The use of microbes for the treatment of medical conditions is not new; history tells us of the many benefits of microbes to man. Their potential in the prevention and treatment of a wide range of disease conditions has been recognised since the days of Jenner, who in 1796 inoculated a young boy with cowpox and later with smallpox. The use of modified non-virulent species, attenuated bacteria/viruses and purified parts of micro-organisms has made vaccination one of the greatest achievements of medicine, and has spared millions of people from the effects of devastating diseases. Similarly, the use of microbes as mini-cell factories for the production of antibiotics, vaccines, biopharmaceuticals and a number of important excipients (for example, non-animal derived albumin) has contributed widely to improvements in health care.

Recent developments have suggested the use of non-pathogenic bacteria – such as *Lactobacillus* species, commonly found in live yoghurt – for more cost-effective mucosal based immunisation. Live brewers’/bakers’ yeast strains have also been shown to play their part, with enteric-coated products already on the market via health food shops – and in some countries on prescription – to treat conditions such as ulcerative colitis and irritable bowel syndrome (IBS).

Exploring other applications, microbes – and in particular yeast cells – were proposed in the early 1970s by Joseph Shank, working for Swift and Co, as preformed microcapsules for the encapsulation of a wide range of active ingredients. His work, and later that of Nahida Pannell of AD2 Ltd (Birmingham, UK), concentrated on industrial uses of yeast cells, for example, in carbonless copy paper and flavour delivery. The flavour delivery technology progressed and has now been taken to market in a collaboration between Micap plc (Merseyside, UK) and Firmenich SA, the Swiss-based flavour and fragrance company. Microencapsulation within the natural double-walled capsule of yeast cells allows volatile flavours to be processed and cooked, ultimately delivering the flavour where it is required in the mouth. Examples of practical applications include enhanced flavours in fried coatings, soups and sauces.

This initial work focused on using the yeast-based microencapsulation technology as a novel means of turning a volatile liquid into a powder for ease of handling. When it was observed that the yeast capsules released their flavour payload on contact with the mucosal surfaces of the mouth more readily than in saliva alone, the possibility of targeted drug delivery using yeast cell capsules was first
proposed. When recovered from the tongue surface, the yeast cells were found to be substantially intact after effective flavour delivery had been achieved. The technology has since undergone development for oral drug delivery, and is able to achieve a loading of active ingredient up to 40% by weight of a lipophilic liquid or lipophilic component dissolved in a suitable carrier.

**YEAST AS A RAW MATERIAL**

Yeast has been cultivated by man for uses such as beer- and wine-production and bread-making for thousands of years. It is now available in large quantities produced in the main by a handful of global ingredient suppliers such as DSM, Lallemand and Lesaffre. Another major source of yeast is provided by the mostly US and South American biofuel (ethanol) producers, where the widespread availability of sugar crops, together with government subsidies, makes the production of ethanol from fermented yeast for addition to motor fuel a viable proposition.

Yeast are unicellular fungi and, although there are many species which reproduce by budding or fission, by far the most commercially important belong to the genus *Saccharomyces*, which is probably also the best-characterised of the yeast genera. Ovoid in shape, an average *Saccharomyces* cell is approximately 5 micron in diameter, compared with other yeast species which can be as large as 20 micron in diameter. The yeast form is defined by the cell wall – a physically rigid structure whose function is primarily to protect the yeast internal membrane and organelles from the environment (Figure 1).

The cell wall is composed of complex and highly cross-linked glucan, mannan and, to a lesser degree, chitin which is associated with the bud scars remaining after a daughter cell has budded from the mother. The wall is approximately 100-200nm thick and comprises 15-25% of the dry mass of the cell. The cell wall surrounds the much thinner (<10 micron) plasma membrane – a typical bilayer unit membrane comprising phospholipids, sterols and neutral lipids, represented mainly by triacyl glycerols and sterol esters.

Currently, *Saccharomyces* yeast strains are produced to food-grade standards for beer, wine, spirits and bread.
manufacture; some strains are also produced to nutraceutical standards. Unlike the case with a newly developed drug delivery polymer, the safety of yeast for application via the oral and topical route (yeast is used widely in cosmetic formulations) is well-established; this should lead to easier acceptance of the material in drug delivery applications. Yeast is currently used in cGMP-based processes for the production of vaccines and biopharmaceuticals, and cGMP grade yeast for the encapsulation and delivery of active pharmaceutical ingredients will soon be available.

THE MICROENCAPSULATION PROCESS

Lipophilic active ingredients are partitioned into the central core of the yeast cell – a process driven by the lipid bilayer membrane of the cell. Although the mechanism of stabilisation has not been fully determined, it appears that lipid material from the membrane and cell organelles goes through a conformational change, forming micellular-like structures within the cell, and the active ingredient is held as many droplets approximately 20nm in diameter.

In practise, the process involves preparation of a yeast-slurry in water; the slurry is stirred using a top-paddle stirrer and the liquid active ingredient is added. The stirrer speed is carefully controlled to obtain the correct droplet size for optimum diffusion, and the vessel is heated to around 40°C; this gently melts the yeast cell membrane, without destroying its structure, enabling penetration of the active ingredient at a practical rate. Within 1-4 hours, more than 85% of the active ingredient will be accumulated inside the yeast cell capsules. The yeast cell wall acts like a molecular sieve, so that only molecules with a narrow molecular footprint can diffuse into the cell. In practise, this means that molecules with molecular weights of below 1,000 daltons can be used. At the end of the encapsulation process, the non-viable yeast cells are harvested by centrifugation, washed with water and if necessary dried by spray, fluidised-bed or freeze-drying processes. A typical spray-dried product will comprise agglomerates of many yeast cells with a diameter of 30 micron. The agglomerates easily disperse down to single cells of approximately 5 micron diameter when added to water. Alternatively, a dry powder can be formulated into tablets or filled capsules.

DRUG DELIVERY APPLICATIONS

Oral Delivery
The yeast microencapsulation system is designed for the encapsulation of high concentrations of lipophilic active pharmaceutical ingredients, and is able to deposit a localised concentrated active on the mucosal surface of choice. Coating the yeast particles with conventional materials (for example, enteric coating systems) allows targeted delivery of the yeast and subsequent delivery of a lipophilic active anywhere from the mouth to the lower bowel. Once released, the yeast cells disperse within the GI tract fluids and, at the mucosal surface, deliver their contents for absorption into the systemic circulation system. In this case, a typical example is illustrated by the anticholesterol agent, fenofibrate; when delivered to the duodenum, it demonstrates the ‘low burst’ effect and enables prolonged delivery of the active ingredient resulting in a very high level of bioavailability (Figure 3).

Topical Delivery
Based on work with targeted release at mucosal surfaces, it was considered possible that other biological materials may also stimulate release of active pharmaceutical substances. While release has not been detected with epidermal skin layers, it has been found that contact with other live microbes can trigger release.

In a comparison with an antifungal topical cream already on the market, it was found that a yeast-microencapsulated formulation containing the same
concentration of active ingredient was at least five times more effective, and that the speed of kill was also greatly enhanced (Figure 4). The yeast microcapsules can be incorporated into a variety of conventional cream bases and can be modified to form an aqueous-based cream without the addition of other excipients.

**TASTE-MASKING**

The yeast-based microencapsulation technology was originally developed for products such as volatile lipophilic flavours. Sensory analysis of encapsulated flavours confirmed that, compared with non-encapsulated flavours, the encapsulated product gave both an enhanced flavour (many times more intensity) and that flavour perception was prolonged. The improved flavour delivery properties, and opportunities for the targeting of flavour and drug release, may be due at least partly to the characteristics of the yeast cell-surface. This is, overall, a negatively charged material primarily due to the presence of phosphomannannans and often possesses protruding nodules (bud scars). Combining the effective delivery of flavour with the delivery of drugs can produce an effective taste-masking solution. With careful choice of flavour and manipulation of lipophilicity compared with the active substance, it is possible to control the release of the flavour in the mouth, while retaining the drug in the yeast capsule. It is also possible to allow both flavour and drug to release together.

In either case, because of the strength of flavour delivery, only the pleasant taste is perceived. As the yeast microencapsulation system retains the active ingredient in the yeast cell capsule in water and other aqueous mixtures, it is possible to produce not only a dry powder or tableted formulation for taste-masking, but also a syrup or similar water-based formulation. In this form, the product will be particularly suitable for paediatric patients.

**CONCLUSION**

Most people are scarcely aware that microbes exist unless they become infected by one. Microbes – as ‘bugs’ – are nearly always perceived as unpleasant because of their role in disease or food spoilage. Yet over the last 200 years, and in particular since the 1940s, microbes have played an important part in improving the health of mankind. Developments in microencapsulation using yeast and/or bacteria as preformed targeted drug delivery vehicles will continue this pathway of progress. Opportunities exist today for oral, topical and taste-masking applications using yeast microencapsulation technology. Furthermore, because of the ability to manipulate the chemical structure of the yeast cell wall and membrane (using extensive knowledge of the genetic and physical make-up of yeast), more precise targeted delivery may become a reality in the future.

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