Dynamic Extraction Technology

A new multi-stage liquid-liquid extraction process is just starting to prove itself in the fast and effective production of large-scale, trial-grade samples of pharmaceutical products.

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It is becoming increasingly important for industries to shorten the time taken to develop manufacturing processes for new products, increase efficiency, reduce costs and reduce solvent usage. Dynamic Extractions Ltd (DE) is building on the R&D expertise at the Brunel Institute for Bioengineering (BIB) at Brunel University (Uxbridge, UK) and has developed a range of rapid, continuous multi-stage liquid-liquid extraction centrifuges which use the same principle of purification at all scales. These provide scale-up from analytical to pilot scale, capable of processing up to 10kg/day of crude extract with 100% sample recovery in the working day. The prospect of full process (manufacturing) scale dynamic extraction is being investigated.

THE MAGIC TUBING

The dynamic extraction process centres on what we call 'the magic tube' element of a dynamic extraction centrifuge. It has the magical properties of being able to stratify two layers of immiscible liquids along its length, have discrete and regular waves of mixing and settling travelling along this tubing (much like waves travel across the sea), and hold a chosen phase stationary against the motion of the other mobile phase. Add to this that there is no solid support, as in HPLC, and that 100% sample recovery is guaranteed, then it really does become truly magical. The Dynamic Extraction Centrifuge sits in one room with intriguing moving parts, flashing lights and noises, while the operations end is just a simple liquid handling exercise. Operation is simple – first, the magic tube is filled with the phase intended to be the stationary phase, and the sample loop is filled with the crude extract (typically diluted 50%w/w with the stationary phase). Then the mobile phase flows in and – *'Hey Presto!'* – the crude sample mixture injected with the mobile phase then elutes in its fractionated component parts over a period of 4-15 minutes. The process can be operated in batch mode with a cycle time of about 25 minutes, or in extract mode where the stationary phase is completely saturated with sample and the mobile phase selectively extracts components from the stationary phase.

THE FIRST LANDMARK

At the time of writing, the first major landmark of the technology has just been reached – its ability to fractionate sufficient quantities of >98% material for clinical trials in economical time periods. For example, with the current Pilot Scale Centrifuge, each run load was about 0.3kg of 50%w/w crude extract (complete with precipitates) and resulted in 25g of pure active pharmaceutical ingredient (API) being purified with 70% being >98% pure (see Figure 1). But first some background...

The robustness and ease of technology transfer of the process has been confirmed through the successful outcome of a recent contract from the Johns Hopkins University in the US, and funded by the US National

Cancer Institute, for scaling up and purifying a potential new cancer preventative drug extracted from broccoli seeds. This is a glucosinolate preparation with two very similar elements to separate – differing only by a methylene group (glucoraphanin – GR, and glucoiberin – GI). Dynamic Extractions Ltd is currently the only company in the world with the expertise to scale up their laboratory conditions to produce a quantity of the purified material for use in clinical tests. Johns Hopkins can currently only purify 1.5grams per day (1).

The robustness of the technology transfer was confirmed when we were able to demonstrate a similar resolution separation within three days on a middle-range dynamic extraction centrifuge – that is, the same extract, phase system and protocol, but with a different centrifuge, a different scale and different operators. We then received a contract to undertake a loading study. The results of this study were reported at the end of August at CCC 2004 (the Third International Conference on Countercurrent Chromatography) in Tokyo (2). The main outcome was that, starting with a crude extract (which could even contain undissolved particulates), concentrations of 50%w/w could be injected in up to 30% of the system volume. The volume loaded only depended on whether such high volumes affected the hydrodynamics of the system.

SCALE-DOWN TO SCALE-UP

Loading studies at large scale are expensive in terms of time (human resourses), setting up, sample and solvent usage. We utilised the approach used by the Biochemical Engineering Department at University College London – namely, to do all the optimisation and load studies quickly at the millilitre scale, and then use this information to predict conditions at the litre scale. An example of chromatograms of a fractionation of a crude extract of broccoli seed from both the Mini-DE centrifuge (17ml capacity) and the Midi-DE centrifuge (0.93 litre capacity) is shown in Figure 2. The resolution between the glucoraphanin and glucoiberin is seen to be identical. This represents a scale-up from Mini-DE to Midi-DE of 80x on sample throughput. Considering the process has not been optimised to the extent that a production process would be, this was better than expected from a linear scale-up on tubing bore (22x) and on capacity (55x).

Following a successful purification of 50g of >98% pure glucoraphanin, Dynamic Extractions Ltd was asked to prepare sufficient quantities for clinical trials. The larger 4.6 litre capacity Maxi-DE centrifuge was chosen for this with sample loading optimised on the 5ml capacity Mini-DE centrifuge. This was a 920x scale-up on capacity and, again, similar chromatograms were achieved at both scales. However, it was noted that the larger scale gave better resolution, yield and purity than the smaller scale had predicted.

Figure 1: Typical purity yield characteristic for the Maxi-DE centrifuge (4.6 litre capacity) running at 0.4 litres/min and with a 5% sample loading at 50%w/w. 70% of sample was recovered with >98% purity (the 'heart cut') – the rest was concentrated and reprocessed for a second run

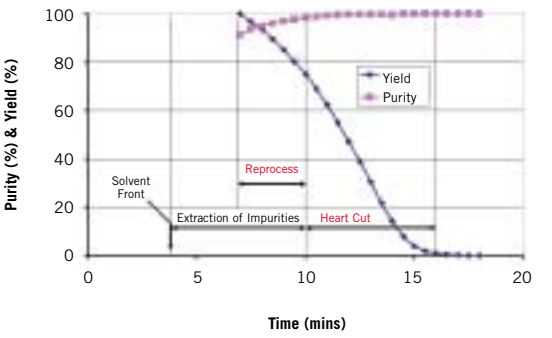
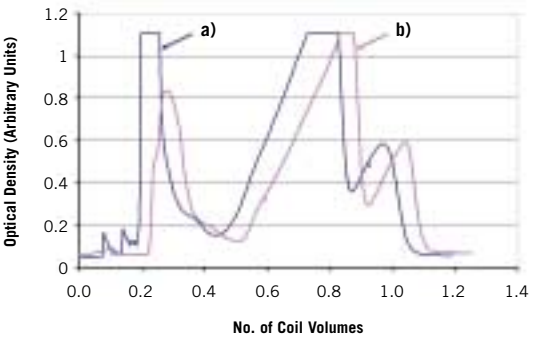


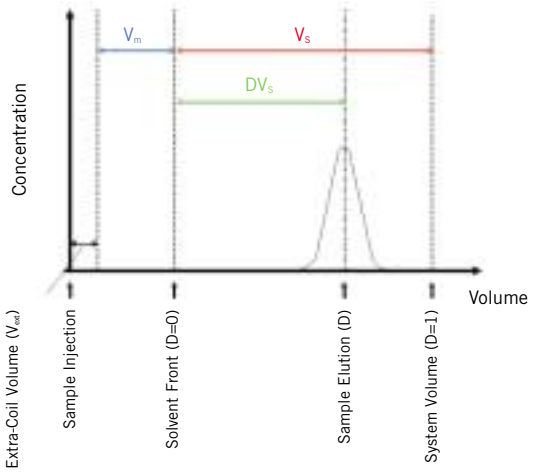
Figure 2: Comparison of the elution profiles of an extract from broccoli seed on: a) the MIDI-DE centrifuge (Vc= 0.93 litres, F=40ml/min, bore=3.73mm) and b) the MINI-DE centrifuge (Vc=17ml, F=0.5ml/min, bore=0.8mm) – both with a sample loading of 1% coil volume @ 50%w/w



REVEALING SOME OF THE MAGIC

As with any 'black box' technology, it is not always necessary to know what is happening inside the black box – as long as the process works predictably under your control from outside of the box.

Figure 3: Schematic representation of when a sample of distribution ratio (D) will elute in a DE centrifuge, when the volume of stationary phase (Vs) and mobile phase (Vm) is known



The tubing is wound on a drum (we call it a bobbin), and the drum is rotated in planetary motion (imagine a toothed planetary gear rotating around a stationary gear). The locus of a point on the extremities of the planetary gear, to which the drum is attached, is a cardioid. On one extreme, there is a very large acceleration field produced on the part of the drum furthest from the point of rotation; this settles the two phases. On the other extreme, at the point nearest the centre of rotation, there is a lower acceleration field and the phases get mixed up. These zones of mixing and settling are synchronous with the coil rotation, and travel along the coil accordingly. The motion sets up a pumping action between the phases. In fact, Wood *et al* describe the coil planet centrifuge used in this way as a constant pressure pump (3), and have found the stationary phase volume retention behaviour to be quite predictable as a function of flow.

Material will elute from the tubing depending on its distribution ratio in the aqueous/organic phase system used. Predictable knowledge of the distribution of the two phases in the system leads to the ability to predict exactly when a particular component with a particular distribution ratio will elute, as shown in Figure 3.

HISTORY OF THE PROCESS

In 1998, the Brunel Institute for Bioengineering (BIB) at Brunel University received a BBSCC/DTI LINK award for the 'Industrial Scale-up of Countercurrent Chromatography'. This research showed the feasibility of scale-up from analysis to pilot-scale production of drugs using a dynamic liquid-liquid extraction /chromatography process. Once the feasibility was demonstrated, two further grants were obtained for research into the engineering challenges of building planetary centrifuges at both small and large scales. This resulted in a mini-centrifuge which could be linked to a mass spectrometer, and a maxi (pilot-scale) unit capable of processing kilograms of purified material per day. The success of the research has led to BIB receiving a £1 million SRIF2 (Science Research Investment Fund) award from the Brunel University's HEFCE (Higher Education Funding Council for England) allocation to set up an Advanced Bioprocessing Centre to advance the technology further and develop an applications portfolio in the pharmaceutical, agrochemical and fine chemical industries.

DYNAMIC EXTRACTION

Dynamic Extraction (DE) is a recent development of countercurrent chromatography (CCC). While CCC has been known and used for many years, and a wide range of compounds have been separated, it has remained essentially a laboratory tool. It has not been the first method of choice when developing commercial separations. Separations using the old CCC technology took hours, equipment was unreliable, method development required experience and the choices of instrumentation were far too diverse. With DE technology, separations take minutes rather than hours, the equipment is more robust and scale-up to pilot scale has been shown to be both quick and easy.

To distinguish these designs and machines from former CCC instruments, and to highlight the added advantages they provide, we have introduced the term 'dynamic extraction' for CCC utilising rotating coils which can be used for process-scale separations. What BIB has done is to combine the science with solving the engineering problems of large-scale production. The Institute has excellent engineers, which it has linked with a sound understanding of the processes and problems of existing machines.

Dynamic Extractions Ltd is a company that has evolved from the success of the Institute's research programmes on the scale-up of countercurrent chromatography. It is focused on the development of novel separation technology – for use by the international pharmaceutical

industry – by providing improved separation solutions to their existing and future development and manufacturing needs.

Dynamic Extractions Ltd has also received a Small Business Research Initiative Grant of £125,000 for research on the further scale-up of the technology, and a DTI SMART award for developing prototype coils that suit industry's needs. In addition, one of the BIB staff has been seconded to Dynamic Extractions Ltd for one year under the EPSRC (Engineering and Physical Sciences Council) RAIS (Research Associates Industrial Secondments) scheme.

Figure 4: a) the Mini-DE (5-17ml capacity, mg scale, W35xH35xD42cm); b) the Midi-DE (0.1-1.2 litre, g scale, W65xH51xD170cm); and c) the Maxi-DE (5-15 litre, kg scale, W150xH190xD170cm)

The company is currently marketing three centrifuges – the Mini-DE (5-17ml capacity, mg scale); the Midi-DE (0.1-1.2 litre, g scale) and the Maxi-DE (5-15 litre, kg scale). Figure 4 shows the prototypes of these centrifuges.

CONCLUSION

As a result of work by Dynamic Extractions Ltd, the pharmaceutical industry now has a high resolution, high-throughput separation process that will shorten the time-to-market for new drugs and chemical entities. The process is tolerant of particles and high loading concentration/volume, and can therefore replace a number of processing steps, greatly simplifying the production process, as well as regulatory issues.

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Acknowledgements

The authors would like to acknowledge that the new technology described in this article has resulted from a team effort by staff at Dynamic Extractions Ltd and Brunel Institute for Bioengineering, with financial support from both the BBSRC (Biotechnology and Biological Sciences Research Council) and EPSRC.

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