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STERILE PRODUCTS AND THE ROLE OF RUBBER COMPONENTS

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Introduction

The selection of the appropriate packaging materials and processes for sterile products presents a series of challenges of considerably greater complexity than for non-sterile products. A sterile product may be defined as a product which is totally devoid of all forms of life, both vegetative and sporing. Most important in this area is contamination by bacteria, fungi or moulds and yeasts. In all pharmaceutical products it is also becoming highly desirable to work towards much lower bioburdens. This involves the application of microbiological standards to both raw materials and finished products. These normally exclude certain pathogens such as *Salmonella typhi* and *Clostridium botulinum,* but it should be noted that even some non-pathogens can also cause problems.

In order to limit or exclude microbiological contamination in pharmaceutical products, a number of approaches are possible:

1 rigid (microbiological) specifications for all raw materials (especially water) and how they are 'packed and stored'
2 strict adherence to the code of good manufacturing practice (GMP) (facilities, personnel, procedures and documentation)
3 use of special processing techniques, e.g. control of air quality, dedicated positive pressure production areas
4 use of antimicrobial preservatives (where acceptable) which are essential to most multi-dose forms of product
5 use of a sterilisation process which may involve a terminal sterilisation or an aseptic process
6 the selection of the appropriate form of packaging, noting the advantages of certain (unpreserved sterile) forms of unit dose.

Various guidelines, including those on general or current GMP (CGMP), are available related to specific aspects of the above from both official organisations, such as the FDA, and specialised societies such as the UK-based Parenteral Society and the US-based PDA.

A terminal sterilisation process usually involves the filling and closing of product containers under conditions of a high-quality (low bioburden/particulate) environment where the product, container and closure are usually also of high microbiological quality but not sterile. This is followed by a final complete sterilisation process. In an aseptic processing operation, the drug product, container and closure are subjected to separate sterilisation processes and then brought together. Since maintenance of sterility relies on the processing, compliance with high standards is critical to any aseptic operation. Each function therefore requires thorough validation and control at every stage of the process.

Antimicrobial preservatives can be added to many pharmaceutical products in order to give protection against microbial contamination. A wide range of preservatives is summarised in Table 12.1. It should be noted that preservatives should not be regarded as protection against poor processing techniques but as a means of minimising bioburden during poor storage and usage.

There are a number of official tests for preservative challenge, i.e. preservative efficacy challenge tests, which can be found in the British Pharmacopoeia (BP), United States Pharmacopeia (USP), and European Pharmacopoeia (EP). There also are 'in house' challenges which may be more rigorous than the compendial tests.

Since preservative efficacy may vary according to the product, analysis of preservative content often has to be supported by a preservative challenge test. This may also be relevant with certain emulsion systems where the preservative may partition between the different oil and water phases. For simple distribution phenomena, the partition or distribution law normally applies.

Because of problems which include toxic hazards and sensitisation, some preservatives are under suspicion, while others have been withdrawn from use. This has often encouraged the use of non-preserved sterile unit dose presentations. Preservatives have also suffered from absorption, adsorption and, where soluble and volatile, subsequent loss by evaporation.

For example, preservatives suffering from adsorption include phenyl mercuric nitrate and acetate; benzalkonium chloride; thiomersal. Preservatives suffering from absorption include phenol; chlorocresol; cresol; and 2 phenyl ethanol.

These preservatives have a solubility in certain rubbers and plastics and will continuously diffuse through the polymeric matrix due to their volatile nature. These

Table 12.1 Antimicrobial preservatives

Substance	*Approx. % used*	*Comments*
Phenyl mercuric nitrate (PMN) and acetate (PMA)	0.002	Various usages but mercurials queried
Benzalkonium chloride	0.01	Oral, ophthalmic, topicals
Ethanol	15.0	Oral, topicals
Benzoic acid	0.1–0.5	Oral, topicals
Parabens	0.1–0.2	Creams, mixtures
Chlorhexidine	0.01	Eye products
2 Phenyl-ethanol	0.4	Eye products
Thiomersal	0.1–0.2	Eye/nasal products
Phenol	0.5	Certain multidose injections
Cresol	0.3	
Chlorocresol	0.1	
Benzyl Alcohol	1.0	
Bronopol	0.02–0.05	Creams

problems illustrate the view that sterile products generally put greater stress on all packaging materials. Thermal processing can cause physical changes to plastics, glass and multilayer materials, as well as the increased potential of interaction, exchange and extraction. Thermal defects may arise from differential expansion and contraction, as well as instant or delayed cracking of glass-based materials due to the effects of thermal shock.

Interaction between product and pack may involve:

- surface interactions
- leaching or migration of material from the pack into the product
- loss of constituents in the product into the pack, i.e. container or closure.

The history of injectables

Injectable products are sterile liquid drug preparations that are administered parenterally, i.e. introduced into the patient's body through the skin. Although injections are a relatively recent form of therapy, the history of the development of the technique can be traced back to the early seventeenth century. William Harvey described the circulation of blood in 1616, and later attributed death caused by snake bites to the distribution of the poison throughout the body via the blood.

The first recorded attempt to inject medication intentionally was in 1665 by Sir Christopher Wren, then Professor of Astronomy at Oxford and later to become a famous architect. Wren worked on animals, but later attempts by a Johann Taylor were with humans. Unfortunately the crude nature of the apparatus, the absence of pure drugs and ignorance caused the practice to fall into disrepute.

However, during the late eighteenth century and throughout the nineteenth century interest was spasmodically revived. Jenner used intradermal administration in the late eighteenth century for his smallpox vaccination and a Frenchman, Pravaz, introduced a plunger-type syringe in 1853 with leather components. Although the work of Pasteur and Lister pointed out the need for the development of aseptic techniques, it did not result in any immediate practical changes. Syringe materials were not suitable for heat sterilisation and the drugs were often heat sensitive, so by 1880 physicians were preparing their own injections at the time of use.

By the 1890s progress had been made in the use of bacteriological filters towards sterilisation, and at about the same time a French pharmacist called Simousin developed the first ampoule. These changes resulted in the manufacture of parenteral solutions passing from the hands of the individual pharmacist or physician to pharmaceutical companies.

Nevertheless, pyrogenic reactions continued to be associated with parenteral therapy. Florence Siebert demonstrated in 1923 that the pyrogenic inducing bodies came from the water used to prepare the solutions, and care in using a pyrogen-free

water eliminated the fever problem. This lead to the official acceptance by the American Formulary in 1926 of injectable solutions, which is the true beginning of universal parenteral therapy.

Today parenteral products are divided into two types, namely large and small volume parenterals. Large volume solutions in containers of 100 ml or more are essentially for intravenous use, usually over an extended period of time. Such solutions are also for irrigation and dialysis. Small volume parenterals are for immediate injection by various routes, such as subcutaneous, intramuscular and intravenous.

Injections are often the most efficient way of administering a wide variety of essential drugs. There are of course both advantages and disadvantages associated with this method of drug administration over the oral form; these are detailed in Table 12.2.

Sterilisation of parenteral products

The basic pharmacopoeial accepted sterilisation processes include:

1 dry heat
2 moist heat
3 irradiation, including gamma irradiation and beta irradiation
4 gases
5 product and air filtration.

Dry heat

Dry heat sterilisation involves high temperatures such as 160°C for 3 h; 170°C for 2 h; 180°C for 1 h; 300–320°C for 3–4 min. Although glass and metal can withstand these temperatures, the use of dry heat sterilisation for rubber and plastics needs to be considered with care. There are relatively few ‘engineering’ type plastics and a limited number of rubbers that can meet these high dry temperatures and retain satisfactory physical properties.

Moist heat

Temperatures of above 100°C can be achieved by moist heat under pressure in an autoclave and, as with dry heat, there is a time—temperature relationship:

- 106–108°C (6–8 h)
- 115–118°C (30 min)
- 121–124°C (15 min)
- 134–138°C (3 min).

Moist heat sterilisation can be used to sterilise packaging components for aseptic filling or to terminally sterilise both the product and the pack. Because of this, various factors have to be noted.

Table 12.2 Parenteral products administration

Advantages	*Disadvantages*
Faster effect	More expensive
Maintenance of high drug levels	Professional administration
Little or no inactivation	Unpleasant for patient
Injected directly into target	

1 The product expands according to its coefficient of expansion as the temperature rises (note that alcohol expands more than water).
2 The air space or ullage above the product also expands according to its nature (air or nitrogen) and its pressure.
3 The combined expansion will depend on the product/ullage ratio and the pressure build-up will depend on whether the pack is rigid with limited expansion (e.g. glass) or flexible and extensible (e.g. various relatively thin-walled plastics).

4 Whether the packaging material is resistant to moisture (metal, glass) or absorbs and loses moisture according to the temperature and level of moisture present (e.g. certain plastic materials).

The above invariably means that closures may dimensionally change during the sterilisation process (especially screw-based systems). Also, certain materials may extend during the heating cycle (plastics) and hence become distorted when recooled. To minimise this distortion, an overpressure or balanced pressure autoclave is essential for some materials, i.e. bottles or bags. Control of this distortion depends on the plastic involved, the design of the pack, the nature of the product, the volume to ullage (air space) ratio, the time-temperature cycle involved, the overpressure and when and how it is applied. These have to be optimised by trial and error experimentation, as other factors (i.e. how the autoclave is loaded and the items spaced etc.) also play a part. This always assumes that the closure remains effective throughout the cycle and does not 'vent'. In this context this cannot happen with welded packs and, in general, closures made by effectively applying an aluminium overseal over a rubber stopper are superior to the older systems based on metal screw caps (older glass IV packs).

As indicated above, the properties of certain plastics may be temporarily modified by the combination effects of moisture and temperature during the autoclaving cycle. In general, the physical properties of rubber formulations are not affected by the moist heat sterilisation other than the fact that closure systems may absorb moisture (depending on the rubber formulation/materials employed) during the autoclave cycle. This can be an issue for lyophilised products or aseptically filled dry powders where long drying cycles for the rubber closures are sometimes employed to prevent desorption of moisture from the closure into the product.

Irradiation

Sterilisation by irradiation typically uses either gamma or beta radiation (electron beam). These two processes are significantly different in that gamma irradiation is a lengthy process involving penetrating rays, whereas beta irradiation is in comparison a short exposure process where the rays are much less penetrating, i.e. it tends to be a surface sterilising process. Typically gamma irradiation is achieved by a cobalt 60 source at a dose of 25 kGy. The items to be sterilised are slowly passed through the process, over a period of up to 24 h. These rays will penetrate most materials, including aluminium foil, paper, board, glass, rubber and plastics. Gamma irradiation has increased in popularity for the terminal sterilisation of medical devices and the sterilisation of packaging components for aseptic process.

Gaseous sterilisation

Although various gases can be employed, e.g. formaldehyde, ethylene oxide, most pharmaceutical processes relate to the latter. Since ethylene oxide (and its residues) are toxic and it forms explosive mixtures with air/oxygen, special precautions are essential to safe handling. Ethylene oxide is therefore mixed with an inert gas (usually CO_2) and needs a certain temperature (usually 55°C) and the presence of moisture to be effective, together with materials which are either porous (paper, Tyvek, board) or permeable to the gas (PVC, PS, PE, etc.). This means that there is a solubility or retention factor related to their use and a period must be allowed to reduce residues (by degassing).

Filtration

Finally, filtration should be mentioned as a means of producing sterile products or gases. In the case of aqueous-based liquids (of low viscosity), terminal filtration usually employs a special filter of 0.2 μm (or minimum 0.22 μm) pore size. Although a single filter can achieve effective sterility, there is a general trend towards a two filter process, i.e. 0.45 μm then 0.22 μm, where applicable. In the case of more viscous products, filtration may need an increase in temperature (which usually reduces the viscosity) and/or additional pressure. Filtration techniques generally assume that the product can be produced with low bioburden products.

Packaging materials

The efficacy, stability and safety of a parenteral drug on storage and administration depends largely on the nature and performance of the packaging components. In general the requirements of a modern parenteral product can be summarised as follows:

- the drug is medically effective
- the complete item is easy and quick to use
- the inside of the container and its contents must be sterile

- the drug and inside of the container must be free from pyrogens and toxic substances
- there must not be excessive contamination by particulates
- minimum interaction or exchange between product and pack.

All the above requirements must be maintained throughout the product shelf life.

Four main materials are used for the primary (direct contact with drug) or secondary packaging (part of the container or administration set but not in direct contact with the drug):

1 glass
2 plastics
3 aluminium
4 rubber.

The first three materials will only be touched on briefly since they are covered in full in earlier chapters.

Glass

Glass is available in four types, i.e. types I, II, III, NP (USA) or I, II, III, IV (Europe), the different grades relating mainly to their chemical 'neutrality'. Applications include:

- ampoules—single or double ended, open or closed (always single use containers)
- vials—normally produced from pregraded tubing and used as single dose or multidose containers, in sizes of 2.5 ml to 100 ml, with neck sizes of 13 mm to 20 mm
- bottles—various sizes and closure systems, produced by conventional glass moulding techniques.

Plastics

There are many different types of plastics and an even greater number of grades to meet virtually every product requirement. The main economical plastics used in pharmaceutical applications are the economical 'four' i.e. polyethylene, polypropylene, polystyrene and polyvinylchloride.

Plastics are used in virtually every pharmaceutical application (oral, topical, ophlthalmic, parenteral applications), either as a single material or in combination with other materials, as coatings or laminations.

Aluminium

Aluminium is used as an overseal to effect a seal between the rubber disc or plug and vial. An overseal must be rigid, yet sufficiently ductile and malleable to be clamped onto the vial. Since the overseal is a secondary closure, problems of drug compatibility do not occur. The aluminium itself is usually coated on the outer surface with an epoxy resin-based lacquer. This protects the aluminium from oxidisation, or from slight surface corrosion during autoclaving. Alternatively, the product may be coloured by using coloured anodised aluminium and a clear lacquer. The range of colours enable coding of products and further differentiation can be achieved using a D-I-D overseal (decoration-identification-differentiation) which enables instructions, logos or product names to be printed on the overseal.

Rubber and elastomers

Rubber components are now used extensively for many parenteral packaging and administration applications including injection vials and prefilled syringes. Because of its varied chemical nature and risk of extractables, rubber is regarded by many as the most critical of the primary packaging materials, especially as a wide range of constituents can be involved (see below).

Up to the beginning of the twentieth century, closures were typically made from cork or glass stoppers. In the early 1900s solid rubber 'corks' or bungs, made using natural rubber as the base elastomer, replaced the cork and glass stoppers. The use of rubber bungs (a popular nomenclature used to describe rubber closures) provided a number of specific advantages summarised in Table 12.3.

Rubber formulations

In this chapter the word 'elastomer' is used to describe the base polymer and 'rubber' to describe the fully compounded finished component. A rubber formulation is a complex blend of ingredients, and a typical high extract sulphur cured natural rubber formulation is given in Table 12.4.

Elastomer

The choice of elastomer has the greatest effect on a formulation. The most common elastomers that can be used for closures for injectable products are given in Table 12.5. Of these elastomers, natural rubber, synthetic polyisoprene, butyl, chlorobutyl and bromobutyl rubber are typically used for the manufacture of rubber closures and stoppers used in the packaging and administration of parenterals.

Table 12.3 Special properties of rubber

Property	*Advantage gained*
Flexible	Conforms to shape of vial etc.
Resilient	Reseals after needle puncture
Non-thermoplastic	Tolerates most heat sterilising and other processes
Good compression set	Retains seal throughout product life
Can be varied by ingredient choice	Formulations can usually be developed compatible with most drugs

Table 12.4 Typical sulphur cured natural rubber formulation

Category	*Ingredient*	*Mass % (w/w)*
Elastomer	Natural rubber	60.00
Filler	Calcium carbonate	25.0
Pigment	Red iron oxide	4.0
Plasticiser	Paraffin oil	5.0
Processing aid/activator	Stearic acid	1.0
Activator	Zinc oxide	2.5
Vulcanisation system	Accelerator (e.g. sulphonamide, dithiocarbamate, thiuram)	1.5
	Elemental sulphur	1.0

Table 12.5 Elastomer characteristics

Polymer	*Characteristic*
Natural rubber	Good physical properties
Synthetic polyisoprene	Good physical properties
Butyl	Low permeability
Halobutyl	As butyl, but with lower water extractables
Nitrile	Mineral oil resistance
EDPM	Resistance to high pH solutions
Silicone rubber	High permeability
Neoprene	Lower oil resistance than nitrile

Natural rubber

This was the first type of polymer used in pharmaceutical applications, and was found to have desirable characteristics in that its resilience provided sealing properties and this resilience could be developed to allow the rubber to be pierced by a hypodermic needle, resealing after removal. This high level of resilience is partially due to its chemical structure, it being a straight chain elastomer (Figure 12.1).

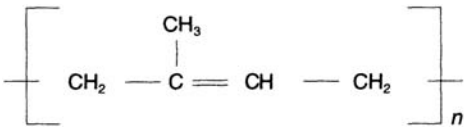

Figure 12.1 Natural rubber

During the curing or cross-linking process only 10–20% of the available double bonds react and this gives rise to the potential for breaking of the chemical chains upon exposure to factors such as heat, oxygen or ozone. This can result in surface tackiness, crazing and ultimately total degradation of the rubber.

Although natural rubber has been used for many years, there is an increasing awareness of an issue described as 'latex protein allergy' whereby naturally occurring proteins and natural rubber latex can cause allergic reactions and, in the most severe cases, anaphylactic shock. Items made from latex natural rubber typically include surgical and examination gloves, anaesthesia masks, and dental dams. During the period 1989–1993 the US Food and Drug Administration (FDA) received reports of over 1000 cases of injury and fifteen cases of death associated with latex allergy (Dillard and MacCollum, 1992). The cases of death related to a single supply of barium enema catheters which were believed to have been produced using poor manufacturing conditions. These were recalled and subsequently replaced by catheters made using synthetic rubber.

Rubber closures and components for the packaging and administration of parenterals are normally made from so-called dry rubber. Where such closures have been produced using dry natural rubber, the bioavailability of proteins has not been proved (Slater, 1993). It is postulated that the difference between latex and dry natural rubber is related to the processing of the base elastomer and rubber compound (Russell-Fell, 1993). The processing of dry natural rubber involves acid coagulation followed by a crumbling/creeping operation with extensive washing in water and drying at 100–130°C. During manufacture dry natural rubber compounds are typically compression moulded at high temperatures up to 160°C. A study of the extractable protein content of fourteen dry natural rubber samples and five dry natural rubber products found limits so low as to be at the limit of detection of the Lowry method (Yip *et al.,* 1995). There has been one reported case (Towse *et al.,* 1995) of an allergic reaction involving local erythema following an injection of insulin. The paper concluded that there was strong circumstantial evidence that the patient's allergic response was caused by latex antigens contained in the insulin vial and/or syringe. Reputable suppliers have been pursuing alternatives to dry natural rubber: one common approach is to introduce synthetic polyisoprene and to describe such materials as natural rubber latex free.

Butyl and halobutyl rubber (chlorobutyl/bromobutyl)

Butyl rubber shown in Figure 12.2, has been commercially available since 1942, and chlorobutyl and bromobutyl have been commercially available since 1960 and the 1970s respectively. All three polymers offer very low permeability to gases. The vulcanisation of butyl rubber requires a high level of curatives to effect cross-linking; the introduction of halogenated butyl rubber resulted in greater reactivity of the base polymer. As a direct result it was possible to use a lower level of curatives for halobutyl polymers and also to explore so-called unconventional vulcanisation systems that yielded a significantly lower level of extractables.

In certain cases it is desirable to combine the properties of the previously described natural rubber with those of the halobutyls. With straight butyl rubbers this was impossible due to the prolonged cure time of butyl which meant that the natural rubber would be overcured, resulting in an unusable 'non-homogeneous' mix.

Cure ingredients

During cure (or vulcanisation), the individual polymer chains become chemically linked together to form a three-dimensional structure. This minimises the tendency for permanent distortion under load at room temperature or for the rubber to 'melt' at high temperatures, such as at steam sterilisation. Certain additives are necessary as vulcanisation agents to provide the chemical cross-links, which are created at elevated temperatures, typically from 140°C to 200°C. Heat is applied to the rubber while it is being compressed in metal moulds, so that the forming and vulcanisation processes occur simultaneously. The most commonly used vulcanisation system in the general rubber industry is based on sulphur. Sulphur systems can be devised to fit most rubber processing conditions and can be applied to most polymers commonly used for pharmaceutical applications. Activators, usually zinc oxide and stearic acid, are necessary to activate the accelerators, but here the precise quantity is less critical. The detailed mechanism of the rubber curing process is complicated, but it is generally accepted that the sulphur combines with a zinc salt of the accelerator to produce a thio-intermediate which, in turn, reacts with the rubber. The inevitable residue of zinc-accelerator salts is slightly water soluble and can be extracted by aqueous solutions, if only at

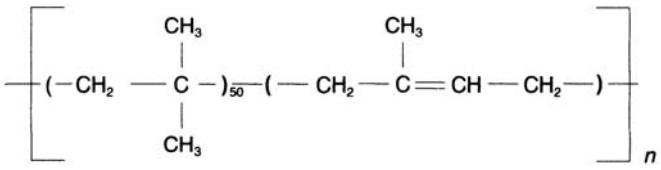

Figure 12.2 Butyl rubber

the level of a few parts per million. This tendency to contaminate is a significant disadvantage of a sulphur cure, although many sulphur cured rubbers have a long history of satisfactory use with aqueous injectables.

One such contaminant associated with sulphur-based vulcanisation systems is the organic accelerator 2-mercaptobenzothiazole (2-MCBT) and associated derivatives. In 1981 (Petersen *et al.,* 1981) the presence of 2-(2-hydroxyethylmercapto) benzothiazole (HEB) was detected in the contents of a disposable hypodermic syringe. It was identified that the extractant was a reaction product formed between a 2-MCBT derivative and ethylene oxide used for sterilisation. Subsequently the oxidation product of HEB, 2-(carboxymethylthio)benzothiazole (CMB), was detected in the serum of premature babies receiving prolonged intravenous therapy (Meek and Pettit, 1985). So-called modem vulcanisation systems do not use sulphur as the cross-linking agent nor use 2-MCBT or derivatives, and are consequently free from this particular problem. These new vulcanisation systems show a considerable reduction in aqueous extractable matter and are often described as having low water extractables.

Fillers

Fillers are added to the elastomer in order to add bulk, lower cost and/or to improve physical properties such as hardness, strength and abrasion resistance. Typical fillers are materials such as carbon black, talc, china clay and whiting. Carbon black has been shown to contain polynuclear aromatics (PNAs) and there is concern regarding their carcinogenicity (Lee and Hites, 1976). However, despite extra controls there has been a move away from the use of carbon black as a filler in applications involving the primary packaging of parenterals. Its use continues as a pigment or colourant in rubber formulations but at substantially lower levels than that as a filler.

The use of calcined clay as a filler has shown to lead to the release of soluble aluminium from rubber closures into the parenteral solution (Milano *et al.,* 1982). Various techniques for the determination of soluble aluminium in rubber closures have been proposed (Mondimore and Moore, 1983). There has been concern about aluminium since the 1970s, when a link was identified between high aluminium levels in tap water used for renal dialysis equipment and accumulation of the element in the brain. The injection of parenteral solutions into the body effectively 'bypasses' the normal defence mechanisms and under these circumstances may present a challenge to the normal metabolic processes (Massey and Taylor, 1989). In response to these challenges, suppliers have developed rubber formulations that are essentially free from materials containing aluminium compounds.

Protecting agents

These are added to reduce the ageing effects of the rubber and include paraffin wax added to form a protective bloom and antioxidants such as phenolic compounds. These protecting agents are required for natural rubber and are not necessary for modern synthetic rubber formulations.

Plasticisers

Petroleum oils (either naphthenic or paraffinic), are added to rubber to reduce the rubber hardness and improve processing characteristics. There are well-documented examples of other materials being used as softening agents in plastics and rubbers. The leaching of plasticisers from infusion bags such as di(2-ethylhexyl)phthalate (DEHP) are well documented. Another plasticiser, tris(2-butoxyethyl) phosphate (TBEP), was found to leach from a rubber stopper into a blood sample collected using an evacuated tube device (Shah *et al.,* 1982; Shang-Qiang *et al.,* 1983). The presence of TBEP was shown to cause displacement of certain drugs from plasma to erythrocytes and hence to distort the apparent concentration of drug in plasma.

Colourants

The main colourants used are inert inorganic materials such as iron oxide, titanium dioxide and, at small loadings, as previously noted, carbon black. With these materials, white, grey, black, brown, pink and red shades can be developed. Other colours are obtained by organic pigments, which can give bright vibrant colours but carry a cost premium.

The hypodermic syringe

The simple disposable syringe is well known and is supplied sterile by the manufacturer. These were introduced to overcome the problems of sterility within hospitals when multi-use non-disposable syringes were used. These syringes are supplied empty and are used in conjunction with vials or ampoules. The development of the disposable syringe has some interesting milestones in its history.

A metal and glass syringe was manufactured by Gemrig and Co. (Philadelphia) in 1857. Here the barrel was made of glass and the plunger rod from a metal, probably German silver. The piston or plunger had a frictional surface manufactured from leather. The 'Luer-Syringe' was invented in 1896 in Paris by Karl Schneider of H Walfing Luer and this was a major step towards the disposable syringe we know today. Obviously, the use of leather for the piston was one area in need of improvement and eventually the move to 'rubber' was made.

The disposable syringe comprises three main component parts, i.e. barrel, plunger rod, piston, and these are described below.

Barrel

The barrel of the disposable syringe is manufactured primarily from a sterilisable grade of polypropylene with an added nucleating agent to improve the clarity. Over the length of the barrel a scale is printed indicating the various dosage levels available from that particular size of syringe. At the open end of the barrel a feature known as the finger grip is incorporated which provides a firm platform for use when injecting into the patient. The administration end is fitted with either a needle or a standard medical luer which enables a wide variety of hypodermic needles to be attached.

Plunger rod and piston

The plunger rod is used to move the piston up and down the barrel. The piston is usually manufactured from a natural or synthetic rubber and is attached by either a simple latch or a screw thread to the plunger rod, and provides the effective seal required to withdraw drug from the vial/ampoule and administer into the patient. The seal is normally made via two, but occasionally three, circumferential ribs which, when inserted into the barrel and assembled on the plunger rod, exert radial interference and thus affect the seal. The seal integrity for such syringes can be evaluated using an aspiration test whereby the capability of holding a vacuum within the barrel is determined. It is this unique sealing characteristic of rubber that has resulted in its wide-ranging use.

In recent years a new challenge has emerged for large-volume disposable syringes in the form of syringe pumps. These are used to administer drugs automatically over periods of 24, 48 or even 72 h, and the break-loose force and sliding friction characteristics of the piston and plunger down the barrel are of paramount importance. The breakloose force is the measure of the force needed to start the piston travelling down the barrel, and the sliding friction is the force needed to maintain that movement, which should be constant with a smooth gliding action. The tendency for a piston to judder down the barrel ('stiction') makes a syringe unsuitable for use in modern syringe pumps. These syringe pumps are capable of delivering a variable amount of fluid and are equipped with overpressure alarms and an alarm to indicate an almost empty syringe. The latter alarm requires careful control of tolerances over the syringe itself to provide a constant final 'hard height' calculated from the plunger rod handle to syringe body fingergrip. These syringe pumps may be coupled to electronically controlled systems to provide controlled drug delivery.

Ampoules and vials

Technical details of the basic ampoule and vial have been given in earlier chapters. Here we detail the historical development of these packaging containers and also identify some recent design improvements which increasingly bridge the gap between a container and drug delivery device.

In 1886 Stanislaus Limousin devised a container for storing sterile solutions. This container was named an 'ampoule' and was manufactured with a long tapering neck at one end. After the ampoule had been filled the 'tip' of the glass was sealed using heat. Today the single dose ampoule has changed little from the original Limousin design.

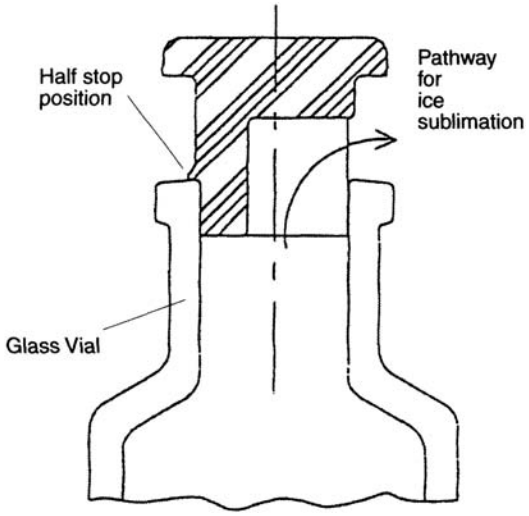

Figure 12.3 Half stop position

The next major step was to introduce a rubber closure to seal a glass vial. This enabled multiple insertions through the rubber closure by a needle while retaining seal integrity, due to the flexible nature of rubber. The rubber stopper is held securely in place by an aluminium overseal which can be crimped onto the vial, thus making an effective seal between the rubber and glass. The aluminium itself is available in a number of designs depending on the application intended. These designs include the Flipoff® overseal (providing tamper-evidence) and fully removable overseals.

Advancements in vial/closure design have resulted in novel prefilled vial systems giving a range of benefits over the 'standard' package. One such system is the Becton Dickinson Pharmaceutical Systems Monovial INF comprising a glass vial holding the drug in either a freeze dried or a liquid state and an integral transfer set. This system allows for the aseptic reconstitution of the drug and subsequent transfer into the infusion bag. The transfer set consists of a protective overcap manufactured from low density polythene, covering the main co-polyester needle guard capsule with an integral needle. The vial is filled and stoppered using one of two stopper designs, depending on whether the drug is lyophilised or in liquid form. The four-part transfer device is then assembled on the vial. In use the overcap is removed and the needle inserted into the infusion bag. Pressure is then applied to the transfer set which forces the rubber stopper down into the vial suspended by the transfer set. A seal is maintained by a sec ondary rubber 'O' ring on the transfer which moves down into the vial neck: subsequent mixing and transferring are then facilitated. This system reduces the number of basic steps performed by the nurse from sixteen with a traditional system to only seven for the Monovial system.

Abbott Laboratories also manufactures a novel vial reconstitution method, the ADD-vantage™ system. Here a purpose-made infusion bag has an integral 'vial port' which is accessed by removal of a protective cap via pulling an integral 'pull-ring'. This allows the unique drug vial to be screwed into the port. Activation of the system is achieved by pulling back the inner cap on the vial, inverting the bag and mixing.

Lyophilised products

Lyophilisation is the process whereby a pharmaceutical formulation containing the active ingredient dissolved in a solvent, usually water, is first frozen and then the water is removed under a reduced pressure by sublimation. The use of lyophilisation is increasing, partly because many of the new biopharmaceutical preparations are unstable when stored in solution. To provide a long shelf life, it is important that the stopper has low permeability to air and moisture. It is recognised that the main source of moisture into the lyophilised cake is through a process of desorption from the rubber stopper itself into the hygroscopic cake. Factors affecting the functional aspects of a stopper selection are:

1 partial insertion or half stop position (Figure 12.3)
2 component stability on the vial
3 insertion force
4 seal integrity
5 reconstitution and the withdrawal of active ingredient.

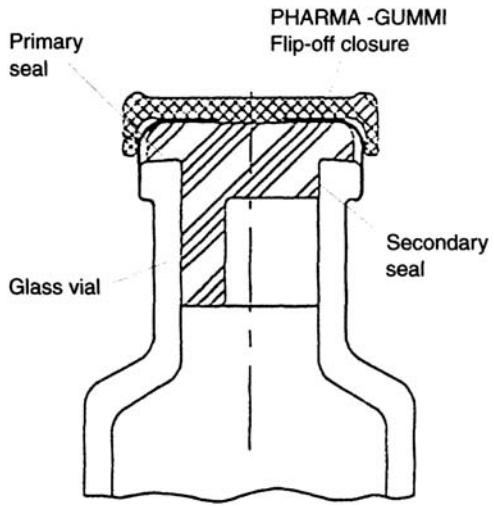

Figure 12.4 Stopper seal integrity

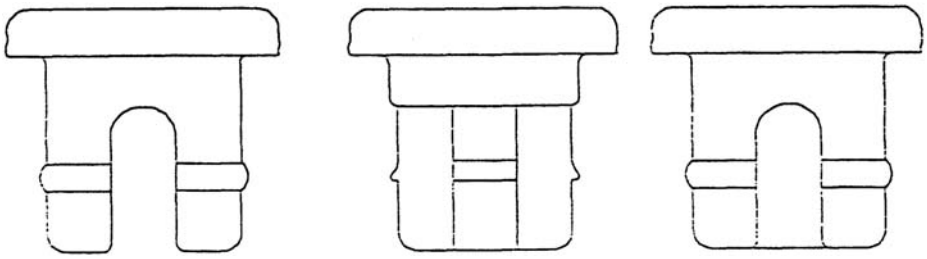

Figure 12.5 Lyophilised stopper designs

The half stop position, shown in Figure 12.3, is the fundamental role of the lyophilisation closure. The partially inserted closure must allow a sufficient pathway for ice sublimation to occur while being able to form an effective seal upon full insertion. Mechanical stability is provided by interference between the plug portion and the neck of the glass or plastic vial. Obviously a trade-off exists between mechanical stability and final insertion force. The seal formed between rubber and the glass container has two functions: initially it must provide a short-term seal prior to overcrimping and may require retention of a nitrogen headspace within the vial. After crimping, the seal must maintain integrity for normally 2 to 3 years. The first requirement is more a factor of geometry, whereas retention of seal integrity over time is more a function of correct rubber formulation selection to include suitable compression set characteristics and overcrimp procedure. In terms of seal integrity there are both primary and secondary seals formed between rubber and the glass container as shown in Figure 12.4.

The design of the lyophilisation closure significantly affects the reconstitution step. Certain stopper designs can retain part of the cake within the flutes of the plug section, resulting in only partial reconstitution. There are essentially three main designs of stopper with the variation occurring in plug portion, as shown in Figure 12.5:

1 two or three leg/peg
2 cruciform design
3 igloo or slot design.

The leg or peg design suffers from relatively poor stability in the half stop position. This results from the relative ease with which the rubber pegs can be forced inwards. The cruciform design has excellent stability in the half stop position because there is significant interference between the rubber and glass in that position. There are some concerns with this design because of cake retention in the flutes and central cone.

The igloo or slot design is widely regarded as being the optimum lyophilisation stopper design. Excellent half stop stability is achieved by rubber-glass container interference in this position. The slot prevents cake retention and thereby provides complete reconstitution.

Lyophilisation stoppers are predominantly made from butyl or halobutyl elastomerbased rubber formulations chosen because of their relatively low permeability to gases.

Prefilled syringes

A prefilled syringe combines the function of a primary container with that of a conventional syringe and prior to use contains the drug. The prefilled syringe has several advantages:

- immediate administration from one item
- can be used anywhere
- labour saving
- measured accurate dose
- low risk of errors in medication
- lower risk of contamination
- marketing advantage.

Removable seal system

This is ostensibly the most simple, with the design being very similar to the disposable syringe except that the barrel is manufactured from glass and is prefilled with the drug solution. This solution is sealed by the piston at one end and a rubber needle shield or plug at the other. In use, the shield or plug is discarded and the device is ready. Some examples of this prefilled syringe type are as follows. Becton Dickinson Pharmaceutical Systems offers the Hypak® prefilled syringe in two versions, the bulk, unsterilised Hypak® and the sterile, ready to fill Hypak® SCF (sterile, clean, fill). Bünder Glas GmbH offers a removable seal system called Variject.

Diaphragm puncture system

With the diaphragm puncture system there is a glass cartridge, one end of which is sealed with the rubber piston and the other with an aluminium overseal and rubber septum. The filled cartridge either is placed into a separate syringe body or forms the body of the syringe. When the piston is depressed the first action is to pierce the rubber septum with one end of a double ended needle. Further movement of the piston administers the drug.

Bünder Glas GmbH produces a range of systems of this type under the trade names Dipsoject, Diviject and Koniject. MIN-I-JET®, manufactured by International Medication Systems (IMS), patented in 1965, is also a diaphragm puncture system.

Single-component bypass chamber system

The glass tube is sealed at one end by a rubber plunger and at the other by a floating rubber piston. When the plunger is depressed, the pressure build-up moves the rubber piston from the glass tube into the plastic end cap. Bypass or flow-past grooves in the plastic end cap allow the parenteral product to be expelled. Duphar BV of Holland markets its prefilled syringe under the name of Dumpharject®, and this works on the bypass chamber principle.

Two-component drug system

Two-component drug systems are on offer from a number of suppliers with dualchamber syringes for liquid-liquid and powder-liquid administration. The two compartments are separated by a ‘floating’ rubber piston and the design of the glass body includes bypass channels to allow liquid transfer.

There is a risk with the standard design that, if the nurse is a little careless, the rear piston will be pressed too quickly past the bypass slot. This could result in inadequate dissolution of the solid, possibly with leakage through the needle. To overcome this, the Vetter Lyoject® has a plunger rod that is partially threaded running through a mating thread at the top of the syringe. The rear piston is advanced slowly by screwing in the plunger. By the time the plunger reaches the end of the threaded portion, mixing is complete and the drug can be administered in the normal way by pressing the plunger.

BD Hypak® has addressed the problem of incomplete dissolution of the dry powder product by introducing a piston with a grooved front rib. This creates turbulence as the water flows past it, thus dissolving the solid more readily. Bünder Glas GmbH also offers a bypass syringe suitable for liquid-liquid or liquid-dry applications under the tradename Variject 2 system.

Auto-injectors

Single use

An auto-injection device for self-administration has been developed for military use by STI. A heavy spring fires the needle about 25 mm into the thigh muscle before drug injection takes place. This is designed to penetrate easily through four layers of battle dress clothing. Such systems are marketed by STI under the tradenames Mediject®, Astopen® and Combopen®, and by Duphar as MultiPen®. Twocomponent wet/dry systems can also be administered using the STI Binaject™ auto-injector. With this relatively new technology it is imperative that personnel are trained in the correct use.

Auto-injection devices have been developed for self-administration of sumatriptan for the treatment of migraine. Glaxo Wellcome has marketed such a device as Subject®.

Multi-use

Diabetics have traditionally been forced to perform self-injection, so auto-injectors have been developed for use in this area and look like a modern felt-tip pen. Quantities of insulin can be pre-set by turning a knob on the pen which has features including an audible click, clear resistance prior to each dose and a visual indication on the scale thus helping to eliminate dosing errors. Quick and easy dosing is possible with such systems. Auto-injectors are marketed by a number of companies under the following tradenames: Novopen®, Novepen®II, NovoLet™, Optipen® II and D-Pen®. Pressurised needleless systems are also now available.

Selection of rubber formulation and component design

A large number of factors should be considered when selecting a rubber closure for the sealing of a new drug or package:

- active drug substance
- solvent used
- preservative
- pH and buffer system
- process of sterilisation and, if aseptic, fill or terminal sterilisation
- size of pack and fill volume
- single or multi-dose
- sensitivity to metal ions
- importance of gas or moisture vapour permeability or desorption
- the total manufacturing process of filling and capping the vials
- product shelf-life requirements
- route of administration
- disposal of the pack and toxicity of contents.

In the past the main focus was on compatibility, primarily because of the relatively high level of extractives present in rubber formulations. The focus has now moved to include aspects such as stopper cleanliness, processability and presentation to the filling line. These issues are of increasing importance with the industry interest in barrier or isolator technology. This technology is gaining importance within the pharmaceutical industry as a preferred method of manufacture for aseptic processing. The entire filling area is covered by a physical barrier and the vial washing depyrogenation tunnel and primary packing component lines can be integrated to provide a complete aseptic filling system. Access to the filling line is provided by a series of specially designed ports and intervention by the operator is provided via glove boxes. The flow of components such as stoppers and plungers has become critical since they must be supplied into the isolator in a sterile and clean condition. This requirement has refocused pharmaceutical companies on the potential to source such components in a ready-to-use or readyto-sterilise format (see below).

Compatibility with the drug

The problems with interaction of a parenteral product and the rubber closure are well documented in the literature. So-called ‘high extract’ formulations are typified in the 1978 paper by Pikal and Lang where a haze was identified as being caused by paraffin wax and sulphur. The organic accelerators which are used in conjunction with sulphur have also been a source for contamination. Wells *et al.* (1986) identified amine impurities that originated from the decomposition of amine salts of

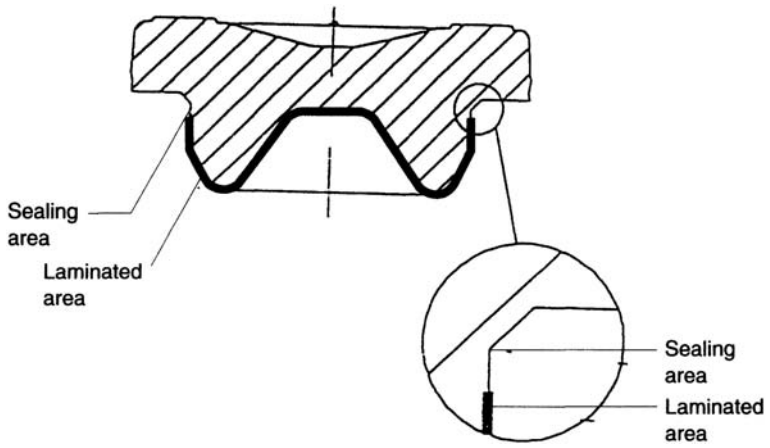

Figure 12.6 FluroTec® closure

dithiocarbamates. These impurities were detected in a water-soluble Vitamin E product intended for intravenous injection. The case of Merck Sharp and Dohme Research Laboratories' development of sodium cefoxitin is an excellent example (Portnoff *et al.,* 1983).

A haze was found to form on reconstitution of the stored lyophilised product. The haze was identified as containing paraffin, silicone oil and zinc. Despite screening of fifty different rubber formulations, the only viable solution at that time was to use the West Company's 'Teflon coated' injection stopper. With the development of so-called 'low extract' rubber formulations, MSD has been able to approve non-Teflon faced closures for this application. These 'low extract' rubber formulations are normally vulcanised using unconventional cross-linking systems and so avoid the problems of sulphur and organic accelerators.

The problem of haze reported by Pikal and Lang (1978), Wells *et al.* (1986) and Portnoff *et al.* (1983) has been studied further by Jahnke *et al.* (1990a, 1990b, 1991), who investigated volatiles from the rubber closures. Jahnke reported a complex mixture of volatile hydrocarbons from the butyl and halobutyl polymers routinely used to make vial closures. The oligomer identified from butyl rubber was 1-isopropenyl2, 2, 4, 4-tetramethylcyclohexane and that from chlorobutyl rubber was 1-(1chloromethylethenyl)-2, 2, 4, 4-tetramethylcyclohexane. Both are referred to as the C_{13} oligomer, and its contribution to haze formation on reconstitution of lyophilised powders has been confirmed.

Problems of closure-related compatibility can be resolved by the use of a stopper laminated with a fluoropolymer on the sealing face. Such products are commercially available as FluroTec® closures for injection, infusion and lyophilisation closures as well as plungers for prefilled syringes. To provide an integral seal, the upper part of the sealing area is not coated, as shown in Figure 12.6. The use of such closures has been proposed by Danielson (1992) as a means of 'reducing or eliminating much leaching of toxic compounds'. Franke *et al.* (1994) have been able to relate the haze formation in reconstituted solutions of parenteral antibiotic powders to volatile components from the closure adsorbed onto the powder surface during storage. To avoid this phenomenon, marketed products such as cefodizime-disodium are commercially packaged using fluoropolymer laminated closures.

Seal integrity

It has been highlighted earlier in the chapter that rubber, due to its chemical structure, has excellent resilience properties. This characteristic forms the basis for its widespread use for piston and closures. A review article by Morton (1987) highlighted that there is debate within the pharmaceutical industry as to where the 'true' seal is formed in a parenteral vial package. The true seal is defined as the primary seal and Morton confirmed the location of both primary and secondary seals as shown in Figure 12.7.

The primary seal is formed between the underside of the closure flange and top of the vial; the secondary seals are formed between the vial side wall and the plug portion of the closure (termed flange seal and bore seal). It has been postulated that correct selection and specification of the aluminium overseal is as important as the primary packaging materials, since it forms an integral part of the sealing mechanism.

Container/closure seal integrity has been reviewed by the Parenteral Drug Association (1983) and the Parenteral Society (1992). Variation in the overall height of the combined vial and closure will result in different closure-seal forces being applied at the crimping head. Quality control tests are proposed such as the dye intrusion challenge, liquid loss or bacterial

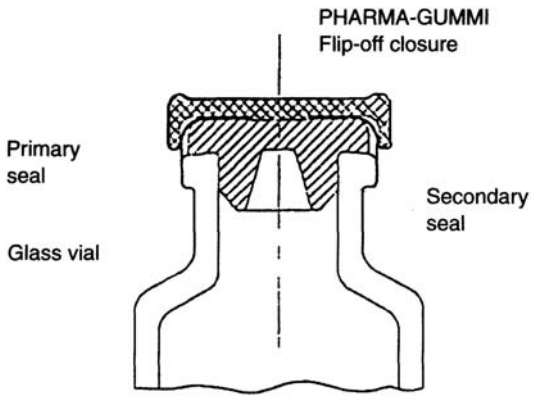

Figure 12.7 Primary and secondary seals in a parenteral vial

challenge test. Methods for 100% on-line testing are also recommended to include high-voltage detection and seal force monitoring (Morton and Lordi, 1988).

The bore and flange seal interaction are used extensively during the filling of pharmaceutical products. Prior to over-capping with an aluminium overseal to effect the primary seal, the manufacturer must rely on the secondary bore seal to provide a shortterm seal during processing.

Self-sealing and fragmentation

An important characteristic of rubber is its resilience, giving rise to so-called selfsealing properties. This property is the ability of the rubber to allow penetration by a hypodermic needle and 'snap' shut when the needle is removed, thus maintaining an effective seal. This is an important consideration for a rubber stopper's coring or fragmentation performance. As the needle passes through a rubber stopper there is the potential for the frictional detachment of rubber particles and for larger fragments to be cut out by the 'heel' of the needle. Each of these in turn depends on the sharpness of the needle point (angle and use of side grinds) and the needle heel (angle and quality of cut). The quality of metal to construct the needle and absence of burrs also play an important role, as does the correct selection of rubber formulation. Determination of this self-sealing characteristic is covered in a number of applicable ISO standards together with the European Pharmacopoeia as follows:

- ISO 8362–2, Injection Containers for Injectables and Accessories [Closures for Injection Vials]
- ISO 8536–2, Infusion Equipment for Medical Use [Closures for Infusion Bottles].

Penetration force is a measure of the force required to insert a needle through the rubber septum. When a needle pierces a rubber closure it inherently generates a number of rubber particles and this phenomenon is termed fragmentation.

Another important authoritative standard is the European Pharmacopoeia: section VI. 2.3.1 relates specifically to rubber closures and is divided into chemical and physical sections. The chemical tests on autoclave extract determine the following properties:

- appearance of solution
- pH
- UV absorbance
- reducing substances
- heavy metals
- soluble zinc
- ammonium
- residue on evaporation
- volatile sulphides.

If the rubber closure or component is intended to be pierced by a hypodermic needle, then the physical test regime of the European Pharmacopoeia applies. This includes test method limits for penetrability, fragmentation and self-sealing categorisation (type I or type II closures), and methods for the determination of penetration force and fragmentation.

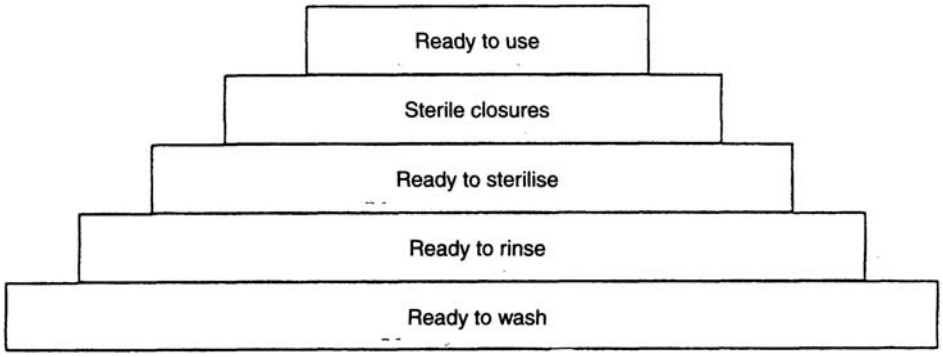

Figure 12.8 Stepwise approach to ready-to-use/sterile closures

Cleanliness of rubber closures

Rubber closures by their very nature need to be subject to some degree of cleaning prior to use to remove particulates and to remove endotoxins from the stopper surface. Indeed, when aseptically packaging a 'sterile' product the closure must be 'sterile and pyrogen-free' itself, or it will contaminate the product.

Within the pharmaceutical and parenteral industry rubber closures can be supplied at differing degrees of cleanliness, with each level placing reduced requirements on the customer for further treatment. The various levels are shown in Figure 12.8.

Ready to wash closures

This level would represent the 'as manufactured' closure and as such was the accepted standard of presentation to the customer in the 1970s. It was accepted that in this state the closures required further processing, cleaning and lubrication with silicone oil by the pharmaceutical company before sterilisation. The closures in the as-supplied condition could be assumed to have moderate to high levels of microbial, pyrogen and particulate contamination.

Ready to rinse closures

Closures supplied ready to rinse have undergone a minimum hot water wash by the closure manufacturer to remove the potential hazards detailed above. For some applications this is considered sufficient and only the addition of surface lubricants is necessary as a post-treatment. However, if the closure is to be used in a 'sterile' package then depyrogenation must be carried out.

Ready to sterilise (RTS) closures

Ready to sterilise closures must as a prerequisite have been pre-lubricated, have a defined maximum particle burden and be pyrogen-free. Proved Clean® closures provide the required definable particle burden by objectively documenting the particulate cleanliness of the rubber closure for particles down to 25 μm in size. To achieve a low level of particulate contamination it is necessary to manufacture within a controlled environment and to complete the final washing stage and packing within a cleanroom environment. Experience has shown that each step of the manufacturing process should be optimised to provide the lowest particulate burden. For example, the polythene bags used to store cleaned stoppers have been identified as a major source of particulate contamination. This was one factor that led to the development by the West Company of a sterilisable bag manufactured using a combination of HDPE and Tyvek to facilitate steam penetration and drying. Rubber closures are routinely supplied in these sterilisable bags ready for steam sterilisation by the pharmaceutical company.

Particles found on the surface of rubber closures have been categorised as exogenous and normally originate from the environment associated with stopper processing. Endogenous particles released by the stopper are normally in the 2–5 μm range and typically include blooms, liquid migration and outgassed materials (Dölcher, 1990). The release of endogenous materials can be significantly reduced by the use of FluroTec® closures with the fluoropolymer laminate. To ensure that the closure is pyrogen-free it is subjected to a rinse in pyrogen-free water or water for injection (WFI). This water can be prepared by FIN-AQUA distillation.

Ready to use (RTU) closures

The 'ultimate' level of closure cleanliness encompasses all the requirements of RTS closures and also is subject to a sterilisation process. The sterilisation process for rubber closures can be achieved by a number of methods summarised below (Table 12.6).

The use of the RTU closures can offer a significant opportunity to reduce the total acquisition cost of a product.

Table 12.6 Methods of sterilisation for closures

Sterilisation method	*Advantages*	*Disadvantages*
Steam sterilisation (autoclave)	Preferred method, established effectiveness	Increased closure moisture level giving potential problems in lyophilised products
Ethylene oxide (ETO) sterilisation	No closure moisture level increase	Ethylene oxide sorbs into closures
Gamma irradiation	No closure moisture level increase	Not suitable for all rubber formulations

Lubrication

Most closures are lightly coated with silicone oil, such as a polydimethyl siloxane, as a means of reducing particulate formation as it acts as a lubricant between closures. It also reduces considerably the inherent tackiness in many rubber formulations. The main advantage of a silicone oil coat is that it facilitates the stoppering operation by lubricating the passage of the closures through assembly machines and insertion into the barrel or vial opening. The actual quantity of silicone can be adjusted to suit individual customer requirements. A vial closure silicone oil level of 0.005–0.020 mg silicone oil per cm^2 of rubber surface has been found to be optimum. Lower levels result in poor tracking and stoppering characteristics, while higher levels can result in 'pop-out' of the stopper from the vial. It has also been noted that silicone oil can contribute to particle counts as measured by a Coulter counter (Mannermaa *et al.,* 1992), and is a potential cause of haze formation, especially on reconstitution of stored lyophilised products (Wells *et al.,* 1986). A silicone resin coating can be applied to the surface of a closure followed by a cross-linking or baking step to adhere the resin. The coating provides the same advantages as silicone oil and is claimed to reduce significantly the level of endogenous particles released from the rubber.

Conclusions

Sterile products clearly place extra challenges on the pack in terms of the materials employed and the processes/procedures involved in the filling and packing operations. They also involve the greatest level of risk to the user/patient since the administration of sterile products involves direct injection, thus bypassing the body's natural defences. Because of this increased risk for the patient, the pharmaceutical company is exposed to a greater degree of responsibility and risk via 'product liability' claims.

The administration of these sterile products and ease of use have become a much more important criterion in recent times. This trend is expected to continue, as will the move towards drug delivery devices intended for easier application, with an increasing focus on self-application. These containers/drug delivery devices will inevitably use an increasing number of plastic components so that any complexity is involved in the assembly of the system rather than in the use of the delivery device. Rubber components have a significant role to play in the successful development of such devices, since there is no real substitute for rubber.

Advances by reputable manufacturers have removed some of the perceived disadvantages of rubber, in particular the refinement of rubber formulations to reduce the level of extractives. The development of a fluoropolymer-based laminate under the FluroTec product range to provide real barrier properties has simplified the complex process of rubber closure selection and offered a level of security not available with a conventional closure. The use of such laminated closures is predicted to increase in the future, in particular these are likely to be specified as the closure system for biopharmaceutical applications.

The trend toward the use of isolator technology for sterile products to improve the quality level and reduce the manufacturing cost has placed a new emphasis on the importance of the primary packaging components. Existing systems for sterilisation and depyrogenisation of glassware fit readily with isolator technology, but there are recognised difficulties in the provision of sterile pyrogen-free rubber closures into the isolator. Solutions range from the supply of sterile rubber closures and metal crimps in disposable bags, each fitted with a special transfer port, to closures being supplied in specially designed recyclable containers. Alternatively, it is possible to gain access into an isolator using a special receptacle and a manual or

automatic transfer port. The use of the latter system would not require any further advances by closure manufacturers, but such systems are likely to be suitable only for small volume production. The supply of closures into isolators would appear to suit ideally the use of gamma sterilisation, and although this method has been used to supply sterilise closures there are certain regulatory requirements that affect existing products. Changes in the sterilisation method will require a degree of evaluation by running stability batches and also validation of the sterilisation method, in particular the impact on the physical and visual properties of the closures.

References

Danielson, J.W., *J. Parenteral Sci. Technol.,* 1992, **46,** 43.

Dillard, S.F. and MacCollum, M.A., Reports to FDA: allergic reactions to latex containing medical devices, *International Latex Conference: Sensitivity to Latex in Medical Devices,* 1992, 23 (abstract).

Dölcher, D., PDA *International Conference,* Arlington, February 1990.

Franke, H., Hencken, P., Ross, G. and Kreuter, J., *Eur. J. Pharm. Biopharm.,* 1994, **40,** 379.

Jahnke, R.W.O., Kreuter, J. and Ross G., *J. Parenteral Sci. Technol.,* 1990a, **44,** 282.

Jahnke, R.W.O., Kreuter, J. and Ross G., *Int. J. Pharm.,* 1991, **77,** 47.

Jahnke, R.W.O., Linde, H., Mosand I.A. and Kreuter, J., *Acta Pharm Technol.,* 1990b, **36,** 139.

Lee, M.L. and Hites, R.A., *Anal. Chem.,* 1976, **48,** 1890.

Mannermaa, J.P., Muttonen, E., Yliruusi, J. and Juppo, A., *J. Parenteral Sci. Technol.,* 1992, **46,** 73.

Massey, R.C. and Taylor, D. (eds), *Aluminium in Food and the Environment,* Royal Society of Chemistry, Cambridge, 1989.

Meek, J.H. and Pettit, B.R., *The Lancet,* 1985, 1090.

Milano, E.A., Waraszkiewicz, S.M. and Dirubio, R., *J. Parenteral Sci. Technol.,* 1982, **36,** 116.

Mondimore, D. and Moore, C., *J. Parenteral Sci. Technol.,* 1983, **37,** 79.

Morton, D.K., *J. Parenteral Sci. Technol.,* 1987, **41,** 145.

Morton, D.K., Lordi, N.G., *J. Parenteral Sci. Technol.,* 1988, **42,** 57.

Parenteral Drug Association Inc., *Aspects of Container/Closure Integrity,* Technical Information Bulletin No. 4, 1983.

Parenteral Society, *The Prevention and Detection of Leaks in Ampoules, Vials and Other Parenteral Containers,* Technical Monograph No. 3, 1992.

Petersen, M.C., Vine, J., Ashley, J.J. and Nation, R.L., *J. Pharm. Sci.,* 1981, **70,** 1139.

Pikal, M.J. and Lang, J.E., *J. Parenteral Drug Assoc.,* 1978, **32,** 162.

Portnoff, J.B., Henley, M.V and Restaino, F.A., *J. Parenteral Sci. Technol.,* 1983, **37,** 180.

Russell-Fell, R., *International Conference: Latex Protein Allergy,* 1993, 3.

Shah, P.V., Knapp, G., Kelly, J.P. and Cabana, B.E., *Clin. Chem.,* 1982, **28,** 2327.

Shang-Qiang, J. and Evenson, M.A., *Clin. Chem.,* 1983, **29,** 456.

Slater, J.E., *International Conference: Latex Protein Allergy,* 1993, 7.

Towse, A., O'Brien, M., Twarog, F.J., Braimon, J. and Moses, A.C., *Diabetes Care,* 1995, **18,** 1194.

Wells, C.E., Juenge, E.C. and Wolnick, K., *J. Pharm. Sci.,* 1986, **75,** 724.

Yip, E., Poon, N.K. and Lang, M.K., *International Conference: Latex Protein Allergy,* 1995, 33.