An investigation into protein stability following low-frequency ultrasound application

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Low-frequency ultrasound (US) has been shown to be effective in enhancing protein and DNA permeation (Boucaud et al 2002; Tezel et al 2004). US can be applied to the skin as a pre-treatment (i.e. before drug application) or concurrently with the drug. The possible advantage of the latter is potentially higher protein permeation into the skin while a possible disadvantage is protein degradation due to heat and other processes generated by US (e.g. cavitation). This study was conducted to determine the stability of a model protein when exposed to US. A solution of bovine serum albumin (MW 66 kDa) in a thermally insulated container was exposed to US (20 kHz) 70% amplitude for a total sonication time of 5 min using a calibrated sonicator (VCX500) in 3 different modes of US application: continuous mode, 5 s on 5 s off pulse mode (50% duty cycle) and 0.1 s on 0.9 s off pulse mode (10% duty cycle). Temperature changes in the coupling medium were measured throughout the experiments. At the end of the experiments, gel electrophoresis was performed and band intensities were analysed using Scion Image software (Maryland, USA) to assess changes in protein structure. A control experiment where a temperature change of 50% duty cycle US was mimicked was conducted to determine the contribution of heat changes in protein stability, if any. It was found that continuous mode produced the greatest rate of increase in temperature (_50_C) in 5min. 50% Duty cycle (5 s on, 5 s off) pulse mode caused a similar temperature rise, but over a duration of 10min. In contrast for the 10% duty cycle (0.1 s on, 0.9 s off) the maximum temperature rise was _15_C. Analysis of band intensities following SDS-PAGE revealed that continuous mode caused the most severe BSA degradation, followed by 50% duty cycle, then by 10% duty cycle (Table 1). Interestingly the control experiment, which mimicked the temperature rise of 50% duty cycle, showed only a small percentage of degradation. This shows that BSA degradation upon sonication was not wholly due to the heat generated during US application but was related to other effects of US such as cavitation. We conclude that US treatment causes degradation of protein and damage is more likely when low frequency US is applied continuously compared with application in pulses. Thermal effects contribute to protein degradation but are not solely responsible for the degradation. Severe protein degradation during simultaneous sonophoresis may result in loss of activity. Therefore, ultrasound pre-treatment will be considered for our future experiments.

Table 1 Percentage of BSA left after sonication

Ultrasound protocol	Percentage of BSA (n = 4)
Continuous mode	48.4_8.6
5 s on 5 s off pulse mode (50% duty cycle)	71.9_10.5
0.1 s on 0.9 s off pulse mode (10% duty cycle)	87.2_15.5
Control	91.5_7.2

Boucaud, A. et al (2002) J. Control. Release 81: 113–119
Tezel, A. et al (2004) Pharm. Res. 21: 2219–2225