the degree to which a drug is distributed in body tissue rather than plasma (volume of distribution). It is generally believed that only the unbound fraction of a drug is available for interactions with the relevant receptors. Thus, accurate determination of unbound drug concentrations at the site of action is essential during therapeutic drug monitoring [70]. However, since the drug concentration is difficult to assess in the skin, the degree of protein binding of a drug is commonly determined in blood, and the resulting unbound drug concentration from blood is used as a surrogate parameter to estimate the unbound drug concentration at the site of action. Such unbound drug concentrations derived from blood are often biased due to different association strengths of the drug with plasma or tissue proteins, and consequently, the resulting volumes of drug distribution differ. Drugs strongly bound to plasma proteins (e.g., penicillin) are restricted to blood, whereas drugs with weak association to plasma proteins that are therefore present in an unbound state in plasma can also be distributed beyond the vascular system. Further, tissue-binding properties of a drug also affect its volume of distribution. Some drugs although highly bound to plasma proteins (e.g., tricyclic antidepressants) have an even greater affinity to tissue proteins and thus also show a large volume of distribution.

With dermal OFM sampling, the amount of the unbound topical drug fraction can be assessed directly in skin ISF. Results from dermal OFM studies showed that the unbound protein concentration in skin ISF significantly differs from that in blood, which highly influences the therapeutic effect of the respective drug at its site of action (unpublished data).

### 57.5.2 Development of New Topical Formulations

Dermal OFM sampling in combination with other techniques such as skin biopsies or in vitro release testing covers a large part of the topical drug development process and guides the development of topical drugs from early drug formulation development to clinical studies. Development of a new topical formulation involves preclinical drug development and clinical studies.

A major aspect of drug development is compliance with regulations of drug licensing authorities, such as EMA or FDA. New Drug Applications (NDA) and Abbreviated New Drug Applications (ANDA) [32] are the FDA's regulatory pathways for drug approval, where NDA regulates the approval of new drugs and ANDA the approval of generic drug products.

NDA regulations describe a number of in vitro and ex vivo tests to determine the major toxicities of a novel compound prior to first use in human and preclinical animal studies to demonstrate the complex interplay of metabolism and drug exposure. Dermal OFM is a promising tool in several of these required stages of the topical drug approval process.

Besides NDA and ANDA, there is an additional pathway, called 505(b)(2) [71], which can accelerate the approval of a new drug. Following this pathway, a connection between the 505(b)(2)-eligible product, or its active ingredients, and a reference product needs to be established. This can be done, for example, with the use of results from bioanalytical testing, preclinical studies, or even clinical trial results. A dermal OFM clinical study offers an elegant way to bridge the developed drug to the reference product based on dermal PK profiles.

In early drug development, dermal OFM can be used to assess the properties of an active pharmaceutical ingredient (API) in a realistic matrix by sampling pure dermal ISF from skin in vivo. This can be performed, e.g., by sampling pure, undiluted ISF with OFM-recirculation from the skin of anesthetized pigs [20]. Results from stability and degradation assessments of the API are of great value for API design optimization and toxicity assessment.

An ex vivo dermal OFM study can be performed to investigate the stability and metabolism of the API in skin and to predict clinical efficacy. The drug effect of a new chemical entity to be tested may be predicted by assessing skin penetration and dermal metabolism of the API. Such dermal ex vivo OFM studies determine the transport across the skin barrier in a highly sensitive manner due to the absence of blood circulation in the skin explants that would drain compounds from the skin.

Further, dermal OFM proves its value when performing a preclinical proof-of-concept studies which elucidate the mode of action of an API in vivo. Dermal OFM has already been used to