INTRODUCTION

Immunization against infectious disease is recognized as the most cost-effective method for controlling and eradicating microbial infections (UNICEF, 2002). However, despite the introduction, over the years, of both human and animal vaccines, against a variety of viral and bacterial pathogens, many diseases yet remain unconquered. This is due, in part, to problems associated with vaccine delivery, stability, and cost (Nelson et al., 2004). Other major hurdles to be overcome, include stimulating immunity at the most effective site, thereby reducing the need for repeated injections to overcome short-lived immunological memory, and stimulating the necessary Cytotoxic T-Lymphocyte (CTL) responses, and antigenic variation among the causative agents.

Vaccination against debilitating infectious diseases has proven remarkable results in the prevention of these diseases and has contributed significantly to an increase in life expectancy, especially in children, in many parts of the world (Sally et al., 2003). Despite these impressive results and remarkable accomplishments, there is still a need to further improve on vaccine research and development to combat deadly and emerging infectious diseases, and to provide complete protection, mainly in developing nations.

Majority of pathogens invade the body via one or more of the mucosal routes. Oral, nasal, pulmonary and urino-genital are the most common pathways for entry of infectious pathogens into human host. Therefore, the importance of generating a ‘first-line of defence’ at the site of entry has been well recognized. In order to have adequate mucosal protection, there are several factors that can influence the effectiveness of vaccines. The most critical factor in mucosal vaccine effectiveness is the route of administration and potentiality for the antigen to be processed by the antigen-presenting immune cells, such as
macrophages and dendritic cells. Presently, most vaccines are administrated via the parenteral route or other invasive routes. Invasive mode of vaccine administration can trigger the systemic immune response but may not essentially provide an adequate mucosal immune protection. On the other hand, effective mucosal vaccines will not only elicit superior local immune protection, but also has been shown to elicit systemic response analogues to that of parenterally delivered vaccine. Hence, it is critically important to examine the development of mucosal vaccination strategies that can effectively trigger systemic, as well as, mucosal immunity.

The objective of vaccination is to provide effective immunity by establishing adequate levels of antibody and CTL responses in many situations, and a primed population of cells, which can rapidly expand on renewed contact with the antigen. The first contact with an antigen should avoid the pathogenic effect of the organism, yet provide an adequate stimulus to the immune system. A successful human vaccine is one that is able to induce rapid and long-lasting protection, ideally, after administration of a single dose. However, because the vaccine contains a non-replicating antigen, booster doses are required.

Additional criteria for a successful vaccine include the following:

a) the vaccine should be effective early in life
b) the antigen should be stable and have a long shelf-life, even at relatively extreme temperatures
c) simultaneous delivery of several antigens should be possible
d) the vaccine should be easily administered, with minimal risk (orally, for example)
e) the vaccine should be readily adaptable to existing immunization programmes.
The excellent advancement of genetic engineering and the recombinant DNA technology has been responsible for the current increase in the production of proteins and peptides for the therapeutic use and also for the development of a new generation of recombinant vaccines. These proteins and peptides have received much attention in recent years as drug candidates.

Although several such products are being rapidly made available, their therapeutic utility is limited by the lack of convenient methods for their effective delivery. Unfortunately, despite the great potential of these new therapeutic and antigenic peptides and proteins, only a small number has received approval by the FDA and other regulatory agencies. This has been, in general a consequence of the lack of an appropriate route of administration, which would permit the therapeutic potential of these molecules to be exploited. In fact most of them still need to be administered repeatedly in an injectable form. Therefore, the design of effective delivery systems and the search for new routes of administration for these new generation drugs and vaccines are important challenges for the pharmaceutical scientist.

Over 36 million children born each year still do not have access to immunization services. More than 2 million of these children will die before they are 5 years, while the rest will survive; half a million of them as chronic carriers of hepatitis-B, having acquired the virus from their mothers or siblings. To this number will be added those who acquire chronic infection in their later childhood or adulthood, roughly, another half a million. Thus, about 1 in every 25 children surviving the first five years of life, will be destined to carry a deadly virus during their lifetime, and to spread it to an unsuspecting contact-peers and ultimately their own children and grandchildren. About a quarter of these carriers would develop chronic liver disease or hepatocellular carcinoma and die.
While most healthy adults recover completely from hepatitis B infection, a percentage of infected individuals become chronic carriers. From patients, who undergo liver transplantation due to hepatitis B induced liver damage, a significant proportion remains at risk from subsequent liver disease mediated by re-infection of transplanted liver with endogenous hepatitis B virus (HBV). Therefore there is a need for improvement in the management of chronic HBV infection and vaccination is likely to be the cheapest and potentially, the most beneficial treatment (Michel et al., 2005).

New vaccines based on recombinant proteins and DNA, are safer than traditional vaccines, but they are less immunogenic. To increase the immunogenecity, the vaccines require the use of adjuvants. The only adjuvant suitable for human use are aluminium salts and gels. Aluminium adjuvanated vaccines have a number of limitations e.g., difficulty to achieve cell-mediated immunity and adverse stimulation of local Ig-E responses can occur. For these reasons, a number of alternate technologies have been investigated, whereby the immunogenecity of such vaccines can be increased, while at the same time, the number of doses required can be reduced.

More immunogenic vaccines based on the inclusion of adjuvant moieties within an antigen delivery system may represent a rational design approach to the development of more effective vaccines (Bramwell et al., 2005). Antigen delivery systems such as microspheres, liposomes and niosomes have been shown to be versatile in their ability for the incorporation of a diverse range of antigens. The possibility for the use of co-adjuvants in these systems further enhances their potential as potent systems for the maximization of immune responses (Bramwell et al., 2005).

HBV infection is easily preventable with highly effective, very low risk, easy to use vaccines that have been shown to greatly reduce the prevalence of the carrier
state among the population within a few years of initiation of an immunization program.

Over the recent years the effort of the World Health Organization (WHO) to highlight the need to use existing vaccines more effectively, within childhood immunization programmes, has drawn attention, amongst other problems, to the dropouts during a course of three or four doses of immunization. This results in poor coverage and high costs for multiple vaccine delivery, especially for new vaccines, which are likely to involve higher production costs.

In countries, where health-care resources are limited, there is a significant fall-off between the number of individuals receiving the first vaccine dose and those receiving the full courses. Although there are a variety of reasons for this, the most significant include poor education and a consequent lack of awareness of the need to return for booster doses, fear of injections, illness at the scheduled time of immunization, and fear of vaccine-related secondary effects. Other reasons for this poor coverage, include lack of finances, deficiencies in the immunization infrastructures, and the logistical barriers to distributing vaccines and immunizing populations in remote areas. Improvements in vaccine formulations that make vaccine delivery easier and safer, decrease dependency on the cold chain or reduce the number of immunization interventions needed, could have a significant impact on this area.

Despite the efforts of the WHO, UNICEF and others, over the recent years and the tremendous success of the Expanded Programme on Immunization (EPI), millions of children still die each year from vaccine preventable diseases. Disease prevention with EPI vaccines depends largely on the population at risk.

Traditional parenteral methods of immunization are expensive due to the need for a sterile manufacturing process and qualified medical personnel for vaccine
administration. There is also the risk of needle-borne infections (e.g. HIV or hepatitis) due to the use of contaminated needles (Kane et al., 1999; Simonson et al., 1999; Jodar et al., 2001). While immunization accounts for only a small percentage of injections given globally, the safety of the immunization process is absolutely essential, as vaccines are administered to a predominantly healthy population. Developing needle-free vaccination methods, such as jet-injectors, oral, nasal, aerosol, or patch delivery technologies, to make administration of vaccines safer is therefore a priority (Clements et al., 2004). In addition, children normally associate the site of needle injections with pain, resulting in a drop in the rate of compliance. Thus, there is an urgent need for the development of a new generation of safer vaccines, that can be effectively administrated by simple, economic and practical immunization procedures (Partidos et al., 2002).

Oral administration has been appointed as the only economically feasible approach to mass vaccination. Impressive logistical advantages of orally administered vaccines were exemplified by two national vaccination days in 1996, when 121 million Indian children were vaccinated against polio at 650,000 centres (Bloom and Widdus, 1998). However, it has been shown that it is very difficult to obtain a protective immune response following oral vaccination, the live-attenuated polio vaccines are being one of the few exceptions (Holmgren and Czerkinsky, 2005). For this reason, only few vaccines currently approved for human use are being administered orally.

Unfortunately, a simple oral formulation is not easily achieved for the new generation of subunit vaccines, which hold the greatest promise for disease prevention in the 21st century (Thanavala et al., 2005). Several explanations have been appointed to justify the disappointing results found for oral administration of subunit vaccines, being almost exclusively biotechnological products. One of the most important reasons is related with the adverse environment of the
gastrointestinal tract (GIT), rich in acids and enzymes, which are able to destroy the antigen.

**The potential of microspheres for oral immunization**

Microspheres have shown potential as carriers for the delivery of vaccines to the mucosal surfaces especially the intestine and lung. As we are aware, majority of human and animal pathogens enter the host via a mucosal surface. In contrast, the majority of the currently available vaccines have been developed for systemic immunization. Although numerous studies have provided convincing evidence that protection can be obtained by oral (or intranasal) administration, the poor uptake of immunogens delivered by these routes has been proved to be a major difficulty. It is a major goal of the WHO Global Programme for Vaccines and Immunization to promote and support the research and development of oral vaccines. Microspheres have received a great deal of attention as antigen carriers for oral administration due to their reported ability to protect the antigen denaturation by bile salts, low pH and high levels of degradative enzymes in the gut and intestine. WHO has encouraged research involving the use of microspheres to avoid problems in delivery to mucosal surfaces, such as proteolytic degradation, as well as, a possible increase in immunogenecity.

The controlled drug delivery offers numerous advantages compared with conventional dosage forms, viz. improved efficacy, reduced toxicity and improved patient compliance and convenience. Consequently considerable interest has been generated in the pharmaceutical field about the encapsulation of vaccines and drugs in bio-degradable proteinaceous or polymeric microspheres. Microencapsulation promises an increase of shelf- life of a vaccine and offers the flexibility of controlled release kinetics for the administered drug (Ravi Kumar, 2000).
Drug release kinetics from microspheres primarily depends on microsphere size and composition. In addition, microsphere size plays a crucial role in targeting a particular site in the body e.g. microspheres of size less than 10μm are absorbed by the intestinal lining in peyer’s patches.

**Recent scenario in the vaccine field**

The development of single-dose vaccines using inactivated biological products now appears to be an achievable goal. A rapid advance in the manufacture of microspheres using bio-degradable polymers has been paralleled by studies designed to maximize the efficiency of entrapment of high molecular weight immunogens. The Controlled Release Tetanus Vaccine project, sponsored and directed by the WHO Global Programme for Vaccine and Immunization and the Children’s Vaccines Initiative, are unique examples of international collaboration between scientists, interested in controlled release drug delivery and vaccinologists, wishing to develop new forms of antigen presentation systems. The major results of this project offer fresh insights for the development of other controlled release vaccines, particularly against diseases for which, effective immunization is hampered by the need for multiple deliveries, poor immunogenecity, or both. Several aspects of the safety of microsphere-based vaccines remain to be carefully addressed, although there is every expectation that controlled release vaccines manufactured using the PLGA or chitosan polymer system will prove both safe and effective. It is particularly important to keep in mind that using microspheres, several antigens can be administered together, avoiding undesired interactions and thus facilitating the task of combination vaccines. In addition, as pointed out above, microspheres may represent the safest way to deliver DNA vaccines to mucosal surfaces. Thus there is considerable excitement within the vaccine field, with the use of controlled release systems offering novel opportunities for the production of vaccines that could prove more effective against diseases for which, vaccines already exist, as
well as, for opening avenues to fight pathogens for which vaccines are not yet available.

Ideally, any new vaccine or antigen delivery system, should be capable of being administered orally with all the added advantages. These include easy self-administration, without any need for trained personnel and reduced cost. In addition to increased vaccine efficacy, the cost of immunization would drop due to the reduced need for health care workers and logistics, which together can account for over 80% of the total cost of immunization.

After success of oral polio drops in mass immunization programmes, there is an urgent need for developing an oral vaccine against hepatitis B which is a major killer disease in the developed and developing countries.