Drug Discovery Advances Inextricably Linked to Specialty Gases

Stephen Harrison,
Linde Gas, Munich, Germany.

The development and commercialisation of new medical drugs is a complex and costly process, but increasing pressure is being placed on drug companies to accelerate the timeline from discovery of new drugs, through to clinical trials and then to their release onto the market. The pharmaceutical industry continues to demand ever more advanced products aimed at improving health and the quality of modern-day life.

The pharmaceutical and biotechnology fields are among the most complex and innovative industries in the world. It can take years of research and hundreds of millions of dollars to formulate a product that can safely and efficiently cure a patient.

This is not the only challenge. Progressing a laboratory-scale product to commercial viability has its own set of issues, such as the likely requirement to construct new manufacturing facilities, or refit existing ones, since manufacturing processes vary from drug to drug. To add further complication, the drive to reduce the risks associated with pharmaceutical treatments has resulted in stricter legislative requirements and increased regulatory burdens on pharmaceutical companies.

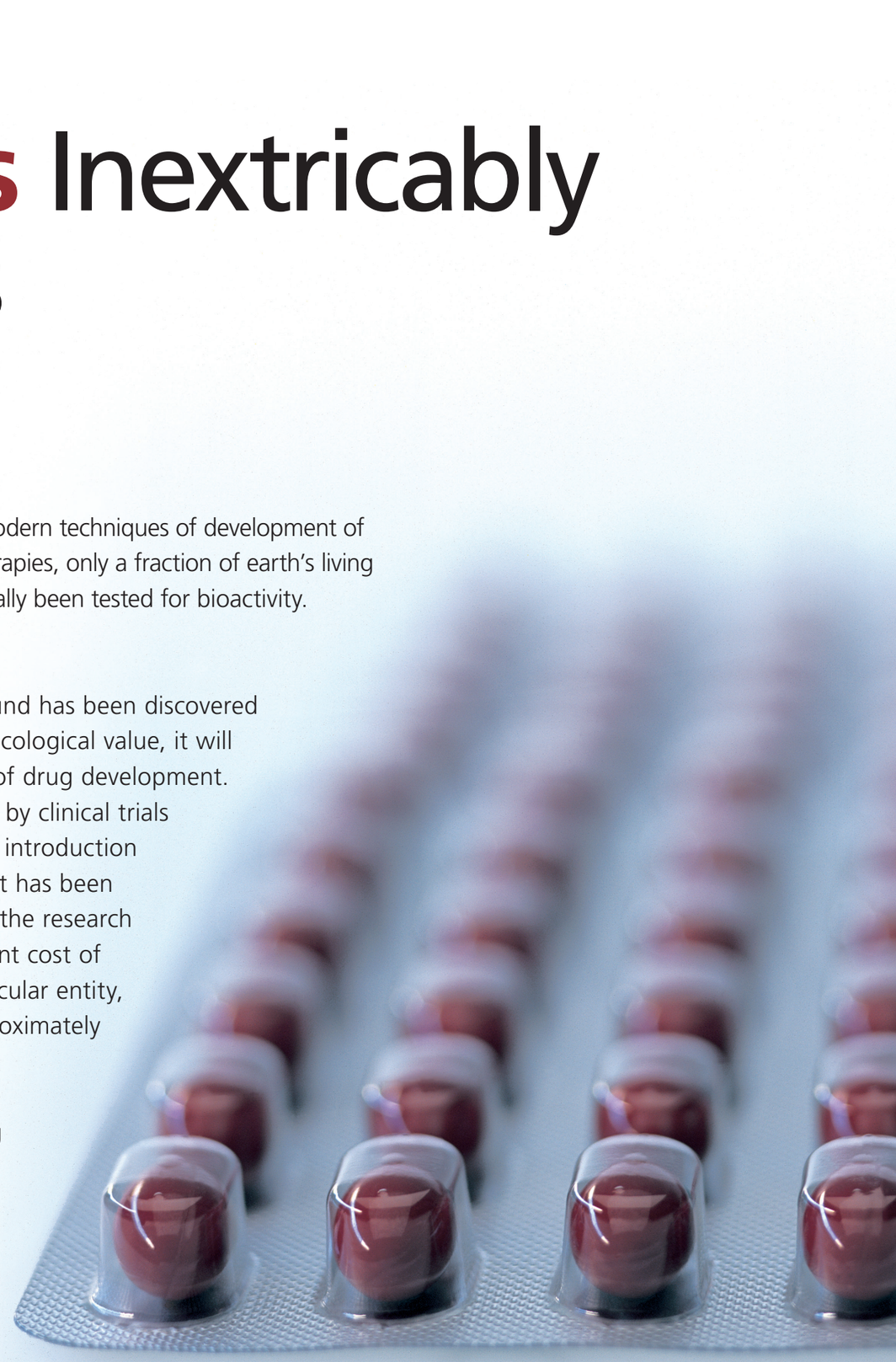
A drug is essentially a molecule that performs some beneficial effect in humans or animals. The process of drug discovery therefore revolves around identifying one molecule among millions of candidate molecules, followed by synthesis, characterization, screening and assays for therapeutic efficacy.

Natural products still play a major role as starting material for drug discovery. According to a report published in 2007, which covers drug development between 1981 and 2006, of 974 small molecule new chemical entities, 63% were naturally derived or semi-synthetic derivatives of natural products. For certain therapy areas, such as antimicrobials, antineoplastics, antihypertensive and anti-inflammatory drugs, the numbers were higher. Despite the implied potential of natural products as a source of novel chemical structures for modern techniques of development of antibacterial therapies, only a fraction of earth's living species has actually been tested for bioactivity.

Discovery

Once a compound has been discovered to have pharmacological value, it will form the basis of drug development. This is followed by clinical trials and, ultimately, introduction to the market. It has been suggested that the research and development cost of each new molecular entity, or drug, is approximately US$1.8 billion.

The industry has come a long way from the origins of drug discovery and development, which dates back to the



early days of human civilization. Throughout human history, medicine and medical care has been critical for the advancement of humankind. In the past many drugs have been discovered, either by identifying the active ingredient from traditional remedies or simply by serendipitous discovery. From the earliest herbal remedies and "folk medicines", drug discovery and development only embarked on the scientific route towards the end of the 1800s.

Key discoveries of the 1920s and 1930s, such as insulin and penicillin, were mass-manufactured and distributed broadly. Switzerland, Germany and Italy had particularly strong industries, with the UK, USA, Belgium and the Netherlands following suit, but the industry really picked up in earnest from the 1950s, as a result of the development of systematic scientific approaches, an advanced understanding of human biology (including DNA) and sophisticated manufacturing techniques.

The idea that the effect of drugs on the human body is mediated by specific interactions of a drug molecule with biological macromolecules (in most cases proteins or nucleic acids) led scientists to the conclusion that individual chemicals are required for the biological activity of the drug. This made for the beginning of the modern era in pharmacology, as pure chemicals, instead of crude extracts, which are essentially mixtures, became the standard drugs.

Numerous new drugs were developed during the 1950s and mass-produced and marketed through the 1960s. These included the first oral contraceptive, Cortisone, blood-pressure drugs and other heart medications. Monoamine Oxidase (MAO) inhibitors, chlorpromazine (Thorazine), haldol (Haloperidol) and tranquilisers ushered in the age of psychiatric medication. Discovered in 1960, Valium (diazepam) was marketed from 1963 and rapidly became the most prescribed drug in history, prior to all the controversy over dependency and habituation. Cancer drugs were a feature of the 1970s, followed in the 1980s by drugs for heart disease and AIDS.

Today, pharmaceutical manufacturing is a concentrated industry, with a few large companies leading global production. Drug development has progressed from the "hit- and-miss" approach of earlier centuries, to rational laboratory techniques, disciplined experimental design and sophisticated analytical equipment. The high cost associated with drug discovery, coupled with the ongoing need for new medicines, has driven the development of cutting edge analytical equipment.

One of the Fastest Growing Industries

The departure point for drug discovery is identifying a useful new drug candidate molecule from among millions of different molecules. Modern techniques are able to screen candidate molecules down from thousands to just one, in a time span as short as eight weeks.

Identifying and understanding the target receptor and lead compound, or drug candidate, as a means to assault a particular disease, is highly complex and intricate. Researchers need sensitive, fast and stable analytical equipment.

"The pharmaceutical industry is one of the fastest growing industries and a significant portion of its sales revenue is reinvested into research and development of new products — an area that requires a wide variety of specialty gases and equipment," says Katrin Åkerlindh, Global Product Manager for Specialty Gases & Specialty Equipment at Linde Gas. "Both the pharmaceutical and biotech industries are heavily dependent on gases and chemicals, from high-purity gases for laboratory use, to process gases for production processes such as chemical synthesis, sterilization gases and gas mixtures to grow biological cultures.

"Linde Gas has been very successful in developing and providing solutions to meet the evolving needs of the pharmaceutical industry in terms of impact, commercial manufacture, good manufacturing practice controls, scale-up issues and process validation. Maintaining the integrity of the gas from the product source to point of use is one of the biggest challenges. Therefore, it is essential that the gas supply system be designed and built with the goal of excluding impurities and ensuring traceability through the supply chain."

Analytical Instruments

Research and development take place in pharmaceutical laboratories using analytical instruments such as gas chromatographs with multiple detectors, liquid chromatographs coupled with mass spectrometers (LC–MS), ultraviolet/visible (UV/vis) spectrometers and nuclear magnetic resonance (NMR) spectrometers. These significantly accelerate research and development cycles, ultimately bringing beneficial drugs onto the market faster than ever before. Åkerlindh says the effective operation of these instruments depends on the use of the appropriate gases or gas mixtures.

One of the key items of analytical equipment harnessed to test these chemical compounds is liquid chromatography–mass spectrometry (LC–MS), which is used to test and separate the molecules of chemical compounds in order to qualitatively identify the individual compounds.

LC–MS defines and detects the structure of the molecular compound, firstly by separating the compounds in the liquid-phase chromatograph and then by detecting them in the mass spectrometer. Specialty gas grades of nitrogen are used in LC–MS as a curtain gas. LC–MS units equipped with electrospray ionization use nitrogen for nebulizing, drying and also as a curtain gas.

NMR spectroscopy also has a key role to play in determining chemical structure. It is able to generate a 3D image or visualization of the compounds in solution, which allows the structure of molecules to be defined right down to atomic level. This allows researchers to develop a good understanding of the molecule and its function in the human body.

NMR spectroscopy involves placing a sample in a strong homogeneous magnetic field and irradiating it with radio waves of defined frequency. The emitted signals provide information about the local molecular environments of nuclei in the sample, from which structures can be derived. NMR can also be used for determining interactions between molecules and is particularly useful for determining the nature of binding interactions between ligands and macromolecules.

"This information is very important in drug design," says Åkerlindh. "By understanding how bioactive molecules interact with a target protein or nucleic acid it is, in principle, possible to design ligands with improved affinity and specificity that may make useful drug leads. In addition to this structure-based approach to drug design, NMR is also useful as a screening tool in drug discovery programmes to identify ligands that bind to target macromolecules."

NMR spectroscopy is built on very strong magnetic fields and magnets, which need to be cooled down to an extremely low temperature and the liquid helium supplied to achieve this is used at a temperature of −269 °C. This temperature is so low that the liquid helium dewar is normally surrounded by a liquid nitrogen dewar at −196 °C to minimize helium boil off.

X-ray crystallography also has an extremely relevant place in biological and scientific history, as one of the most powerful tools available to researchers for visualizing candidate molecules. The technique was largely pioneered in the 1950s by British biophysicist, Rosalind Franklin, who was responsible for much of the research and discovery work that led to the understanding of the structure of deoxyribonucleic acid or "DNA". X-ray crystallography is a method of determining the arrangement of atoms within a substance, in which a beam of X-rays strikes a molecular or atomic structure and causes the beam of light to spread into many specific

directions. Based on the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the substance. From this electron density, the mean positions of the atoms in the substance can be determined, as well as their chemical bonds, their disorder and various other information.

Essential organic molecules, which exist in the bodies of humans and animals, are large, complex and structured in a variety of different shapes, such as chains, spirals or spikes.

"It's fascinating that the same molecule with the same chemical formula and composition can exist in mirror images of itself," comments Linde's Steve Harrison, Global Head of Specialty Gases & Specialty Equipment. "When broken down into its constituent atoms, the basic structure is exactly the same. So although the chemical formula of the molecule might be the same, the molecule can exist in different shapes, referred to as 'chiral variations'. While chiral molecules are mirror images of each other and have the same chemical composition, one of these mirror images might have no effect on a human body at all — or even a damaging effect, while the other chiral molecule occurring in a different shape, could be a 'wonder drug'.

"This reinforces why some of the visualization techniques achieved with today's analytical equipment are so critical. We need to know more than the molecule's chemical composition, but also the chiral variation. Visualization techniques such as NMR or X-ray crystallography move us one gigantic step beyond chemical analysis."

Supercritical fluid chromatography (SFC) is a relatively recent chromatographic technique, having been commercially available since the early 1980s. What differentiates SFC from other chromatographic techniques is the use of a supercritical fluid as the mobile phase. SFC is used for the analysis and purification of low to moderate molecular weight, thermally unstable molecules. It can also be used for the separation of chiral compounds. Principles are similar to those of high performance liquid chromatography (HPLC), however, SFC typically utilizes high purity carbon dioxide as the mobile phase.

It is interesting to note that the potential breakthrough antibiotic candidate, platensimycin, initially found in soil microbes, was identified and developed using a combination of high-performance liquid chromatography (HPLC), two-dimensional NMR and X-ray crystallography.

Medical research has produced an array of cures for many human and animal diseases over the decades, yet the fight against cancer continues and is an extremely active field of research. The latest generation of anti-cancer

drugs harnesses platinum as a constituent of active molecules to kill the diseased cells. This advancement has been enabled through the use of modern sophisticated analytical techniques such as NMR spectroscopy.

Trusting the Validity of the Result

"With the immense cost associated with bringing a new drug to market and the speed at which companies are today required to do this, finding the correct molecular candidate and then being able to trust in the validity of the result is absolutely essential," says Åkerlindh. "The purity level of the specialty gases involved in this process, as well as the integrity of gas from the product source to point of use, is therefore critical."

Linde Gas also supports the drug discovery industry with liquid cryogenic gases used to store molecular compounds and biological material, or biopharmaceutical drug candidates, which require extremely low temperatures. Nitrogen freezers are ideal for this type of storage, achieving temperatures down to −196 °C. Specifically, the Linde Group's UK subsidiary company, BOC, has recently begun to offer state-of-the-art cryogenic bio-storage facilities. Although BOC has been supplying liquid nitrogen to customers for the past 50 years, the establishment of proprietary cryobanks is a sign of a definitive shift in the pharmaceutical industry that is seeing business opting to focus on their core competencies and outsourcing other requirements to appropriate service providers. This builds on our experience and expertise developed in Linde NL over the past decade.

Drug Integrity

Amid the many breakthroughs and improvements constantly taking place in the drug arena, a fundamental problem has arisen with the potential to affect people all over the world. This is the issue of counterfeit drug production and a prime example is malaria medication.

While there are a number of very effective and authentic malaria drugs on the world market, counterfeiting exists in some areas of Asia and Africa.

"This makes it critical for authorities to test the pharmaceuticals on sale in their markets, randomly and periodically, to ensure they are not potentially harmful counterfeits," comments Harrison. "Since some of these anti-malarial drugs are relatively simple compounds or molecules, simple chemical analysis techniques, based on wet chemistry, can be used to check for the existence of these drugs commonly used to prevent and treat malaria.

"This illustrates how analytical techniques are also critical to identifying and avoiding malpractice in the manufacture, sales and distribution of drugs, and how a mix of traditional chemistry or modern instrumentation can be appropriate, according to the situation."

Harrison says that modern drug discovery could not take place without today's advanced analytical instruments and that these instruments could not operate effectively and reliably without high purity specialty gases.

Gas quality can often affect the accuracy of these instruments. Therefore one of Linde's flagship offerings in the realm of drug discovery is its HiQ line-up of pure gases, gas mixtures and precision engineered gas supply systems. Carrier gases and calibration mixtures with known degrees of accuracy, purity and composition are an essential part of the HiQ specialty gases product programme.

Linde's traceable VERISEQ pharmaceutical grade gases are suitable for the manufacturing of pharmaceuticals and active pharmaceutical ingredients (APIs).

"Where there is a demand for gas products in such areas as production, growth of biological cultures, environmental mixtures, sterilization or chemicals, we are able to offer the right product for each application,' Harrison says

"In some cases, cylinder or liquid gas supply might be unsuitable. This may be for safety reasons or due to difficulty in cylinder transportation. For these situations, Linde has a range of small and reliable gas generators that

produce gas on-site. The main advantage of this is that because the gas is produced on site, it allows complete control over gas production.

"Another advantage is that there is no need to store large amounts of compressed or liquefied gas, since there is access to newly-produced gas. Gas generators are small and allow for flexibility in laboratory set-up owing to their portability."

The HiQ specialty gas generator programme includes high-purity no-maintenance hydrogen generators (up to 99.9999% purity), liquid chromatography–mass spectrometry (LC–MS) nitrogen generators up to 99.999% purity and Ultra Zero air generators.

Stephen Harrison is a British Chartered Engineer (MIChemE) with a career in industrial gases spanning 20 years, 10 of which have been focused in the area of specialty gases. He has worked in an international capacity for both Linde Gases and previously BOC, and now leads Linde's global Specialty Gases and Specialty Equipment business from Munich, Germany. Stephen has a Masters degree in Chemical Engineering from Imperial College, London, UK.

E-mail: **press@linde-gas.com**
Website: **http://hiq.linde-gas.com**