DRUG DELIVERY SYSTEMS AND PHARMACEUTICAL BIOTECHNOLOGY

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About this lecture

This lecture is in two main parts, viz, *Drug delivery systems* and *Pharmaceutical biotechnology*. The former will be considered first. However, as you will observe in the course of the lecture, there is significant overlap between the two topics.

Introduction to Drug Delivery Systems

Ever since man began to treat various disease states with medicinal agents, the need to present medicines in the most suitable physical form for delivery to their sites of action has persisted. Some of the earliest drug delivery forms include solutions, suspensions and powders. Soon, cachets, pills, tablets and emulsions followed.

In the early years and probably up to the middle of this century, formulation and production of drug delivery systems were far more of an art than science. The main objective then was to deliver the therapeutic agent in an administrable form. Little attention was paid to such desirable formulation objectives as masking unpleasant taste and odour, better aesthetics, controlled and/or prolonged drug action, and enhanced patient acceptance. In the last four decades, however, particularly with the advent of the use of synthetic polymers in pharmaceutical formulation, there have been concerted and accelerated efforts to move towards perfection in drug delivery. Today, far more progress than was thought possible 60 years ago has been made in this field.

Several of the new drug delivery systems are still not available in the West African market due to their high cost. This situation is, however, expected to change in the years ahead. Therefore, there is a need to continually keep abreast of rapid developments in the highly dynamic area of drug delivery technology. This lecture is an effort in this direction.

In this lecture, no attempt will be made to discuss all the new drug delivery systems that have been invented nor will any of them be treated in great detail since a rapidly expanding field such as this cannot be adequately treated in a presentation of this nature. However, some of the more novel, exciting and promising advances that have been attained in drug delivery design will be highlighted. All the systems that will be examined have gone beyond the

conceptual stage. They are either at the laboratory stage, undergoing clinical trials or already in the market.

What is drug delivery?

Drug delivery is a term or concept that refers to the mode, approach, formulations, technologies and systems for conveying a medicinal substance to a location/site in the body where it is intended to produce the desired therapeutic result in a safe manner. Drug delivery is of two types: ***conventional*** and ***advanced/controlled***. The former have the following features:

- A large quantity of the drug is delivered to the site of action which may lead to side effects
- There is usually wide fluctuation (peak and trough pattern) in the blood drug concentration especially for repeated dosing during the day
- Repeated dosing often results in less patient compliance due to missed doses.

Most of the dosage systems in the Nigerian market, especially analgesics, antimalarials, antibiotics, are conventional delivery systems. Their modes of formulation and manufacture have been well-established several decades ago.

On the other hand, advanced drug delivery systems (ADDS) are designed and produced to release the drug contained in it:

- At a predetermined rate
- Over a prolonged period
- To a predetermined site
- From a single dosage unit.

Designing ADDS often requires sophisticated techniques and creative biomedical engineering, and therefore, involves time, considerable resources and a multidisciplinary approach.

The Delivery Quartet

Before we go on to look at specific delivery systems, it is desirable to mention briefly here what has been proposed as a fundamental approach to drug delivery design. It is based on what has been referred to as the *Drug Delivery Quartet*, and was advanced by Prof SS Davis of the University of Nottingham in the early 1980s. It is illustrated in Fig 1. Essentially, this quartet of D's suggests that anyone setting out to design a new drug delivery system would necessarily have to consider the other components of the quartet, namely, the type of drug, the nature of the disease and the destination of the drug. Although drug delivery design has become sophisticated since then, the delivery quartet still remain relevant as a basic approach.

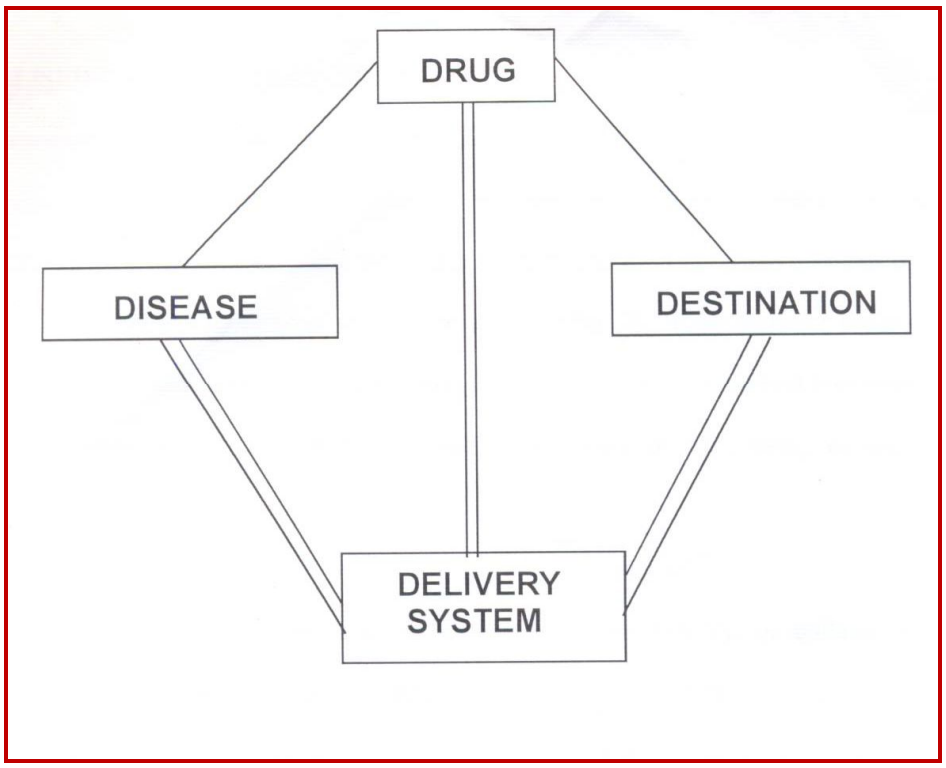

Fig 1: The Drug Delivery Quartet

Anatomical, physiological and biochemical considerations

Drug delivery research employs diverse approaches. The goal is to optimise drug efficacy rather than modifying the intrinsic pharmacological activity of the drug. The objective, therefore, is to optimize, in the words of Professor Gordon Amidon, *'the delivery of drug to the receptor or to the right tissue or to reduce side effects in other tissues, by controlling the pharmcokinetics, pharmacodynamics, drug targeting, dose rate or even simply patient compliance"*. To achieve this, pharmaceutical researchers
have realised the imperative for a more detailed and clearer knowledge of human anatomy, physiology and biochemistry down even to the molecular level. This has spurned specialist areas of research such as cell biology, molecular biology, biotechnology and nanotehnology. Thus, drug delivery research is now a multidisciplinary effort with multidisciplinary research teams who focus on specific therepeutic objectives. It is clear, therefore, that it is no longer sufficient to focus on understanding/modifying the *in vitro* or *in vivo* physicochemical characteristics of the drug or drug product in order to attain a drug delivery objective.

The results of the multidisciplinary approach is that an enzyme or other biochemical (endogenous chemical) at a site of action can be manipulated to play a major role in delivering a drug to the site. Similarly, the electrostatic charge of the cells of a particular tissue can be advantageously used to achieve

site-specific delivery. Yet another example is the blood-brain barrier (BBB) which for decades was poorly understood but is well-known for hindering delivery of drugs to the brain. Consequently, several attempts to treat brain disorders and other diseases whose origin are traceable to the brain have been frustrated. As an illustration, the neurotransmitter, dopamine, which is released at specific parts of the brain, is largely restricted to this area by the **BBB**, and plays a significant role in protecting man from depression. Thus peripheral receptors are not affected by dopamine and even if the drug is injected directly into peripheral circulation, it cannot cross the **BBB.** One of the efforts in drug delivery research is directed at acquiring a better understanding of the **BBB** with a view to formulating drug delivery systems that can deliver therapeutic agents to the brain.

SPECIALISED DRUG DELIVERY SYSTEMS

Micro- and nano-particulate systems

These are usually fine and ultrafine solid particles, solution, micelles or emulsion droplets that may or may not be coated reproducibly with extremely thin polymeric films or shells.

Microencapsulated systems: When the microparticles or droplets are coated, they are generally referred to as *microcapsules* and usually consist of a core and a surrounding outer membrane (see Fig 2). Their diameters usually range from as low as 0.05 to as high 1000μ. When used in drug delivery, the drug is usually in the core and the coating/membrane serves as the rate controller of drug release. Thus, drug release can be modulated by the varying the number, size and/or behavior of the pores in the membrane. Membrane properties are influenced by the type of polymer used and the manner the polymer is applied to the microcapsule core. Examples of synthetic polymers have been used for membrane/coating include polylactic acid, polylactic glycolic acid, gelatin, ethyl cellulose, polyvinyl alcohol, cellulose acetate phthalate and styrene maleic anhydride, etc (see Fig 2).

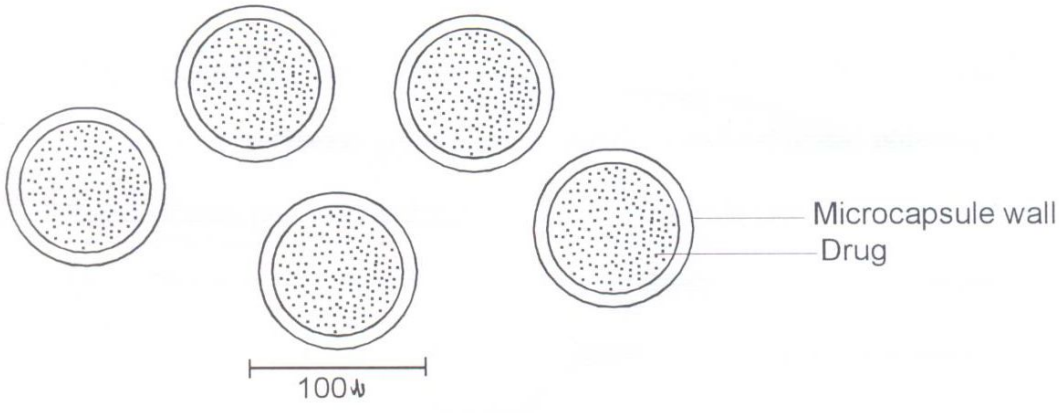

Fig 2: Illustration of cross-sections of simple microcapsules

The process of preparing microcapsules is known as *microencapsulation.* Two main methods are generally used. In the first method known as coacervation, or co-precipitation, one approach is to suspend the drug particle in a polymer solution and a non-solvent for the polymer is then added. This results in the precipitation (coacervation) of the polymer around the individual drug particles. The coated particles are filtered and then dried. The second method is generally referred to as interfacial or emulsion polymerization. An emulsion is prepared with a monomer (which is meant to form the polymer coating or shell of the microcapsule) together with the drug to be encapsulated in the disperse phase. Usually, an initiator agent is then added to the continuous phase of the emulsion and this sets off a polymerization reaction at the droplet (i.e., disperse phase) interface. The resulting polymer, formed around each droplet, constitutes the shell or coating of the microcapsules and the coated droplets are the microcapsules. Microcapsules, after filtration and drying, generally appear as free flowing powders.

Drug release occurs by diffusion or leaching through the wall/coating (Fig 3) or, in the case of coating based on water soluble polymers such as gelatin and some cellulose derivatives, by swelling and dissolution of the microcapsule shell.

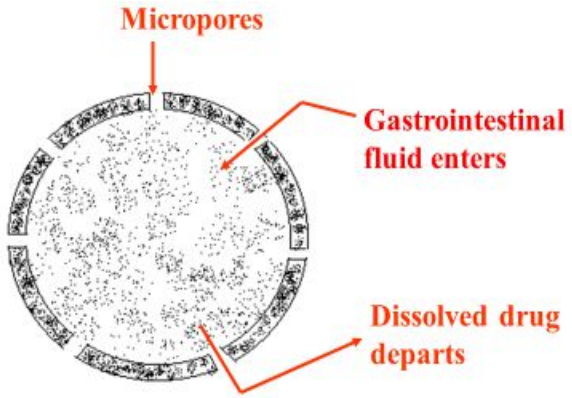

Fig 3: Illustration of drug release from microcapsules by diffusion/leaching

The general applications of microcapsules include the following:

- *To retard the release and change the bioavailability of the encapsulated drug.*

- *To mask the bitter taste and drugs, impart a more attractive colour and alter the shape of drug particles to a more free-flowing one (spherical).*
- *To protect labile materials from heat, light and moisture.*
- *To minimize gastric irritation by drugs, e.g., aspirin microcapsules.*
- *To separate incompatible drugs in a dosage form. For example, one of the drugs may be microencapsulated and the other is not.*
- *To change liquids to free flowing powders in order to facilitate handling, formulation and manufacture.*
- *To trap carcinogens present in ingested food or formed in situ in the gastrointestinal tract. Magnetic microcapsules incorporating the agent, polyethleneimine, have been tested for this purpose.*
- *To facilitate drug targeting (details later).*
- *To aid the design of bioartificial organs (details later)*

There are variants/subtypes of microparticles and microencapsulated systems. These include

- *Liposomes:* Otherwise known as multilamellar vesicles (MLV), or unilamellar vesicles (ULV) depending on the number of concentric walls/membranes, they consist of one or more phospholipids bilayers alternating with aqueous drug compartments (Fig 4).

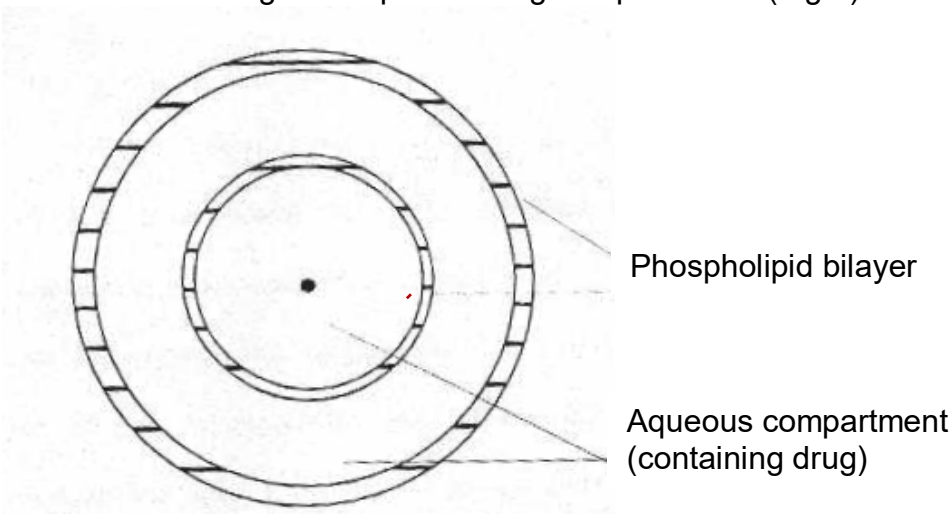

Fig 4: Illustration of a cross-section of a two-compartment liposome

The phospholipids used in liposome preparation include cholesterol, lecithin, phosphatidyl choline (PC) as well as the dimyristoyl, dipalmitoyl and distearyl esters of PC. Drug is situated in the inner most compartment and the walls/membranes modulate drug release and as the wall is digested by lysosomal enzymes, drug release follows.

- *Microspheres*
- *Microemulsion*
- *Micelles*

Nanoparticles: These differ from conventional microcapsules only in being considerably smaller. They are, in fact, of colloidal dimensions with particle diameter in the range 10 – 100nm. Polymeric materials used are usually polyalkylcyanoacrylates such as polyisobutylcyanoacrylate. They have been used to encapsulate drug molecules, globulins and toxoid, e.g., tetanus toxoid and human immunoglobin G for parenteral use in animals.

Other variants/subtypes of nanoparticles include

- *Nanosomes*
- *Niosomes*
- *Pegylated nanoparticles*
- *Coated nanoparticles*
- *Solid Lipid nanoparticles (SLNs)*
- *Nanosphere*
- *Nanosuspension*
- *Nanogels*

We will now consider the other specialized drug delivery systems according to route of administration

ORAL ROUTE

Osmotic pump device ('Oros)

The Oros was first introduced by Alza Corporation of the United States of America in 1981. Some sustained release preparations, particularly those which manifest gradual erosion of the dosage form, will effect drug release by a first-order process. Thus, the rate of drug release would decrease in direct proportion to the amount of drug still left in the dosage form and this can be presented by the first-order equation:

$$dR/dt = KA \quad \text{..... (1)}$$

where dR/dt is the drug release rate, A is the amount of drug still left in the dosage form at any particular time and k is a constant. A zero order release process (see Eq 2) should provide the ideal release mechanism for a truly controlled release product since the release rate is constant and independent of the amount of drug in the dosage form:

$$\underline{dR}/dt = K \quad \text{..... (2)}$$

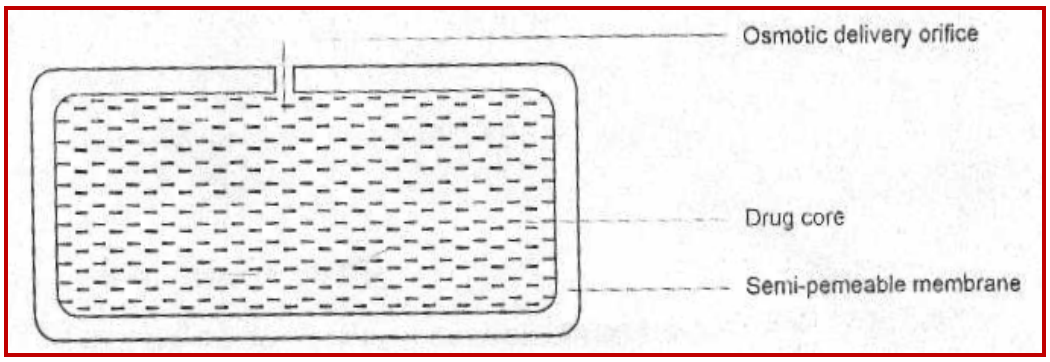

Fig 5: *Osmotic pump (Oros) Device*

The ***Oros*** device is designed to release drug by a **zero-order** fashion. It consists of a core coated with a semi-permeable membrane through which a tiny orifice has been drilled by means of laser (Fig 5). The core is composed of the drug and an osmotically active substance (e.g., NaCl, KCl and glucose). Water from gastric fluid transverses the semi-permeable membrane by osmosis at a steady rate determined by the permeability of the membrane and the solubility of the tablet core formulation. As the core is gradually dissolved and a saturated solution of the drug is formed, and the hydrostatic pressure created by the entry of water forces the saturated solution out through the orifice. Since the membrane does not allow any expansion of the volume of the dosage form, the drug release rate is constant (i.e., zero-order), being equal to the rate of osmotic passage of water into the core. However, the release rate ultimately decreases parabolically to zero when there is no longer excess undissolved drug in the core. The osmotic pump device has the advantage (over earlier sustained release formulations) in that its drug release is independent of such physiological factors as pH and gastrointestinal motility. Constant drug release can be sustained for up to 20 h, thus permitting a once-daily dosage regimen.

Former Glaxo and Pfizer introduced Oros forms of salbutmol and prazosin, respectively, in the 1980s. Twelve other Oros formulations including nifedipine and vitamin C were reported to be under development. However, the manufacturers of Osmosin ® (an Oros indomethacin) were compelled to withdraw the product from the market in August 1983 following a warning by the UK Committee on the Safety of Medicines (CSM). It was stated that Osmosin® did not reduce the adverse reactions usually associated with indomethacin, and more seriously, two reports of intestinal perforation by doctors suggested that "dose-dumping", i.e., exposition of certain areas of the gut to abnormally high concentrations of drug, might have occurred Nonetheless, Oros technology is being applied in the development of a system that releases at specified intervals ("timed pulses") to simulate usual dosage intervals. Hormonal therapy will benefit from this development. Perhaps, so also will insomniacs since the device could release one dose at bedtime and then another some hours later when they would probably wake up.

3. Floating dosage forms (FDF)

This is also known as **hydrodynamically balanced system (HBS).** The main feature of this dosage form is that it has a bulk density lower than the density of gastric fluids and therefore floats or is buoyant in gastric fluid but does not affect gastric emptying rate.

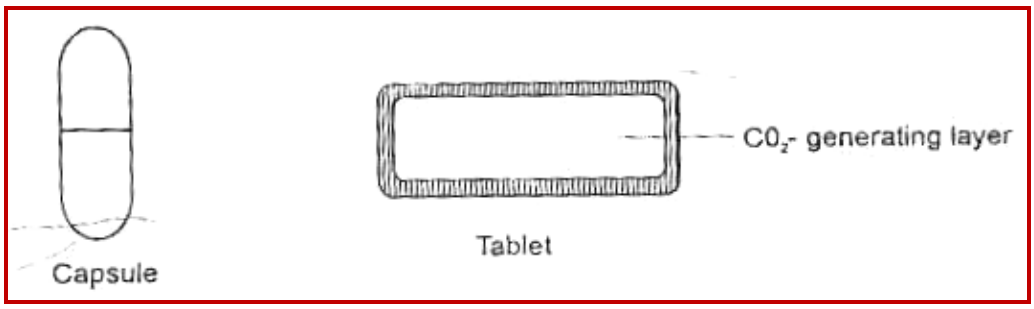

Fig 6: *Floating dosage systems*

Two forms are available: capsule and tablet (see Fig 6). The capsule contains the drug dispersed in a gel-forming colloid or polymer which absorbs moisture from gastric fluid and swells, causing the caspule to float (Fig 7). As a result, the capsule remains longer in the stomach but slowly releases drug by diffusion through the polymer gel thus producing prolonged drug release in the stomach. The tablet form consists of two layers. The inner layer contains a carbon dioxide-generating blend (calcium carbonate or sodium bicarbonate and citric acid) in addition to a gel-forming polymer. The outer layer contains a gel-forming polymer and the drug. Following moisture absorption in the stomach, the polymer in both layers gel, and carbon dioxide is generated and trapped within the inner layer. Due to the reduced bulk density, the tablet floats (Fig 7).

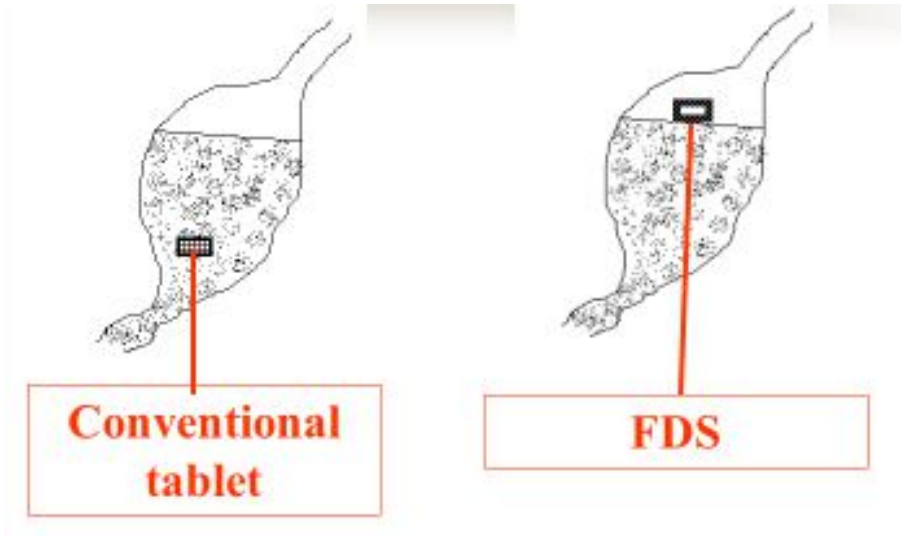

Fig 7: Illustration of conventional and FDS in the stomach

Drug release pattern (diffusion) is similar to that described for the capsule. The dosage is used for drugs that are optimally absorbed in the stomach.

A product with the brand name Madopar® (levodopa and benserazide) was released into the market in the late 1980s by Roche in a capsule form for the treatment of Parkinson's disease. Early attempts to produce a controlled release formulation of levodopa failed largely because the formulation while providing the desired slow release, presented the drug too far down the gut for maximum absorption, given that levodopa was best absorbed from the duodenum and jejunum. The Madopar capsule swells to twice its size in the stomach where it is retained and slowly releases drug for long periods (up to 8 hours).

TRANSMUCOSAL ROUTE

4. Mucoadhesive dosage system (MADS)

This unique dosage system utilises both the adhesive and gel-forming properties of some water-soluble polymers in which the drug is dispersed. This system is used in the treatment of both topical and systemic ailments by application to the appropriate mucosa. The polymers used, e.g. hydroxypropyl cellulose, are bioadhesive, i.e., they adhere well to mucosal surfaces.

It is used either as a compressed disc or as a powder (Fig 8). The directly compressed disc incorporating the drug, bleomycin, has been used to treat cervical and uterine cncer in Japan with good results. The drug in the polymer matrix slowly diffuses, after dissolution, from the dosage form into systemic circulation. Duration of release is up to two weeks. MADS can also adhere to oral mucosa and in this way, insulin has been administered successfully. Topically, a MADS containing triamcinolone acetonide is now being marketed in Japan as Aftach® for the treatment of aphthous stomatitis. Similiar preparations containing lidocaine (for toothache), prostaglandin F2 (to facilitate tooth movement in orthodontics) and nifedipine have been developed. MADS in a powder form has also been successfully used to deliver insulin via the nasal mucosa.

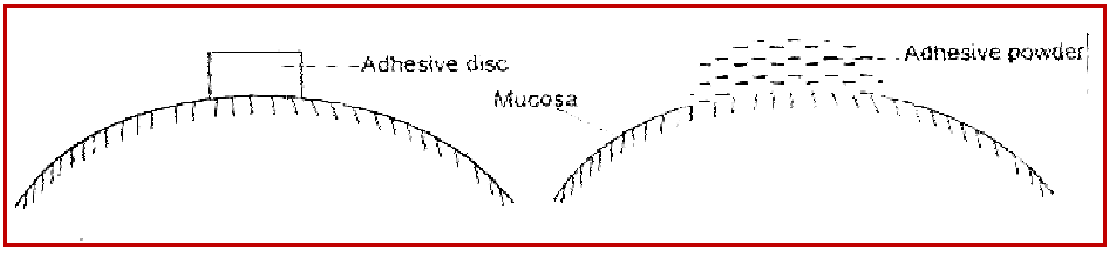

Fig 8: Mucoadhensive dosage systems

OCULAR ROUTE

Initially, explorative attempts at controlled ocular delivery into systemic circulation were made using soft contacts lenses as drug reservoirs. The lens was placed in the sterile drug solution from which drug is absorbed into its polymer matrix. When the lens was then placed in the eye, the drug diffused out over a duration of up to 24 hours. Studies with drugs such as prednisolone and pilocarpine indicated that contact lens application of drugs produce two or three-fold higher levels of drug than with eye drop application. However, the use of contact lens as a delivery system suffers from certain drawbacks, the most important of which is that the drug release mechanism is first order rather than the preferred zero order release which produces controlled drug delivery. Thus, it releases drug at a high rate initially which then declines rapidly with time.

Alza Corporation subsequently introduced into the market the Ocusert ®, a controlled release opthalmic device designed to overcome the problems posed by the use of contact lens in drug application. The delivery system consists of a drug (pilocarpine) reservoir in an alginate matrix surrounded by two membranes of ethylene-vinyl acetate copolymer which control the drug release rate of the device (see Fig 9). It is oval or elliptical in shape and the membrane is elevated and pigmented white with titanium dioxide to facilitate handling during insertion in the eye. It is inserted in the conjuctival sac (cul-desac) under the upper or lower lid. Two types of the Ocusert pilocarpine device are available: the Ocusert Pilo-20 and the Ocusert Pilo-40 which are designed to release pilocarpine at the rates of 20 and 40 ug h^{-1}, respectively. For each type, the duration of controlled release is one week. Pilocarpine effects miosis and lowers intraocular pressure in open-angle glaucoma.

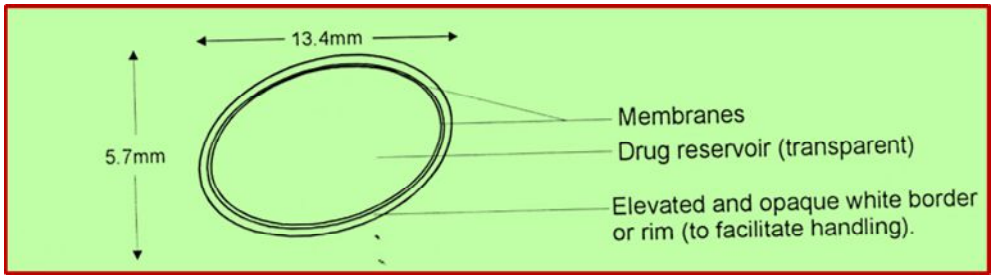

Fig 9: Top view of pilocarpine Ocusert device

The advantages of the Ocusert pilocarpine system over the drop therapy are numerous. They include:

- Reduced local side effects and toxicity due to the fact that the eye is exposed to approximately 1/4 to 1/8 of the pilocarpine when compared with drop therapy.

- Ocusert, once inserted, provides continuous therapeutic drug level but drops do not, thus resulting in a period of raised intraocular pressure in between applications.
- The Ocusert is a considerably more efficient delivery system than the drop which is poorly retained in the conjunctival sac. Thus large doses of the drop are given but very little is absorbed as the bulk is lost with tears.
- The Ocusert enhances patient convenience and compliance since it is administered weekly.

However, the Ocusert is expensive. The patient must also periodically check to see that the device is still in place. Furthermore, not all patients can tolerate the presence of the device in the eye. Nonetheless, the Ocusert system was later developed for other ophthalmic drugs including antibiotics such as chloramphenicol.

TRANSNASAL ROUTE

These should be distinguished from inhalation (aerosol) systems where the drugs is meant either to exert a local effect on the lungs or permeate the lung tissues to exert a systemic effect. Transnasal products are designed to deliver drugs into systemic circulation via the nasal mucosa taking advantage of the thin membrane covering of the mucosa and also the underlying dense network of blood vessels.

Research in this area has advanced and some progress has been made. Drugs that are considered appropriate for transnasal administration are those which are unsuitable for the oral route due to their instability in the GIT and poor absorption through the gut wall. Many products of biotechnology including a number of peptides and proteins used as biologic response modifiers are potential candidates for the transnasal route in spite of the large size of their molecules. Preliminary studies with insulin and calcitonin have produced promising results. The MADS-insulin product (in the powder form) mentioned in the section on "*Mucosal route*" provides an example of successfully tested insulin transnasal delivery system. The luteininizing hormone releasing factor analogue, Buserelin (Hoechst) has been introduced into the market in the 1980s as a nasal spray for the systemic treatment of endometriosis.

TRANSDERMAL ROUTE

Traditionally, medications applied to the skin are designed to exert topical effects only. Thus these medications are designed to penetrate the skin but not beyond. Cutaneous absorption is the penetration of a drug into various layers of the skin. On the other hand, percutaneous or transdermal absorption refers to the passage of therapeutic agents through the skin into the bloodstream. Indeed, some drugs have been found to readily penetrate the skin and enter into blood circulation. It is, therefore, reasonable to expect that transdermal application of drugs for systemic

therapy, can in some cases,, substitute oral therapy. The advantages of transdermal drug therapy may be summarised as follows:

- ♥ The transdermal route eliminates the various factors which influence oral therapy such as pH changes, food intake and intestinal transit time.
- ♥ Drugs given by this route usually avoid hepatic first-pass metabolic degradation.
- ♥ There is improved patient compliance.
- ♥ When problems arise during therapy, termination of drug input through the skin (by removal of the transdermal device) can be achieved instantly.
- ♥ This route generally provides a steady and controlled delivery of drug into systemic circulation.
- ♥ There are reduced side effects due to a decrese in dose frequency and magnitude.

A typical transdermal patch, illustrated in Fig 10, is a composite system made up of a drug reservoir sandwiched between a backing membrane and a microporous rate-controlling membrane, the drug delivery rate of the patch can be varied. However, the membrane is designed such that it provides the rate-limiting step in the transdermal absorption process. Examples of transdermal devices in overseas market include Nitrodisc® (Searle), Transderm Nitro® (Ciba Geigy). Nitro Drug® (Key Pharmaceuticals) and Deponit® (Schwarz). These are all glyceryl trinitrate systems for angina therapy, and each of them provides therapeutic levels of the drug in blood for 24 hours.

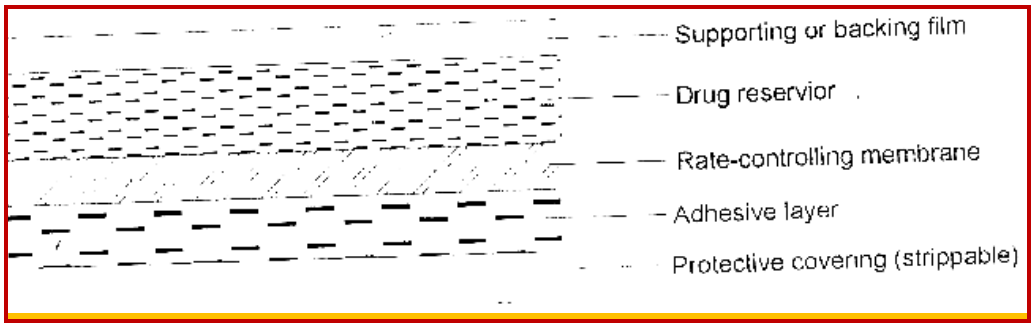

Fig 10: An illustration of the cross-section of a typical transdermal patch

Deponit® is a 0.3mm thick 7-layer device with increasing amount of drug from the outer-most layer to the innermost layer, i.e., the layer closest to the skin. The Deponit® approach has been tried for other drugs including oestradiol, beta-blockers, calcium antagonists, antirheumatics and chemotherapeutic agents for the treatment of cancer.

MISCELLANEOUS ROUTES/SYSTEMS

'Dry Emulsion'

Here, a typical *w/o* emulsion is prepared with the drug dissolved in the aqueous phase. A hydrophilic silica is then added to the emulsion which adsorbs the aqueous phase. A hydrophobic silica which adsorbs the oily phase is also added. The result (see Fig 11) is a large number of dry particles, approximately spherical (corresponding to the aqueous globules or disperse phase of the w/o emulsion), each consisting of a large hydrophilic silica grain incorporating the drug and surrounded by numerous smaller hydrophobic silica grains incorporating the oily phase. This system, which is administered orally, releases drug in a sustained release fashion.

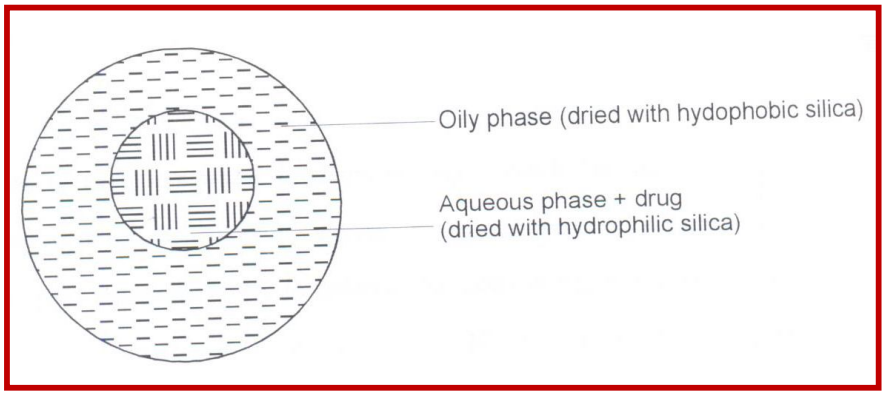

Fig 11: A illustration of a cross-section of a '*dry emulsion*' the internal and external phases

Multiple emulsions

These are usually water-in-oil-in-water (*w/o/w*) emulsions as illustrated in Fig 12, but oil-water-oil (*o/w/o*) emulsion can also be prepared. In order to prepare w/o/w emulsion, two emulgents are required; a w/o emulgent and o/w emulgent. The w/o emulgent, e.g., sorbitan mono-oleate, is first mixed thoroughly with the oily phase such as liquid paraffin in a mixer. This is followed by the slow addition of the aqueous phase (containing the drug) to the oil/emulgent mixture with vigorous mixing to form a w/o emulsion. With the aid of a homogenizer or colloid mill, the w/o emulsion is then dispersed in an aqueous solution of an o/w emulgent such as Tween (polysorbate) 80 to obtain the desired *w/o/w* emulsion.

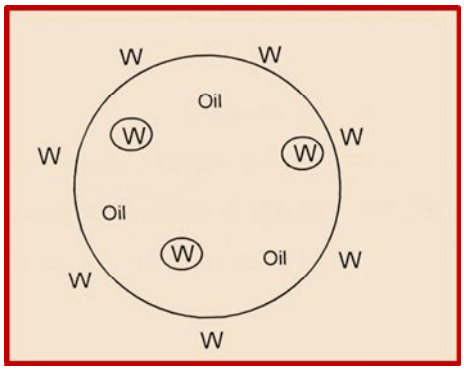

Fig 12: An illustration of multiple emulsion layout

Multiple emulsions are mainly useful in parenteral therapy to effect drug localization and prolongation of drug action. The drug in the innermost phase diffuses slowly through the other phases to provide sustained release.

Implant

These are sterile solid dosage forms prepared either by fusion or direct compression of the drug with or without excipients. Generally, implants are formulated to provide controlled drug release over an extended period. This objective is usually achieved with the aid of excipients, especially polymers, which serve as a matrix for the incorporated drug. They are often manufactured in the form of thin long cylinders of varying dimensions (Fig 13) for implantation intramuscularly or subcutaneously by surgical incision or with the aid of special injectors such as the Kearn and Perloff injectors. Ring-like implants for insertion in uterine cavity have also been introduced (see Fig 13).

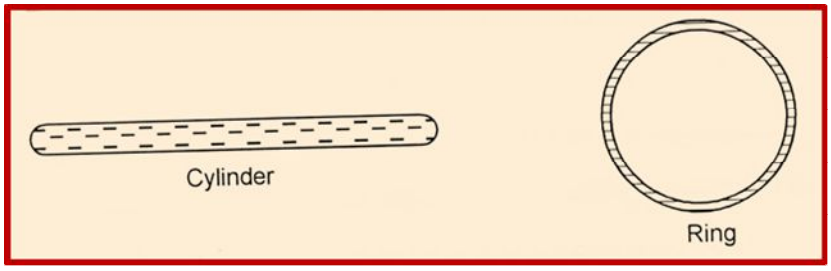

Fig 13: Main shapes of implants

The bulk of the implant is a polymer matrix (which may or may not be biodegradable) from which the dispersed drug (after dissolution that has penetrated the matrix) is slowly but steadily released into tissue fluids and then into blood circulation over a period of days, weeks, months or even years, depending on the formulation. Examples of implants in use include:

- *Zoladex*® (goserelin acetate): manufactured by Zeneca for the treatment of prostate cancer. Goserelin is an analogue of the hypothalamic hormone, luteinizing hormone releasing hormone (LHRH). The implant is approximately the size of a rice grain and its effect lasts for one month.
- *Norpant*® contains 36mg levonorgestrel and was first marketed in 1987 by Leiras. It was originally developed by the Population Council, an international non-profit organization, and consists of five match stick-size implants which are inserted subcutaneously in the upper arm and remain effective for five years. User failure is virtually nil and side effects are lower than with oral equivalents due to controlled release of the drug from the dimethylsiloxane polymer matrix. An advantage of this implant over the injectable prolonged release equivalents such as Depo-Provera® is that unlike the latter, it can be removed if problems arise.
- *Progestasert®:* contains progrestresterone and acts for one year following intrauterine insertion and has been Approved by WHO and FDA for contraception.
- *Compudose*® (Eli Lilly): is an implant with the active constituent, 17β-oestradiol, embedded in a silicone matrix and is used as a growth promotant for cattle

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TARGETED/SITE-SPECIFIC DELIVERY SYSTEMS

In 1906, Paul Erlich advanced the concept of the 'magic bullet' as an ultimate objective in drug delivery. Such a "bullet" drug, with the aid of carrier molecules, seeks out the precise part of the body its desired effect is to be exerted. There was also an advertisement, some time in the 1970's, of the Dutch beer, Heinekens, which claimed that the beer reaches certain parts of the body which are inaccessible to other brands of beer! Probably, only a few of those who saw the advert would have thought of the import of this fantastic claim.

The concept of "drug targeting", which continues to generate considerable interest in the pharmaceutical research world, arises from a situation where a drug administered i.v., for example, enters the blood stream and is distributed to varying extents throughout the body when the actual desire is to deliver or direct the drug selectively to its site of action. This site could be an organ structure, a cell subset or even an intracellular region. In such a case, pumping the drug throughout the whole body is not only wasteful but more fundamentally, it is also likely to lead to undesirable side-effects. On the other hand, restricting the distribution of the drug to the specific target site should allow for an increase in efficacy with an attendant decrease in toxicity. Thus in treating liver cancer, for example, the lipsomes containing the cytostatic drugs are directed, say, by

selected antibodies attached to the liposomes to the lysosomes of the cancerous cells where lysosomal lipases disrupt the liposomal wall and release the drug. It is possible for the targeting to be so precise that cancerous cells are destroyed without normal cells or other organs being exposed to the drug in high concentrations.

The benefits of drug targeting may be summarised as follows:

- There is reduced drug waste. This benefit has become particularly important with the advent of very costly biotechnology products - mainly endogenous and endogenous-like protein and peptides - which exhibit therapeutic properties in minute quantities (a few micrograms or less).
- The drug reaches the site where it is needed without other tissues being exposed to harm that could result in adverse reactions and side-effects. This is particularly important in cancer therapy because most antitumour agents have very low therapeutic indices and are therefore very toxic. (The therapeutic index of a drug is the ratio of its toxic dose to the therapeutic dose). Ideally, a drug should have a large therapeutic index, an indication that the gap between the toxic and therapeutic dose levels is big. However, many drugs fail to reach the market due to their low therapeutic indices as the risk/benefit ratio is often considered unacceptably high even for critical disease states such as terminal cancer. Often, toxicity is due to the 'swamping' of the body with the drug since conventionally administered drugs literarily roam the body system as they find their way to their receptor sites.
- Drug targeting may also make it possible to deliver a drug to a tissue or cell region not normally accessible to the free or untargeted drug. For example, most intracellular infections are protected from antibiotics. Furthermore, such infected cells may also constitute a "depot" from which bacteria are periodically released causing recurrence of systemic infections. Some success has been achieved using targeted biodegradable ampicillin and gentamycin nanoparticles to eradicate intracellular infections.

The following four main approaches were the early ones used in the development and formulation of targeted drug systems:

(a) ***Prodrug:*** A prodrug is the therapeutically inactive derivative of a drug which converts in vivo to the active form. The substances that have been chemically conjugated or complexed with drugs to form prodrugs are macromolecules such as dextran, polyamino acids, dihydropyridine (a nicotinic acid derivative) and agarose. The anti-tumour agent, mitomycin C, has been complexed with some of these macromolecules to form prodrugs which are either positively or negatively charged depending on the complexation conditons. The charged non-drug moieity of the prodrug is designed to selectively direct the drug to the target site. For example, the positively charged dextran-mitomycin C prodrug is directed to, and

becomes adsorbed onto, the negatively charged tumour cell surface by an electrostatic force. The free drug is detached from the prodrug by an appropriate enzyme either before, or after tumour cell penetration by the prodrug. Some other property of the moieity other than surface charge may also be used to achieve specific targeting. Metronidazole monosuccinate-dextran prodrug has also been investigated, while brain-specific delivery of prodrugs of oestrogens, testosterone, phenylethylamine and depamine, prepared by conjugation with nicotinic acid deriviatives, have been accomplished.

(b) ***Natural or passive targeting:*** In this approach, microcapsules or colloidal particles (nanoparticles) incorporating the drug are administered either intravenously, intrarterially or intraperitoneally. They are prepared by a microencapsulation technique. The microcapsule wall or colloidal material used is one that does not invoke the immune-response system. Unless they are speciffically targeted to other organs, these particles will end up in the lung and the reticuloendothelial system (RES), i.e., the liver, spleen and bone marrow. In the lungs, most particles greater than 10μ become trapped in the capillary bed by a process of mechanical filtration. All the other particles will be taken up by a process of phagocytosis (Fig 14) by the highly endocytic cells of the RES, especially the Kupffer cells of the liver. Thereafter, depending on the nature of the microcapsule material, the drug is released either by diffusion through the microcapsule shell, dissolution of the shell or degradation of the liposomal shell by lysosomal enzymes. Thus the lungs and RES are considered the natural "sink" or "graveyard" for non-actively targeted particles and therefore obvious focus for passive targeting.

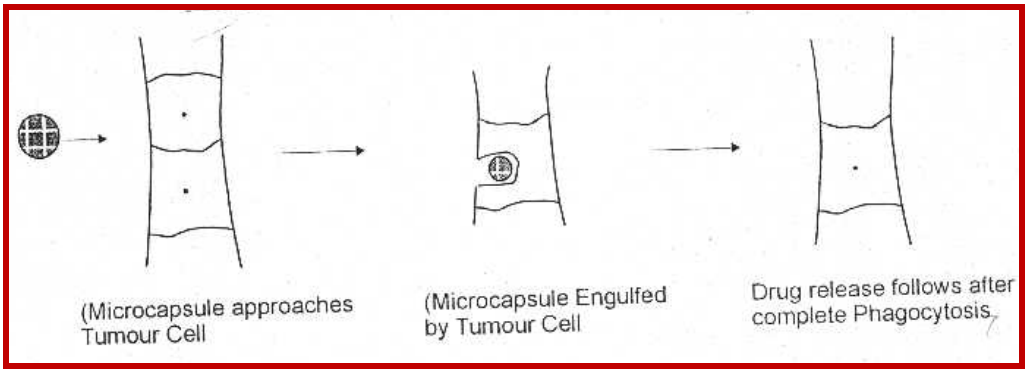

Fig 14: Illustration of passive targeting via phagocytosis

Examples of passively targeted systems which are still undergoing trials include the following liposomal preparations.

- Antimony compounds, amphotercin B, griseofulvin and pentamidine in the treatment of leishmaniasis, and
- Primaquine in the treatment of malaria.

These lipsosomal preparations were found to be therapeutically more effective than the respective free drugs due to the highly phagocytic power of the liver and the fact that the life cycles of both leishmania and plasmodium involve a hepatic stage.

(c) ***Active or ligand-mediated targeting:*** This approach to drug targeting is designed to ensure that as little as possible of the administered drug delivery system is entrapped or engulfed by the lung and the RES. This is usually achieved by tagging the microcapsules or nanoparticles with a tissue-specific ligand (an "address") such as antibodies, sugar residues, apoproteins and hormones. An analogy can be drawn with a postal packet, where the envelope is the microcapsule wall or shell, the content of the envelope is the drug, the address on the envelope is the ligand and the addressee is the target tissue or organ. A ligand's selection is based on its recognition by, and specificity for, the target site. Expressed in another way, a ligand is to a drug delivery system what a "homing device" is to an intercontinental ballistic missile used in wars. Thus, in selecting a ligand for a drug system, e.g., for the treatment of cancer, the antigen produced by the tumour should be known. To target such a drug to the tumour-bearing site, the drug microcapsules are 'addressed' with the antibody for the antigen. At the target site, drug release from the microcapsules or manoparticles proceeds either by dissolution or enzymatic degradation of the shell. In some cases, drug release at the target involves a phenomenon known as *chemo-embolism*. In chemo-embolism, microcapsules, co-administered intra-arterially with an anti-cancer drug, produce infarction and sustained drug action by blocking temporarily the target organ's arterioles, so permitting enhanced drug penetration of the tissues and prolonged drug retention. A product that acts this way has the brand name, Spherex®. It consists of microphores and was introduced 1990 into the European market.

(d) ***External or extracorpereal guidance systems*****:** This approach employs external means to direct drug to, or cause drug release at, the target site. Such external manipulation could involve the use of magnetic fields, temperature, light or electromagnetic radiations. For example, magnetic particles (magnetite) have been incorporated with medicament in microspheres or nano-particles, and following intravenous administration of this preparation, site-specific delivery is achieved by creating a 2-dimensional magnetic field at the target tissue or organ with the aid of an externally located magnet. This localise or concentrates the administered drug system at the target. Studies using human serum albumin with magnetic particles (ferromagnetic albumin microspheres, 1-7um in

diameter) have shown that high concentrations (3.7ug/g within one hour) of the anti-cancer, doxorubicin, was achieved at the tumour sites of rates bearing Yoshida sarcoma, with no drug in other organs. Furthermore, this resulted in the total remission of the turmour.

ELECTROMECHANICAL SYSTEMS

These consistitute one of the so-called futuristic or ultimate drug delivery systems, since the intention of the designers is to achieve perfection in the mode of drug delivery. Generally, following the administration of a dosage form the subsequent release and activity of the drug are practically out of the control of the patient, but depend on the formulation of the product. Due to anatomical and physiological factors, the response elicited will vary from one patient to another with the result that it could be lower or higher than optimum requirements. This is a highly undesirable therapeutic situation, particularly, if it involves a drug with a narrow therapeutic range such as digoxin. In such an "open loop" situation, the best that can be achieved is to "titrate", by trial and error, each patient to an individualized dosage regimen.

The use of electromechanical drug (EMD) systems, which release drug in an "open loop" fashion to the site of action to meet the instantaneous (minute by minute or even second by second) requirements of the patient, is intended to eliminate the irritating problem of variability in patient response to drugs. Drug release from EMD systems does not occur at standard rates predetermined during the design and formulation of product. The EMD system constantly monitors the patient's condition, and then releases the right amount of drug to meet the momentary needs of he patient. It takes the form of a computerized miniature device strapped to the side of the body with the drug sensor inserted into a vein. An EMD system for insulin administration with an in-built glucose sensor that constantly monitors blood glucose level has been developed for diabetics. However, there are still a number of technical problems that have to be overcome in order to extend the application of this technology to other drugs.

A variation of the electromechnical approach is the System for the Automatic, Feedback Controlled Administration of Drugs (SAFCAD). SAFCAD monitors the level of pharmacological response, rather than the level of drug in body tissues or fluids. It then releases drug, as may be necessary, to maintain the desired level of response. Thus parameters such as blood pressure and degree of anaesthesia can be monitored constantly by appropriate sensors in this device. Encouraging results were obtained when SAFCAD was used for the delivery of the hypotensive drugs, trimetaphan camsylate and sodium nitroprusside (in hypotensive surgery) as well as the short-acting anaesthetic, thiopentone. The thiopentone SAFCAD, which monitors the degree of anaesthesia with

the aid of data from an on-line computerized analysis of EEG signals, has made it possible to carry out prolonged surgery (6 hours) without risk of respiratory arrest (to the patient0, which would usually occur when thiopentone is used in an extended surgical operation

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MICROENCAPSULATED CELL SYSTEMS

The EMD insulin system discussed in the preceding section may be likened to a non-biological artificial pancreas. By applying a microencapsulation method, known as the living cell immobilization technique, it has now become possible to prepare bioartificial organs for use in organ replacement therapy. Laboratory studies so far have shown that this delivery system has very promising clinical potentials as a unique approach in the treatment of diseases such as diabetes and those of the liver. The microencapsulation method (coacervation) used has been described previously under the section on “microencapsulated systems”. In this instance, viable (living) cells from an organ rather than drug are encapsulated. The cells are entrapped within biocompatible, durable and semipermeable microcapsule shells of controlled and selective permeability. A bioartificial pancreas (i.e., microencapsulated islets of Langerhans for anti-diabetic therapy has been prepared in this way (see Fig 15). The microcapsule shell consists of the polymer, sodium alginate, covered with a thin film of a polyamino acid (poly-L-lysine) and finally with another coating of sodium alginate. The selective permeability of the microcapsule shell is such that it allows small molecules (mol.wt. 6×10^4) such as nutrients (including glucose), oxygen, and insulin to diffuse through, but prevents the entry of larger molecules such as immunoglobins or antibodies (mol wt: 1.5×10^5). In this way, the wall is able to protect the encapsulated cells from destruction by the recipient’s immune system (antibodies).

In diabetic rats injected intraperitoneally, this bioartificial pancreas restored normoglycaemia in 2 days and maintained it for more than 2 years. Moreover, such diabetic complications as cataracts, weight loss and polyuria were absent. In addition to convenience, the bioartificial pancreas, unlike the EMD insulin system, simultaneously releases at a controlled rate, the other hormones produced by the pancreatic islets, namely, glucagon and somatostatin. These hormones, along with insulin, work together to finely tune or control the blood glucose level.

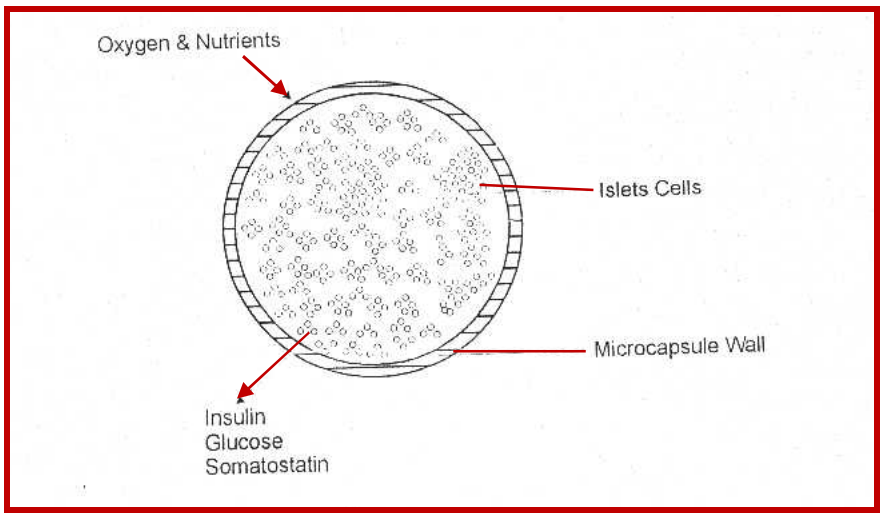

Fig 15: Microencapsulated Islets of Langerhans (Bioartificial pancreas)

Perhaps, it is pertinent to mention here also that microencapsulated cell technology or living cell immubilization technique has been applied in biotechnology to produce some natural products, mainly endogenous proteins and peptides such as erythropoietin, clotting factors, etc. For example, the first commercially produced monoclonal antibodies, from encapsulated hybridoma cells, are being marketed under the trade name, Encapsel®. The microcapsule shell (alginate-polylysine-alginate), which is the same as that described for the bioartificial pancreas, has also been used for the preparation of human interferon.

PERORAL DELIVERY OF THERAPEUTIC PROTEINS AND PEPTIDES

Nearly all proteins and peptides used in therapeutics, including vaccines, hormones, blood factors, etc, are administered parenterally rather than via the oral route. Two main factors are responsible for this. First, proteins and peptides are highly susceptible to degradation by the acids and proteolytic enzymes of the stomach. Second, even if they are shielded from the hostile environment of the stomach and find their way into the intestine, their absorption is very poor because their molecules are simply too large to diffuse across the intestinal wall into systemic circulation. However, parenteral delivery is associated with several disadvantages. These include cumbersomeness, invasive administration, anaphylaxis, poor patient acceptance and high cost. Insulin-dependent diabetics often discover that they have to live with some of these drawbacks the rest of their lives. Consequently researchers have begun to re-examine the possibility of oral delivery of bioactive proteins and peptides as a better alternative to parenteral delivery.

As far back as the early 1920's, there was evidence suggesting that large molecules, and even powder particles would be absorbed in certain regions of toad and rabbit intestines. Researchers, therefore, believe that peroral delivery could be feasible if the proteins can be protected from gastric degradation and then made available at specific absorption sites of the intestine. A significant stimulus to oral delivery research is provided by the on-going development of new types of vaccines. Vaccine candidate currently being investigated include proteins, polysaccharides and/or peptide fragments corresponding to specific antigenic determinants on the infecting agents, as well as synthetic peptides corresponding to certain areas of protein antigen. They will probably make their entry into the market shortly, and because they have smaller and less complex molecular constitutions than conventional vaccines, they are potentially more suitable candidates for peroral delivery.

Potential beneficiaries of an effective peroral delivery system for bioactive/therapeutic proteins include human and veterinary medicine as well as aquaculture (fish farming). For example, a large proportion of the infant mortality in developing nations is attributable to diseases that can be prevented by immunisation. However, immunisation coverage is often inadequate due to high cost and low patient acceptance of injectable vaccines by rural dwellers. An acceptable oral alternative would overcome these problems.

Interestingly, the peroral approach is also being explored to control morbidity in aquaculture (fish farming). Fish are susceptible to several diseases and the incidence can greatly increase in localised environments such as fish ponds. Whilst treatment with chemicals nd antibiotics provide a short-term remedy when there is a disease outbreak, immunisation is a more cost-effective, safer and longer lasting solution to fish morbidity. Furthermore, there are some fundamental similarities between the pH and enzyme profiles of human and fish digestive systems. Several methods of immunising fish have attracted attention. These include parenteral (intraperitoneal) administration to individual fish, hyperosmotic immersion, and direct ingestion of the vaccines. Varying but profound problems have been associated with these methods, amongst which are cumbersomeness, considerable stress to the fish, huge vaccine wastage and low immune response.

Microencapsulation technology provides an important avenue for researchers involved in peroral delivery of macromolecules in both human medicine and aquaculture. As illustrated in Fig 16, the approach entails the immobilisation of the therapeutic protein/peptide in the core (e.g., alginate) of the microcapsule with the microcapsule wall or membrane designed and formulated in such a way as to resist assault by gastric acids and enzymes as it transits through the stomach. Some degree of

success in affording gastric protection has been achieved by using a cationic polymer, chitosan, (whose membrane pores close up at low pH) as the capsule wall. Additional shielding of the microcapsule against hostile gastric environment can be provided by further treating both the capsule core and wall with a pH-sensitive (enteric) polymer such as hydroxyprophyl methylcellulose acetate succinate. The membrane is programmed to open and thus release the protein content of the core at a specific region of the small intestine by a pH-dependent mechanism. In this way, site - specific or targeted release is achieved for optimum intestinal absorption. To further increase absorption, special absorption enhancement agents have been tested with varying degrees of success. Examples of the successful use of microcecapsulation/immobilisation technology in peroral delivery of large molecules are still few. Meanwhile the race to develop oral

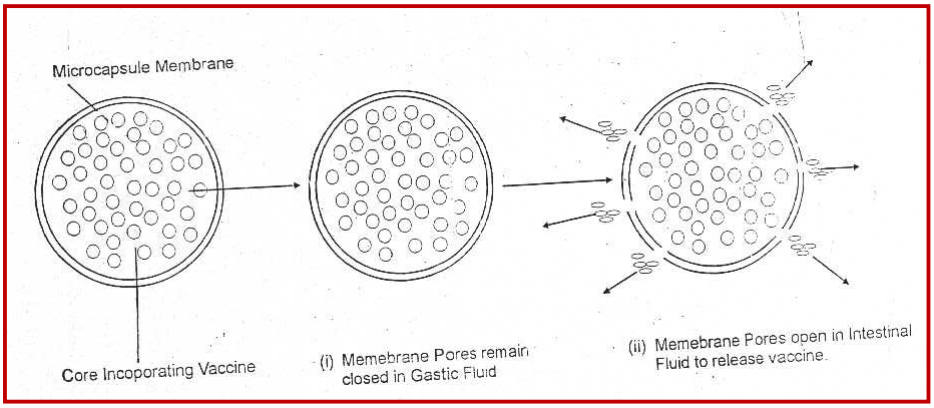

Fig 16: An illustration of possible approach to oral vaccine delivery

delivery systems for bioactive/therapeutic proteins and peptides has intensified especially among researchers in drug delivery specialty firms as estimates of the world-wide market for the this type of products are as high as US $30 billion annually.

Future of Drug Delivery – one prediction

It will be difficult to answer this question accurately with regard to drug delivery systems. However, it may just be adequate to reproduce here excerpts of the response of a drug delivery expert, Gordon Amidon, Professor of Pharmaceutics at the University of Michigan, Ann Arbor, U.S.A. when he was asked a similar question in 1995 *(Pharmaceutical News, Vol. 2, no 6, pp. 32-33, 1995)*: "*Today, I think one of the areas of great activity is in the area of gene therapy, since gene therapy is like a large biotechnology macromolecule; delivery is the problem, andusing such delivery systems such as liposomes or the natural delivery systems of viruses have their advantages and disadvantages. The goal will be to*

develop a delivery system as efficient as the virus, but would be non-infectious. As we understand targeting, cell membrane binding, uptake and cellular trafficking of peptides and proteins, we will undoubtedly be able to more precisely target drugs, including genes, for therapeutic purposes. I think that the enormous advances in molecular biological research are beginning to penetrate into the areas of drug delivery systems. Let me give you some examples: the cloning of transporters that are responsible for drug distribution; drug elimination or metabolizing enzymes that are responsible for some of the oral absorption problems of drugs; and in the identification of cell surface receptors and markers that may be used for targeting of drug selectivity. I think that drug delivery system research should be coupled with the drug discovery system research and that we will increasingly see a blurring of fields with the requirement that multi-disciplinary research teams focus on specific therapeutic problems. I think we are seeing this integration within the pharmaceutical industry today and to a lesser, but significant, extent in academics, where multidisciplinary teams focus on broader research problems. On the other hand, as an academic, this trends is a little bit unsettling, because it means that you can no longer function at the cutting edge of research as an individual researcher. You still may be able to do research, but in today's environment, research and research resources are going to require a more multidisciplinary focused effort to achieve a specific goal. On the other hand, I think that the positive side is that the advances in biological research and drug therapy can accelerate dramatically over the next ten years. If I put on my visionary hat and thought 25, 50 or more years down the road, I would have to say I see a decreasing importance for oral delivery, the preferred route and most important route for drug delivery today. What I would envision in my imagination would be an implantable drug delivery system device that would contain drug reservoirs that could be filled externally by syringe once a month, or once every six months, with sensors to measure some drug level or biological electrical response or ion concentration combined with microcomputers. These computer chips would not only store data and compute dosage schedules, but be able to transmit signals to external receivers. Once a week or once a month, you transfer your data to a computer that may be done via modem or by satellite to a computer that then evaluates your therapy and you get a printout back saying what you should do - go and get your reservoirs filled, or go and get your dose rates reprogrammed. Well, if we include engineering in its broadest sense, which could include the use of biological materials such as the implanting of cells that have been engineered to produce drugs at a certain rate or cells that might be responsive to certain specific stimuli if we take engineering broadly, then yes, I think that this is possible. The time frame is long though - over a 25 - or 50 - year period or longer".

PHARMACEUTICAL BIOTECHNOLOGY

Review of relevant genetics

To enable us properly treat genetic engineering and pharmaceutical biotechnology, it is necessary to refresh your knowledge of basic aspects of genetics as it relates to the above subjects.

Chromosomes

The body's cell is the location of the hereditary material of the body known as *chromosomes*. Specifically, chromosomes are situated in the nucleus of the cell as shown below in Fig 10.

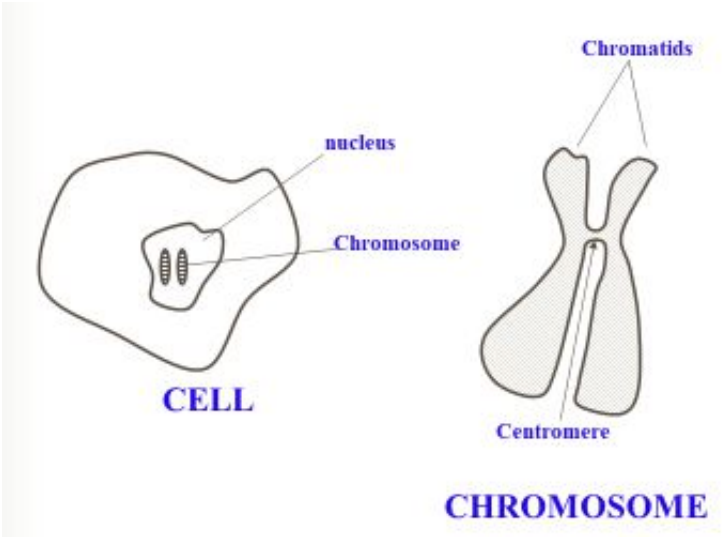

Fig 1: Body cell and chromosome

A chromosome in a cell is visible under the light microscope using appropriate staining techniques, as long thin threads of approx. 0.25μm to 50μm in length. In actual fact, a chromosome consists of two strands, known as *chromatids* linked at one point by a bridge referred to as *centromere*. The human cell contains 23 pairs of chromosomes. One of the pairs is referred to as *sex chromosomes* while the other 22 pairs are called *autosomes*. The sex chromosomes determine the sex of the individual in procreation. The pair of chromosomes is represented as XY for males and XX for females with one of the chromosomes from the father and the other from the mother. For males, the Y chromosome is always from the father. Each chromosome is made of a *bundle of protein units* around which is coiled a long molecule of DNA (deoxyribonucleic acid).

DNA: DNA is a component of chromosomes. It consists of two strands that coil to form a double helix structure (Fig 2).

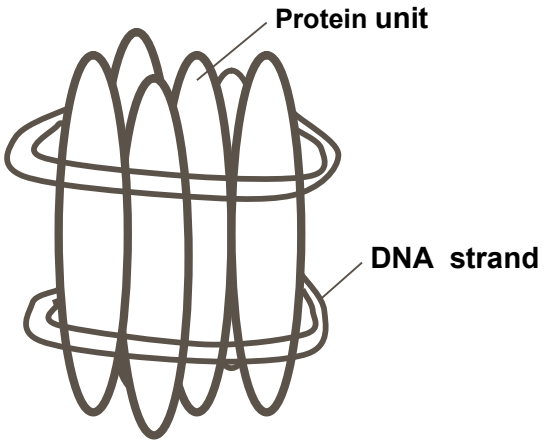

Fig 2: *The fine structure of chromosome*

The strands are held together by means of hydrogen bonds formed between complementary pairs of bases called nucleotides (Fig 3). There are four bases: *adenosine (A),* thymine (T), guanine (G) and cytosine (C). In the DNA structure, pairing is limited only toA-T (or (T-A) and G-C (or C-G). The DNA does reproduce itself by breaking of the hydrogen bonds into two half-strands which then couple free nucleotides or bases to form two new identical DNA strands. This process is called *replication.*

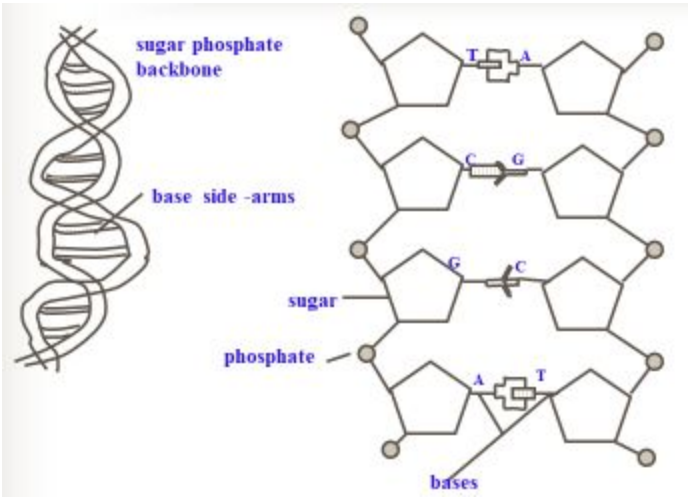

Fig 3: Structure of DNA

The DNA contains an organism's hereditary traits and propels the metabolic activities in body cells. Thus replication of DNA plays a crucial role in the transmission:

√ hereditary material from parents to offspring
√ instruction for protein synthesis

- ***Genes and the genetic code***. Simply stated, genes represent segments of DNA. Thus the human DNA contains thousands of genes (in the range 20,000 - 25,000). For example, the gene for insulin consists of a sequence of 153 bases or nucleotides on the DNA molecule, and it directs or instructs specific (cells/Islet of Langerhans) in the pancreas to manufacture insulin which regulates glucose metabolism. Within genes are contained the information that determines the characteristics or traits of living organisms, e.g. height, hereditary disease, skin colour, facial appearance, etc. This information is in the form of a code, often referred to as DNA or genetic code. Deciphering this code for any particular trait continues to present a tremendous challenge for genetic scientists. The sequence of bases, for example, the base sequence A-C-C-T-G on a gene (or DNA) represents information which is different from that coded by the sequence A-T-C-C-G.

PHARMACEUTICAL BIOTECHNOLOGY

Biotechnology is a relatively young branch of science that derives from, and is essentially based on, the manipulation of the genetic material of living organisms to generate products that have found applications, especially in medicine and agriculture. Biotechnology, in several instances, have revolutionized practices in these two fields. The term, ***'pharmaceutical biotechnology'***, is used for biotechnology when it has to do with products and processes that have therapeutic and/or diagnostic applications.

Some major biotechnology applications and processes

The following biotechnology applications and processes provide illustrations of the significance of this field in pharmacy and medicine:

✓ ***Recombinant DNA Technology***

This technology was invented in 1973 by Drs Stanley Cohen (Stanford University) and Herbert Boyer (University of California, San Francisco). It involves cutting DNA and pasting it elsewhere, i.e., in another cell in order to generate huge quantities of genes. This technology is being used to grow large amounts of genes in bacterial cells. The choice of the bacterial cell is dictated by its ability to multiply rapidly.

✓ ***Cloning***

The technique of large-scale production of genes by means of DNA or gene transplant is known as *cloning*. The new genes produced are clones

or exact copies of the original gene from which they were grown. In 1996, the world was greeted with the pleasant/disturbing news of the cloning of a sheep in England. The cloned sheep was named '*Dolly*' and was an exact genetic copy of the original sheep. Cloning over the years has raised several moral, religious, safety, legal and socio-medical issues. Cohen, Boyer and many scientists were so greatly alarmed with the consequences of reckless mixing of genes from different organisms that an unprecedented moratorium was instituted in the mid-1970s with the result that recombinant DNA experiments were temporarily suspended until guidelines for safe experimentation were subsequently put in place. With the creation of '*Dolly*', fears increased in many circles worldwide that scientists might even attempt to clone man. However, there is an absolute prohibition of experiments on human cloning in advanced countries of the world.

✓ ***Production of biopharmaceuticals***

In 1980, geneticists transplanted a human gene into the DNA of a bacterial cell. The gene introduced into the bacterial cell is the one that codes for the protein, interferon. Fig 4 illustrates the bio- engineering process known as genetic engineering. Interferon is a natural body chemical that enhances human resistance to viral infections.

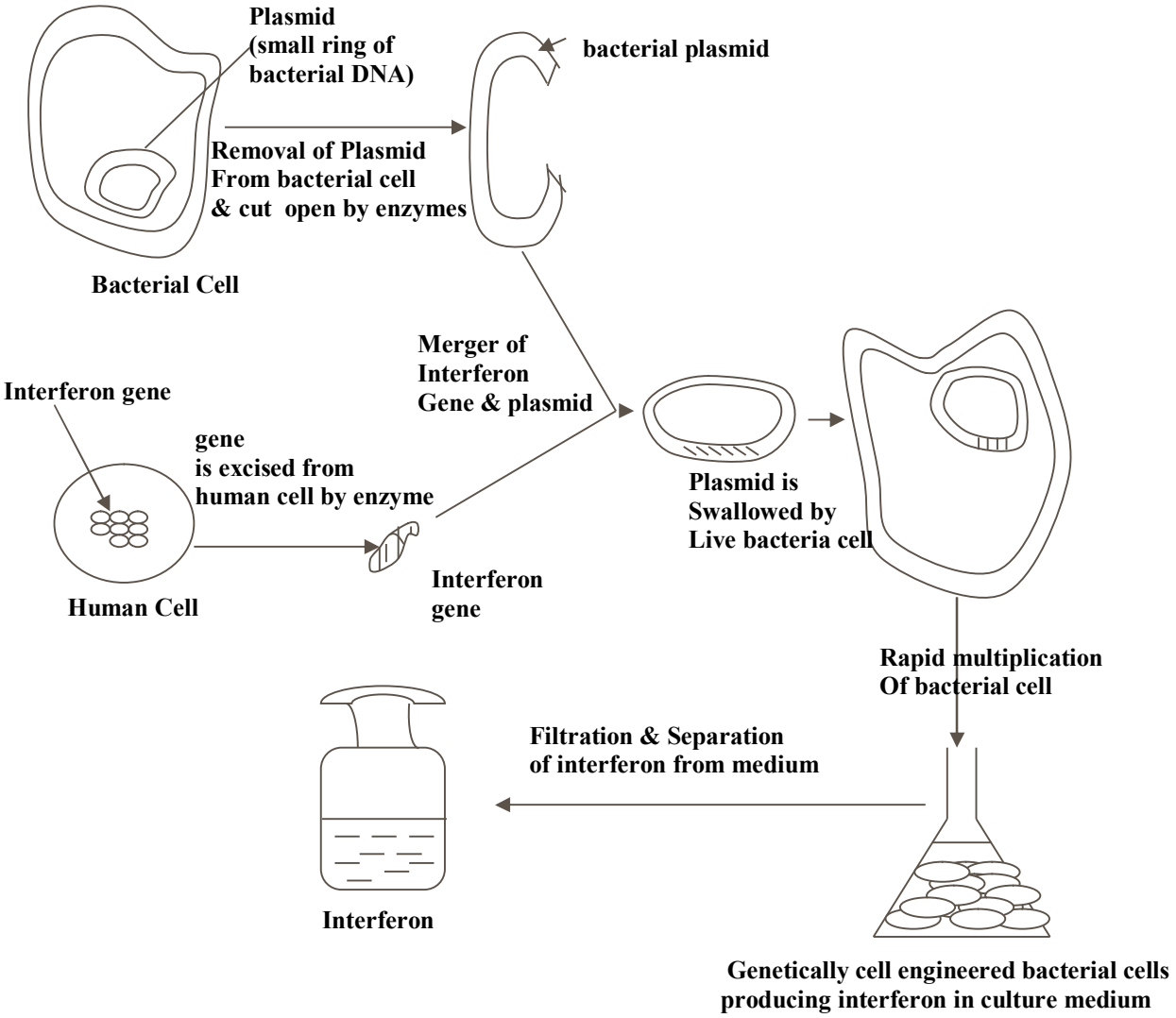

Fig 4: Production of interferon using recombinant DNA technology

As shown in the figure, the bacterium takes up a 'foreign' human interferon gene which influences the bacterium to begin to produce interferon. The choice of bacteria is based on the fact that bacterial cells multiply rapidly. Previously, interferon was extracted from human tissue but the amount obtained was always very small and, therefore, prohibitively expensive. The procedure was also time-consuming. In contrast, large amounts of interferon are produced cheaply and quickly by genetic engineering. There are now several products of genetic engineering in the market and they include including vaccines, hormones and enzymes. Interestingly, genes are also being transplanted into plant cells to enhance disease/pest resistance, nutrition/protein composition, etc.

In the late 1980s, further advancement of genetic engineering led to what has been described as *Hybridoma Technology*. In this technique, two genetically different cells are made to fuse or merge to form hybrid cells containing genes from the original cells (see Fig 13)

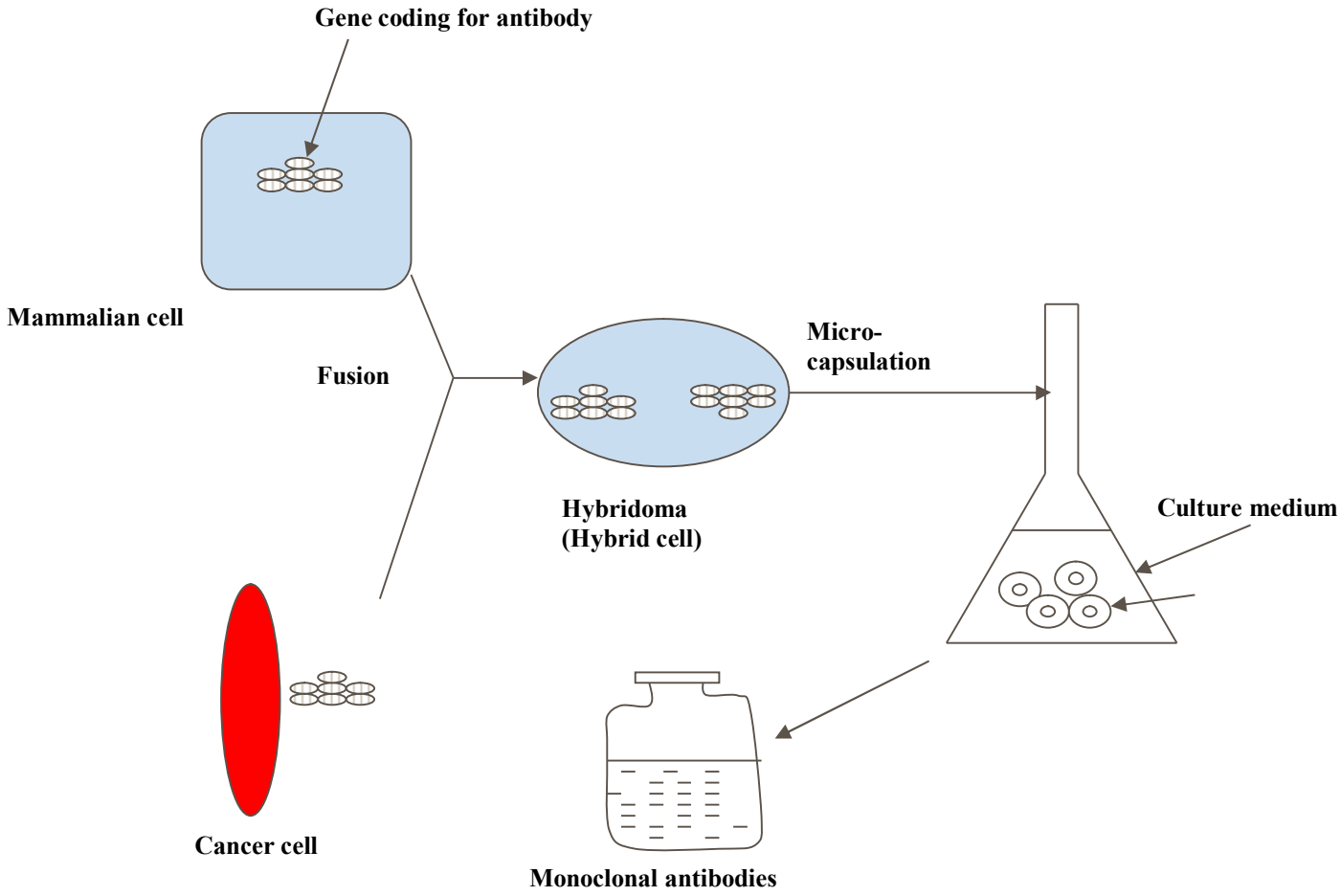

Fig 5: Schematic illustration of production of biopharmaceuticals by Hybridoma Technology

This technology has been used to produce specific human monoclonal antibodies. The first of such product was first marketed as *Encapsel*® Hybridoma technology employs a mammalian cell that has the gene that codes for the desired biochemical substance such as a specific antibody. Fusion of this cell with a cancer cell (which has a gene for extremely rapid cell multiplication/proliferation) results in a rapidly multiplying/dividing hybrid cells from which large amounts of the antibody can be generated cheaply and rapidly. The significance of the foregoing technological developments is that several diseases, including those of metabolism, are best treated with genetically engineered products usually referred to as *biopharmaceuticals*. *For millions all over the world*, they are life-saving medicines. In addition to human insulin (as

against porcine insulin which was usually extracted from the pancreas of pigs). Some biopharmaceutical drugs and vaccines approved by United States FDA are listed in Table….

DNA Fingerprinting

Conventional fingerprinting emerged in the 1930s as a police tool for detecting crimes. It is taken *only* from the tip of the finger and provides a unique identity of the individual. However, it can be altered by severe injury or surgery. An individual also has a unique DNA pattern known as DNA fingerprint. Unlike the conventional fingerprint, the DNA fingerprint cannot be altered by any known means. This is because a DNA fingerprint is the same for every cell, tissue, and organ of a person. Even the DNA fingerprint of a person can be obtained from hair or finger nail. Thus, DNA fingerprinting is fast becoming the most acceptable and primary technique for identifying and distinguishing among individual persons.

DNA fingerprinting has several **applications** which include:

- *Identifying and distinguishing among individual human beings* (as indicated above) to resolve criminal cases, establish parentage and identify deceased persons whose corpses may have been mutilated or defaced beyond normal recognition. A recent case in point was the establishment of the true mother of a little Lagos girl, Mary, after a six-year dispute among three women who all claimed to be her mother.
- *Diagnosis of inherited disorders* in adults, children and unborn babies. These genetic disorders include haemophilia, cystic fibrosis, sickle cell anaemia, familial Alzheimer's, etc. In the case of pre-natal and new-born babies, early detection enables the medical staff to prepare themselves and counsel the parents on the right treatment of the child. The DNA fingerprint clearly shows gene defects.

- *Developing cures for inherited disorders* depend on the correct location of inherited disorders on chromosomes and this, in turn, is predicated on information contained in DNA fingerprints. This is usually achieved by examining the DNA fingerprints of relatives who have a history of a particular disorder, or by comparing large groups of people with and without the disorder. This leads to the identification of certain DNA patterns associated with the disease. This is a primary step in developing an eventual genetic cure for these disorders.

A clear illustration of the great impact DNA fingerprinting has made on criminal investigations is the great and dramatic success in resolving rape cases in the United States. More and more, there is no need for a victim to testify about whether a sexual act took place. A case of mistaken identity? No way! This is because DNA from a semen sample can be used to link a suspect to that semen

sample. Importantly, the innocent suspect can be clearly exonerated. In fact, it has been reported that many rapists now plead guilty in the face of accurate, incontrovertible DNA evidence.

Some DNA enthusiasts have even gone on to propose national computer databases of every person's DNA type. That way, for example, when a rape is committed, there is no need to find a suspect. Simply obtain the semen sample and compare to the database of everyone's DNA pattern and then determine whose it is. It is that quick and easy. However, there are many who feel some unease about such databases because of the potential abuses it can be put to.

Stem Cell Technology

This is a very recent technology that is generating considerable. Stem cells are ***unspecialized cells*** of the body that serve as the ***building blocks*** for ***all of the specialized cell types*** in the body. In the other words, they create all the other cells in the body. Because they have the potential to become any type of cell or tissue, and therefore, stem cell technology provides a ready approach to achieving cloning. By transplanting stem cells that have been modified genetically, i.e., altering its gene constitution by inserting a specific gene and implanting in a target organ/tissue in the body, the transplanted cells can generate the desired cells/tissues/organs which they are coded for. There are two main sources of stem cells – ***somatic*** and ***embryonic.*** While research using the former has generally been permitted, embryonic stem cell research is not allowed in some countries especially the U.S. for ethical reasons. Progress/successes in the application of this technology include:

- In 2005, Japanese researchers have succeeded in cloning a human kidney by cultivating human stem cells extracted from adult bone marrow into rat embryos.
- The use of stem cells and gene therapy in 2005 to regenerate teeth has shown promising results, thus making it possible in the near future to add new tissue to decayed or damaged teeth, and even grow new teeth from scratch.
- Australian scientists have used human stem cells to produce red blood cell in 2005. These synthetically produced red blood cells, in theory, would overcome the concerns about dangerous infections (e.g., HIV) that can be transmitted from blood donors to patients.

Human Genome Project

Researchers are trying to discover the sources of close to 4,000 disorders caused by defects in single genes. This is sequel to the recent success in locating and 'mapping' all of the 20,000 – 25,000 genes (about 3 billion letters or nucleotides) on our chromosomes with the objective of producing a precise genetic 'map' of man, usually referred to as the *human genome*. This is the task of the *Human Genome Project*, an international collaborative effort of scientists from U.S., Europe and Asia which spanned a period of 13 years (1990 – 2003),

gulped over US $200 million annually but ultimately succeeded in mapping all human genes. Similar exercises pertaining to specific human races across Asia, Africa and Latin America are currently being pursued. It is hoped that within the next 10 - 15 years, it will be possible to identify and treat most diseases afflicting man. This will definitely revolutionise medicine, and improve man's quality of life.

Gene Therapy

Gene therapy is a technique whereby the absent or faulty gene is replaced by a working gene so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease. It has been estimated that each of us carries on the average, six (6) defective/altered/flawed genes but we remain blissfully unaware of this fact unless we, or one of our close relatives are amongst the many millions who suffer from a genetic disease. It has been estimated that 10% of all people has, or will develop at some later stage, an inherited genetic disorder, and approx 2,800 specific conditions are known to be caused by defects (mutations) in just one of the patient's genes.

As stated earlier, humans, like most other animals and plants have two copies of every chromosome, and hence also two copies of every gene. In each pair, one is inherited from the father and the other from the mother, but they do not necessarily possess identical DNA sequences. If a person carries two similar DNA sequences at a specific site on a chromosome, the person is said, in genetic parlance, to be *homozygous* while carriage of two different DNA sequences attracts the label, *heterozygous.*

The importance of this phenomena is that it can mean the difference between health and ill-health. Perhaps the best known example is the inherited disease, *sickle-cell_anaemia,* which is due to a defective protein, haemoglobin. This protein constitutes a significant portion of red blood cells and transports oxygen. The defective protein is traceable to a defect in one gene. For sickle cell anaemia to manifest, the defective gene must be present in the two copies. Where only one copy of the defective gene is present, the person is usually healthy and is referred to as a 'carrier'. About 9% of blacks in the United States are *heterozygous (AS)* for sickle cell anaemia while less than 1% are homozygous (SS) and show symptoms of the disease. Data from some parts of Africa show a heterozygous population of close to 45%.

Some interesting facts have emerged from Africa regarding the heterozygous sickle-cell status. They are:

- The status confers some immunity from malaria. Heterozygous people have been found to show higher resistance to malaria attack.
- Women carriers (AS) appear to be more fertile than women who have two normal genes (AA).

DNA is indeed an intriguing miracle material as it contains all of man's genetic instructions that determine our individual traits. Increasingly, scientists are

discovering that the root causes of several previously mysterious diseases are *abnormal or defective genes.* The result is that researchers can now make more precise diagnoses and predictions; design more effective drugs; prevent many painful disorders. Even more exciting is that the door is being thrown open for the development of the *ultimate therapy*, i.e., replacing a defective gene with a normal gene to permanently correct a patient's genetic disorder.

It has been estimated that each of us carries on the average, six (6) defective/altered/flawed genes but we remain blissfully unaware of this fact unless we, or one of our close relatives are amongst the many millions who suffer from a genetic disease. It has been estimated that 10% of all people has, or will develop at some later stage, an inherited genetic disorder, and approx 2,800 specific conditions are known to be caused by defects (mutations) in just one of the patient's genes. Let's just cite a historical example: the *powerful Queen Victoria* of England a couple of centuries ago was a carrier of the defective gene responsible for *haemophilia*, and through her it was transmitted to the royal families of Russia, Spain and Prussia. Minor cuts and bruises which is hardly causes any problem to most people, can prove fatal to *haemophiliacs,* who lack the proteins (Factors VIII and IX) that play a role in clotting of blood, and are coded for by the defective genes. Happily, genetically engineered Factors VIII and IX are now available. Prior to this, the proteins were isolated from blood and where the blood was HIV – infected, haemophiliacs often became infected with the AIDS virus.

Amazing advances have been made in this direction in the case of several genetic disorders including two fatal diseases affecting children, namely, *cystic fibrosis* and *Duchenne muscular dystrophy*. Even genetic flaws that predispose people to more widespread though still poorly understood diseases such as various forms of heart disease, breast and colon cancer, diabetes, arthritis, etc, have been identified. Some of these diseases were not previously thought to be genetic in origin. Nearly 1,000 gene therapy clinical trials are currently going on worldwide. Here are some highlights of some significant achievements in gene therapy:

- Several children suffering from severe combined immunodeficiency disease (SCID) commonly known as the ***'bubble babies' syndrome***, a rare life-threatening affliction due to total immune deficiency. The result is that the sufferer is completely unable fight any infection and has to be encased in a plastic bubble to shield him from pathogenic microbes. In 2005, a harmless virus was used in a London hospital to deliver stem cells containing a corrective gene (from a donor) to the 4-year old boy. The child is now out of the 'bubble', has acquired normal immunity, and is living a normal life.
- In 2005, a technique known as electroporation whereby electricity is used to open up skin pores and deliver an immune-boosting gene to fight melanoma (a deadly skin cancer) was reported.

- A clinical trial in the U.S. in 2005, based on a single injection of healthy genes into six men to tackle impotence by restoring normal male function proved very promising.
- In 2005, an experimental gene therapy in mouse was found to grow hair, thus holding up hope for the cure of baldness.
- A newly developed viral vector has been successfully used to introduce a blood pressure lowering gene, atrial natriuretic peptide (ANP), into cells. The gene brought about the maintenance of blood pressure for 4 months
- A 3-year old girl suffering from Batten disease, one of a group of degenerative brain conditions which leaves its young victims blind, speechless, and paralysed before killing them was operated on to insert a gene. The girl was cured.
- Introgen invented a mouth wash that contains, among other ingredients, a benign form of the common cold virus which carries genes, known as tumour suppressors, that rids the body of cancerous cells. This form of therapy specially targets the pre-cancerous growths in the mouth.
- Sickle cell disease has been successfully treated in mice gene therapy

TECHNIQUES FOR APPLYING GENE THERAPY

There are two main techniques for applying gene therapy. They are:

√ ***Ex-vivo,*** whereby carriers or vectors such as inactivated virus is used to convey the gene to the desired site in the body. A recent news report in November 2001 stated that two researchers in Massachusells General Hospital and Havard Medical School attached a gene to a *harmless, inactivated herpes virus* which acted as a vehicle to transport the gene to the injured nerve cells of rat pups which were inflicted with injuries roughly comparable to human injuries following a car accident or deep tissue wound'. The gene 'switched on' or triggered the production of a natural compound called heat shock protein (Hsp) 27 which helps to 'rescue immature neurons from injury-related death. This therapy effectively prevented the death of injured nerve cells in the pups. It is believed that this particular discovery could pave the way for similar treatments in humans that could help patients with spiral cord injuries, conditions such as Huttington's disease, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) as well as 'prevent both sensory and motor neuron loss in patients with nerve damage associated with diabetes or AIDS.

√ ***In vivo,*** where surgical procedures are used to obtain and replace cells. This approach has been largely used in cancer therapy. There are a variety of strategies including immunopotentiation, oncogene inactivation, tumour suppressor gene replacement, molecular chemotherapy and drug resistance genes.

ABUSES, SAFETY, REGULATORY AND ETHICAL ISSUES

- Gene therapy does have risks and limitations and has been known to go amiss. For example, 3 out of the 11 children who underwent gene therapy to cure the 'bubble babies' syndrome developed leukaemia (one of them has now died) because the transplanted gene went to the wrong location on the DNA and triggered off the cancer. This was not the case for the other children. They were successfully cured.
- The use of inactivated virus as a vector for gene transplant, and also stem cells can lead to infection if not properly tested and handled
- Only somatic gene therapy is currently acceptable. Germline gene therapy is clearly off-limits. However, there are fears, though remote, that even somatic gene therapy could possibly affect sperm and egg cells and this could lead to inheritance consequences.
- Should cosmetic/enhancement gene therapy, in which a gene is implanted in order to try to enhance or improve a normal trait or specific characteristic, e.g. to increase height or become younger, or longer hair, be acceptable? It is now believed that GM (genetically modified) athletes or super-athletes are possibilities in the near future. This would arise from genetic 'doping', analogous to doping with performance-enhancing drugs such as steroids. Some are even forecasting that in the next 20 years, the Olympic Games could even be in three sections instead of the present two – conventional Olympics and Paralympics. The section could be Olympics for GM athletes. Scientists are currently working to identifying genes believed to power naturally high-performing athletes.
- Some people are even of the view that gene therapy should be restricted to life-threatening disorders where no current alternative effective treatments are available.
- Some people have questioned whether gene therapy should not been limited to ***disorders*** as against ***disabilities.*** Is disability a disease, and does it need to be cured or prevented? In any case, it may be difficult, in some cases, to decide whether a condition is a disorder or a disability.
- Gene therapy, currently, is exorbitantly expensive. Most people, on their own, cannot afford it.

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COURSE: PCT 523

TOPIC: Pharmaceutical Evaluation of Dosage Forms – Liquids and Semi-solids; Incompatibility in Liquid Dosage Forms

LECTURER: Professor AO Okhamafe

INTRODUCTION

Pharmaceutical evaluation is required because it is necessary to ascertain if a pharmaceutical product has attained minimum standards for its use by a patient. Often the minimum standards are those specified in the general and individual monographs of the various official pharmacopoeias such as BP, USP, EurP, etc. However, most manufacturers seek to set standards that exceed those contained in the pharmacopoeia.

Another reason why evaluation is required is to ensure consistency in the quality of a product emanating from a firm. For example, while a batch of manufactured pharmaceuticals may have met minimum pharmacopoeial standards, it is possible for the same product but of another batch from the same manufacturer to differ significantly, say, in colour, viscosity or refractive index, without compromising pharmacopoeial standards. Such a situation is unsatisfactory as doubts may be created in the minds of the consumers as to whether the product is genuine or not.

LIQUIDS

Most liquid dosage medicines come in one of three general categories: *proprietary, non-proprietary* and *extemporaneous*. *Proprietary products* are those manufactured medicines made and/or sold by an individual or firm having the exclusive rights of manufacture or sale.

Proprietary rights cover both name and other distinguishing marks on the packaging as well as the content of the product. *Non-proprietary medicines,* on the other hand, do not enjoy similar protection regarding exclusiveness and therefore, other manufacturers are not similarly restricted. Such products bear generic or official pharmacopoeial names. *Extemporaneous medicines* are usually medicines prepared off hand (so to say) on a small scale in a hospital or community pharmacy to be dispensed to the patient. They are freshly prepared, that is not more than 24 hours before they are issued to be used and usually used within a period not longer than 4 weeks.

The following standards often apply to liquid medicines depending on the type of product and application.

1. Labeling

The labeling requirements are not comprehensive and national laws governing the statements to be stated on labels should also be complied with. The information on the label often indicate the proprietary/brand name printed boldly in large characters/fonts with a circled R (®) or TM in superscript adjacent to the name. This suggests that the name is a registered trademark. Other information items presented include the generic name, composition of the preparation based on a unit dose, (e.g., 5ml spoonful), quantity, batch no, address of the manufacturer and warnings such as

'*Store in a cool, dark place', 'Shake well before use'* and *'Keep out of the reach of children'*, etc, as may be appropriate. The manufacture and expiry dates also appear on the label. So also the content of active ingredients which is expressed as milligram or microgram weight per unit dose (usually 5ml spoonful).

In U.K., the symbol, *POM*, appear on the label of those medicines that should only be given out by the pharmacist while the symbol, *CD*, indicates that the preparation is a controlled drug and, therefore, subject to regulations governing controlled drugs. In most advanced countries, it has become mandatory that the excipients or additives in the preparation, e.g. colourant, flavour, preservative, stabilizer, etc, should also be shown on the package. This is done to enable consumers avoid products that contain ingredients they are known to be allergic to.

In all cases, labels must be firmly affixed to the container. Adherence to labeling standards provides a means of determining the quality and/or genuineness of a product. Any deviation from the norm must necessarily raise suspicions as to the satisfactory nature of the product. Most fake and sub-standards products in the Nigeria market often show deviation and/or defects in labeling.

2. Packaging

Medicines are required to be packed into containers and stored in such a way as to prevent contamination and, as far as possible, deterioration. For some products, special conditions of storage are required, including the type of container and limits of temperature.

In general, medicines are packed in well-closed containers and stored at 25^0C. Where the monograph or a pharmacopoeia specifies that a preparation be *'protected from moisture'*, it means should be kept in an air-tight container. *'Protect from light'* means that the product be stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded. The term, *'tamper-evident container'* means a closed container fitted with a device that reveals irreversibly whether the container has been opened.

3. Description/Appearance

Liquid preparations are often categorized according to their description and/or appearance. Thus, those for *cutaneous application* are preparations of a variety of viscosities intended for local or transdermal delivery of active ingredients. They may be solutions, emulsions or suspensions and usually contain one or more active substances in a suitable vehicle. They may also contain suitable antimicrobial preservatives, antioxidants and other excipients such as stabilizers emulsifier and thickeners. *Emulsions* may show evidence of phase separation but are easily re-dispersed on shaking. *Suspensions* show sedimentation but are re-dispersable on shaking to again yield a suspension which is sufficiently stable to enable a homogeneous preparation to be measured out.

Liquid preparations for *oral use,* like those for cutaneous use, can be solutions, emulsions or suspensions. Further classification results in preparations known as

elixirs, mixtures, drops, etc. Any deviation from its standard appearance, consistency, re-dispensability (in the case of emulsions and suspensions) provide a basis for the evaluation of liquid preparations. Visual comparative tests (BP) are often carried out using identical tubes of colourless, transparent, natural glass with a flat base. The volumes of liquid prescribed are for use with tubes 16mm in internal diameter. Tubes with a larger internal diameter may be used but the volume of liquid examined must be increased so that the depth of liquid in the tubes is not less than that obtained when the prescribed volume of liquid and tubes 16mm in internal diameter are used. Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background or, if necessary against a black background. The examination is carried out in diffuse light.

4. **Content**

Content of active ingredient is often the most critical factor in the evaluation of pharmaceutical products. For preparations other than those of fixed strength, the quantity to be taken for an assay is usually expressed in terms of the active ingredients rather than in terms of the entire formulation which includes excipients. This means that the quantity of the active ingredients expected to be present and the quantity of the preparation to be taken are calculated from the strength stated on the label. In assays, the approx. quantity to be taken for examination is indicated but the quantity actually used must not deviate by more than 10% from that stated. The product monograph in the pharmacopoeia specifies the content range or limits which take account of normal analytical errors, of acceptable variations in manufacture, and of deterioration to an extent considered acceptable. In determining compliance with a numerical limit, the calculated result of an assay is first rounded to the number of a significant figures stated, unless otherwise prescribed by the pharmacopoeia.

When the result of an assay is required to be calculated with reference to the dried or anhydrous substance or on some other specified basis, the determination of loss on drying, water content or other content is carried out by the method prescribed in the relevant assay in the monograph. The words, “dried substance” or “anhydrous substance”, etc, appear in parenthesis after the result.

5. pH

The pH of some liquid preparations must fall within a specified pH range for a number of reasons, e.g., to maintain chemical and therapeutic activity of the active ingredient, preservative, irritability, product stability, etc. pH may be evaluated using indicators the colours of which change over approximately the same range of pH. pH strips may also be useful.

6. **Weight per ml**

This parameter is a measure of the specific gravity or density of the liquid preparation. The specific gravity bottle is a handy piece of apparatus for achieving this purpose. A product that has a significant proportion of its vehicle lost through evaporation could become more viscous or can lead to crystallization of its solute components and even precipitation of solids.

7. **Refractive Index**
   The refractive index of a substance with reference to air is the ratio of the *sine* of the angle of incidence to the *sine* of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in measurement. Refractive indices are stated in terms of the wavelength of the sodium D-line (589.3 nm) at a temperature of 19.5 to 20.5 unless otherwise specified. Commercial refractometers are available for measurements and they give readings that are accurate to at least the third decimal place

8. **Viscosity**
   A capillary viscometer is used for determining the viscosity of Newtonian liquids while a rotating viscometer is used for Newtonian and non-Newtonian liquids. The capillary viscometer usually consists of a glass U-tube made of a clear borosilicate glass and constructed in accordance with specified dimensions. For the capillary viscosity measurement, the time taken for the meniscus of the sucked liquid to fall from a mark on the tube to another mark 5mm below is compared to that of water or a specified liquid at the same temperature.

SEMI-SOLIDS

Most semi-solid preparations are used for topical or cutaneous application. In other words, they are intended for local or transdermal delivery of active substances, or for their emollient or protective action.

1. **Description/Appearance**
   They consist of a simple or compound basis in which, usually, one or more active substances are dissolved or dispersed. According to its composition, the basis may influence the activity of the preparation. The basis may consist of natural or synthetic substances and may be single phase or multiphase. According to the nature of the basis, the preparation may have hydrophilic or hydrophobic properties, and may contain suitable excipients such as antimicrobial preservatives, antioxidants, stabilizers, emulsifiers, thickness and penetration enhancers. Several categories of semi-solid products include ointments, creams, gels, pastes, poultices and medicated plasters. According to their structure, ointments, cream and gels generally show viscoelastic behaviour and non-Newtonian in character, e.g., plastic pseudophastic or thixotropic type flow at high sheer rates. Pastes frequently exhibit dilatancy.

2. **Labeling and Packaging**
   The general principles stated for liquid preparations also apply to semi-solid products. In addition, it should be stated on the label that the preparation should not be allowed to freeze. They mostly come in plastic tubes with narrow openings to minimize exposure to the atmosphere. In some cases, especially ointment balms, they are packed in wide-mouthed jars. Instructions for use, *"For External Use Only"* and other required information are usually stated on the package. The total weight or volume, as appropriate, appears on the container which often is enclosed in a paper packet while the content of active ingredient is expressed as percent concentration (w/v, v/w or w/w).

3. Content

Assay of the active ingredient follows the same principles as stated earlier for liquid preparations. The assay procedure must ensure that the active ingredient(s) is (are) fully released for measurement.

INCOMPATIBILITY IN LIQUID DOSAGE FORMS

What is *Incompatibility*?

Incompatibility occurs when *one drug* is mixed with ***other drug(s)*** or ***excipients(s)*** to produce a product ***unsuitable*** for administration either because of some modification of the effect of the active drug, such as ***an increase in toxicity***, or because of ***some physical change*** such as ***immiscibility*** or a ***decrease in solubility***. Such incompatibility can also cause ***unsightly, non-uniform products*** from which it is difficult to remove the correct dose. Incompatibility is largely an ***interaction*** phenomenon.

From the above definition, one can infer that incompatibility may take place before drug administration (i.e., *in vitro)* or after (i.e., *in vivo).* However, the scope of this topic is limited to the former, i.e., ***incompatibility before drug administration***. Broadly, this incompatibility may result in ***precipitation of the drug from solution***, ***loss of potency*** and/or ***instability***. However, with the increasing unpopularity of and consequently, decline in, ***extemporaneous dispensing***, this aspect of pharmaceutical incompatibility has diminished somewhat in importance. Hence, ***today's pharmacist*** is unlikely to see many incompatibilities because he/she mainly dispenses proprietary preparations or prepares medicines from official formulae (such as those from B.P. and U.S.P.). Nonetheless, there are still situations where ***the pharmacist must be alert***. They include:

i) The possibility of incompatibility occurring when proprietary medicines are diluted or mixed together or placed in an unsuitable container or package.

ii) If the doctor prescribes a special formula containing a list of names and quantities of ingredients, then the pharmacist should be on the lookout for possible incompatibilities.

iii) Obviously, any pharmacist employed as a formulator in the pharmaceutical industry should be aware of possible incompatibilities in new or proposed formulations.

iv) Drugs may be added to intravenous fluids being administered to patients in a hospital, and this is often done without the pharmaceutical oversight needed to avoid incompatibilities and instabilities arising.

Types of Incompatibilities

Incompatibility is of two types:

a) *Therapeutic Incomatibility*: Occurs when a medicine contains two or more antagonistic substances, the effect of which counteract or enhance each other or when the action of one component in the body affects the action of another component. E.g., a medicine containing an expectorant and a cough suppressant drug would be considered to be a *therapeutic incompatibility*

b) *Pharmaceutical Incompatibility*: Arises when the components of the medicine ***interact either physically or chemically*** to give an unsuitable product. This lecture will focus on this type of incompatibility.

CLASSES OF INCOMPATIBILITY

1) **Physical Incompatibility:** This is usually demonstrated in pharmaceutical formulations in the following ways:
   a) ***Immiscibility*** – Oils are immiscible with water, a problem that may be resolved by emulsification or solubilisation. Also, addition of a high concentration of electrolyte, e.g., Potassium Citrate Oral Solution B.P.C., to a solution containing a volatile oil, e.g., lemon oil, 'salts out' the lemon oil. To disperse the oil evenly, quillaia tincture is added as a suspending and emulsifying agent.
   b) ***Insolubility*** – When a preparation contains a potent *insoluble* drug, precipitation can easily result due to changes in physical chemical conditions such as pH or temperature. In this case, sometimes it is possible to substitute a chemically equivalent amount of a soluble derivative of the drug, e.g., an alkaloidal salt for an alkaloid. Another scenario is when high concentrations of electrolytes (e.g. NaCl or KCl) is added to soap emulsions. This cracks the emulsion by 'salting out' the emulgent.

2. **Chemical Incompatibility:** This is illustrated briefly by amongst others, the following (some of which are actually physicochemical processes):

   a) ***pH effects*** – Drugs are often salts of weak acids or bases. These salts are usually soluble in water while most of the unionized acids are bases which are practically insoluble. Therefore, if, for example, the salt of a weakly basic drug is made alkaline, the free base is precipitated. Similarly, precipitation of free acid may occur if a solution of a weakly acidic drug is acidified. However, precipitation depends on the solubility of the unionized drug, pH of the solution and pKa of the acid or base. Also, for polymeric (disperse) systems, pH below 3 causes alginic acid to be precipitated from sodium alginate while strong acids precipitate carboxymethyl cellulose (CMC) from sodium CMC.

   b) ***Soap emulsions & polyvalent cations*****:** Emulsions prepared with monovalent cations (e.g., alkali metal such as Na^+ or K^+, NH_4^+ and triethanolamine soaps) are incompatible with salts with polyvalent cations. Double decomposition would occur, yielding a polyvalent soap which causes phase inversion in the emulsion.

   c) ***Complexation:*** Perhaps, one of the most widely known examples of complex formation or complexation is the chemical binding or chelation of tetracycline with polyvalent cations such as Fe^{2+}, Mg^{2+} and Al3+ as well as anions such as trichloroacetate or phosphate ions. This is important because the cations may be found in liquid haematinics and/or antacids while phosphates may be used to buffer some liquid preparations. Furthermore, it has been found that some macromolecular (polymeric) excipients form complexes in which drugs and preservatives are bound to, or trapped within, the macromolecules. Although complex formation is sometimes used to achieve prolonged (depot) release, it can also adversely affect the amount of drug available for therapeutic activity as well as preservative efficiency. Thus suspending agents (e.g., polysaccharides such as tragacanth), emulgents (e.g. polyethylene glycol [PEG] esters and ethers) and solubilisers/surfactants (e.g. the polysorbates such as the Tweens) can exhibit this phenomenon.

   d) ***Ionic compounds of high molecular weight:*** Some high molecule weight drugs may have ions that are associated with their pharmaceutical or therapeutic properties and consequently, if drugs of opposite ion types are mixed with them, they could result in salts of very high molecule weight which almost always would

be insoluble in water and lack the useful properties of ether of the ions. Examples include mixing chlorpromazine hydrochloride with phenobarbitone sodium or benzylpenicillin potassium. Similarly, an organic colouring agent such as tartrazine which is a sodium salt of an organic acid (an anionic compound) should not be mixed with cationic dyes such as methylene blue or crystal violet as this will slowly yield a precipitate that is observable only after long storage, and ultimately results in a loss of colour.

Incompatibility with containers

When formulating a dosage form, it is important to remember that the dosage form will require some form of final container or package before presentation to the patient. Incompatibility can occur between the formulation and the container. An example includes the adsorption of chloroquine from chloroquine oral solution by some plastic containers.

PCT 413

PHYTOMEDICINES – Formulation and Dosage Form Evaluation

Definition

A herbal medicine is one that contains ingredients of vegetable matter or its constituents as a finished product for therapeutic use, and may include whole plant parts or other plant material. Often, it is also known as phytomedicine or phytopharmaceutical.

Introduction

The traditional use of herbal preparations dates from the earliest written records and have been used through the ages. Traditional medicine originally involved not only the use of herbal medicines, but also the use of animal parts and minerals. However, of these three types of materials, herbal medicines are the most widely used. Used as self-care or as an alternative form of treatment to conventional medicines, there is a large market and demand for medicinal plants and herbal products. In India, for example, there were 2,860 hospitals providing traditional medicines in 2003. It is obvious that most ancient forms of herbal medicine are now coming back to use but with more purified forms using new technologies of processing. According to the World Health Organization (WHO), worldwide use of herbal medicine is 3 – 4 times higher than conventional medicine, or put another way, 80% of the world's population rely on medicinal plants for their primary health care. WHO has been promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries. Natural products have also been successful used in drug development. Over 50% of the best-selling pharmaceuticals in use today are derived from natural products.

Annual sales of herbal medicines have been rising with revenues in U.S. alone exceeding US$4.2 billion in 2004. The European herbal trade is estimated at Euro3.2 billion (approx. U.S. $4.3 billion). This rapid growth in the use of herbal medicines underscores the need to formulate policies and guidelines to maintain the quality of herbal healthcare products and to promote the industry.

Classification of Herbal Medicines

For practical purposes, a WHO document in 2003 classified herbal medicines into 4 categories, based on their origin, evolution and the forms of current usage. While these categories are not always mutually exclusive, they have sufficient distinguishing features for a constructive examination of the ways in which safety, efficacy and quality can be determined and improved.

Category 1: ***Indigenous herbal medicines.*** They have the following features:

- Historically used in a local community or region
- Known through long usage by the local population in terms of its composition, treatment and dosage
- Detailed information on them (including folk medicines) may or may not be available.
- It can be used freely by the local community or region without any regulatory restrictions
- However, if they are being marketed or promoted beyond the local community or region, they have to meet the safety and efficiency requirements laid down in the national regulation for herbal medicines.

Category 2: ***Herbal medicines in systems.*** They have the following characteristics:

- Have been in use for a long time
- They are well documented with their special theories and concepts, and accepted by the country. Examples include Ayurveda in India

Category 3: ***Modified herbal medicines.***

- These are herbal medicines described in the above two categories
- Additionally, they have been modified in some way - either shape, or form including dose, dosage form, mode of administration, herbal medicinal ingredients, methods of preparation and medical indications.
- They have to meet the national regulatory requirements of safety and efficacy of herbal medicines

Category 4: ***Imported products with a herbal medicinal base***

- All types of imported herbal medicines, including raw materials and products, are covered here.
- They must be registered and marketed in the countries of origin
- Safety and efficacy data have to be submitted to the national authority of the importing country.
- They need to meet the regulatory requirement of safety and efficacy of herbal medicines in the recipient country.

FORMULATION OF PHYTOMEDICINES

Herbs work in a holistic fashion, not targeting one area of the body but actually an array of sites to produce a particular health benefit. Their actions are caused by a multitude of compounds working together to produce therapeutic effects.

Herbs have been used in a variety of dosage forms since they were first discovered to have medicinal qualities. These include the fresh or dried plant parts themselves such as leaves, stems, flowers, seeds or fruits. However, for stability, standardization and convenience, herbal medicines should be formulated into dosage forms and meet the requirements of quality, efficacy, safety and stability. Commercial herbal dosage forms include:

- Infusions or teas where the unit dose (e.g., a sachet) is steeped in hot water.
- Decoctions or extracts, obtained by boiling the herb, is made by dissolving or dispersing in water to form a concentrate
- Powder – pulverized herbs, which may be formulated into granules or compressed into tablets or filled into capsules.
- Tinctures – made by extracting herbs with alcohol, glycerol or vinegar
- Herbal ointments/creams – herbs dissolved or dispersed in waxes/petroleum jelly
- Liniments made with alcohol and vegetable oil
- Poultices (See Isaiah 38: 21)
- Herbal oil – formulated with a base oil (olive, sesame, almond oils, etc)

Herbal Standardization

A key requirement in the manufacture of acceptable phytomedicines is that the herbal extracts should be standardized. **Standardization** means adjusting the solution of the extract so as to maintain consistency and repeatability in its composition, and should be distinguished from **concentration** which is merely adjusting a solution so as to increase its strength, density

and/or efficacy. When a herbal product is not standardized, you have absolutely no idea how much of the herb's key compound(s) you are actually getting, or if these levels are the same from product capsule or tablet to the next. Thus standardized herbal extracts are extracts guaranteed to contain a 'standardized' level of active compounds. Stating the content of active compounds allows for accurate dosages to be made by manufacturers and practitioners.

The best scenario for determining the quality of an herb is the level of active components or key biological markers. Regardless of the form the herb is in, it should be analyzed to ensure that it contains these isolated key components at an acceptable standardized level. More accurate dosages can then be given for maximum beneficial effect. The potency or strength of herbal extracts are generally expressed in either of two ways:

- If they contain known active principles, their strengths are commonly expressed in terms of the content of these active principles

- Where the active principles are not known or quantified, then strength is expressed in terms of their concentration. E.g., a **1:5** tincture means **one part** of the herb (in grams) is soaked in **five parts** of liquid. A **4:1** concentration, on the other hand, means that **one part** of the extract is equivalent to or derived from, **four parts** of the crude herb.'

To illustrate the importance of using a ***standardized*** herb product, let us look at *Saw Palmetto Berry*. Studies have shown that the best *Saw Palmetto* extracts are standardized to contain 85 – 95% fatty acids and sterols, at a dosage of 160mg twice daily. To get that equivalent with dried powered berries, an individual would have to consume 40 to 50 500mg tablets each and every day. In terms of key compounds, **one bottle** of standardized *Saw Palmetto* extract is equal to **17 bottles** of the non-standardized product.

PRODUCTION OF PHYTOMEDICINES

In general, the production of phytomedicines follows the same process as for orthodox medicines. Preferably, the dry standardized extract is used, if available. These can be formulated into the various dosage forms such as tablets, capsules, syrups, ointments, etc.

(Read your lecture notes on formulation and production of dosage forms, including tablets, capsules, syrups, ointments, etc.)

EVALUATION OF PHYTOMEDICINES

WHO has set out guidelines for ***Good Agricultural and collection Practices*** for Medicinal Plants **(GACP)** and ***Good Manufacturing Practices*** **(GMP)** for orthodox medicines. The quality control system for production should be in place. The implementation of a credible concept of quality assurance, e.g., identifying and eliminating potential sources of contamination, should be a primary goal of the manufacturer rather than the implementation of all individual technical aspects. The following should be considered while studying the WHO guidelines:

- Control of raw materials (refer to ***GACP*** and ***Quality Control Methods for Medicinal Plant Products***)

- Control of starting materials and intermediate substances

- In-process control (standard operating procedure **(SOP)** for processing methods should be mentioned)

- Finished product control (It should be performed with reference to the control of raw materials, starting materials and intermediate substances).

The purpose of quality control is to ensure quality of the products by adhering to appropriate specifications and standards. Information on appropriate standards can be found in official pharmacopoeias, monographs, handbooks, etc. The ***European Pharmacopoeia (Ph. Eur)***, for example, contains over 120 specific monographs on herbal substances and general monographs on herbal drugs, herbal drug preparations and herbal teas. Where there are no pharmacopoeial specifications for a particular herbal substance, the manufacturer would have to draw up its own specifications following the ***Ph. Eur*** format.

Specifications for herbal dosage forms would need to include the following:

- Description
- Identity tests for each active substance
- Assay for known therapeutic constituents/markers.
- Degradation products
- Tests specific to the dosage form, e.g., hardness, uniformity of mass, disintegration test for tablets.

Note that these are in addition to pre-formulation tests (such as foreign matter, loss on drying, total ash) assay, pesticide and fumigant residues, microbial levels and mycotoxins.

Pharmacovigilance

Post –marketing surveillance (PMS) is particularly necessary as an evaluation tool with regard to the safety of herbal products. Manufacturers should:

- have access to an appropriately qualified person responsible for pharmacovigilance at all times.
- have in place an adequate pharmaceovigilance system to maintain detailed records of all suspected adverse drug reactions (ADR) occurring worldwide.
- report to the licensing authority all serious suspected ADRs within 15 calendar days.
- include all other suspected ADRs as part of periodic safety update reports (PSURs).

Currently, only manufacturers of licensed herbal products are required to record and submit data on safety aspects; manufacturers of unlicensed herbal products have no such obligation.

Assignment: ***Read up materials on formulation and evaluation of solid dosage forms from course lecture notes, textbooks and pharmacopoeias. IT IS IN YOUR INTEREST!***