Preparative Enantioselective Chromatography

Edited by

Geoffrey B. Cox

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Editorial Offices:
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Tel: +44 (0) 1865 776868
Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014-8300, USA
Tel: +1 515 292 0140
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Contributors

Henri Colin	Novasep SAS, Site Eiffel, Boulevard de la Moselle, BP 50, Pompey, France
Geoffrey B. Cox	Chiral Technologies, Inc., 800 North Five Points Rd., West Chester, PA 19380, USA
Olivier Dapremont	Aerojet Fine Chemicals, P.O. Box 1718 Rancho Cordova, CA 95741, USA
Félicie Denet	Novasep SAS, Site Eiffel, Boulevard de la Moselle, BP 50, Pompey, France
Jules Dingenen	Johnson & Johnson Pharmaceutical Research and Development, Department of Global Chemical Development – Preparative Separation Techniques, Tournhoutseweg 30, B 2340 Beerse, Belgium
Eric Francotte	Novartis Institutes for BioMedical Research, Discovery Technologies, WKL-122.P.25, Postfach CH-4002, Basel, Switzerland
Charles M. 'Pete' Grill	Pfizer, Inc., MS 9185-314, Eastern Point Road, Groton, CT 06340, USA
Michel Hamende	UCB Pharma, Chemin du Foriest, B 1420 Braine-l'Alleud, Belgium
Olivier Ludemann-Hombourger	Novasep SAS, Site Eiffel, Boulevard de la Moselle, BP 50, Pompey, France
Vera G. Mata	Laboratory of Separation and Reaction Engineering, School of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
Laurence M. Miller	Amgen, Inc., One Kendall Square, Building 1000, Cambridge, MA 02139, USA
Luke A. Miller	GlaxoSmithKline, CASS, Discovery Research, Research Triangle Park, NC 27709, USA

Luís S. Pais School of Technology and Management, Bragança Polytechnic Institute, Campus de Santa Apolónia, Apartado 1134, 5301-857 Bragança, Portugal

Alírio E. Rodrigues Laboratory of Separation and Reaction Engineering, School of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

Cristina Suteu Chiral Technologies Europe, Parc d'Innovation, Blvd Gonthier d'Andernach, 67404 Illkirch, France

Manon S. Villeneuve GlaxoSmithKline, DR/CASS/Analytical Sciences, Research Triangle Park, NC 27709, USA

Christopher J. Welch Merck & Co., Inc., Analysis & Preparative Separations Group, Department of Process Research, Merck Research Laboratories, Ry 800 C-362 Rahway, NJ, USA

Preface

GEOFFREY B. COX

Life is chiral. The peptides, proteins and carbohydrates from which we are all made are generally available in only one configuration, made up of enantiopure building blocks. It is not so surprising that the enantiomers of even small molecules have different properties (physiological or pharmacological) when placed in the chiral environment of living organisms. This fact, underlined by the thalidomide tragedy played out in Europe some years ago, is at the basis of the current regulatory environment where the enantiomers of any drug candidate have to be tested individually for toxicity, activity and side effects and where, for the many cases in which the properties differ, only an enantiopure drug is deemed to be acceptable.

Enantioselective chromatography is generally accepted to be the most rapid route to the preparation of small quantities of pure enantiomers. In recent years, it has begun to be realised that chromatographic processing does not have to be the excessively expensive and wasteful process that many chemists and engineers have been educated to believe; given careful optimisation, chromatographic processes can be cost-competitive with the other routes to enantiopure materials.

Against this background of the expansion of use of preparative enantioselective chromatographic techniques throughout the industry, the need is clear for a book that brings together experiences from people who have been involved in the development, improvement and implementation of chromatographic purification at all scales from laboratory to production and using all techniques, HPLC, SFC and multicolumn operation. The book is not written by academics for teaching, although one can argue the benefits of having fresh graduates who understand something of the preparative separations they will be asked to perform. It has been written by those with a job to do for those who will work with them today and who tomorrow will have to follow on after them. These scientists will need the depth of understanding and practical experience that is contained within these pages. From a wider viewpoint, and especially at the larger scale of operation, there are many who are looking for answers about preparative and production-scale chromatography. In the scope of the various chapters, from dealing with the smallest to the largest scale, these people, too, can find the information they seek.

Many of the authors who have contributed to this book have been in and around the pharmaceutical industry at the cutting edge of the development of enantioselective preparative chromatography. All are recognised by their peers as being experts in their field. The aim of the book is to bring this expertise to answer many of the questions that are often raised:

- What is going on in the preparative column?
- What chiral stationary phase should one use?

- How can one develop preparative separation methods; are they different from analytical methods?
- How does one carry out preparative chromatography; what equipment should be used?
- What is involved in recycling, in SFC and multicolumn techniques such as SMB?
- What about production-scale chromatography; is it possible, economic and should it be done in-house or outsourced?

The book contains many case studies to illustrate the principles, although as these studies are from work carried out in the pharmaceutical industry the true identities of the compounds concerned are often shrouded in secrecy. This does not matter greatly, since the purpose is to illustrate what to do and how to do it rather than discuss the intimate details of molecular interactions (which in any case only too often descends into optimistic arm-waving when attempting to find explanations of why an enantioselective separation occurs – or does not occur). Collectively, we have tried to address the needs of most of the people involved in enantioselective chromatography, from the analytical or discovery chemist who needs to isolate a few milligrams of a new compound, through those who need to isolate the few grams or few tens of grams for further study, to the development chemists and engineers who have the job of isolating materials for clinical trials and eventually taking the decision if the chromatographic route is viable for manufacturing.

My thanks go to the contributors to the book who have somehow fitted this extra task into their very busy lives and who have accepted the prolonged gestation period as I have fitted my tasks as editor into mine. My thanks also go to Blackwell's editor, Paul Sayer, without whose patience and encouragement this book would still be something necessary but unavailable.

1 Chiral chromatography in support of pharmaceutical process research

CHRISTOPHER J. WELCH

1.1 Introduction

Preparative chiral chromatography has recently become a preferred method for rapidly accessing enantiopure compounds in the pharmaceutical industry [1–8]. While preparative chromatographic enantioseparation has been practiced for a number of years by specialized researchers, the current widespread interest in the approach can be attributed in part to advances in equipment and stationary phases, but more importantly, to an increasingly widespread realization of the cost-effectiveness of this technique. In many instances, developing and executing a chromatographic enantioseparation is faster and less labour-intensive than more traditional approaches for accessing enantiopurity. Consequently, preparative chiral chromatography is increasingly used in place of, or in conjunction with, the more traditional methods of organic synthesis. We herein present a general introduction that focuses on some of the current areas of interest in the field of preparative chromatographic enantioseparation, which we hope will be useful to newcomers and experienced practitioners alike.

1.2 A brief introduction to chirality

If one imagines the set of all possible organic molecules with molecular weight less than 1000, it can readily be appreciated that most of these structures are *chiral*, i.e. they cannot be superimposed on their mirror images. Only those comparatively simple and symmetrical structures that show a plane, centre, or alternating axis of symmetry are *achiral*. The two mirror image forms of a chiral molecule are termed *enantiomers*, and a mixture composed of equal proportions of the two enantiomers is termed a *racemic mixture* or *racemate*. A mixture containing an excess of one enantiomer is said to be *enantioenriched*, while a mixture containing exclusively one enantiomer is said to be *enantiopure*. *Enantioenrichment* is typically reported in terms of *% e.e.* or *enantiomeric excess*. This is a somewhat antiquated term indicating the excess of pure enantiomer relative to the racemate, and given by the formula e.e. $=$ (major $-$ minor)/(major $+$ minor), where major and minor denote the relative amounts of the more and less prevalent enantiomers. Enantioenrichment is also sometimes expressed in terms of *e.r.* or *enantiomeric ratio*, which is simply the ratio of the major to the minor enantiomer. Clearly, enantiopurity is an ideal that is only approximated in the real world, although the term 'enantiopure' is routinely used somewhat loosely to denote substances that are $>98\%$ e.e. A process whereby a racemic mixture is separated into its two component enantiomers is termed a *resolution*, and a process where enantioenriched mixture is converted to a racemate is termed a *racemization*. The interested reader is directed to Eliel's *Stereochemistry of Organic Compounds* [9] for a more comprehensive description of key stereochemical terminology.

1.3 Why chirality is important

Most of the molecules of importance to living systems are chiral, e.g. amino acids, sugars, proteins and nucleic acids. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. When a chemist synthesizes a chiral molecule in an achiral environment using achiral starting materials, an equal mixture of the two possible enantiomers (i.e. a racemic mixture) is produced. In order to make just one enantiomer, some enantioenriched starting material, reagent, catalyst, or template must be present in the reaction medium. Oftentimes, only a single enantiomer of a chiral molecule is desired, as is the case when the target molecule is a chiral drug that will be used in living systems. Drug molecules can be likened to tiny keys that fit into locks in the body and elicit a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomer of the 'key' molecule should be used (the mirror image of our car key will not start our car). In general, the use of both enantiomers in a racemic formulation of a chiral drug may be wasteful, and sometimes even introduces extraneous material that may lead to undesired side effects or adverse reactions.

The importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades. As technologies for measuring and making enantiopure materials have improved, the production of enantiopure pharmaceuticals has become commonplace, with many of the top selling drugs in the world now being sold in enantiopure form. Consequently, the subject of chirality and the pharmaceutical industry is a topic of considerable recent interest and importance [10–12].

1.4 Accessing enantiopurity: a brief overview of approaches

Chiral chromatography is but one of a number of methods for providing enantiopure compounds. While more detailed descriptions of each of these approaches can be found in individual textbooks, or in a generalized text on the subject of stereochemistry, some discussion of the *pros* and *cons* of these various approaches is appropriate for placing chromatographic enantioseparation in the appropriate context.

1.4.1 *Enantiopure starting materials: the chiral pool*

The most straightforward approach to accessing enantiopure materials is to use starting materials that are available from the 'chiral pool' of naturally occurring enantiopure materials (e.g. amino acids, sugars, terpenes). This approach is preferable when the starting materials are inexpensive and readily available, and when the synthetic sequence from the chiral pool material to the target compound is direct and straightforward. Synthesis from chiral pool starting materials is a tried and true approach that has been used for many years. The approach sometimes suffers from the disadvantage of requiring numerous synthetic steps to achieve the goal of proper insertion of stereochemistry into the target molecule, and so it is not practical for all syntheses. The art of transforming the chirality of natural materials into the required stereochemistry of biologically relevant target molecules has a long and rich history, which is evidenced in much of the body of classical natural product

synthesis. The subject is specifically treated in Hanessian's classic, *The Chiron Approach to Natural Product Synthesis* [13].

It is worth noting here that the chiral pool of naturally occurring enantiopure starting materials is continually being augmented by newly available commodity chemicals that are produced via new chirotechnology approaches. Consequently, much of modern enantioselective synthesis can be viewed as drawing from an 'expanded chiral pool' [14]. When viewed in this light, the importance of keeping up to date with newly emerging chirotechnology approaches and their associated enantiopure products can readily be appreciated.

1.4.2 *Removable enantioenriched auxiliaries*

An alternative approach to synthesis from chiral pool starting materials, also with a decades-long history, involves the use of removable enantioenriched auxiliaries to influence the stereochemistry at a newly formed stereogenic centre. After removal of the auxiliary, an enantioenriched product is obtained. Classic examples of this approach can be found in the work of chemists such as Evans [15,16], Meyers [17,18] and Seebach [19]. A wide variety of chiral auxiliaries have been used, but they most often are derived from enantiopure chiral pool starting materials such as amino acids. Advantages of the chiral auxiliary approach include predictability and dependability. Major disadvantages include the need for a full equivalent of the chiral auxiliary and the need to attach the auxiliary to the substrate and subsequently remove it after reaction. This requires a minimum of two additional steps, and sometimes considerably more. Consequently, this technique can be rather ungainly, especially at industrial scale, and has been criticized as an approach with poor *atom economy* [20,21] since a full equivalent of auxiliary is used and converted to waste for each stereocentre that is set. This problem can sometimes be addressed through recovery and recycling of the auxiliary. Nevertheless, the auxiliary approach is less frequently used today than in the past.

1.4.3 *Enantioselective catalysis*

Most of the disadvantages of the auxiliary approach are overcome with enantioselective catalysis [22] – a substoichiometric amount of an enantioenriched material is used to control product stereochemistry, and, in contrast to the auxiliary approach, the chemical steps needed for appending and then later removing the stereochemically determining component are eliminated. With enantioselective catalysis, the stereochemical outcome of the new bond formation is determined in the catalytic reaction that leads to the newly formed stereocentre. In addition, a single catalyst molecule can effect multiple transformations, millions in the best cases [23]. In such cases with high catalyst turnover, even relatively expensive catalysts can be used for economical production of enantiopure products. Although enzymes remain the prototypical enantioselective catalysts, and are extensively used in enantioselective synthesis, considerable progress in the development of *synthetic* enantioselective catalysis has occurred in recent years [24]. A wide variety of such catalysts are now available for many different kinds of synthetic transformations. In many cases these catalysts show a degree of substrate generality that renders them quite useful and predictable tools for enantioselective synthesis.

1.4.4 *Resolution technologies: introduction*

Resolution techniques in which the two enantiomers comprising a racemic mixture are physically separated are an important and extensively utilized family of approaches for accessing enantiopure materials on a large scale. The major resolution approaches, classical resolution via diastereomeric salt formation, resolution of conglomerate crystals, kinetic resolution and chromatographic resolution, will be described in more detail below. Initial considerations suggest that all resolution approaches suffer from a common fundamental drawback of being inherently wasteful, as at most only half of the material is recovered (the half corresponding to the desired enantiomer, the other half being 'waste'). However, recycling of the undesired enantiomer is sometimes possible, enabling higher yield and reduction of waste. Coupling of such resolution and racemization approaches can lead to truly impressive processes for generating enantiopurity [25], and such approaches have long been a mainstay of successful industrial-scale synthesis of enantiopure materials. The most widely utilized resolution technologies are summarized below.

1.4.4.1 *Resolution technologies: classical resolution* Classical resolution of enantiomers via diastereoselective salt formation is an important technique with a long history [26–28]. In this approach, a single enantiomer of a resolving agent is mixed with the two enantiomers of a racemate, leading to two diastereomeric salts with differing solubility properties, which are subsequently separated by crystallization. As resolving agents, naturally occurring acids or bases from the chiral pool have traditionally been used (e.g. tartaric acid, quinine), although in recent years, reagents derived from the 'expanded chiral pool' have also been used. Classical resolution remains one of the preferred methods for industrial-scale resolution. In a related technique, a chiral derivatization reagent can be used to prepare covalent diastereomeric derivatives, which are then separated by crystallization, chromatography or other means, and which afford the purified enantiomer upon deprotection.

1.4.4.2 *Resolution technologies: conglomerate resolution* Racemic materials can crystallize in several ways, as illustrated in Fig. 1.1. Most racemates crystallize as racemic compounds in which the two component enantiomers (R and S in the figure) are regularly arrayed within the crystal lattice in equal numbers. Thus, analysis of any single crystal always reveals a 1:1 ratio of the two component enantiomers. A few percent of all racemates

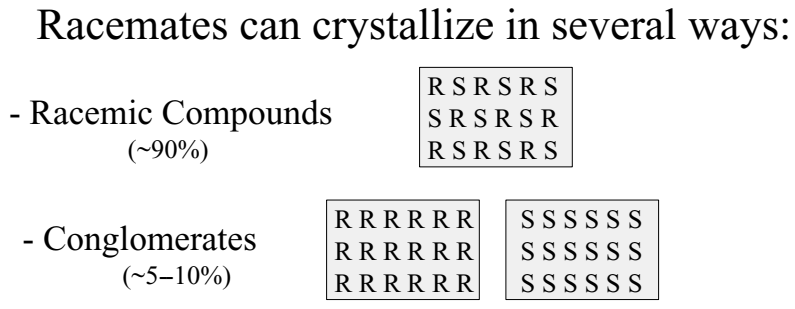

Figure 1.1 Racemates can crystallize as either racemic compounds, where both enantiomers are present in the crystal lattice, or more rarely as conglomerates, where two enantiomorphous crystal types are formed.

crystallize as conglomerates, in which two enantiomorphous crystals are present, each containing only one of the component enantiomers [26]. Consequently, analysis of any single crystal of a conglomerate shows a very high level of enantioenrichment.

The technique of *triage*, the manual sorting of conglomerate crystals, is the method by which Pasteur initially separated the enantiomers of the conglomerate, sodium ammonium tartrate [25]. Manual sorting of conglomerate crystals is, of course, an unsuitable technique for generating enantiopure materials on large scale (although some interesting physical sorting devices have been proposed [29,30]). Large-scale resolution processes are sometimes based on preferential crystallization approaches, where a supersaturated solution of the racemate is seeded with a single enantiomorphous form of a conglomerate crystal, with the ensuing kinetically controlled crystallization affording the desired crystals of a single enantiomorphous form. It is important to understand that this is a *kinetically* controlled process, and that if the contents of the crystallization vessel were allowed to come to equilibrium, a 1:1 mixture of the two enantiomorphous crystal forms would result. Seeding with a single crystal form of the conglomerate and harvesting the product crystals before the undesired enantiomorphous crystal begins to form can afford material with very high enantiopurity.

As with any resolution technology, recycling the undesired enantiomer is an important consideration for process economy. The industrial process developed more than 30 years ago for preparation of the drug, Aldomet^{TM}, is based on a conglomerate resolution that takes place in a continuous crystallization reactor with periodic crystal harvesting and a recycling of the undesired enantiomer via racemization [31]. While conglomerate resolutions can be attractive for commercial processes, most compounds are unsuitable for resolution with this technique.

1.4.4.3 *Resolution technologies: kinetic resolution* Kinetic resolution is another resolution technology that is frequently used to access enantiopure materials at industrial scale [32]. In this approach, the enantiomers of a racemic mixture undergo reaction with a chiral reagent or catalyst at differing rates, leading to a build-up of one of the enantiomers of either product or starting material over time. In kinetic resolution, frequently carried out with enzyme catalysts, the enantiopurity of the product varies with time, and the reaction must be harvested at the appropriate point to afford maximum yield and enantioselectivity of the desired enantiomer. Consequently, method development is often not as straightforward as with other resolution technologies, making kinetic resolution generally less well suited for small-scale, short-term needs. However, kinetic resolution can be very useful for industrial-scale manufacturing, provided that the economics are favourable. The all-important parameter for kinetic resolutions is the stereoselectivity factor (s), which is the ratio of the rate constants for reaction of the two enantiomers. While in principle, enantiopure material can be generated whenever $s > 1$, in practice high enantiopurity can only be achieved at the expense of greatly reduced yield when s is small. On the other hand, high s values allow access to enantiopure material with very little loss in yield. As with all resolution approaches, recovery and recycling of the undesired enantiomer is important. However, unlike other resolution approaches, the two enantiomers produced in a kinetic resolution are chemically differentiated. For example in the kinetic resolution of a chiral ester, one enantiomer remains unchanged while the other is hydrolysed to the corresponding alcohol. In cases where stereoselectivity is very high, it may be possible to obtain both of these materials with high enantiopurity, and it is sometimes possible to convert *both* of

these materials to a common downstream product (e.g. using one sequence that affords inversion of the alcohol, and another sequence that affords retention with the ester). In the best examples of this approach, the two reactions can be executed simultaneously in the same pot without the need for any physical separation [33].

1.4.4.4 *Resolution technologies: chromatographic resolution* The technique of chromatographic enantioseparation depends upon the differential adsorption of enantiomers by an enantioenriched adsorbent material called the chiral stationary phase (CSP) (Fig. 1.2), which is packed into a column through which an eluent flows [34]. Selective adsorption of one enantiomer results in increased retention of that enantiomer on the column. The degree of adsorption of each enantiomer by the column is described by the retention factor k', which equals $[(t - t_0)/t_0)]$, where t is the retention time of the enantiomer and t_0 is the retention time of an unretained analyte or void marker [35]. The retention factor k' is proportional to the equilibrium adsorption constant, and is in effect a ratio of the time that the analyte spends adsorbed to the CSP relative to the time spent in the eluent.

The ratio of retention factors for the two enantiomers is given by α, the separation factor, also called the chromatographic selectivity. A complete lack of separation of the two enantiomers corresponds to a selectivity of 1.0. Separation factors of 1.05 or even a little less can be measured using highly efficient analytical columns; however, preparatively useful HPLC separations typically have α of at least 1.2, and hopefully 1.5 or better. Very large separation factors in excess of 100 are possible, although rare. In general, bigger separation factors are advantageous for preparative resolution of enantiomers, although other factors come into play, as will be discussed in a following section. Since the separation factor is the ratio of two numbers that are each proportional to adsorption equilibrium constants, α can be directly related to $\Delta\Delta G$, the difference in free energy of adsorption for the two enantiomers, by the equation $\Delta\Delta G = -RT \ln \alpha$, where R is the universal gas constant and T is absolute temperature in Kelvin. Variable temperature chromatographic studies are sometimes used to further extract the enthalpic ($\Delta\Delta H$) and entropic ($\Delta\Delta S$) contributions to this free energy term, which are sometimes of value in studies of the mechanism of enantioselective adsorption [36].

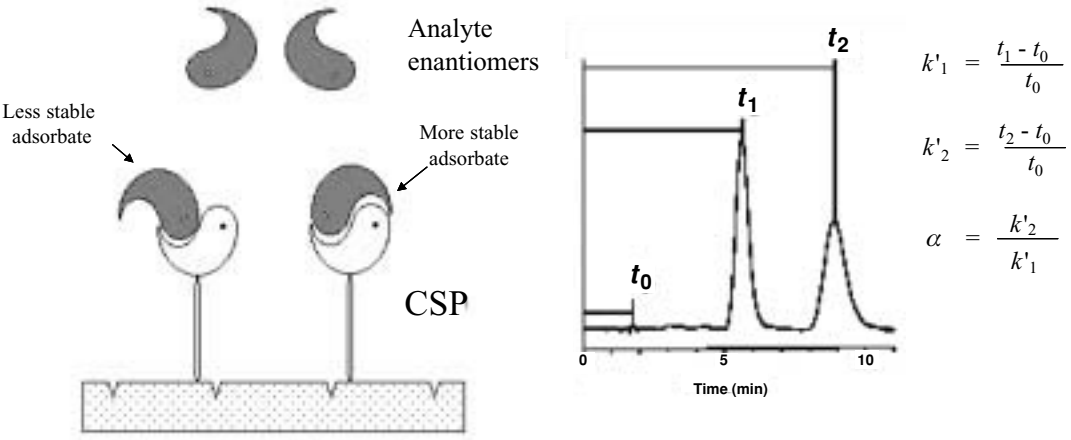

Figure 1.2 Chromatographic resolution of enantiomers depends upon differential adsorption of enantiomers by an enantioenriched adsorbent material called the chiral stationary phase (CSP).

The chromatographic parameter N, or *efficiency*, is given in terms of *theoretical plates*, and is a measure of the ability of the column to elute a nice, sharp, chromatographic peak. Most preparative chromatography takes place with columns that are considerably less efficient than those used for analytical HPLC [37]. Nevertheless, chromatographic efficiency is quite important for preparative separations. It should also be noted that in contrast to analytical HPLC, symmetrical peaks are no longer the goal. Small columns are routinely used for small-scale chromatographic enantioseparation [38,39], and because of the ready scalability of chromatography, such studies can be used to accurately model separations at larger scale.

1.4.5 *Chromatographic productivity is the key metric for preparative chromatography*

In addition to the familiar parameters describing any chromatographic separation, preparative chromatography is most concerned with the additional parameter, productivity, which measures how much purified material can be prepared with a given quantity of stationary phase per unit time. Owing to the predictable scalability of chromatographic processes, a measurement of productivity developed using a small column can accurately predict the requirements for separation on a much larger scale. Productivity is typically expressed with units of kg/kg/day (kilograms of purified enantiomer per kilogram of stationary phase per day), with a poor separation having a productivity of about 0.1 kg/kg/day or lower, and a good separation having a productivity in the range of 1 kg/kg/day. A truly remarkable separation might have a productivity greater than 10 kg/kg/day. Chromatographic productivity can easily be estimated by carrying out loading studies, where injection of progressively larger amounts of material onto an analytical HPLC column packed with the adsorbent of interest is performed. In the example illustrated below, the production rate (the rate at which enantiopure material can be produced) for various sizes of HPLC column can easily be calculated for a separation with a given productivity (Fig. 1.3). Estimates of chromatographic

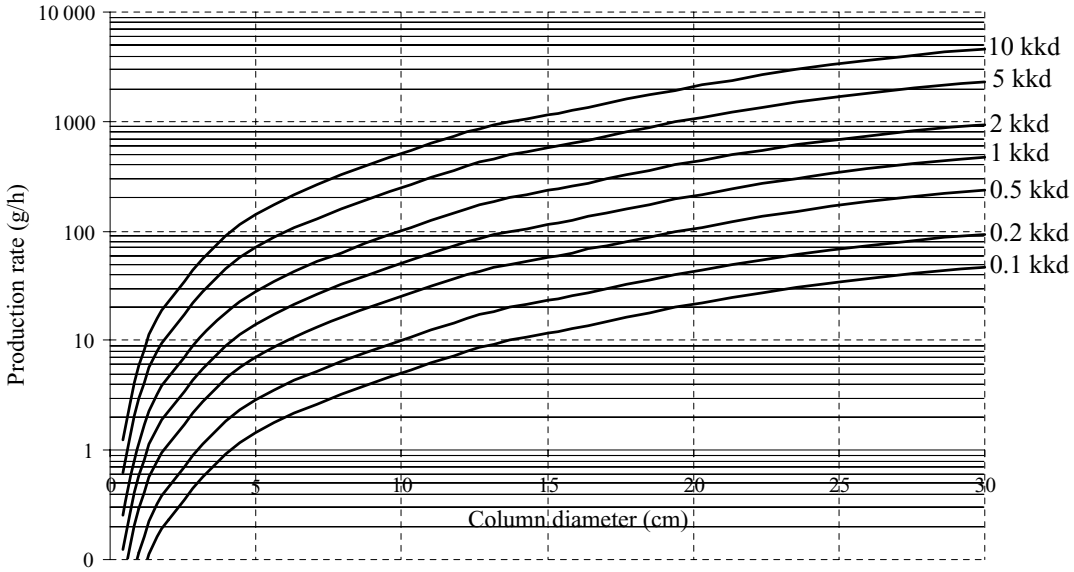

Figure 1.3 Production rate depends upon column size and chromatographic productivity. kkd = kg/kg/day.

productivity obtained from modelling with small columns allow the user to estimate the equipment, labour, and materials that will be required for carrying out a separation at any given scale.

An additional parameter to keep in mind when developing a chromatographic resolution is specific solvent consumption, which measures the amount of solvent required to purify a given amount of material. A low productivity resolution might require the use of as much as 10 000 L of solvent to obtain a single kilogram of purified material, whereas an outstanding resolution might require only a few hundred litres. Not surprisingly, solvent is generally recycled in larger scale resolutions, and solvent distillation and recycling is an important parameter contributing to the overall economics of a preparative chromatographic resolution.

1.4.6 *Stationary phases for preparative chiral chromatography*

Although many CSPs are available for use in analytical columns, only a few materials are available or widely used for preparative enantioseparation. By far, the most commonly used preparative CSPs at this time are the modified cellulose and amylose CSPs invented by Yoshio Okamoto and commercialized by Daicel Chemical Industries, Ltd. [40]. One of these materials, Chiralpak AD, based on the 3,5-dimethylphenylcarbamate derivative of amylose, is by far the most versatile preparative CSP on the market.

Several other types of CSP are routinely used for preparative chiral chromatography, and there are new commercial CSPs introduced each year. In addition, there are ongoing advances in the technology of CSP design and development [41–48]. While most commercial CSPs are used because they demonstrate some general ability to separate enantiomers, the chemist considering an industrial-scale resolution may be more interested in the ability of a CSP to separate the enantiomers of one particular compound, and there has been considerable recent interest in the preparation and evaluation of tailor-made CSPs for carrying out given separation tasks [49–51].

1.4.7 *Advantages of preparative chiral chromatography over other approaches for accessing enantiopure materials*

Preparative chromatography offers a number of advantages over competing techniques for rapidly accessing enantiopure materials, these advantages stemming from the speed and the relatively small amount of labour required to develop a chromatographic resolution method. Each of the various approaches for accessing enantiopure material (chiral pool, catalysis, auxiliary, classical resolution, preferential crystallization) employs a search protocol which first requires that the researcher develop a chiral assay, and then subsequently use this assay to screen for the appropriate combination of catalyst, conditions, reagent or resolving agent to achieve the desired result. Following the identification of a lead, the process is gradually optimized, and then investigated on pilot scale before going to large-scale production.

Chiral assays are typically performed using chiral chromatography, and method development often relies on automated or semi-automated analysis of a number of different commercial chiral chromatography columns and solvent combinations for their ability to separate the target enantiomers [52,53]. Such method development screening tools often turn up column and mobile phase combinations with a reasonably large separation factor

that can readily be translated, with just a few hours of labour, into a preparative chromatographic resolution. In such instances, the chromatographic option for accessing a few grams or even a few kilograms of material can already be in place before the development work for the competing approaches has even begun. When only small amounts of material are required, this method development advantage often makes chromatographic resolution the fastest and the most cost-effective option.

Clearly, there are more important considerations than speed and labour economy in the development of a process that will be implemented on an industrial scale, where overall process economy dictates which approach will be used. In such instances, an approach that requires much greater development labour may be selected if it can provide resolved material at a saving of just a few cents per kilogram.

1.4.8 *Simulated moving bed enantioseparation*

Most enantioseparation demands for pharmaceutical development can be met with traditional elution chromatography approaches utilizing 'touching band' separations (i.e. separations in which the two enantiomers are still baseline or nearly baseline resolved). Moving from the touching band situation to the sample overload situation results in a complex peak, where peaks become merged to a degree. Chromatographic productivity using such an approach can be superior to the touching band method, provided one has investigated the appropriate place for fraction cutting within the complex chromatographic peak. In addition, the solvent requirements for such separations can be reduced, although a considerable disadvantage is the need to collect and reprocess a middle fraction consisting of a mixture of the two peaks. The technique of simulated moving bed (SMB) chromatography, by using multiple columns, can allow for continuous chromatography with continuous reprocessing of the mixed fraction. SMB chromatography has recently gained attention as a useful tool for industrial-scale chromatographic separations [3–5,54], and can often lead to gains in productivity and reduced solvent consumption, which can improve the economics of a separation process at the appropriate scale [55]. A single column innovation has been developed [56] that utilizes recycling of the mixed fraction with augmentation with fresh feed solution so that a 'steady-state recycling' situation can be attained.

It should be noted that the increase in productivity and the solvent savings of SMB chromatography relative to touching band elution chromatography is most dramatic for those separations having poor chromatographic selectivity. The 'SMB advantage' is much reduced for the most enantioselective, highly productive separations. As a general rule, highly productive chromatography requires either highly selective separation media or high-performance separation equipment, but not both.

1.5 Green enantioseparation

The subject of 'Green Chemistry' has recently received considerable attention [57], although many of the central tenets of waste reduction, process economy, and elimination of risks and hazards have been embraced by industrial chemists for many decades. First impressions might incline one to the conclusion that chromatography, with its intensive use of solvent, is decidedly 'un-green'. However, industrial-scale separations, in order to

be economically viable, almost always utilize solvent recycling; so in order to be fair, one should focus only on waste solvent, and not recycled solvent [58]. Smaller scale preparative chromatography utilized in developmental research may eliminate the need for carrying out development using more traditional approaches, thereby saving considerable labour, and sometimes even resulting in a net decrease in waste generation.

The use of supercritical fluid chromatography (SFC) for preparative enantioseparation has enjoyed considerable recent attention [59–61], and is the method of first choice in our own laboratories. In this technique, supercritical or subcritical carbon dioxide replaces petrochemical derived hydrocarbons, resulting in reduction in solvent utilization by as much as 90% or more. Preparative SFC is not a net generator of carbon dioxide, a known greenhouse gas. Instead, it utilizes carbon dioxide which is condensed from the atmosphere, and then later returned to the atmosphere. Furthermore, with preparative SFC the product is recovered in a more concentrated form relative to HPLC, greatly reducing the amount of solvent that must be evaporated, and resulting in considerable savings in labour. In addition, because of the low viscosity of the supercritical fluid eluent, separations may be conducted at flow rates that would be impossible with liquid solvents, an advantage that can contribute to the oftentimes higher productivity of preparative SFC enantioseparations relative to HPLC methods. Cumulatively, these advantages make preparative SFC enantioseparation an attractive and potentially 'greener' addition to conventional HPLC approaches, and a technique with a promising future.

1.6 What is the appropriate role of preparative chromatography in organic synthesis?

The incorporation of preparative HPLC into organic synthesis is by no means a new phenomenon. R.B. Woodward, perhaps the most famous synthetic chemist of the twentieth century, was an early advocate of preparative HPLC, using some of the very first commercial HPLC equipment to separate complex mixtures of closely related isomeric intermediates required for his famous vitamin B_{12} synthesis. Woodward's experience prompted him to state in 1973 that

> *The power of these high pressure liquid chromatographic methods hardly can be imagined by the chemist who has not had experience with them; they represent relatively simple instrumentation and I am certain that they will be indispensable in the laboratory of every organic chemist in the near future.* [62]

Interestingly, it is only within the last few years that Woodward's prediction of the widespread adoption of preparative HPLC as an enabling technique for organic synthesis has begun to be borne out. The biochemical and natural product fields have embraced preparative HPLC for a number of years, and the HPLC purification of peptides, recombinant proteins, natural products and oligonucleotides has become commonplace [63]. The delayed realization of the Woodward prediction of widespread use of preparative HPLC within the field of organic synthesis may be in part due to Woodward being significantly ahead of his time, but can also be traced, in part, to a historical reluctance of synthetic chemists to utilize preparative chromatography. Many causes underlie this reluctance, ranging from

unavailability of equipment and expertise to a matter of aesthetic appeal, with the utilization of chromatography being viewed as 'inelegant' – a 'crutch' that helps an incomplete synthesis to cross the finish line, and something to be avoided when possible.

To be fair, most synthetic chemists have embraced the use of preparative chromatography in the form of flash chromatography, especially since the 1978 publication of Still's influential paper on the topic [64]. Nevertheless, despite its routine use in medicinal chemistry and early development, the use of flash chromatography in a final process came to be viewed (rightly so in many cases) as a hallmark of an inferior process. Thus, a generalized rule of thumb of 'first, eliminate all chromatography' came to be held by those engaged in developing large-scale processes from medicinal chemistry syntheses, and the 'eliminate chromatography at all costs' outlook is still occasionally encountered. Clearly, such shorthand notions are of limited use, and the question of whether or not to use preparative chromatography within a synthesis is purely an economic one, where the costs of different competing processes are calculated based on assumed production scales over time.

In recent years there has been a growing appreciation of the value that preparative chromatography can bring to organic synthesis, and rather than a crutch, the technique can be more of a 'fulcrum', empowering and enabling a synthesis. While industrial-scale processes which will be repeated on multi-ton scale over many years are still generally chromatography-free, there have been a growing number of examples in recent years where large-scale chromatography has proven to be the preferred approach on a production-cost basis.

Furthermore, the vast majority of the syntheses developed in academia and industry are performed on a relatively small scale, and destined to be repeated infrequently, if ever. This includes early preclinical drug candidates, the large majority of which are unsuccessful. In such cases, there is little practical advantage to developing a chromatography-free process, particularly when one considers the developmental labour required to do so. Understandably, the use of the enabling technology of preparative chiral chromatography is becoming increasingly accepted in pharmaceutical development. Given the fact that only a small fraction of all lead compounds in early pharmaceutical development survive the increased scrutiny of later stage preclinical investigation, a high value is placed on methods that can provide the compound needed for preclinical investigation quickly and with minimum labour and materials costs. A downside to this chromatographic shortcut is that should the candidate survive preclinical evaluation, a route with potential for larger scale manufacturing will be needed, and quickly. Thus, a balance between synthetic quality and speed often characterizes modern pharmaceutical process research, where needs to provide material quickly are weighed against needs to develop a process that is suitable for industrial scale.

1.7 Fording the river at the easiest point: some observations on the appropriate placement of a chromatographic resolution within a chiral synthesis

While the paradigm for developing and carrying out an enantioselective synthesis is now quite well established, strategies for the efficient use of preparative chiral chromatography within a synthesis are still evolving. As with the planning of any resolution, a certain

degree of flexibility should be maintained regarding placement within the overall synthetic scheme. Even more so than with enantioselective synthesis or non-chromatographic resolution approaches, seemingly minor changes in compound structure, for instance a change in protecting group, can dramatically influence chromatographic resolution and productivity. Consequently, a development strategy that examines a variety of intermediates and structural variants is more likely to afford a convenient and highly productive enantioseparation than a strategy that demands the resolution of one particular compound.

Developing a synthetic process can be likened to navigating in an unknown territory, with the objective of proceeding from starting materials to product with a minimum of effort. If we imagine a river dividing this territory, with racemates and achiral compounds on one bank, and enantioenrichment on the other, then a resolution can be likened to a river crossing. And, just as a navigator would never attempt a difficult river crossing without first scouting upstream and down, the sensible chemist closely examines the available options for chromatographic enantioseparation within a synthetic scheme, ever watchful for a place where the river can be forded with a single step. Clearly, the person deciding where the river should be crossed should have a firm grasp of the subject of preparative chromatography, otherwise forced crossings at rapids or canyons can inevitably lead to disaster and wasted effort.

1.8 Origins of preparative chiral chromatography

The year 2003 marked the centennial of Tswett's invention of chromatography, a technique that plays an essential supporting role in most areas of chemistry and biochemistry [65,66]. It is interesting to note that chromatography began life as a preparative method, and only later evolved into what would become the most widely used method for chemical purity analysis. Given the current pivotal role of chromatography throughout the chemical sciences [67], it is somewhat surprising to find that it was initially quite slow to 'catch on'. There was very little utilization of the technique from the time of Tswett's original work with separation of plant pigments until about 1940, when 'rediscovery' of the technique led to rapid developments in modern liquid and gas chromatographic techniques and theory [52]. Pumped flow liquid chromatography systems with UV detection were becoming known by the early 1960s, and preparative liquid chromatography using automated fraction collection and solvent recycling was known by the mid-1970s [68]. Since that time, preparative chromatography has grown in importance for conducting laboratory and industrial-scale purifications of organic compounds and biomolecules.

Like chromatography itself, chiral chromatography also has a long and rich history. It is interesting to note that much of the initial research into chiral chromatography was motivated by the desire to resolve enantiomers *preparatively*, with the adoption of chiral chromatography as the preferred method for measuring enantiopurity coming only later. Willstätter initially proposed the idea that the two enantiomers of a racemic dye might be differentially adsorbed by a biopolymer such as wool or silk in 1904 [69], and there followed several subsequent reports dealing with this phenomenon [70,71]. It was not until 1938 that the first chromatographic separations of enantiomers were reported by two different groups: one reporting a partial separation of the enantiomers of a camphor derivative using a lactose stationary phase [72], and the other reporting a partial separation of the enantiomers of an organometallic chromium complex using a stationary phase consisting of optically

active quartz powder [73]. Subsequently, Prelog and Wieland described the preparative separation of the enantiomer of Tröger's base using starch as a stationary phase [74], and Senoh and co-workers reported the chromatographic separation of amino acid enantiomers using paper chromatography [75]. Pauling's idea that polymerization in the presence of a 'template' molecule could lead to a stationary phase possessing some selectivity for the template molecule was demonstrated for enantiomers in 1952 [76], and the first 'brush type' bonded phase consisting of a chiral selector immobilized on a silica support was described by Klemn and Reed in 1960 [77]. The first separation of amino acid enantiomers using ligand-exchange chromatography was reported by Davankov and Rogozhin in 1971 [78], the same year that the first attempt at the development of a CSP designed specifically for the enantioseparation of a particular target molecule (3,4-dihydroxyphenylalanine) was reported [79]. In 1974, Cram and co-workers described the preparation of a completely synthetic CSP that showed high enantioselectivity for amino acid enantiomers [80]. As an outgrowth of studies on NMR chiral solvating agents [81], Pirkle and co-workers reported the first in a long line of CSPs in 1979 [82]. The first commercial CSP was introduced in 1980, followed rapidly by widespread general acceptance and utilization of chiral chromatography, and by the introduction of a number of commercial CSPs. Interestingly, the explosion of general research interest in chirotechnology [83] (the science of making and measuring enantiopure materials), which began in the early 1980s and continues unabated to this day, can in some measure be attributed to the availability of tools for the rapid and reliable quantitation of enantiopurity. Where it once took days or weeks to obtain oftentimes questionable results, the ready availability of commercial CSPs meant that accurate and reliable enantiopurity measurement could be obtained in a matter of minutes.

1.9 Practical tips for preparative chromatographic enantioseparation

(1) Screen broadly: A broad selection of high-quality stationary phases will increase the chances of developing a straightforward resolution for any compound.
(2) Invest in good chromatographic equipment: Quality equipment will pay for itself in labour savings over time.
(3) Invest in automation: Automated sample injection and fraction collection are a must – even in academic laboratories.
(4) Monitor and maximize productivity: The productivity of any separation method is easily measured, and developing a sense of how to maximize productivity is the key to successful preparative chromatography.
(5) Preparative chromatography begins where the pretty peaks end: beginners often inject too little sample onto the column. Do not be afraid to push injection size to the limit.
(6) Maximize the use of the column: Ideally, one should endeavour to use most of the column most of the time. For enantiomer separations the strategy of overlapping injections is frequently used so that *something* (either peak A or peak B) is always eluting from the column.
(7) Maximize flow rate: Increased productivity will result when columns are operated towards the upper end of their specified safe flow rate or pressure range. Less

viscous solvents allow greater flow, and are thus preferred as eluents. Operating large columns at low flow rates is a common beginner's mistake, which is unproductive and wasteful.

(8) Analyte solubility: Solubility of the analyte in the chromatographic eluent is an important factor in preparative separation. Poor solubility can render even a highly enantioselective separation unproductive. Make sure to consider solubility when selecting a chromatographic method, and when selecting the appropriate intermediate for chromatographic resolution.

(9) Compound stability: Compounds in solution are more prone to undesired reaction and decomposition than are compounds in the solid state. If chromatographic fractions are to remain in solution for prolonged periods of time, make sure that the chemical and stereochemical stability studies have been performed to ensure that there will be no surprises with formation of new impurities or with racemization or epimerization. Esterification, transesterification and ketal formation reactions are frequent problems when using alcohol modifiers, especially when strong acids such as trifluoroacetic acid are present.

(10) Beware crystallization of feed: By necessity, chromatographic feed solutions are highly concentrated, usually near the saturation point. As such solutions sit for prolonged periods of time, it is not uncommon to observe formation of crystals, even in cases where the compound has never before been crystallized. Formation of crystals in the feed mixture not only disrupts chromatography and potentially damages injector pumps or other hardware, but once crystallization has taken place it may be virtually impossible to return to the original operating conditions. It is therefore often prudent to prepare feed solution only as needed.

(11) Beware solvent impurities: Since large quantities of solvent are evaporated in product recovery, impurities in the solvent can become concentrated in the recovered sample and may be difficult to remove. Evaporation and analysis of bulk solvent allows one to anticipate this problem.

(12) Look for upgrade: Many compounds can be 'upgraded' by crystallization, meaning that a sample of lower enantiopurity can be crystallized to afford material with the desired enantiopurity. When such an upgrade can be accomplished simply and with minimal loss of material, it can greatly increase the overall productivity of a resolution. Chromatographic productivity for producing 95% ee material can be several-fold the productivity for producing 99% ee material.

(13) Beware of temperature effects: Chromatography can be very dependent upon temperature, and sometimes temperature can dramatically influence preparative separations. If operating in an environment that could experience temperature swings, it may be a good idea to investigate temperature effects. Temperature effects can sometimes be used to advantage in preparative chromatography [84].

(14) Tell vendors what you like, what you don't like, and what you need: Materials and equipment for preparative chromatography are still at an early stage of evolution. You can help to move the process along by interacting with the vendors and suppliers.

(15) When all else fails, consider derivatization: although seldom required today, the old trick of derivatization often renders compounds more easily separated [85,86].

(16) If you cannot find the right CSP, consider making your own.