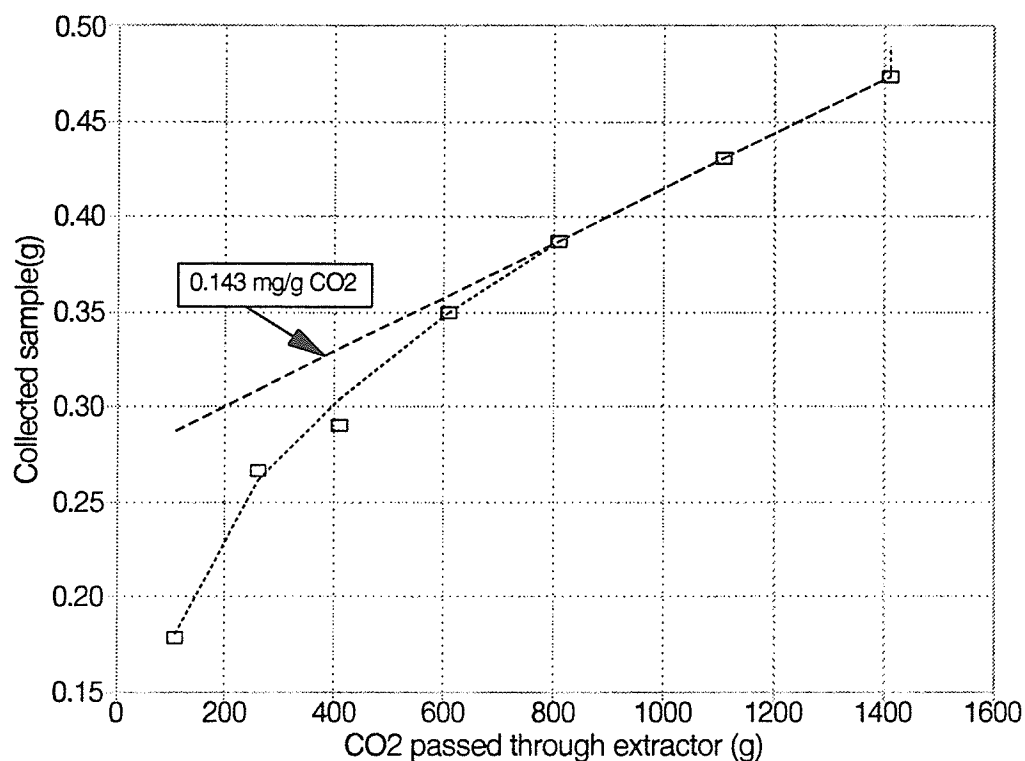
Figure 4.18: Typical extraction profile for SC CO_2 re-extraction of defatted freeze-dried egg yolk residue with injected ethanol entrainer (nominal 5% w/w in CO_2) at 40°C, 36 MPa. The solubility of the phospholipid-rich extract has been calculated from the final slope after the presumed initial extraction of cholesterol and triglyceride.

typically displayed the dual-slope characteristic (Figure 4.18). In one case (a re-extraction with 3% w/w methanol) the re-extraction was stopped before the constant-solubility phase was reached. For each extraction profile displaying the dual-slope characteristic, the slope of the final portion of the curve was determined by curve-fitting. Selection of the points to fit was made "by eye". The solubility values determined for re-extraction with the various entrainer types and concentration are summarized in Table 4.6. Based on the results of re-extractions of defatted residue with CO_2 premixed alcohol

Table 4.6: Effect of entrainer type and concentration on lipid solubilities during later stages of SC CO_2 re-extraction of defatted freeze-dried egg yolk residue with injected entrainer.

Extraction Conditions		Entrainer Type and Concentration (w/w in CO_2)	Solubility, mg/g CO_2 (mean ± st. dev.)
Temperature (°C)	Pressure (MPa)		
40	36	3% MeOH	0.058[a]
40	36	3% EtOH	0.077 ± 0.001[a]
40	36	5% EtOH	0.165 ± 0.031[b]
40	36	10% EtOH	0.23 ± 0.016[c]

means with different superscripts are significantly different ($\alpha = 0.05$)

entrainers (previous subsection) these latter-phase lipid solubilities were presumed to be estimates of the solubilities of phospholipid-rich extracts.

Due to the limited data available, the solubilities measure for late-stage re-extraction of defatted residue with 3% w/w methanol in CO_2 could not be distinguished from those obtained for re-extraction with 3% ethanol (Table 4.6). The estimated solubilities of 0.06-0.08 mg/g CO_2 were similar to those determined using premixed entrainers. Labay (1991) found that extraction of egg lipids with premixed ethanol entrainer resulted in slightly higher triglyceride, cholesterol, and phospholipid solubilities as compared to extraction with methanol entrainer. However, whether this apparent 'solubility increase' was in fact experimental error due to incomplete removal of ethanol from the recovered samples is not clear. Increasing ethanol entrainer concentration from 3% to 5% w/w approximately doubled residual lipid solubility (from 0.077 to 0.165 mg/g

CO_2). This was again consistent with the results of Labay (1991). Increasing entrainer concentration from 5 to 10% w/w again significantly increased lipid solubility (to 0.23 mg/g CO_2) although not as dramatically. It must be noted that once the entrainer concentration becomes appreciable, the actual solvent volume is being appreciably changed when entrainer is altered. Therefore, increasing entrainer concentration to obtain only a slight increase in solubility is unlikely to be practical.

Analysis of the extracts from the entrainer-injected extractions was not as revealing as had been hoped for. In contrast to the preliminary results cited in the previous subsection, no appreciable amounts of phospholipid (> 10%) were found in any of the extracts. However, peak splitting and extraneous peaks observed during chromatographic analyses suggested possible sample degradation during storage before analysis. Several analyses of defatted residue indicate total lipid (sum of cholesterol, triglyceride, and PC concentrations) of 100-120+%. In addition several extract analyses indicated total lipid compositions of only 40-50% or less when the extracts should theoretically have been 100% lipids. The author believes that the extract samples, stored dry under nitrogen for 3-4 months prior to analysis may have auto-oxidized, particularly the phospholipid components, despite the addition of BHT. The analytical results for these extractions have therefore not been reported in detail.

Clearly, it would be of interest to repeat these entrained CO_2 re-extractions and attempt better preservation of the samples for analysis. The author suggests pooling of defatted egg yolk residue samples to obtain a uniform starting material for re-extraction.

Secondly, the interval between defatting, re-extraction, and sample analysis should be minimized, to avoid degradation. Finally, the samples should perhaps be stored in chloroform (as opposed to in dry form or with traces of alcohol entrainer) as suggested by Christie (1987) as a precaution against auto-oxidation.

5. CONCLUSIONS

5.1 MODIFIED SYSTEM PERFORMANCE

1) Enclosing the extraction vessel in an airbath under PC temperature control and adding a temperature equilibration coil to the CO_2 inlet stream has provided stable uniform extraction temperature control to within $\pm 1°$ over the range of 10 to 80°C.

2) Vessel filling and cleaning has been facilitated by placing the extractor vessels on free-standing bases within the airbath and connecting them to the extractor process tubing with quick-connect couplings. Also, a 55-mL extractor or a high-pressure view cell may now be easily substituted for the standard 300-mL extractor. This maximizes the extraction system flexibility.

3) Drying in a 45°C water bath with nitrogen flushing for 6-8 h achieved effective removal of ethanol or methanol from model lipid/entrainer sample mixtures. The changes in lipid mass for samples which had been mixed with entrainer and then dried were not significantly different than those for control samples which were simply refrigerated under nitrogen.

4) The entrainer injection pump performed reliably. The rate of entrainer addition was accurately measured by monitoring the rate of mass change of the sample reservoir. The rate of solvent addition for ethanol and methanol was found to be a linear ($\pm 0.13\%$ of mean) function of the controller program rate setting for the range of 3 to 10% w/w, entrainer in CO_2.

5) Automation of the compressor drive lubrication resulted in reliable, trouble-free operation for the duration of the experimental period. Tear-down inspections revealed only normal wear. The only regular maintenance requirement with the modified

system is periodic replenishment of the lubricating oil.

6) The process controller, datalogger, and data analysis software have been tested and developed to a serviceable level for SCE extraction of egg lipids with and without entrainer. The SCE system as modified and instrumented provides a structured, semi-automated environment for SCE processing, data gathering, and conversion of results for display in report form.

5.2 ENTRAINER ADDITION AND RECOVERY

7) Methanol or ethanol (3 and 5% w/w concentration in CO_2) entrainer delivery from premixed cylinders has been shown to exceed the nominal concentration by at least one or two percent in some cases. Recovery data also suggests that entrainer concentration from premixed cylinders may also vary by several weight percent (decreasing rate of delivery) during the course of an extraction.

8) Demonstrated variability in entrainer delivery rates and a lack of a suitable method to monitor actual entrainer concentration during extraction make the use of entrainer-CO_2 premixtures unsuitable for quantitative SCE studies.

9) Entrainer injection has been shown to be a reliable, accurate, and flexible method for the addition of ethanol or methanol entrainers to CO_2 in the concentration range of 3 to 10% w/w.

10) The entrainer recovery in the sampling vial was highly dependent on the amount of solute being recovered. Heavy solute loadings (as in the extraction of freeze-died egg yolk with SC CO_2 and alcohol entrainer) trapped relatively large amounts of entrainer with the solute. Where extracted solute loadings were light (as found for

defatted residue re-extractions) only a fraction of the entrainer was recovered in the sampling vial.

11) Alcohol entrainers are extremely volatile and difficult to recover from the depressurized CO_2 stream. During re-extractions of defatted residue with CO_2 and 3% w/w methanol or 3-10% w/w ethanol, the entrainer could not be quantitatively recovered even in multi-stage trapping comprising the sampling vial, cooled U-tube trap and multiple chilled condenser traps.

5.3 EGG YOLK LIPID EXTRACTION

12) Egg lipid solubility in SC CO_2 is relatively insensitive to extraction temperature in the range of 40 to 75°C, at an extraction pressure of 36 MPa. At 40°C, 36 MPa, the solubility of egg lipids in SC CO_2 is 11.5 mg/g CO_2. The solubility at 55 and 65°C is not significantly different. Extraction at 75°C, 36 MPa produces a slight drop in solubility, to 10.8 mg/g CO_2.

13) The percentage of egg lipid recoverable by SC CO_2 extraction at 36 MPa approaches 45% of the sample mass, regardless of extraction temperature in the range 40 to 75°C. This corresponds approximately to the neutral lipid content of freeze-dried egg yolk.

14) Phospholipids were not detected in any of the non-entrained SC CO_2 egg lipid extracts for extraction temperatures in the range of 40 to 75°C at an extraction pressure of 36 MPa.

15) The ratio of cholesterol to triglycerides in the SC CO_2 extracts is similar to their ratio in the original freeze-dried egg yolk. The average ratio of cholesterol to triglycerides

in extracts recovered during the linear extraction phase at 40°C, 36 MPa was 0.057:1. This was slightly lower than the ratio of 0.074:1 in the egg yolk. Extracts collected during extractions at 55, 65, and 75°C at 36 MPa extraction pressure had average cholesterol:triglyceride ratios not significantly different than the original egg yolk ratio.

16) The cholesterol content of the extracts did not change significantly during the course of the extraction for SC CO_2 extractions performed at 40 and 55°C and 36 MPa. The cholesterol content of the extracts decreased significantly towards the end of the extractions for SC CO_2 extractions performed at 65 and 75°C and 36 MPa.

17) The apparent solubility of triglycerides generally followed the trends observed for the solubility of gross egg lipids. The solubility measured for extraction from freeze-dried egg yolk at 40°C, 36 MPa was 9.2 mg/g CO_2. Solubilities measured for extraction at 55 and 65°C and 36 MPa were slightly higher. The solubility measured for extraction at 75°C, 8.1 mg/g CO_2, was significantly lower.

18) The apparent solubilities measured for cholesterol extracted from freeze-dried egg yolk at 36 MPa vary only slightly for extraction temperatures from 40 to 75°C. The cholesterol solubility measured at 40°C, 36 MPa is 0.56 mg/g CO_2. The solubilities measured at extraction temperatures 55 and 75°C are not significantly greater, while the solubility measured at 65°C, 0.77 mg/g CO_2, is slightly higher.

The following conclusions refer to the re-extraction of defatted egg yolk residue with SC CO_2 and injected alcohol entrainers. Preliminary data indicated that residual cholesterol and triglycerides were removed during the early stages of re-extractions. The solubilities

referred to in the following conclusions were determined for extracts during the later stages of extraction, when preliminary results indicated that phosphatidylcholine contents could be as high as 70-90%. The results are referred to as 'defatted egg component' solubilities to distinguish them from the equilibrium solubilities of lipids extracted from full fat egg yolk:

19) The solubility of defatted egg components in SC CO_2 with 3% w/w methanol entrainer was not found to be significantly different than the solubility of egg lipids in SC CO_2 with 3% ethanol entrainer, for extractions performed at 40°C and 36 MPa.

20) The solubility of defatted egg components in SC CO_2 with 3% ethanol entrainer was 0.077 mg/g CO_2, at 40°C, 36 MPa. The reduced lipid solubility increased with increasing entrainer content, to 0.165 mg/g CO_2 at 5% ethanol w/w in CO_2.

21) Increasing the entrainer content from 5% to 10% ethanol w/w in CO_2 caused a further significant increase in defatted egg component solubility, to 0.23 mg/ g CO_2.

6. RECOMMENDATIONS

1) Drying of entrained lipid extracts and storage of lipid samples should receive additional study to ensure lipid sample integrity is maintained until analysis:

    i) Periodic addition of chloroform during the drying of entrained samples under nitrogen should be evaluated as a method for promoting quantitative removal of residual alcoholic entrainers.

    ii) The stability and storage life of egg lipid samples containing triglycerides, cholesterol, and phospholipids should be compared when stored 'dry' under nitrogen and when stored in chloroform.

2) The potential for isolation of high-purity egg phospholipid extractions by re-extraction of defatted residue with SC CO_2 and entrainers should be further investigated:

    i) The solubility behaviour of pure phosphatidylcholine in ethanol-entrained CO_2 should be measured, and the effect of ethanol concentration and extraction temperature established. This would clarify whether the solubility of PC is actually as low as indicated by the experimental results, or whether a binding mechanism the cause of its slow release from the egg yolk biomatrix.

    ii) The re-extraction experiments with SC CO_2 and ethanol and methanol entrainers should be repeated. Defatted residue samples from several extractions should be pooled before re-extraction to provide a uniform sample material. Extended extractions, with extensive analysis of the lipid extracts should be performed to confirm the extraction profiles of residual mono-, di- and triglycerides, fatty acids, cholesterol and phospholipids.

3) The original SCE screening system metering valve should be replaced. The replacement valve should feature a fine metering tip, with replaceable stem and seat elements, such as the Autoclave Engineers "60VM" series 2-way angle valve.

4) The existing Sensym transducer for the dry test flowmeter pressure displays excessive drift and should be replaced, eg: with a Sensym model 142SC01 pressure transducer (0-6.9 kPa range). The new pressure transducer should ideally be calibrated in absolute rather than gage pressure. However, this would require a means of maintaining calibration. Otherwise, a mercury barometer (eg: a Fortin-type mercury barometer such as the Princo model 469 sold by Anachemia Science) should be made available near the extraction laboratory to enable accurate measurement of atmospheric pressure. In this case, gage pressure readings could continue to be converted to absolute equivalents.

5) A means to maintain the accuracy of the dry test flowmeter's calibration should be provided - either a wet test flowmeter (eg: the Precision Scientific model 63126 sold by Canlab) or a piston-type displacement prover (calibrator). Another economical possibility for a calibration device would be a high-capacity bubble meter (0 - 10 L/min) such as those sold by Supelco.

6) Automating control of CO_2 flowrate and extraction pressure would greatly reduce the need for operator intervention. Since direct program control would probably overtax the PC controller, an intelligent controller(s) interfaced to the PC controller would probably be required. In addition, automatic control would require instantaneous flowrate measurement, rather than the current indirect calculation from averaged time rate of flow. A mass flowmeter would give accurate, reliable flowrate information, is

easily interfaced to the PC/controller, and could be calibrated using the same equipment as the dry test flowmeter. One suitable mass flowmeter would be the FMA-5610 (flow range 0-10 L/min) mass flowmeter sold by Omega Engineering.

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APPENDIX A:

Calculations

and

Instrument Calibrations
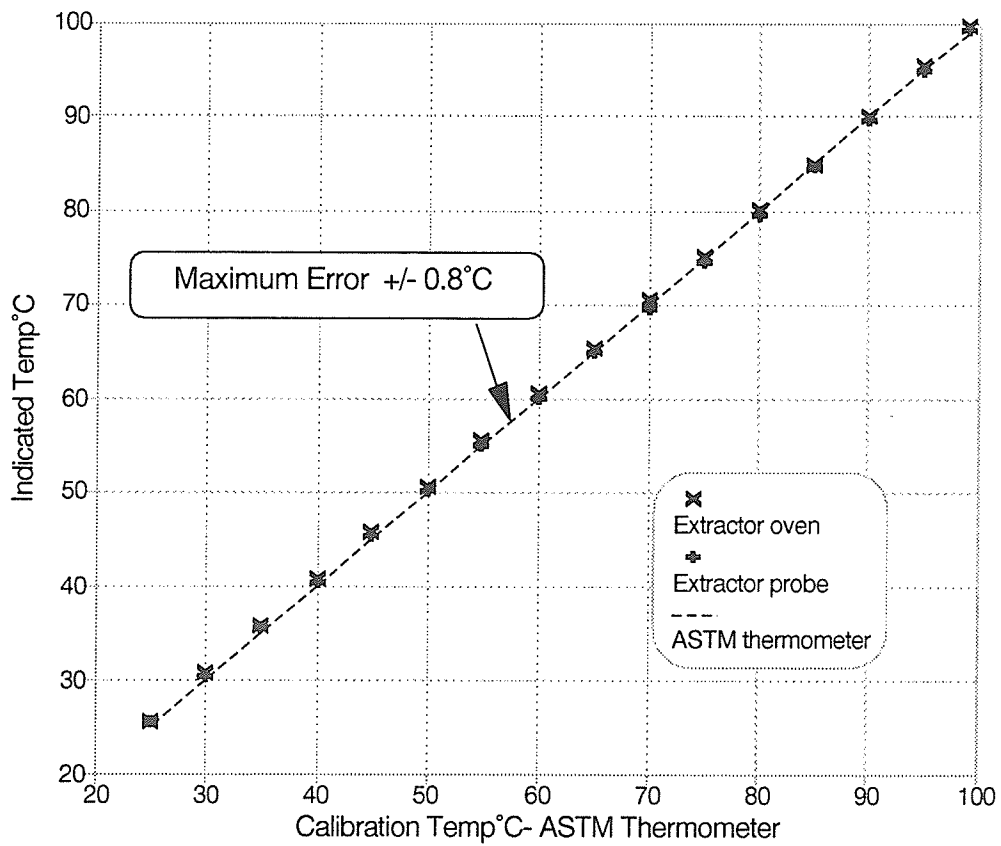
A1. VALIDATION OF ITERATIVE SOLUTION TO IUPAC EQUATION

As mentioned in the section on the IUPAC equation of state for CO_2 in the Literature Review, an iterative algorithm to solve the equation for temperatures in °C and pressures in MPa was developed, and then tested against the IUPAC tabulated values. Table A-1 contains selected temperature-pressure conditions, together with their corresponding IUPAC tabulated CO_2 densities, the densities calculated from the iterative algorithm, and the percentage differences in the results. The errors determined are generally negligible when it is considered that the IUPAC tables themselves were considered to have estimated error margins in the range of ± 0.1-0.3% for the ranges 0.1 to 100 MPa, -60 to 800°C.

A2. CHOLESTEROL SOLUBILITY VALUE CONVERSIONS

A2.1 Introduction

In the section of the Literature Review concerning the composition of egg lipids and their extraction with SC CO_2, the conditions and reported cholesterol solubilities in CO_2 for several empirical studies were listed. Because of the lack of a standard method of reporting supercritical solubility, and the sketchy empirical information sometimes available in the literature, the conversion of experimental values to one basis for comparison can be difficult and sometimes subject to errors of interpretation. In this section, the actual reported cholesterol solubility values are reported in their original units, and the calculations used to convert them to mg/g CO_2 equivalent are described. These examples are useful not only for checking this author's accuracy in converting the specific literature values cited, but as a general guide to interpreting the solubility

Table A-1: Comparison of calculated densities for selected pressure and temperature combinations: IUPAC tables (Angus *et al.*, 1976) and solution of IUPAC equation by QuickBASIC program using algorithm of Appendix C.

Temperature °C	Pressure MPa	Calculated Density (g/mL)		Difference %
		IUPAC Tables	Iterative Algorithm	
826.85	50.0	0.21248	0.21236	-0.05
-43.15	50.0	1.2197	1.2196	-0.01
-26.85	50.0	1.0293	1.0294	0.01
76.85	35.0	0.80127	0.80134	0.01
56.85	35.0	0.87518	0.87526	0.01
26.85	35.0	0.98080	0.98086	0.00
-43.15	35.0	1.1983	1.1982	-0.01
626.85	15.0	0.08582	0.08580	0.00
76.85	15.0	0.45012	0.44885	-0.29
-3.15	15.0	1.0131	1.0131	0.00
386.85	7.5	0.06044	0.06044	0.00
56.85	7.5	0.17860	0.17856	-0.06
26.85	7.5	0.73394	0.73405	0.01
-53.15	7.5	1.1806	1.1805	-0.01
26.85	1.0	0.00177	0.00177	0.00
56.85	1.0	0.00161	0.00161	0.00
76.85	1.0	0.00152	0.00152	0.00
-3.15	1.0	0.00197	0.00198	0.00
25.0	36.0		0.88747	-
35.0	36.0		0.95715	-
38.5	36.0		0.99081	-
55.0	36.0		0.82527	-
75.0	36.0		0.94519	-
25.0	0.101325		0.001809	-

notations common in the literature.

A2.2 Chrastil (1982)

Material Studied: cholesterol

Units Reported: g cholesterol/L CO_2 (at experimental conditions)

Reported Value(s): @ 40°C, 200 atm = 20.3 MPa, S_1 = 2.35 g/L CO_2

Now from Appendix E:

@ 20.0 MPa, 40°C, d_{CO_2} = 0.841 g/mL ⎫   => at 20.3 MPa by interpolation
@ 20.5 MPa, 40°C, d_{CO_2} = 0.845 g/mL ⎭ d_{CO_2} = 0.843 g/mL

The cholesterol solubility is therefore:

$$S = 2.35\,g/LCO_2 * \frac{1000\,mg}{g} * \frac{1\,LCO_2}{1000\frac{mL}{L} * 0.847\frac{g}{mL}} = 2.8\,mg/g\,CO_2$$

A2.3 Wong and Johnston (1986)

Material Studied: cholesterol

Units Reported: mole fraction

Reported Value(s): @ 40°C, 203.5 bar = 20.4 MPa, 10^5y = 15.4,

Now

$$S\,(mg/g\,CO_2) = y\left(\frac{mol\,chol}{\underset{\text{neglect w.r.t. } CO_2}{mol\,chol} + mol\,CO_2}\right) * \frac{\frac{387\,g\,chol}{1\,mol\,chol}}{\frac{44\,g\,CO_2}{1\,mol\,CO_2}} * \frac{1000\,mg\,chol}{1\,g\,chol}$$

$$= y * 8795$$

$$=> S = (15.4x10^{-5}) * 8795 = 1.4\,mg/g\,CO_2$$

A2.4 Supercritical Processing, Inc. (1989)

<u>Material Studied:</u> anhydrous butterfat

<u>Units Reported:</u> ng/μL CO_2 (at experimental conditions)

<u>Reported Value(s):</u> @ 40°C, 3000 psi = 20.7 MPa, S_2 = 62 ng/μL CO_2

@ 20.5 MPa, 40°C, d_{CO2} = 0.845 g/mL $\Big\}$ => at 20.7 MPa by interpolation
@ 21.0 MPa, 40°C, d_{CO2} = 0.850 g/mL $\Big.$ d_{CO2} = 0.847 g/mL

The cholesterol solubility is therefore:

$$S(mg/g\ CO_2) \begin{array}{l} = S_2(ng/\mu L\ CO_2) * \dfrac{100\,\mu L}{1\,mL} * \dfrac{1\,mg}{10^6\,ng} * \dfrac{1\,mL}{0.847\,g} \\[2mm] = 0.097\,mg/g\ CO_2 \end{array}$$

A2.5 Crerar (1989-90)

<u>Material Studied:</u> cholesterol

<u>Units Reported:</u> mg/g CO_2

<u>Reported Value(s):</u> @ 40°C, 17.3 Mpa, S = 0.49 mg/g CO_2

A2.5 Bulley and Labay (1991)

<u>Material Studied:</u> freeze-dried egg yolk

<u>Units Reported:</u> mg/g CO_2

<u>Reported Value(s):</u> @ 40°C, 20.0 MPa, S = 0.16 mg/g CO_2
@ 40°C, 20.0 MPa, S = 0.16 mg/g CO_2

A2.6 Lee *et al.* (1991)

<u>Material Studied:</u> cholesterol

<u>Units Reported:</u> mole fraction

<u>Reported Value(s):</u> @ 40°C, 200 atm = 20.3 MPa, y = 1.6 x 10^{-4} mole fraction
@ 40°C, 300 atm = 30.4 MPa, y = 2.5 x 10^{-4} mole fraction

=> @ 20.3 MPa, S = (1.6 x 10^{-4}) * 8795 = 1.4 mg/g CO_2
@ 20.3 MPa, S = (2.5 x 10^{-4}) * 8795 = 2.2 mg/g CO_2

A2.7 Lim *et al.* (1991)

Material Studied: anhydrous milk fat

Units Reported: mole fraction

Reported Value(s): @ 40°C, 241 atm = 24.4 MPa, y x 10^6 = 26.45 mole fraction

=> @ 24.4 MPa, S = (26.45 x 10^{-6}) * 8795 = 0.23 mg/g CO_2

A2.8 Pasin *et al.* (1991)

Material Studied: liquid egg yolk

Units Reported: log [ng/μL CO_2] (at experimental conditions)

Reported Value(s): @ 40°C, 3000 psi = 20.7 MPa, log [S_2] = 1.9
$$=> S_2 = 79 \text{ ng/μL } CO_2$$
@ 40°C, 4500 psi = 31.0 MPa, log [S_2] = 1.9
$$=> S_2 = 79 \text{ ng/μL } CO_2$$

@ 20.7 MPa, 40°C, d_{CO2} = 0.847 g/mL (see previous)
@ 31.0 MPa, 40°C, d_{CO2} = 0.916 g/mL

The cholesterol solubility for 20.7 MPa is therefore:

$$S(mg/g\ CO_2) = (79\,ng/\mu L\ CO_2) * \frac{100\,\mu L}{1\,mL} * \frac{1\,mg}{10^6\,ng} * \frac{1\,mL}{0.847\,g}$$
$$= 0.093\,mg/g\ CO_2$$

and the cholesterol solubility for 31.0 MPa is:

$$S(mg/g\ CO_2) = (79\,ng/\mu L\ CO_2) * \frac{100\,\mu L}{1\,mL} * \frac{1\,mg}{10^6\,ng} * \frac{1\,mL}{0.916\,g}$$
$$= 0.086\,mg/g\ CO_2$$

A2.9 Yun *et al.* (1991)

Material Studied: cholesterol

Units Reported: mole fraction

Reported Value(s): @ 40°C, 200 atm = 20.3 MPa, $y = 7.0 \times 10^{-5}$ mole fraction

=> @ 20.3 MPa, $S = (7.0 \times 10^{-5}) * 8795 = 0.62$ mg/g CO_2

A3. INSTRUMENTATION CALIBRATION RESULTS

A3.1 Introduction

This section contains calibration data for pressure and temperature instrumentation used in the SCE screening system. Several calculations used to implement the calibration equations in the data acquisition program are also included.

A3.2 Flowmeter Pressure

The Sensym LX1801 GBZ pressure transducer, which was used to measure the gas pressure inside the dry test flowmeter, was calibrated against a water column (Table A-2). The calibration curve was highly linear (linearity 0.35%), and showed little sign of hysteresis (Figure A-1). The calibration equation, determined by least-squares regression of the data, was:

$$P_{FM} = \frac{V_{FM} + 7.189}{0.1453} \qquad \ldots \text{(A.1)}$$

where:

P_{FM} is the flowmeter internal gage pressure, kPa

V_{FM} is the transducer output voltage, VDC (excitation circuitry as shown in Appendix D)

Table A-2: Calibration of Sensym LX1801 GBZ pressure transducer by water column.

February 20, 1991

Water temp: 22.5°C   Transducer Resting Output: 7.116 VDC

Dens. H_2O: 20°C   9790 N/m^3
                            25°C   9781 N/m^3
                            15°C   9800 N/m^3

@ 60 F (15.56 C), 1 in H_2O = 0.24884 kPa
@22.5 C, dens H_2O = (9790 + 9781)/2 = 9785.5 N/m^3
@15.56 C, dens H_2O = 9800 + (15-15.56)(9790-9800)/(15-20) = 9798.88 N/m^3
@ 22.5 C, 1 in H_2O = 9785.5/9798.88*0.24884 = 0.2485 kPa

Applied Pressure (in. H_2O)	Applied Pressure (kPa)	Transducer Output Unloading (VDC)	Loading (VDC)	Fit Output (VDC)	Error (VDC)	Linearity %
70.00	17.40	9.68		9.72	-0.03	-0.33
67.75	16.84	9.63		9.63	-0.01	-0.09
65.25	16.21	9.53		9.54	-0.01	-0.11
64.19	15.95	9.47		9.51	-0.03	-0.35
60.75	15.10	9.37		9.38	-0.02	-0.17
58.25	14.48	9.28		9.29	-0.01	-0.09
52.44	13.03	9.10		9.08	0.02	0.20
50.38	12.52	9.03		9.01	0.02	0.21
42.75	10.62	8.76		8.73	0.02	0.28
36.50	9.07	8.50		8.51	-0.01	-0.10
30.50	7.58	8.28		8.29	-0.01	-0.11
27.88	6.93	8.18		8.20	-0.01	-0.17
22.63	5.62	7.99		8.01	-0.02	-0.23
20.81	5.17	7.93		7.94	0.01	-0.12
18.56	4.61	7.85		7.86	-0.01	-0.07
16.19	4.02	7.77		7.77	0.00	-0.03
14.38	3.57	7.70		7.71	-0.01	-0.15
11.81	2.94	7.61		7.62	-0.01	-0.13
9.88	2.45	7.54		7.55	-0.01	-0.14
8.31	2.07	7.48		7.49	-0.01	-0.14
7.00	1.74	7.45		7.44	0.01	0.08
4.75	1.18	7.37	7.37	7.36	0.01	0.08
6.75	1.68		7.43	7.43	0.00	0.01
8.75	2.17		7.51	7.51	0.01	0.08
11.13	2.76		7.60	7.59	0.01	0.11
15.00	3.73		7.74	7.73	0.01	0.07
20.00	4.97		7.92	7.91	0.01	0.07
25.97	6.45		8.14	8.13	0.01	0.17
34.38	8.54		8.45	8.43	0.02	0.20
44.13	10.97		8.80	8.78	0.02	0.21
50.38	12.52		9.02	9.01	0.02	0.18
58.25	14.48		9.31	9.29	0.02	0.20
63.83	15.86		9.51	9.49	0.02	0.20
68.38	16.99		9.67	9.66	0.02	0.00

Transducer Output vs. Applied Pressure
          Regression Output:
Constant   7.18939
Std Err of Y Est   0.015302
R Squared   0.999651
No. of Observations   34
Degrees of Freedom   32

X Coefficient(s)   0.1452542
Std Err of Coef.   0.0004797
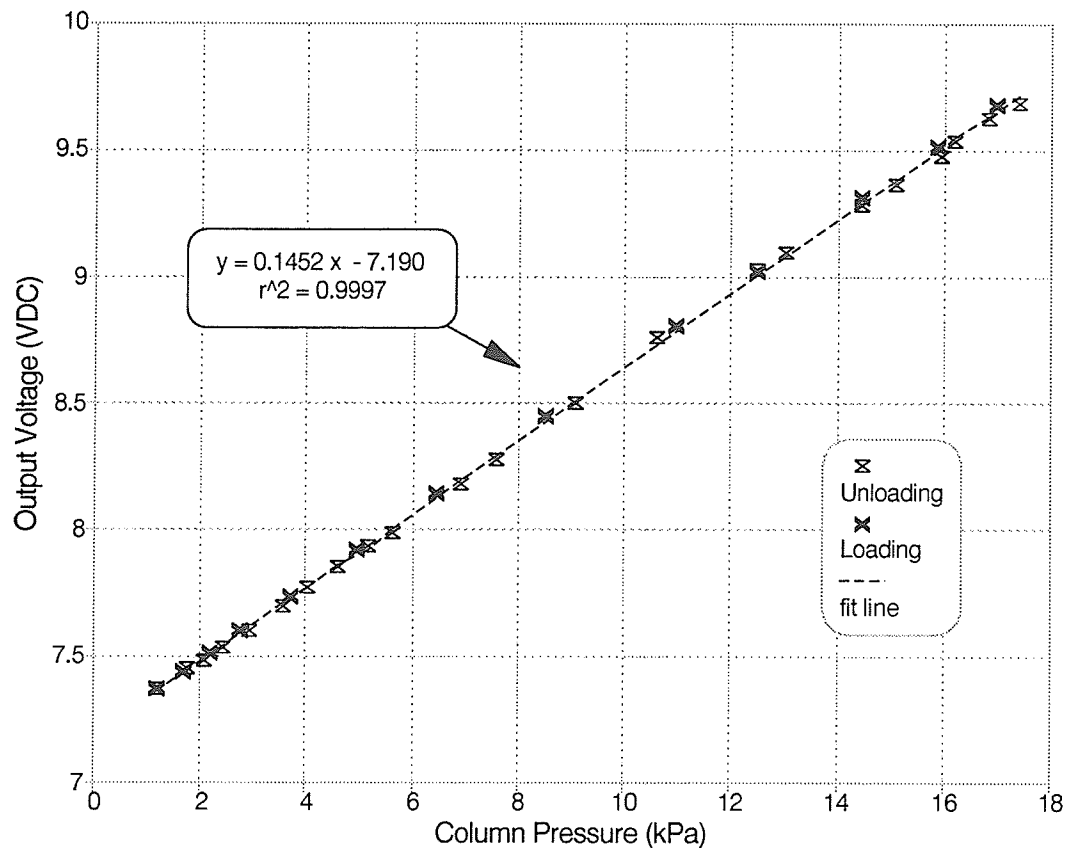
Figure A-1: Calibration curve for Sensym LX1801 GBZ pressure transducer, showing output voltage as a function of applied water column pressure.

Using the slope from Eq. A.1, the maximum observed deviation of the data (

-0.3 VDC) from the calibration line translates to:

Maximum error = ± | -0.3 / 0.1452 | / 100 = ± 0.02 kPa

The transducer took considerable time to stabilize after being powered up - about

4-6 h before the resting output (port open to ambient air) remained at about 7.116 V. It

will be noted that this value does not quite match the regression constant in Table A-1,

because of the difficulty of establishing the accurate 'zero level' for a water column.

Also, there was some slow day-to-day baseline drift ($\pm$ 100 mV max) in the resting output of the transducer. In order to accurately zero the transducer output for a run, therefore, the regression constant in Eq. A.1 was replaced by the resting output of the transducer at the start of the run:

$$P_{FM} = \frac{V_{FM} + V_{FM_0}}{0.1453} \qquad \ldots (A.2)$$

where:

V_{FM0} - is the FM transducer resting output, VDC.

A3.3 Extraction Pressure

The Data Instruments pressure transducer could not be calibrated for its full working range because no calibration standard of this size was available. Instead, a 7 MPa-capacity dead weight pressure tester belonging to the Department of Mechanical Engineering was used to calibrate the low end of the transducer range (Table A-3, Figure A-2), and a pressure gage of reputable accuracy belonging to the Industrial Technology Centre (See Appendix B) was used as a comparison standard for the 0-35 MPa pressure range (Table A-4, Table A-3). As shown in Figure A-2, the transducer showed some loss of linearity and evidence of hysteresis at low pressure ranges, below say 2 MPa, but linearity is 1% or better at the high ranges. (Such behaviour might be expected in a diaphragm gage designed for ranges up to 35 MPa and beyond). In the comparison tests at higher pressures (Table A-4, Figure A-3), the Data instruments transducer exhibits good linearity (1%), with a maximum error from the regression line of $\pm$ 2 atm ($\pm$ 0.2 MPa). Note that the comparison results were calculated in both MPa and atmospheres,

Table A-3: Calibration of Data Instruments AB pressure transducer and digital readout against dead weight pressure tester for low range (0-7 MPa).

- pressure on piston includes 25 psi self weight
- barometric pressure: 970 mbar = 97.06 kPa
- readout was initially zeroed with dead weight tester valve opened

Applied Pressure (psig)	Applied Pressure (psia)	Applied Pressure (atma)	Applied Pressure (MPa)	Indicated Pressure (atmg)	DI AB Transducer Indicated Pressure				Indicated Pressure (MPa)	Fitted Ind Press (atma)	Error (atma)	Linearity %
					Incr. 1 (atma)	Decr. 1 (atma)	Incr. 2 (atma)	Decr. 2 (atma)				
25	39.1	2.7	0.27	3	4.0				0.4	2.8	-1.1	-40.62
50	64.1	4.4	0.44	4	5.0				0.5	4.8	-0.2	-4.19
100	114.1	7.8	0.79	7	8.0				0.8	8.1	0.2	1.98
150	164.1	11.2	1.13	10	11.0				1.1	11.5	0.5	4.53
200	214.1	14.6	1.48	14	15.0				1.5	14.8	-0.1	-0.81
300	314.1	21.4	2.17	20	21.0				2.1	21.6	0.6	2.78
400	414.1	28.2	2.85	27	28.0				2.8	28.3	0.3	1.13
600	614.1	41.8	4.23	41	42.0				4.3	41.7	-0.2	-0.58
800	814.1	55.4	5.61	54	55.0				5.6	55.2	0.2	0.36
1000	1014.1	69.0	6.99	68	69.0	69.0			7.0	68.6	-0.4	-0.53
800	814.1	55.4	5.61	54		55.0			5.6	55.1	0.2	0.30
600	614.1	41.8	4.23	41		42.0			4.3	41.6	-0.4	-0.87
400	414.1	28.2	2.85	27		28.0			2.8	28.1	0.1	0.38
300	314.1	21.4	2.17	20		21.0			2.1	21.3	0.3	1.60
200	214.1	14.6	1.48	14		15.0			1.5	14.5	-0.4	-2.91
150	164.1	11.2	1.13	10		11.0			1.1	11.2	0.2	1.74
100	114.1	7.8	0.79	7		8.0			0.8	7.8	-0.2	-2.42
50	64.1	4.4	0.44	3		4.0			0.4	4.4	0.4	9.79
25	39.1	2.7	0.27	2		3.0			0.3	2.7	-0.3	-9.71
25	39.1	2.7	0.27	2			3.0		3.0	2.6	-0.4	-14.90
50	64.1	4.4	0.44	3			4.0		4.0	4.3	0.3	7.29
100	114.1	7.8	0.79	7			8.0		8.0	7.7	-0.3	-3.90
150	164.1	11.2	1.13	10			11.0		11.0	11.0	0.1	0.83
200	214.1	14.6	1.48	14			15.0		15.0	14.4	-0.5	-3.59
300	314.1	21.4	2.17	20			21.0		21.0	21.2	0.3	1.24
400	414.1	28.2	2.85	26			27.0		27.0	28.0	1.0	3.72
600	614.1	41.8	4.23	41			42.0		42.0	41.6	-0.4	-0.95
800	814.1	55.4	5.61	54			55.0		55.0	55.1	0.2	0.30
1000	1014.1	69.0	6.99	68			69.0	69.0	69.0	68.7	-0.3	-0.40
800	814.1	55.4	5.61	54				55.0	5.6	55.0	0.1	0.10
600	614.1	41.8	4.23	41				42.0	4.3	41.6	-0.4	-0.93
400	414.1	28.2	2.85	27				28.0	2.8	28.1	0.2	0.61
300	314.1	21.4	2.17	20				21.0	2.1	21.4	0.4	2.10
200	214.1	14.6	1.48	14				15.0	1.5	14.7	-0.3	-1.86
150	164.1	11.2	1.13	10				11.0	1.1	11.3	0.4	3.24
100	114.1	7.8	0.79	7				8.0	0.8	8.0	0.0	0.07
50	64.1	4.4	0.44	4				5.0	0.5	4.6	-0.4	-7.72
25	39.1	2.7	0.27	2				3.0	0.3	2.9	0.0	-1.22

Regression Output:

Constant	0.177498
Std Err of Y Est	0.412037
R Squared	0.99958
No. of Observations	38
Degrees of Freedom	36
X Coefficient(s)	9.787807
Std Err of Coef.	0.033456
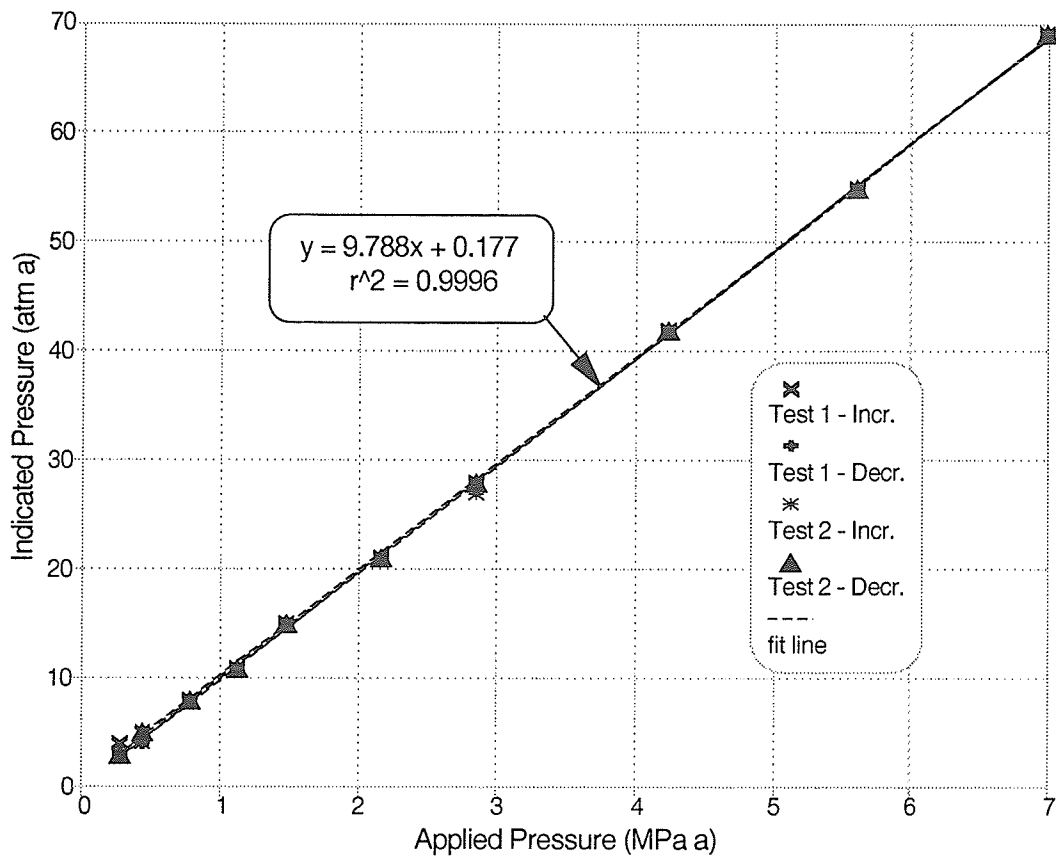
Figure A-2: Calibration curve for low-pressure (0-7 MPa) range for Data Instruments AB pressure transducer and digital readout.

since the DI transducer readout is calibrated in atm. The calibration equation for the 0-35

MPa range (atm units) was:

$$P_{DI,MPa} = \frac{P_{DI,atm} + 0.0863}{10.0665} \qquad \ldots (A.3)$$

where:

$P_{DI, atm}$ - is the indicated pressure in atm

$P_{DI, MPa}$ - is the actual (absolute) pressure, MPa

Table A-4: Comparison test of Data Instruments AB pressure transducer and digital readout against Transducer Inc. pressure transducer for high range (0-35 MPa).

Model: AB   Range: 0-5000 psi (35 MPa)

Comparison Transducer: Transducer Inc, located at ITC, Winnipeg

Model: GP59F5000   Range: 0-5000 psi (35 MPa)
- both transducers initially warmed up for 30 minutes prior to calibration
- DI transducer was zeroed at atmospheric pressure prior to first test
- barometric pressure: 0.9944 bar =   0.09944 MPa

ITC Readout Pressure (psig)	ITC Readout Pressure (psia)	ITC Readout Pressure (MPaa)	DI AB Pressure Transducer Readout			DI AB Pressure Transducer Readout			DI AB Pressure Transducer Readout			Lst-Sqrs Fit Pressure (atma)	Maximum Error (atma)	Linearity % of Pressure
			Pressure Trial 1 (atmg)	Pressure Trial 2 (atmg)	Pressure Trial 3 (atmg)	Pressure Trial 1 (atma)	Pressure Trial 2 (atma)	Pressure Trial 3 (atma)	Pressure Trial 1 (MPaa)	Pressure Trial 2 (MPaa)	Pressure Trial 3 (MPaa)			
500	514.4	3.5	35.0	36.0	35.0	36.0	37.0	36.0	3.65	3.75	3.65	35.6	-0.4	-1.02
1000	1014.4	7.0	69.0	70.0	70.0	70.0	71.0	71.0	7.09	7.19	7.19	70.3	0.3	0.48
1500	1514.4	10.4	104.0	103.0	104.0	105.0	104.0	105.0	10.64	10.54	10.64	105.0	1.0	0.99
2000	2014.4	13.9	138.0	139.0	138.0	139.0	140.0	139.0	14.08	14.18	14.08	139.7	0.7	0.53
2500	2514.4	17.3	173.0	174.0	173.0	174.0	175.0	174.0	17.63	17.73	17.63	174.4	0.4	0.26
3000	3014.4	20.8	208.0	208.0	207.0	209.0	209.0	208.0	21.18	21.18	21.07	209.1	1.2	0.55
3500	3514.4	24.2	243.0	243.0	241.0	244.0	244.0	242.0	24.72	24.72	24.52	243.8	1.9	0.76
4000	4014.4	27.7	279.0	278.0	276.0	280.0	279.0	277.0	28.37	28.27	28.07	278.5	1.6	0.56
4500	4514.4	31.1	314.0	312.0	311.0	315.0	313.0	312.0	31.92	31.71	31.61	313.2	1.3	0.40
5000	5014.4	34.6	351.0	347.0	345.0	352.0	348.0	346.0	35.66	35.26	35.06	347.9	2.0	0.56

Regression of DI Transducer Readout (atma) on ITC Readout (MPaa)

Constant	-0.08628278
Std Err of Y Est	1.202270579
R Squared	0.999884233
No. of Observations	30
Degrees of Freedom	28
X Coefficient(s)	10.0664984
Std Err of Coef.	0.02216796

Regression of DI Transducer Readout (MPaa) on ITC Readout (MPaa)

Constant	-0.0087426
Std Err of Y Est	0.12182007
R Squared	0.99986423
No. of Observations	30
Degrees of Freedom	28
X Coefficient(s)	1.019987955
Std Err of Coef.	0.002246168
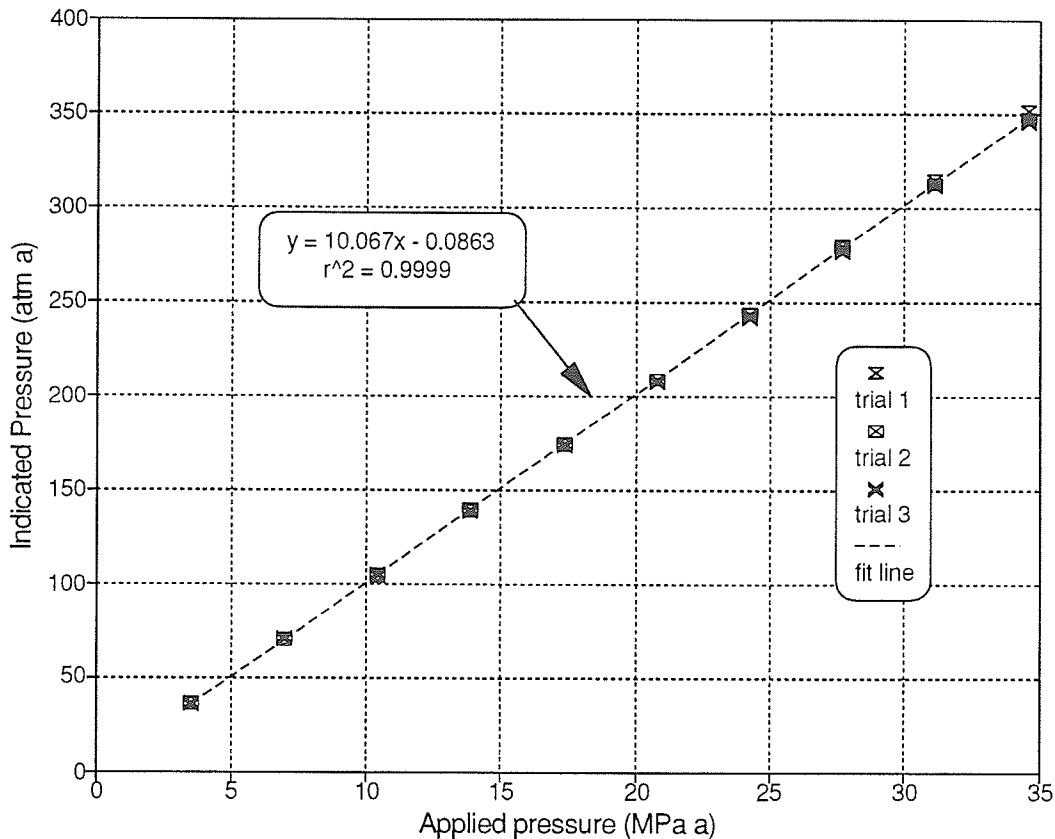
Figure A-3: Comparison curve for hi-pressure (0-35 MPa) range for Data Instruments AB pressure transducer and digital readout against Transducer Inc. gage.

The DI pressure transducer readout was of the DMM (digital multimeter) style, ie: a reading of 356 atm corresponded to a signal-conditioned (bridge temperature compensation, amplifier) transducer output of 356 mV. To interface the 0-400 mV output of the readout to the 0-10 V input of the KS102, a preamplifier (differential amplifier) circuit was constructed (See Appendix D) with a gain of approximately 21. The amplifier circuit was then calibrated by applying millivolt inputs and measuring the responses (Table A-5). The amplifier circuit (Figure A-4) shows good linear response, with negligible error so long as the input remains below about 450 mV (corresponding

Table A-5: Calibration of Data Instruments AB Pressure Transducer Pre-amplifier interface to KS102 Data Acquisition Board

September 4, 1988

Input voltages (measured using a Fluke 8068A digital multimeter) were applied to the differential amplifier circuit, and the outputs measured using the analog voltmeter on the KS102 , to determine the actual gain and offset being obtained:

Input Voltage (VDC)	Increasing Output Voltage (VDC)	Increasing Output Voltage (VDC)	Fit Voltage (VDC)
0.0036	0.194		0.195
0.0134	0.408		0.407
0.0282	0.728		0.727
0.0473	1.140		1.141
0.0791	1.828		1.829
0.0995	2.270		2.270
0.1298	2.925		2.925
0.1557	3.486		3.486
0.1686	3.765		3.765
0.1978	4.397		4.396
0.227	5.034		5.028
0.255	5.648		5.634
0.271	5.977		5.980
0.300	6.621		6.607
0.323	7.019		7.105
0.352	7.740		7.732
0.374	8.219		8.208
0.408	8.955		8.943
0.426	9.342		9.333
0.449	9.827		9.830
0.471	10.299		10.306
0.501	10.406	10.406	
0.449		9.836	
0.397		8.706	
0.353		7.754	
0.300		6.616	
0.242		5.356	
0.201		4.476	
0.137		3.075	
0.100		2.274	
0.043		1.043	

Regression Output:

Constant	0.117464
Std Err of Y Est	0.017449
R Squared	0.99997
No. of Observations	30
Degrees of Freedom	28
X Coefficient(s)	21.63189
Std Err of Coef.	0.022209
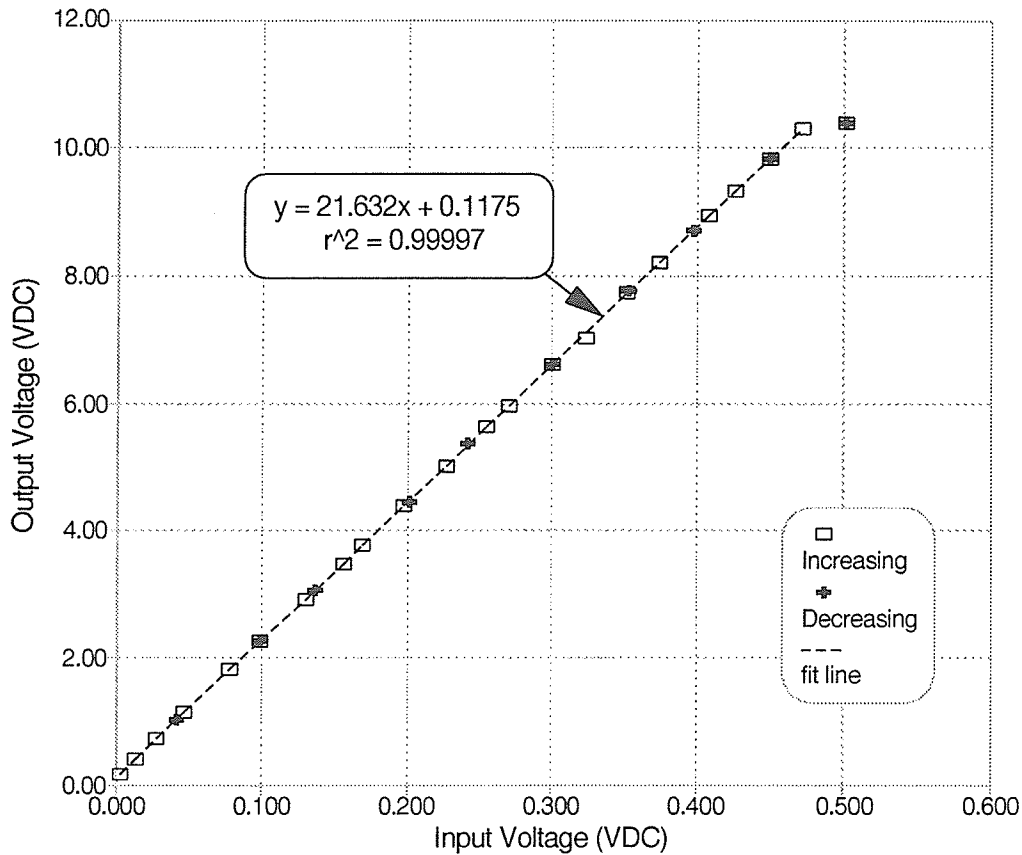
Figure A-4: Calibration curve for Data Instruments pressure transducer preamplifier interface to KS102 data acquisition board.

to the 10 VDC maximum for the KS102 voltmeter input). The response equation for the amplifier was determined to be:

$$V_{in} = \frac{V_{out} - 0.1175}{21.632} \qquad \ldots (A.4)$$

where:

V_{in} - is the voltage input to the amplifier; $V_{in} = 0.001 * P_{DI, atm}$

V_{out} - is the amplifier output measured by the KS102; $V_{out} = V_{DI, KS102}$.

Combining equations A.3 and A.4, therefore, the calibration equation for the extraction

pressure transducer, as interfaced to the KS102 acquisition system, is:

$$P_{DI,MPa} = 4.5922 \cdot V_{DI,KS102} - 0.5310 \qquad \ldots (A.5)$$

A3.4 Temperature

The thermocouple temperature sensors for the datalogger and control systems were calibrated against mercury-in-glass thermometers in stirred temperature baths, using the normal KS102-PC setups to read the measured temperatures. The Type T thermocouples from the datalogger (monitoring the metering valve, sample collector bath, dry test flowmeter, and entrainer evaporation bath temperatures) were calibrated for the range -20 to 100°C (Table A-6). The thermocouple indicated temperatures agreed well with the values read from the thermometer (Figure A-5a), with the maximum discrepancy not exceeding ±0.6°C. The J-type thermocouples used in the controller system (sensing oven temperature and extractor vessel internal temperature) were calibrated for the range 25 to 100°C (Table A-6). The thermocouple values showed good agreement with the temperatures measured by thermometer (Figure A-5b), differing by a maximum of ±0.8°C error.

Table A-6: Calibration of T- and J-type thermocouple sensors and KS102 cold-point compensation/linearization against ASTM 9C mercury-in-glass thermometer.

Calibration of T-type thermocouples from SCE process monitoring/data acquisition system:

- calibrated in Lauda RM6 circulating bath w/ 50% ethylene glycol coolant
- calibrated against ASTM type 9C mercury thermometer, range -5 to 110°C, div. 0.5 °C for the temperature range 10 to 100°C, and a general-purpose (ASTM 1C) mercury thermometer, range -20 to 150°C, div. 1°C for the temperature range -20 to 10°C

Bath Setpoint °C	Glass Thermometer °C	Indicated Temperature at PC from KS102 Acquisition Board				Error from Calibration Line			
		Metering Valve °C	Evap. Bath °C	Flow Meter °C	Sampler Bath °C	Metering Valve °C	Evap. Bath °C	Sampler Bath °C	Flow Meter °C
-20	-21.0	-20.3	-20.3	-20.2	-20.1	0.7	0.7	0.8	0.9
-10	-11.5	-10.9	-11.0	-10.9	-10.9	0.6	0.5	0.6	0.6
10	10.5	10.8	10.6	10.9	11.1	0.3	0.1	0.4	0.6
20	21.0	21.1	21.1	21.0	21.4	0.1	0.1	0	0.4
30	30.5	30.6	30.7	30.6	31.1	0.1	0.2	0.1	0.6
40	40.5	40.7	40.4	40.7	40.7	0.2	-0.1	0.2	0.2
50	51.0	50.7	50.7	50.8	51.1	-0.3	-0.3	-0.2	0.1
60	60.0	60.3	60.2	60.4	60.4	0.3	0.2	0.4	0.4
70	70.0	70.1	69.9	69.9	70.4	0.1	-0.1	-0.1	0.4
80	79.0	78.8	78.8	78.9	79.5	-0.2	-0.2	-0.1	0.5
100	98.0	98.1	97.8	97.8	98.4	0.1	-0.2	-0.2	0.4

Calibration of J-type thermocouples from SCE temperature control system
- calibrated in stirred, heated water bath
- calibrated against ASTM type 9C mercury thermometer range -5 to 110°C div. 0.5°C

Glass Thermometer °C	Indicated Temperature		Error from Calibration	
	Oven Sensor °C	Extractor Probe °C	Oven Sensor °C	Extractor Probe °C
99.0	99.6	99.2	0.6	0.2
95.0	95.5	94.9	0.5	-0.1
90.0	90.1	89.8	0.1	-0.2
85.0	84.9	84.6	-0.1	-0.4
80.0	80.1	79.5	0.1	-0.5
75.0	75.1	74.5	0.1	-0.5
70.0	70.5	69.6	0.5	-0.4
65.0	65.3	64.9	0.3	-0.1
60.0	60.5	59.9	0.5	-0.1
55.0	55.5	55.0	0.5	0.0
50.0	50.6	50.2	0.6	0.2
45.0	45.8	45.4	0.8	0.4
40.0	40.7	40.4	0.7	0.4
35.0	35.7	35.6	0.7	0.6
30.0	30.7	30.5	0.7	0.5
25.0	25.7	25.7	0.7	0.7
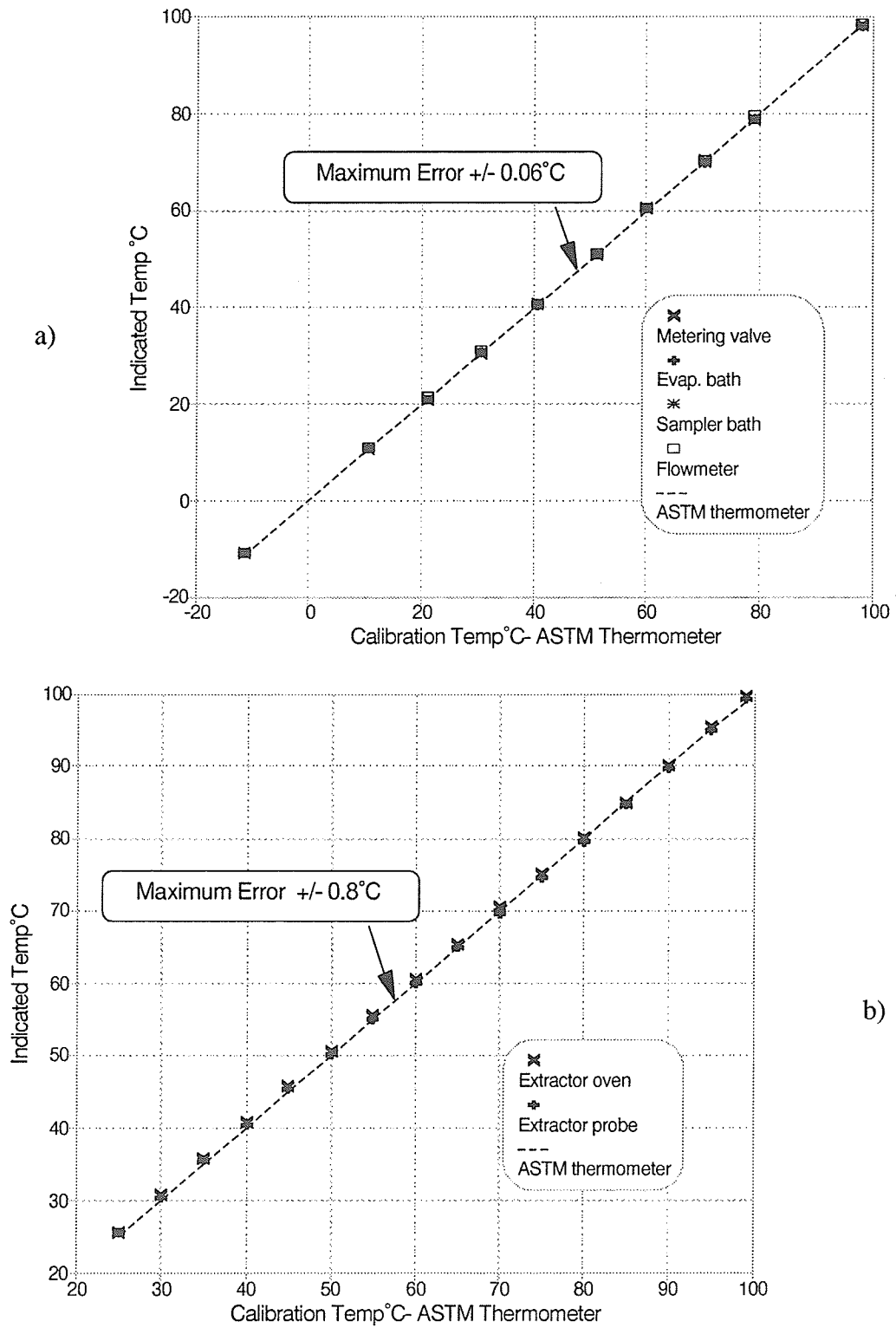
Figure A-5: Calibration curves of thermocouple sensors with KS102 cold-point compensation/linearization calibrated against ASTM 9C mercury-in-glass thermometer: a) Type T thermocouples b) Type J thermocouples.

A4. MISCELLANEOUS CALIBRATION/TEST RESULTS

A4.1 Entrainer Injection Rate

After being rough calibrated, the entrainer injection system was used for several extractions, at different set concentrations, with ethanol and methanol feed solvents. During these extractions, the rate of entrainer depletion from the solvent reservoir was measured, as an accurate method of monitoring the rate of entrainer addition. After these runs, the actual entrainer and CO_2 flowrates were calculated from the slopes of the entrainer addition vs. time and CO_2 passed vs. time curves, respectively, tabulated, and compared with the set entrainer flowrates (calculated by the controller program from the set entrainer percentage and the average CO_2 flowrate) in Table A-7. The plot of actual addition rate against the set rate showed that the actual flowrate was a linear function of the set rate ($r^2 = 0.9992$). A regression of actual flowrate on set rate showed that all data points came very close to lying on a straight line (maximum $\pm$ 0.13% error), despite differences in flowrate, entrainer type, and extraction bed matrix. The regression line was therefore applied as a correction to the controller flowrate measurement:

$$\dot{m}_{E,actual} = 1.018 \cdot \dot{m}_{E,HPLC} + 0.016 \qquad \ldots (A.6)$$

where:

$\dot{m}_{E,\,actual}$ - is the actual entrainer mass flowrate, g/min

$\dot{m}_{E,\,HPLC}$ - is the mass flowrate indicated by the HPLC injector pump software

Table A-7: Final calibration of entrainer injection system using entrainer addition data from extraction runs.

Date	Extraction Conditions Temp. °C	Press. MPa	Feedstock	Entrainer	Nominal Injection %	Target CO2 Flowrate (g/min)	Actual CO2 Flowrate (g/min)	Set Entr'r Flowrate (g/min)	Actual Flowrate (g/min)	Fit Flowrate (g/min)	Actual Entrainer %	Calibrated Entrainer %	Error %
June 02	40	36	Re-extr'n of egg residue	EtOH	3.0	5.0	4.95	0.1531	0.1726	0.1719	3.37	3.36	0.01
June 11	40	36	Re-extr'n of egg residue	EtOH	3.0	5.0	5.03	0.1556	0.1745	0.1744	3.35	3.35	0.00
June 15	40	36	Re-extr'n of egg residue	EtOH	5.0	5.0	4.90	0.2579	0.2825	0.2786	5.45	5.38	0.07
June 07	40	36	Re-extr'n of egg residue	EtOH	5.0	5.0	4.94	0.2600	0.2769	0.2807	5.31	5.38	-0.07
April 30	40	36	Glass beads	MeOH	3.0	10.0	9.76	0.3000	0.3092	0.3214	3.07	3.19	-0.12
May 23	40	36	Re-extr'n of egg residue	MeOH	3.0	10.0	9.88	0.3056	0.3263	0.3271	3.20	3.20	-0.01
May 25	40	36	Re-extr'n of egg residue	MeOH	3.0	10.0	10.03	0.3102	0.3307	0.3318	3.19	3.20	-0.01
June 09	40	36	Re-extr'n of egg residue	EtOH	10.0	5.0	4.86	0.5400	0.5735	0.5657	10.55	10.43	0.13
June 22	40	36	Re-extr'n of egg residue	EtOH	10.0	5.0	4.97	0.5522	0.5714	0.5782	10.31	10.42	-0.11
May 17	40	36	Glass beads	MeOH	10.0	10.0	9.86	1.0956	1.1235	1.1313	10.23	10.29	-0.06

Regression Output:

Constant	0.016034
Std Err of Y Est	0.004806
R Squared	0.999190
No. of Observations	8
Degrees of Freedom	6
X Coefficient(s)	1.017983
Std Err of Coef.	0.011836

A4.2 Drying Efficiency of Entrained Samples

To test whether the 45°C water bath/nitrogen flushing was an efficient means of removing entrainer from the lipid samples collected after extraction runs with SC CO_2 and entrainer, tests were conducted using model samples containing known amounts of lipid and entrainer. Twelve vials of collected lipid were used; three vials were kept as controls (stored at -10°C under nitrogen), three vials had no entrainer added and were used as blanks in the drying process. Three vials had methanol and BHT added, and three vials had ethanol and BHT added. The nine vials were then evaporated under nitrogen for 8 h in the 45°C water bath, cooled, and the final masse of lipid in each vial was compared to the original mass (Table A-8). The non-entrained vials actually lost mass, possibly due to the presence of small quantities of moisture or volatiles. The entrained samples mostly showed small increases in mass, indicating incomplete removal of entrainer. However the increases were small, with none above 2.8 wt%, and were therefore felt to be acceptable.

Table A-8: Efficiency test for drying model entrained samples under nitrogen in 45°C water bath.

May 10, 1991

- recovered lipid samples (~ 2 g/vial) from the May 08 extraction of freeze-dried egg yolk with SC CO2 were divided into four groups:

    Vials 2,3 and 4 were simply stored under nitrogen at -10°C for the duration of the experiment

    Vials 5,6, and 7 were dried for 8 h under N2 in the 45°C bath

    Vials 8,9 and 10 had 4 mL of EtOH and 0.5 mL of 1.002 mg/mL BHT (in MeOH) added, thoroughly mixed, and then dried for 8 h under N2 in the 45°C bath

    Vials 11,12, and 13 had 4 mL of MeOH and 0.5 mL of 1.002 mg/mL BHT (in MeOH) added, thoroughly mixed, and then dried for 8 h under N2 in the 45°C bath

- masses of vials and contents were determined by a Mettler analytical balance to ±0.1 mg

Vial #	Vial Mass (g)	Mass of Vial & Sample (g)	Sample Mass (g)	Treatment	Mass of Vial, Sample & Entr'r (g)	Mass of Vial, Sample, Entr'r & BHT sol'n (g)	Mass of Vial & Dried Sample (g)	Change in Mass (g)	Change in Mass (% of Sample)
2	18.112	20.1107	1.9987	control	-	-	20.0954	-0.0153	-0.8
3	18.178	19.7838	1.6058		-	-	19.7734	-0.0104	-0.6
4	18.082	20.0056	1.9236		-	-	19.9818	-0.0238	-1.2
5	18.040	19.7893	1.7493	no entr'r	-	-	19.7451	-0.0442	-2.5
6	18.139	19.9969	1.8579		-	-	19.9836	-0.0133	-0.7
7	18.142	19.7488	1.6068		-	-	19.7089	-0.0399	-2.5
8	18.094	19.8740	1.7800	EtOH	23.0491	23.7427	19.9042	0.0302	1.7
9	18.133	19.8562	1.7232		22.9904	23.7113	19.8634	0.0072	0.4
10	18.170	19.6335	1.4635		22.7027	23.4530	19.6410	0.0075	0.5
11	18.083	19.6721	1.5891	MeOH	22.6711	23.3890	19.6533	-0.0188	-1.2
12	18.205	19.6242	1.4192		22.5572	23.3160	19.6634	0.0392	2.8
13	18.053	19.5517	1.4987		22.4422	23.1587	19.5637	0.0120	0.8

A4.3 Evaporative Losses from Entrainer Injection Reservoir

Since the rate of entrainer addition was monitored by monitoring the rate of mass change in the entrainer reservoir, there was some concern that evaporation from the reservoir might impair the accuracy of measurement. Because the reservoir flask had to 'float' free from the pump intake (so the reservoir mass could be measured without interference), the reservoir could not be tightly sealed. Instead, an aluminum disk, loosely fitting around the intake tube and covering the flask mouth, was used to minimize the open area. The mass change of the flask, sitting in the laboratory while set up as for normal entrainer injection, was recorded, and evaporation-time curves calculated from the data (Table A-9, Figure A-6). It was determined that the rate of evaporation , about 2.5 g in 10 h, was cut almost in half, to 1.5 g in 10 h by the use of the cap, and that this rate of loss was negligible compared to the rate of injection of entrainer during a typical run.

Table A-9: Measurement of evaporation rate of methanol from entrainer pump reservoir under typical extraction conditions with and without anti-evaporation cap.

May 20, 1992

Measurement of Evaporation of MeOH from Reservoir

Test 1: Room Temp: 25.2 C

- no cap on Erlenmeyer flask reservoir

Time (min)	Reservoir Mass (g)	MeOH Evap (g)	MeOH Fit
0.0	1046.605		-0.001
10.0	1046.559	0.046	0.041
26.0	1046.498	0.107	0.108
59.5	1046.362	0.243	0.248
241.0	1045.595	1.010	1.009
600.0			2.513

Regression Output:

Constant	-0.00098
Std Err of Y Est	0.005315
R Squared	0.999906
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.00419
Std Err of Coef.	0.000029

=> for a 10 h run, could lose 2.51 g MeOH

for 3% MeOH injection, the amount of MeOH injected would be:
$$10 \text{ g/min} * 600 \text{ min} * 3/(100\text{-}3) = 185.6 \text{ g MeOH, ie: } 1.35 \text{ \%error}$$

for 10% MeOH injection, the amount of MeOH injected would be:
$$10 \text{ g/min} * 600 \text{ min} * 10/(100\text{-}10) = 666.7 \text{ g MeOH, ie: } 0.38 \text{ \%error}$$

Test 2: Room Temp: 26.3 C

- free-fitting aluminum cap on Erlenmeyer reservoir

Time (min)	Reservoir Mass (g)	MeOH Evap (g)	MeOH Fit
0.0	1051.107		0.030
10.0	1051.069	0.038	0.054
20.0	1051.035	0.072	0.079
30.0	1051.004	0.103	0.103
76.0	1050.866	0.241	0.215
551.0	1049.743	1.364	1.367
600.0			1.486

Regression Output:

Constant	0.030235
Std Err of Y Est	0.018465
R Squared	0.999198
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	0.002426
Std Err of Coef.	0.00004

Note: temp dropped to 23.5 C b/t 76 min and 551 min

=> for a 10 h run, could lose 1.49 g MeOH

for 3% MeOH injection, the amount of MeOH injected would be:
$$10 \text{ g/min} * 600 \text{ min} * 3/(100\text{-}3) = 185.6 \text{ g MeOH, ie: } 0.80 \text{ \%error}$$

for 10% MeOH injection, the amount of MeOH injected would be:
$$10 \text{ g/min} * 600 \text{ min} * 10/(100\text{-}10) = 666.7 \text{ g MeOH, ie: } 0.22 \text{ \%error}$$
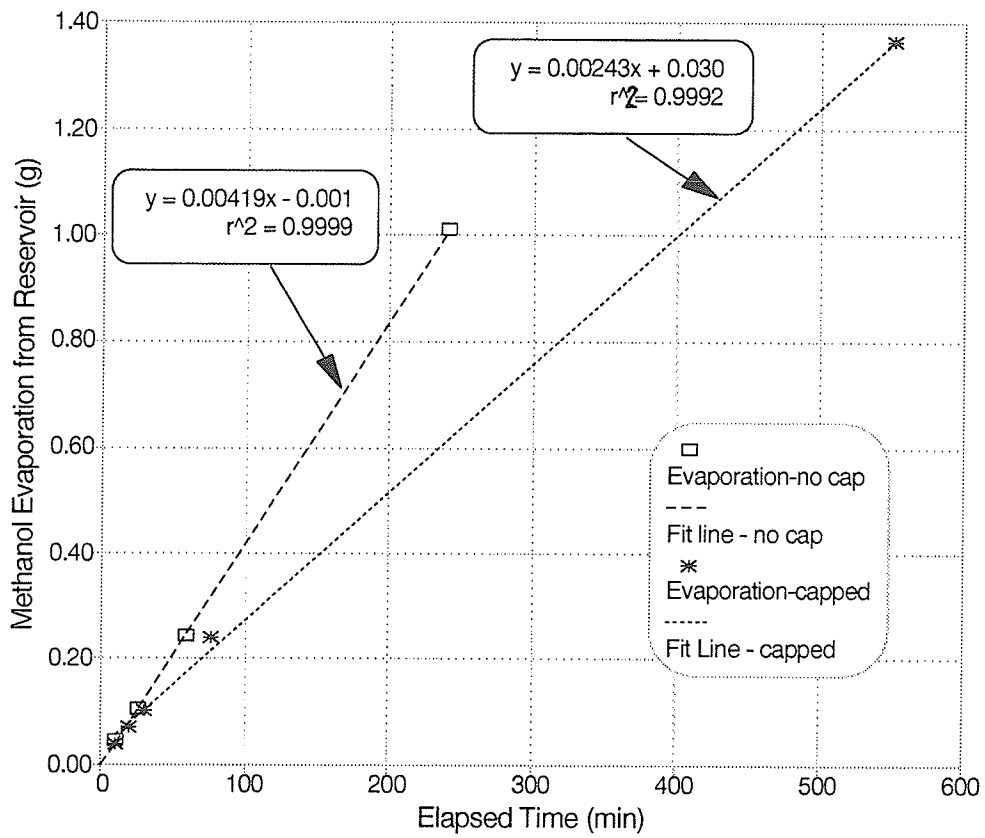
Figure A-6: Graph of evaporative loss as a function of time for methanol from entrainer pump reservoir under typical extraction conditions with and without anti-evaporation cap.

A5. STATISTICAL ANALYSES OF EXPERIMENTAL DATA

A5.1 Mass Change During Entrainer Evaporation

A5.1.1. SAS listing

```
DATA EVAPDAT;
  INPUT VIALNUM STYPE MASSCHG;
  CARDS;
2 1 -0.8
3 1 -0.6
4 1 -1.2
5 2 -2.5
6 2 -0.7
7 2 -2.5
8 3 1.7
9 3 0.4
10 3 0.5
11 4 -1.2
12 4 2.8
13 4 0.8
PROC GLM;
  CLASS STYPE;
  MODEL MASSCHG=STYPE;
  MEANS STYPE/ DUNNETT ('1');
RUN;
```

where:
      1 - refers to control sample stored in refrigerator
      2 - refers to blank samples placed in evaporator without entrainer
      3 - refers to samples mixed with EtOH and evaporated
      4 - refers to samples mixed with MeOH and evaporated

A5.1.2 SAS output

General Linear Models Procedure
Class Level Information

Class	Levels	Values
STYPE	4	1 2 3 4

Number of observations in data set = 12

Dependent Variable: MASSCHG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	16.34916667	5.44972222	3.83	0.0573
Error	8	11.39333333	1.42416667		
Corrected Total	11	27.74250000			

R-Square	C.V.	Root MSE	MASSCHG Mean
0.589318	-433.9580	1.193385	-.27500000

General Linear Models Procedure

Dunnett's T tests for variable: MASSCHG

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 8 MSE= 1.424167
Critical Value of Dunnett's T= 2.880
Minimum Significant Difference= 2.8059

Comparisons significant at the 0.05 level are indicated by '***'.

STYPE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
3 - 1	-1.073	1.733	4.539
4 - 1	-1.139	1.667	4.473
2 - 1	-3.839	-1.033	1.773

A5.2 Effect of Temperature on Egg Lipid Solubility in SC CO$_2$

A5.2.1. SAS listing

```
DATA TEMPEFF;
  INPUT TEMP SOL;
  CARDS;
40 11.0
40 11.1
40 11.1
40 12.2
40 11.8
40 10.6
40 12.2
40 11.3
40 12.1
40 12.6
40 11.1
40 11.4
40 12.7
40 10.4
55 12.2
55 12.1
65 12.5
65 12.2
75 10.3
75 11.4
;
PROC GLM;
  CLASS TEMP;
  MODEL SOL=TEMP;
  MEANS TEMP/ LSD TUKEY CLDIFF NOSORT;
  MEANS TEMP/ DUNCAN;
RUN;
```

where:
        TEMP - is extraction temperature in °C
        SOL - is gross lipid solubility in mg/g CO$_2$

A5.2.2 SAS output

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TEMP	4	40 55 65 75

Number of observations in data set = 20

Dependent Variable: SOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.89621429	0.96540476	2.06	0.1464
Error	16	7.50928571	0.46933036		
Corrected Total	19	10.40550000			

R-Square	C.V.	Root MSE	SOL Mean
0.278335	5.898208	0.685077	11.6150000

T tests (LSD) for variable: SOL

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05   Confidence= 0.95   df= 16   MSE= 0.46933
Critical Value of T= 2.11991

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit
40 - 55	-1.705	-0.607	0.491
40 - 65	-1.905	-0.807	0.291
40 - 75	-0.405	0.693	1.791
55 - 40	-0.491	0.607	1.705
55 - 65	-1.652	-0.200	1.252
55 - 75	-0.152	1.300	2.752

65	- 40	-0.291	0.807	1.905
65	- 55	-1.252	0.200	1.652
65	- 75	0.048	1.500	2.952 ***
75	- 40	-1.791	-0.693	0.405
75	- 55	-2.752	-1.300	0.152
75	- 65	-2.952	-1.500	-0.048 ***

Tukey's Studentized Range (HSD) Test for variable: SOL

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 16 MSE= 0.46933
Critical Value of Studentized Range= 4.046

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
40 - 55	-2.089	-0.607	0.875
40 - 65	-2.289	-0.807	0.675
40 - 75	-0.789	0.693	2.175
55 - 40	-0.875	0.607	2.089
55 - 65	-2.160	-0.200	1.760
55 - 75	-0.660	1.300	3.260
65 - 40	-0.675	0.807	2.289
65 - 55	-1.760	0.200	2.160
65 - 75	-0.460	1.500	3.460
75 - 40	-2.175	-0.693	0.789
75 - 55	-3.260	-1.300	0.660
75 - 65	-3.460	-1.500	0.460

Duncan's Multiple Range Test for variable: SOL

NOTE: This test controls the type I comparisonwise error rate, not
the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 0.46933
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 2.545455

Number of Means   2   3   4
Critical Range 1.285 1.349 1.392

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TEMP
	A	12.350	2	65
	A			
B	A	12.150	2	55
B	A			
B	A	11.543	14	40
B				
B		10.850	2	75

A5.3 Effect of Entrainer Type and Concentration on Egg Lipid Solubility in SC CO$_2$

A5.3.1. SAS listing

```
DATA ENTCONC;
   INPUT CONC SOL;
   CARDS;
1 0.058
3 0.076
3 0.077
5 0.143
5 0.182
10 0.220
10 0.243
;
PROC GLM;
   CLASS CONC;
   MODEL SOL=CONC;
   MEANS CONC/ LSD TUKEY CLDIFF NOSORT;
   MEANS CONC/ DUNCAN;
RUN;
```

where:

CONC - is the nominal concentration of ethanol in CO$_2$, % w/w

except '1'- represents methanol at 3% w/w concentration in CO$_2$

SOL - is gross lipid solubility in mg/g CO$_2$

A5.3.2 SAS output

General Linear Models Procedure
Class Level Information

Class	Levels	Values
CONC	4	1 3 5 10

Number of observations in data set = 7

General Linear Models Procedure

Dependent Variable: SOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03249393	0.01083131	31.69	0.0090
Error	3	0.00102550	0.00034183		
Corrected Total	6	0.03351943			

R-Square	C.V.	Root MSE	SOL Mean
0.969406	12.95507	0.018489	0.14271429

T tests (LSD) for variable: SOL

NOTE: This test controls the type I comparisonwise error rate not
the experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 3 MSE= 0.000342
Critical Value of T= 3.18245

Comparisons significant at the 0.05 level are indicated by '***'.

CONC Comparison		Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
1	- 3	-0.0906	-0.0185	0.0536	
1	- 5	-0.1766	-0.1045	-0.0324	***
1	- 10	-0.2456	-0.1735	-0.1014	***
3	- 1	-0.0536	0.0185	0.0906	
3	- 5	-0.1448	-0.0860	-0.0272	***
3	- 10	-0.2138	-0.1550	-0.0962	***
5	- 1	0.0324	0.1045	0.1766	***
5	- 3	0.0272	0.0860	0.1448	***
5	- 10	-0.1278	-0.0690	-0.0102	***
10	- 1	0.1014	0.1735	0.2456	***
10	- 3	0.0962	0.1550	0.2138	***
10	- 5	0.0102	0.0690	0.1278	***

Tukey's Studentized Range (HSD) Test for variable: SOL
NOTE: This test controls the type I experimentwise error rate.
Alpha= 0.05 Confidence= 0.95 df= 3 MSE= 0.000342
Critical Value of Studentized Range= 6.941

Comparisons significant at the 0.05 level are indicated by '***'.

CONC Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
1 - 3	-0.1296	-0.0185	0.0926	
1 - 5	-0.2156	-0.1045	0.0066	
1 - 10	-0.2846	-0.1735	-0.0624	***
3 - 1	-0.0926	0.0185	0.1296	
3 - 5	-0.1767	-0.0860	0.0047	
3 - 10	-0.2457	-0.1550	-0.0643	***
5 - 1	-0.0066	0.1045	0.2156	
5 - 3	-0.0047	0.0860	0.1767	
5 - 10	-0.1597	-0.0690	0.0217	
10 - 1	0.0624	0.1735	0.2846	***
10 - 3	0.0643	0.1550	0.2457	***
10 - 5	-0.0217	0.0690	0.1597	

Duncan's Multiple Range Test for variable: SOL

NOTE: This test controls the type I comparisonwise error rate, not
the experimentwise error rate

Alpha= 0.05 df= 3 MSE= 0.000342
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 1.6

Number of Means   2   3   4
Critical Range .0657 .0660 .0660

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CONC
A	0.2315	2	10
B	0.1625	2	5
C	0.0765	2	3
C			
C	0.0580	1	1

A5.4 Effect of CO2 Flowrate on Egg Lipid Solubility in SC CO_2

A5.4.1. SAS listing

```
DATA FLOWREFF;
  INPUT FLOWR SOL;
  CARDS;
2 11.1
2 11.1
2 12.2
2 11.8
2 10.6
2 12.2
2 11.3
2 12.1
2 12.6
2 11.1
2 11.4
2 12.7
2 10.4
1 10.8
1 11.5
1 9.7
;
PROC GLM;
  CLASS FLOWR;
  MODEL SOL=FLOWR;
  MEANS FLOWR/ LSD TUKEY CLDIFF NOSORT;
  MEANS FLOWR/ DUNCAN;
RUN;
```

where:

       1 - refers to high flowrate range (9.60-10.60 g CO_2/min) used in present study

       2 - refers to low flowrate range (4.13-5.16 g CO_2/min) used by Labay (1990)

A5.4.2 SAS output

General Linear Models Procedure
Class Level Information

Class   Levels   Values

FLOWR   2   1 2

Number of observations in data set = 16

Dependent Variable: SOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.05391026	2.05391026	3.51	0.0819
Error	14	8.18358974	0.58454212		
Corrected Total	15	10.23750000			

R-Square	C.V.	Root MSE	SOL Mean
0.200626	6.699264	0.764554	11.4125000

T tests (LSD) for variable: SOL

NOTE: This test controls the type I comparisonwise error rate not
the experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.584542
Critical Value of T= 2.14479

Comparisons significant at the 0.05 level are indicated by '***'.

FLOWR Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit
1 - 2	-1.968	-0.918	0.132
2 - 1	-0.132	0.918	1.968

Tukey's Studentized Range (HSD) Test for variable: SOL

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.584542
Critical Value of Studentized Range= 3.033

Comparisons significant at the 0.05 level are indicated by '***'.

FLOWR Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
1 - 2	-1.968	-0.918	0.132
2 - 1	-0.132	0.918	1.968

Duncan's Multiple Range Test for variable: SOL

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 14 MSE= 0.584542
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 4.875

Number of Means   2
Critical Range 1.048

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	FLOWR
A	11.585	13	2
A			
A	10.667	3	1

A5.5 Effect of Temperature on Cholesterol & TG Solubility in SC CO_2

A5.5.1. SAS listing

```
DATA CHOLTRI;
   INPUT TEMP TRIS CHOLS;
   CARDS;
40 8.81 0.66
40 8.76 0.65
40 9.30 0.64
40 9.52 0.59
40 9.50 0.60
40 9.11 0.57
40 9.47 0.43
40 9.38 0.33
55 10.70 0.73
55 10.21 0.62
55 11.01 0.59
65  11.88 0.87
65 9.33 0.69
65 9.38  0.87
65 9.52 0.78
65 9.76 0.66
75 8.07 0.71
75 8.08 0.51
;
PROC GLM;
   CLASS temp;
   MODEL CHOLS=TEMP;
   MEANS TEMP/ LSD TUKEY CLDIFF NOSORT;
   MEANS TEMP/ DUNCAN;
PROC GLM;
   CLASS temp;
   MODEL TRIS=TEMP;
   MEANS TEMP/ LSD TUKEY CLDIFF NOSORT;
   MEANS TEMP/ DUNCAN;
RUN;
```

A5.5.2 SAS output

Class   Levels   Values

TEMP   4   40 55 65 75

Number of observations in data set = 18

General Linear Models Procedure

Dependent Variable: CHOLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.14450361	0.04816787	4.06	0.0287
Error	14	0.16627417	0.01187673		
Corrected Total	17	0.31077778			

R-Square	C.V.	Root MSE	CHOLS Mean
0.464974	17.05780	0.108980	0.63888889

T tests (LSD) for variable: CHOLS

NOTE: This test controls the type I comparisonwise error rate not
the experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.011877
Critical Value of T= 2.14479

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
40 - 55	-0.2462	-0.0879	0.0703	
40 - 65	-0.3485	-0.2153	-0.0820	***
40 - 75	-0.2360	-0.0513	0.1335	

55 - 40	-0.0703	0.0879	0.2462
55 - 65	-0.2980	-0.1273	0.0434
55 - 75	-0.1767	0.0367	0.2500
65 - 40	0.0820	0.2153	0.3485 ***
65 - 55	-0.0434	0.1273	0.2980
65 - 75	-0.0316	0.1640	0.3596
75 - 40	-0.1335	0.0513	0.2360
75 - 55	-0.2500	-0.0367	0.1767
75 - 65	-0.3596	-0.1640	0.0316

Tukey's Studentized Range (HSD) Test for variable: CHOLS

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.011877
Critical Value of Studentized Range= 4.111

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
40 - 55	-0.3024	-0.0879	0.1265	
40 - 65	-0.3958	-0.2153	-0.0347	***
40 - 75	-0.3017	-0.0513	0.1992	
55 - 40	-0.1265	0.0879	0.3024	
55 - 65	-0.3587	-0.1273	0.1040	
55 - 75	-0.2525	0.0367	0.3258	
65 - 40	0.0347	0.2153	0.3958	***
65 - 55	-0.1040	0.1273	0.3587	
65 - 75	-0.1010	0.1640	0.4290	
75 - 40	-0.1992	0.0513	0.3017	
75 - 55	-0.3258	-0.0367	0.2525	
75 - 65	-0.4290	-0.1640	0.1010	

Duncan's Multiple Range Test for variable: CHOLS

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 14 MSE= 0.011877
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 3.453237

Number of Means   2   3   4
Critical Range 0.178 0.186 0.192

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TEMP
	A	0.7740	5	65
	A			
B	A	0.6467	3	55
B	A			
B	A	0.6100	2	75
B				
B		0.5587	8	40

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TEMP	4	40 55 65 75

Number of observations in data set = 18

Dependent Variable: TRIS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.62657028	3.20885676	7.98	0.0024
Error	14	5.63145750	0.40224696		
Corrected Total	17	15.25802778			

R-Square	C.V.	Root MSE	TRIS Mean
0.630918	6.645398	0.634229	9.54388889

T tests (LSD) for variable: TRIS

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.402247
Critical Value of T= 2.14479

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
40 - 55	-2.330	-1.409	-0.488	***
40 - 65	-1.518	-0.743	0.033	
40 - 75	0.081	1.156	2.232	***
55 - 40	0.488	1.409	2.330	***
55 - 65	-0.327	0.666	1.659	
55 - 75	1.323	2.565	3.807	***
65 - 40	-0.033	0.743	1.518	
65 - 55	-1.659	-0.666	0.327	
65 - 75	0.761	1.899	3.037	***
75 - 40	-2.232	-1.156	-0.081	***
75 - 55	-3.807	-2.565	-1.323	***
75 - 65	-3.037	-1.899	-0.761	***

Tukey's Studentized Range (HSD) Test for variable: TRIS

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 0.402247
Critical Value of Studentized Range= 4.111

Comparisons significant at the 0.05 level are indicated by '***'.

TEMP Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
40 - 55	-2.657	-1.409	-0.161	***
40 - 65	-1.794	-0.743	0.308	
40 - 75	-0.301	1.156	2.614	
55 - 40	0.161	1.409	2.657	***
55 - 65	-0.680	0.666	2.012	
55 - 75	0.882	2.565	4.248	***

65	- 40	-0.308	0.743	1.794	
65	- 55	-2.012	-0.666	0.680	
65	- 75	0.357	1.899	3.441	***
75	- 40	-2.614	-1.156	0.301	
75	- 55	-4.248	-2.565	-0.882	***
75	- 65	-3.441	-1.899	-0.357	***

Duncan's Multiple Range Test for variable: TRIS

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 14 MSE= 0.402247
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 3.453237

Number of Means   2   3   4
Critical Range   1.033 1.084 1.118

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TEMP
	A	10.640	3	55
	A			
B	A	9.974	5	65
B				
B		9.231	8	40
	C	8.075	2	75

A5.6 Effect of Extr'n Temperature on Extract Cholesterol:Triglyceride Ratio

A5.6.1. SAS listing

```
DATA CTRATDAT;
   INPUT EXPNUM TREATNO RAT;
   CARDS;
1 0 0.080
2 0 0.054
3 0 0.080
4 0 0.075
5 0 0.075
6 0 0.079
7 0 0.075
1 40 0.070
1 40 0.069
1 40 0.065
1 40 0.059
1 40 0.060
2 40 0.044
2 40 0.034
3 55 0.064
3 55 0.057
3 55 0.051
4 65 0.068
4 65 0.069
5 65 0.085
5 65 0.076
5 65 0.064
6 75 0.081
6 75 0.059
7 75 0.090
7 75 0.079
7 75 0.075
;
PROC GLM;
   CLASS TREATNO;
   MODEL RAT=TREATNO;
   MEANS TREATNO/ DUNNETT ('0');
RUN;
```

---

where:
    TREATNO - is the temperature for SC CO_2 extraction of egg yolk in °C
except   '0'- represents control samples (from freeze-dried egg yolk before extraction)
    RAT - is the mass ratio of cholesterol to triglyceride from sample analysis

A5.6.2 SAS output

General Linear Models Procedure
Class Level Information

Class   Levels   Values

TREATNO   5   0 40 55 65 75

Number of observations in data set = 27

General Linear Models Procedure

Dependent Variable: RAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00188087	0.00047022	4.20	0.0112
Error	22	0.00246210	0.00011191		
Corrected Total	26	0.00434296			

R-Square	C.V.	Root MSE	RAT Mean
0.433084	15.54876	0.010579	0.06803704

Dunnett's T tests for variable: RAT

NOTE: This tests controls the type I experimentwise error for coMparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 22 MSE= 0.000112
Critical Value of Dunnett's T= 2.658

Comparisons significant at the 0.05 level are indicated by '***'.

TREATNO Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
75 - 0	-0.01366	0.00280	0.01926	
65 - 0	-0.01806	-0.00160	0.01486	
55 - 0	-0.03607	-0.01667	0.00274	
40 - 0	-0.03174	-0.01671	-0.00168	***

A5.7 Changes in Extract Composition During SC CO_2 Extraction of Egg yolk

A5.7.1. Statistical model

The triglyceride and cholesterol contents of SC CO_2 extracts of freeze-dried egg yolk were determined from analysis and plotted as functions of the lipid specific recovery. Extractions and analyses had been performed in duplicate (except single run analyzed at 55°C) for extraction temperatures of 40, 55, 65, and 75°C at a constant extraction pressure of 36 MPa. Separate linear regressions were calculated for the cholesterol and triglyceride contents as functions of the lipid specific recovery (% of original sample mass recovered) at each extraction temperature, using QuattroPRO. The null hypothesis (H_0) was that the cholesterol and triglyceride contents of the extracts did not change during the progress of the extraction (ie: regression lines had zero slopes). For each case, the null hypothesis was accepted or rejected according to the one-tailed t statistic:

Regression Equation:

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x \qquad \dots (A\text{-}7)$$

where:

$\hat{y}$ - is the least-squares regression estimate of extract cholesterol (or TG) content (y)

$\hat{\beta}_0$ - is the least-squares regression intercept

$\hat{\beta}_1$ - is the least-regression slope coefficient

The null hypothesis was:

H_0: $\beta_1 = 0$

where β_1 is the *actual* slope of the extract cholesterol (or TG) content *vs.* specific recovery function. The alternative hypothesis was:

H_A: $\beta_l < 0$

(or H_A: $\beta_l > 0$, if $\hat{\beta}_l$ was positive)

ie: the cholesterol (or TG) content of the extract decreased (or increased) during the course of the extraction. The test statistic t was calculated from the equation:

$$t = \frac{\hat{\beta}_1}{s_{\hat{\beta}_1}}$$ ... (A-8)

where:

$s_{\hat{\beta}_l}$ - is the standard error of slope $\hat{\beta}_l$

and $\hat{\beta}_l$ and $s_{\hat{\beta}_l}$ were taken from the QuattroPRO regression reports. The test statistic t in each case was compared against the critical value $t_\alpha = t(\alpha, n\text{-}2)$ from tabulated values (McClave and Dietrich, 1988). Here the Type I error probability α was set to 0.05, and n was the number of data points in each regression. The rejection region for the null hypothesis was then:

$t < -t_\alpha$ (or $t > t_\alpha$ when H_A: $\beta > 0$)

The calculations and results are shown in Table A-10:

Table A-10: Tests of significance for regressions (trend lines) calculated for changes in triglyceride and cholesterol concentrations in SC CO_2 extracts during extractions at 40, 55, 65, and 75°C at an extraction pressure of 36 MPa.

Extraction		Extract Cholesterol Content						Extract Triglyceride Content					
Temp. (°C)	Press. (MPa)	Trend Line Slope $\hat{\beta}_l$	# of data points n	Slope Standard Error $s_{\hat{\beta}_l}$	Test Statistic t	Critical Value $t(0.05, n\text{-}2)$	Test Results	Trend Line Slope $\hat{\beta}_l$	# of data points n	Slope Standard Error $s_{\hat{\beta}_l}$	Test Statistic t	Critical Value $t(0.05, n\text{-}2)$	Test Results
40	36	0.0117	19	0.0191	0.614	1.740	0	0.118	19	0.0713	1.657	1.740	0
55	36	-0.032	5	0.0163	-1.946	2.353	0	-0.001	5	0.140	-0.007	2.353	0
65	36	-0.055	12	0.0153	-3.602	1.812	-	0.184	12	0.148	1.242	1.812	0
75	36	-0.098	12	0.0310	-3.156	1.812	-	0.329	12	0.0646	5.104	1.812	+

'-" - significantly decreasing   '0'- no significant change   '+' - significantly increasing

APPENDIX B:

List of Material Suppliers

and

Equipment Manufacturers

B.1 LIST OF MATERIAL SUPPLIERS

ACS - Advanced Computer Solutions International Ltd.   ACS-1000™ PC-XT
Suite 330 - 2105 Luna Rd.   compatible computer
Carrollton, TX
75006 USA
(214) 247-5151
Canadian rep:
Doal Electronics
Box 228
Rosenort, MB
R0J 1W0

Active Components   electronic components:
106 King Edward St.   logic ICs
Winnipeg, MB   connectors
(204) 786-3075   transistors
                                        solid state relays
                                        optical switches
                                        thyristors

Adams A Supply (1969) ltd.   lapping compound
879 Wall St.   markout bluing dye
Winnipeg, MB
(204) 786-7481

AFG Glass Centre   tempered fireplace glass
308 Colony St.
Winnipeg, MB
(208) 783-0413

Aime's Auto Parts   salvage auto parts:
15 Aimes Ave.   engine oil pump
Winnipeg, MB
(204) 257-0858

Aldrich Chemical Company, Inc.   Pyrex™ glass wool
1001 West St. Paul Ave.
Milwaukee, WI
53233 USA
(800) 558-9160

Anachemia Science
4-214 Debaets St.
Winnipeg, MB
(204) 661-6734

mercury barometer
(proposed)

Atlas Alloys
1424 Wilson Pl.
Winnipeg, MB
(204) 284-4480

316 stainless steel stock
drill rod

Borland International, Inc.
1800 Green Hills Rd.
P.O. Box 660001
Scotts Valley, CA
95067-0001 USA
Canadian rep:
University of Manitoba Bookstore
140 University Centre
Ft. Garry Campus - University of Manitoba
Winnipeg, MB
(204) 474-8231

QuattroPRO™ v. 3.0
spreadsheet program

Brinkman Instruments Inc.
Cantiague Rd.
Westbury, NY
11590 USA
(516) 334-7500
Canadian rep:
Canlab Division
Baxter Diagnostics Corporation
(which see)

Lauda™ circulating
refrigerated bath

Brooks Instrument Division
Emerson Electric Canada Ltd.
P.O. Box 150
Markham, ON
L3P 3J6
(416) 475-4628

Brooks 5860 mass
flowmeter

Caledon Laboratories Ltd.
40 Armstrong St.
Georgetown, ON
(416) 449-7750

LC grade solvents

Canada Metal Co. Ltd.
1221 St. James St.
Winnipeg, MB
(204) 774-7455

brass stock

Canadian Tire Associate Stores
2195 Pembina Highway
Winnipeg, MB
(204) 269-9630

lubricating oil
emery paper
onion chopper

Canlab Division
Baxter Diagnostics Corporation
590 Moray St.
Winnipeg, MB
(800) 668-4666

hypodermic syringes and
    needles
Sartorius™ balance
filter flask
Whatman filter paper
aspirator vacuum pump
magnetic stirrer
wet-test flowmeter
    (proposed)

Cole-Parmer Instrument Company
7425 N. Oak Park Ave.
Chicago, IL
60648 USA
(800) 323 4340

Barnstead/Thermolyne™
    heating mats

Computerland
240 Graham Ave.
Winnipeg, MB
(204) 985-5300

Epson™ FX-100 dot-matrix
    printer

Consolidated Alcohols Ltd.
55 Mill St.
Toronto, ON
M5A 3C4
(416) 861-2440
Authorized Manitoba distributor:
Manitoba Liquor Control Commission
1555 Buffalo Pl.
Winnipeg, MB
(204) 284-2501

Absolute ethanol

Corona Data Systems Inc.
Westlake Village, CA
Canadian rep:
Scarsdale Computer Corporation
1 Scarsdale Rd.
Don Mills ON
M3B 2R2
(416) 441-1900

Corona PC-2^{TM}
   PC compatible computer[*]

Crawford Fitting Co.
Solon, OH
Local Rep:
Winnipeg Valve and Fitting Ltd.
341 Bedson St.
Winnipeg, MB
(204) 837-7879

Swagelok^{TM} fittings
Swagelok Quick-Connects
Whitey^{TM} valves
Sno-Trik^{TM} high-pressure
   valves and tubing
Snoop^{TM} leak detector fluid
Cajon^{TM} conversion fittings
Nupro^{TM} filters
Nupro^{TM} check valves

Data Instruments, Inc.
100 Discovery Way
Action, MA
01720 USA
Local Rep:
Intertechnology Inc.
1 Scarsdale Rd.
Don Mills, ON
M3B 2R2
(416) 564-0828

Model AB diaphragm
   pressure transducer

Datamedia Corp.
New Jersey, USA
Obtained from:
Computer Services
University of Manitoba
Winnipeg, MB
(204) 474-9485

computer terminal

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[*] - This computer was purchased ca. 1983; the present status and whereabouts of Corona Data Systems are unknown. However, this is essentially a typical PC compatible which could as easily be sourced elsewhere.

Dynamic Machine Corporation
Hydraulic and Pneumatic Division
1407 Dugald Rd.
Winnipeg, MB
(204) 237-6199

hydraulic components:
  Parker™ valves
O-rings

Export Packers Company Ltd.
70 Irene St.
Winnipeg, MB
(204) 477-1830

bulk frozen egg yolk

Environment Canada
Atmospheric Environment Service
Winnipeg, MB
(204) 983-2050

hourly local barometric
  pressure readings

Fisher Scientific Ltd.
10720-178th St.
Edmonton, AB
T5S 1J3
(800) 661-9981

Ainsworth™ balance
Gooch flat-bottomed
  filtration funnel
Shimadzu™ UV/vis HPLC
  detector

Forever Industries (1971) Ltd.
1301 Dugald Rd.
Winnipeg, Manitoba
(204) 661-6959

perforated steel sheet mesh

Hewlett-Packard (Canada) Ltd.
6877 Goreway Drive
Mississauga, ON
L4V 1M8
(416) 678-9430

HPLC

Industrial Technology Centre
Division of Manitoba Research Council
1329 Niakwa Rd. E.
Winnipeg, MB
(204) 945-6000

loaned high-pressure gage

Jerguson Gage & Valve Co. 15 Adams St. Burlington, MA 01803 USA (617) 272-3600 Canadian rep: Peacock Inc. 1180 Aerowood Dr. Mississauga, ON L4W 1Y5 (416) 625-7100	Jerguson™ high pressure gage
Johnston Industrial Plastics Ltd. 3-4 Stevenson St. Winnipeg, MB (204) 633-9256	Teflon™ stock
Mallinkrodt Specialty Chemicals Canada Inc. 1020 Cardiff Blvd. Mississauga, ON L5S 1P3 (800) 465-9824	HPLC grade solvents: methanol, chloroform Reagent-grade acetone
Matheson Gas Products Canada Inc. 530 Matson St. E. Whitby, ON L1N 5R9 (416) 863-3397	entrainer/carbon dioxide mixtures

Microsoft Canada Inc.
320 Matheson Blvd. W.
Mississauga, ON
L5R 3R1 CANADA
(800) 563-9048
Local dealer:
University of Manitoba Bookstore
140 University Centre
Ft. Garry Campus - University of Manitoba
Winnipeg, MB
R3T 2N2
(204) 474-8231
	QuickBASIC v.4.0 & 4.5
	    Basic compiler/
	    programming
	    environment
	MS-DOS v. 5.0 operating
	    system

Omega Engineering, Inc. One Omega Drive Box 4047 Stamford, CT. 06907 USA (800) 622 2378	thermocouples data aquisition equipment thermal mass flowmeter (proposed)

Princess Auto Ltd.
535 Panet Rd.
Winnipeg, MB
(204) 669-4252
	surplus farm, electronic,
	industrial components:
	   gearhead motors
	   hydraulic filter, fittings
	   oil pressure indicator kit

Russelsteel 1510 Clarence Ave. Winnipeg, MB (204) 475-8584	steel sheet and shapes
B A Robinson Co. Ltd. Wholesale Lighting Div. 167 Sherbrook St. Winnipeg, MB (204) 784-0075	Phillips Lumiline-II™ incandescent lights

SAS Institute Inc.
Cary, NC
27512-8000 USA
Licenced by:
Computer Services
University of Manitoba
Winnipeg, MB
(204) 474-9485
	SAS™ PC v. 6.04
	   statistical analysis
	   software for personal
	   computers

Sensym, Inc. 1244 Reamwood Ave. Sunnyvale, CA 94089 USA Canadian rep: Banke Electronics Ltd. Unit 1-72 Dynamic Dr. Scarborough, ON M1V 3Z5 (416) 292-5152	Sensym™ low pressure transducers

Sigma Chemical Co.
P.O. Box 14508
St. Louis, Mo.
63178 USA
(800) 325-8070

BHT
lipid standards

Spectrex Ltd.
5250 Ferrier St.
Suite 508
Montreal, PQ
H4P 1L6
(514) 738-3377

0.5-mm ϕ glass beads[†]

Sub Cool Ltd.
Refrigeration & Air Conditioning
95 Mutchmor Close
Winnipeg, MB
(204) 663-3404

air conditioning module for
  recirculating airbath

Sunbeam Appliance Service Co.
948 St. James St.
Winnipeg, MB
(204) 774-1859

deep frier (water bath)

Supelco Canada Ltd.
46-220 Wyecroft Rd.
Oakville, ON
L6K 3V1 CANADA
(416) 842-8210

HPLC and GC components
  and accesories:
Rheodyne press. relief
  valves
Valco Switching valves
stainless steel tubing
glass beads
hi-capacity bubble flowmeter
  (proposed)

[†] Spectrex Ltd. is no longer distributing glass beads. Suggest sourcing glass bead column packing from a chromatographic supply house eg: Supelco (see entry).

Superpressure Division
Newport Scientific Inc.
Jessup, MD
Canadian rep:
Finnan Engineered Products Ltd.
1149 Bellamy Rd. N., Unit 22
gage
Scarborough, ON
M1H 1H7
(416) 438-6070

United Electric Co. Newport
Spacepak™ temperature
controllers
Ashcroft™ Duragage™
pressure gage
Marshalltown™ pressure

Tescom™ pressure regulator

Taurus Computer Products
110 - 1140 Morrison Drive
Ottawa, ON
K2H 8S9
(613) 596-3910

KS102™ multifunction I/O
data aquisition boards for
PCs‡

United Refrigeration & Heating Wholesale
1365 Erin St.
Winnipeg, MB
(204) 775-9731

small-diameter flexible
copper tubing

Virtis Company
Rt. 208
Gardiner, NY
12525
Canadian rep:
Allen Crawford Assoc. Ltd.
5835 Coopers Ave.
Mississauga, ON
L4Z 1Y2
(416) 277-0331

benchtop freeze drier

Waters
Division of Millipore (Canada) Ltd.
3688 Nashua Dr.
Missisauga, ON
L4V 1M5
(800) 268-4881

HPLC supplies:
Sep-pak™ silica cartridge
columns

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‡ Taurus Computers is no longer in operation, and the KS102 is no longer being manufactured. However, Omega Engineering (see entry) manufactures PC data acquisition boards with similar capabilities.

Welders Supplies Ltd.
150 McPhillips
Winnipeg, MB
(204) 786-6031

Linde gas products:
   standard grade siphon
     carbon dioxide
   standard grade nitrogen
   regulators

Windsor Plywood
(The Plywood People)
2634 Pembina Hwy.
Winnipeg, MB
(204) 269-9114

fir plywood
rigid foam insulation

B.2 SCE EQUIPMENT MANUFACTURERS/DISTRIBUTORS

Applied Separations
930 Hamilton St.
Allentown, PA
18101 USA
(215) 770-0900

Speed SFE analytical-scale system
   diaphragm pump
   68 MPa max. pressure
   50 mL extraction vessel
   max. oven temperature 200°C

Autoclave Engineering of Canada
4129 Harvester Road
Burlington, ON
L7L 5M3 CANADA
(416) 632-6961

Screening system
300 mL extractor
Metering pump 0 - 7.7 g/min CO_2

Components: valves, vessels, fittings

Hewlett-Packard Comapany
Palo Alto, CA
Canadian rep:
Hewlett-Packard (Canada) Ltd.
6877 Goreway Drive
Mississauga, ON
L4V 1M8 CANADA
(416) 678-9430

Analytical-scale system
HP 7680A
7mL, 1.5 mL extractor
packed bed sample trap
autosampling
coupled to PC w/ graphic
control/monitoring software
Metering pump

Marc Sims SFE
2200 Powell St., Suite 745
Emeryville, CA 94608
94608 USA

PDU extraction unit
10 L extractor
 5 L separator
      40 L condenser for sample
Extraction press. 2 - 33 MPa
Extraction temp. ambient - 100°C
Separator press. 2 - 8 MPa
Metering pump max flow 1000 g/min CO_2
          max press. 34 MPa
Recycle system, computer control

Milton-Roy
Ivyland, PA USA
Canadian rep:
Analytical Technology Marketing Corp.
Ste. 320, 55 University Ave.
Toronto, ON
M5J 2H7 CANADA
(416) 862-7737

Screening system
150 or 55 mL extractor
2 x 150 mL separator
Extraction press. max. 34.5 MPa
Extraction temp. 25 - 100°C
Metering pump, dual head ajustable stroke
   max flow 7.7 g/min CO_2 per side
Optionallly connect entrainer to one side

Critical Extraction Monitor
   UV/vis, inline mounting

Nova-Swiss
Nova Werke AG
Vogelsangstrasse 24,
CH-8307 Effretikon
Switzerland
052 51 11 11

Small PDU
4 L extractor
2 L separator
Extraction press. 2 - 95 MPa
Extraction temp. ambient - 100oC
      Separator press. 2 - 20 MPa
Diaphragm compressor

Sitec Sieber Engineering AG
U.S. rep:
Sulzer Biotech Systems
Div. of Sulzer Bros., Inc.
230 Crossways Park Drive
Woodbury, NY
11797 USA
(516) 921 7373

Large screening unit
1 L extractor
optional 4 L, 10 L, 50 L, 100 L, 200 L
1.2 L separator
2 L condenser for sample
Extraction press 7.5 - 32 MPa
Extraction temp. ambient - 90°C
Separation press. 4 - 7 MPa
Diaphragm compressor
   max flow 16 L/h CO_2
   max pressure 32 MPa
1 L liquid extraction column

Superpressure Division
Newport Scientific Inc.
Jessup, MD
Canadian rep:
Finnan Engineered Products Ltd.
1149 Bellamy Rd. N., Unit 22
Scarborough, ON
M1H 1H7 Canada
(416) 438-6070

Large Screening system
845 mL extractor
502 mL separator
Extraction press. max. 69 MPa
Adjustable stroke diaphragm compressor
    max flow 32 g/min CO_2 (est.)
    max press. 69 MPa

Screening system
300 mL extractor
Extraction press. max. 69 MPa
Diaphragm compressor
    max flow 16 g/min CO_2 (est.)
    max press. 69 MPa

Components: valves, fittings, vessels,
               compressors

Suprex Corporation
125 William Pitt Way
Pittsburgh, PA
15238 USA
(800) 800-5205 ext. 247

AutoPrep 44 fully automated analytical
    scale SCE system

Valco Instruments Co. Inc.
P.O. Box 55603
Houston, TX
77255 USA
(713) 688-9345

SFE flat rotary valves, on/off valves,
    manual and automatic, rated to 48 MPa
extraction vessels, volumes 250 µL to
    50 mL, rated to 69 MPa

Zeton, Inc.
4129 Harvester Rd.
Burlington, ON
L7L 5M3 CANADA
(416) 632-3123

Screening system
232 mL extractor
 78 mL separator
Extractor press. 69 MPa
Extractor temp. max 85°C
Separator press. max. 40 MPa
Separator temp. max. 85°C
Diaphragm compressor
    max flowrate 250 mL/min CO_2

B.3 SUPERCRITICAL EXTRACTION
RESEARCH FACILITIES

Norac Technologies Inc.
4222-97th St.
Greystone Pavillion
Edmonton, AB
T6E 5Z9 CANADA
(403) 461-7163

Screening to pilot size
  facilities, eg:
400 mL extractor screening
  system
1 L extractor large
  screening system
  solid/liquid
4 L extractor small PDU
10 L extractor PDU
  solid/liquid
100 L extractor pilot plant
  solid/liquid

Phasex Corporation
360 Merrimack St.
Lawrence, MA
01843 USA

Screening to pilot size

St-Hyacinthe Food Research & Development Centre
Research Branch, Agriculture Canada
St-Hyacinthe, PQ
CANADA J2S 8E3

Autoclave Engineers, Inc.
Pilot Plant
4 L extraction/2 L
  separation
or 500 mL extraction/
  /300 mL separation
69 MPa 200°C maximum