An Assessment of Cross-contamination Issues in the Context of Chemical and Pharmaceutical Processes Using a Continuous Oscillatory Baffled Reactor

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Abstract

Past research in oscillatory baffled reactors has shown that there are significant technological and business advantages in using such reactor technology in fine chemical and pharmaceutical industries: shorter reaction times, fewer by-products, uniform product quality and higher yields, while at the same time with a significant saving in space, capital and running costs.

This project focused on the robustness and adaptability of the continuous oscillatory baffled reactor (COBR) for a large spectrum of chemical reactions that are performed in very different fields of industry: from cosmetics and fine chemicals to pharmaceutical products. In particular, the emphasis was on cross contamination issues which may occur when different reactions are performed in a tandem fashion in this reactor.

The experimental results indicate that the COBR is well suited to a broad spectrum of chemical reactions, as well as for crystallization operations. During the continuous production of a fine chemical and two active pharmaceutical ingredients in tandem the conditions inside the reactor remained stable and were easily controlled. The minimal amounts of contaminants present and the high quality of the products obtained are a testament to the consistent operation and robust nature of the COBR.

The three production phases were interspersed with a well-defined cleaning procedure. The established cleaning protocol is simple, efficient and fast, while the amount of waste generated is minimized. The cleaning kinetics is of first order, which is consistent with previous work.

The results reported in this thesis show that the COBR, which incorporates quality-by-design principles, is a suitable alternative to current mixing technologies and can be readily assimilated into a variety of fine chemical and pharmaceutical manufacturing processes.

Dedicated to my family, in particular to my father
and to Reem, for her unreserved love and support

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I would like to acknowledge the importance of Prof. Xiong-Wei Ni, my supervisor, to the work presented in here. Prof. Ni has always accompanied and guided me with his sound advice, giving me the motivation necessary when I most needed it.

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Special thanks to my family, who throughout my stay in Scotland were always there to support me. My father, who in sickness has never lost the willingness to fight for his life, is my role model. He taught me how insignificant mundane problems are, and with his recommendations and advice showed me how to succeed in life.

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List of Abbreviations

API – Active Pharmaceutical Ingredient

C&T – Cosmetics & Toiletry

CFD – Computational Fluid Dynamics

CIP – Cleaning-in-place

COBR – Continuous Oscillatory Baffled Reactor

CPI – Critical Path Initiative

CSTR – Continuous Stirred Tank Reactor

DPIV – Digital Particle Image Velocimetry

EPSRC – Engineering and Physical Sciences Research Council

FDA – Food and Drug Administration

FEP – Flourinated Ethylene Propylene

GLP – Good Laboratory Practice

GMP – Good Manufacturing Practice

HPLC – High Performance Liquid Chromatography

ICH – International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

IR – Infrared Spectroscopy

ISPE – International Society of Pharmaceutical Engineers

MHLW - Ministry of Health, Labour and Welfare

MHRA – Medicines and Healthcare products Regulatory Agency

NMR – Nuclear Magnetic Resonance Spectroscopy

OBR – Oscillatory Baffled Reactor

OTC – Over-the-counter

PAT – Process Analytical Technologies

PFA – Perfluoroalkoxy

PFR – Plug Flow Reactor

PIV – Particle Imaging Velocimetry

PTFE – Polytetrafluoroethylene

PSD – Particle Size Distribution

QbD – Quality by Design

RPC – Reciprocating Plate Column

RTD – Residence Time Distribution

SEM – Scanning Electron Microscopy

STR – Stirred Tank Reactor

TCPO – bis(2,4,6-trichlorophenyl) oxalate

TDI – Total Daily Intake

WARF – Wisconsin Alumni Research Foundation

WHO – World Health Organization

XRD – X-Ray Diffractometry

List of General Nomenclature

Symbol

A_p	Projected area of the particle	[m^2]
BL_1	Limit of the target residue	[$g.L^{-1}$]
BL_2	Residue limit in the API used in the manufacture of the finished drug	[$g.L^{-1}$]
C	Concentration of the chemical species at a given time	[mg mL^{-1}]
C_0	Concentration of the chemical species at the start of washing	[mg mL^{-1}]
C_D	Drag coefficient	
D	OBR diameter	[m]
d_i	Internal diameter	[m]
d_o	Orifice diameter	[m]
f	Frequency	[s^{-1}]
F_b	Buoyant force	[N]
F_d	Drag force	[N]
F_g	Force of gravity	[N]
g	Gravitational acceleration	[$m.s^{-2}$]
L	Baffle spacing	[m]
m	Mass	[kg]
Re	Oscillatory Reynolds number	
Re_n	Net flow Reynolds number	
SF	Safety Factor	
St	Strouhal number	
u	Superficial net flow velocity	[$m.s^{-1}$]
u_t	Terminal velocity	[$m.s^{-1}$]
u_m	Mixing velocity	[$m.s^{-1}$]
u_o	Oscillatory velocity	[$m.s^{-1}$]
u_n	Net flow velocity	[$m.s^{-1}$]
V_r	Reactor volume	[L]
V_w	Washing volume	[L]
x_0	Centre-to-peak oscillation amplitude	[m]

Greek Symbols

α	Baffle free area	
δ	Baffle thickness	[m]
μ	Dynamic viscosity	[N.s.m^{-2}]
ρ	Density	[kg.m^{-3}]
ρ_l	Density of the fluid	[kg.m^{-3}]
ρ_p	Density of the particle	[kg.m^{-3}]
v	Fluid kinematic viscosity	[Pa.s]
ω	Angular oscillation frequency	[rad.s^{-1}]
Ψ	Cleaning index	
Ω	Wash index	

List of Publications

Caldeira, R.; Ni, X., Evaluation and Establishment of a Cleaning Protocol for the Production of Vanisal Sodium and Aspirin Using a Continuous Oscillatory Baffled Reactor. *Organic Process Research & Development* **2009,** 13, (6), 1080-1087

Caldeira, R.; Ni, X., Suitability Assessment of the Continuous Oscillatory Baffled Reactor for the Pharmaceutical Industry: Production, Crystallization and Cross Contamination Studies for Two Model APIs. *Organic Process Research & Development, in print.*

CHAPTER 1
INTRODUCTION

A good engineer is a person who makes a design
that works with as few original ideas as possible.

Freeman Dyson, theoretical physicist and mathematician

1.1 - Mixing technology, OBR and COBR

Fine chemicals and drugs manufacturing has long been associated with batch processing because production runs are segregated into lots, making it relatively easy to pinpoint out of specification product. In addition, laboratory scale experiments on new pharmaceutical compounds are often done in batch and the shortest way of migrating a process to full-scale is to mimic what happens at a small-scale (Kossik 2002; Jr. and Lodaya 2004; Swichtenberg 2008). The mindset of pharmaceutical companies is then geared towards batch-wise production, even if it compromises quality and yield, whilst increasing downstream purification units with associated wastes as well as processing time of pharmaceutical products (Plumb 2005; TL LaPorte 2007).

The drive towards implementing innovative continuous manufacturing technologies in the pharmaceutical production environment has gathered significant momentum recently. A recent report by AstraZeneca showed that crystallisation of an active pharmaceutical ingredient (API) for one of its best selling drugs was done in 12 minutes in a continuous crystallizer in comparison of 9 hours and 40 minutes in a traditional batch stirred tank crystallizer of 6.5 m^3 to achieve the same solid concentration with better specifications, e.g. filterability (Lawton et al 2009). This example has offered a paradigm shift to the long-established batch manufacturing processes. In fact, there are several drivers making the use of continuous reactors more appealing than its batch counterpart, such as minimizing the footprint and chemicals

inventory in newly built plants while maintaining the production volume flexibility that a continuous process is able to deliver, therefore keeping initial investment and production costs down (Jr. and Lodaya 2004; Pellek and Arnum 2008; Thakore and Bhatt 2008).

In terms of retro-fit, i.e. just to replace batch stirred tank crystallizers by continuous crystallizers in existing plants, this allows for better and greater control on product quality and eliminates in some cases the need for milling processes. In the AstraZeneca case, this represented an estimated 50% reduction in capital costs, and further avoidance of >£300k per annum costs in personnel, milling losses and other inefficiencies (Lawton, Steele et al 2009). Continuous processing minimizes the downtime associated with batch operations, can run continuously for either a short or a long period of time depending on the throughput (T/yr) required. The operational conditions are monitored and controlled continuously along the length of the continuous reactor/crystallizer. In fact, continuous reactor technologies offer a more simple, predictable and controllable solution for pharmaceutical manufacturers together with using less energy and solvents, while minimizing the production of waste (Liu, Lipták et al 1997; Angelucci 1999; Vervaet and Remon 2005; Crosby 2006).

In addition to this, regulatory bodies have recently published guidelines with the purpose of making manufacturers to fully understand and optimize the way their pharmaceutical products are developed, evaluated and manufactured, such as the Critical Path Initiative (CPI) issued by the FDA or the Q8 guideline published by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), which focuses on Quality by Design (QbD) (Plumb 2005; Pellek and Arnum 2008; Swichtenberg 2008). These regulatory efforts present the perfect opportunity for continuous process technology to be embraced and to be successfully implemented. One example is that of Genzyme's manufacturing process, which utilizes continuous reactors that have been approved by FDA and been running since 2007 (Ni 2009).

One of the important aspects of both CPI and QbD is the prevention of cross-contamination. The design of modern pharmaceutical equipment has to take into account that there is a current trend for new drugs to be tailored for narrower population

targets and so manufactured in smaller production runs (Gassmann et al 2004; Voelter-Mahlknecht and Mahlknecht 2004). In order to maximize the plant productivity it is then a requirement to use the same parts, such as chemical reactors, to manufacture different products. In this case avoiding cross-contamination is of critical importance. The cleaning of a continuous reactors utilizes a single-path cleaning-in-place (CIP) procedure that does not require cleaning to be done in contact with the outside environment, which reduces process downtime, waste, risk of cross-contamination and brings operational costs down (Perka et al 1993; Gambrill 1995; Chew, Tonneijk et al 2006).

One successful development in the continuous process technology design is the Continuous Oscillatory Baffled Reactor (COBR). The COBR is a reaction/mixing device and consists of a glass or stainless steel tube with periodically spaced circular orifice baffles within, perpendicularly orientated in relation to the flow. The fluid inside the tube is mixed by means of oscillatory movement, caused by a motor placed at one or both ends of the reactor.

Coupling a baffled tube with oscillatory movement results in excellent mixing of fluids. When a fluid that is being oscillated at a reasonable velocity encounters a sharp barrier, disruption of the flow occurs and eddies around the annular baffles are formed: as illustrated in Figure 1.1, a doughnut-shaped vortex is produced downstream of each baffle pulling the fluid to the tube wall when the oscillatory cycle begins and when the direction of flow is reversed the vortex is pushed back in to the centre of the tube, while a new vortex is formed on the other side of each baffle (Brunold et al 1989; Gelicourt 2000). This continuous generation and cessation of eddies provides a vigorous axial and radial mixing within each baffled region defined by two adjacent baffles, which can be considered as a perfectly mixed continuous stirred-tank reactor (CSTR) (Mackley and Ni 1991; Nelson 2001). As a consequence, the entire COBR behaves as a series of perfectly mixed CSTRs – very sharp residence time distribution measurements are achieved using this technology (Ni 1995; Ni and Pereira 2000; Ni et al 2003).

There are two designations that are frequently used throughout this thesis: OBR (oscillatory baffled reactor) and COBR. The former refers to batch operation, while the latter to continuous operation, as suggested by the name.

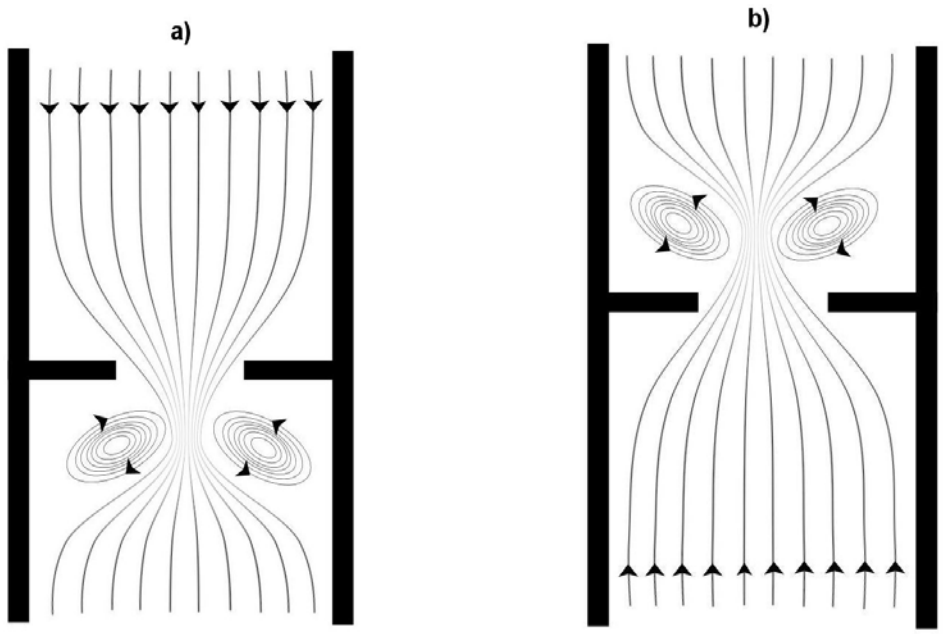


Figure 1.1 Flow pattern in an OBR operating with oscillation;
a) down stroke, b) up stroke

The characteristic oscillatory flow of an OBR is defined by two dimensionless groups, the oscillatory Reynolds number (Re_o) and the Strouhal number (St) (Brunold et al 1989):

$$\mathrm{Re}_o = \frac{\rho D x_0 \omega}{\mu} \tag{1.1}$$

$$St = \frac{D}{4\pi x_0} \tag{1.2}$$

When dealing with the flow in a COBR, the net flow Reynolds number is also pertinent dimensionless group:

$$\mathrm{Re}_n = \frac{\rho u D}{\mu} \qquad (1.3)$$

where ρ is the density of the bulk fluid (kg.m^{-3}), D is the OBR diameter (m), x_0 the centre-to-peak oscillation amplitude (m), ω the angular oscillation frequency (rad.s^{-1}), μ dynamic viscosity (N.s.m^{-2}) and u the superficial net flow velocity (m.s^{-1}).

1.2 - Motivation and Structure of the Project

It is believed that a central role in the adoption of a new technology by its potential users is that research done behind closed doors needs to be published to be legitimized (Technologies 2008; Thomas 2008). In this way, proactive companies that are prepared to adopt a proven, yet emerging technology earlier than their rivals can gain a competitive advantage. Examples can be found in diverse technology intensive businesses. For example, the introduction of a rear wing in the 1968 Lotus 49 in Formula 1 proved to be so competitive that by the end of that year most other cars were updated in a similar fashion; the Apple Macintosh 128K was such a commercial success due to its use of a graphical user interface (GUI) that Microsoft applied the same interface principles in its Windows package as a GUI for its own MS-DOS operating system (Scarso 1996; Rios 2007).

Having proved that the OBR and COBR design is a better alternative to conventional reactors in a number of diverse applications such as heat and mass transfer (Hewgill et al 1993; Mackley and Ni 1993; Mackley and Stonestreet 1995), polymerization (Ni et al 1999; Ni et al 2003), fermentation (Gaidani et al 2005; Masngut et al 2006), crystallization (Ni et al 2004; Caldeira and Ni 2009; Ni and Liao 2010), biodiesel production (Harvey et al 2003) or fine chemicals (Winder 2003; Ni 2006), the time has arrived to demonstrate that this relatively young technology is also suitable for extended pharmaceuticals and fine chemicals syntheses, in order to establish the robustness, versatility and adaptability of COBR – not only several chemical

reactions will be studied using this reactor technology, but also will these be done in a sequential fashion, with cleaning procedures in between. Each product must meet the stringent quality standards set by regulatory agencies. The synthesis of different products in tandem also allows for the degree of cross-contamination in the manufacturing process to be assessed, and the efficiency and kinetics of the cleaning to be evaluated.

The structure of this thesis is as follows: after the introduction in Chapter 1, the literature survey is given in Chapter 2, covering the oscillatory baffled reactors, fouling encrustation and cross-contamination, cleaning approaches, quality by design, the relevant regulatory matters in the chemical and pharmaceutical industries and acceptable limits of cross-contamination. The experimental setup, materials used and analytical techniques are presented in Chapter 3, and the chemistry screening together with a brief introduction for each of the chemical reactions performed is described in Chapter 4. Chapter 5 is dedicated to the results and discussion of the production campaigns performed in the COBR for the selected chemistries. Conclusions and suggestions for future work to be carried out are outlined in Chapter 6.

CHAPTER 2
LITERATURE REVIEW

Learning and innovation go hand in hand. The arrogance of success is to think that what you did yesterday will be sufficient for tomorrow.

William Pollard, physicist and priest

This chapter presents literature studies covering the fields of the oscillatory baffled reactors, fouling and encrustation, cleaning methods and strategies, the relevant regulations to which the chemical and pharmaceutical industries must abide to in order to market up-to-standard products as well as the levels of cross-contamination acceptable in a finished product.

2.1- OBR and COBR

The origins of oscillatory flow can be traced to Van Dijck's patent on a reciprocating plate column (RPC) as a means to improve extraction or washing processes by bringing into close contact two immiscible or slightly miscible liquids (Dijck 1935). One of his original drawings is shown in Figure 2.1.

According to the classification put forward by Lema and co-workers (2001) there are two types of oscillatory flow reactors concerning the way in which the contents inside are forced into motion: reactors in which the movement is provoked by the cyclic introduction of feed via a pump (plug or membrane) and reactors in which the oscillation is created via the characteristic cyclic motion of a part of the reactor. Both the RPC and OBR/COBR belong to the latter group - in RPCs and laboratory scale batch OBRs, oscillatory flow movement is created by the cyclic movement of the plates

and annular baffles; in COBRs and some batch OBRs it is the oscillation piston connected to one or both ends of the reactor that creates the oscillatory movement (Dijck 1935; Mackley and Ni 1991).

Since Van Dijck's pioneering work hundreds of scientific papers have been published in this research field, but it was not until the last two decades that the research on oscillatory flow mixing and its application in the chemical industry has grown substantially and there has been a constant stream of research papers published in this area since the early 1990's, as is illustrated in Figure 2.2.

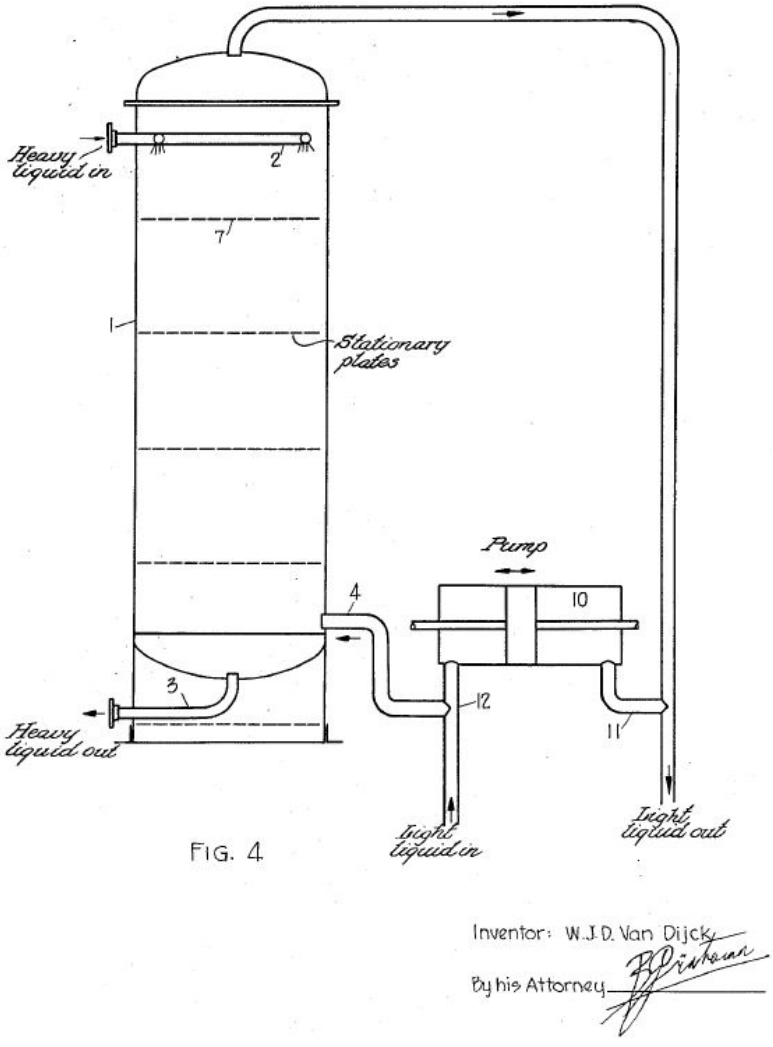


Figure 2.1 Scheme of Van Dick's reciprocating plate column

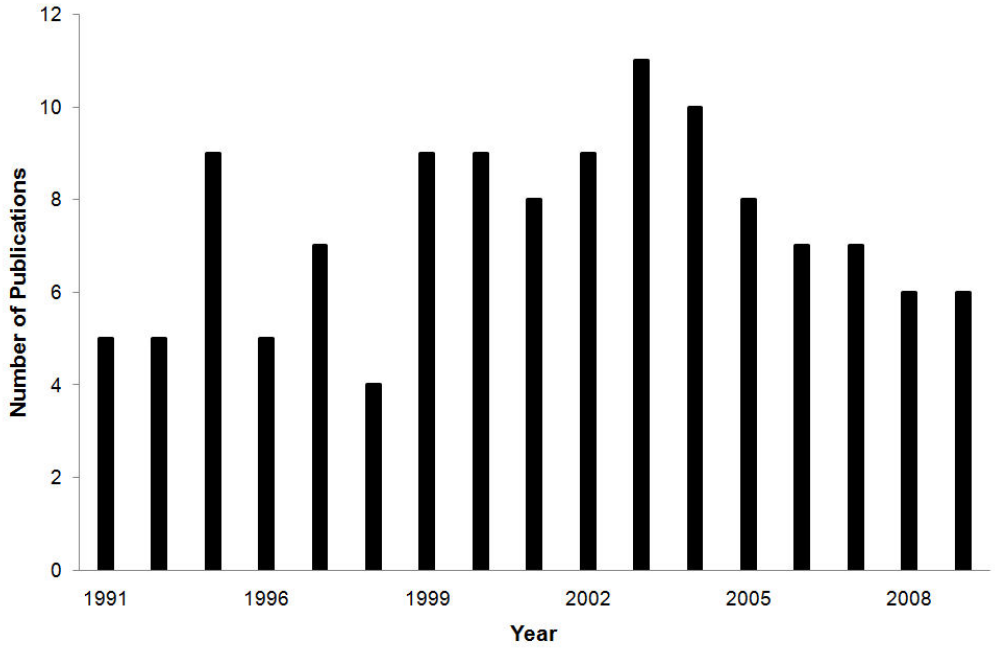


Figure 2.2 Number of publications containing the words “oscillatory” and “baffled”, according to ISI Web of Knowledge (www.isiknowledge.com)

2.1.1 – OBR

A review of the several studies carried out in OBR is presented in the following sections. Fundamental research in oscillatory flow in a baffled tube has been carried out covering flow patterns, mixing, the effect of several OBR geometrical parameters on mixing (e.g. baffle orifice diameter, baffle spacing, baffle thickness, reactor diameter), the effect of oscillation frequency and amplitude on mixing, heat and mass transfer characteristics, residence time distribution and turbulent properties, which include both experimental and numerical studies. These scientific publications set the basis for this further study in both batch OBR and COBR, regarding their applicability and suitability to a number of different chemical reactions and processes.

2.1.1.a – Flow characteristics

Brunold et al (1989) first reported that large scale eddy mixing can be generated in regions between sharp edges present in rectangular or cylindrical cross-section ducts

when oscillatory flow was introduced. A year later Howes and Mackley (1990) observed axial dispersion in a series of tracer experiments and concluded that the OBR was able to operate at near plug-flow conditions. Ni and Gelicourt (2002) found that the axial dispersion increased with the density of the solution when they studied the axial dispersion in a baffled column with 50 mm diameter. Both axial and radial dispersion were characterized in a batch OBR using the Laser Induced Fluorescence technique (Fitch and Ni 2003). Electrical Impedance Tomography was used for on-line measurements and the analysis of the dispersion enabled the description and prediction of the characteristics of the oil-water emulsion present in an OBR (Vilar et al 2008).

Gao and colleagues used the Particle Imaging Velocimetry (PIV) technique to determine local velocity components and found that complete mixing was consistently achieved under low oscillatory Reynolds numbers (Gao et al 2002). PIV was also used by Fitch and co-workers (2005) in conjuction with computational fluid dynamic to study the mixing effects of fluid viscosity in an OBR and concluded that good mixing was achieved in the OBR if the ratio between the plane-averaged axial and radial velocities was lower than 3.5.

2.1.1.b – Geometrical parameters and operating conditions

Eddy formation and the impact of the OBR geometrical parameters on these characteristics was first studied by Knott and Mackley (1980) and followed by Brunold et al (1989). Both studies reported that the cycle of eddies formation and disruption was optimal for a baffle spacing of 60% in a tube with an internal diameter of 25 mm. Since then a multitude of other studies investigated the impact of the baffle free area (α), baffle spacing (L), baffle thickness (δ), and of the frequency (f) and amplitude (x) of oscillation on mixing efficiency. Those geometric parameters are shown in Figure 2.3.

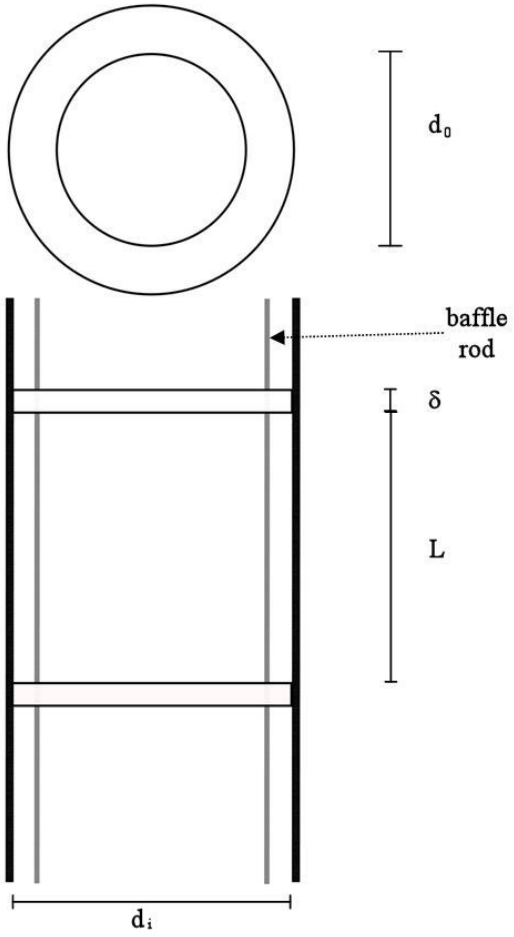


Figure 2.3 Schematic representation of an OBR. d_i – reactor internal diameter, δ – baffle thickness, L – baffles spacing, d_0 – orifice diameter

The influence of the baffle free area (α), defined as d_0^2/d_i^2, on mixing time was evaluated by Brunold et al (1989). They reported that α should be 34% in order for optimal mixing to occur. Later on, Ni et al (1998) varied α from 11 to 51% and found that a ratio of 20-22% was the optimum. As a result, a ratio of 21% was used in the experimental work for this project.

The ideal baffle spacing (L) is the one that allows the generated eddies to fully develop in between the baffles, whilst at the same time creating the conditions for good mixing to occur. In other words, if L is too small the formation of eddies in the inter-baffle space due to the oscillatory motion of the flow is severely disturbed; however, if L is too big, eddies formed won't be able to cover the inter-baffle space in its entirety thereby rendering mixing less efficient. While Brunold et al (1989) suggested that L should be 1.5d, Ni and Gao (1996) recommended that L should be 1.8d for better mixing.

Another important parameter in the design of an OBR is the baffle thickness (δ). If the baffles are too thick and eddies remain on the edges of the baffles for too long, the formation of vortex can be compromised. These findings were corroborated by Ni et al (1998) who suggested that thinner baffles helped reducing mixing times. Ni and Stevenson (1999) also reported that the existence of a gap between the outer baffle and the inner tube diameter affected the mixing patterns and resulted in a lengthening of mixing time in an OBR.

The operational parameters of the OBR, e.g. frequency (f) and amplitude (x_0), have a decisive input on the way in which fluid behaves inside the OBR, for example, on controlling both droplet and particle sizes in suspension polymerization of acrylamide and methylmethacrylate (Gough, Ni et al 1997; Ni et al 1998; Ni et al 1999; Ni et al 2001; Ni et al 2003). The impact of f and x_0 on backmixing was also studied by Takriff and Masyithah (2002) and later by Reis and co-workers (Reis et al 2004; Reis et al 2010), who demonstrated that the oscillation, with a particular relevance to x_0 affected the backmixing phenomena due to the variation in mixing length.

2.1.1.c – Heat and mass transfer

Heat and mass transfer characteristics of the OBR were the subject of study by different researchers. Stephens and Mackley (2002) used two different OBR configurations – one where annular baffles were oscillated within the tube and other where oscillatory motion was applied to the fluid in a tube with fixed annular baffles – and demonstrated that the heat transfer coefficient depended on the intensity of oscillation. Zhang et al (2005) also showed the significance of frequency and amplitude with regards to heat transfer, and concluded that an increase in oscillatory velocity led to an enhancement of heat transfer in the OBR.

The effect that periodic oscillatory motion inside a baffled tube has on mass transfer of oxygen into water was discussed by Hewgill and co-workers (1993). They found that the volumetric oxygen transfer coefficient offered a 6-fold increase when a

tube was set up in the presence of both baffles and fluid oscillation. In a comparison between the performance of an OBR and a STR in a yeast re-suspension operation made by Ni et al (1995), it was concluded that the volumetric oxygen transfer coefficient was 75% higher in the OBR than that in a conventional STR.

2.1.1.d – Numerical and simulation studies in OBR

Howes (1988) first studied the dispersion of unsteady flow in baffled tubes using a numerical code. This work was later further developed by Roberts (1992) into the study of the flow in 2-D baffled channels.

Computational fluid dynamics (CFD) has been used extensively in recent times in order to get a better insight into the flow characteristics in the OBR. Examples of this are the published work by Ni et al (2002) and by Chew et al (2004). In the former, CFD was used to generate a 3-D numerical simulation of the flow in a batch OBR, which were in good agreement with laboratory experiments. In the later study, the flow patterns of both OBR and STR are compared using CFD and the spatial and temporal distribution analysis showed that the OBR is an attractive mixing technology alternative for process intensification. The effect of viscosity on mixing performance in a batch OBR was investigated by Fitch et al (2005) using CFD together with Digital Particle Image Velocimetry (DPIV) in order to provide guidance notes for future industrial applications of this mixing technology when dealing with viscous fluids.

Mathematical models were also developed by Hounslow and Ni (2004) in which the coalescence and breakage of methylmethacrylate droplets were modelled and results compared agreeably with experimental data. Zhang and co-workers similarly developed a mathematical model with the objective of optimizing the heat transfer in the OBR (Zhang et al 2005).

2.1.1.e – Applications

The fundamental research on batch OBRs was complemented with studies on the applicability of this novel mixing technology, namely with regards to chemical reactions, energy efficiency, polymerization, crystallization, fermentation, mass and heat transfer, product engineering and process intensification.

Ni and Mackley (1993) followed a simple chemical reaction in the batch OBR and STR and found that the former was much more energy-efficient alternative. Later Ni et al (2001) compared the flocculation of bentonite and of a bacterial species using the same type of reactors. A similar level of flocculation was obtained in the OBR with much lower strain rate, when compared with a STR.

In suspension polymerization, Ni et al (1999; 2003) reported that by changing both the amplitude and frequency of the oscillation, they were able to control the size and shape of the polymers synthesized. These conclusions were later confirmed for the inverse phase suspension polymerisation of acrylamide (Ni et al 2003).

More recently, Gaidhani et al (2005) used the batch OBR in the production of pullulan (a polysaccharide polymer) and compared the microbial growth rates in the OBR and the STR. The results showed that the growth rate was much faster in the batch OBR due to the enhanced mass transfer rate. These results were later supported by Masngut et al (2006), who investigated solvent fermentation from palm oil mill effluent, cell growth, glucose consumption and solvent production.

Ni and Liao (2008) studied the crystallization of L-glutamic acid and found that the metastable zone width was narrower in the OBR than that in the STR, due to the more uniform mixing and better heat transfer characteristics. Following from this work, the effects of mixing, crystal seeding and baffle material on the crystallization of L-glutamic were examined, and by finely controlling OBR parameters the desired crystal polymorph were obtained (Ni and Liao 2010). The OBR performed well against the STR in a protein refolding operation due to its superior mixing and linear scale-up characteristics (Mackley et al 2001).

2.1.2 - COBR

The expansion of the OBR is the COBR, which follows the same principles but works in a continuous fashion. This section reviews the fundamental research for the COBR, as well as some of its applications.

2.1.2.a – Residence time distribution (RTD)

Dickens et al (1989) examined the RTD profiles of a COBR and near plug-flow characteristics were achieved, with similar magnitudes of axial and radial velocities. Similar results were observed by Mackley and Ni (1991) and by Ni (1994). With the aid of a thin beam of light reflecting from small particles suspended in the fluid, Baird and Rao (1995) were able to observe well defined flow patterns. Particle velocities inside a COBR were measured by Liu et al (1995) and it was shown that narrower RTDs are achievable for particles with a density distribution. Ni and Pereira (2000) confirmed earlier results obtained by Mackley et al (1996), affirming that plug-flow behaviours dominated in the COBR for a range of laminar-flow net Reynolds numbers.

The breakage of oil droplets dispersed in water was studied by Mignard et al (2004) using a high speed camera and the impact of frequency and amplitude of oscillation in a COBR was investigated. Studies regarding mean size distribution of droplets in a COBR were carried out by Pereira and Ni (2001) and it was found that frequency and amplitude of oscillation were more important than the net flow in shaping the mean size distribution of the droplets.

2.1.2.b – Heat and mass transfer

Mackley et al (1990) reported that when a net flow was superimposed with oscillatory motion in a baffled tube a significant increase of the convective heat transfer coefficient was observed. Mackley and Stonestreet (1995) also performed heat transfer experiments where they remarked that under certain oscillation conditions a 30-fold

increase in the Nusselt number, the ratio between convective and conductive heat transfer, was observed under low net flow Reynolds numbers.

2.1.2.c – Scale-up/down

The versatility of this mixing technology was illustrated by Harvey et al (2003) when a scaled-down version of the design was studied and the good mixing and particle suspension characteristics found in bigger versions were retained. These results are particularly interesting for speciality chemicals production and high-throughput screening. Reis et al (2004) confirmed the same oscillatory flow mixing principles of the COBR using a mini screening reactor (total volume = 4.5 ml). In a series of experiments using 3 different sets of baffled tubes (from 1 to 4.5 m long and 24 to 150 mm of internal diameter) working either in batch or continuous, Smith and Mackley (2006) showed that mixing intensity was largely independent of the internal diameter of the tubes, thus making this type of reactor technology easy to scale up linearly, from laboratory scale to pilot-plant and up to full industrial size.

2.1.2.d - Applications

The fundamental research on the COBR created a knowledge base for exploring applications. One of the first examples was in wastewater treatment area. The treatment of organic matter in aqueous solution was achieved using a photo-oxidation reactor, whereby the excellent gas-liquid mixing kept the solid catalyst-coated particles in suspension (Harvey and Stonestreet 2001). In a saponification reaction for the continuous production of sterols using the COBR mixing technology, an eight-fold reduction of reaction time and a 100-fold footprint minimization were achieved when comparing with a full-scale batch reactor, maintaining the same production rates and the quality of the batch operaton (Harvey et al 2001). A significant reduction of residence time was also achieved in biodiesel production, thereby intensifying the existing process (Harvey et al 2003). Using a COBR micro-bioreactor, Reis et al (2006) reported a 50% decrease in residence time in the production of γ-decalactone, when compared to the

STR. This result was accomplished due to the enhanced mass transfer capabilities through the increase of the interfacial area between the two immiscible liquid phases involved in this process.

In the production of a speciality chemical product the existing two STRs were replaced by a COBR at a James Robinson Ltd. plant (Figure 2.4) (Ni 2006). The total process time was reduced drastically from 12 hours to 40 minutes, the reaction yield was increased and the reactor footprint was only 5% of the existing one (1200 m^3 compared to 60 m^3), even though the daily output remained unchanged (Winder 2003).

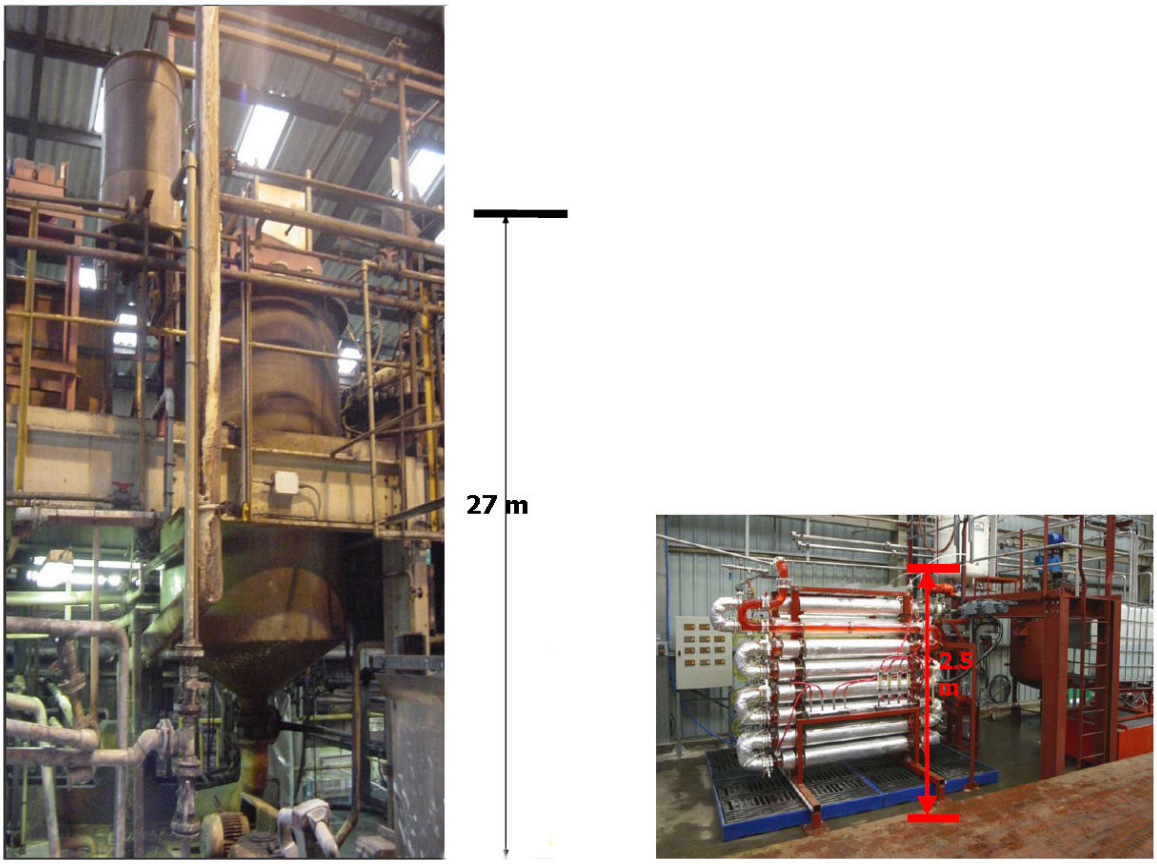


Figure 2.4 The COBR installed in James Robinson Ltd's plant is considerably smaller than the STR it replaced

Genzyme has recently built the largest-patent protected continuous API manufacturing plant in the world, where a COBR is used in one of the synthesis steps to produce hundreds of tonnes of a pharmaceutical compound per year. This reactor design was chosen primarily because of the reduction in reaction time (30-fold reduction) and due to the 99% footprint reduction when the COBR was implemented (2008).

Novartis has recently partnered with the Massachusetts Institute of Technology (MIT) to form the Novartis-MIT Centre for Continuous Manufacturing with the objective of researching new technologies that can transform current batch-based manufacturing processes into fully continuous processes, from start to finish (Trout 2009). Continuous reactor technologies offer a more simple, predictable and controllable solution for pharmaceutical manufacturers together with using less energy and solvents, while minimizing the production of waste (Liu et al 1997; Angelucci 1999; Vervaet and Remon 2005; Crosby 2006). For example, a 10% solvent consumption reduction would result in £400k per annum savings for a AstraZeneca's chemical at a production scale case-study (Lawton et al 2009). These and other factors will continue to play a significant role in the drive to adopt continuous mixing technology.

2.2 – Fouling and cross-contamination

2.2.1 – Fouling and Encrustation

If the residues existent inside the reactor are not cleaned systematically and periodically fouling might occur and encrustation of these particles on the reactor surface can seriously disrupt the normal operation of a manufacturing plant – for instance deposition on the bottom of batch reactors can have an important effect on the mixing patterns and, therefore, the quality of the product; a simple blockage in pipes connecting reactors usually results in the disruption of the production schedule, with a possible negative impact in the plant's cash-flow and reputation. Fouling is defined as the accumulation of solid particles at interfaces, such as reactor walls or baffles. Fouling is a phenomenon which can take place without a temperature gradient in a great many

natural, domestic, and industrial processes, but in the presence of such a gradient, fouling or encrustation might be enhanced (Minton 1986).

Considering the liquid-solid interface in the reactor, fouling can occur as a result of different phenomena. Accordingly, the following classification is based on the key physical or chemical process essential to the particular fouling phenomenon. Six primary categories have been identified (Minton 1986; Mwaba 2003), of which four may have relevance for this project:

1. Precipitation Fouling: crystallization from solution of dissolved substances onto the heat transfer surface as the fluid becomes supersaturated with the fouling material, and is sometimes called encrustation or scaling (e.g. for acetylsalicylic acid or paracetamol, the crystals formed on sub-cooled surfaces might induce the formation of fouling);

2. Sedimentation Fouling: the accumulation of finely divided solids suspended in the bulk fluid onto the reactor's surface. In a minority of instances settling by gravity prevails, and the process may then be referred to as particulate fouling. This type of fouling might occur in the synthesis of methyl diantilis: the solid catalyst spheres might become entrapped in the reactor and accumulate on the walls and baffles, thereby disrupting normal operation;

3. Chemical Reaction Fouling: the deposit formation on the reactor surface by chemical reactions in which the surface material itself is not a reactant. Such might be the case with the synthesis of cyalume;

4. Corrosion Fouling: the accumulation of indigenous corrosion products on the heat transfer surface, which is caused by the reaction between the species in the fluid and the stainless steel, glass or fluoropolymer that form the reactor surface. Corrosion products may act as catalysts for other fouling mechanisms, e.g. as the reactor surface becomes corroded, there is an increase in nucleation sites, promoting undesired sedimentation and crystallization.

The net rate of the formation of the deposits is the generally accepted starting point for all fouling models. Two competing processes are involved in this – formation and removal of the fouling.

The formation of fouling and encrustation involves the following processes acting in sequence:

- processes in the body of the fluid;
- transport to the reactor surface;
- attachment/formation of the deposit.

Removal involves one or more of the following:

- dissolution (material leaves in molecular or ionic form);
- erosion or reentrainment (material leaves in particulate form);
- spalling (material leaves as a large mass).

Whereas spalling, or sloughing, takes place at random times and at random locations inside the reactor, in a normal process dissolution and erosion are taking place continuously across all the deposit's surface.

The fouling characteristics of a fluid in contact with a reactor surface are a function of a variety of parameters:

- properties of the material used in the reactor;
- geometry of the reactor surface;
- temperature difference of the boundary region between the fouling liquid and the reactor surface;
- rheology of the system;
- characteristics of the fouling fluid.

The sequence in which fouling or encrustation occurs for every category of fouling mechanism is the following (Minton 1986; Mersmann 2001; Mwaba 2003):

1) Initiation of the process (delay, nucleation, induction, incubation, surface conditioning);
2) Transport (mass transfer);
3) Attachment (surface integration, sticking, adhesion, bonding);
4) Removal (release, reentrainment, detachment, scouring, erosion, spalling, sloughing);
5) Aging.

1) Initiation - After starting up a process with a clean reactor there is usually a short period before any appreciable fouling can be registered. This is called the initiation period and it is the time when conditions that make the deposition of fouling material onto the surface of the reactor more prone are established. In the case of precipitation fouling, it is closely associated with the nucleation process of product crystals, which increases with increasing degrees of supersaturation. For chemical reaction fouling, the lag period usually decreases as the temperature on the internal surface of the reactor is increased. If the surface roughness of the reactor is increased, there is a tendency for the lag time to be decreased. This can be explained by the fact that there is an increase in the availability of sites for nucleation, adsorption, and chemical surface-activity, while the small indentations at the surface act as a shelter for deposits, which are then protected from the mainstream flow. Surface roughness also increases eddy transport to the wall.

2) Transport - This period of the fouling sequence starts when the components from the bulk of the fluid travel to the surface of the reactor. There are two ways in which this can occur: for sedimentation, precipitation and chemical reaction fouling, the fouling material itself is brought from the fluid to the surface, but for the corrosion fouling mechanism it is not the fouling material that is transported but an ionic species, which contributes to the corrosion reaction in the surface of the reactor (e.g. when an strong acid or base is being used). This in turn results in the formation of reaction products that cause fouling - the transported ionic species acts as a trigger for the deposits' growth. Deposition of fouling substances can either be controlled by diffusion or by adhesion. If the rate of diffusion to the walls of the reactor is

higher than the rate at which they are adhering to the surface, the deposition is adhesion controlled. On the other hand, if the rate of adhesion is much higher than the rate of diffusion then the deposition is said to be diffusion controlled. During this early stage of deposits formation and encrustation there is a tendency for the creation of nuclei on the surface of the reactor or, simply by the action of gravity, the particles have a natural tendency to settle on a surface where the fluid dynamics of the system are not sufficient to get those particle back into suspension. This creates the optimal conditions for the formation a crust on the surface of the reactor. To avoid fouling and encrustation phenomena the local fluid velocity throughout the reactor has to be greater than the minimum velocity at which settling of particles will take place;

3) Attachment - Apart from the sedimentation fouling process, the attachment of the deposit takes place in the reactor's wall region, where the solid species is formed. The adherence of the different particles to a solid surface depends not only on the characteristics of the particles but also on the type and smoothness of the surface. Glass, glass-lined material and polished stainless steel are generally more favoured than regular steel to be used as reactor surfaces. The pipes and ducts should be as smooth as possible and without traps that might capture any type of particle.

4) Removal - The speed at which removal phenomenon takes place is directly proportional to the mass of deposit and the shear stress on the surface of the reactor and the properties of the bulk fluid, and inversely proportional to the absorption force of the fouling deposit when interacting with the walls of the reactor. The characteristics of the deposit influence the particular removal process and it can happen that more than one removal process may occur during the period in which fouling is taking place. Dissolving the deposit is a way of removing it, and must be accompanied by a change in the characteristics of the stream or of the deposit (e.g. changing solvents, the temperature or the pH may influence the solubility of the fouling deposit). Sloughing and spalling of materials might happen due to changes in the characteristics of the deposit or due to changes in stream conditions, but the

essential event for sloughing to occur is a change in the character of the deposit after its formation that weakens its attachment to the reactor surface. Re-entrainment of deposited material by the flowing fluid involves fluid mechanical forces and the mutual interaction between the elemental particles of the deposit. The most severe problem that can happen in this process is when the detachment of crusts takes place: plugging of tubes or pumps may occur, leading to operation shutdowns. Not only is this a concern in terms of lost operation time, but there is also an increase in energy consumption used for the hot water or solvent needed to dissolve the crusts;

5) Aging - The deposit starts to age as soon as it contacts with the reactor surface and may comprise changes in the chemical or crystal structure. These events may, with time, lead to a strengthening of the deposit in the surface but also can trigger the opposite effect – this is visible when a deposit is removed suddenly without a change in the normal operating conditions of the reactor.

Fouling and encrustation or even a simple contamination issue can seriously undermine a chemical or pharmaceutical plant's operation, and on certain occasions might lead to a complete halt of the manufacturing process. Genzyme faced this scenario recently, when one of its plants had to shut down due to a virus contamination. This provoked a shortage of two of Genzyme's most lucrative drugs, which prompted the US Food and Drug Administration (FDA) to consider issuing a $175M fine and to allow patients access to competitors drugs, even though these had not been approved for human consumption at that time. In that period of time Genzyme's stock market value dropped sharply, with investors considering suing the company (Clarke 2009; Pollack 2010). It is then of paramount importance to prevent these situations from arising in order for a chemical or pharmaceutical plant to operate safely.

One of the major concerns in the chemical industry, especially for those dealing with active pharmaceutical ingredients (APIs), is the possibility of having their products

contaminated. As delineated by the International Conference on Harmonisation (ICH) Guidelines (FDA 2003; FDA 2006), an impurity is a component present in the product that is not defined as its chemical entity. In the case of finished products, this definition is extended to include any excipients or the product in itself (Basak et al 2007).

2.2.2 – Cross-contamination

In the case of chemical and pharmaceutical products, there are two types of contaminants: the ones associated with the production process and the ones related to the formulation of the finished product or to its aging. Of particular interest to this work are the impurities that are formed during the manufacture of the chemical and pharmaceutical products. These can be classified as organic contaminants, inorganic contaminants and residual solvents (Roy 2002; FDA 2003; FDA 2006; Basak et al 2007).

Contaminants of organic origin in the product include starting materials, intermediates, by-products and degradation products of the chemical reaction, and catalysts. It is very rare to obtain a 100% yield in any chemical process and although end products are generally isolated with the use of solvents and other purification techniques, there is always the possibility that a residual portion of the aforementioned types of impurities can still be found in the product – in fact, these are the most common sources of impurities and manufacturers have to take extra care to design processes that avoid them, or at least diminish their occurrence to acceptable levels. It's interesting to note that in the case of some pharmaceutical products, enantiomeric forms of the product other than the one considered to be the most effective can be classed as an organic impurity, and as such have to be removed from the final product.

Contaminants classed as inorganic include reagents, ligands and catalysts, heavy metals and other substances (such as activated carbon or filter aids). Contamination of the product by heavy metals can occur when non-demineralized water is used in stainless steel where an acidification or acid hydrolysis is taking place. The use of USP water and glass-lined reactor is essential to prevent contaminations of inorganic origin.

The very nature of solvents and its widespread use in chemical processes means that it's very difficult to completely remove residues of this kind. However, due to their toxicity factor, the presence of solvent residues in the finished products must be avoided (class 1 – known or suspected human carcinogens, environmental hazards) or reduced to acceptable levels (classes 2 and 3 – non geno-toxic animal carginogens and solvents with low toxic potential hazard, respectively), as described in the ICH guideline Q3 (Roy 2002; Basak et al 2007).

The contaminations may also result from a series of diverse factors - e.g. introduction of the wrong ingredients; introduction of out-of-date, damaged or contaminated ingredients; microbial growth inside the piping or vessel and, of particular interest to this project, contamination from a previous batch if the vessel or piping was badly cleaned. It is of utmost importance to control and reduce the occurrence of these events, both through the intelligent design of the chemical reactor – avoiding the use of pipes of different diameter and "dead-volume" areas where contaminants can settle – and through the use of a cleaning procedure that guarantees a degree of cleaning of the reactor that is up to the stringent standards set by regulatory organizations (Gambrill 1995; Argentine et al 2007; Basak et al 2007).

2.3 – Fouling Prevention and Cleaning

Until fairly recently the typical industrial approach to cleaning was empirical and not based on concrete and studied measures to tackle this issue (Perka et al 1993). However, with the increased stringency concerning the purity of produced APIs and other fine chemicals enforced by regulatory agencies around the world, there is a current drive towards better cleaning standards and practices. Another factor influencing chemical companies to take actions to reduce waste and energy use is public opinion. In an age when global warming and climate change are receiving so much media attention, companies are putting an extra effort to be perceived as environmentally-friendly, therefore raising their profile; even in a sector traditionally regarded as being very polluting such as the pharmaceutical sector, there are good examples of this trend, with

companies such as Bayer and Bristol-Myers Squibb leading the way in the efforts to reduce toxic emissions and generated waste (Marwaha 2007).

Prevention of fouling and waste generation can be done by monitoring the operation conditions and synthetic route more closely, in order to maximize the efficiency of the process. For example, in cooling crystallization fouling and encrustation problems can be greatly reduced by minimizing the temperature difference between the jackets and the solution in the reactor. In theory, encrustation can be eliminated if this temperature differential is inferior to the width of the metastable zone, because larger temperature differences can increase the possibility of spontaneous crystal formation to occur. Using a reactor in which the surface-to-volume ratio is small increases the heat transfer efficiency and reduces this risk (Mullin 2007; Wieckhusen 2008; Tung, Paul et al 2009).

Nonetheless, in large-scale conventional reactors it's almost impossible to completely eliminate these factors and therefore process down-time is required, lengthening turnaround times and reducing yields, thereby increasing operational costs (Chew et al 2006). In order to proceed to the cleaning stage between different batches, conventional mixers normally have to be disassembled and often require off-line time-consuming cleaning of parts to prevent contamination. That might also be another source of contamination, since typically these cleaning procedures are done while the vessel is in contact with the outside atmosphere (Gambrill 1995). Although the cleaning of vessels has always been a potential source of contamination and waste, it is necessary to put in place a more cost effective solution to reduce waste generation and cleaning times. While the cleaning of STRs is seen as time-consuming, the cleaning of plug-flow reactors is perceived to be a costly procedure (Coker 2001).

2.3.1 – The Cleaning Continuum

Given the variety of facilities, manufacturing processes and final products in the chemical and pharmaceutical industry and in the absence of a universal approach to cleaning, a cleaning continuum has been established. This concept can be defined as an "organizational model that helps to draft the operational details of a specific cleaning validation programme" (Potdar 2007). By coupling several opposed extremes that represent current industry cleaning practices, the cleaning continuum serves as a guide in order to establish the most appropriate cleaning validation protocol (Table 2.1).

Table 2.1 The cleaning continuum

(Note: The extremes which are more attuned with this project are in bold)

Manual	.....	**Automated Cleaning**
Clean-out-of-Place (COP)	.....	**Clean-in-Place (CIP)**
Dedicated Equipment	.....	**Non-Dedicated Equipment**
Product Contact Surfaces	.....	Non-Product Contact Surfaces
Minor Equipment	.....	**Major Equipment**
Low Risk Drugs	.....	**High Risk Drugs**
Highly Characterized	.....	Poorly Characterized
Sterile	.....	**Non-Sterile**
Soluble	.....	Insoluble
Campaigned Production	.....	Non-Campaigned Production

An advantage of using automated cleaning is that it is easier to reproduce and will yield more reproducible results. This type of operation suits continuous reactors. The validation of the automated procedure is done using sampling and analytical

techniques that have been extensively used in the industry with proven results (Madsen 1998).

The cleaning of continuous reactors can be done using a single-path cleaning-in-place (CIP) procedure that does not require cleaning to be done in contact with the outside environment, which reduces process downtime, waste, risk of cross-contamination and brings operational costs down. Moreover, the cleaning conditions (e.g. temperature, pH, high detergent concentration) used while performing CIP can be more extreme due to the fact that there is no direct operator contact with the cleaning solution (Perka et al 1993; Gambrill 1995; Bismuth and Neumann 2000; Chew et al 2006).

Chemical reactors are often not used as dedicated equipment, in the sense that they may be used to perform different tasks, according to the plant's requirements. Cross-contamination is a possible occurrence if the cleaning procedure is not well designed or performed and so particular attention is put in the detection of residues, in both the cleaning and production phases.

The cleaning procedure can be designed towards detecting residues and contaminant species inside the reactor when using closed continuous reactors, in which reactants, products and possible by-products of the reactions to be performed will be in direct contact with its walls. When considering a manufacturing process, the chemical reactor plays a central role and as such it is considered as a "major equipment". Therefore, materials of construction have to be carefully selected to ensure excellent chemical and temperature resistance. Due to their non-reactive characteristics and smooth surfaces (low friction coefficients make the deposition of residues more difficult to occur) materials such as glass, stainless steel or fluoropolymers are usually chosen when considering the design of a reactor to be used in the manufacture of fine chemicals and APIs (Madsen 1998).

In order to pass regulatory norms and get their products on the market, manufacturing companies have to be able to characterise extensively the chemical entities that are included in the process. These pose various degrees of health risks and knowing the safe limits of exposure constitutes an added layer of protection for both the

operators of the reactor and final users of the products being made. This is why it is important to determine appropriate cleaning acceptance criteria, based both on the allergenicity/toxicity/mutagenicity/potency of the chemical entities and on the characteristics of the reactors being used in the specific manufacturing processes (Madsen 1998; LeBlanc 2000).

Sterile facilities have to ensure that while in operation no microbial life or endotoxin will contaminate the system. Generally, the reaction conditions present in a typical fine chemical or API production campaign are considerably harsh either because of the high temperatures or strong acids or bases are used. As such, the possibility of contamination of the reactor by the aforementioned agents is not a very likely event to happen. On the other hand, the residues that might indeed remain in the reactor before the cleaning stages start are normally expected to be soluble and will be easier to rinse out, making cleaning a more simple process.

The planned experimental work to be undertaken in the COBR for this project includes week-long production campaigns of three different chemical entities. Apart from testing the capability of the COBR as a suitable alternative chemical reactor from the production point of view, the cleaning procedure to be put in place must be adequate, verifiable and reproducible (Scarso 1996; Rios 2007).

2.4 – From quality-by-analysis to quality-by-design

The chemical and pharmaceutical industries are traditionally batch-oriented. Processes devised and registered with regulatory agencies decades ago are still in use and are not easy to be changed. Due to factors such as the existence of patents or due the simple fact that processes in place “work” and generate a constant stream of profit there is no urgency in making these same manufacturing processes more efficient and making the shift from batch to continuous unless this change is driven by the ever more stringent quality criteria being put in place or by the fierce competition existent in the chemical and pharmaceutical industries (Malhotra 2009).

Quality concerns and regulatory bodies dictate that a plant that operates in a batch fashion must test each product batch and check if it conforms to all the quality parameters. In a world where quality parameters are increasingly more stringent this "quality-by-analysis" approach might serve as a safety blanket, but it also means that expensive testing equipment must be used (Malhotra 2008). The testing procedures frequently take a long time to be performed and all the while the whole batch cannot be moved out of the plant, adding further financial strain because adequately dimensioned facilities have to be built in order to store the plant's production. Furthermore, if a batch that has been stored - while samples are being tested - does not meet the required quality criteria, all of it has to be discarded or reprocessed. The treatment of waste APIs that are not good enough to be marketed is in itself an expensive process and regarded as anti-ecological.

Together with compliance and supply chain issues, a different approach to product quality has also been driven by the expiration of patents and the dwindling number of new molecular entities that are approved to market each year by regulatory agencies. Chemical, and in particular, pharmaceutical companies are being forced to find ways in which to optimize manufacturing processes in order to remain competitive (Malhotra 2008; Cook, Venkateshwaran et al 2009). The "quality-by-design" (QbD) approach, whereby entire processes are designed from the beginning with a profound knowledge of the chemistry and the variables involved, is associated with continuous manufacturing.

Designing product quality into a continuous process instead of addressing quality at the end of the production stage ensures that the chemical or API being manufactured is of the highest and most consistent quality. Process Analytical Technologies (PAT) is an essential feature of QbD and includes all analysis (chemical, physical, microbiological, etc) done in-process, in a timely manner. According to the FDA, PAT is a "system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. The goal of PAT is to enhance understanding and control the manufacturing process, which is consistent with our current drug quality system: *quality cannot be tested into products; it should be built-in or should* ***be*** *by design*." (CDER 2004).

In addition to the above-cited guideline, other published material shows how important the drive for the adoption of continuous manufacturing has been in recent times. This is the subject of the following section.

2.5 - Regulatory Bodies and Good Manufacturing Practices

In order to sell their products, pharmaceutical companies must be aware of current regulations of the markets where they want to operate. For example, if the product is to be marketed in the USA, it has to be compliant with the requirements set by the FDA; similarly, if it wishes for its product to be marketed in Japan, it has to comply with the Japanese Ministry of Health, Labour and Welfare (MHLW) regulations. Global manufacturers have also to operate in compliance to rules and guidance set out by the International Society of Pharmaceutical Engineers (ISPE) and the World Health Organization (WHO).

Because of the increasing globalization effects, there has been an effort in recent years towards harmonization of these rules in international markets. Following the birth of the International Conference on Harmonization (ICH) in April 1990 there has been a continuous effort to bring "together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration"[1]. For example, the "Rules Governing Medicinal Products in the European Union", Volume 4, published by the European Commission concerning Good Manufacturing Practice (GMP) is in accordance with "FDA Good Manufacturing Practices for Finished Pharmaceuticals"; additionally, these two sets of rules are consistent with ISPE good automated manufacturing practice. Japan's pharmaceutical affairs law also had to undergo major changes in order to make it comparable to the Western world rules and regulations on GMP (Bonanomi 2006).

[1] *In* www.ich.org

In the European Union and the European Economic Area[2] a framework of laws and regulations is in place in order to ensure the good functioning of the chemical products market. Since 1965, when the Council Directive 65/65/EEC on "the approximation of provisions laid down by law, regulation or administrative action relating to medicinal products" was put in place, there has been a continuous effort to regulate the market, in the form of several other directives and amendments to directives.

Nowadays, as a result of Title IV of Directive 2001/83/EC (last amended in 2004), Governments of Member States have to ensure that pharmaceutical manufacturers are authorized to operate, in accordance, among other requirements, to GMP. The guidelines and principles of GMP for medicinal products for human use are described in Directive 2003/94/EC and the European Community Guide to GMP includes elements from ISO 9001:2000 with additional requirements specific to medicines; in the UK, these regulations and other are described in the Rules and Guidance for Pharmaceutical Manufacturers and Distributors, compiled by the Inspection and Standards Division of the Medicines and Healthcare products Regulatory Agency (MHRA), and is also commonly known as the "Orange Book" (MHRA 2007).

In order to be authorized to produce and market chemicals, a company must comply with the country's regulatory body's requirements in order to reduce the risk to the client due to inadequate safety, quality or lack of efficacy. In particular to the case of medicinal products and APIs, the manufacturer has to have in place "a comprehensively designed and correctly implemented system of Quality Assurance incorporating Good Manufacturing Practice and thus Quality Control"[3].

With particular relevance to this work there is the definition and scope of GMP, the requirements on premises and equipment[4], documentation[5], production (with special attention to the "Prevention of Cross-contamination in Production" sub-section)[6], the regulations on buildings and facilities (namely those pertaining to the design and

[2] This includes the EU countries plus Iceland, Liechtenstein and Norway
[3] *in* Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2007, p. 43, London, Pharmaceutical Press.
[4] Described in Chapter II, Part I, Section 3 of the "Orange Book"
[5] Described in Chapter II, Part I, Section 4 of the "Orange Book"
[6] Described in Chapter II, Part I, Section 5 of the "Orange Book"

construction sub-section)[7], process equipment[8], guidelines for documentation and records[9] and for the production and process controls (with particular attention to the contamination control subsection)[10].

As defined in the "Orange Book", GMP is "that part of Quality Assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the Marketing Authorization or product specification.

GMP is concerned with both production and quality control. The basic requirements of GMP are that:

(i) all manufacturing processes are clearly defined, systematically reviewed in light of experience and shown to be capable of consistently manufacturing medicinal products of the required quality and complying with their specifications;

(ii) critical steps of manufacturing processes and significant changes to the process are validated;

(iii) all necessary facilities for GMP are provided including:

a. appropriately qualified and trained personnel;
b. adequate premises and space;
c. suitable equipment and services;
d. correct materials, containers and labels;
e. approved procedures and instructions;
f. suitable storage and transport;

(iv) instructions and procedures are written in an instructional form in clear and unambiguous language, specifically applicable to the facilities provided;

(v) operators are trained to carry out procedures correctly;

(vi) records are made, manually and/or by recording instruments, during manufacture which demonstrate that all the steps required by the

[7] Described in Chapter II, Part II, Section 4 of the "Orange Book"
[8] Described in Chapter II, Part II, Section 5 of the "Orange Book"
[9] Described in Chapter II, Part I, Section 6 of the "Orange Book"
[10] Described in Chapter II, Part I, Section 8 of the "Orange Book"

defined procedures and instructions were in fact taken and that the quantity and quality of the product was as expected. Any significant deviations are fully recorded and investigated;

(vii) records of manufacture including distribution which enable the complete history of a batch to be traced, are retained in a comprehensible and accessible form."

The relevant regulations for this work and the adequate measures taken are described in Table A.1 in the Appendix A.

In addition to this, regulatory bodies have recently published guidelines with the purpose of making manufacturers fully understand and optimize the way their pharmaceutical products are developed, evaluated and manufactured, such as the Critical Path Initiative (CPI) issued by the FDA or the Q8 guideline published by the ICH guideline which focuses on QbD. These regulatory efforts present the perfect opportunity for continuous process technology to be embraced and to be successfully implemented (ICH 2006; FDA 2009).

2.6 - Acceptable Limits of Cross-Contamination

In order to have a sound cleaning procedure, there is the need to set limits with regard to contamination, most critically in APIs and their finished products. While the FDA merely affirms that residue limits must be logical, practical, achievable, and verifiable in its publication "Guide to Inspections of Validation of Cleaning Processes" (1993), the same regulatory agency, in light of the ICH, has published a set of limits for impurities in drug substances and drug products (FDA 2003; FDA 2006) (Tables 2.3 and 2.4).

Table 2.2 Applicable thresholds for impurities in new drug substances. Notes: 1- Higher reporting thresholds should be scientifically justified; 2 - Lower thresholds can be appropriate if the impurity is unusually toxic. *Source: FDA, USA.*

Maximum Daily Dose (g)	Reporting Threshold (1,2)	Identification Threshold (2)	Qualification Threshold (2)
≤2	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2	0.03%	0.05%	0.05%

Table 2.3 Applicable thresholds for degradation products in new drug products**.** Notes: 1- Higher reporting thresholds should be scientifically justified; 2 - Lower thresholds can be appropriate if the impurity is unusually toxic; 3 – whichever is lower; TDI – Total Daily Intake. *Source: FDA, USA.*

Maximum Daily Dose	Reporting Threshold (1,2)	Identification Threshold (2)	Qualification Threshold (2)
≤ 1 g	0.1%		
> 1 g	0.05%		
< 1 mg		1.0% or 5 µg TDI (3)	
1 mg - 10 mg		0.5% or 20 µg TDI (3)	
>10 mg - 2 g		0.2% or 2 mg TDI (3)	
> 2 g		0.10%	
< 10 mg			1.0% or 50 µg TDI (3)
10 mg - 100 mg			0.5% or 200 µg TDI (3)
>100 mg - 2 g			0.2% or 3 mg TDI (3)
> 2 g			0.15%

The regulatory bodies do not set limits of acceptable contamination for specific manufacturing processes. That is the responsibility of the manufacturer, and the limit set must be sufficient to guarantee that the bulk API or finished product conform to the quality requirements. It is, therefore, of utmost importance to clearly define residue limits both in APIs, finished products and also in the manufacturing equipment used due to the possible effects those can have in public health (LeBlanc 2000; Basak et al 2007).

A particularly relevant study about acceptable contamination levels was carried out by LeBlanc (2000) and limits are defined in the following way:

Considering BL_1 as the limit of the target residue (in this case, the target residue is the bulk active API_A) in any finished drug product in which the residue may ultimately be found, it is calculated as:

$$\mathrm{BL}_1 = \left(\frac{\text{minimum daily dose of } \mathrm{API}_\mathrm{A}}{\text{minimum daily dose of } \mathrm{Prod}_\mathrm{B}} \right) \times 1000000 \times \mathrm{SF} \qquad (2.3)$$

API_A – Bulk active A.

$Prod_B$ – Finished product B

SF – Safety Factor (= 0.001)

Taking BL_2 as the limit of the residue in the API used in the manufacture of the finished drug:

$$\mathrm{BL}_2 = \mathrm{BL}_1 \times \left(\frac{100}{\%\ \mathrm{API}_\mathrm{B} \text{ in } \mathrm{Prod}_\mathrm{B}} \right) \qquad (2.4)$$

Cross-contamination levels can then be set and safety limits of operation can be established when the production of different chemicals are involved, as in this project, ensuring that the manufactured chemical and pharmaceutical entities respect the quality guidelines currently in place.

2.7 – Summary

The relevant background information covering OBR and COBR, fouling and cross-contamination, cleaning strategies, regulatory guidelines and acceptable limits of cross-contamination is reviewed in this chapter. While it is evident that extensive work has been carried out in order to understand the characteristics and possible applications

for the OBR, the work herein described is the first to investigate the robustness and adaptability of the COBR to different synthesis of chemical and pharmaceutical entities in tandem. It is also the first to establish a reliable, fast and cost-efficient cleaning procedure that satisfies the current regulatory guidelines in a GLP/GMP environment.

Chapter 3

EXPERIMENTAL APPARATUS

An experiment is a question which science poses to Nature,
and a measurement is the recording of Nature's answer.

Max Planck, physicist

This chapter is dedicated to the description of the equipment used in the experiments carried out in the OBR and COBR, as well as in the analytical assessment. There are three main sections in this chapter: the first addresses the OBR experimental setup, the second focuses on the planning, design and setup of the continuous work and the last section describes the analytical methods used to assess the quality of the work executed.

3.1 – The Setup of OBR

A description of the OBR setup used for batch studies of the various chemistries is presented in this section.

The OBR was 25 mm in diameter and had a total working volume of 60 mL. Figure 3.1 shows the OBR with the baffle set, when dismounted from the supporting frame. Figure 3.2 represents the scheme of the used OBR.

Figure 3.1 Photo of the batch OBR

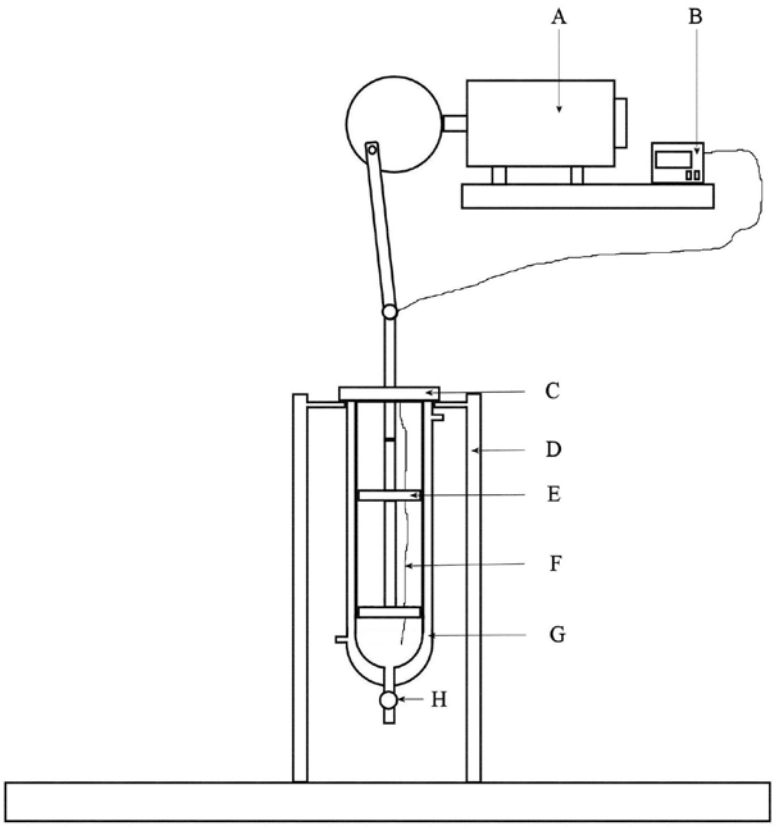


Figure 3.2 Scheme of the OBR

A – Sew Eurodrive motor; B – Temperature reading unit; C – PTFE lid; D – Supporting frame; E – Baffle set; F – Thermocouple; G – Glass-jacketed OBR; H – Discharge valve

The reactor was made of jacketed glass in order to perform temperature-controlled experiments and has a burette-style valve at the bottom for sampling. The used baffle set was made of stainless steel plates; the baffles had a thickness of 3 mm and the internal diameter was 13.5 mm. The distance between the baffles is 33.5 mm. The oscillation was provided by a SEW Eurodrive motor and was controlled by a SEW Eurodrive frequency inverter. The hot water that circulated inside the OBR jacket during the experiments was provided by a Grant W28 hot water bath and a K-type thermocouple (limit of error ±0.55 ^{o}C) fitted through the OBR top was used for temperature control.

3.2 – The Setup of COBR

A COBR was purposefully designed and built to specifications in order to carry out the selected synthesis of the fine chemical and active pharmaceutical ingredients. The reactor has an internal diameter of 15 mm and a total working volume of circa two litres. Figure 3.3 is a photo of the reactor, which is supported by an aluminium frame. The COBR was made of jacketed glass with PFA (perfluoroalkoxy) baffles and composed of 15 glass sections, each 700 mm long. Each baffle was 22.7 mm apart from the others and 3mm thick with an internal diameter of 7.5 mm. PFA was the baffle material of choice because it belongs to a class of compounds known to have the lowest surface energy of all solid materials and a fully fluorinated, micro-void free surface, which makes this a non-stick material (McKeen 2006). Those characteristics coupled with the fact that PFA is virtually chemically inert and the possibility of using it at temperatures up to 260 ^{o}C (Fleming et al 2001) make it ideal to be used in the type of chemical reactions to be undertaken in the COBR, where strong acids and high temperature are going to be used. The use of any other common and cheaper materials for the construction of the reactor would carry the risk of chemical or heat degradation. Product deposition and encrustation is also a phenomenon that is reduced by using PFA.

Figure 3.3 Photo of the COBR

The following scheme (Figure 3.4) details the COBR setup, with the relative location of the valves, ports, thermocouples and pressure gauges. The assembly of the COBR was carried out by NiTech Solutions Ltd.

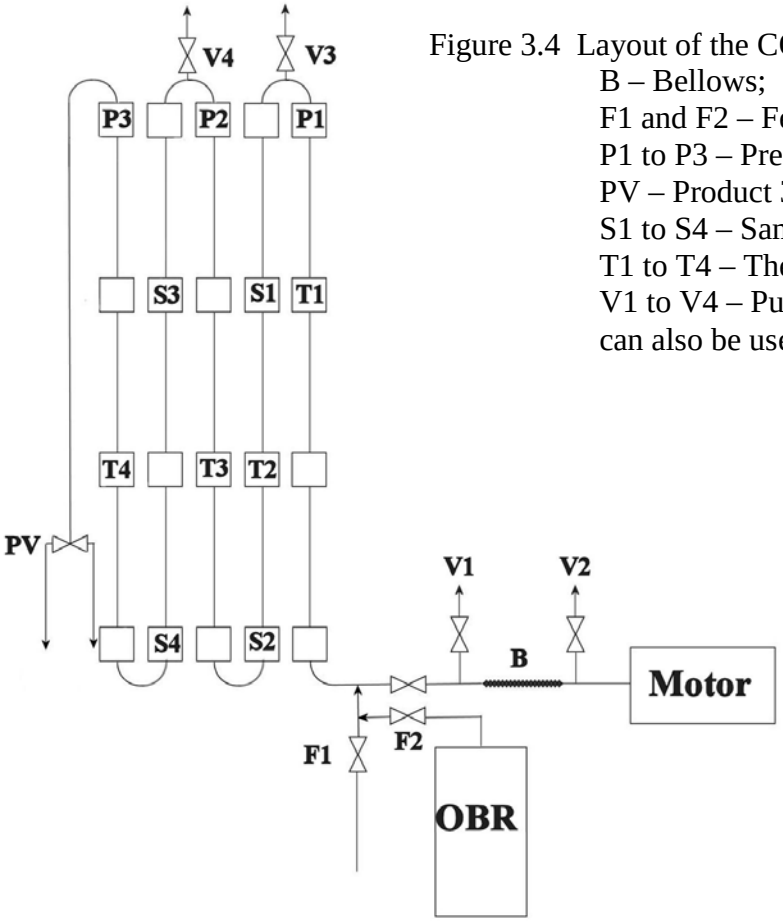


Figure 3.4 Layout of the COBR:
B – Bellows;
F1 and F2 – Feed valves;
P1 to P3 – Pressure gauges;
PV – Product 3-way valve;
S1 to S4 – Sampling valves;
T1 to T4 – Thermocouples;
V1 to V4 – Purge valves (V3 and V4 can also be used as sampling valves).

Oscillation was provided by the Copley Controls Corp. XTA38 servotube actuator connected to the PTFE bellows, which provide the linkage to the COBR (Figure 3.5). The actuator is controlled by a purpose built control unit, which allows a fine control over frequency and amplitude.

Figure 3.5 The Copley Controls XTA38 actuator.
Source: Copley Controls – Division Analogic Corporation

The COBR was equipped with two entry ports and two discharging ports. The latter ports were controlled via a 3-way valve. The sampling of the reaction mixture inside the reactor was done via four sample ports located along the reactor. Located at each top bend there are the purge valves which can also function as sample ports. In order to control the operational conditions four K-class thermocouples (limit of error ±0.55 ^{o}C) and three pressure gauges (0-4 bar) were fitted at different locations along the COBR shown as T_1-T_4 and P_1-P_3 in Figure 3.4. All the 36.5 mm long sleeves that connect the glass tubes, valves, taps and pressure gauges were made of stainless steel 316 with BSP threads.

The product hoses were made of temperature and chemical resistant non-corrugated FEP (Flourinated Ethylene Propylene) and have an internal diameter of 14.7 mm. The entry ports are connected to chemically resistant Chem-Durance® peristaltic pump ready tubes, with an internal diameter of 7.9 mm.

Two Watson-Marlow 520S pumps (Figure 3.6) were employed to pump the starting materials and solvents into the COBR, ensuring that the range of operational flowrates used are covered. One of the COBR's entry ports was connected to a purpose-built feeder DN80 glass-jacketed OBR, which is supported by an aluminium frame located inside the fume hood.

Figure 3.6 The Watson-Marlow 520S peristaltic pump, used to pump starting materials to the COBR. *Source: Wtson-Marlow Bredel Pumps – Spirax-Sarco Engineering Group*

The OBR feeder had a total volume capacity of 1000 ml and can be sealed with a PTFE lid, as a first barrier for developing fumes. It was equipped with 3 entry ports (internal diameters of 22.2 mm, 17.2 mm and 12.7 mm, respectively) and a PTFE tap-controlled outlet port. The baffle set was made of PTFE connected by stainless steel rods, with an interval of 113 mm. The baffles had an internal diameter of 34.7 mm and are 3.4 mm thick. These materials were chosen due to their high chemical and heat resistance, and were found adequate for the chemistry being studied. The motor that

drives the baffle set was a Parvalux RS718-010 - a powerful and small-footprint unit - and was controlled by a Eurotherm 650 frequency inverter. Figure 3.7 shows the assembled OBR in the supporting frame. This setup was placed inside a fully ventilated fume hood for safe operation.

Figure 3.7 The batch OBR feeder

The hot water was provided to the COBR and the OBR feeder via two water baths: Grant W28 and Grant Y28, both with 28 litres of capacity and a maximum working temperature of 99 ^{o}C (Figure 3.8).

Figure 3.8 Grant W28 water bath

3.3 – Materials

This section describes the materials used for each of the experiments carried out in this project.

3.3.1 - Bis(2,4,6-trichlorophenyl) oxalate

All the reactants used for this experiment were supplied by Sigma-Aldrich (St. Louis, USA). The 2,4,6-trichlorophenol 98% (code T55301-50G) used was is powder form and had a light brown appearance. Triethylamine >99% (code T0886-100ML) was a colourless liquid with an amine-like odour. Oxalyl chloride >99% (code 221015-25G) was a pungent, clear liquid. The solvent used, anhydrous toluene 99.8% (code 244511-1L), was a colourless liquid. Anhydrous hexane 95% (code 296090-1L), used for the vacuum filtration, was a clear liquid.

3.3.2 - Methyl diantilis

Methanol and amberlyst-15 were sourced from Sigma-Aldrich (St. Louis, USA). 3-ethoxy-4-hydroxybenzyl alcohol was acquired from Fluorochem (Glossop, UK). Acting both as reaction medium and reactant, anhydrous methanol 99.8% (code 322415-1L) was a colourless liquid. The catalyst used, amberlyst-15 (code 216380-25G), was in the form of light yellow spherical pellets that had to be treated with methanol before being used in the experiment. 3-ethoxy-4-hydroxybenzyl alcohol (code 018670) had a white powder appearance.

3.3.3 – Vanisal sodium

Vanillin and sodium hydrogen sulphite was purchased from Sigma-Aldrich (St. Louis, USA). The purified water used was sourced from Heriot-Watt University (Edinburgh, UK). Vanillin 99% (code V1104-100G) had a very light yellow powder

appearance and sodium hydrogen sulphite 40% solution (code 13438-1L-R) was a clear liquid.

3.3.4 – Acetylsalicylic acid

The salicylic acid, acetic anhydride and sulphuric acid used for this experiment were acquired from Sigma-Aldrich (St. Louis, USA). The purified water, used to decompose excess acetic anhydride and in the vacuum filtration, was sourced from Heriot-Watt University (Edinburgh, UK). Salicylic acid >99% (code 247588-500G) had a white crystalline appearance. The acetic anhydride >99% (code 320102-1L) used was a pungent, colourless liquid. Sulphuric acid 95-98% (code 320501-1L) was a clear, thick liquid.

3.3.5 – Paracetamol

The p-aminophenol and acetic anhydride used were acquired from Sigma-Aldrich (St, Louis, USA). The purified water, used as a solvent and also in the vacuum filtration, was sourced from Heriot-Watt University (Edinburgh, UK). The p-aminophenol 98% (code A71328-1KG) used had a light brown powder appearance. The acetic anhydride used had the same origin as the one utilized for the acetylsalicylic acid experiments.

3.4 – Analytical Equipment

The following section describes the analytical instrumentation that was used to analyze the samples taken from the experiments. The equipment covers a wide range of analytical techniques: Infrared Spectroscopy, Nuclear Magnetic Resonance

Spectroscopy, High Performance Liquid Chromatograpy, Scanning Electron Microscopy, X-Ray Diffractometry and Particle Size Distribution.

3.4.1 – Infrared Spectroscopy

Infrared spectroscopy (IR) was used to analyze the Bis(2,4,6-trichlorophenyl) oxalate and methyl diantilis produced. The equipment used at Heriot-Watt University was a Perkin Elmer Spectrum FT-IR System (Figure 3.9). For the cyalume analysis the KBr pellet technique was used, whereas to analyse the fragrance tetrafluorocarbon-washed glass disks were used.

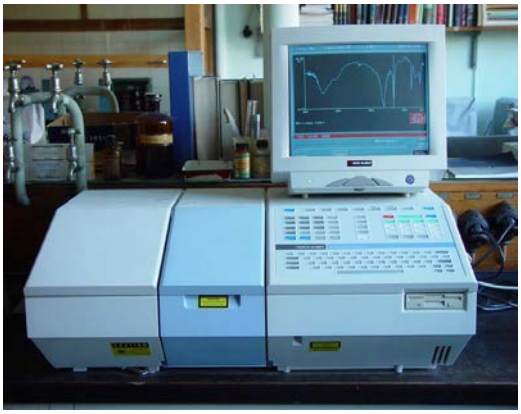


Figure 2.9 The Perkin Elmer Spectrum FT-IR system used (Organic Chemistry laboratory, Heriot-Watt University)

3.4.2 – Nuclear Magnetic Resonance Spectroscopy

The produced vanisal sodium was analyzed using Nuclear Magnetic Resonance (NMR) spectroscopy. The equipment used at Heriot-Watt University was a Bruker DPX400 NMR spectrometer (^{13}C at 100 MHz) equipped with pulsed field gradients, as illustrated in Figure 3.10.

Figure 3.10 Bruker DPX400 NMR spectrometer

3.4.3 – High Performance Liquid Chromatography

The High Performance Liquid Chromatography (HPLC) equipment used at NiTech Solutions Ltd was a Varian Prostar 230 (Figure 3.11) and the method used for analysing both acetylsalicylic acid, paracetamol and vanisal sodium was an adaptation of the method developed by Franeta et al (2002): the chromatography column was a reverse-phase YMC ODS-AQ (250 x 4.6 mm; 5 μm packing), the UV detector was set at 254 nm and the mobile phase was composed by acetonitrile (A) and water with phosphoric acid at pH 2.6 (B). In each run the chromatography program was started with 10% v/v of A and 90% v/v of B, with a flow rate of 1 mL min^{-1}. The mobile phase composition was then gradually transformed to 100% of A over a period of 20 minutes and the program was stopped at 25 minutes.

Figure 3.11 Varian Prostar 230 used for HPLC analysis

3.4.4 – Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used to observe in detail samples of the acetylsalicylic acid and paracetamol crystals produced. These were imaged using a Hitachi S-2700 Microscope (Figure 3.12), available at the Chemistry Department in Heriot-Watt University. The crystals were gold coated and then observed at a magnification of 30× and 200×, using 10 kV accelerating voltage.

Figure 3.12 Hitachi S-2700 Microscope used for SEM analysis

3.4.5 – X-Ray Diffractometry

X-Ray Diffractometry (XRD) was carried out to determine the diffraction patterns of the acetylsalicylic acid and paracetamol produced. A Bruker D8 Discover transmission X-Ray diffractometer with a Cu-Kα target, pictured in Figure 3.13, was used. The tube voltage and current was 40 kV and 40 mA, respectively and the θ angle increments of two degrees. The gathered data was obtained using the wide angle x-ray scattering (WAXS) technique and the EVA data handling package was used to process the results. It is available in the Chemistry Department at Heriot-Watt University.

Figure 3.13 Bruker D8 Discover transmission X-ray diffractometer used for XRD analysis

3.4.6 – Particle Size Distribution

A Malvern Mastersizer S equipped with a liquid sample holder, available at the Institute of Petroleum Engineering in Heriot-Watt University (Figure 3.14), was used for particle size distribution studies of the paracetamol crystals produced. After testing

several liquid media, Multipar H oil was found to be the best in order to suspend the studied particles.

Figure 3.14 Malvern Mastersizer S used for particle size distribution studies

Chapter 4
CHEMISTRY SCREENING

Just as houses are made of stones, so is science made of facts;
but a pile of stones is not a house and a collection of facts is not necessarily science.

Henri Pointcaré, mathematician, philosopher of science

The selection process for the chemical entities to be studied in a batch OBR and, subsequently, in continuous fashion using a COBR began by screening different chemistries. A series of criteria were established in order to narrow down the potential candidates to a maximum of three. These were:

1. Type of chemistry

   Considering that the OBR/COBR mixing technology is suitable for a wide range of chemistry, pharmaceutical and food processes (see Chapter 2), this work is focused on the fine chemicals and pharmaceuticals.

2. Industrial relevance

   This PhD project is sponsored by EPSRC and NiTech Solutions Ltd. via an EPSRC industrial CASE studentship. The chemistries that are relevant to NiTech Solutions Ltd. are industrially driven, with a particular emphasis on the pharmaceutical market.

3. Cost

   The cost of carrying out the experiments was one of the major factors that shape the scope of the project. With a final objective of carrying out a week-long production run for each of the three selected chemical reactions, the overall costs

for the equipment (e.g. COBR), consumables (e.g. solvents and reactants) and analytical tests must be within the limited budget.

4. Reaction time

   Bearing in mind the budget of the project, the reaction time should ideally be less than one hour in literature, ensuring that the reactor will not be too long and thus expensive;

4.1 – Chemistries excluded

With the objective of proving that the OBR/COBR design can be a viable alternative to the stirred tank reactor in the manufacture of fine chemicals and pharmaceuticals both in terms of efficiency of the process and quality of the products obtained, several different chemistries were initially considered for this project. Four of them were excluded from experimental studies due to factors such as cost of starting materials, cost of having to use more equipment associated with the synthetic or isolation steps or simply because the experimental workup required time-consuming steps (eg. sample treatment for analysis) that prevented a single operator to carry out the experiments in a focused and safe manner. These chemistries, described in the following sections, were warfarin sodium, nitisinone, tesmilifene and acamprosate calcium.

4.1.1 – Warfarin sodium

Warfarin sodium is a well-know and established anticoagulant drug and powerful rodenticide (Ashkar et al 2004). The name is derived from the initials of the Wisconsin Alumni Research Foundation, the organism that provided funding for initial research into this chemical entity to Karl Link, a professor at the University of Wisconsin (Link 1959).

Link's work on warfarin was first motivated by a series of unexplained cattle deaths in the 1920's. These deaths were later associated with spoiled hay made from sweet clover that caused cattle death by severe haemorrhaging. Link and his team were eventually successful in isolating the anticoagulant agent and finally proved that synthetic dicoumarol was identical to the naturally occurring chemical entity, in 1940. (Stahmann 1941) Work on several similar chemicals wielded more potent anti-coagulants, and one of those new chemical entities, warfarin, was patented by Karl Link and first marketed as a rodenticide. Further studies investigated its properties as a therapeutic anti-coagulant and warfarin started being prescribed for heart attack sufferers in 1955 (Link 1959).

The synthesis of warfarin sodium involves stirring pure warfarin acid and sodium carbonate (1:1.6 molar ratio) in an absolute ethanol medium at a temperature of 30 ^{o}C for two hours, with the isolation steps requiring a vacuum filtration and oven drying. Figure 4.1 describes the synthetic step (Ashkar 2004):

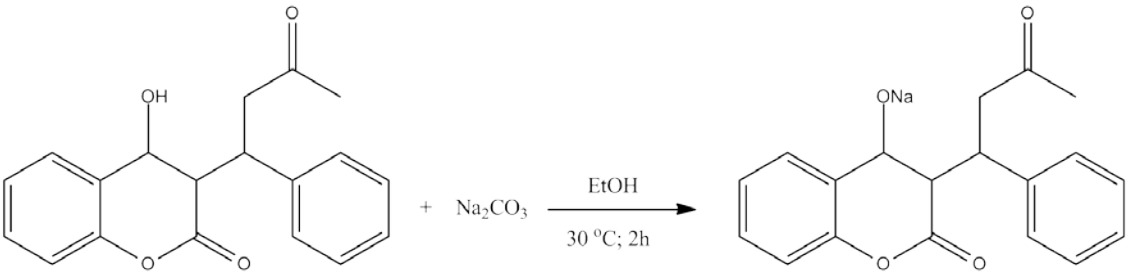


Figure 4.1 Synthesis of warfarin sodium

This synthesis was not selected for experimental studies due to the high cost of purchasing warfarin acid. Also, the stirring time is above what was desirable for this project.

4.1.2 – Nitisinone

Nitisinone's origin can be traced to the 1980's when new research at Syngenta was put in place to find more potent herbicides, but its use as a weed killer was halted after it was found that nitisinone cause eye lesions to rats.

It was subsequently found that it was an efficient drug in the treatment of tyrosinemia. The carriers of this rare hereditary disease are unable to process the amino acid tyrosine. If left untreated, tyrosinemia can lead to kidney and liver failure, mental retardation and eventual death. Until nitosinone was applied in its treatment, the primary course of action would be a liver transplant – with all the health implications that such a complicated procedure can carry (Santra and Baumann 2008; McKiernan 2006; Lock et al 1998).

The synthesis of nitisinone involves the acylation of a solution of cyclohexan-1,3-dione in dichloromethane with the acid chloride, adding triethylamine dropwise (molar ratio 1:1:1.3) for an hour at ambient temperature, as shown in Figure 4.2:

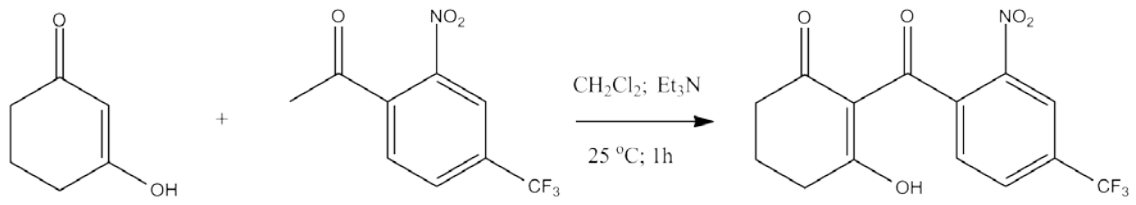


Figure 4.2 Synthesis of nitisinone

Although the reaction in itself is relatively simple to be carried out, the product isolation is not trivial: it involves two consecutive strong acid washes and extractions to remove triethylamine and a subsequent rotary evaporation. A substantial amount of time is then needed in order to prepare samples for analysis, and that incompatible with the smooth running of a continuous seven day production campaign. For this reason nitosinone was deemed not to be a suitable candidate for this project.

4.1.3 – Tesmilifene

Tesmilifene, a drug still in development, has shown promise as an antitumor agent. The mode of action is as yet unknown, but it has been proved that it enhances the activity of several classes of chemotherapy agents against cancers that are resistant to other drugs in patients with symptomatic metastases (Raghavan et al 2005; Lednicer and Mitscher 1999).

The last synthetic step is an alkylation. An acidic solution of p-benzylphenol is reacted with 2-chlorotriethylamine for 30 to 60 minutes, at 55 to 60 ^{o}C, as shown in Figure 4.3 (Brandes and Hermonat 1989):

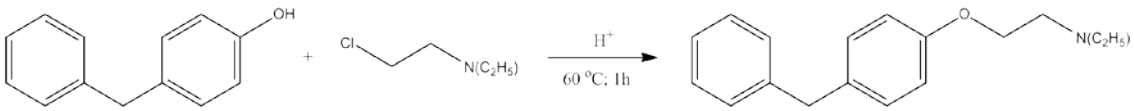


Figure 4.3 Synthesis of tesmilifene

The synthesis is relatively simple to carry out, but during the reaction two layers are formed. Similarly to the nitisinone product isolation, this involves two washes (the first one with sodium hydroxide and the second with hydrochloric acid) and a subsequent vacuum drying step. Thus, preparing samples for analysis is a labour intensive procedure that is not compatible with the running of a production campaign. The synthesis of tesmilifene was not pursued in this project due to this reason.

4.1.4 – Acamprosate calcium

In recent years there has been a drive towards finding effective drugs to control alcohol addiction. Homotaurine, an antagonist of γ-aminobutyric acid (the main inhibitory neurochemical which takes part in the regulation of excitability throughout the mammalian nervous systems), is thought to stabilize the brain's chemical balance that is disrupted by the abuse of alcoholic substances. Acamprosate calcium, the

calcium salt of the n-acetyl derivative of homotaurine has the same therapeutic effects and helps maintaining abstinence by preventing relapse. It was approved by the FDA for human use in 2004 (Williams 2005; Watanabe 2002; Lednicer and Mitscher 1999).

For this acylation calcium hydroxide, acetic acid and homotaurine are stirred in water until dissolution at a temperature of no more than 40 ^{o}C (molar ratio 1:2:2). A slight excess of acetic anhydride is then added and the solution is left to stir between 30 and 40 ^{o}C for an hour. The solution is then concentrated under vacuum (Durlach 1982; Lednicer and Mitscher 1999) Figure 4.4 shows the scheme of the reaction:

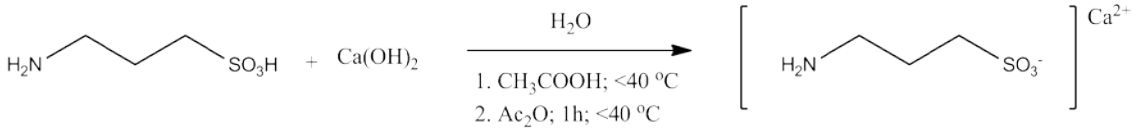


Figure 4.4 Synthesis of acamprosate calcium

Acamprosate calcium is a very interesting molecule from the point of view of its therapeutic effects, and its synthesis and isolation is straightforward. It would be an ideal chemistry to include in this project, but the prohibitive cost of homotaurine excluded the possibility of studying this chemistry further.

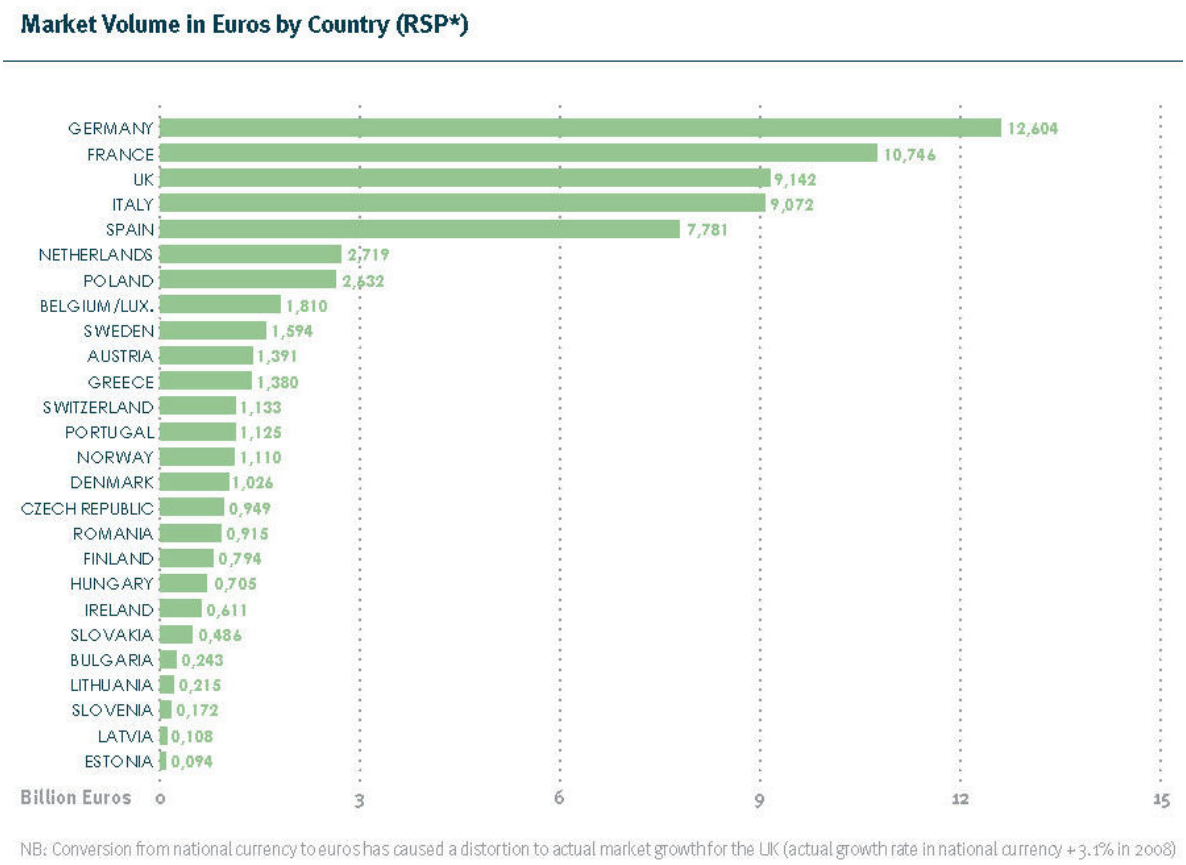
4.2 – Chemistries selected

Five different chemistries were pre-selected for screening. The chemical products studied in batch belong to the cosmetics & toiletries (C&T) and the pharmaceutical markets and include a cyalume (bis(2,4,6-trichlorophenyl) oxalate), two fragrances (methyl diantylis and vanisal sodium) and two APIs (acetylsalicylic acid and paracetamol).

The European pharmaceutical industry alone accounts for 19.2% of all business related R&D investments and represents around 3.5% of the total EU manufacturing value added. Despite being valued at circa €150 billion in 2007, having grown at a

median rate of 7% over the previous ten years, the pharmaceutical market in Europe accounted for less than a third of the total world pharmaceutical market in that year (EFPIA 2009). On the other hand, the European Union Cosmetics & Toiletry (C&T) market was valued at circa €62 billion in 2008. Accounting for 14.8% of this total, the market in the UK had grown by circa 10% from the previous year (COLIPA 2008). Figure 4.5 depicts the European Union C&T market and the relative importance of each continent in the world pharmaceutical market.

In the following sections the fine chemicals and pharmaceutical compounds that were screened for this study are described in more detail. The synthesis of each studied compound was executed according to the literature and the results obtained from the batch experiments can directly be applied to the COBR. In order to perform the experiments in the batch OBR the available protocols had to be adapted and typical procedures used are described hereinafter, followed by the obtained results.



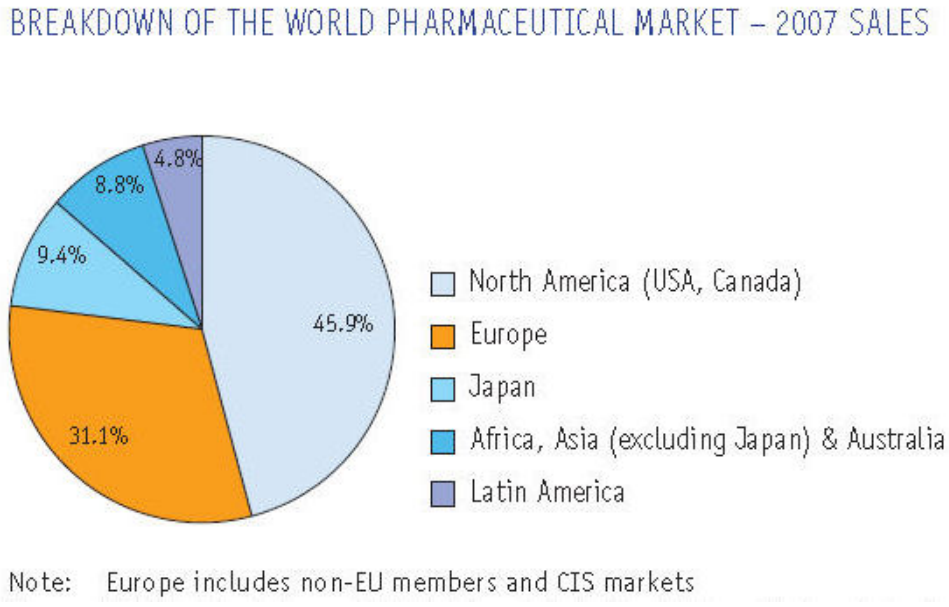


Figure 4.5 European Union Cosmetics and Toiletries Market sales in 2006 and the World Pharmaceutical Market in 2007, *in* COLIPA 2008 and EFPIA 2009 (adapted)

4.2.1 – Bis(2,4,6-trichlorophenyl) oxalate

This compound belongs to the family of cyalumes. Cyalumes are symmetrical diesters that when in contact with peroxides undergo a series of exothermal chemical reactions, producing carbon dioxide and light in the presence of a colorizing molecule. This class of molecules is the result of long years of research that started in the 1960's, when scientists were trying to explain the chemiluminescence phenomenon. One such scientist was Edwin Chandross: when working at Bells Laboratories, he discovered the underlying mechanism behind this type of reaction. Currently the owner of more than 60 patents (Sturchio 2005), he later found out that because he failed to patent his discovery he missed on a very profitable business. The company that patented the "Chemical Light Device" was the American Cyanamid Company (Zandt 1977), which benefited from Edwin Chandross' work and took it one step further - Michael Rauhut tried to improve the efficiency of the chemiluminescent reaction by synthesizing a series of oxalate esters and was ultimately able to raise Edwin's 1% quantum yield to 5% (Wilson 1999; Peters 2002).

Nowadays, the lightstick business is a multimillion industry. It has found uses in the military, fire-fighters, police corps, in outdoors activities such as fishing, hiking and camping and even in our cars and homes. Its popularity is due to its durable and waterproof container that does not depend on batteries and does not create sparks, therefore being one of the most dependable and safe sources of light. Cytec Industries, the company that owns the Cyalume trademark, was worth circa €534M in March 2009 (Fortune 2009).

The chemical reaction that is used to produce bis(2,4,6-trichlorophenyl) oxalate is as shown in Figure 4.6:

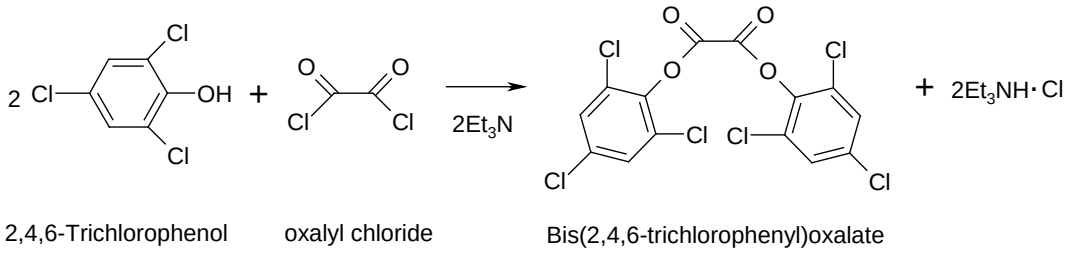


Figure 4.6 Synthesis of bis(2,4,6-trichlorophenyl) oxalate

The synthesis procedure described in literature is similar to the one described in the following section, with the exception that reflux is required for 20 minutes. Refluxing the solvent requires more equipment and makes the setup more complicated, which is a clear process disadvantage. The reported yield for this reaction is 60% (McCapra 2000; Jasperse 2007).

4.2.1.a – Synthesis of bis(2,4,6-trichlorophenyl) oxalate

The procedure used is as follows:

1. Weigh out 5.27 g of trichlorophenol; transfer it to the batch OBR;
2. Add 40 mL of toluene and 3.70 mL triethylamine (using a syringe) and begin oscillation (ν = 2.0 sec^{-1}; x_0 = 40 mm);
3. Cool the reactor to 5 °C using ice-water bath and add 1.30 mL of oxalyl chloride;
4. Plug a hot water bath (T_{water} = 95°C) to the OBR for 10 minutes;
5. Transfer the reaction product to an Erlenmeyer flask and chill it, using a ice-water bath;
6. Perform a vacuum filtration, using hexane;
7. Dry the solid in the oven for two hours at 30°C;
8. Wash the solid with purified water (the cyalume is insoluble, whereas the formed triethylamine hydrochloride is soluble in water);
9. Filter the product using a vacuum apparatus and purified water;
10. Oven-dry the solid (o/n, 30 °C) and weigh the samples.

Due to the toxicity of the chemical compounds involved in this synthesis, it was not possible to take several samples along the reaction time. A 10 mL sample was taken at the end of the reaction and was isolated in the way previously described.

4.2.1.b – Results

Due to the toxicity risk posed by all of the reactants used it was not possible to sample the reaction while it was occurring - the safety guidelines suggested that it was advisable to stop the reaction at a given time so that the product could be analyzed safely. Figure 4.7 shows the temperature profile inside the OBR after the oxalyl chloride was added to the solution, when the hot water bath was plugged to the reactor. The boiling point of the used solvent (toluene) is 110-111 ^{o}C. Refluxing the reaction mixture, as suggested in literature, indicates that the reaction should be performed at higher temperatures than those recorded in this experiment.

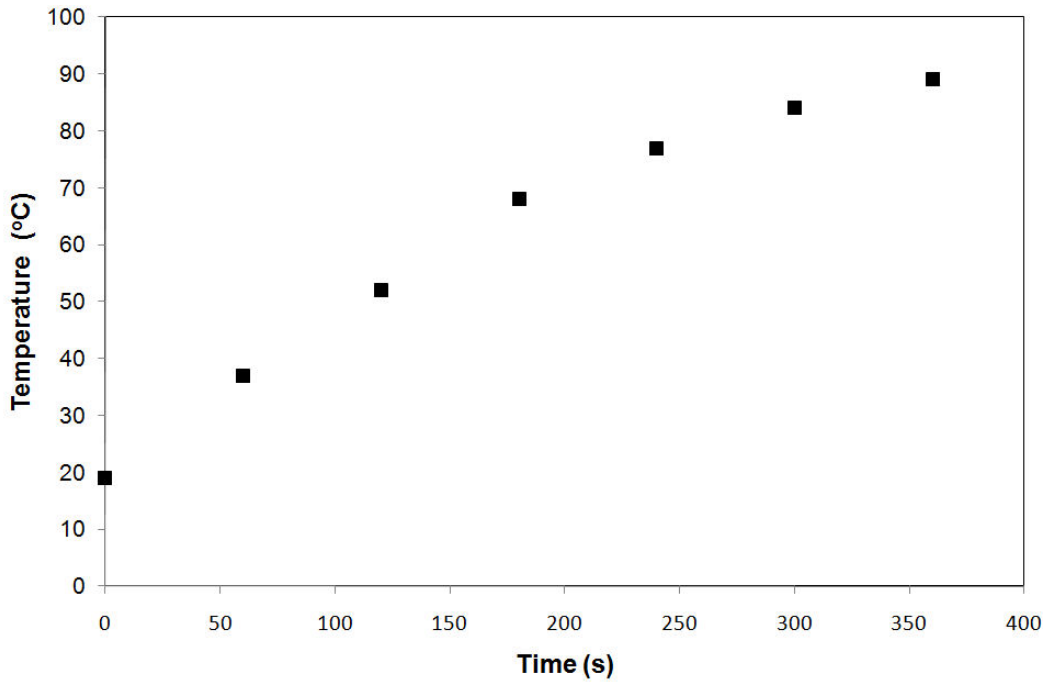


Figure 4.7 Temperature profile inside the OBR

Upon addition of oxalyl chloride the reaction progressed and the mixture inside the reactor changed within the first minute from orange to a brownish colour. The resulting precipitate became a pale yellow powder after the isolation steps. The obtained yield of 67% is superior to that described in literature (60%).

Figures 4.8 and 4.9 compare the IR spectrum obtained for the product sample with the IR spectrum published in literature. It is evident that they are similar, proving that the obtained product is indeed the cyalume. The band at 3435 cm^{-1} indicates the presence of the hydroxyl group, possibly from water residues left from the isolation procedure. Table 4.1 correlates the IR peaks from the spectrum with the molecular structure of the synthesised bis(2,4,6-trichlorophenyl) oxalate.

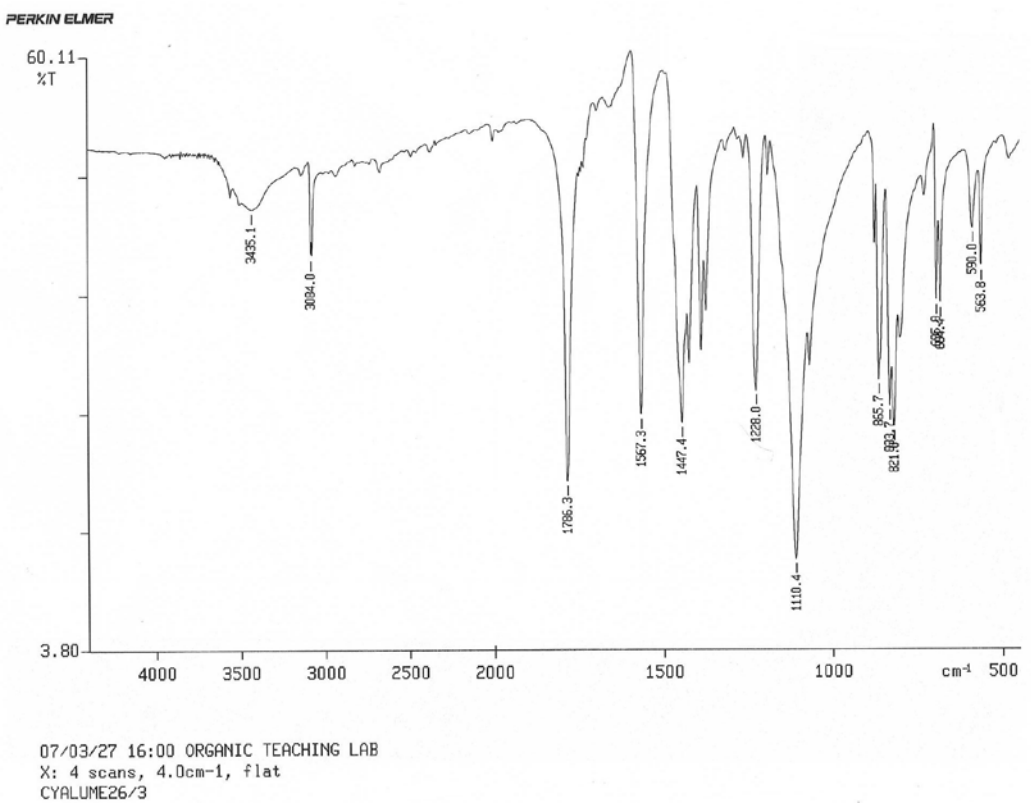


Figure 4.8 IR spectrum of the synthesized Bis(2,4,6-trichlorophenyl) oxalate, KBr disc

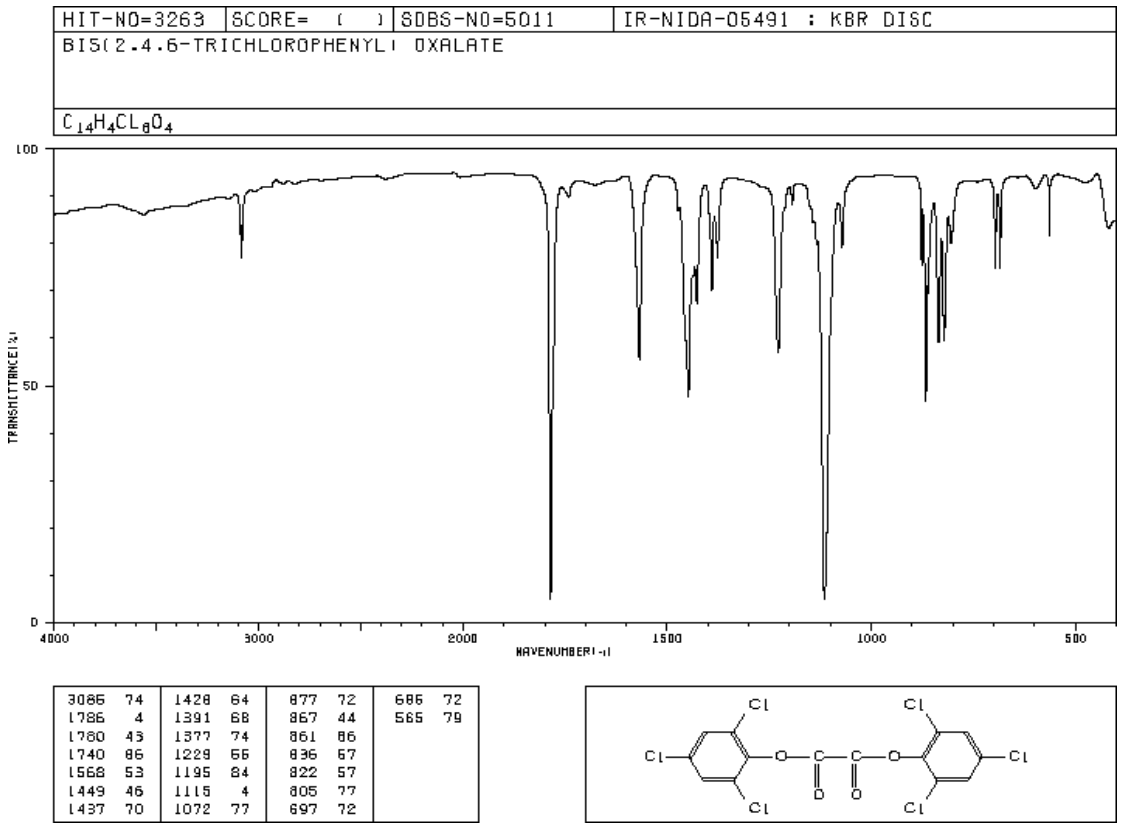


Figure 4.9 IR spectrum for Bis(2,4,6-trichlorophenyl) oxalate, KBr disc. *Source:* National Institute of Advanced Industrial Science and Technology (Japan)

Table 4.1 IR spectrum peaks and its molecular significance[11]

Bond	Experimental data peak (cm^{-1})	Literature data peak (cm^{-1})
Aromatic C-H	3084.0	3086
Ester C=O	1786.3	1786
Aromatic C-C	1567.3	1568
	1447.4	1449
Ether C-O	1228.0	1229
	1110.4	1115

[11] The reference IR bands and peaks are described in Morrison, R. and R. Boyd (1992). Organic Chemistry. New Jersey, Prentice-Hall. Literature peaks *in* National Institute of Advanced Industrial Science and Technology (Japan)

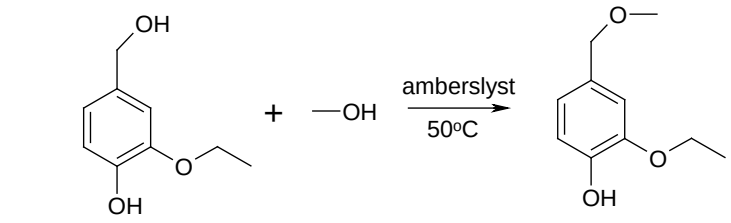
The results gathered for this set of experiments show that performing this synthesis in OBR would have a number of advantages over the method reported in the literature (Jasperse 2007). Not only was the reaction time faster than the 20 minutes cited, but also toluene refluxing was not needed to accelerate and complete the reaction. It is well-known that the superior mixing and heat-transfer capabilities of the OBR confer this reactor design a marked advantage over more traditional types of reactors. This means that less heat is delivered to the system and energy is saved, the risk of decomposing reactants or product is greatly reduced and the experimental apparatus is simplified.

The aforementioned health and safety compliance issues prevented further studies on this chemical species from taking place. In conclusion, it would not be impossible to adapt this synthesis to the COBR, but this chemistry was not chosen for further studies in the COBR given the increased cost of adapting and implementing further safety procedures and equipment.

4.2.2 – Methyl diantilis

The use of perfume and scented materials goes back thousands of years. The origin of the word "perfume" is associated with the burning of incense, one of the oldest rituals where aromatic materials played an important part (*Latin: per fume*). In every great world civilization references to perfumes and fragrances can be found – from the Sumerian to the Greek civilizations, the Egyptians and Romans, in all of these cultures perfume was an important part of life (Pybus 1999; Fortineau 2004). At the end of the 19th century chemistry was replacing alchemy and the work on fragrances prompted Jean-François Houbigant to produce "Fougère Royale", founding modern perfumery (Kraft et al 2000). Nowadays, the perfume industry is a relatively mature one and thousands of fragrances enter the composition of a myriad of different products. In the continuous search for new and attractive chemical compounds, organic chemists regularly synthesize new fragrances.

One such fragrance is 2-ethoxy-4-(methoxymethyl)phenol, more commonly known as methyl diantils. It was born out of the necessity of having a more powerful alternative to vanillin, but, unlike isoeugenol, one that wouldn't lose its colour with time. Named after carnations (*Dianthus caryophllus*), Methyl Diantilis "has a sweet and creamy vanilla odour reminiscent of white chocolate" (Kraft et al 2000; Miles and Connell 2006) and its synthesis was patented by Givaudan Corporation, a leader in the flavours and fragrance industry (Ochsner 1987). The last step in the synthesis of methyl diantilis is the one studied in this project and is given in Figure 4.10:



3-ethoxy-4-hydroxybenzyl alcohol Methyl Diantilis

Figure 4.10 Synthesis of methyl diantilis

The synthetic process for Methyl Diantylis described in literature (Miles and Connell 2006) is very similar to the procedure described next, with the exception that the experiment was conducted under milder temperature conditions thus obviating the need for solvent refluxing.

4.2.2.a – Synthesis of methyl diantilis

The procedure adapted for the OBR includes:

1. Weigh out 3 g of Amberlyst-15 (a strongly acidic cation exchange resin) and wash 6 times with 10 ml of methanol. Transfer it to the batch OBR;
2. Add 45 ml of methanol to the OBR. Start the oscillation (ν = 2.0 sec^{-1}; x_0 = 40 mm);
3. Add 3 g of 3-ethoxy-4-hydroxybenzyl alcohol (a small amount at a time) to the OBR;

4. Plug the hot water bath (T_{water} = 62 ^{o}C) to the reactor for 15 minutes;
5. Perform a vacuum filtration of the product, using methanol;
6. Remove the solvent using a rotary evaporator under vacuum at 30 ^{o}C;
7. Weigh the samples.

The OBR oscillation had to be stopped for a few seconds each time a sample was taken from the reactor because the design of the particular OBR prevented on-line sampling. Each 10 ml sample was taken on regular intervals from step 4) in the procedure, using a small pipette. All samples were isolated as described in steps 5 to 6.

4.2.2 – Results

In line with what happened for bis(2,4,6-trichlorophenyl) oxalate, the synthesis of methyl diantilis was done with a simpler setup and was quicker to complete in the OBR than what is described in literature (Miles and Connell 2006) because the use of a refluxing apparatus was obviated.

As described in the procedure, a pre-treatment of the used catalyst by applying a methanol washing was necessary before the synthesis could be started, which transformed the previously crème amberlyst spheres to “dirty white”.

Figure 4.11 displays the temperature profile of the reaction at sampling times and shows that the temperature inside the OBR remained very stable throughout the experiment, which was carried out without the need for refluxing the methanol.

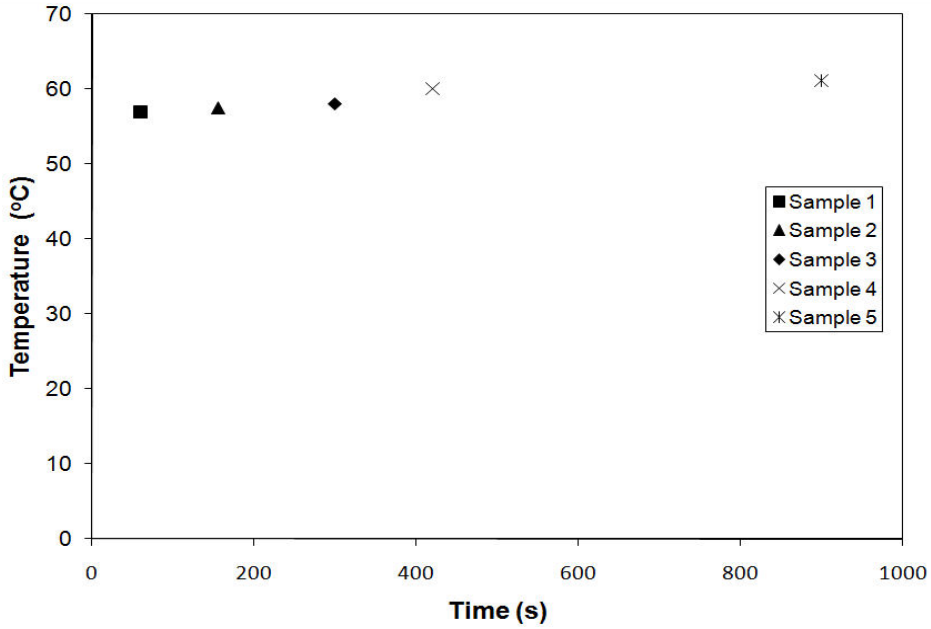


Figure 4.11 Temperature profile and sampling times for the methyl diantilis reaction.

A deep vanilla scent with carnation notes was evident even from the first sample taken, thus implying the presence of methyl diantilis. The peak data available by Miles and Connel (2006) for this chemical compound is the following: IR (neat) 3427,2981,1606 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (m, 2 H), 6.80 (dd, *J* =1.8, 8.0 Hz, 1H), 5.89 (s, 2 H), 4.11 (q, *J* = 7.1 Hz, 2 H), 3.35 (s, 3 H), 1.43 (t, *J* = 7.2 Hz, 3 H). Figures 4.12 to 4.23 show the IR and NMR spectra of the samples, which confirm the smell analysis and indicate that the reaction was completed within 8 minutes from the start, considerably faster than the 20 minutes reaction time described by Miles and Connel (2006). Notwithstanding, the data obtained for some of the samples suggest that the solvent elimination was not complete – as affirmed by Dr. Alan Boyd, of Heriot-Watt University, the extra peaks reveal the presence of methanol that was not totally evaporated from the samples analyzed – and so the vacuum filtration should have been more thorough. The reaction time might indeed be shorter, but the methanol contamination of some of the samples prevents from drawing a more definite conclusion on this matter.

After the isolation steps, the resulting end product was a pale yellow liquid with the characteristic vanilla and carnation odour of methyl diantilis. The identification of

the IR spectrum peaks for the sample where less solvent was found (sample 4) is described in Table 4.2.

Table 4.2 IR spectrum peaks/bands and its molecular significance[1]

Bond	Literature Peak/band (cm^{-1})	Experimental (sample 4) Peak/band (cm^{-1})
Aromatic O-H	3427	3367
Aromatic C-H	2981	2945
Aromatic C-C	1606	1604

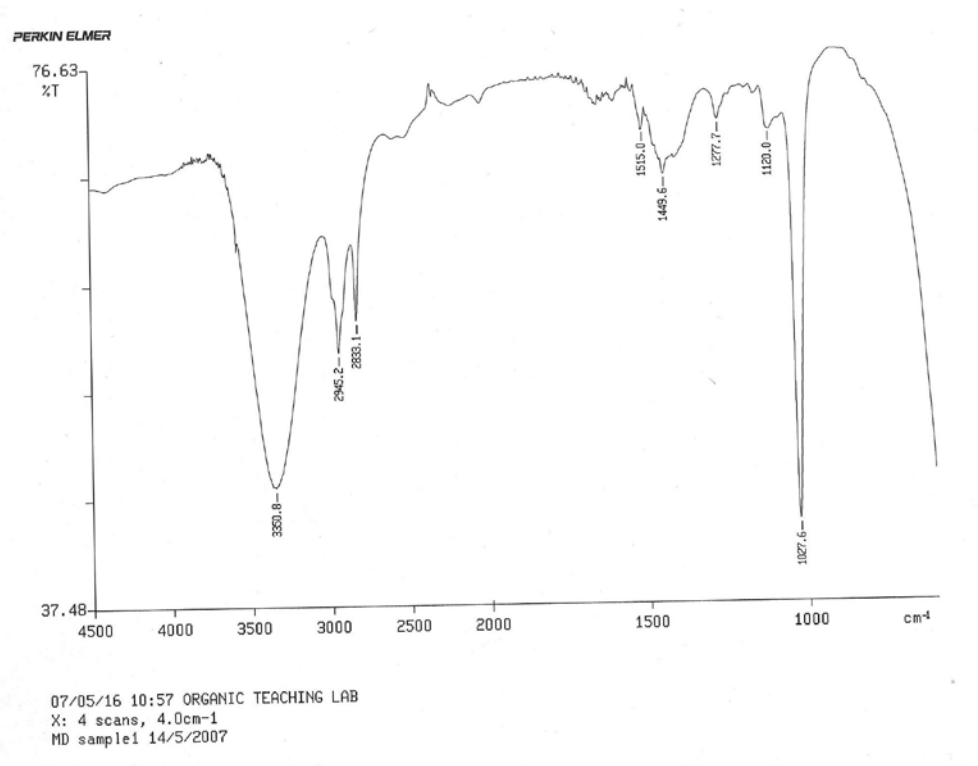


Figure 4.12 IR spectrum of the synthesised methyl diantilis, sample 1

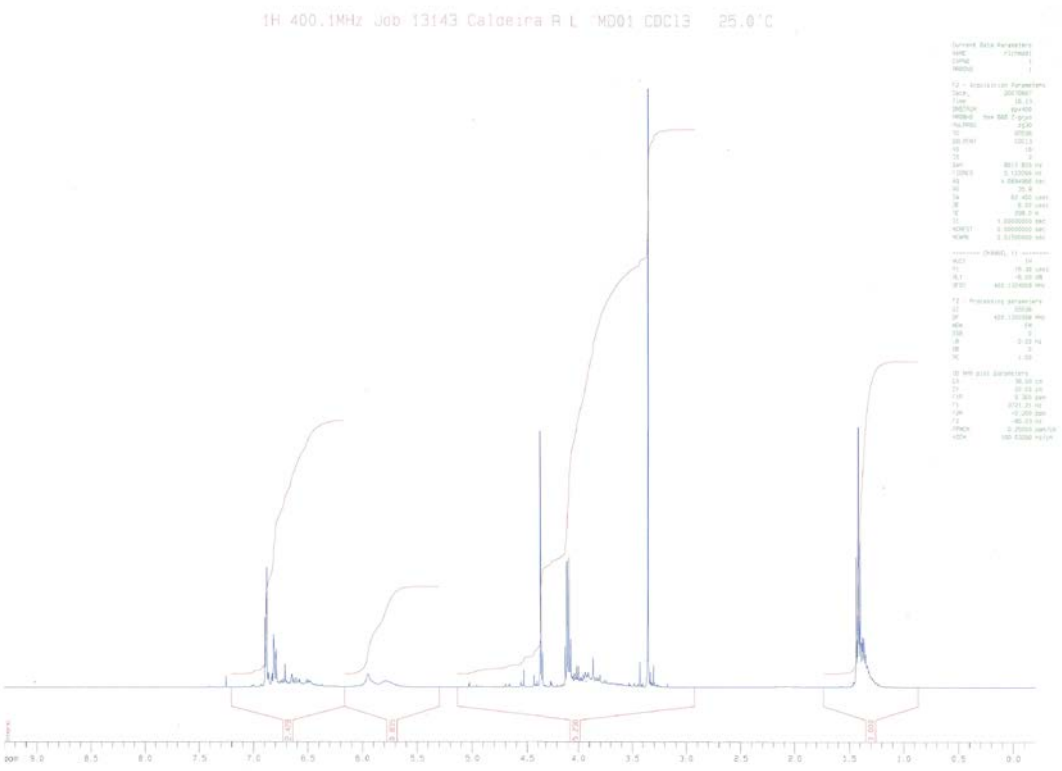


Figure 4.13 ^{1}H 400Mhz NMR spectrum of the synthesised methyl diantilis, sample 1

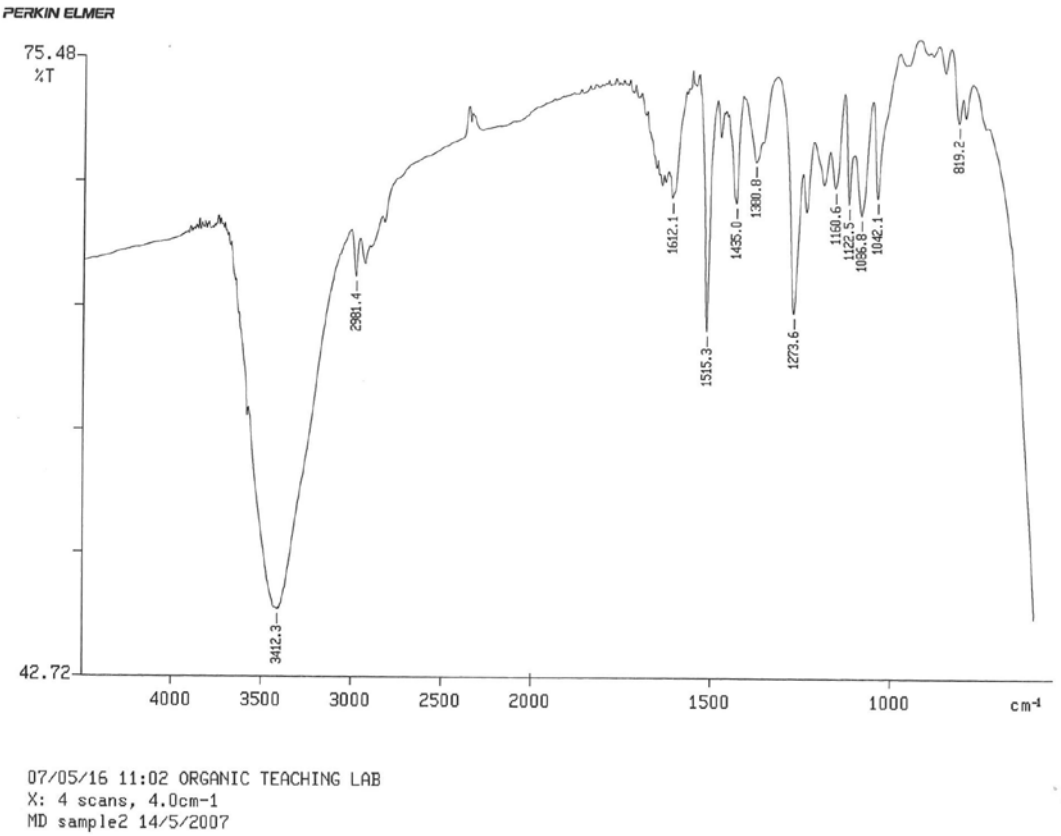


Figure 4.14 IR spectrum of the synthesised methyl diantilis, sample 2

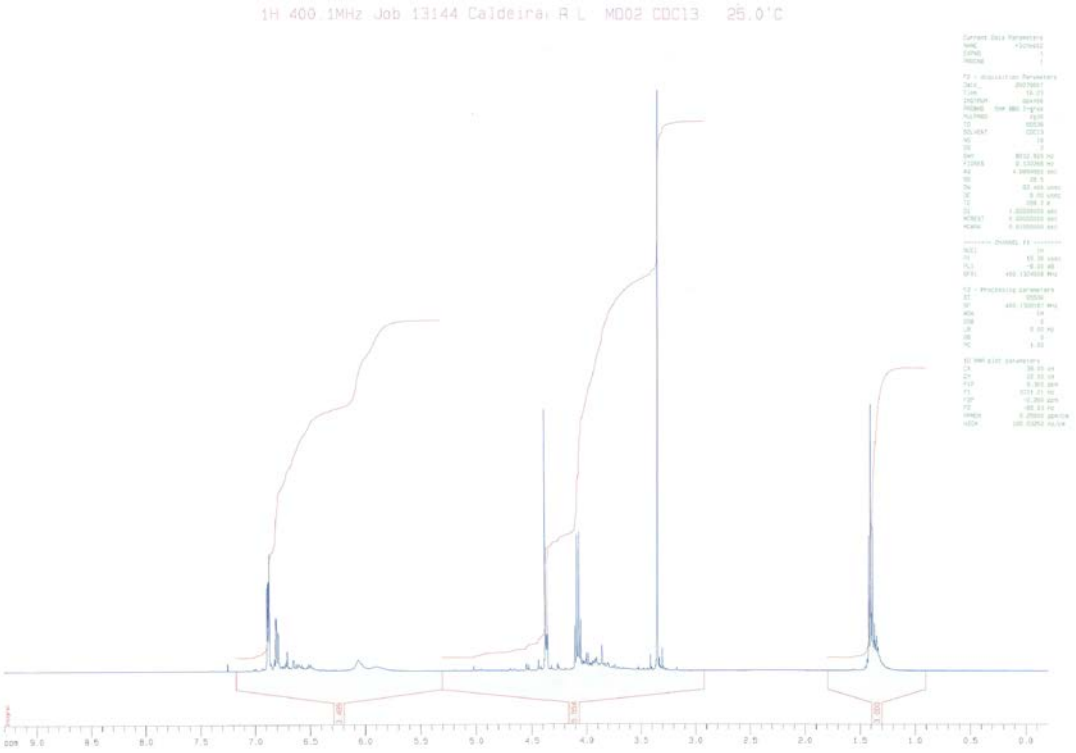


Figure 4.15 ^{1}H 400Mhz NMR spectrum of the synthesised methyl diantilis, sample 2

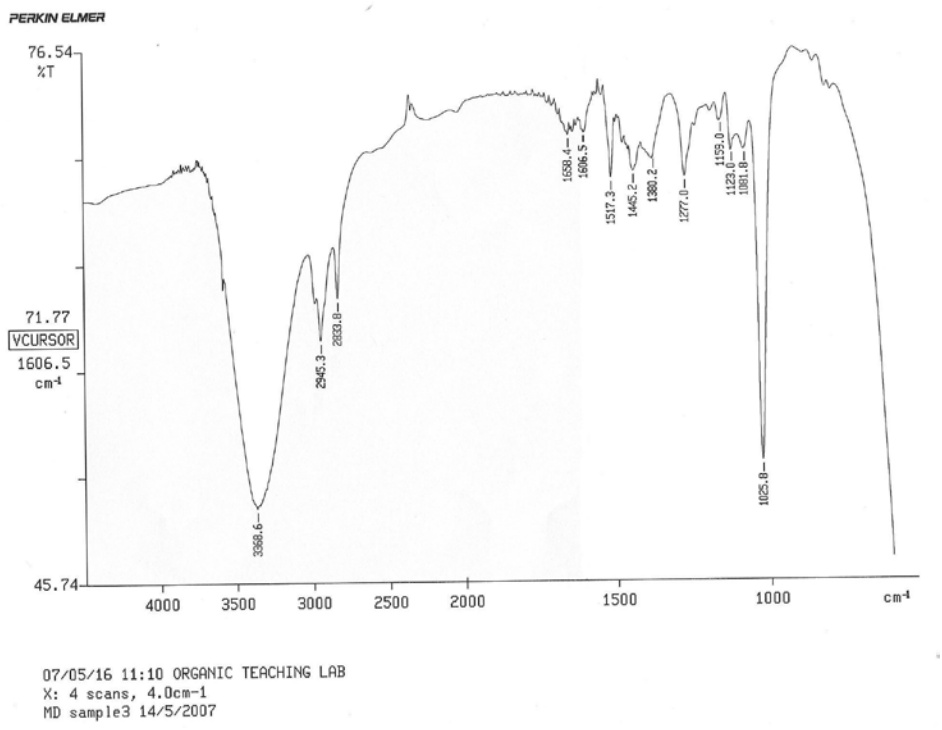


Figure 4.16 IR spectrum of the synthesised methyl diantilis, sample 3

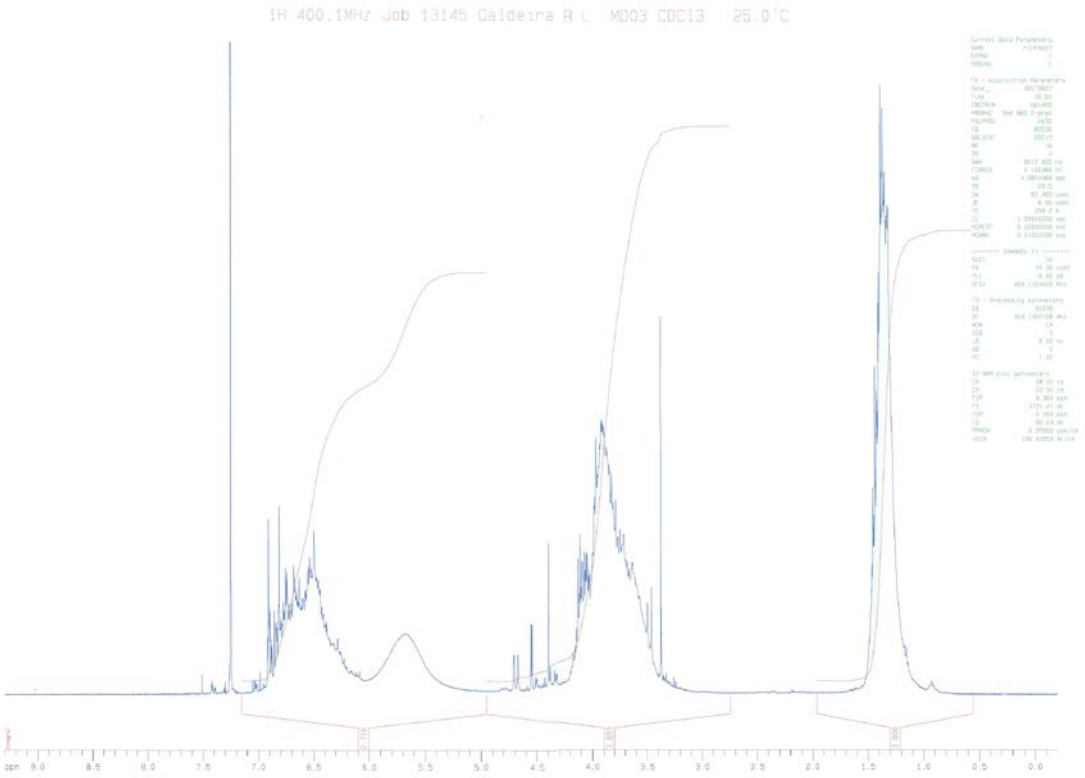


Figure 4.17 ^{1}H 400Mhz NMR spectrum of the synthesised methyl diantilis, sample 3

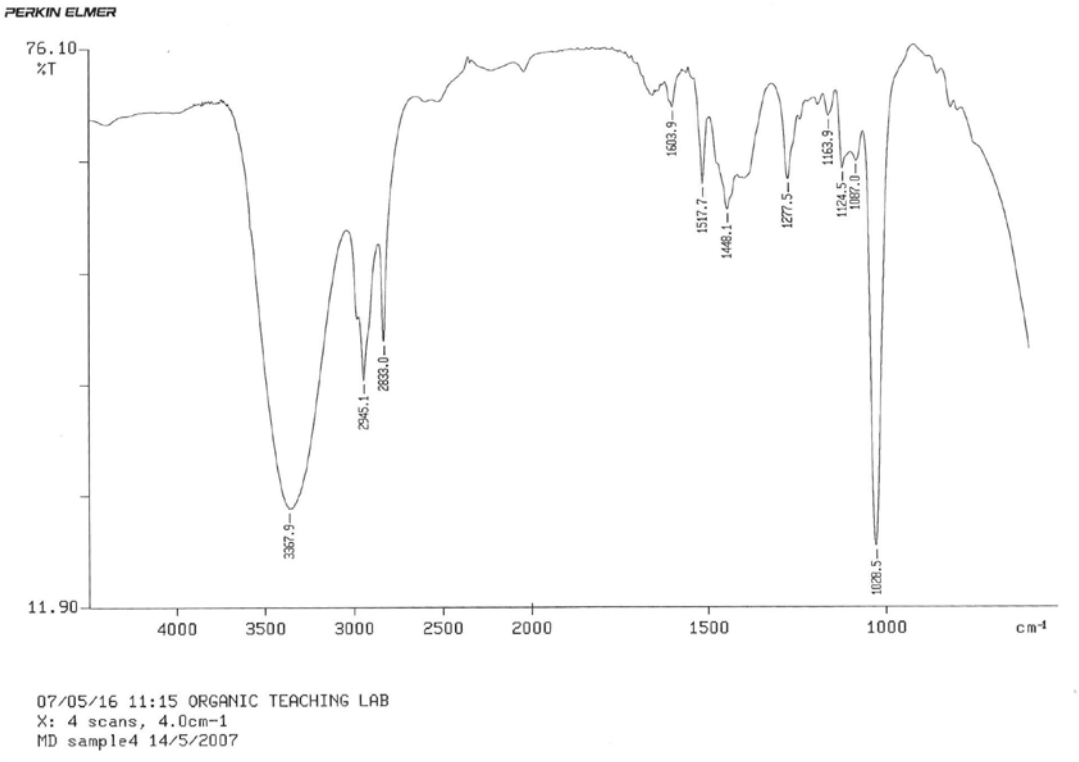


Figure 4.18 IR spectrum of the synthesised methyl diantilis, sample 4

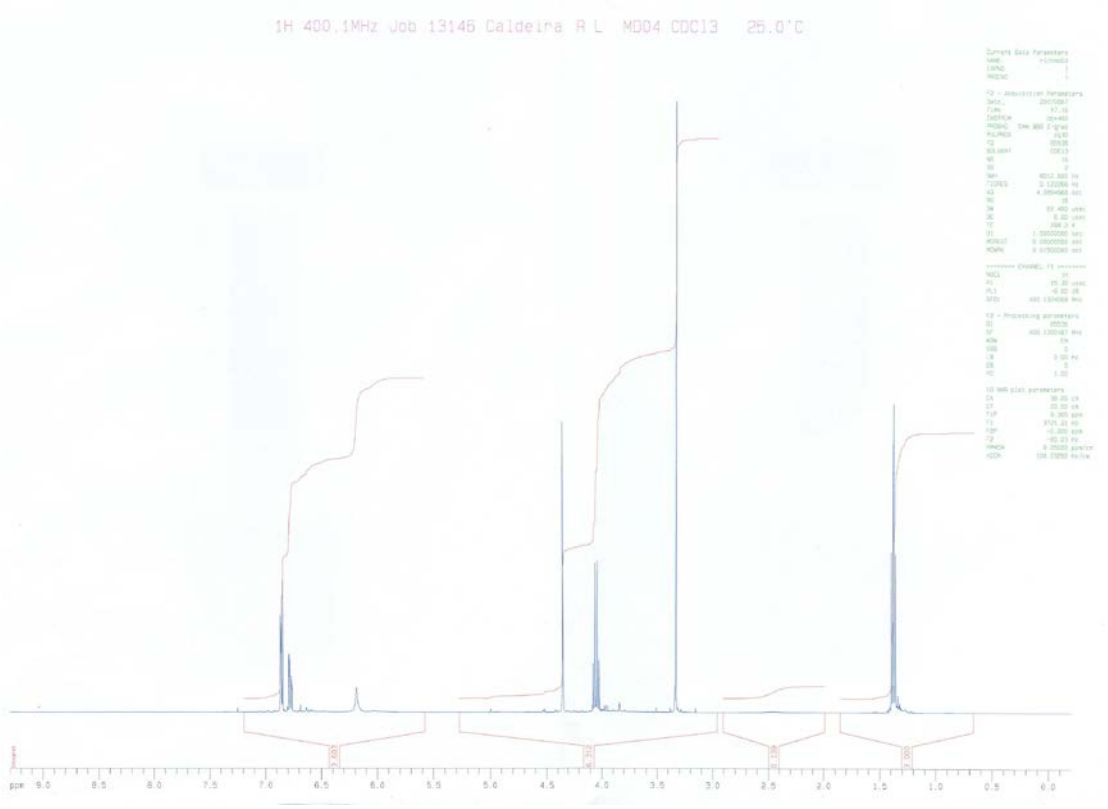


Figure 4.19 ^{1}H 400Mhz NMR spectrum of the synthesised methyl diantilis, sample 4

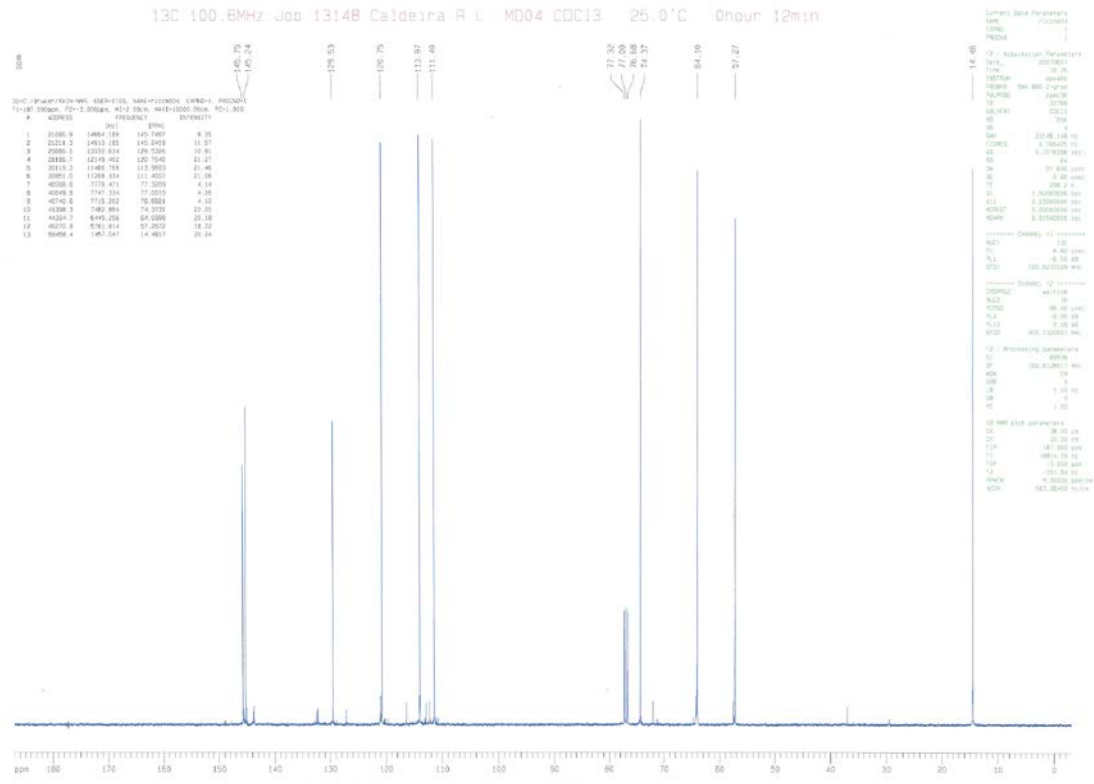


Figure 4.20 ^{13}C 100Mhz NMR spectrum of the synthesised methyl diantilis, sample 4

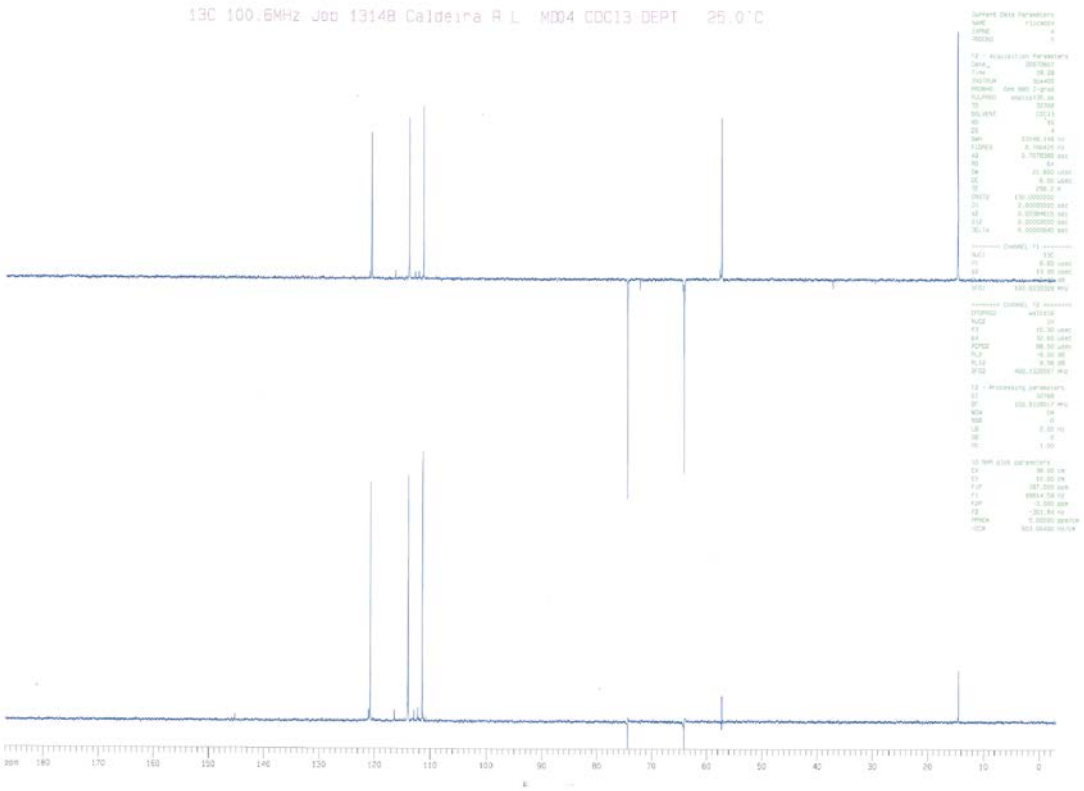


Figure 4.21 ^{13}C 100Mhz (depth) NMR spectrum of the synthesised methyl diantilis, sample 4

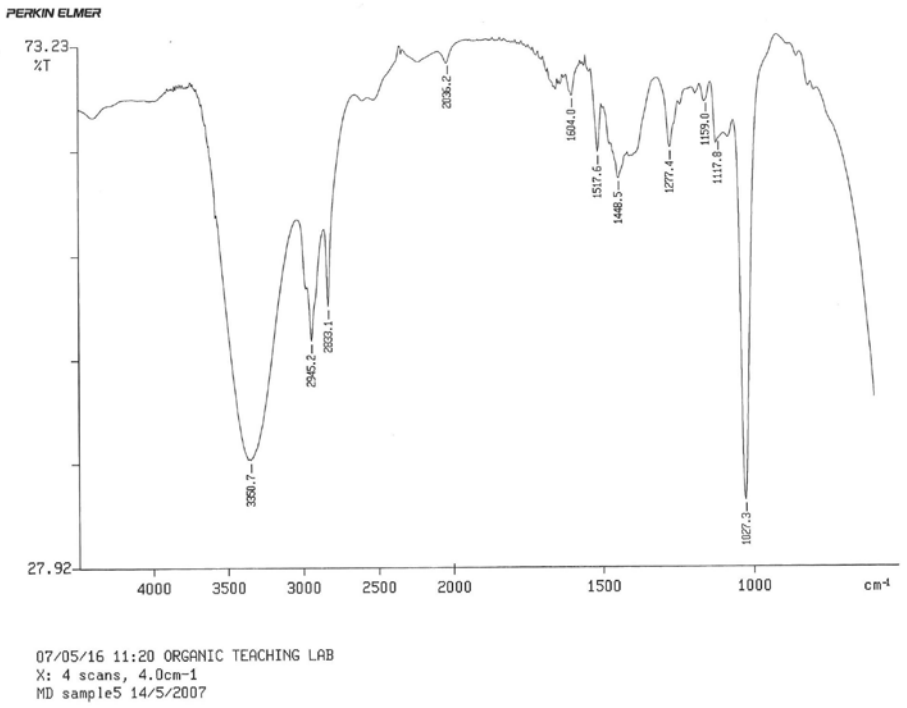


Figure 4.22 IR spectrum of the synthesised methyl diantilis, sample 5

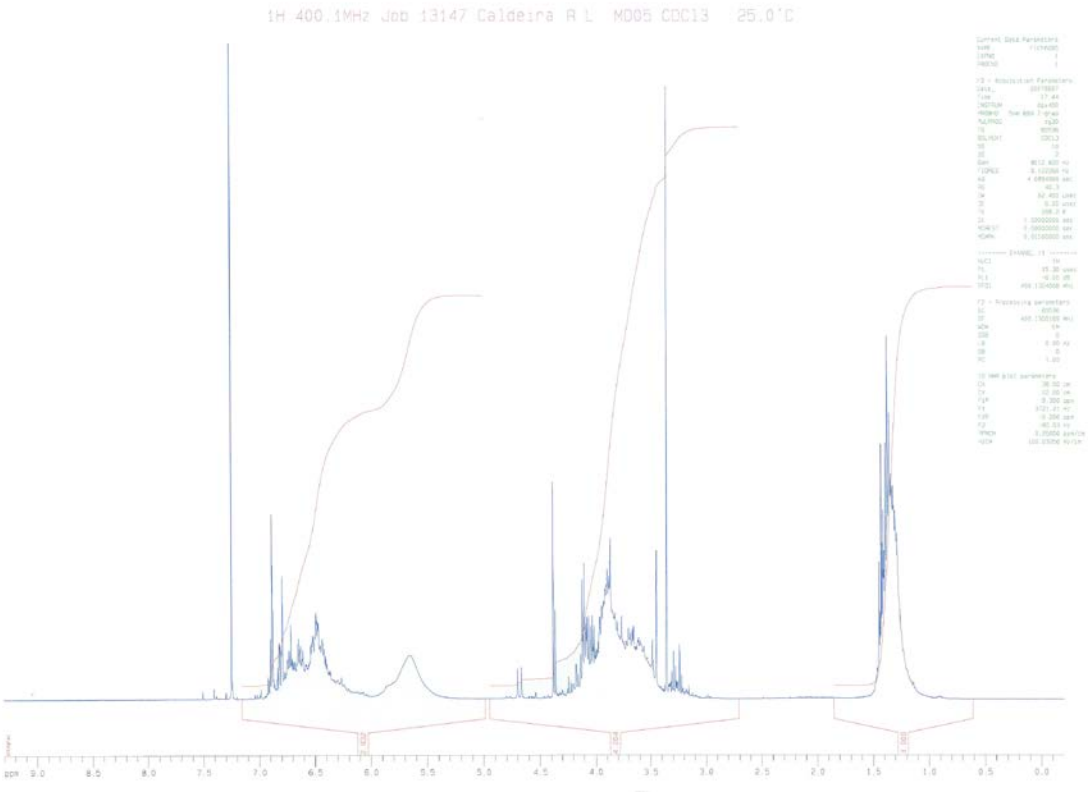


Figure 4.23 ^{1}H 400Mhz NMR spectrum of the synthesised Methyl Diantilis, sample 5

In summary, there are two main advantages of performing this reaction in OBR: it is faster and it is carried out at lower temperatures (energy is saved), which, in turn, makes the experimental apparatus simpler because there is no need to reflux the solvent. Once again it is proved that the more efficient mixing occurring in each of the OBR's cells leads to better heat transfer and thus to faster reaction times than those observed for more traditional reactors

The synthesis of this chemical compound was not pursued further in the COBR due in part to the high costs associated with the purchase of the catalyst, but more importantly the intrinsic need for its methanol pre-treatment which is more time consuming than what was foreseen.

4.2.3 – Vanisal sodium

Vanilla is one of the most popular odours in the fragrance industry. Since the early 1990's perfume makers have been incorporating it in their creations as a significant part of the odour note, and, more recently, Body Shop introduced a pure vanilla fragrance in the market (Fox 2005).

But while in perfumes vanillin - the molecule that is normally used to evocate the smell of vanilla - is relatively stable, that does not occur in soap formulations. Due to the combination of light, heat and the basic conditions in soaps, vanillin and its derivatives undergo chemical reactions that rapidly transform them into polyphenols and other detergent products. The outcome of these events is the discolouring of soaps – from a light tone progressively to brown and eventually black; another consequence is the loss of foaming power (Vidal 2006; Turin 2007).

The most common path to overcome this problem is to use more odour-potent chemical variations of vanillin (such as ethyl vanillin) in low enough concentration, use colouring agents or non-discolouring chemical alternatives to mask the aforementioned problems. This means that either the resulting soap cannot be clear or the soap does not have the desired scent properties.

Flexitral Inc. was founded by Luca Turin - the "Emperor of Scent" - where he has had the opportunity to put to the test the highly controversial vibrational theory in the creation of new odour molecules. As opposed to the widely accepted lock-and-key concept defended by the shape theory, the vibrational theory defends that after fitting in the receptor site, the odour molecule must have a vibrational energy compatible with the different receptor's energy levels so that electrons can travel through the molecule via inelastic electron tunnelling, thus triggering a signal transduction.

This flavours and fragrances company has recently launched in the market two solutions to the aforementioned problem: vanisal and ethyl vanisal. The company claims that these two compounds, which are the product of the treatment of vanillin and ethyl vanillin with a sulfiting agent, do not suffer from discoloration when used in the

composition of soaps and retain the much appreciated vanilla scent (O'Hare 2006; Brechbill 2007; Turin 2007).

The reaction scheme to the production of vanisal sodium is as shown in Figure 4.24:

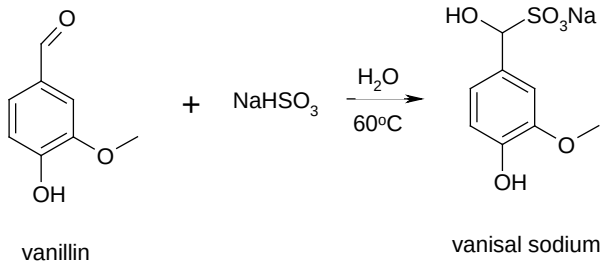


Figure 4.24 Synthesis of vanisal sodium

According to the Flexitral patent, vanisal sodium can be prepared by mixing equimolar amounts of sodium hydrogen sulphite and vanillin in water, either at room temperature or at 50 to 60 ^{o}C. The reaction is complete when there is no undissolved vanillin, an indication that it has reacted to form the sulphite adduct.

4.2.3.a – Synthesis of vanisal sodium

The following steps are followed in order to perform this synthesis:

1) Transfer 35 mL of purified water to the batch OBR;
2) Heat the reactor to 60 °C; Measure 3.86 g of vanillin and 7.2 mL of sodium hydrogen sulphite 40%. Transfer it to the batch OBR;
3) Start the oscillation (ν = 2.0 sec^{-1}; x_0 = 40 mm) and mix for 10 minutes;
4) Transfer the solution to a beaker and put it in the oven (35 °C, overnight) and weigh the samples.

4.2.3.b – Results

For the experiments just 10 to 12 seconds were sufficient after the oscillation had started in the OBR for the mixture to become transparent, a clear indication that the reaction was complete. This suggests that the reaction can indeed progress at lower temperatures using an OBR, thus saving energy in the process. Although the patent that describes the method of manufacture of vanisal sodium (Turin 2007) does not mention reaction times, it was later stated in private correspondence with Luca Turin, the Chief Technical Officer of Flexitral, that the reaction times obtained in this set of experiments are “a little fast, but not wildly so” compared to the benchmark set by the company that markets this chemical entity. The mixing and heat transfer capabilities of the OBR were again tested and, as it had happened for the previously studies chemistries, this reactor design proved to be more efficient when compared to a more traditional design.

Because the solution became transparent so quickly, only one sample was taken after 15 seconds of reaction time. The vanillin scent was evident and after the sample was oven-dried the resulting powder was analysed via NMR. A comparison between the spectrum obtained and the reference for pure vanillin (Figures 4.25 and 4.26) show a new peak at $\delta = 85.49$ ppm, revealing the presence of a previously non-existent hydroxyl group attached to a tertiary carbon atom. This proves that the analysed sample was of vanisal sodium. The dried sample had a brittle white solid aspect, which was also consistent with the appearance of vanisal sodium as described by Luca Turin in the aforementioned private correspondence.

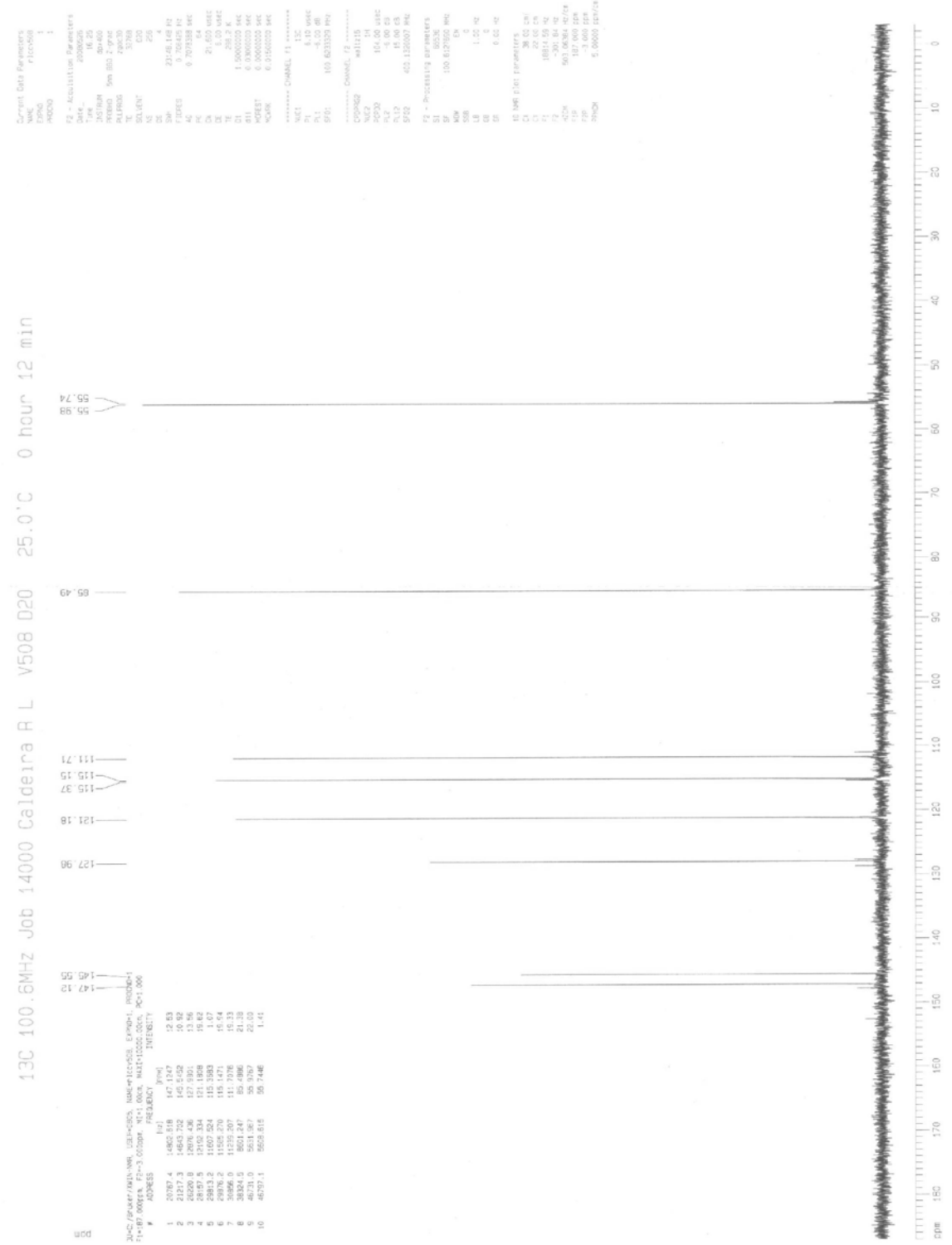
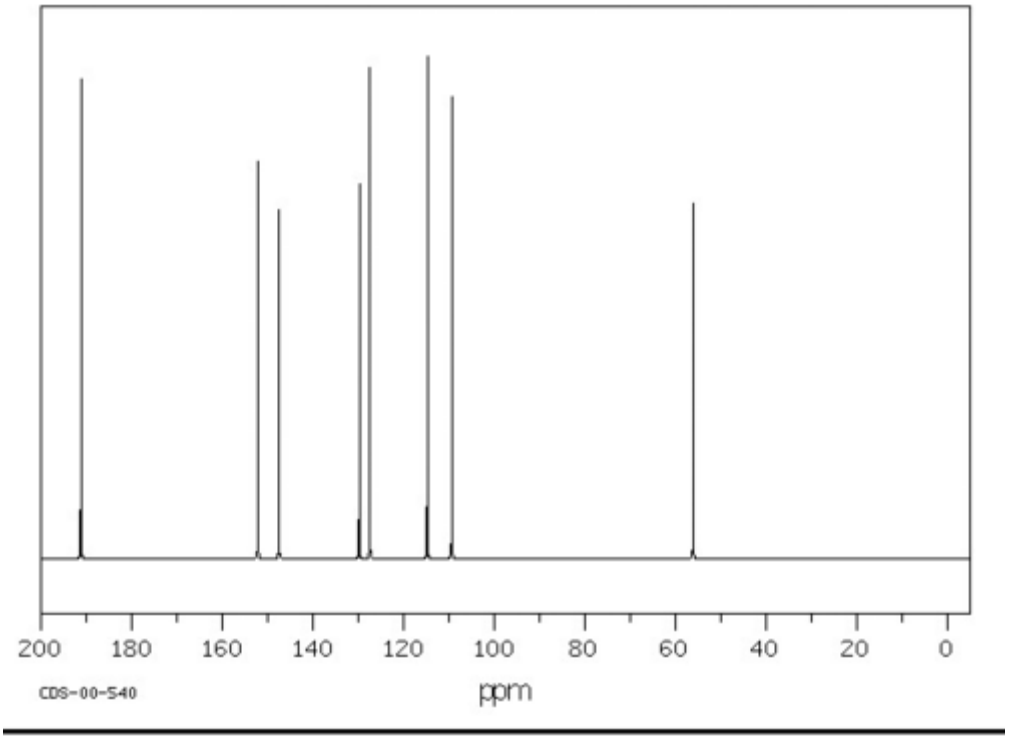
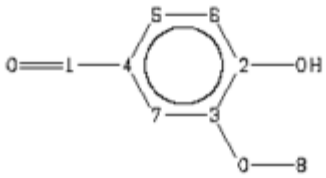


Figure 4.25 NMR spectrum of vanisal sodium





ppm	Int.	Location
191.21	955	1
152.18	791	2
147.50	692	3
129.77	746	4
127.49	975	5
114.75	1000	6 *
109.34	920	7 *
56.10	706	8

Figure 4.26 Reference NMR spectrum of vanillin. *Source: AIST, Japan*

While being relevant for the valuable cosmetics and toiletries market, this was also the first chemical compound that gathered all the conditions and characteristics suitable for further studies in COBR, in order to prove its competence in the manufacture of fine chemicals. It was proved that the reaction is fast, economical and safe to operate from a health and safety point of view.

4.3.4 – Acetylsalicylic acid

Acetylsalicylic acid, or aspirin as it is more commonly known, was born out of the necessity of having an alternative to salicylic acid, which, despite working well to relieve aches and pains, is hard to swallow and causes irritation to the digestive tract (Osborne 1998). When assigned to this task, Felix Hoffman of the Bayer pharmaceutical company came up with a method to make acetylsalicylic acid in 1897, two years after having started his research – a drug that not only was very efficient in the treatment of pain fever and inflammation, but also tasted better – and was granted a US patent to the process in 1900 (Hoffmann 1900; Henderson 2007)[12].

More than a century after the Bayer pharmaceutical company started selling aspirin, it still is one of the most widely used over-the-counter (OTC) drugs in the world. Nowadays over 100 billion tablets are produced and consumed annually, the equivalent to circa 35,000 tons. Despite facing hard competition coming from the generics manufacturers, aspirin is the 2nd top selling Bayer Consumer Health product, with sales close to €720M (BAYER 2009). With around 3500 scientific papers every year dedicated to it, aspirin is no longer regarded simply as a pain reliever or an anti-inflammatory drug. Figure 4.27 shows the current usage trends for aspirin, and recent studies claim that this drug might be used against colon, ovarian and breast cancer and also to improve brain function (Nordenberg 1999).

[12] Despite this being the "official" version of Bayer AG., there is still some controversy to whom should the discovery and commercialization be credited. *in* Sneader, W. (2000). "The discovery of aspirin: a reappraisal." British Medical Journal **2000**(321): 1591-1594.

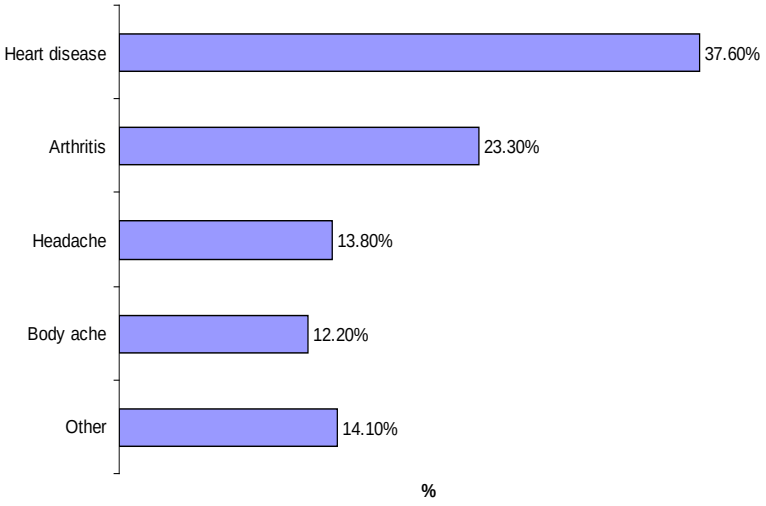
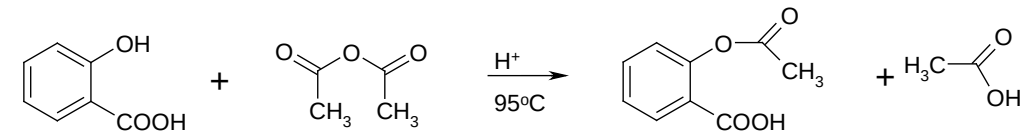


Figure 4.27 – Popular used for aspirin. *Source*: Bayer AG. (adapted)

The synthesis of acetylsalicylic acid, as idealized by Hoffmann, involves the acetylation of salicylic acid by acetic anhydride in an acidic medium, as showed in Figure 4.28:



salicylic acid — acetic anhydride — acetylsalicylic acid — acetic acid

Figure 4.28 The synthesis of acetylsalicilic acid

The traditional process of producing acetylsalicylic acid typically involves mixing a small stoichiometric excess of acetic anhydride with salicylic acid. This reaction is catalyzed by sulphuric acid and, and when performed at 85-90 ^{o}C takes between 20 minutes to 2 hours to complete (Kamlet 1956; McKetta and Cunningham 1977; Metz 2002).

4.2.4.a – Synthesis of acetylsalicylic acid

The synthesis of acetylsalicylic acid involves the following steps:

1. Weigh out 15 g of salicylic acid and transfer it to the batch OBR;
2. Add 40 mL of acetic anhydride and 2 mL of concentrated sulphuric acid;
3. Switch on the pre-heated water bath (T_{water} = 95 °C) to heat the OBR. Switch the oscillation (ν = 2.0 sec^{-1}; x_0 = 40 mm) on and mix for 10 minutes;
4. Transfer the solution to an Erlenmeyer flask. Chill the mixture using an ice-water bath and add 20 mL of purified water to decompose any excess acetic anhydride. Chill it until crystals of aspirin no longer form; mix it gently occasionally to decompose residual acetic anhydride. If an oily substance appears instead of a solid precipitate, reheat the solution until the substance disappears and cool it again.
5. Perform a vacuum filtration of the product, using purified water to wash the crystals;
6. Dry the crystals in the oven (30 °C; overnight);
7. Determine the mass of the crude aspirin crystals.

Each sample was taken at regular intervals from step 3) in the procedure, using a small pipette. All samples were purified in a similar fashion to the way described.

4.2.4.b – Results

Samples were taken from the reactor every 2 minutes for a total duration of 10 minutes. Figure 4.29 shows the temperature profile of the reaction at sampling times.

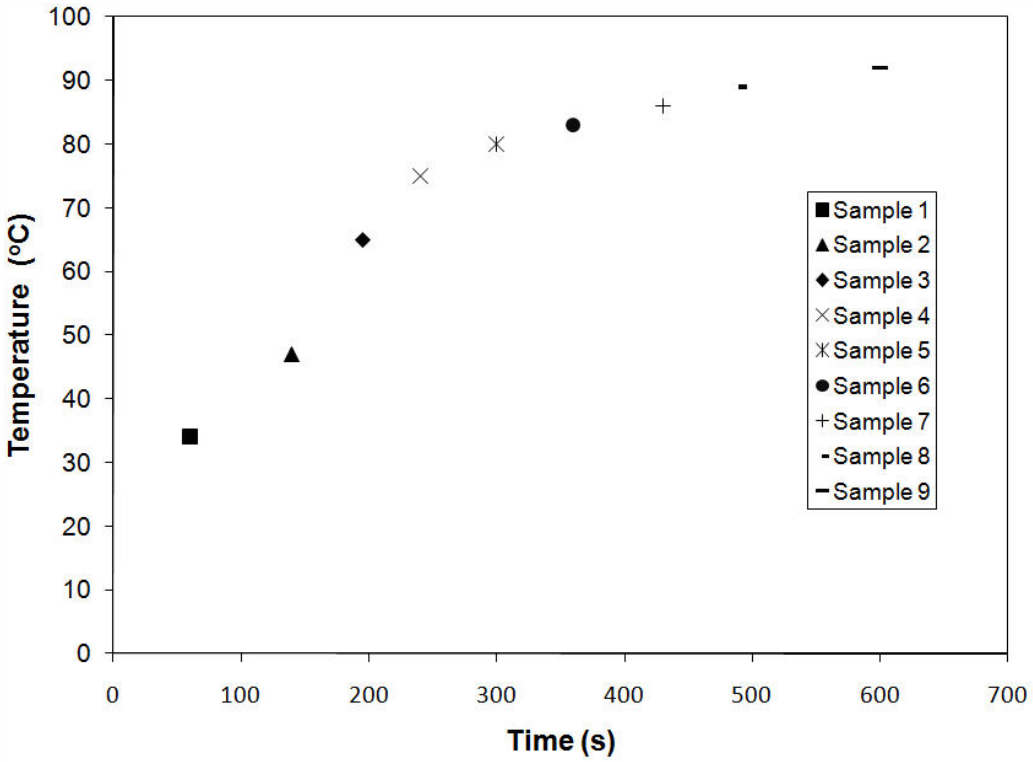


Figure 4.29 Temperature profile for the acetylsalicylic acid samples

The temperature measurements taken at sampling times indicate that the temperature inside the OBR rose steadily, quickly providing a suitable environment for the reaction to proceed rapidly.

It was also observed that 10 seconds after the heated water-bath was connected to the reactor and the oscillation was started the solution was already clear, with no signs of salicylic acid solid aggregates, a sign that the mixing inside the OBR was efficient. In fact, the HPLC chromatograms (Figures 4.30 and 4.31) indicated that even in the first sample (taken 60 seconds after the reaction was started) there was little sign of reactants left or side products, as confirmed in Figure 4.30 – the existent peak at 10 minutes of elution is relative to acetylsalicylic acid, as confirmed by the commercial sample chromatogram (Figure 4.32). The HPLC chromatograms for the remaining samples analysed can be found in the Appendix B. These results show that the reaction proceeded in the OBR at lower temperatures than those indicated to be necessary in the literature.

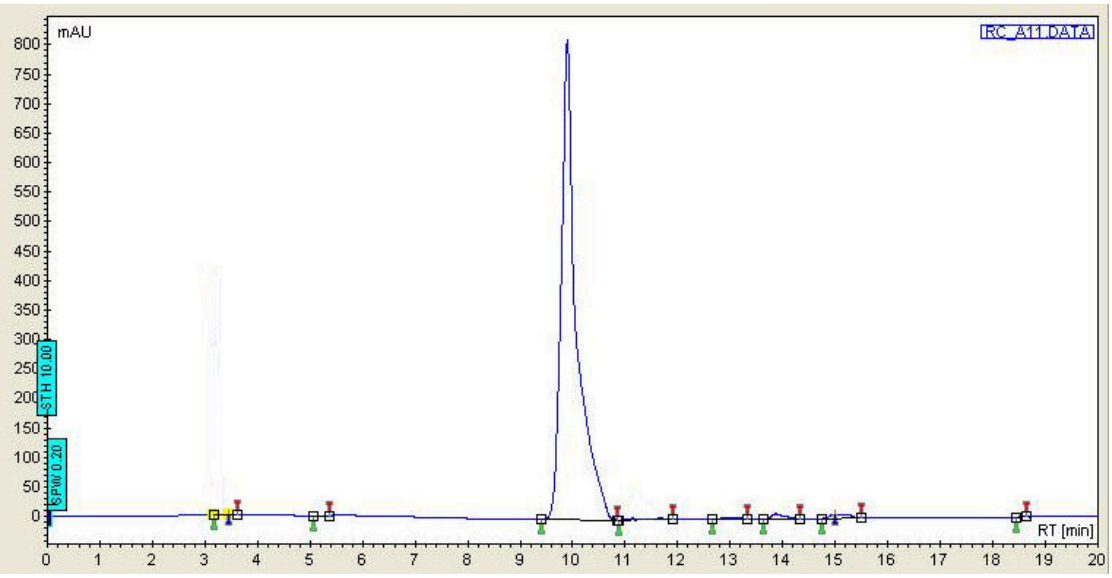


Figure 4.30 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 1

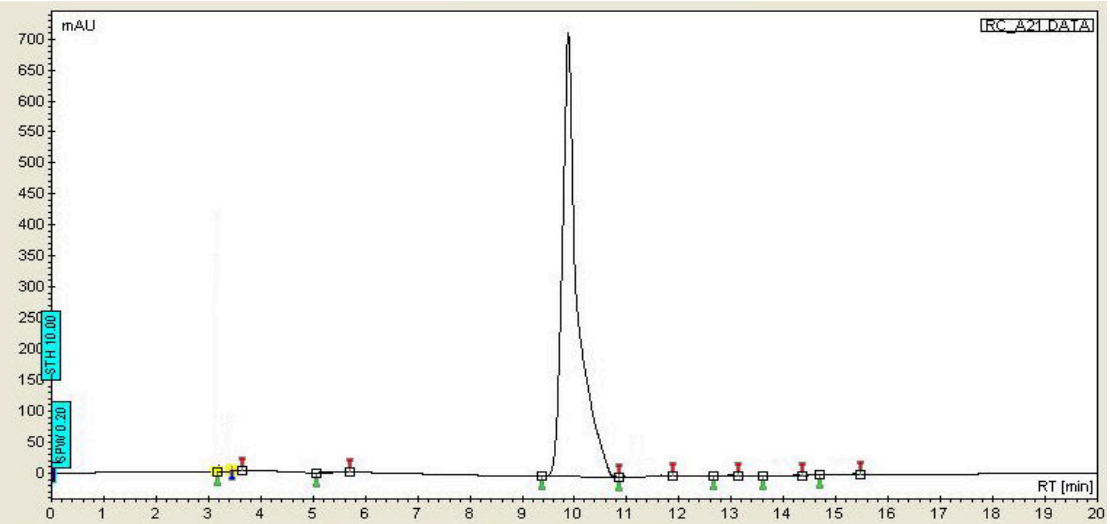


Figure 4.31 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 2

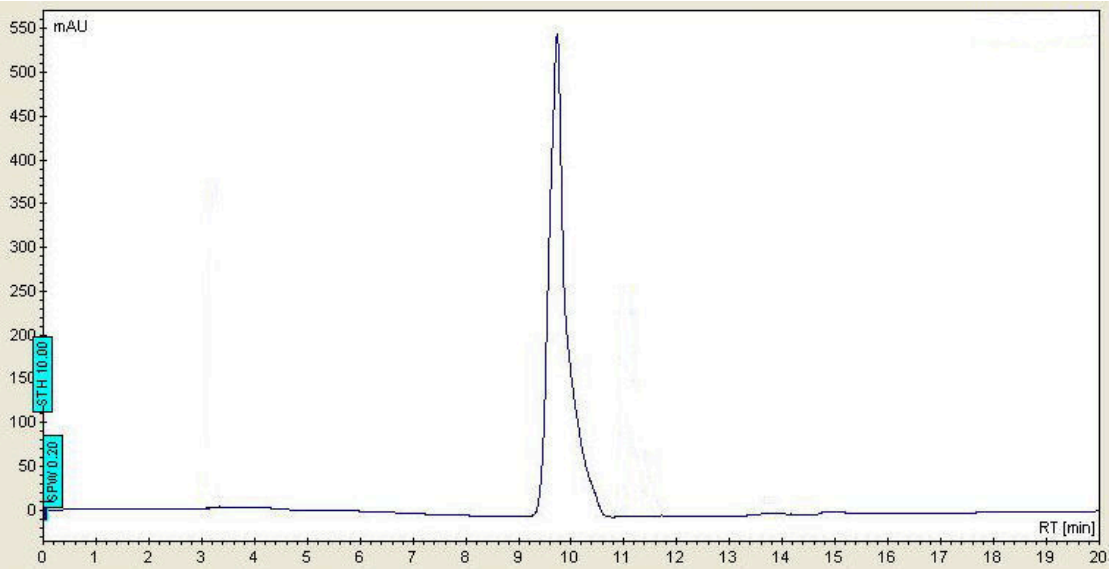


Figure 4.32 HLPC chromatogram of the pure aspirin sample

While it is known that higher temperature accelerates the reaction rate, the results show that the reaction can be undertaken under milder conditions. The product obtained after the crystallization and purification steps was in the form of fairly small crystals and white in colour.

The results obtained from the experiments clearly indicate that the recommended reaction time was greatly reduced. The reaction can be considered to be completed in two minutes, which is shorter when compared with what is found in literature: typical reaction durations range from 30 minutes to 2 hours (Kamlet 1956; McKetta and Cunningham 1977). The OBR proved to be a superior alternative once again, this time in the manufacture of a popular API. The superior heat transfer and mixing capabilities of the OBR confer this design a definite advantage over others in the manufacture of both fine chemicals and pharmaceuticals.

In light of these findings and of the fact that this chemistry fulfils all the pre-requisites set out in the beginning of the chapter, this was the second reaction to be chosen for further studies in COBR.

4.2.5 – Paracetamol

When it was discovered - by mere dispensing accident - that acetanilide had anti-pyretic activity despite causing serious haemoglobin deactivation problems, researchers at Bayer synthesized phenacetin, a chemical derivative of acetanilide. However, due to its damaging effects in the kidneys of long-term patients, phenacetin had to be replaced by an alternative with fewer side effects. It was in 1893 that Joseph von Mering, came up with such drug: paracetamol. Unfortunately, having proved its anti-pyretic and pain-relieving properties this new molecule was put on hold for nearly half a century, when von Mering wrongly affirmed that it had the same side-effects as acetanilide[13] (Ellis 1998).

[13] It is currently believed that the side effects that von Mering observed were due to an impurity, 4-aminophenol *in* Brown, T., A. T. Dronsfield, et al (2005). "Pain relief: from coal tar to paracetamol." Education in Chemistry **42**: 102.

By 1946 Bernard Brodie and Julius Axelrod were given a grant to investigate the effects observed by von Mering and discovered that acetanilide was indeed associated with methemoglobinemia and its therapeutic properties derived from paracetamol, to which it was metabolized in the human body (Brodie and Axelrod 1948). As a result they advocated the use of paracetamol to reduce pain and fever in patients and by 1955 paracetamol was first marketed in the USA by McNeil Laboratories, under the name Tylenol Elixir (Brown et al 2005).

Since then paracetamol has become one of the most widely used drugs in the world. The world paracetamol market is worth circa 800 million Euros, equating to circa 160 thousand tonnes produced worldwide (Chassany 2008). In the UK alone there are over 90 different drugs brands with paracetamol in its composition and the most popular of them all, Anadin, is worth over 65 million Euros, selling 27 million packs every year (ANADIN 2006; Naish 2008).

The synthesis of paracetamol involves the reaction of acetic anhydride with 4-aminophenol in aqueous medium (Figure 4.33):

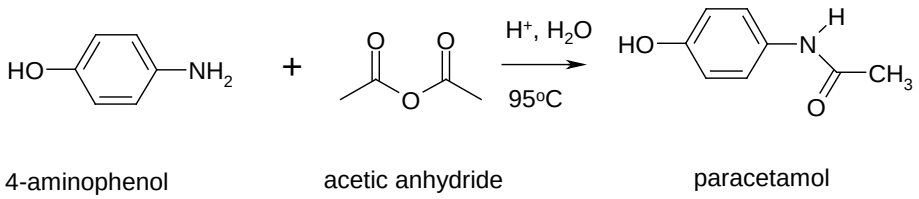


Figure 4.33 The synthesis of paracetamol

In the traditional process for the synthesis of paracetamol, p-aminophenol is mixed with a small stoichiometric excess of acetic anhydride in an aqueous medium at high temperature and in the presence of phosphoric acid, which acts as a catalyst. The benchmark reaction time is circa 20 minutes for laboratory scale synthesis, as described in literature (Pavia et al 1990),

4.2.5.a – Synthesis of Paracetamol

1. Weigh 1.8 g of p-aminophenol; transfer it to the batch OBR;
2. Add 30 g of pure water and 2 ml of phosphoric acid. Mix it until the amine has been dissolved;
3. Switch on the pre-heated water-bath (T_{water} = 80 ^{o}C) to heat the OBR and carefully add 2.4 mL of acetic anhydride to the OBR;
4. Mix the solution for 10 minutes (ν = 2.0 sec^{-1}; x_0 = 40 mm) and transfer the solution to a flask;
5. Chill the mixture in an ice-water bath;
6. Perform a vacuum filtration of the mixture, washing the flask with purified water;
7. Oven-dry the produced crystals (30 °C; 2h);
8. Determine the mass of the crude paracetamol crystals.

Each sample was taken with regular intervals from step 4) in the procedure, using a small pipette. All samples were purified according to the protocol.

4.2.5.b – Results

Samples were taken from the reactor regularly for a total duration of 6 minutes. Figure 4.34 illustrates the temperature profile at the sampling times. It shows that the temperature rise in the OBR was quick, providing the required heat for the reaction to progress.

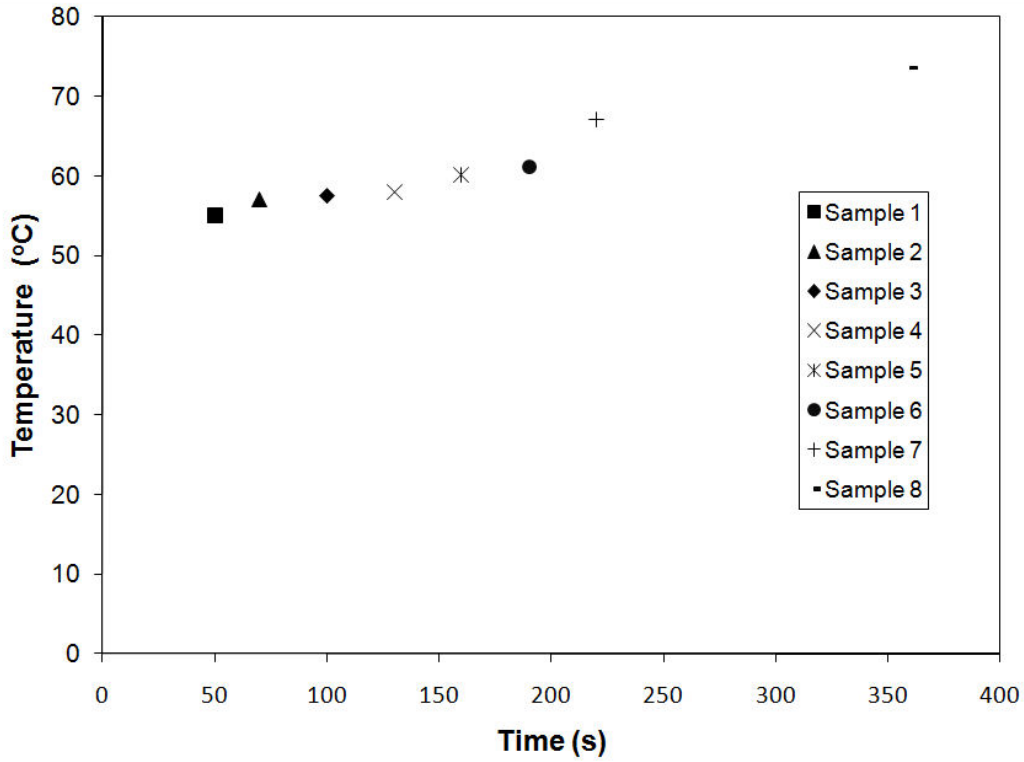


Figure 4.34 Temperature profile for the paracetamol samples

The HPLC data revealed that the reaction was considered complete well before the reaction time cited in literature, and the reaction progressed fast even though it was performed at lower temperatures. The amine was dissolved 30 seconds after the oscillation was started and the last sample was taken at the end of 6 minutes of reaction, at which point the low volume left inside the OBR meant that it was not feasible to take more samples from the reactor. The peak found in each of the chromatograms with an elution time of 7.5 minutes (Figures 4.35 and 4.36) refers to acetaminophen, which is identical to the peak found for the pure chemical compound (Figure 4.37). The HPLC chromatograms for the remaining samples analysed can be found in the Appendix C.

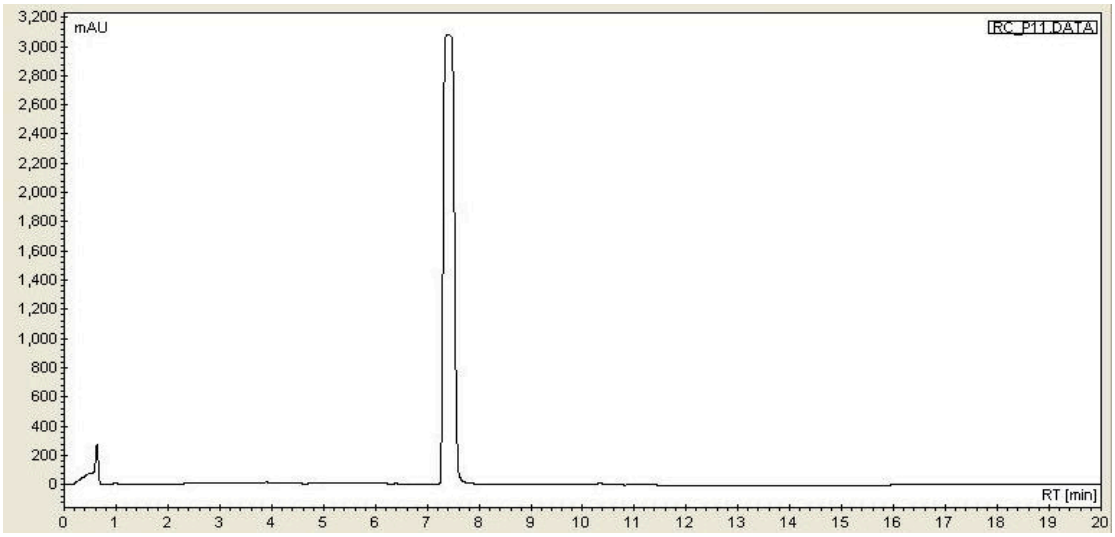


Figure 4.35 HLPC chromatogram of the synthesized paracetamol, sample 1

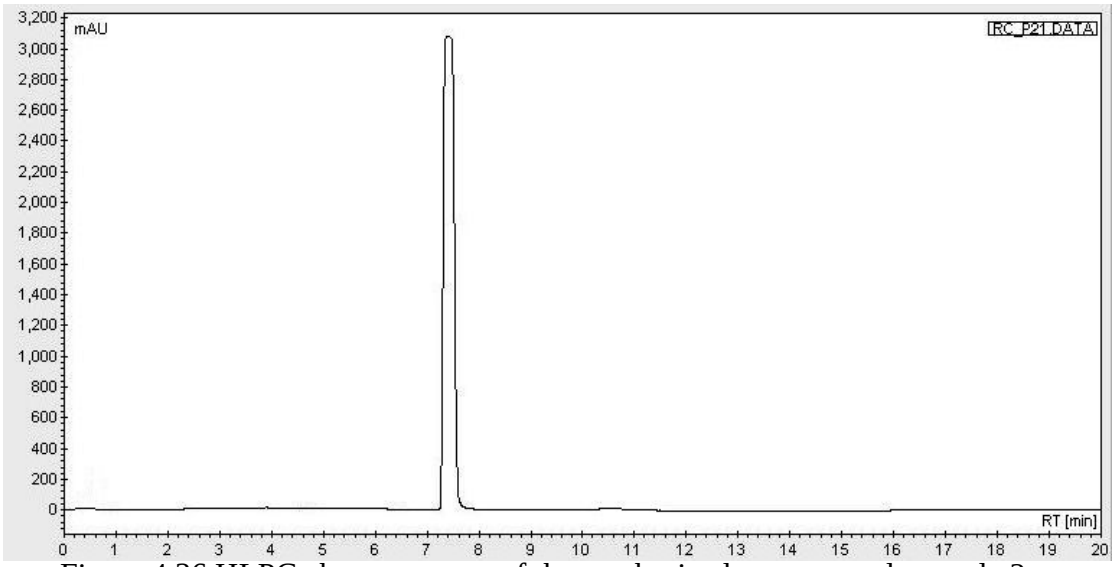


Figure 4.36 HLPC chromatogram of the synthesized paracetamol, sample 2

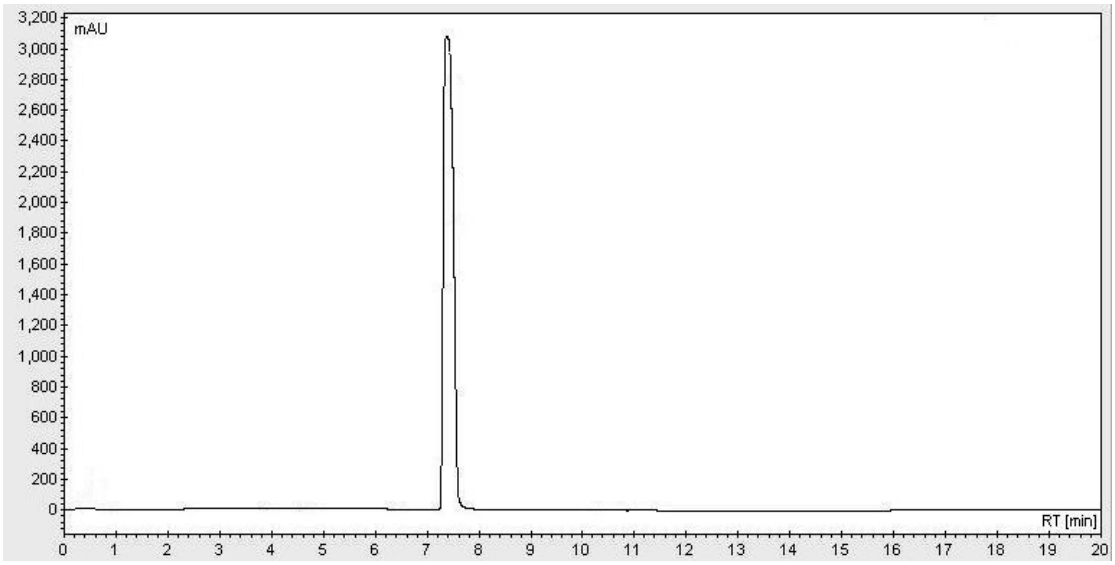


Figure 4.37 HLPC chromatogram of the pure paracetamol sample

In line with the findings for the acetylsalicylic acid experiments, the temperature profile and chromatograms suggest that the reaction can be carried out under milder conditions, given the short reaction times. The end product was in the shape of large white crystals (0.5-1 cm).

This was the third chosen chemistry to be studied in COBR. Paracetamol is one of the most common anti-inflammatory drugs in the world and, in tune with what happens for acetylsalicylic acid and vanisal sodium, it complies with all the selection criteria.

The OBR proved to be a better alternative to the STR in each of the five chemistries studied. The constant pattern of eddie formation and cessation inside each cell of the OBR increase the probability of different particles to collide and react with each other. This translates into better mixing and heat transfer capabilities and allows for faster reaction times, even at lower process temperatures, as it was proven in the experiments described in the previous sections. A finer control of process variables, such as reaction temperature, particle morphology and even particle size modulation can be achieved – this is a significant OBR advantage over the STR that has to be taken in consideration by pharmaceutical and fine chemical companies trying to optimize their manufacturing processes. Table 4.3 compares the chemistries that underwent screening, summarizing which selection criteria each chemical reaction fulfils. On this basis, the chosen chemical compounds to be studied further in COBR were vanisal sodium, acetylsalicylic acid and paracetamol.

Table 4.3 Selection criteria fulfilled by the screened chemistries

	Bis(2,4,6-trichlorophenyl) Oxalate	Methyl Diantylis	Vanisal Sodium	Acetylsalicylic Acid	Paracetamol
Chemistry type	FC	FC	FC	API	API
Relevance	✓	✓	✓	✓	✓
Reaction time	✓	✓	✓	✓	✓
Cost	✗	✗	✓	✓	✓
Safety	✗	✓	✓	✓	✓

Key: FC – fine chemical; API – active pharmaceutical ingredient

4.3 – Summary

This chapter described the experimental results for the five different chemical entities that were screened. While the results obtained were positive with regard to reaction conditions, reaction times and product yields, the chemical entities to be subjected to further studies in COBR were narrowed down to three. All the selection criteria were fullfiled for the following chemistries: vanisal sodium, acetylsalicylic acid and paracetamol.

CHAPTER 5
COBR EXPERIMENTS

Argument is conclusive, but it does not remove doubt,
so that the mind may rest in the sure knowledge of the truth,
unless it finds it by the method of experiment.

Roger Bacon, philosopher

From the results obtained in the screening experiments it was determined that three chemistries, namely vanisal sodium, acetylsalicylic acid and paracetamol, were better suited for the purpose of studying the robustness and adaptability of the COBR, as well as the degree of cross-contamination. One of advantages of this mixing technology is that the results obtained from the batch OBR can directly be applied to the continuous operation because the scale up is linear. This means that the procedure, the operation and the analysis done at the screening stage are transferable here with minimal adaptations.

Critical product and chemical process attributes, such as reaction times, particle size, morphology and campaign waste and turnaround times were given special relevance in the COBR experiments due to the importance of these aspects to the fine chemical and pharmaceutical industries. The experimental procedure and the results gathered for each of these are described in the following sections, preceded by mixing and residence time distribution (RTD) considerations for the used COBR.

5.1 – Mixing and Residence Time Distribution

Inside a COBR with no oscillation being applied a particle is subjected to three different forces: gravity, buoyancy and fluid drag. Therefore, the particle will

continuously accelerate downwards until the fluid drag force balances out the force of gravity, at which point it will fall at a constant velocity until it is deposited in the COBR wall (Levenspiel and Kunii 1991; Coker and Ludwig 2007). This constant velocity is also referred to as terminal velocity and can be derived in the following way:

$$m\frac{du}{dt} = F_g - F_b - F_d = 0 \tag{5.1}$$

where F_g is the force of gravity, F_b the buoyant force and F_d the drag force (N). Thus:

$$F_g - F_b = F_d \tag{5.2}$$

and

$$F_g - F_b = V_p(\rho_p - \rho_l)g = \frac{m}{\rho_p}(\rho_p - \rho_l)g \tag{5.3}$$

$$F_d = \frac{C_D u_t^2 A_p \rho_l}{2} \tag{5.4}$$

Substitution of equations 5.3 and 5.4 in 5.2 gives:

$$\frac{m}{\rho_p}(\rho_p - \rho_l)g = \frac{C_D u_t^2 A_P \rho_l}{2} \tag{5.5}$$

$$u_t = \sqrt{\frac{2g(\rho_p - \rho_l)m}{A_p \rho_p C_D \rho_l}} \tag{5.6}$$

where u_t is the terminal velocity (m.s^{-1}), g the gravitational acceleration (m.s^{-2}), ρ_p and ρ_l the densities of the particle and of the fluid, respectively (kg.m^{-3}), m the mass of the particle (kg), A_p the projected area of the particle (m^2) and C_D the drag coefficient. If the shape of the particle is considered to be spherical with a diameter D, then the particle mass is

$$m = \frac{\pi D^3 \rho_p}{6} \tag{5.7}$$

and its projected area is

$$A_p = \frac{\pi D^2}{4} \tag{5.8}$$

Substitution of equations 5.8 and 5.7 into equation 5.6 gives:

$$u_t = \sqrt{\frac{4g(\rho_p - \rho_l)D}{3C_D \rho_l}} \tag{5.9}$$

In order for particles to remain in suspension, other forces must come into play. The combined velocity imposed in the COBR by the net flow and the oscillatory motion must then be equal or greater than the terminal velocity of the particles, if it is to remain in suspension in the fluid.

Applying oscillatory motion to a fluid in a baffled tube results in the improvement of mixing conditions of the system. Whereas mixing is independent of net flow in a COBR, this does not happen in a traditional PFR, where a minimum Reynolds number has to be maintained for good mixing to occur. One of the advantages of the COBR is that it allows for a small net flow type of operation while maintaining good mixing conditions. It was shown by Harvey and colleagues (2001) that an increase of Re_n will not significantly impact on the rheology and residence time distribution of the system.

Good mixing was achieved in the COBR during the three week-long campaigns due to the imposition of a high oscillatory Reynolds number (Re_o) coupled with a low flow rate. This ensured that good radial mixing and narrow RTD was achieved, guaranteeing that all the non-dissolved particles remained in suspension, thus preventing deposition and, in a worst-case scenario, the clogging of the COBR. The

utilization of a low net flow also allows for savings to be made in expensive raw materials. A comparison between the terminal velocity and the mixing velocity in the COBR for the aspirin and paracetamol campaigns can be found in Table 5.1. vanisal sodium's product is in the form of very fine particles and can thus be easily suspended in solution in the COBR.

Table 5.1 Comparison between terminal velocity (u_t) and mixing velocity (u_m) for the aspirin and paracetamol campaigns

	Aspirin	Paracetamol
ρ_p (kg.m^{-3})	1.350	1.263
ρ_l (kg.m^{-3})	1.108	1.046
D (m*10^{-6})[14]	400	425
C_D [15]	0.8	0.8
u_t (m.s^{-1})	**0.0378**	**0.0379**
x_0 (m)	0.02	0.02
f (s^{-1})	2	2
u_o (m.s^{-1})	0.04	0.04
u_n (m.s^{-1})	0.0002	0.0019
u_m (m.s^{-1})	**0.0402**	**0.0419**

It is evident that u_m was superior to u_t for both cases. This ensured good mixing conditions in the COBR and that the particles remained in suspension for the duration of the campaigns.

[14] These are literature values taken from crystallization experiments for the studies chemistries (*in* Cambeiro, L. et al (2006). "Crystallization of Aspirin Crystals in Polar and Non Polar Solvents in the Presence of Surfactants: An Experimental Approach to Control Size and Shape of Crystals." The New Jersey Governor's School of Engineering and Technology Research Journal. *and* Granberg, R. A. and Å. C. Rasmuson (2005). "Crystal growth rates of paracetamol in mixtures of water + acetone + toluene." Particle Technology and Fluidization **59**(1): 2441 - 2456.

[15] Value taken from Hölzera, A. and M. Sommerfeld (2007). "New simple correlation formula for the drag coefficient of non-spherical particles " Powder Technology **184**(3): 361-365.

5.2 – Vanisal Sodium

Vanisal sodium was the first chemical compound to be produced in the purpose-built COBR. The experimental method is described next. This is followed by the results gathered during a week-long run.

5.2.1 – Experimental Method

The operational procedure is as follows:

1) The COBR was flooded with purified water;
2) The COBR and the OBR feeder were heated up to 60 ^{o}C and oscillation was started (ν = 2.0 sec^{-1}; x_0 = 40 mm);
3) 143 mL of sodium hydrogen sulphite 40% was measured;
4) 77.1 g of vanillin was weighted and 700 mL of purified water was measured. These were transferred to the OBR and mixed until vanillin was dissolved;
5) The COBR was fed with the contents of the OBR feeder along with the sodium hydrogen sulphite via the second feeder line;
6) The feed rate of the contents to the COBR was checked at a flowrate of 2 mL.min^{-1} and temperature readings were taken;
7) Samples were taken and dried for analysis;
8) Steps 3 to 7 were repeated 27 times.

5.2.2 – Results

Vanisal sodium was synthesized in a continuous fashion using the purpose-built COBR for the duration of seven days, 24 hours a day. The temperature measurements from the four thermocouples during the reaction are shown in Figure 5.1 and constant temperatures are seen along the COBR. This indicates that the environment within the COBR is highly consistent and reliable for the reaction, promoting the synthesis of a highly homogenous and uniform product throughout the campaign. Samples were taken regularly from the sampling valves and analyzed and the amount of vanisal sodium produced was plotted against time.

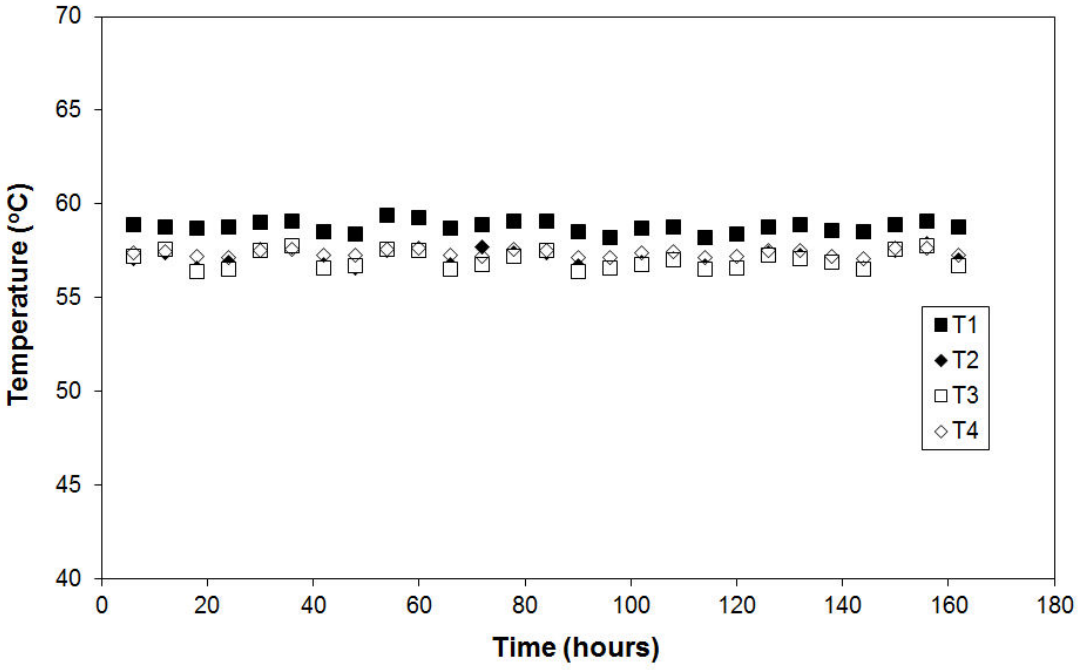


Figure 5.1 Temperature measurements during the vanisal sodium reaction

Figure 5.2 displays the data from the sample locations S1 and S4 along the COBR as shown in Figure 3.4. It is clear that there is a time delay in the product at the start from the furthest sample port in comparison to that at the closest location from the feed line. The production of vanisal sodium was consistent during the whole the operation, in light of the stability of the temperature readings and concentration data. The samples recovered and dried all had the same brittle white solid appearance, which was consistent with both the sample recovered and dried in the OBR experiment (see

Chapter 4) and the description given by Luca Turin in the private correspondence maintained.

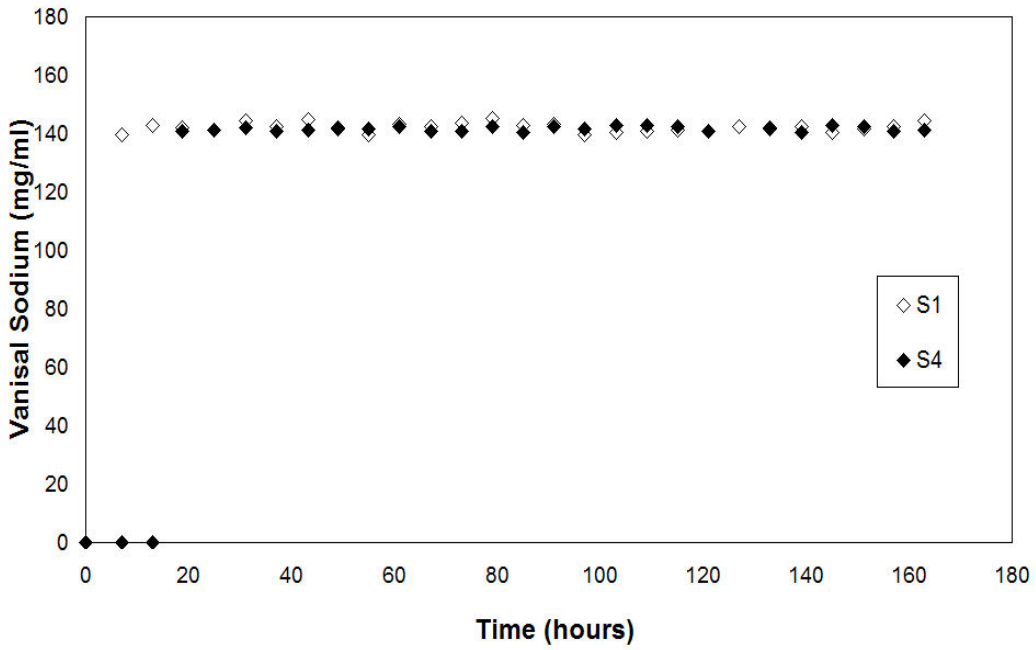


Figure 5.2 Concentration profile for the vanisal sodium production run

The purity of the product was assessed using both NMR spectroscopy and HPLC over 54 samples, with 99.92% and 99.95% purity of vanisal sodium, respectively. The calculated average was 99.94%, which demonstrates the robustness of both analytical methods and the high product purity obtained in this campaign. The percent weight yield, calculated as the ratio between the actual yield over the theoretical yield (Kotz et al 2009), averaged 91.4% over the analysed samples.

The cleaning of the COBR started after the production run had been completed, so that cross-contamination could be assessed and the second campaign could be started. A cleaning-in-place (CIP) procedure was used, as explained previously in Chapter 2. As this is the first time that the cleaning process and procedure in a COBR is reported, two dimensionless groups are introduced that are relevant to the protocol: the cleaning index and the wash index.

In order to assess the cleaning intensity of a cleaning process, it is useful to employ the cleaning index (Ψ), which is defined as the ratio between the net flow Reynolds numbers and the oscillatory Reynolds numbers used in both the cleaning (Re_c) and the operation (Re_{op}) periods. This ratio allows for a comparison to be made between the various cleaning procedures that took place during this project. It also allows for future comparisons to be made, if continuous oscillatory reactors are tested with different chemistries or operating conditions:

$$\Psi = \frac{\mathrm{Re}_c / \mathrm{Re}_O}{\mathrm{Re}_{op} / \mathrm{Re}_O} \tag{5.10}$$

When the same oscillation conditions (*Re*o) were used, as in this campaign, the cleaning index is defined in this case as:

$$\Psi = \frac{\mathrm{Re}_c}{\mathrm{Re}_{op}} \tag{5.11}$$

An important measure of the effectiveness of a cleaning procedure is the amount of waste produced during this operation. More specifically, it is of interest to compare the volume of the used chemical reactor with the volume of the waste generated. This can be studied and understood using the wash index (Ω), which is defined as the ratio between the washing volume (V_w) and the reactor volume (V_r):

$$\Omega = \frac{V_w}{V_r} \tag{5.12}$$

The cleaning procedure was defined having in account previous experience and advice from the pharmaceutical industry (Norton 2009). As such, the cleaning operation was performed by continuously pumping tap water (measured pH = 7.3) in the COBR (60 °C, ~0.5 L min^{-1}), immediately followed by an industrial “free rinsing” cleaning

solution (Liquinox - Solujet, 1% solution), and lastly the USP grade water at room temperature. The oscillation frequency and amplitude were 2 Hz and 40 mm, respectively and the cleaning index was 228. Four samples per minute were taken from the COBR during the cleaning phase and were analysed using the HPLC method previously described. Figure 5.3 shows the remaining concentration of vanisal sodium during the washing process in the vertical axis, while the volume of washing liquids employed is the horizontal axis. This volume is also related to the time of cleaning liquids used as the horizontal axis in Figure 5.4. The dotted line in Figure 5.3 denotes the calculated maximum residual level of vanisal sodium (0.026 $mg.mL^{-1}$) allowable when the COBR is changed over to produce aspirin, in accordance to the limits set by the International Pharmacopeia (2008), including a 100-fold safety factor, as shown:

$$\text{Vanisal sodium limit} = \frac{C_{\text{min asprin in COBR}} - (C_{\text{min asprin in COBR}} \times \text{Max. impurity in aspirin})}{100} \qquad (5.13)$$

Maximum impurities level in aspirin (IP) = 1%

Minimum expected quantity of aspirin in COBR = 265 mg ml^{-1}

It is evident that the COBR was clean well before the cleaning procedure had been completed. The wash index was 5.18, meaning that a total 10.8 L of washing liquids was used until the reactor was clean. To further prove the effectiveness of the cleaning procedure, the reactor was disassembled at ports V3 and V4 (possible dead legs due to valve design) and visually inspected for solid vanisal sodium residues. No residues were found at those locations, confirming that the reactor was clean.

The loss of product, which is defined in this case as the ratio of the amount of vanisal sodium lost in the cleaning over the total vanisal sodium produced, was 0.001%. This is on a substantially lower level than the industry norm of 0.1-0.2% (Norton 2009). This cleaning procedure proved to be complete and efficient and, with that in mind, the same method was applied in the end of each of the following productions (Sections 5.2.2 and 5.2.3). The time used in cleaning is considerably shorter than the total operation time, circa 0.28% of the total running time.

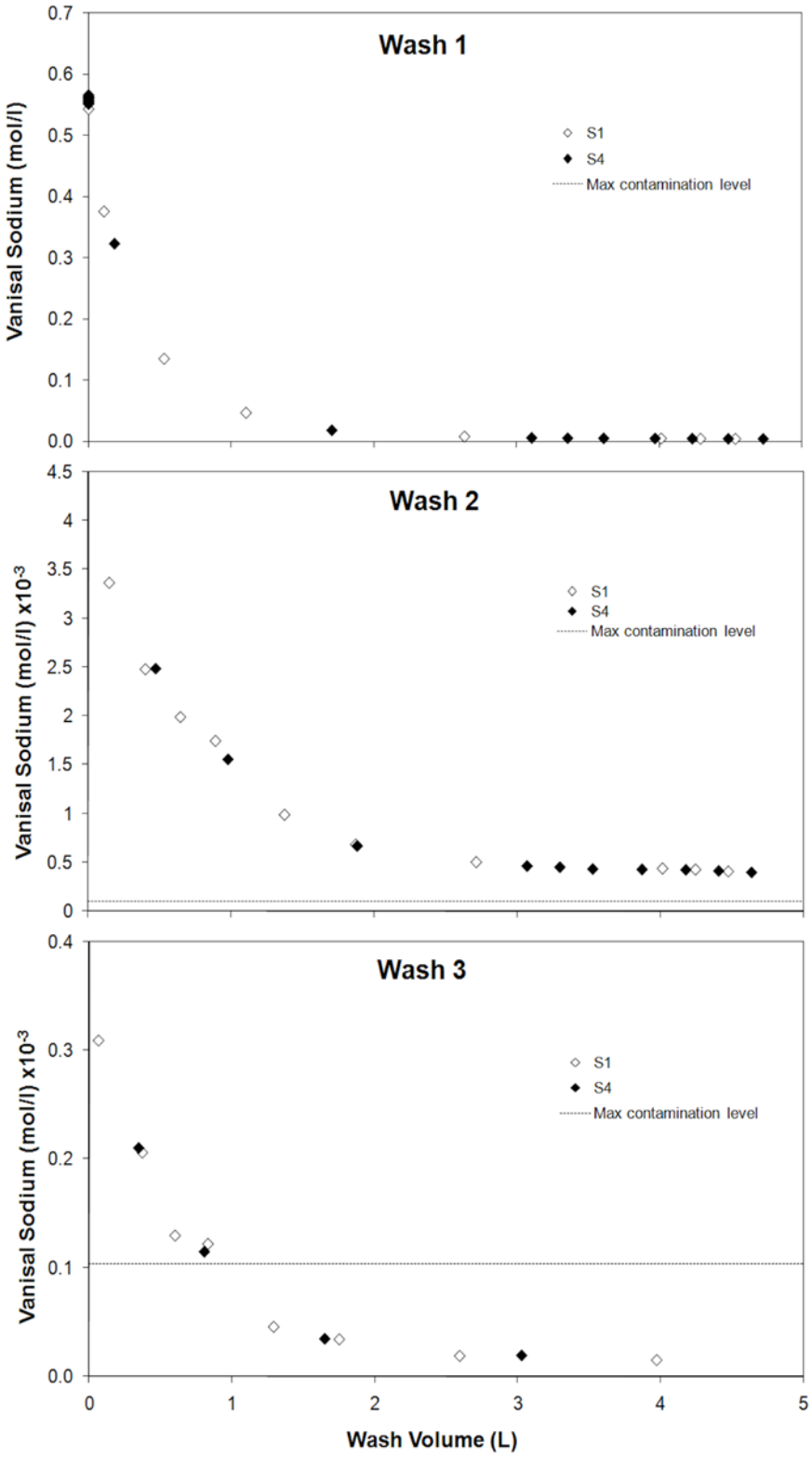


Figure 5.3 Washing data for vanisal sodium

Samples from ports S1 and S2 were taken during the cleaning process and quenched for analysis. The kinetics of the cleaning process were assessed from the washing data gathered from the samples and are shown in Figure 5.4 by plotting $-\ln(C/C_0)$ against time, where C is the concentration of the studied chemical species, vanisal sodium in this case, at a given time t (mg mL^{-1}) and C_0 the concentration of that chemical species at the start of washing (mg mL^{-1}). The linearity of the fits in Figure 5.4 confirms first-order kinetics for the washing process in the COBR, with an averaged rate constant of 0.0046 s^{-1} and $t_{1/2}$ of 150 s. It should be noted that the process of washing using water or detergent may not involve chemical reactions, the reaction kinetics adopted here is a reasonable means to study the washing process.

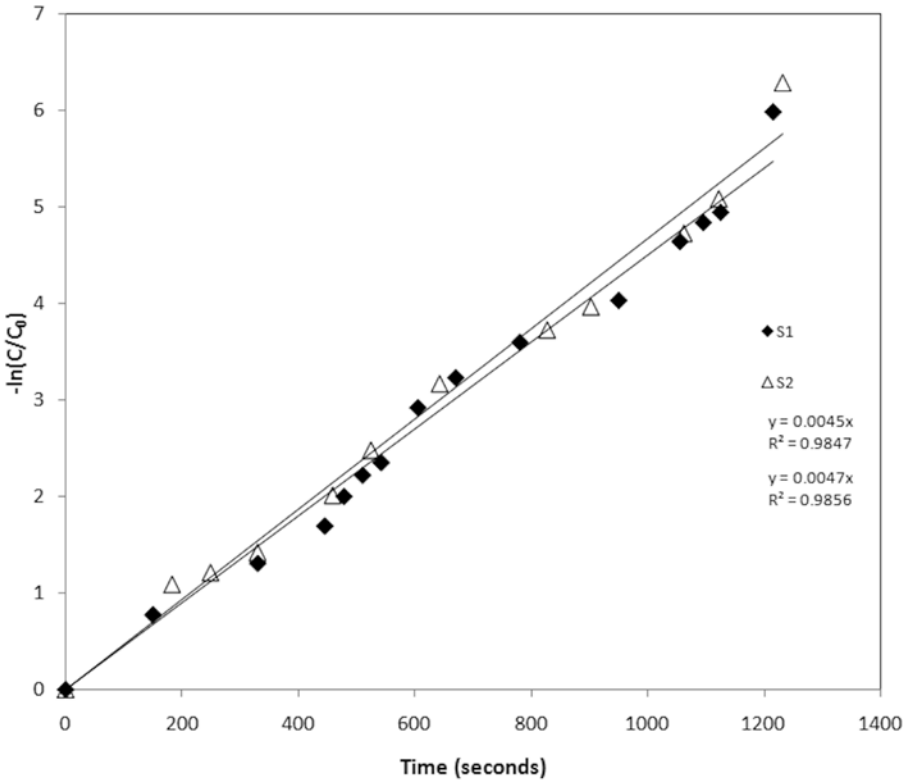


Figure 5.4 First order kinetics plot for vanisal sodium cleaning data

Our data are in line with several other studies involving tubular equipment where cleaning was described as a first-order kinetics (Jennings et al 1957; Bourne and Jennings 1963; Karlsson et al 1998; Cabero et al 1999; Lelievre et al 2002).

5.3 – Acetylsalicylic Acid

The synthesis and crystallization of aspirin was carried out for a period of 7 days, 24 hours a day. In the next section the experimental procedure is described, followed by the results obtained for the production campaign, including the cleaning phase. The kinetics of the reaction were also studied and the results are presented.

5.3.1 – Experimental Method

The experimental procedure used in this campaign includes:

1) The COBR was flooded with acetic anhydride and the first 4 sections were heated up to 60 ^{o}C. The COBR oscillation was started (ν = 2.0 sec^{-1}; x_0 = 40 mm);
2) 263.1 g of salicylic acid was weighted. It was then transfered to the OBR feeder, previously heated up to 90^{o}C. 700 mL of acetic anhydride were added;
3) The COBR was fed with the contents of the OBR feeder, along with sulphuric acid via the second feed line;
4) The feed rate of the contents to the COBR was checked at a flowrate of 2 mL.min^{-1} and temperature readings were taken;
5) A sample was taken for analysis;
6) Steps 2 to 5 were repeated 27 times.

5.3.2 – Results

In this production process the reaction takes place first, which is then followed by crystallization. It was therefore necessary to create a controlled temperature decrease along the COBR for the crystallization to take place, as it is shown in Figure 5.5. It is evident that during the week-long synthesis the temperature measured at each of the four thermocouples was stable and consistent.

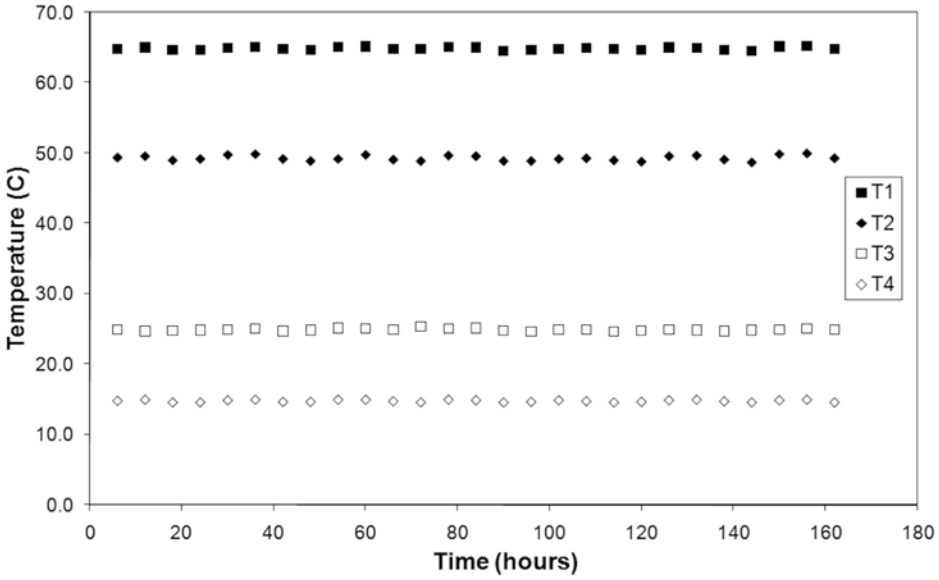


Figure 5.5 Temperature measurements during the acetylsalicylic acid production run reaction

The amount of acetylsalicylic acid produced as a function of time is plotted in Figure 5.6 and once again it is visible that the production of aspirin is consistent over the course of the process. The purity of the product analyzed by HPLC was 99.57% over 54 samples. The operational consistency of the COBR goes in line with what was found for the production of vanisal sodium. The average yield over the 54 samples was 77.4%, which is higher than that the 75% indicated in literature even though the concentration used in this work is lower than that found in literature (Kamlet 1956), as shown in Table 5.2.

Table 5.2 Experimental conditions and results for this work and in the literature (Kamlet 1956)

	Concentration of limiting reactant (Salicylic acid) ($g.L^{-1}$)	Temperature of Reaction (^oC)	Product Concentration ($g.L^{-1}$)	Percent Yield (%)
Kamlet (1956)	661.3	90-92	646.5	75
This work	263.1	90	268.5	77.4

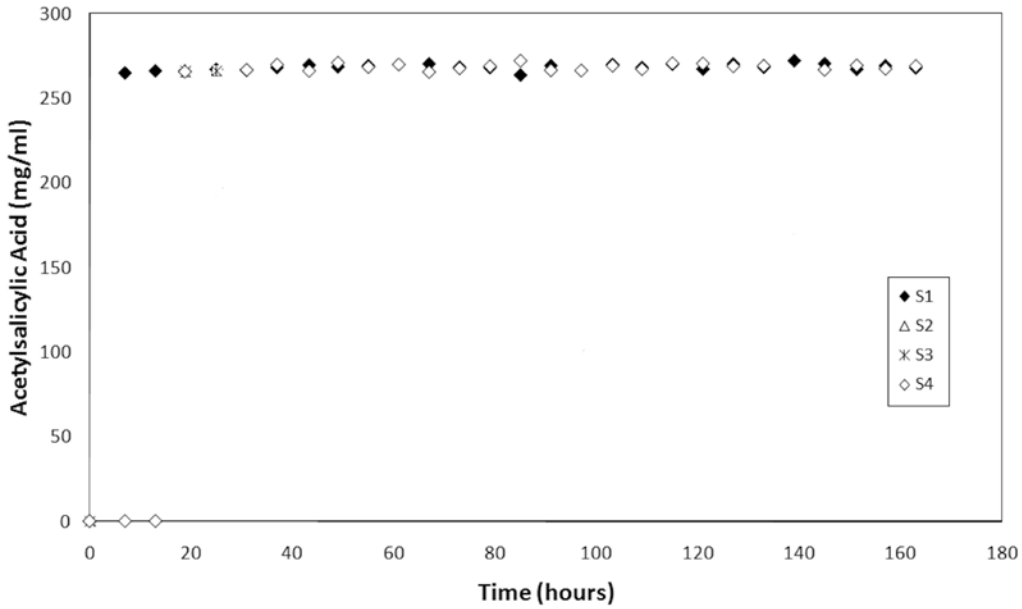


Figure 5.6 Acetylsalicylic acid produced continuously in the COBR

The polymorphism of aspirin has been a subject of controversy in recent times. While some authors claim that there is a second form of the acetylsalicylic acid crystal (classified as form II) in addition to form I, others doubt of its existence (Vishweshwar et al 2005; Amato 2007; Bond et al 2007). Nevertheless, the XRD results in Figure 5.7 confirm that the synthesized acetylsalicylic acid is of the form I, which is consistent with what is found in literature (Cambeiro et al 2006). The SEM images taken from the samples at the location S4 in the COBR are given in Figure 5.8.a for different times of operation. The obtained crystals of aspirin are all of the plate-type form, which again

shows the consistency of the product during the week-long run. These crystals are comparable to the ones obtained by Cambeiro et al (2006) shown in Figure 5.8.b

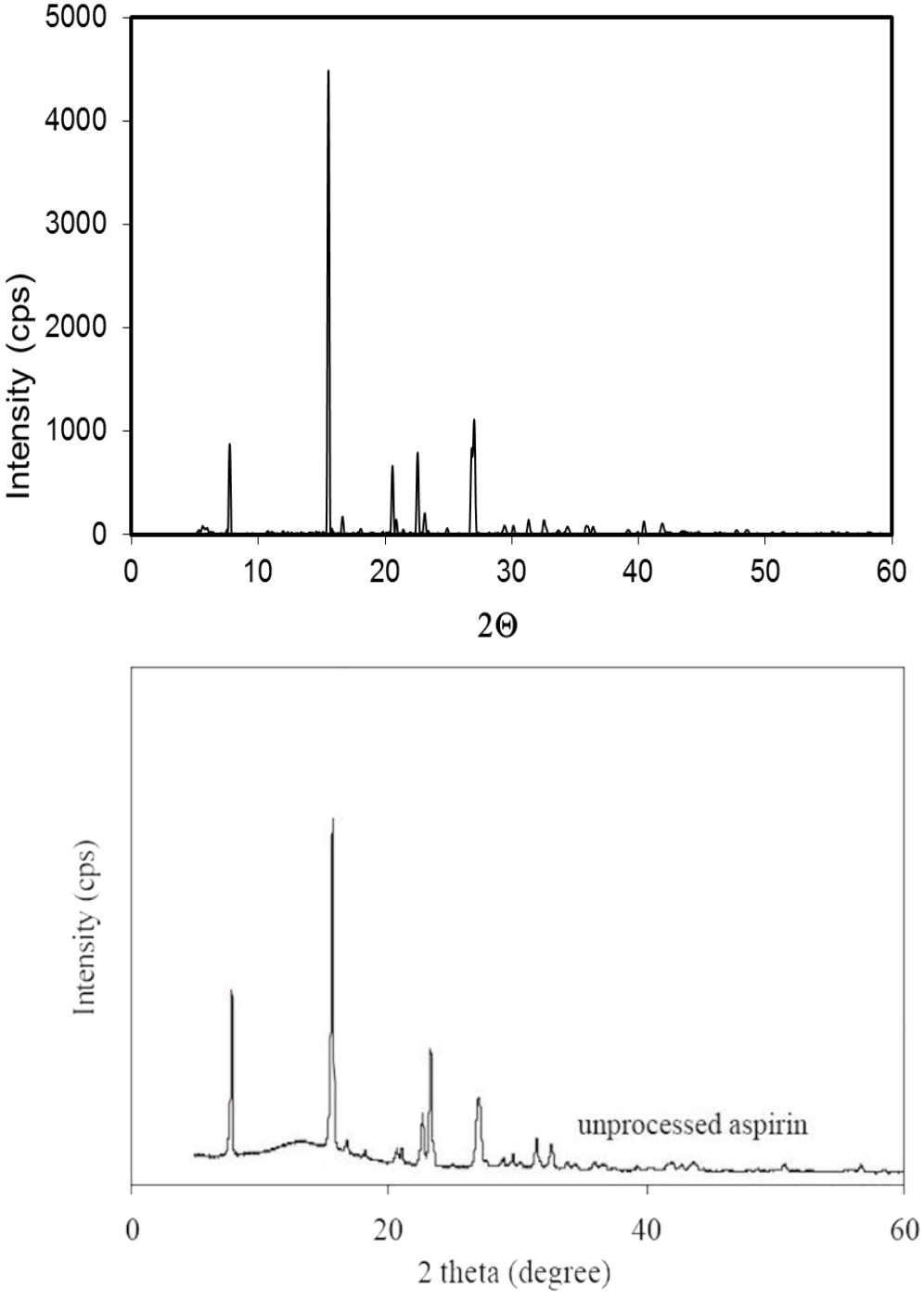
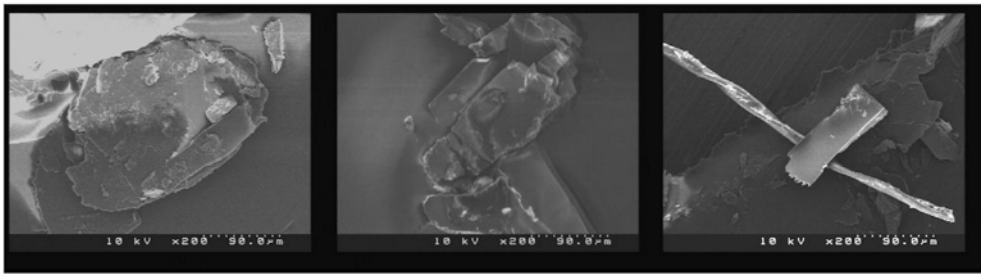
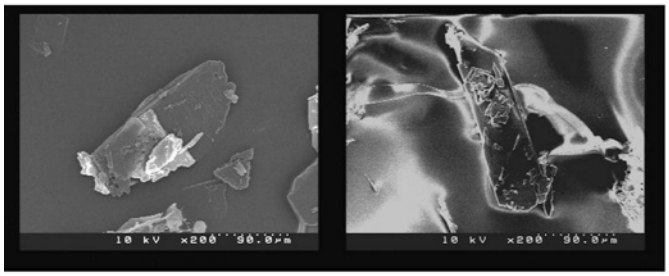


Figure 5.7 a) XRD results for the produced acetylsalicylic acid; b) XRD results for acetylsalicylic acid (form I) in Huang et al (2005)





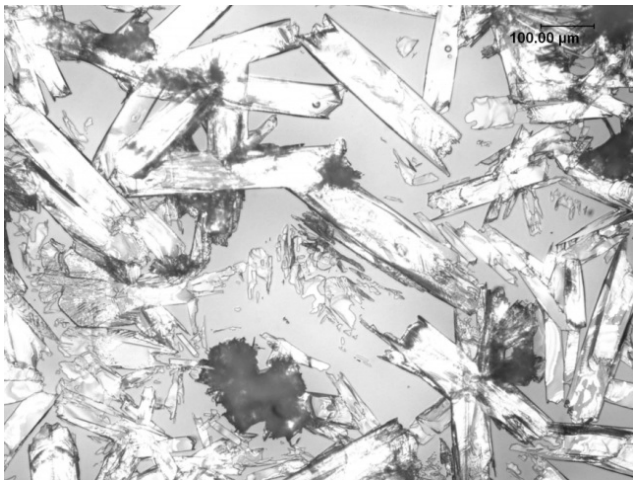


Figure 5.8 a) SEM images of produced acetylsalicylic acid crystals; b) Plate shaped acetylsalicylic acid crystals, *in* Cambeiro et al (2006)

The reaction kinetics between salicylic acid and acetic anhydride in the COBR was studied. This was done by following the concentration of salicylic acid during the reaction. Samples were taken at each of the sampling ports in the COBR, immediately quenched, analyzed using HPLC and averaged over the number of samples at each site. The concentration of salicylic acid as a function of time was then plotted and used to confirm the order of the reaction and evaluate the rate constant of the reaction. Figure 5.9 shows ($1/C_{SA} - 1/C_{SA_0}$) against the time of reaction, where C_{SA0} and C_{SA} are the

concentration of salicylic acid at the start and at anytime during the reaction. The concurrence between the results and the linear regression indicates that the reaction follows a second order kinetics with a rate constant of 0.029 $L.g^{-1}.s^{-1}$.

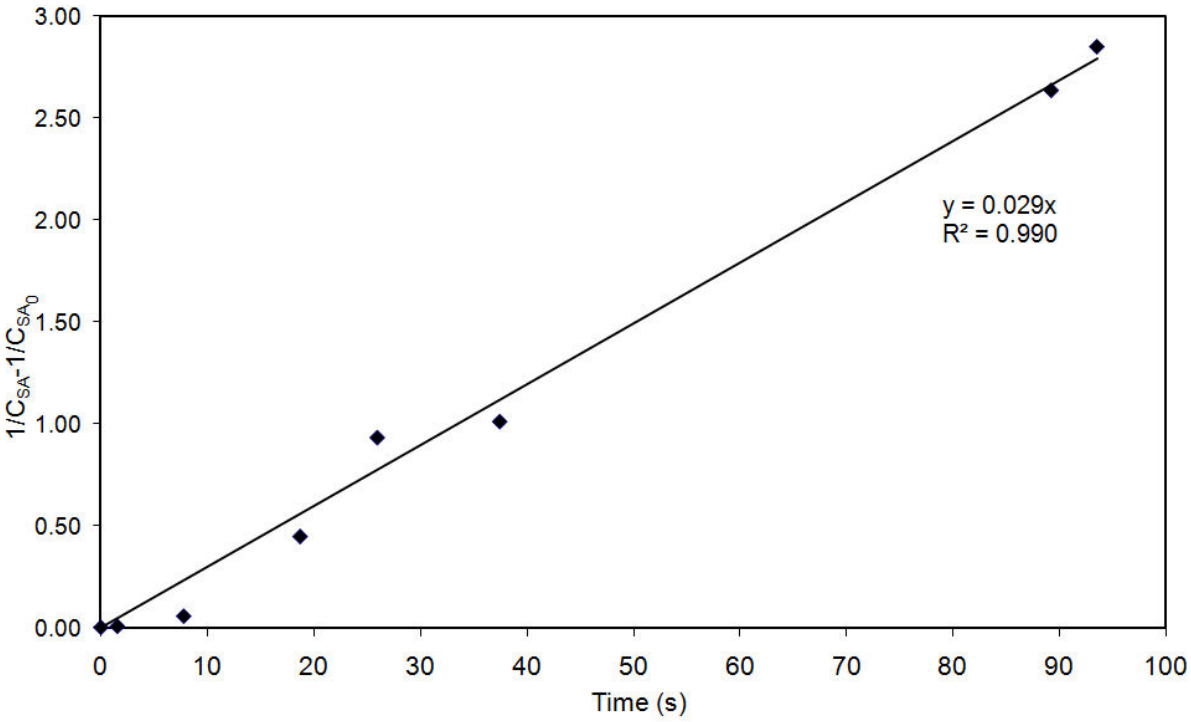


Figure 5.9 Second order plot for the reaction between salicylic acid and acetic anhydride at 90 ^{o}C

Given that the cleaning procedure used for the vanisal sodium run was effective, the same technique was employed for the acetylsalicylic acid. Tap water at 60 ^{o}C was first pumped into the COBR, followed by a Liquinox - Solujet, 1% solution, and finally by the USP grade water, one immediately after the next. The same values of oscillation frequency and amplitude were also used during the cleaning.

It is widely accepted that higher production and cleaning standards are required for pharmaceutical and biochemical products than for the fine chemical counterparts. Yet, there is no published guideline or information on how much higher it should be. That was the reason behind the higher cleaning index used for acetylsalicylic acid, 336, which is 1.5x higher than that used for vanisal sodium. Four samples per minute were taken during the cleaning cycle and analyzed. The results are shown in Figure 5.10, where the dotted line indicates the calculated maximum contamination level for

cleaning validation purposes in the manufacture of paracetamol (2008), which was the next API selected for production in the COBR after this cleaning process. The maximum amount of residual acetylsalicylic acid (BL_2) allowed in the COBR was calculated using the following expression developed by Leblanc (2000):

$$\mathrm{BL}_2 = \left(\frac{\text{minimum daily dose of } \mathrm{API_A}}{\text{minimum daily dose of } \mathrm{API_B}} \right) \times 1000000 \times \mathrm{SF} \qquad (5.14)$$

where BL_2 is the limit of the target residue (in this case, the target residue is the bulk active API_A) in any finished drug product in which the residue may ultimately be found, API_A is the bulk active *A*, API_B is the bulk active *B* and *SF* is the Safety Factor (= 0.001). In this case, this calculated value was 0.113 mg mL^{-1}. Similar to what had happened with the vanisal sodium's case, the COBR was cleaned after the washing was completed. The loss of product due to the cleaning process amounted to 0.005% of the total amount of product. This is again lower than the industry norm of 0.1-0.2% (Norton 2009). The swab sample test was carried out at the V3 location in the COBR (see Figure 3.4 in Chapter 3) according to the method developed by Nozal et al (2000) and is also presented in Figure 5.10 (▲). It is noticeable that the degree of contamination detected in the swab sample is well below the threshold. The result confirms that the cleaning of the reactor was effective. The wash index is 5.07 when the reactor is cleaned, which equates to 10.6 L of liquid waste. The amount of time used for cleaning is much smaller than the corresponding total operation time, only circa 0.19%. The reactor was once again disassembled and visually inspected for residues in sample ports V3 and V4. No residues were found at these locations, another indication that the cleaning procedure was effective.

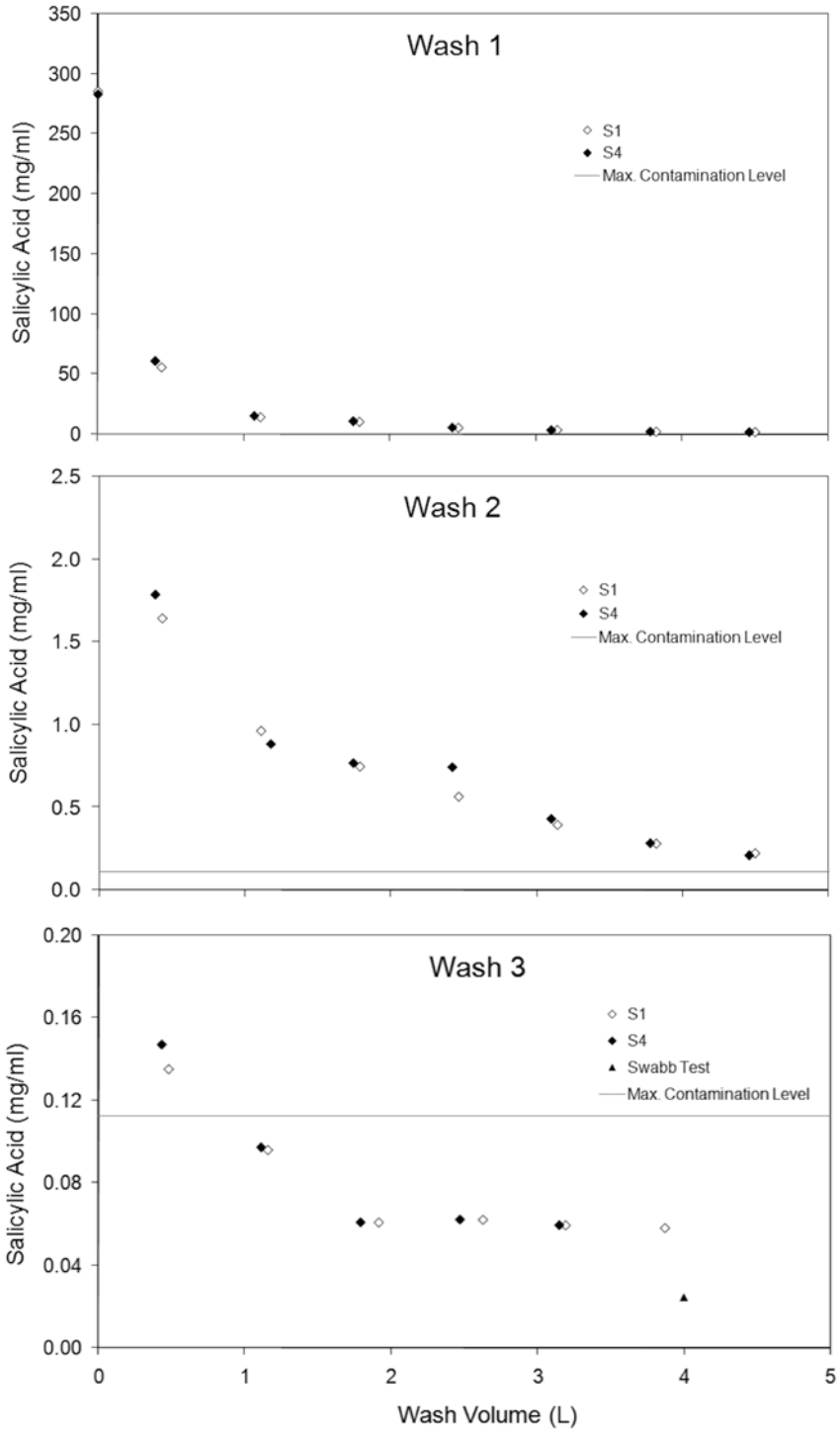


Figure 5.10 Washing data for aspirin

The kinetics of the cleaning process for the acetylsalicylic acid run was also evaluated, and a plot of $-\ln(C/C_0)$ against time is shown in Figure 5.11. The first-order kinetics are well suited to describe the cleaning process, which is in line with what was found for the vanisal sodium case. The mean rate constant is 0.006 s^{-1}. As a result of the higher cleaning index used when compared to the one used in the vanisal sodium cleaning procedure, a higher rate constant was expected for aspirin cleaning ($k_{aspirin}/k_{vanisal\ sodium}$ = 1.3). This indicates that at identical oscillation conditions, the rate constants for the cleaning of the COBR depend on the cleaning intensity that is regulated by the net flow, as suggested previously by Bird et al (1991). This implies that the washing process is mainly a physical process, in which mixing plays an important part.

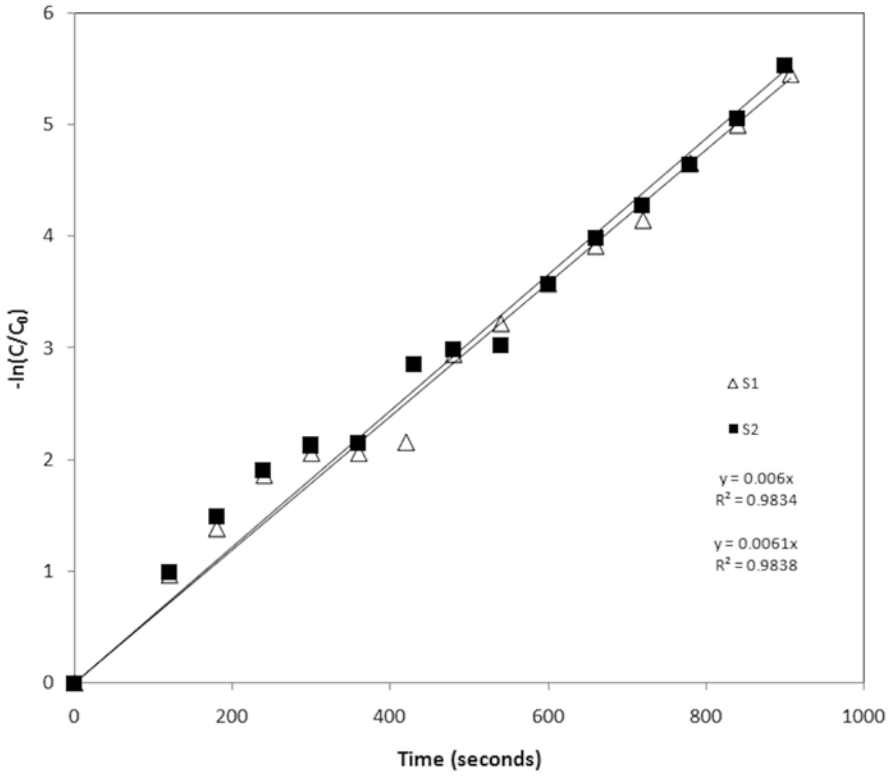


Figure 5.11 First-order kinetics plot for aspirin cleaning data

5.4 – Paracetamol

The synthesis and crystallization of paracetamol was the next and final campaign in this PhD project. A continuous production and subsequent cleaning process of the COBR was carried out and the results are shown below, after the description of the experimental method.

A first attempt of carrying out the synthesis and crystallization campaign of paracetamol with the COBR in a vertical layout and at a flowrate of 2.0 mL min^{-1} (equivalent conditions to the aspirin campaign) proved to be unsuccessful, as crystals started accumulating around the PFA baffles situated just before P2 (see Figure 3.4). As the crystals accumulated, mixing on the subsequent sections was less and less efficient and eventually the pipe was blocked. This was probably due to the differential in pressure in the system - the top sections of the COBR were at a height of 2.80 m, making it difficult for the pump used to move all the fluid inside the reactor at a constant speed throughout the system. Thus, the fluid at the top sections was mixed poorly and the crystals that were being formed at those locations had more probability of interacting with the baffle surface. These conditions led to the continuous adherence of crystals to the baffle and the eventual blocking of that pipe section. The campaign had to be aborted and in light of these findings the COBR was re-orientated horizontally and the flowrate of the system was increased.

5.4.1 – Experimental Method

For this campaign, the following steps were used:

1) The COBR was flooded with purified water;
2) The reactor was heated up to 80 ^{o}C (4 first sections), the OBR feeder was heated up to 90 ^{o}C and oscillation was started (ν = 2.0 sec^{-1}; x_0 = 40 mm);
3) 48 g of p-aminophenol was weighted, 53.33 mL of phosphoric acid, 64 mL of acetic anhydride and 800 mL of purified water were measured;
4) The purified water, p-aminophenol and phosphoric acid were transferred to the OBR feeder;

5) The COBR was fed with the contents of the OBR after all the amine was dissolved, along with the acetic anhydride via the second feed line;

6) Feed flowrate of the contents to the COBR was kept at 20 mL min^{-1} and temperature readings were taken;

7) A sample was taken for analysis;

8) Steps 3 to 7 were repeated 128 times.

5.4.2 – Results

Paracetamol was synthesized and crystallized in the COBR with the same oscillation conditions used for the aspirin campaign and once again a constant temperature profile is required in the process. In Figure 5.6 it is apparent that the reaction and subsequent crystallization of aspirin was finished when the solution reached the first sampling port. In the paracetamol run, the flow rate of the reactants was increased by 10 fold to take that result in consideration. On one hand, this allows more concentration data to be obtained, showing the profile of paracetamol generating; on the other hand, this means that either the amount of starting materials required for the reaction is increased accordingly, or the overall running time is reduced for the same amount of material. In order to keep the project on budget the latter was the only option, i.e. the campaign was reduced from the 7 days initially planned to 4 days, 24 hours a day.

In this run the COBR was operated horizontally in order to demonstrate the versatility of the system. Both reaction and crystallization were performed in this campaign and the variable temperature profile is used. Figure 5.12 shows the temperature profiles inside the COBR. The gathered data is once again in line with the previous experimental results, and very stable temperatures were achieved in the COBR.

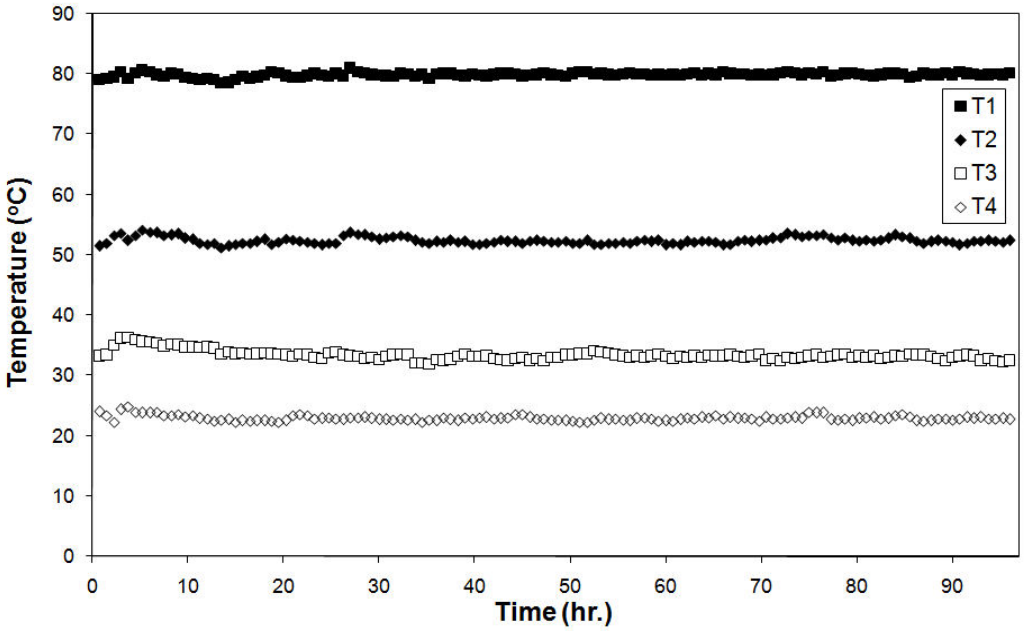


Figure 5.12 Temperature measurements for the paracetamol production run

Samples were taken from the various ports along the COBR and the amount of paracetamol produced along the run was determined. The results are shown in Figure 5.13 and once again the data for each sample port was consistent. The product samples were analyzed using HPLC and the averaged purity was 99.63% over 55 samples. The

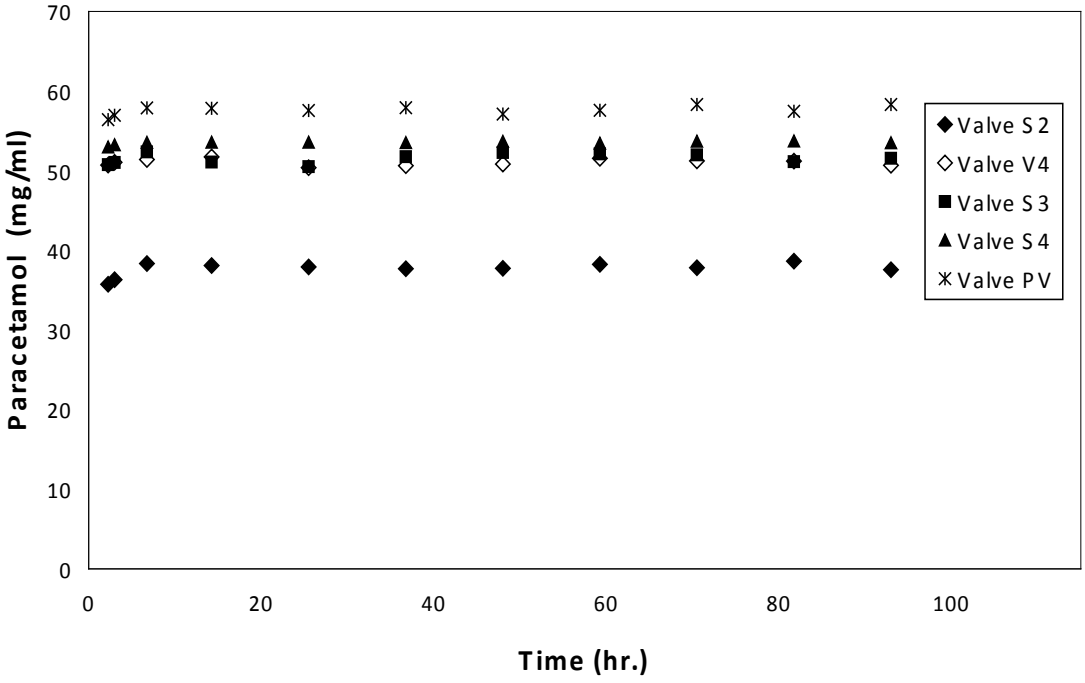


Figure 5.13 Concentration profile of the produced paracetamol

average yield obtained for this campaign was 79%, which is higher than that found for literature (circa 73%) even though the used feed concentration in this production run is lower (Schulman et al 1975), as shown in Table 5.3.

Table 5.3 Experimental conditions and results for this work and in literature (Schulman, Baron et al 1975)

	Concentration of limiting reactant (4-Amiphenol) ($g.L^{-1}$)	Temperature of Reaction (^oC)	Product Concentration ($g.L^{-1}$)	Percent Yield (%)
Schulmann et al (1956)	147.2	85-90	148.6	72.8
This work	52.3	80	58.6	79.4

Crystals were taken from the PV sampling port (Figure 3.4) at different times of COBR operation and the XRD data presented in Figures 5.14 and 5.15 show that crystals are monoclinic (form I) (Yamamura and Momose 2003). The crystals were also imaged using SEM at 30x and 200x amplifications, as shown in Figure 5.16. The morphology of the sampled crystals was consistent throughout the run and its monoclinic structure is consistent with what was found by Lee et al (2006), as shown in Figure 5.17. This is the crystal structure currently in use for drug formulations (Florence and Attwood 2007). It should, however, be noticed that production of different crystalline forms of paracetamol and aspirin using oscillatory baffled reactors might be achieved by regulating different variables (e.g. temperature, used solvent, pH), as demonstrated in previous studies for glutamic acid (Ni and Liao 2008).

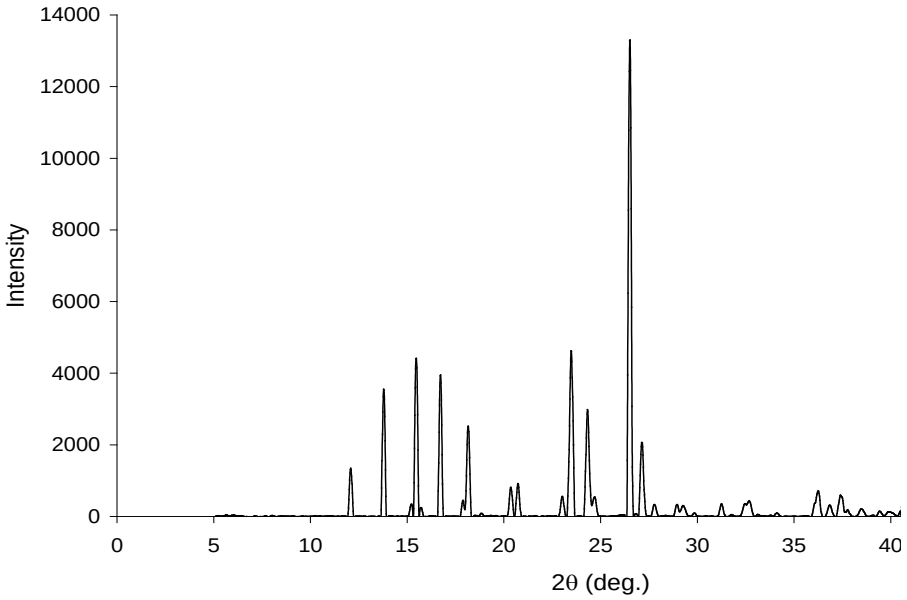


Figure 5.14 XRD data for paracetamol produced in the COBR

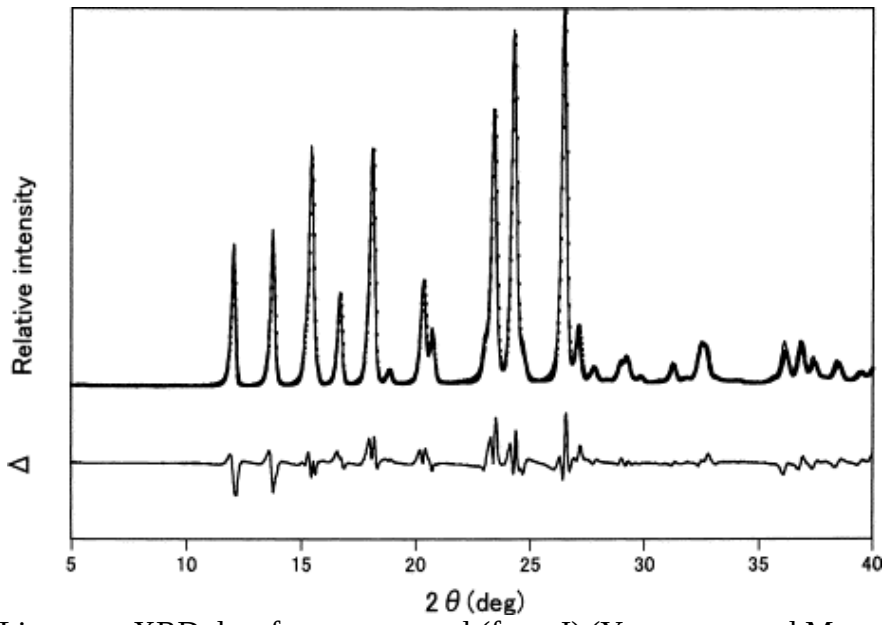


Figure 5.15 Literature XRD data for paracetamol (form I) (Yamamura and Momose 2003)

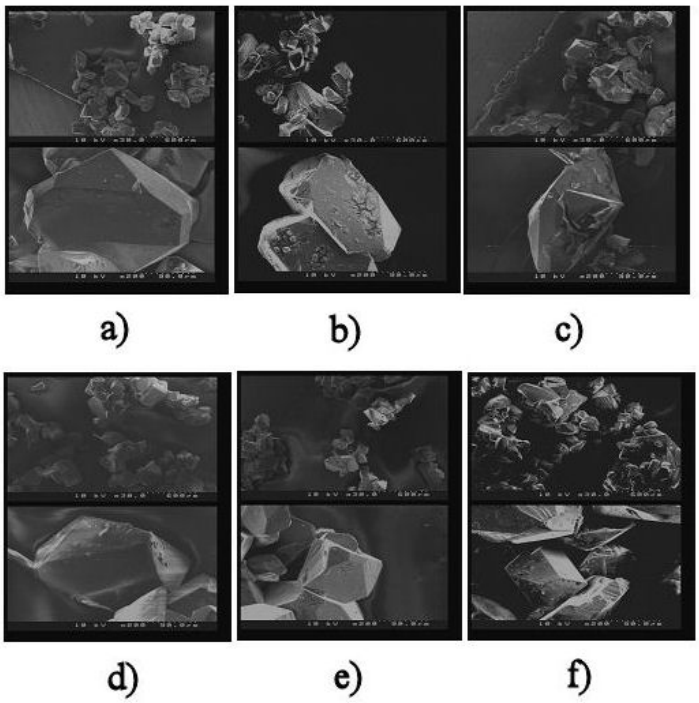


Figure 5.16 SEM images of paracetamol crystals taken at different times of operation.
a) 7.5 hrs b) 26.25 hrs c) 45 hrs d) 63.75 hrs e) 75 hrs f) 93.75 hrs

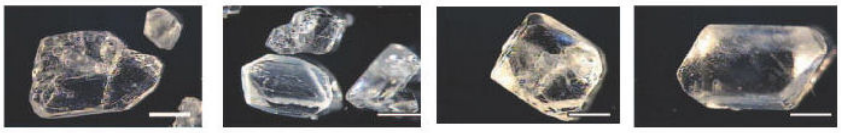


Figure 5.17 Optical micrographs of monoclinic paracetamol crystals,
in Lee et al (2006)

The size distributions of the paracetamol crystals along the COBR were determined using the particle size distribution technique and are presented in Figure 5.18. Paracetamol crystals have a tendency to grow to large sizes and it is evident that the distribution was initially broader with smaller sized particles at the earlier stage of the crystallization and became narrower with bigger sized crystals. This shows that the continued mixing at a determined fixed frequency and amplitude over time has a selective effect on the particle size – this might be explored further in view to control particle size distributions to the required specifications. The Gaussian-type of size distribution obtained in the COBR is the direct result of the good mixing achieved during operation. Table 5.4 complements Figure 5.18 and shows the mean sizes as well

as $D_{v,0.1}$ and $D_{v,0.9}$, the sizes of particles with 10% and 90% of the samples being below the corresponding sizes, respectively. The crystal size distribution results show how well the COBR is suited for crystallization operations and, once again, the consistency of the product obtained during the whole run.

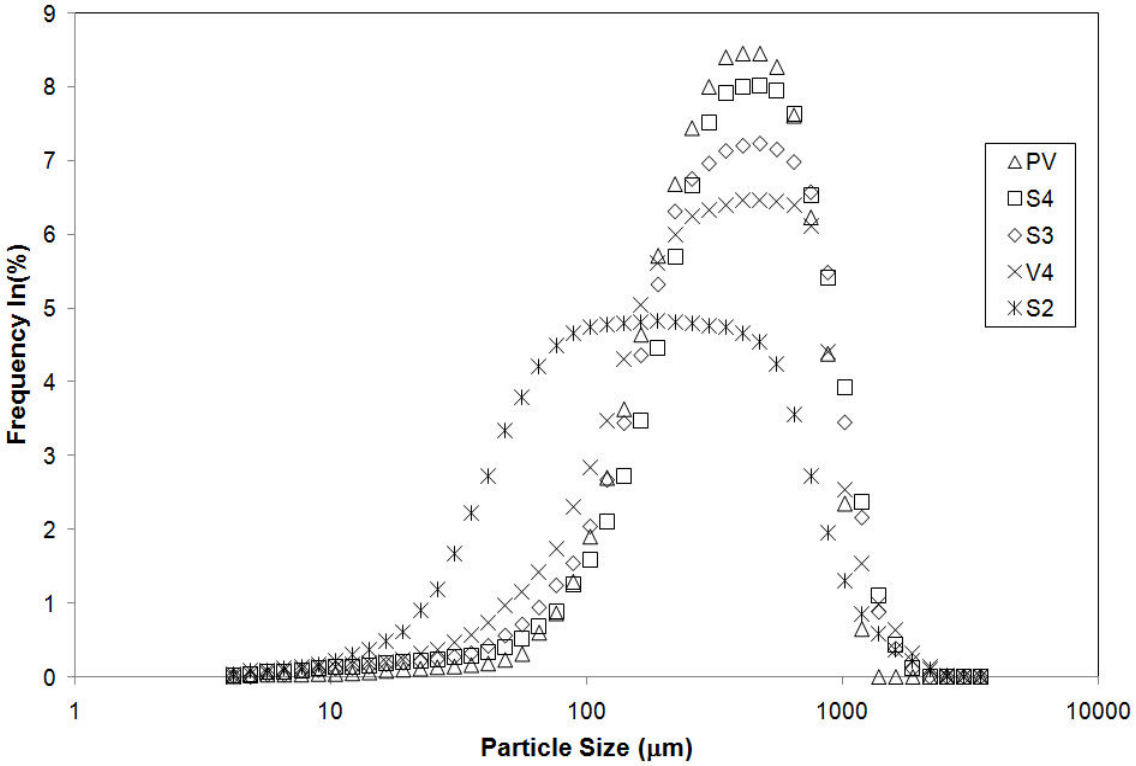


Figure 5.18 Particle size distribution of paracetamol samples (time of sampling = 3 hours)

Table 5.4 Particle size distribution for paracetamol samples

Time of Sampling (hour)	Sampling Location	Mean Size ($D_{v,0.5}$) (μm)	$D_{v,0.1}$ (μm)	$D_{v,0.9}$ (μm)
3	S2	185	45	692
3	V4	342	95	893
3	S3	389	125	936
3	S4	422	141	963
3	PV	439	146	820
7.5	PV	451	149	824
26.3	PV	436	143	817
45	PV	442	151	820
63.8	PV	448	149	826
75	PV	433	142	816
93.8	PV	444	153	824

Reaction kinetics of paracetamol in the COBR was also evaluated. Samples were taken from different locations in the COBR and immediately quenched for HPLC analysis. The decrease in the concentration of 4-aminophenol along the COBR was then recorded and the reaction kinetics extracted as shown in Figure 5.19. Once again the reaction of paracetamol is of a 2^{nd} order with a rate constant of 0.011 ml mg^{-1} s^{-1}. The reaction times found in literature range from 5 to 12 minutes (Young 1963; Schulman et al 1975), which are longer than what is found in this work (circa 3 minutes) due to the superior mixing and heat-transfer capabilities of the oscillatory baffled reactor.

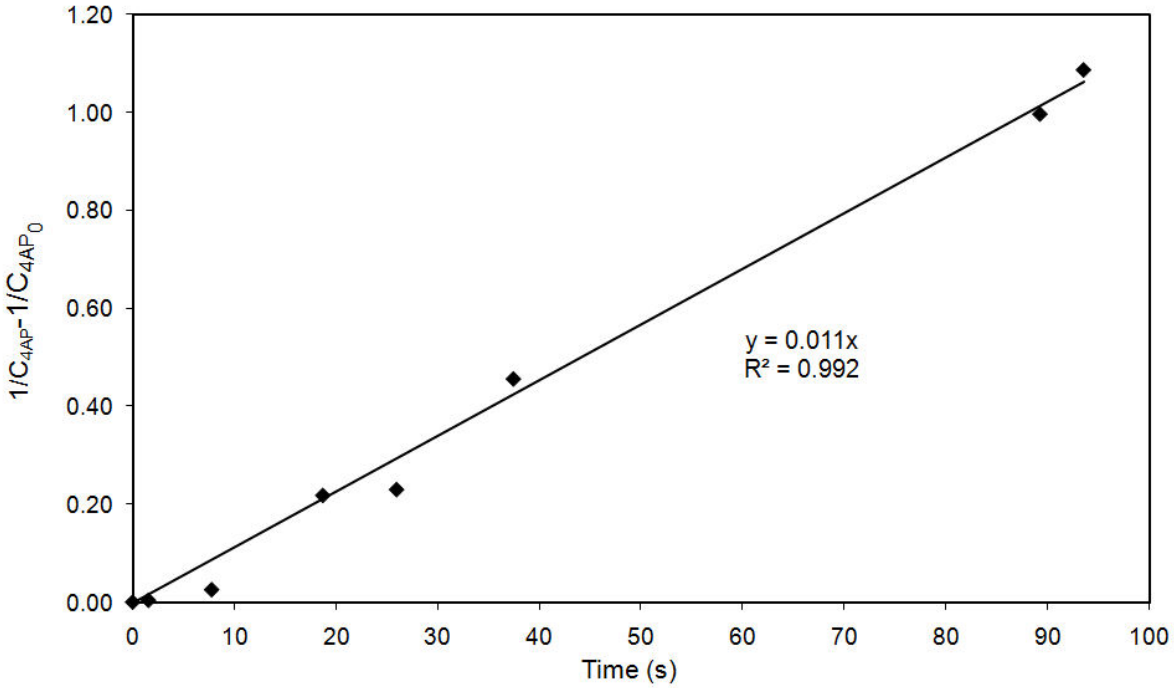


Figure 5.19 Kinetics of the reaction between p-aminophenol and acetic anhydride at 80 ^{o}C

The COBR was again cleaned after the paracetamol experiments in COBR were concluded. The same cleaning procedure was used for the paracetamol run. The cleaning index was 33.9, which reflects the 10-fold increase in the flow rate used in the paracetamol production run in comparison to the aspirin production campaign, which allowed for a paracetamol concentration profile to be evident during the campaign. Figure 5.20 shows the data collected during the cleaning stage. In a similar fashion to what was done for the aspirin cleaning stage, a maximum allowed level of paracetamol in the COBR was calculated as 0.125 mg mL^{-1}, assuming that the production would be reverted back to aspirin using the COBR. It is clear that the concentration of paracetamol that remained in the cleaning solutions was lower than that required by the time the cleaning was completed. The swab test (▲) was also performed and the amount of paracetamol detected was also well below the maximum permitted level. The results show that the method used for cleaning was once more efficient. The loss of product due cleaning was 0.004%, lower than the industry norm of 0.1-0.2%. The washing index was 4.08, which correlates to a generated waste of 8.44 L during the cleaning process.

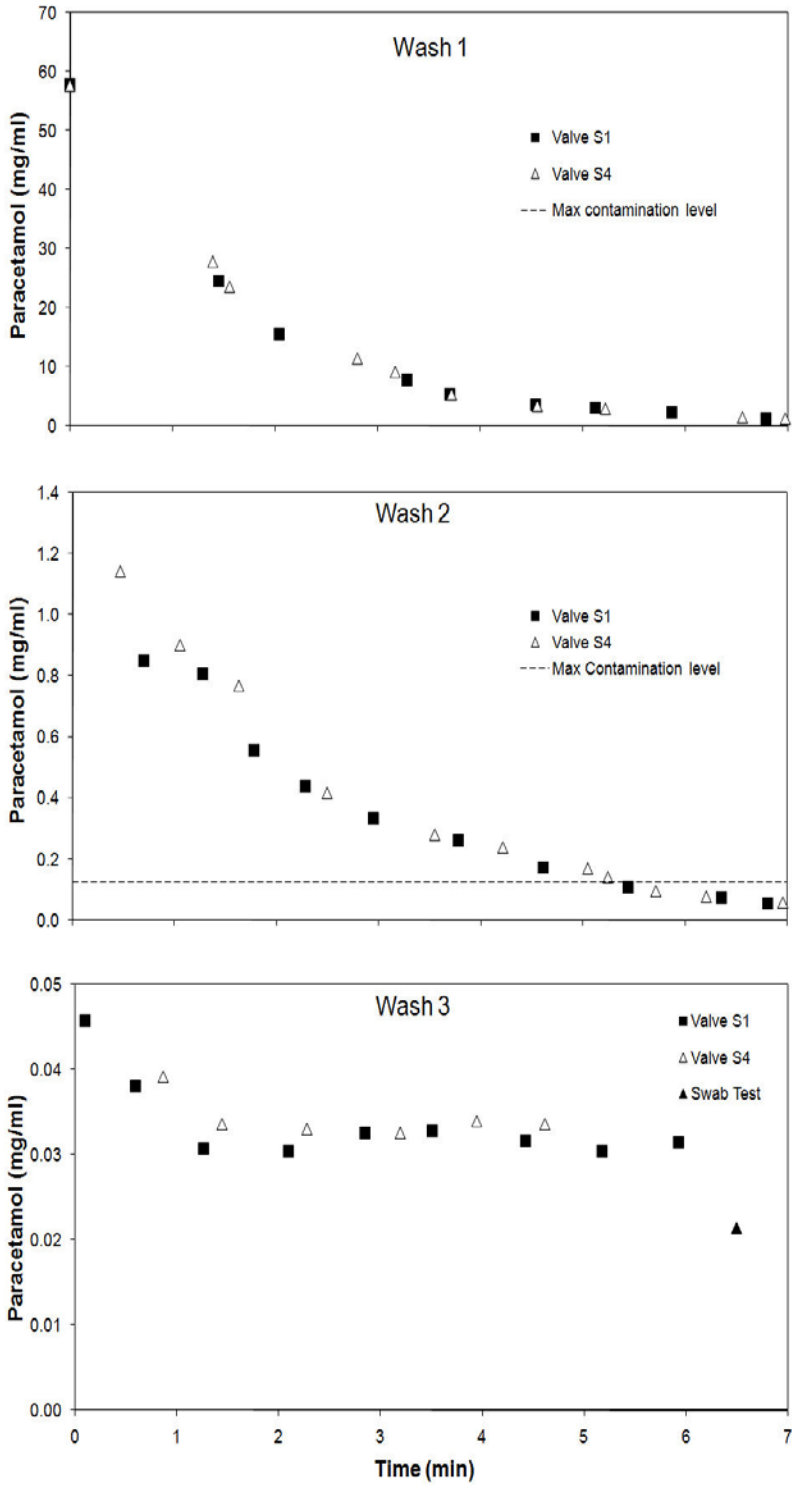


Figure 5.20 Washing data for paracetamol

The kinetics of the cleaning process was evaluated and a plot of $-\ln(C/C_o)$ versus time is shown in Figure 5.21. The first-order kinetics fits well to the experimental data and the mean rate constant is 0.007 s^{-1} for this case.

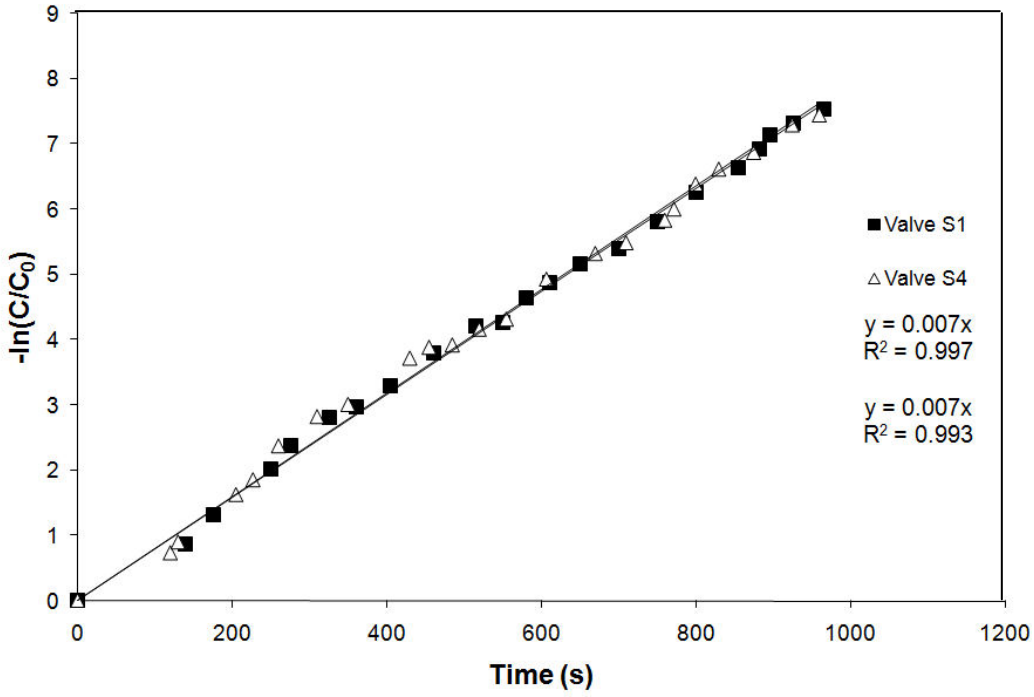


Figure 5.21 Paracetamol cleaning kinetics

This is in line with the previously obtained results and is, once again, concurrent with Bird's view (1991) that the efficient mixing occurring inside the COBR during the cleaning stage plays a critical part in what is considered to be mainly a physical process. As explained in Chapter 2, it should be noted that while there are many factors that have an impact in the cleaning efficiency, such as the nature of the fouling substance, the flow rate, the cleaning temperature, the shape and nature of the cleaned surfaces or the nature and the concentration of the cleaning solution, establishing an explicit correlation between previous studies and this work is still not feasible. Table 5.5 compiles the kinetic data for other cleaning studies in tubular type of reactors for indication only, as the basis of comparison is not the same. On this standing point, the rate constants from this work are comparable to these from the previous studies.

Table 5.5 Comparison of cleaning data from different studies (s.s. – stainless steel)

Authors	Cleaning Solution	Fouling Substance	Net flow velocity ($m.s^{-1}$)	Temperature (^0C)	Cleaned surface	k (s^{-1})
Our data	Tap water Liquinox 1% USP water	vanisal sodium	0.043	60 room temp.	COBR (glass/PFA)	0.0045
Our data	Tap water Liquinox 1% USP water	aspirin	0.064	60 room temp.	COBR (glass/PFA)	0.0061
Bourne (1963)	NaOH 0.03M	tristearin	0.095	---	s.s. strip in a glass pipe	0.023
Karlsson (1998)	NaOH 0.1-0.2mg/mL	β-lactoglobulin	0.033	24	s.s. cell	0.048 to 0.086
Lelievre (2002)	NaOH 0.5%	*Bacillus* spores	0.2 to 3.3	60	s.s. pipe	0.0012 to 0.083

The ratio between the operation and the cleaning times is 427.5 for the paracetamol run, which shows that the cleaning stage used only a very small fraction (circa 0.23%) of the total operation time. As a consequence of this, the productivity is maximized and the operational costs are minimized.

5.5 – Summary

The experimental results obtained for each of the chemistries selected for COBR work are described in this chapter. Reaction time, production campaigns and cleaning results are included and show that the COBR is a suitable continuous reactor for the synthesis of vanisal sodium, acetylsalicylic acid and paracetamol to high standards. The fast cleaning procedure devised for this project was effective. The reactor was cleaned in compliance to the stringent regulatory standards at the end of each production campaign.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Just a drink, a Martini, shaken not stirred.
James Bond, *in* Goldfinger (1964)

6.1 – Conclusions

This thesis reports the first experimental research with the objective of establishing a cleaning protocol for fine chemicals and APIs manufactured in COBR. Taking this into consideration, three different chemical entities were continuously produced in tandem fashion, interspersed with well-defined cleaning procedures. The main conclusions taken from this work are subsequently summarized:

- The reaction times for the studied chemistries were shorter than those described in literature. Some were performed under milder temperature conditions, thus obviating the use of reflux apparatus in the reactor.

- The crystalline forms of the produced APIs, aspirin and paracetamol, were consistently of the most common form I for each of the chemical entities, which is currently used in the pharmaceutical industry for medicine formulation purposes.

- A precise control over the temperature profile was achieved while operating the COBR. In addition, particle size distribution studies revealed the uniformity of the crystal size obtained for the duration of the paracetamol production campaign.

- All three chemical entities were produced to high purity standards, and one of the main strengths of the COBR mixing technology was demonstrated by the presence of minimal amounts of contaminants in the fine chemical and APIs produced.

- Products of consistent quality and yield were obtained for the duration of each of the production campaigns. The precise control of operational conditions in the COBR and the data gathered during the experiments suggests that this type of continuous reactor is well suited for the manufacture of high quality APIs and fine chemical products.

- The cleaning kinetics for the studied chemical entities are of a first-order, which is comparable to previous work. The cleaning protocol is very simple, efficient and fast with a minimal amount of waste generated, in accordance to Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) standards.

- The COBR design can potentially incorporate QbD principles and will play an important role in shaping the current pharmaceutical manufacturing industry.

The data gathered and the conclusions that are taken from this work suggest that the COBR mixing technology is superior to current mixing technologies, not only in terms of the consistency demonstrated by the COBR when performing chemical reactions, but also in terms of crystallization and, not less importantly, the ease of maintenance and cleaning.

The increasing trend towards process intensification and making the pharmaceutical industry leaner, reaching the levels of efficiency already demonstrated by the automotive or the semi-conductor industrial sectors is forcing the market players into investing more into research and development programs. Instead of expending large sums of money into making small improvements on already existing processes, the time has come for a paradigm shift, from the traditional *stirred* to the more efficient *shaken*.

6.2 – Recommendations for Future Work

The experimental work reported here suggests that the COBR is a better alternative to current mixing technologies with regards to the production of fine chemicals and APIs. The washing of the reactor is done with a simple, fast and effective cleaning procedure, thereby allowing for short turn-around times in manufacturing facilities.

There is, however, scope for more experimentation in this field of research in order to better understand and optimize the processes studied. With that in mind, some recommendations for future work are listed below:

- The intrinsic modular characteristics of the COBR design make it perfect for adapting more in-line and on-line process analytical technologies. Investigating ways of installing devices such as UV-Visible spectrophotometer or even real-time HPLC or mass spectrometry apparatus will contribute to a better understanding of the phenomena occurring in the COBR, enabling a more informed modulation of the reaction or oscillation conditions.

- Although the results obtained in the experimental work confirm the suitability of the COBR for performing chemical reactions and crystallization operations, these could be the object of further studies. Investigating how crystallization conditions affect both the size and crystalline structure by varying oscillation conditions, using different solvents or even temperature profiles could provide more data and help understanding these processes.

- An interesting path to follow in the continuation of this research would be to investigate how the material that the COBR is made of interacts with how the processes here studied occur. In other words, replacing the glass pipes and the PFA baffles with a combination of materials such as stainless steel, different kinds of polymers or even using glass baffles could have a positive impact on how the chemical reactions, crystallization and cleaning processes evolve.

- A broader view in how the COBR interacts, complements and integrates in a larger, industrial manufacturing process is needed. Having a project in which the proven capabilities of the COBR are put to test in a real-world pharmaceutical or fine chemicals plant would be the definite assessment of the performance of this mixing technology. If successful, the publication of such results would help in removing the cautiousness with which new technologies are naturally met with by decision-makers in the industry.

- A logical and critical next step in this field of research is to broaden it to other APIs and chemistries. This is probably the most important recommendation for future work, as further successes would increase the COBR's visibility in the pharmaceutical and fine chemicals industries and reaffirm its place as a viable alternative to current mixing technologies.

Appendix – A

cGMP Regulations and Guidelines and Compliance Actions

Table A.1 Applicable regulations and guidelines and compliance actions taken or to be taken.

1. PREMISES AND EQUIPMENT Principle: Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimise the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination, build up of dust or dirt and, in general, any adverse effect on the quality of products.	
Regulations and Guidelines	Measures Taken
1.1 Premises should be situated in an environment which, when considered together with measures to protect the manufacture, presents minimal risk of causing contamination of materials or products.	The equipment is located in a cleaned laboratory; all the chemicals used are labelled and stored according to suppliers indications.
1.2 Premises should be carefully maintained, ensuring that repair and maintenance operations do not present any hazard to the quality of products. They should be cleaned and, where applicable, disinfected according to detailed written procedures.	The laboratory is regularly maintained and properly staffed with trained personnel to perform maintenance and repairs.
1.3 Lighting, temperature, humidity and ventilation should be appropriate and such that they do not adversely affect, directly or indirectly, either the medicinal products during their manufacture and storage, or the accurate functioning of equipment.	Lightning, temperature, humidity and ventilation in the laboratory are adequate to perform the chemical reactions screened.
1.4 Premises should be designed and equipped so as to afford maximum protection against the entry of insects or other animals.	The laboratory is properly equipped for that effect, preventing the entry of insects or other animals.
1.5 Steps should be taken in order to prevent the	There are adequate signs in the laboratory

entry of unauthorised people. Production, storage and quality control areas should not be used as a right of way by personnel who do not work in them.	entrance. The laboratory is an obligatory passage only to storage rooms and the workshop.
1.6 The adequacy of the working and in-process storage space should permit the orderly and logical positioning of equipment and materials so as to minimise the risk of confusion between different medicinal products or their components, to avoid cross-contamination and to minimise the risk of omission or wrong application of any of the manufacturing or control steps.	The working space is carefully laid-down taking in account this guideline, as are the chemical storage cupboards; the COBR will be positioned in such way that permits the reactant and product vessels to be inside the ventilated hood.
1.7 Where starting and primary packaging materials, intermediate or bulk products are exposed to the environment, interior surfaces (walls, floors and ceilings) should be smooth, free from cracks and open joints, and should not shed particulate matter and should permit easy and effective cleaning and, if necessary, disinfection.	The laboratory was built taking in account these considerations.
1.8 Pipework, light fittings, ventilation points and other services should be designed and sited to avoid the creation of recesses which are difficult to clean. As far as possible, for maintenance purposes, they should be accessible from outside the manufacturing areas.	The COBR will be positioned in such way that it will be readily accessible and cleaning of the premises will constitute no problem.
1.9 Production areas should be effectively ventilated, with air control facilities (including temperature and, where necessary, humidity and filtration) appropriate both to the products handled, to the operations undertaken within them and to the external	The COBR will be positioned in such way that permits the reactant and product vessels to be inside the ventilated hood.

environment.	
1.10 Production areas should be well lit, particularly where visual on-line controls are carried out.	The laboratory is well illuminated.
1.11 In-process controls may be carried out within the production area provided they do not carry any risk for the production.	Temperature and pressure sensors will be connected to the reactor, and present no risk for the reactions to be carried out.
1.12 Manufacturing equipment should be designed, located and maintained to suit its intended purpose.	Careful thought was taken in the design and layout of the equipment to be used.
1.13 Repair and maintenance operations should not present any hazard to the quality of the products.	The COBR can be repaired/maintained off-line to original specifications, and the quality of the products should not suffer from any quality concerns.
1.14 Manufacturing equipment should be designed so that it can be easily and thoroughly cleaned. It should be cleaned according to detailed and written procedures and stored only in a clean and dry condition.	The success of this work depends largely on cross-contamination control and elimination, and as such cleaning protocols will be tested and validated.
1.15 Washing and cleaning equipment should be chosen and used in order not to be a source of contamination.	
1.16 Equipment should be installed in such a way as to prevent any risk of error or of contamination.	
1.17 Production equipment should not present any hazard to the products. The parts of the production	Only non-reactive material such as glass, PFA and stainless steel will be in contact

equipment that come into contact with the product must not be reactive, additive or absorptive to such an extent that it will affect the quality of the product and thus present any hazard.	with reactants/products.
1.18 Balances and measuring equipment of an appropriate range and precision should be available for production and control operations.	Balances, digital thermometers and pressure gauges are adequate for the processes to be carried out.
1.19 Measuring, weighing, recording and control equipment should be calibrated and checked at defined intervals by appropriate methods. Adequate records of such tests should be maintained.	This type of equipment is regularly checked, according to University regulations.
1.20 Fixed pipework should be clearly labelled to indicate the contents and, where applicable, the direction of flow.	Provisions will be taken during the assembly of the reactor.
1.21 Defective equipment should, if possible, be removed from production and quality control areas, or at least be clearly labelled as defective.	
2. DOCUMENTATION Principle: Good documentation constitutes an essential part of the quality assurance system. Clearly written documentation prevents errors from spoken communication and permits tracing of batch history. Specifications, Manufacturing Formulae and instructions, procedures, and records must be free from errors and available in writing. The legibility of documents is of paramount importance.	
Regulations and Guidelines	Measures Taken
2.1 Formally authorised Manufacturing Formula and	All the documentation produced in-house

Processing Instructions should exist for each product and batch size to be manufactured. They are often combined in one document. The Manufacturing Formula should include: (a) the name of the product, with a product reference code relating to its specification; (b) a description of the pharmaceutical form, strength of the product and batch size; (c) a list of all starting materials to be used, with the amount of each, described using the designated name and a reference which is unique to that material; mention should be made of any substance that may disappear in the course of processing; The Processing Instructions should include: (a) a statement of the processing location and the principal equipment to be used; (b) the methods, or reference to the methods, to be used for preparing the critical equipment (e.g. cleaning, assembling, calibrating, sterilising); (c) detailed stepwise processing instructions (e.g. checks on materials, pre-treatments, sequence for adding materials, mixing times, temperatures); (d) the instructions for any in-process controls with their limits; (e) any special precautions to be observed.	takes in account these guidelines.
2.2 There should be written procedures for sampling,	The sampling procedures for the COBR

which include the person(s) authorised to take samples, the methods and equipment to be used, the amounts to be taken and any precautions to be observed to avoid contamination of the material or any deterioration in its quality.	will be written taking in account these indications.
2.3 There should be written procedures for testing materials and products, describing the methods and equipment to be used. The tests performed should be recorded.	Product testing will be carried out in-house (IR, NMR, HPLC, PSD) and will be done in accordance to guidelines.
3. PRODUCTION Principle: Production operations must follow clearly defined procedures; they must comply with the principles of Good Manufacturing Practice in order to obtain products of the requisite quality and be in accordance with the relevant manufacturing and marketing authorisations.	
Regulations and Guidelines	Measures Taken
3.1 Production should be performed and supervised by competent people.	Appropriately trained personnel will be in charge of operating the equipment.
3.2 All handling of materials and products, such as sampling, storage, (…) and processing should be done in accordance with written procedures or instructions and, where necessary, recorded.	Sampling, storage and production will all be adequately documented.
3.3 All incoming materials should be checked to ensure that the consignment corresponds to the order. Containers should be cleaned where necessary and labelled with the prescribed data.	This is done in line with Heriot-Watt University practices.
3.4 All materials and products should be stored under the appropriate conditions established by the	There are adequate storage rooms and cupboards in order to keep chemicals well

manufacturer and in an orderly fashion to permit batch segregation and stock rotation.	stored.
3.5 Operations on different products should not be carried out simultaneously or consecutively in the same room unless there is no risk of mix-up or cross-contamination.	Special attention will be made to this, as success of this work depends largely on this and similar guidelines.
3.6 At every stage of processing, products and materials should be protected from microbial and other contamination.	
3.7 At all times during processing, all materials, bulk containers, major items of equipment and where appropriate rooms used should be labelled or otherwise identified with an indication of the product or material being processed	Provisions will be made to clearly label every item necessary.
3.8 Labels applied to containers, equipment or premises should be clear, unambiguous and in an agreed format.	
3.9 Labels applied to containers, equipment or premises should be clear, unambiguous and in the company's agreed format.	
3.10 Any deviation from instructions or procedures should be avoided as far as possible. If a deviation occurs, it should be approved in writing by a competent person.	Provisions will be made to meet this guideline
3.11 Access to production premises should be	There are adequate signs in the laboratory

restricted to authorised personnel.	entrance to prevent the access of unauthorized personnel to the premises.
3.12 Contamination of a starting material or of a product by another material or product must be avoided. This risk of accidental cross-contamination arises from the uncontrolled release of dust, gases, vapours, sprays or organisms from materials and products in process, from residues on equipment, and from operators' clothing.	The layout and operating procedures of the COBR and ancillary equipment take in account this recommendation.
3.13 Cross-contamination should be avoided by appropriate technical or organisational measures, for example: (a) providing appropriate air-locks and air extraction; (b) minimising the risk of contamination caused by recirculation or re-entry of untreated or insufficiently treated air; (d) keeping protective clothing inside areas where products with special risk of cross-contamination are processed; (e) using cleaning and decontamination procedures of known effectiveness, as ineffective cleaning of equipment is a common source of cross-contamination; (f) using "closed systems" of production; (g) testing for residues and use of cleaning status labels on equipment.	The product and reactants vessels are safely kept in a ventilated cupboard; protective clothing and safety goggles and gloves are kept in the laboratory at all times; cross-contamination avoidance and minimization procedures will be put in place when in operation.

3.14 Measures to prevent cross-contamination and their effectiveness should be checked periodically according to set procedures.	

Appendix – B

HPLC Chromatograms (Aspirin OBR Experiments)

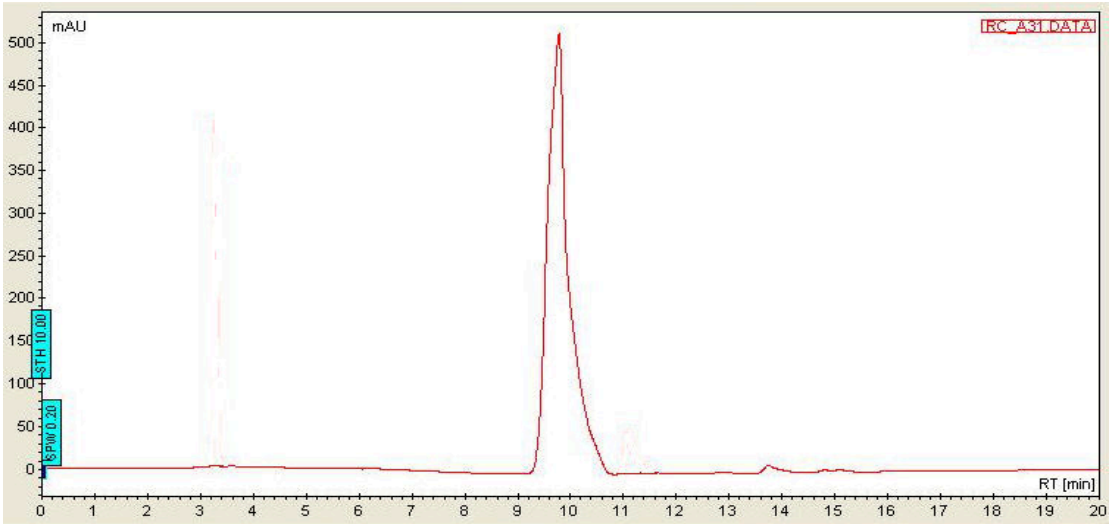


Figure B.1 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 3

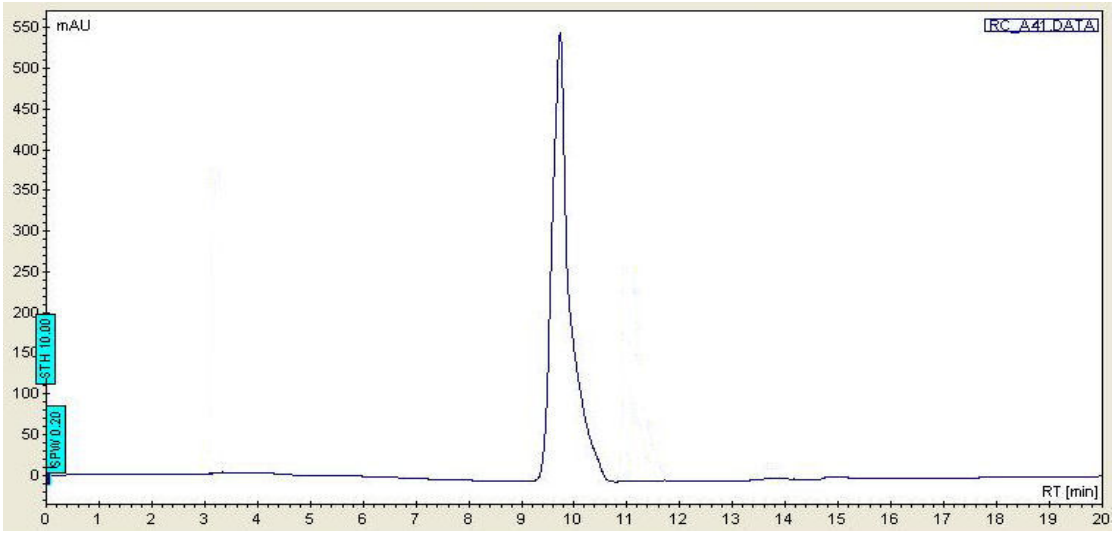


Figure B.2 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 4

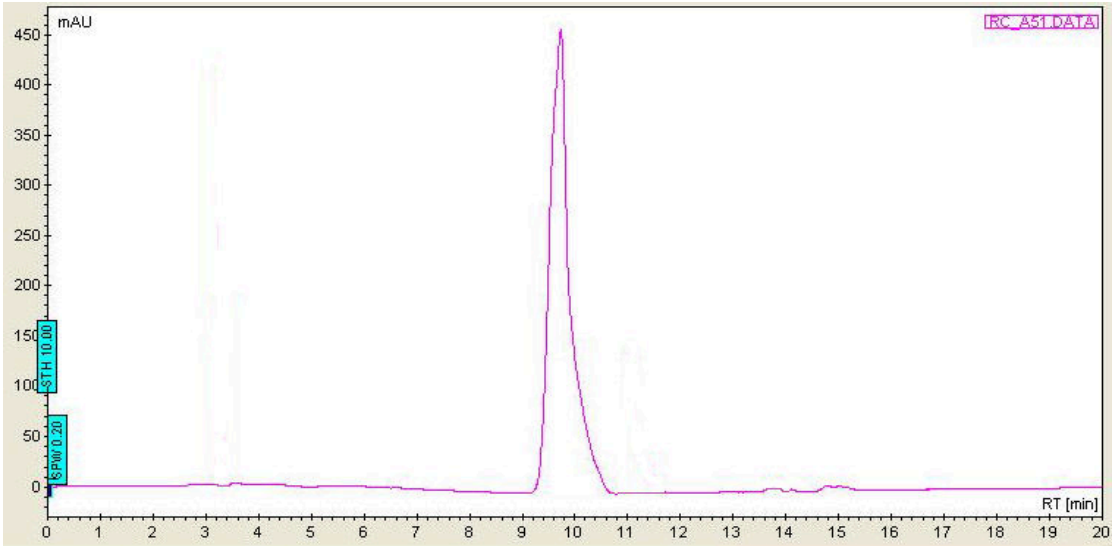


Figure B.3 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 5

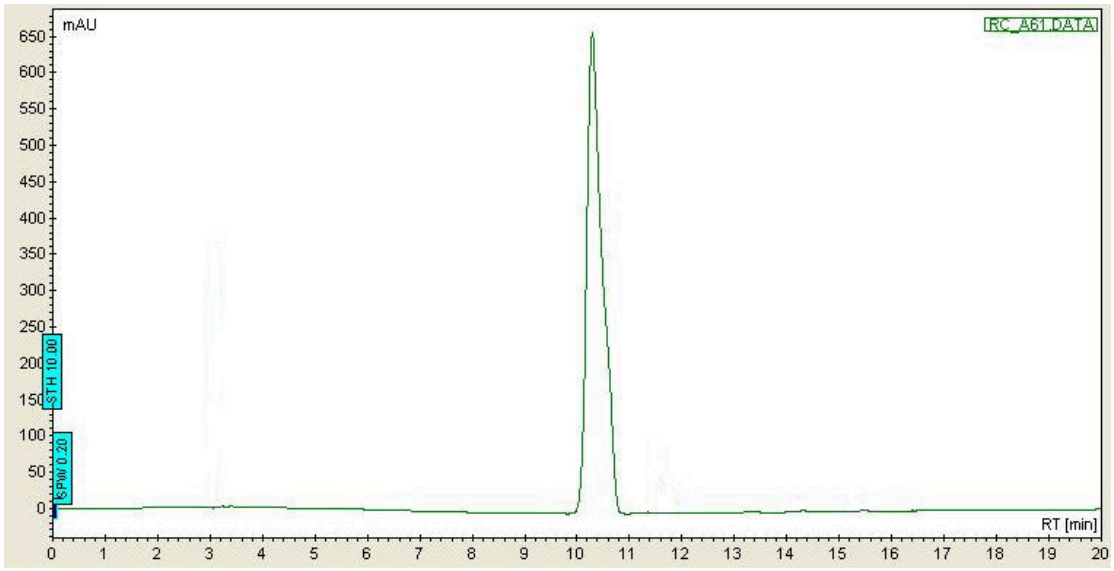


Figure B.4 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 6

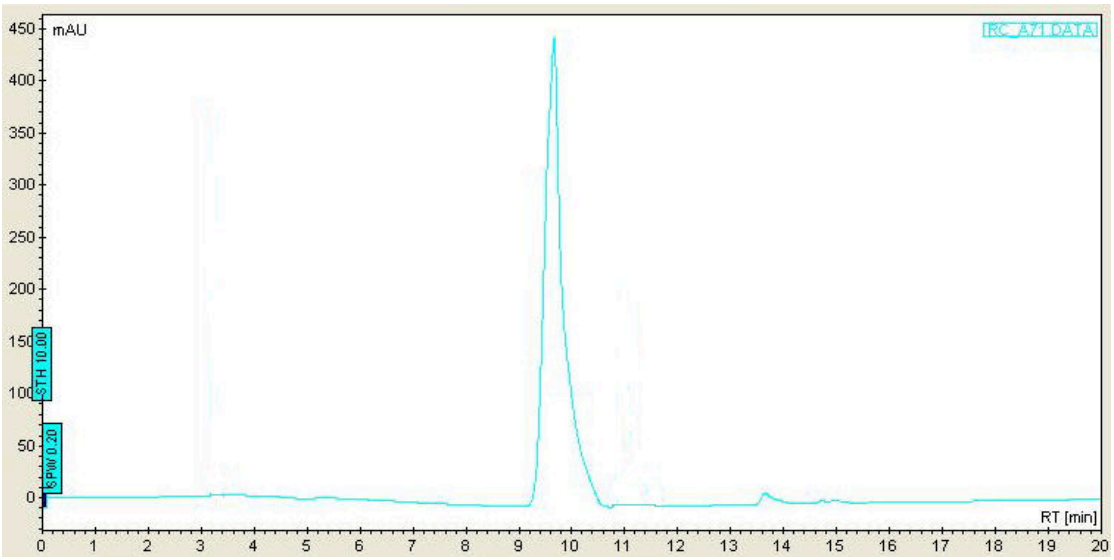


Figure B.5 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 7

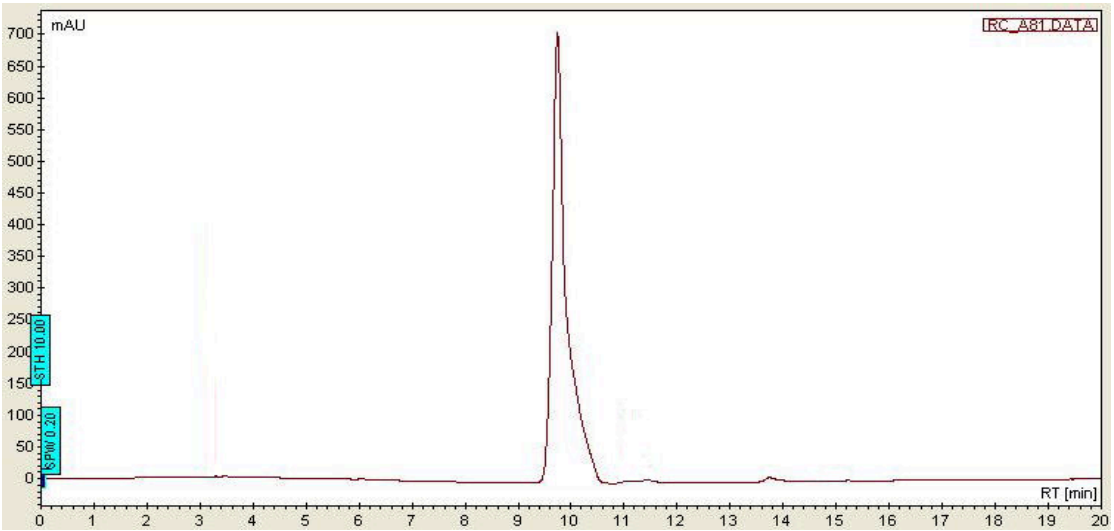


Figure B.6 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 8

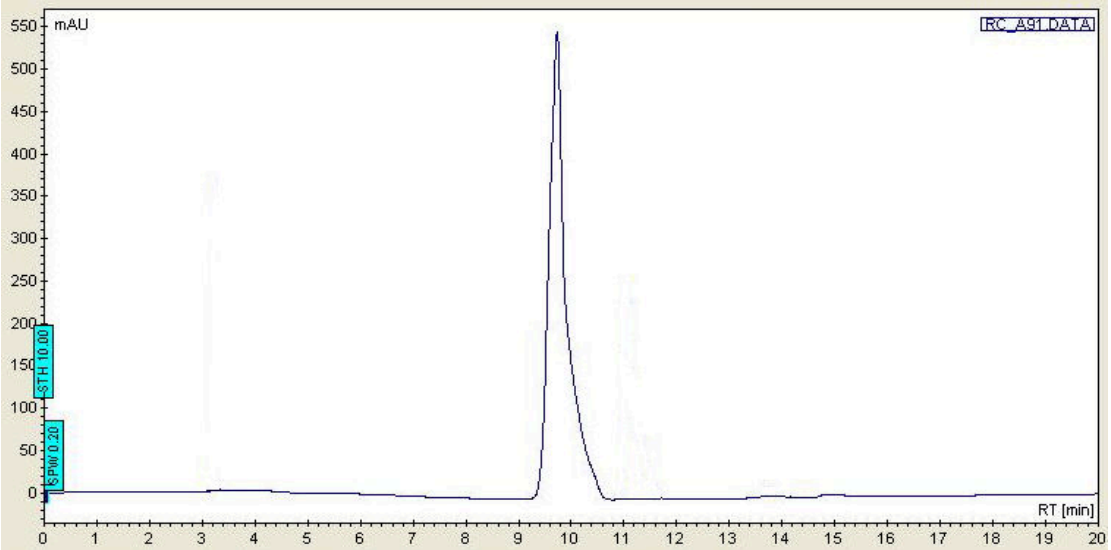


Figure B.7 HLPC chromatogram of the synthesized acetylsalicylic acid, sample 9

Appendix – C

HPLC Chromatograms (Paracetamol OBR Experiments)

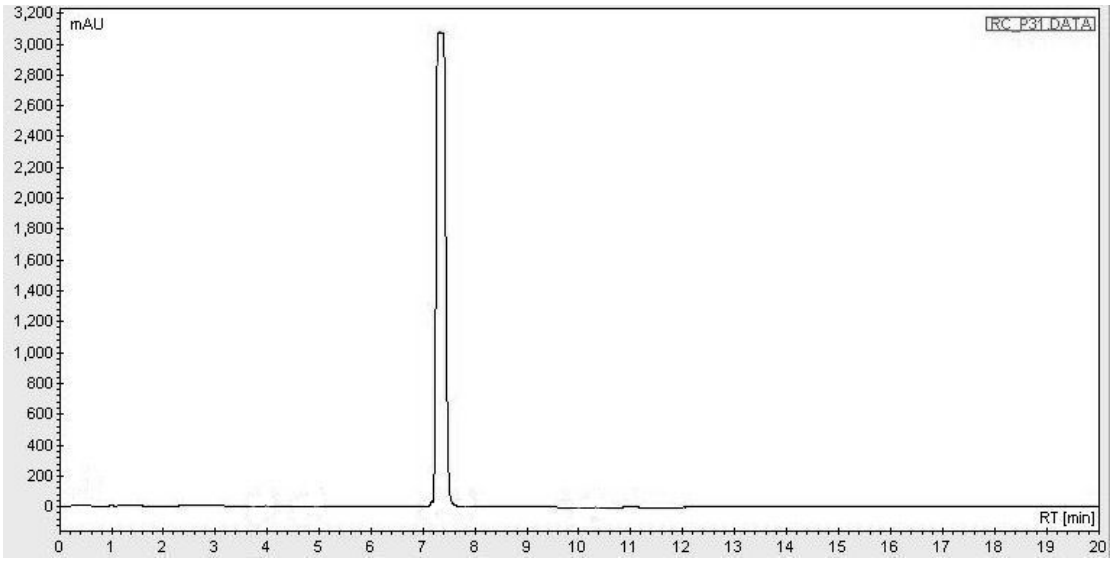


Figure C.1 HLPC chromatogram of the synthesized paracetamol, sample 3

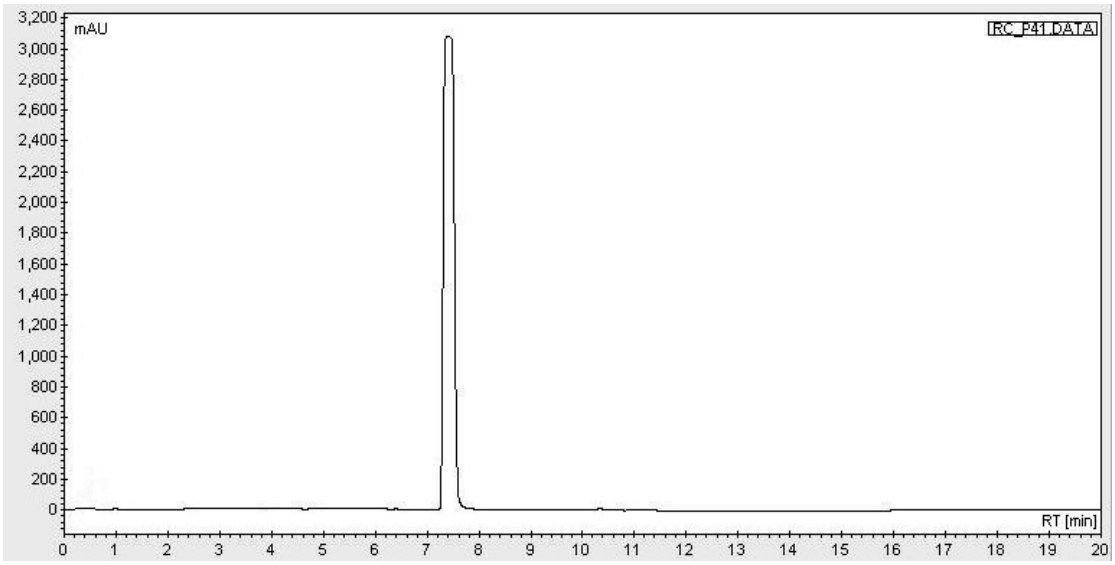


Figure C.2 HLPC chromatogram of the synthesized paracetamol, sample 4

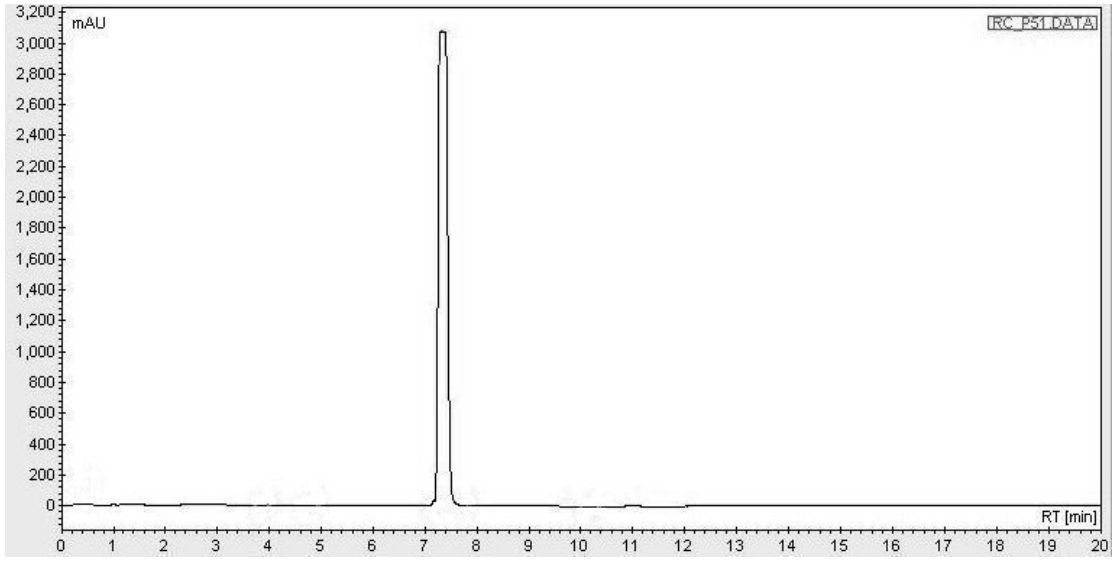


Figure C.3 HLPC chromatogram of the synthesized paracetamol, sample 5

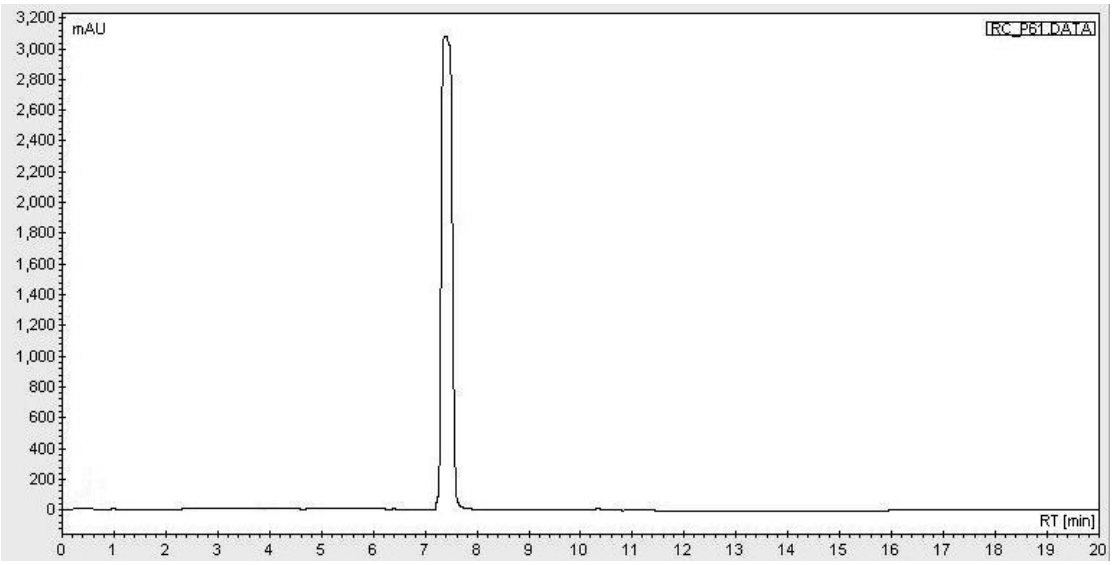


Figure C.4 HLPC chromatogram of the synthesized paracetamol, sample 6

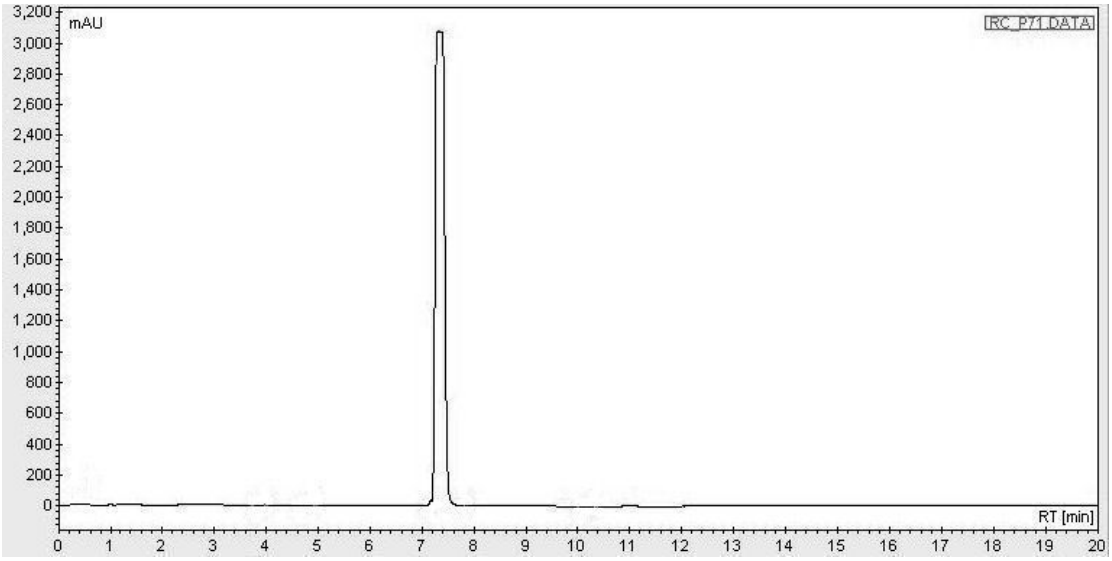


Figure C.5 HLPC chromatogram of the synthesized paracetamol, sample 7

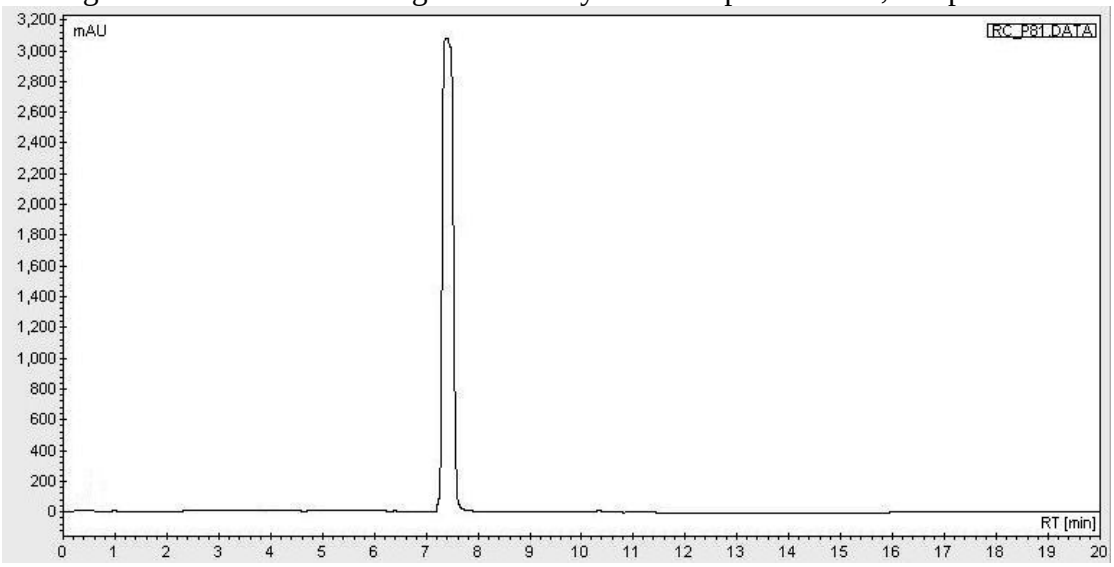


Figure C.6 HLPC chromatogram of the synthesized paracetamol, sample 8

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