(DGME), a penetration enhancer. They first determined specific vibrations of the metronidazole Raman signature, which can be detected in the skin: two vibration bands were selected at 1191 and 1369 cm^{-1}. Metronidazole was applied at the concentration of 18 $\mu g \cdot mL^{-1}$ in DGME, and Raman spectra were acquired at several depths in the skin after 1- and 2-hour application times. Metronidazole was detected down to 23 to 24 µm in the skin after 1 hour and between 15 and 40 µm after 2 hours. These results were confirmed by Raman images obtained on thin slices of the skin samples used in the penetration experiment. Spectral images were reconstructed by integration of the metronidazole vibration (1191 cm^{-1}) intensity. They showed that metronidazole was present at depths of 40 to 45 µm in hair follicles and 25 µm in the SC. Furthermore, the authors used CRM to study the effect of metronidazole–DGME solution on the skin structure. A decrease in the intensity of the 1084 cm^{-1} peak was observed after applying the solution, corresponding to a decrease of the lipid chain organization. Such fluidization of lipid chains in skin can be related to the drug or DGME penetration.

Propylene glycol appears to be a gold standard among penetration enhancers for hydrophobic molecules. It has, however, a limited effect on the penetration of hydrophilic molecules. Ascencio et al. (2016) investigated the penetration of caffeine nanocrystals and propylene glycol as penetration enhancers, applied topically on porcine ear skin in the form of a gel, using CRM. Nanocrystals have been employed as a new alternative to increase the skin penetration of active components. Spectral ranges of 526 to 600 cm^{-1} for caffeine and 810 to 880 cm^{-1} for propylene glycol were chosen for analysis of penetration into the skin. Data reduction using statistical methods showed that propylene glycol penetrates significantly deeper than caffeine (20.7 to 22.0 µm versus 12.3 to 13.0 µm) without any penetration enhancement effect on caffeine, probably due to its size. Considering the measured SC thickness of 18.1 ± 1.0 µm, it was concluded that caffeine did not deeply penetrate SC and accumulated to saturate the upper 70% to 80%, while propylene glycol easily penetrated the SC and reached the stratum spinosum layer.

CRM was also applied to monitor the skin penetration of hyaluronic acids (HAs) on human skin sections (Essendoubi et al. 2016). HA is a highly hydrophilic polymer used as an active in cosmetics formulations. However, the skin penetration of HA is the matter of controversy because of its molecular size. According to the literature, chemical substances with a molar mass greater than 500 $g \cdot mol^{-1}$ may not penetrate skin. In this study, the penetration of three HA derivatives were investigated: Cristalhyal (1000 to 1400 kDa), Bashyal (100 to 300 kDa), and Renovhyal (20 to 50 kDa). HA solutions were deposited on skin surface for 8 hours at 32°C. Raman spectral analyses were then performed from cryo-sections of skin samples. The major bands of HA were found in two spectral windows: 800 to 1660 and 2700 to 3000 cm^{-1}. The results showed a skin permeability of low-molar-mass HA (20 to 300 kDa) and impermeability of high-molar-mass HA (1000 to 1400 kDa). Renvhyal and Bashyal were present in the skin section at an epidermal depth of about 100 µm for Renovhyal and 50 µm for Bashyal. The penetration depth of Cristalhyal was less than 25 µm. Raman images demonstrated that Renvhyal was present in the deepest layers of the epidermis, whereas Bashyal HA was localized in the superficial layer of the epidermis beneath SC, and Cristalhyal was only found in SC.

55.5.2.2 Metabolism of Active Substances Studied by CRM

The use of Raman may provide simultaneous information on the penetration and metabolism of drugs and pro-drugs. For instance, Zhang et al. (2007c) observed the spatial distribution of a pro-drug (1-ethyloxycarbonyl-5-fluorouracil or pro-5-fluorouracil (pro-5FU)) and a drug (5-fluorouracil, 5FU) inside pig skin using CRM. The pro-drug was applied before the drug because this is known to enhance transdermal delivery of 5FU. The pro-drug is converted to the active molecule, an important systemic antitumor drug, by endogenous enzymes or simple chemical hydrolysis once in the epidermis. Aqueous solutions of pro-5FU were topically applied to the SC (5 $\mu L \cdot cm^{-2}$) for 20 hours at two different temperatures (22°C and 34°C). Raman bands at 866 and 637 cm^{-1} were chosen to monitor the relative concentrations of pro-drug and drug, respectively. The presence of