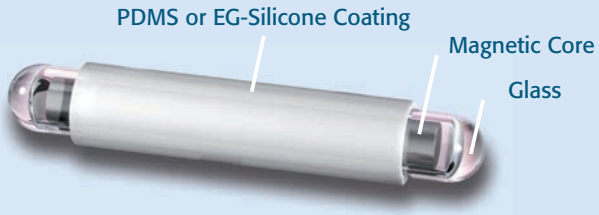
Extractables & Leachables

Medical Implants – A closer look

In order to assess the quality and compatibility of a polymer based implant, it is vital to perform simulated in-vivo studies to provide qualitive and quantitive information concerning the compounds that are leached out of the device. These compounds are referred to as Leachables. Since they are present at very low concentrations, analysts often turn to Stir Bar Sorptive Extraction (SBSE) followed by Thermal Desorption GC-MS/MS for an accurate answer. In the following, we report on a joint effort to test polymer based tibial knee inserts for Leachables.

A look inside: Implants at work



The patented GERSTEL Twister® serves as extraction medium based on the Stir Bar Sorptive Extraction (SBSE) technique. The Twister is coated with a relatively large volume of sorbent, either polydimethylsiloxane (PDMS), mainly for non-polar compounds, or Ethyleneglycol silicone (EG Silicone Twister), which provides enhanced extraction of various polar compounds in addition to non-polar compounds. Analyte extraction is performed while the Twister stirs the sample or, in the case of the Twicester, with one Twister immersed in the sample, but held in place on the vial wall, while another Twister stirs the sample.

Pharmaceutical products in general should serve to maintain the health of the patient. Under no circumstances should they endanger or adversely impact the patient's health. Manufacturers are required to perform extensive testing on pharmaceutical products, including the packaging used, over an extended period during the development phase and regularly once it has been approved and is in production. If unallowed Leachables from the packaging are found, or if they are found in too high concentrations in the product, it will not be approved, for example, by the US FDA, which means it cannot be sold in the US.

In simulated "worst case" scenarios, Extractables studies are performed on complete, undamaged packaging and the extracted chemical compounds determined. The packaging is exposed to solvents of different polarities at elevated temperature to extract a wide range of compounds. Those compounds identified as potentially hazardous that could leach into the product are then studied further in formalized Leachables studies based on the actual product using validated methods [1].

Implants must be safe as well

Implants are generally made of both metal and polymer materials and they must be analyzed for the presence of contaminants. Further, it should be studied whether additional contaminants could be formed while the product is inside the patient (*in vivo*), endangering the health of the patient. Up until now, manufacturers of medical implants have not had an easy way to perform such analyses on medical device implants. However, since implants remain inside the body for an extended period of time in direct contact with body fluids, these analyses are even more important. Leached contaminants from implants can gain direct access to the patient's blood, unlike most pharmaceuticals, which are ingested orally and have to go through the digestive tract.

Unlike the situation for pharmaceutical products, until now there are no legally binding regulations for implants and prosthetics, or indeed any standardized methods for performing Extractables or Leachables studies according to Gyorgy Vas. For this reason, the researcher who works for the US based company Intertek, followed regulations for performing E&L studies on pharmaceutical packaging when describing the leaching properties of implants. They further adhered to guidelines of the „Product Quality Research Institute" (PQRI), a US non-profit organization, which recommends a Maximum dose for genotoxic or carcinogenic leachables of 150 ng/day [2].

Not identical but comparable

In its guidelines, the PQRI refers to results obtained from testing of inhaler products. As Gyorgy Vas and his colleagues report in their article in *Journal of Pharmaceutical and Biomedical Analysis* [3], they based their studies on the PQRI guidelines, which represent a more "conservative" approach in the sense of erring on the side of caution in order to ensure the greatest possible safety for the patient. The researchers report how they determined leachable compounds in medical implants, in this case tibial knee inserts, based on Stir Bar Sorptive Extraction (SBSE) followed by GC/MS analysis. The goal of their study was to develop a systematic procedure, by which antioxidant-related leachables can be determined accurately. These are formed in knee implants during sterilization with gamma radiation.

Of Additives and Guidelines

The tibial knee inserts that were investigated by Vas et al. are produced from ultra-high molecular weight polyethylene (UHMWPE). After the molding process, these are cross-linked and sterilized using a very high dose of gamma radiation. During this process, the mechanical properties of the polymer are improved, but free radicals are formed, which can attack the material and negatively impact its chemical and mechanical long term stability. To prevent oxidation processes in the material and improve product stability, an antioxidant is added, such as pentaerythritol tetra-

Twisters are desorbed in the Thermal Desorption Unit (TDU) shown in this picture or in the Thermal Desorption System (TDS). The process is automated.

Image/s: GERSTEL, Istockphoto
Author: Guido Deussing

Stir Bar Sorptive Extraction using the GERSTEL Twister is followed by thermal desorption in the Thermal Desorption Unit (TDU), cryofocusing, and GC/MS analysis – fully automated using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO control.

kis (3-[3,5-di-tert-butyl-4-hydroxyphenyl] propionate). The purpose of adding this compound known as PBHP is to remove free radicals, prevent oxidation processes and improve the long-term stability of the product. However, PBHP can degrade and the degradation products formed leach from the implant into the surrounding tissue. A total of 16 such PBHP degradation and by-products have been identified in simulated in-vivo experiments with knee implants that were between ten and thirty years old. Because of these findings, the researchers stress that implants need to be tested for leachables. Studying the leaching behavior of larger implants, however, requires large volumes of aqueous simulants. This results in the extracted analytes being present at very low levels. As reported by Vas *et al.*, a concentration step is required prior to GC/MS or LC/MS determination in order to enable quantification as per the PQRI guidelines. Typically, such a concentration step would be time consuming and would require highly pure organic solvent. These factors make the whole process expensive, challenging, and environmentally unfriendly.

Turning to Stir Bar Sorptive Extraction (SBSE)

Evaporative concentration of organic solvent extracts inevitably leads to loss of volatile analytes. Additionally, a solvent peak typically masks peaks of interest in the chromatogram and a solvent could easily introduce interfering compounds leading to errors and uncertainty. Stir Bar Sorptive Extraction (SBSE), on the other hand, relies on the GERSTEL Twister to extract organic compounds from an aqueous solution with high recovery without any use of solvent.

Following an extensive evaluation of various widely used methods, Dr. Vas and his team decided to use SBSE and the PDMS Twister as extraction medium combined with GC/MS analysis. The Twister extracts and concentrates analytes in a single step, no further sample preparation is required before GC/MS analysis. Thanks to the large PDMS sorbent volume and the efficient stirring of the sample by the Twister stir bar, analyte recovery is far higher than what is achieved with Solid Phase Micro-Extraction (SPME). In the words of Vas et al.: "Case-by-case, we have found SBSE to be significantly more sensitive than SPME for the determination of organic compounds in aqueous matrices".

Technical details

This is how the scientists approached the project: Whole implants were immersed in 500 mL of an aqueous extraction solution (90:10 water/acetone). Extraction times of 24 hours and 30 days were used. The resulting extract was stirred with a Twister for two hours at 1,000 rpm, during which time the organic compounds in the extract solution were absorbed into the PDMS coating of the Twister. The Twisters were collected from the aqueous extract, briefly rinsed with deionized (DI) water, dried using a lint-free paper cloth, placed in a TDU liner, and transferred to a TDU liner tray. The next step was thermal desorption in the Thermal Desorption Unit (TDU), fully automated with the GERSTEL MultiPurpose Sampler (MPS). The desorbed analytes were cryogenically trapped in the GERSTEL Cooled Injection System (CIS), a PTV-type inlet, mounted in an Agilent 7890 GC (Agilent Technologies). Analytes were determined using an Agilent Triple Quad MS/MS detector. As an aside, three different sources of DI water were tested. All had detectable background concentrations of antioxidants. Monitoring and controlling the blank level, and choosing the right DI water source, is clearly very important in order to obtain accurate results at these low concentration levels.

End of a successful study

The SBSE-GC-MS/MS method developed and validated by Vas *et al.* enabled them to determine Leachables in medical implants at trace level concentrations equivalent to leaching rates below 150 ng/day. The scientists reported that no genotoxic or carcinogenic compounds were determined to leach from the tested implants at rates higher than 150 ng/day in the 24 hour extraction tests. For the PHBP degradation products, the linearity of the developed method was tested over the 1–150 ng range (equivalent to 40–6000 pg/mL) for all relevant components. Calibration curves with internal standard were prepared using the extraction media as the matrix. The linear range for each component was 1.0–150 ng with good RSDs and the method was subsequently used to analyze eleven tibial knee implants. Unlike other methods, which can only analyze fragments, the developed method can analyze the entire implant, which is the approach preferred by regulatory authorities to eliminate sampling errors, according to Dr. Vas. Most of the sample preparation was automated; only minor manual steps were needed. The method had the added benefit of eliminating the use of expensive, toxic solvents that are also expensive to dispose of and which can negatively impact the laboratory air quality.

References

[1] GERSTEL Solutions No. 10 (2010) 20-22
[2] Summary of the PQRI Leachables and Extractables Reconnedations (www.pqri.org/workshops/leach_ext/imagespdfs/posters/PQRI_Recommendations_Poster.pdf)
[3] J. Pharm. Biomed. Anal. 74 (2013) 62-70

Extractables and Leachables

In the case of pharmaceutical packaging, Leachables and Extractables studies are required in order to determine if packaging will leach contaminants into the product under normal storage conditions or if contaminants can be extracted by the product under well-defined more aggressive conditions.