Bound water in reconstructed skin samples: quantification by NMR

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INTRODUCTION Reconstructed skin samples are routinely used for *in vitro* safety and efficacy evaluation of cosmetic ingredients[1]. Full thickness skin models are composed of a dermal part (collagen gel containing dermal fibroblasts) and of a stratified epidermis formed of differentiated keratinocytes. NMR measurements combined with Magnetization Transfer (MT) is an attractive tool to quantify the bound proton fraction and the exchange rate between water and macromolecules[2]. In this work, a standard MT-NMR technique was used to characterize dermis extracted from reconstructed skin samples. A linear relationship between the bound proton fraction and the solid weight fraction was found.

MATERIAL AND METHODS Reconstructed skin samples were obtained from EPISKIN (Lyon, France). Examined samples were from three groups : G1: dermis cultured alone, G2: dermis cultured with epidermis and examined alone i.e. after epidermis removal, G3: dermis cultured with epidermis and examined as a whole. Each sample was positioned in a blood-test tube sealed with Parafilm. MR measurements were performed on a 4.7 T scanner controlled by a Tecmag sequencer and equipped with 400 mT/m gradient bore using a loop-gap resonator (16 mm diameter). For all samples, their weight was controlled (Scout Pro 123, 1 mg accuracy) right before and after NMR examination, then after 24 h left to dry at ambient atmosphere.

Longitudinal relaxation curves were collected by IR (31 delays in log-scale, range 15 µs-15 s) and transverse relaxation by CPMG (inter-echo spacing 0.75 ms, 1600 echoes) with 100 µs duration RF pulses. Off-resonance saturation was performed [2] with 27 offset RF frequencies *df* and 6 f_1 ($f_1=\gamma B_1/2\pi$) amplitudes of a 5 s saturation pulse. For each (*df*, f_1) pair, both saturated M_{ZA} and reference M_{0A} (longitudinal magnetization of the free protons) were assessed as FID intensity after a 100 µs 90° RF pulse (TE=175 µs), with a 15 s delay for magnetization recovery. The reproducibility of each measurement was better than 5%. Two-pool model parameters[2] were adjusted from the measured saturation M_{ZA}/M_{0A}. For each sample, T_{1A} and T_{2A} were determined from IR and CPMG measurements respectively, T_{1B} was set equal to T_{1A}, and the remaining parameters (T_{2B}, R, M_{0B}) chosen to minimize RMSE (Root Mean Square Error) between model and data.

RESULTS Sample weights were in the range 70-210 mg and solid weight fractions in the range 4.5%-17%. Longitudinal relaxation data could all be fitted as a sum of two exponential decays, as expected for M_{ZA} and M_{ZB} cross- relaxation[3]. However the confidence on the short time and on the short-time amplitude was poor ; the long time was determined with better than 5% accuracy and chosen as T_{1A}. Transverse relaxation data were best fitted as the sum of three exponential decays, with more than 85% of the decay amplitude attributed to the shortest time constant, chosen as T_{2A}. The two-pool model could be fitted to off-resonance saturation (Fig 1), RMSE was in agreement with measurement reproducibility. The bound proton fraction M_{0B} thus obtained showed a linear correlation with the solid weight fraction in the samples (Fig 2, R^2=0.73 for all samples), while T_{2B} was stable (13.5±0.35 µs) and RM_{0B} in the range of 0.5-3 s^{-1}, relatively disperse whatever sample group and weight fraction.

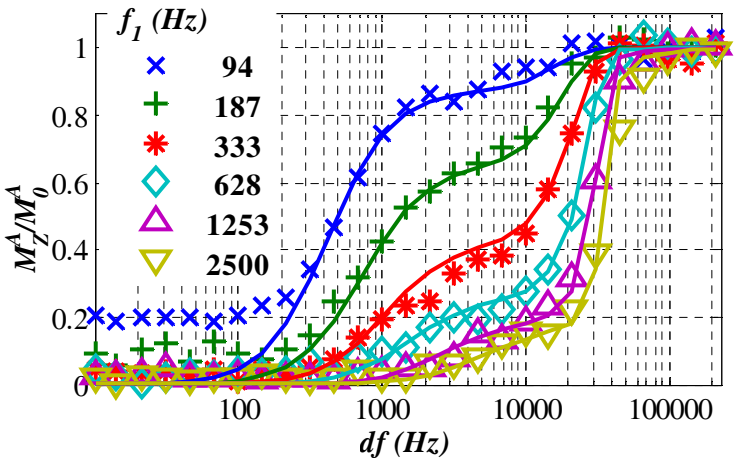

Fig 1 : Off-resonance saturation in a G2 sample

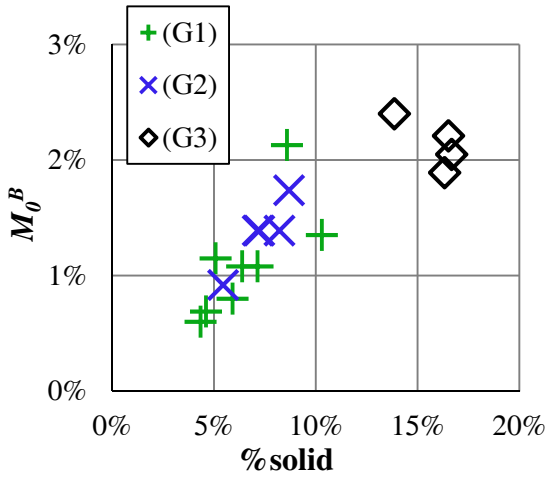

Fig 2 : M_{0B} as a function of the solid weight fraction

DISCUSSION CONCLUSION Non-destructive characterization of reconstructed skin samples is possible by MT-NMR, the M_{0B} value is compatible with 0.2 g of bound water per g of collagen, in fair agreement with the 4 water molecules per tripeptide description of collagen hydration[4]. Work in progress shows the possibility to use saturation by binomial excitation for easier implementation in a standard GE sequence[5] and quantitative mapping of M_{0B}.

REFERENCES

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