

Supplementary Information

Methods

H₂S release from ADT-OH permeated across murine skin

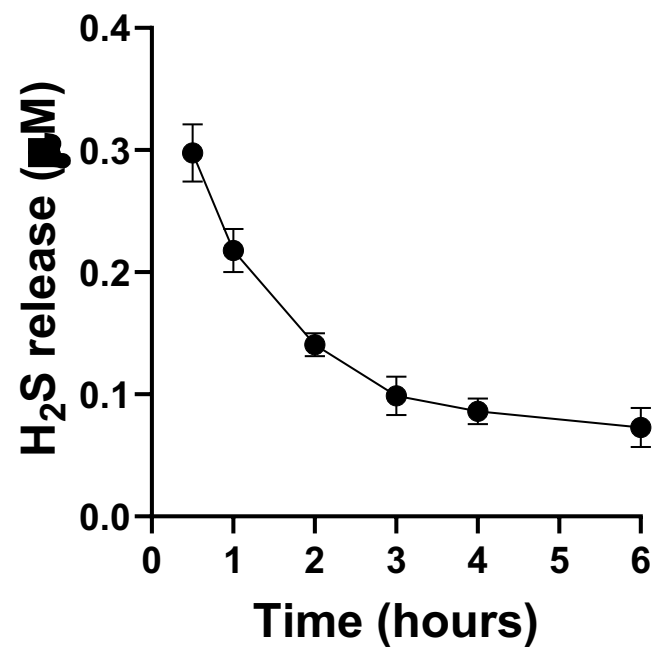
Free H₂S is strong reducing agent and reacts with the tetrazolium dye 3- (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) and forms purple colour formazan (32). Media collected following murine skin permeation studies containing was diluted 1 in 2 with fresh cell culture media and added to HUVEC cells. Over the course of 6 hours, 100 µL of media was removed and was added to 50 µL MTT (5 mg/mL), allowed to incubate for 3 hours and changes in absorbance were recorded on a plate reader at 570 nm. The reaction was carried out in a humidified incubator at 37 °C with 5% CO₂ atmosphere to mirror the cell culture conditions and minimise evaporation. The H₂S calibration curve was created by preparing serial dilutions of freshly dissolved Na₂S and by measuring the reducing capacity. The H₂S generation is reported as hourly change in absorbance with respective H₂S concentrations.

Figure 1.

H₂S is rapidly released from ADT-OH on HUVEC cells. Hourly H₂S release values detected using an MTT assay are plotted with curve-fitting results to highlight the donor compound decomposition on HUVEC cells.

Table 1. pH of all formulated HPMC gels loaded with up to 10% v/v PG or liposomes to give a final loading of 0.025% ADT-OH observed values similar to that of human skin.

Supplementary Figure 1



Supplementary Table 1

Formulation	pH
0 % v/v PG	5.5 ± 0.1
2 % v/v PG	5.67 ± 0.06
10 % v/v PG	5.77 ± 0.06
Deformable liposomes	5.8 ± 0.1