skin. More information and detailed TS technique protocols for in vitro and in vivo can be found at (Melero et al. 2011; Wagner, et al. 2000, 2002; EMA 2018; N'Dri-Stempfer et al. 2009). Since the results of the TS depend on many influencing parameters during the performance and the variety of conditions impede the comparison between published works, a standardization of the method is needed. Crucial parameters that can be a source of variation in the TS results (natural, e.g., anatomical region, age, gender, sex, time of day, presence of skin wrinkles, season or hydration state, as well as methodological factors), will be discussed systematically next.

53.3 TECHNICAL ISSUES: STANDARDIZATION OF THE TECHNIQUE AND CRITICAL PARAMETERS TO OBTAIN REPRODUCIBLE RESULTS

53.3.1 Decisions and Requirements before the Experiment: in vivo and in vitro Studies

The following questions are essential in the beginning: in vivo or in vitro, human or nonhuman (animal) subjects. In the case of in vivo experiments, human experiments are the gold standard, but animals are reasonably easier to obtain and the results can be scaled up to humans (Abd et al. 2016). Independent of the experimental setup, prior evaluation from an authorized ethical committee must be obtained, including informed consent signed by participants. Since in vivo testing in animals should be reduced to a minimum and replaced by other appropriate models, in vitro techniques are of great interest (Eskes and Zuang 2005). Ex vivo human skin is still considered the best surrogate for human volunteers.

Even for in vitro experiments, different experimental setups can be chosen. Diffusion cell, a setting that was developed to study drug permeation through the skin, consists of a donor (application of the formulation) and acceptor compartment (filled with buffer/acceptor solution) separated by excised skin as a membrane. Apart from the widely known static Franz diffusion cell (FDC), there are also flow-through diffusion cells available, facilitating better sink conditions. Sink conditions are mandatory for permeation studies to ensure that the permeation through the skin is not influenced by the solubility of the applied drug in the receptor solution. That means the highest concentration of the permeated drug during the experiment in the receptor solution must be below 10% of its saturation solubility in the receptor solution (WHO 2006).

Besides the diffusion cells, there is also a skin penetration model, the Saarbruecken Penetration Model (SB-M). Here, the formulation is applied on the excised skin, which is the only acceptor compartment, and the skin humidity is preserved by a soaked filter paper beneath it (Selzer et al. 2013a). After a predetermined time, the amount of drug in the skin can be determined in the SC by TS and in the underlying deeper skin layers by cutting with a cryomicrotome and subsequent analysis (Wagner et al. 2000).

The realization of sink conditions in a FDC can often only be achieved by supplementing with solubility enhancers. As a result, the SC properties can be altered by damage, saturation, and hydration. This issue also points out the importance of the in vitro setup chosen; in comparison to the FDC, nonphysiological skin hydration is prevented in the SB-M (Wagner et al. 2000, 2002; Selzer et al. 2013a).

Institutions such as the organization for economic co-operation and development (OECD), U.S. food and drug administration (FDA), and scientific committee on consumer safety (SCCS) provide guidelines containing general instructions on accepted techniques (OECD 2004a,b; SCCS 2018; WHO 2006; FDA 1997). TS is the most widely used experimental setup to determine the permeation of substances within the SC, equally feasible in vitro as well as in vivo (Brain, Walters, and Watkinson 2002).

While most human in vivo studies are performed on the volar forearm, in vitro studies most commonly utilize abdominal or breast skin obtained from surgery. It has to be considered here that the absorption rate through skin can be up to fortyfold different (Feldmann and Maibach 1967). Nevertheless, in vitro experiments have shown to be sufficient in predicting in vivo skin absorption