Chemical research is now hardly conceivable without the properties of the nanoworld. This is especially true for the nanocapsules of Katharina Landfester, Director at the Max Planck Institute for Polymer Research in Mainz. These objects are tiny – with a diameter of only a few hundred nanometres. But they are still much larger than typical molecules. Chemical reactions – for example, polymerization – can therefore take place on their surfaces. If there are many nano-objects in play, they offer a huge surface area for reactions. It is precisely this strength of the nanoworld that Landfester uses in her miniemulsions of finely dispersed nanodroplets.

Anyone who immerses themselves in Katharina Landfester’s research projects will realize just how fascinating and diverse chemistry can be. Basic research and application often go hand in hand. The chemistry professor now holds more than 50 patents – and nanocapsules play an important role in all of them. The applications range from encapsulated colour pigments that therefore no longer clump together to perfume microcapsules for detergents or encapsulated corrosion protection for metals (that is released only when damage occurs) to the vision of a completely new kind of medicine. Her nanocapsules serve, so to speak, as tiny "transport submarines" in the blood [Fig. A]. Landfester first talks about how the chemical "magic kit" that she has been developing since her time as a junior researcher works.

**BASIC MIXTURE**

The basis is "miniemulsions". An emulsion is a finely dispersed mix of small oil or fat droplets in an aqueous environment – or vice versa. In miniemulsions, these droplets are particularly small or – in Landfester’s case – even nanoscopic. The nanoworld is located between the microworld with micrometre-sized objects and the world of atoms and most molecules. The diameter of atoms is measured in tenths of a nanometre (see techmax 28).

We encounter emulsions many times in everyday life. For example, in food and cosmetics. Milk is a mixture of fine fat droplets in a watery solution; with butter or skin cream, it is the other way round. Emulsions are technically impossible. But chemistry and physics help make the impossible possible. This is what you experience when you make a salad dressing. The vinegar as the aqueous phase and the salad oil initially remain separate from each other. Whisking quickly makes for finer oil droplets. But the vinaigrette becomes stable only after a bit of mustard is added.

Aqueous and fatty phases mix poorly because of the properties of their molecules. In a water molecule, the oxygen atom attracts the electrons of the two hydrogen atoms. This gives the molecule electrically negative and positive "poles". As a polar...
solvent, water is therefore good at dissolving molecules that also have electrically charged sections. When the water molecules attach, hydrogen bonds are formed. These play an important role in Landfester’s research. With their positively charged hydrogen atoms, the water molecules “dock” to the negatively charged part of other molecules.

Substances with such “hydrophilic” (water-loving) properties are water-soluble. On the other hand, non-polar solvents such as fats and oils lack these properties dominated by electrical charges. Their molecules interact with each other through Van der Waals forces. Water molecules therefore cannot attach themselves as well to fat molecules. This explains the low solubility or insolubility of fats in water. Fat molecules are therefore referred to as “hydrophobic” (water-hating). There are also “amphiphilic” substances, the molecules which have both lipophilic (fat-loving) and hydrophilic parts. As contact mediators, they can thus ensure that fat droplets distribute finely in water (i.e. emulsify) at the interface between the two phases. This happens when washing greasy dishes thanks to the surfactant molecules of the washing up liquid. “One example of a common surfactant is the sodium dodecyl sulphate in detergents”, explains Landfester.

The special thing about her miniemulsions is that extremely tiny nanodroplets form a large total surface. On this playing field, chemical reactions can take place much more efficiently than between the unmixed aqueous and oily phases. If these are layered on top of each other separately, they come into contact only on the comparatively small cross-sectional area of the vessel. “But in a miniemulsion of the same volume, the total interface between the two phases is roughly equivalent to the size of a football field”, says Landfester.

Landfester explains how the production of miniemulsions works in the laboratory using the equipment. “The first step actually starts with a turbomixer – basically a heavy-duty blender”, she says [Fig. B]. This results in a pre-emulsion. This is a high-pressure homogenizer – much like the one used to homogenize milk. In the homogenizer, the pre-emulsion is shot through a narrow gap at a pressure of up to 2000 bar against a kind of impact wall. This breaks up the droplets down to the nanoscale. In comparison: A pressure of 1000 bar prevails at a depth of 10 km – roughly the deepest point of all oceans in the Mariana Trench. If the team needs smaller quantities, they can use an ultrasound device for this purpose.

SPONTANEOUS SPHERE FORMATION
But how does Landfester’s team use the tiny droplets as chemical nanoreactors? The Mainz team started with technical polymers and the necessary polymerization reactions. These reactions generally always link the same basic chemical building blocks – the monomers – to form long polymers. Mono means “one” in ancient Greek, and poly means “many”. “In our case, however, the polymer may be formed only on the capsule wall”, says Landfester. One example where this works is nylon. “Nylon works in principle in the miniemulsion, although not particularly well. It’s also not that scientifically interesting for us”, says Landfester.

Nanocapsule production with a similar reaction that produces polyurethane works much better [Fig. C]. Both plastics basically have common polymerization reaction links two different monomer building blocks to form a long polymer chain. Polyurethane is formed in a polyaddition reaction, whereas the polyamide fibres of nylon are formed in a polycondensation reaction. In a polycondensation reaction, a by-product must always be split off so that the functional groups of both monomers can be joined. This is not necessary with a polyaddition reaction.

The decisive factor for Landfester’s strategy is that one type of building block is more soluble in the oily phase, whilst the other is more soluble in the aqueous phase. As a result, they come into contact with each other only at the interface. This means that the polymerization reaction takes place only there. Because the resulting polymer is amphiphilic, it remains between the aqueous and oily phases and rebuilds the sphere of the entrapped droplet.

In this way, the nanocapsule forms itself in a self-organised way. In polyurethane, one of the two monomer building blocks is a diol, an organic compound that contains two alcoholic groups and is thus often water-soluble. The second monomer is a disocyanate, which dissolves better in the oily phase. As soon as both monomers come into contact at the interface, the polyaddition reaction starts. That’s because the disocyanate is highly reactive.

COMPATIBLE TRANSPORTERS
However, such technical plastics are not suitable for nanocapsules that are supposed to deliver medically active ingredients to the target. Use in the body requires biocompatible alternatives for the capsule material. And something else is important: ‘Most active substances are water-soluble’, says Landfester. This is typical for biological molecules. One example is the mRNA molecule in the COVID vaccine of BioNTech [see Biomax 36]. “We thus have to turn our miniemulsion around – so to speak”, she says [Fig. B right]. The team had to create nanocapsules with an aqueous content emulsified in an oily liquid during the reaction.
In other words, a high-tech cream. For the material of the capsule, the Mainz researchers experimented with different biocompatible substances that can be polymerized. These substances must have reactive groups that are suitable for forming chemical bonds to other molecules. This is how the Mainz team arrived at carbohydrates and proteins. "Sugars have OH groups with which you can make such interfacial reactions", says Landfester. "And with proteins, it is NH_2 groups on certain amino acids that you can use for the reaction".

Proteins are particularly interesting for the goal of delivering medications in the body exactly where they are supposed to work. This is because the protein surfaces of nanocapsules can be provided with "chemical shipping addresses" that can recognize certain cells. These can be either immune cells (that are to be activated against a virus or a type of cancer) or tumour cells. This is referred to as targeting.

Proteins are already polymers. The interfacial reaction that is to form nanocapsules from proteins must therefore do something other than polymerize. It must cross-link neighbouring polymer strands together so that they form a stable capsule. We encounter the result of cross-linking proteins in everyday life. For example, in the form of gelatine. Various cross-linking reactions are used for the biocompatible nanocapsules. "Click reactions" are a particularly interesting approach. A conventional reagent designed to start cross-linking works "non-specifically" (i.e. not in a targeted way). As a result, there is a risk that it will also react with the active ingredients that are to be encapsulated. "Many active ingredients also have OH or NH_2 groups", explains Landfester. "That means that we would inadvertently cross-link them with the capsule". But that should not happen. And this is where the click chemistry can be advantageous: it works purposefully and quickly.

There are various cross-linking reactions that involve click chemistry. One is the alkyne–azide reaction. The decisive factor here is the N_3 group of azides with which the proteins are first "functionalized" – or prepared for cross-linking. In this process, the NH_2 groups of the protein are used to attach a molecule that has an N_3 group at the end. This azide group then reacts with a di-alkyne, which takes on the role of a "cross-linking molecule". The triple bond of the di-alkyne opens and ensures that ring molecules...
A click cross-linking reaction has another advantage: no by-products potentially harmful to human health are produced. In addition, the cross-linking must proceed in such a way that it does not alter the biological function of the capsule proteins themselves. This is important for the smart function of targeting in the body. However, there is still a challenge with the nanocapsules: they must be so thick that the active ingredient molecules remain safely encapsulated as long as they are not at the target. Such a capsule with a diameter of about 100 nanometres is minuscule. Consequently, the capsule wall is only 15 to 20 nm thin – an extreme film. "Now try to make that thick", says Landfester. The thinner such a polymer network is, the more permeable it becomes. As a result, there is a risk that the active ingredient molecules will exit the capsule before they reach their destination.

Fortunately, there are ways to make these super-thin capsule walls thicker. As a first step, they can be cross-linked even more strongly (i.e., the molecular lattice can be made more closely meshed). "But that is often not enough", says the chemist. Additional hydrogen bonds can provide a remedy. "They pull the network of protein strands even closer together and make it denser", says Landfester. Above all, the strands sometimes arrange themselves so neatly next to each other that they begin to form crystals. This partial crystallinity also makes plastic films more waterproof, for example. "When plastic bags crinkle, you can even hear this partial crystallinity", says Landfester, explaining an effect that everyone is familiar with.

**ACCUARATE DELIVERY**

However, a nanocapsule that can transport an active ingredient safely enclosed is only the first step. The next is targeting (i.e., successfully addressing a target in the body). This requires solutions where chemistry meets biology and medicine. Landfester’s team has been working with these disciplines in a large collaborative research centre since 2013. Physics is also involved. One goal is to provide the capsules with surface molecules that are recognized only by the pathogens to be fought in the body. However, the capsule must be chemically camouflaged in such a way that it is not incorrectly recognized and absorbed in the blood by phagocytes.

"Considering that there are 1600 proteins in the blood, this is a considerable challenge", says Landfester. In the meantime, the team has found solutions for such a stealth function. Apolipoproteins play an important role in this. These molecules lead to the nanocapsules not being recognized by the cells.

In targeting, which is supposed to fight cancer cells, there is a smarter alternative than directly poisoning the tumour cells: the immune system itself is to be made fit against the hidden tumour cells. For this purpose, the nanocapsules are prepared on the surface in such a way that the T-cells of the immune system recognize and destroy them. The capsule contains molecules that train the immune system to recognize characteristics of the tumour cells. The T-cells thus learn to seek out and destroy these enemies in the body.

In fact, these “nanotherapeutics” already work in diseased mice in the laboratory. Landfester is optimistic that the use of such therapeutics will soon also be possible in humans. Basic chemical research can therefore bring progress in a wide variety of areas. That is what is so fascinating about it.

**Keywords**

Alkyne-azide reaction, click chemistry, emulsion, miniemulsion, nanocapsule, polyaddition reaction, polycondensation reaction, polymer, polymerization reaction, proteins, targeting

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