

Persulfated Ascorbic Acid Glycoside as a Safe and Stable Derivative of Ascorbic Acid for Skin Care Application

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Table S1 ^1H and ^{13}C NMR data of 2-O- α -D-glucopyranosyl-L-ascorbic acid persulfate (AAGS) and ascorbic acid persulfate (AscS) ($\text{DMSO-}d_6$).

$^1\text{H}^a$	δ (ppm)		$^{13}\text{C}^b$	δ (ppm)	
	AAGS	AscS*		AAGS	AscS*
1	-	-	1	176.5	173.1
2	-	-	2	114.2	95.7
3	-	-	3	173.6	154.2
4		4.29 (m)	4	114.2	92.4
5	3.94-4.10 (m)	4.52 (q)	5	70.4	70.2
6a		4.14 (q)	6	63.3	63.1
6b	3.70-3.83 (m)	3.73 (t)			
1'	5.53 (d, $J = 2.9$ Hz)	-	1'	97.3	-
2'	4.52-4.62 (m)	-	2'	74.0	-
3'	4.52-4.62 (m)	-	3'	75.4	-
4'	4.31 (brt)	-	4'	71.0	-
5'	4.13-4.18 (m)	-	5'	74.0	-
6'a	3.94-4.10 (m)	-	6'	65.9	-
6'b	3.70-3.83 (m)	-			

^aMeasured in $\text{DMSO-}d_6$ at 300.13 MHz. Values in parts per million (δ_{H}).

^bMeasured in $\text{DMSO-}d_6$ at 75.47 MHz. Values in parts per million (δ_{C}).

*Literature assignments (1)

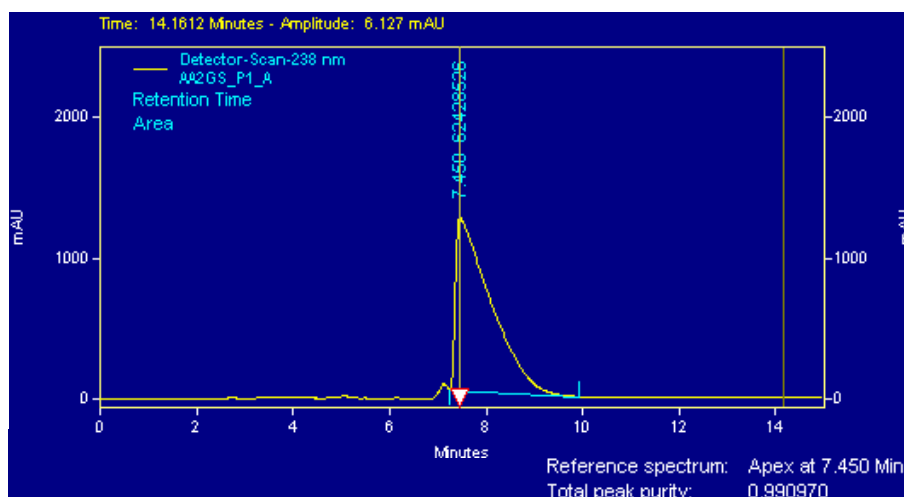


Figure S1 Obtained chromatogram for AAGS by HPLC-DAD analysis (column C18; mobile phase: 25 mM of tetrabutylammonium and acetonitrile (38:62)).

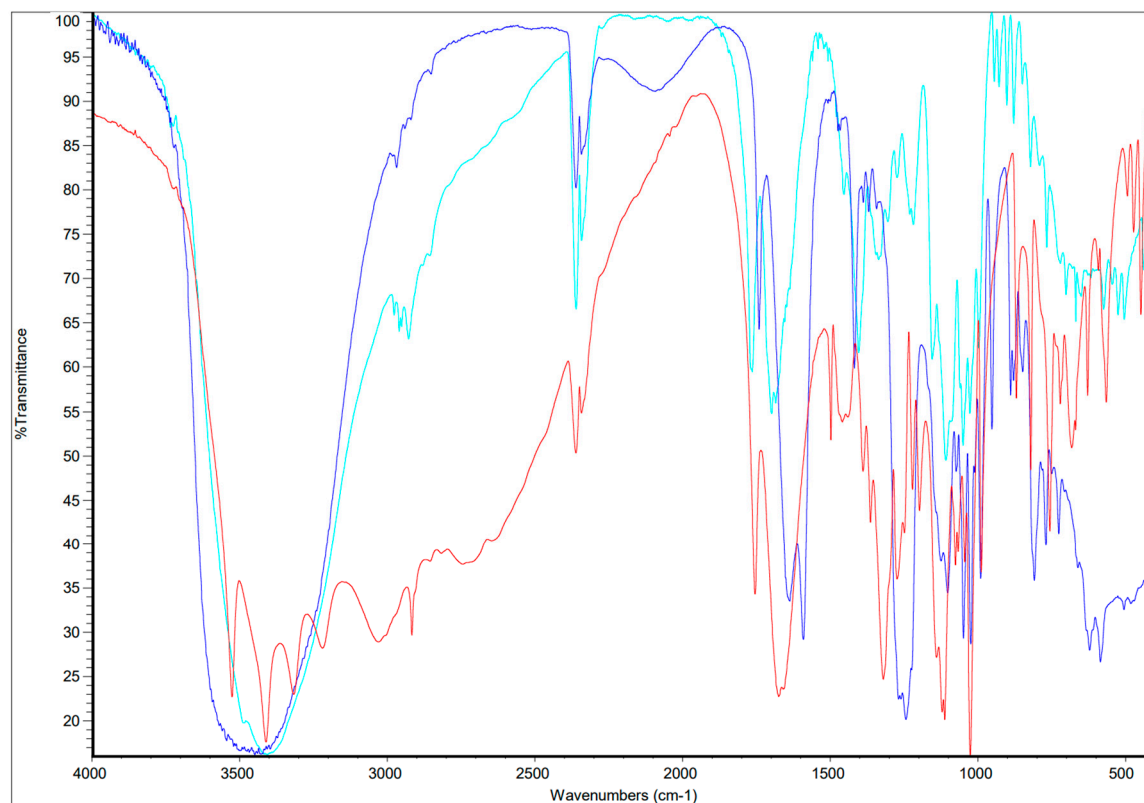


Figure S2 IR (KBr) spectra: dark blue= 2-O- α -D-glucopyranosyl-L-ascorbic acid persulfate (AAGS), light blue= ascorbic acid 2-glucoside, and red= ascorbic acid.

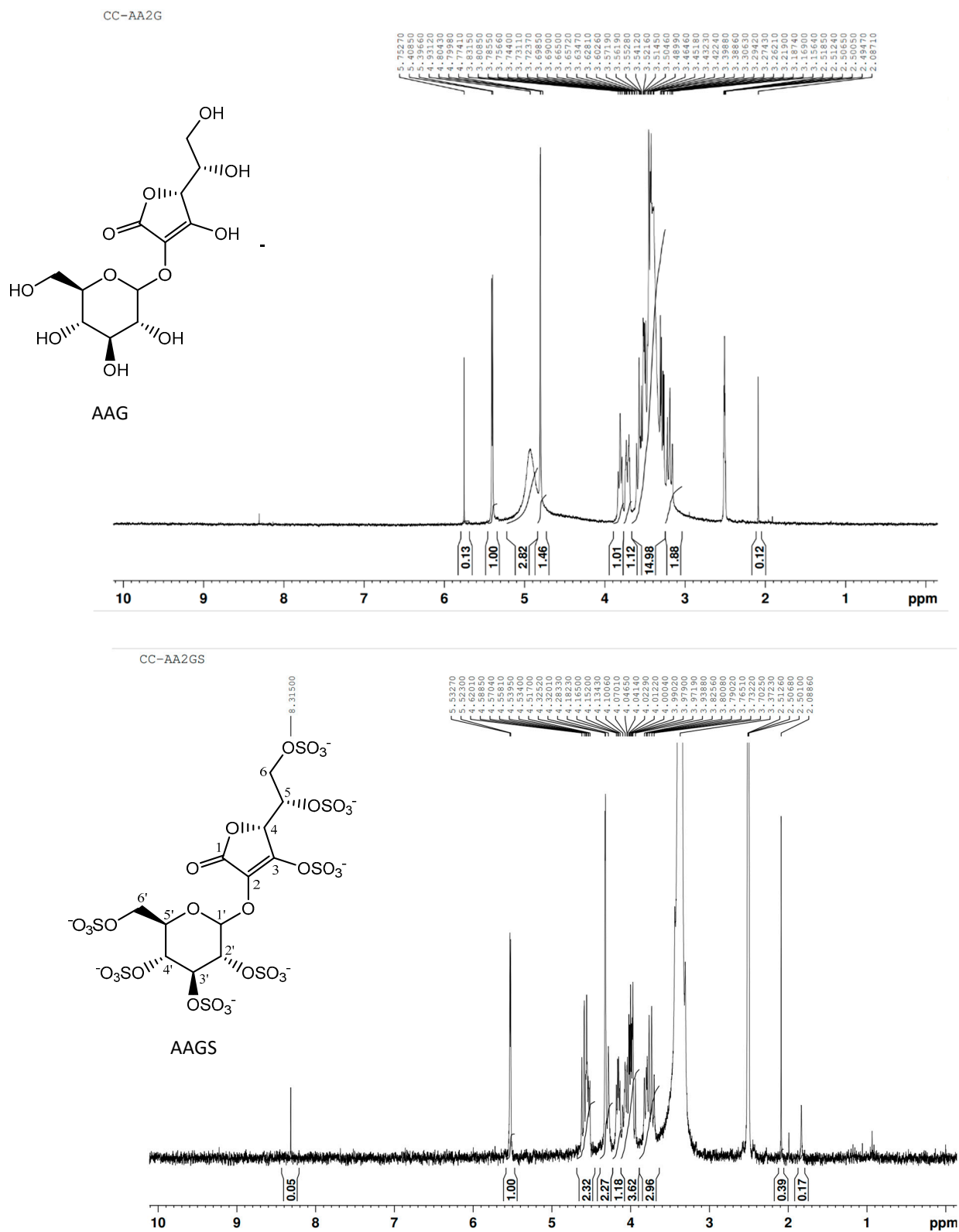


Figure S3 ^1H NMR spectra of ascorbic acid 2-glucoside (AAG) and 2-O- α -D-glucopyranosyl-L-ascorbic acid persulfate (AAGS).
 *Measured in $\text{DMSO-}d_6$ at 300.13 MHz.

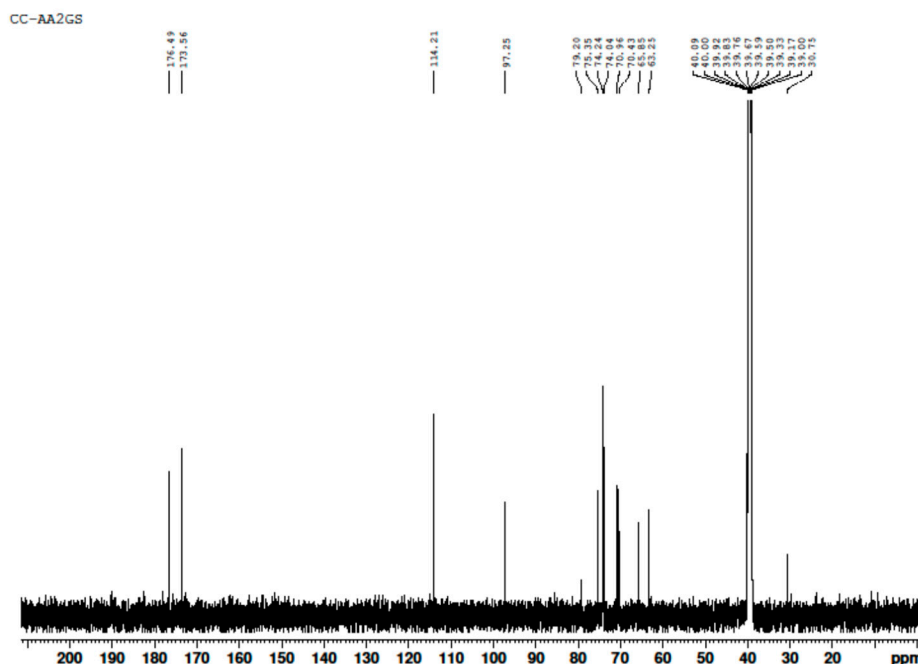


Figure S4 ^{13}C NMR spectrum of 2-O- α -D-glucopyranosyl-L-ascorbic acid persulfate (AAGS). *Measured in DMSO- d_6 at 75.47 MHz.

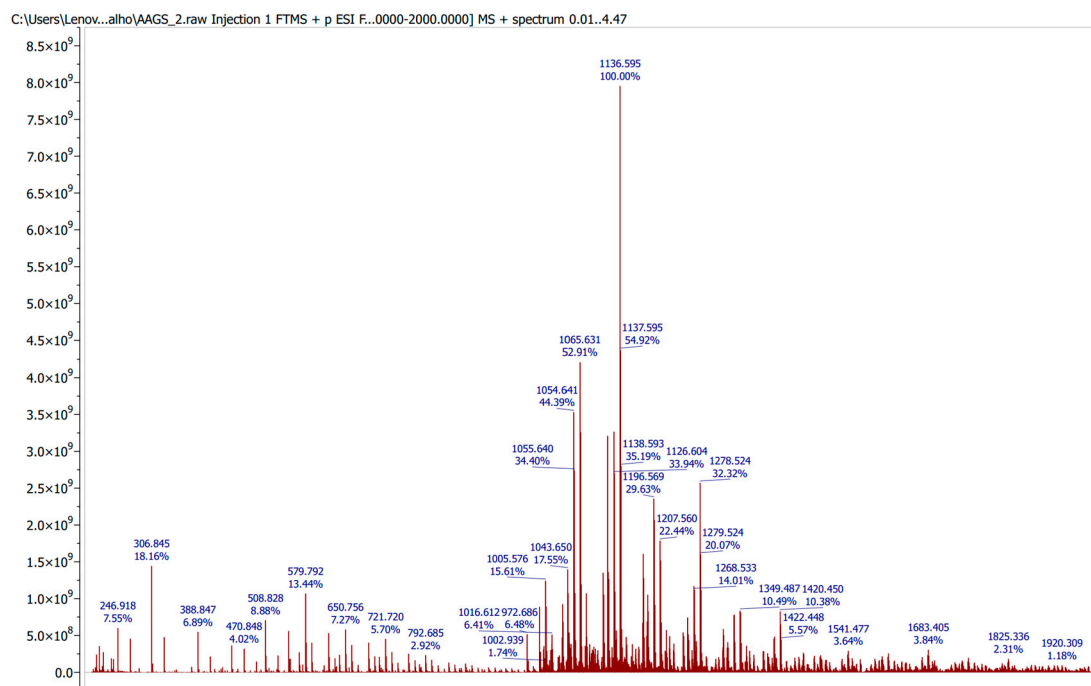


Figure S5. ESI-HRMS of 2-O- α -D-glucopyranosyl-L-ascorbic acid persulfate (AAGS). **Experimental conditions.** Analysis was done on an Orbitrap Exploris 120 mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by *Orbitrap Exploris Tune Application 2.0.185.35* and *Xcalibur 4.4.16.14*. The capillary voltage of the electrospray ionization source (ESI) was set to 3.4 kV and 2 kV for positive and negative mode. The capillary temperature was 320°C. The sheath gas and auxiliary gas flow rate were at 5 (arbitrary unit as provided by the software settings). The resolution of MS scan was 60 000. Data dependent MS/MS was performed on HCD using nitrogen as gas with collision energy settings of 30 V. The m/z range were 200-2000 Da. The resolution of SIM MS scan was 60,000. MS data handling software (*Xcalibur QualBrowser* software, Thermo Fischer Scientific) was used to search for predicted metabolites by their m/z value and MS/MS value.

Table S2 - DPPH scavenging activity of AA, AAG and AAGS given by EC₅₀ values (μM).

Compounds	EC ₅₀ ± SD (μM)
AA [#]	1.54 ± 0.21
AAG	9.15 ± 0.13
AAGS	5.28 ± 0.36

* EC₅₀: Concentration of a compound required to scavenge 50% of the initial DPPH radical.

SD: Standard deviation

[#] EC₅₀ of AA in DPPH assay (literature value) = 16.3 ± 0.569 μM (2).

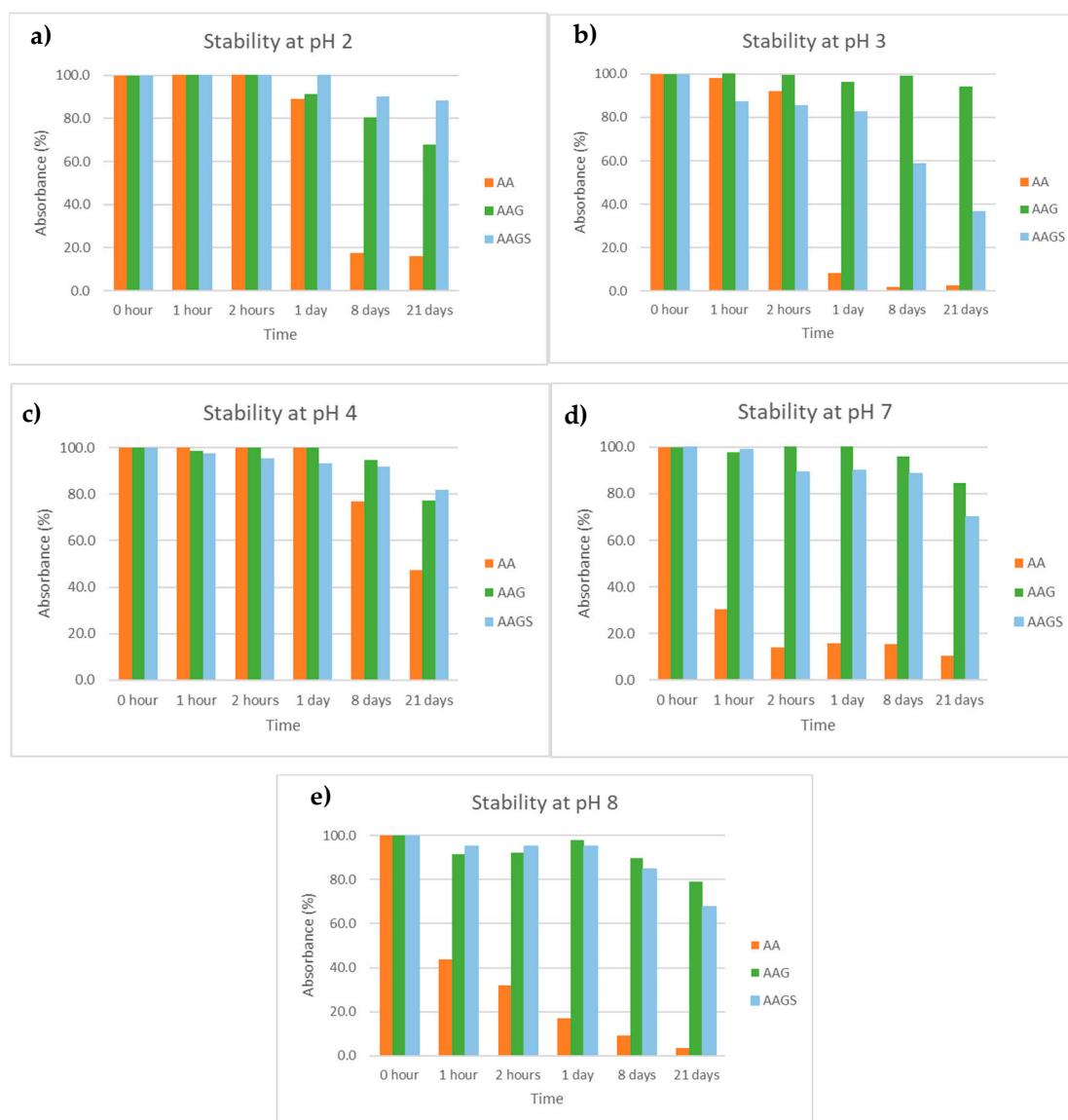


Figure S6 Stability studies at different pH for compounds AA; AAG and AAGS. a) pH 2, b) pH 3, c) pH 4, d) pH 7 and e) pH 8. Results are expressed as % of absorbance normalized to the absorbance at the time t=0, in triplicate.

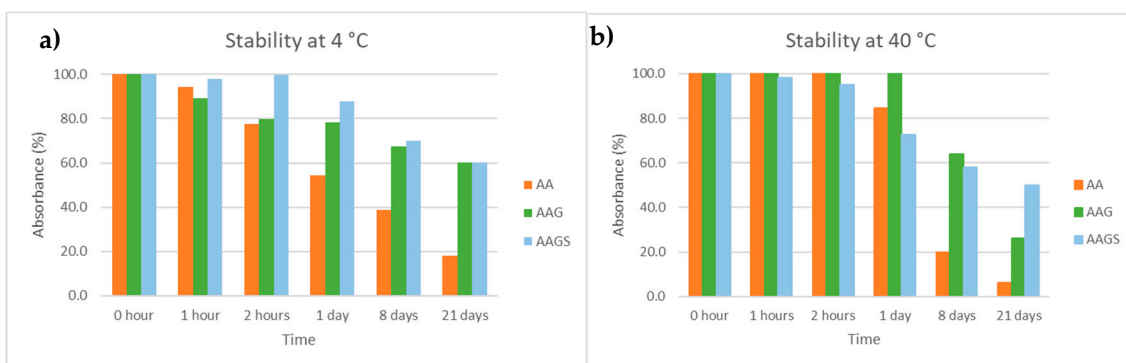


Figure S7 Stability studies for compounds AA; AAG and AAGS at pH 5, at a) 4 °C, and b) 40 °C. Results are expressed as % of absorbance normalized to the absorbance at the time t=0, in triplicate.

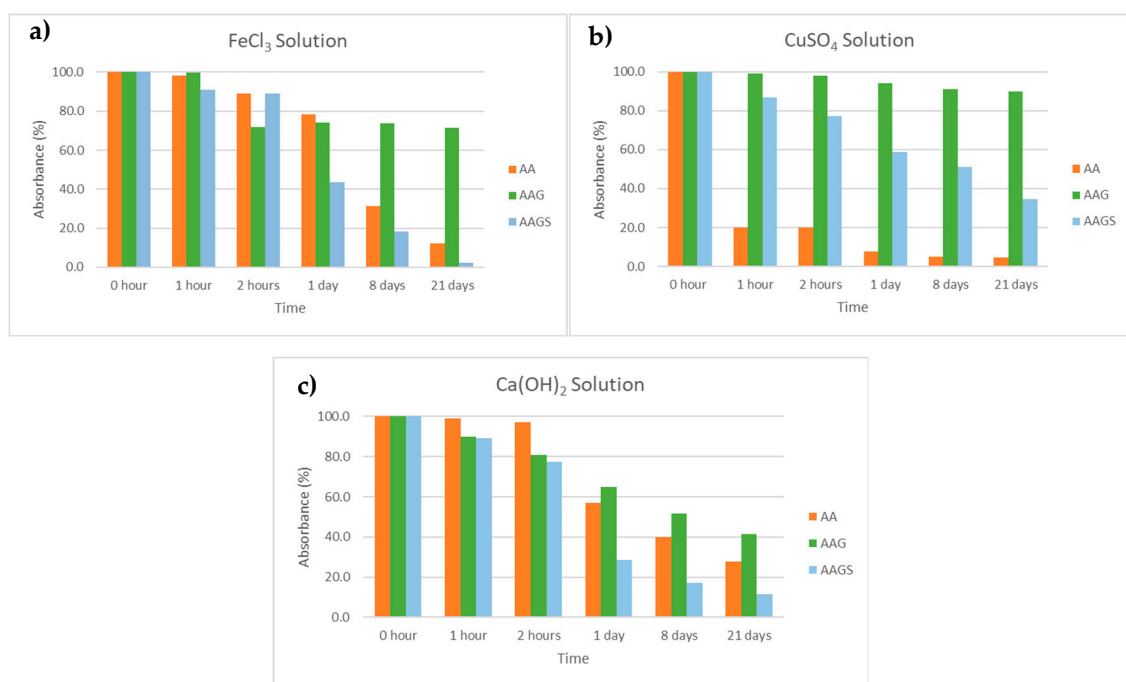
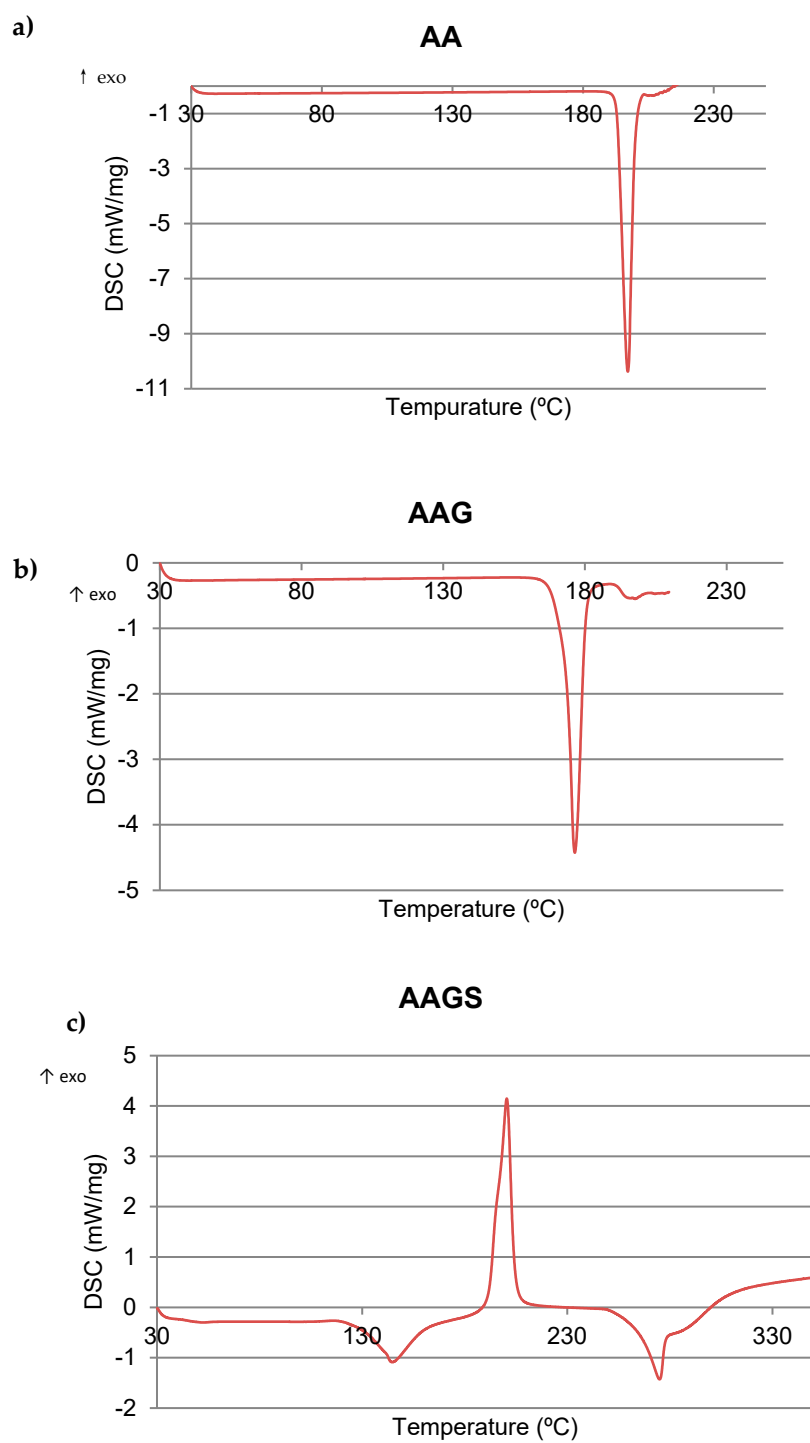
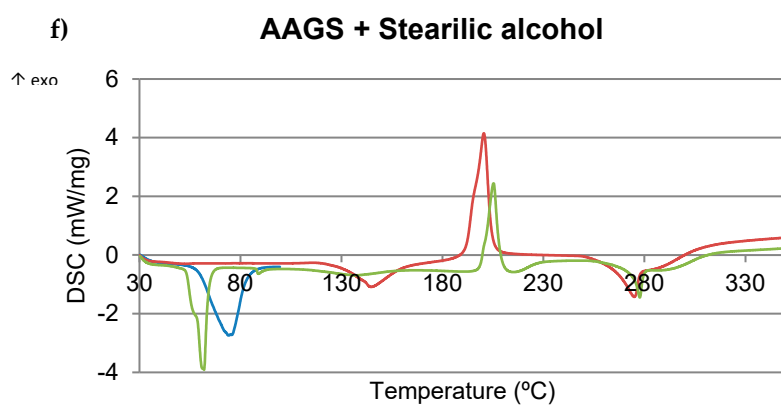
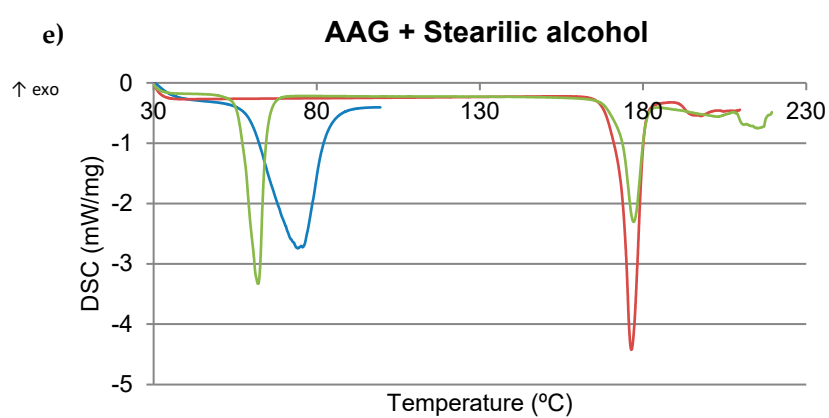
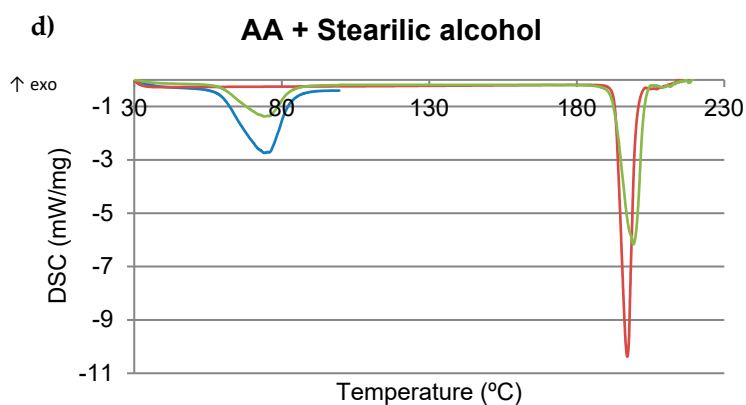
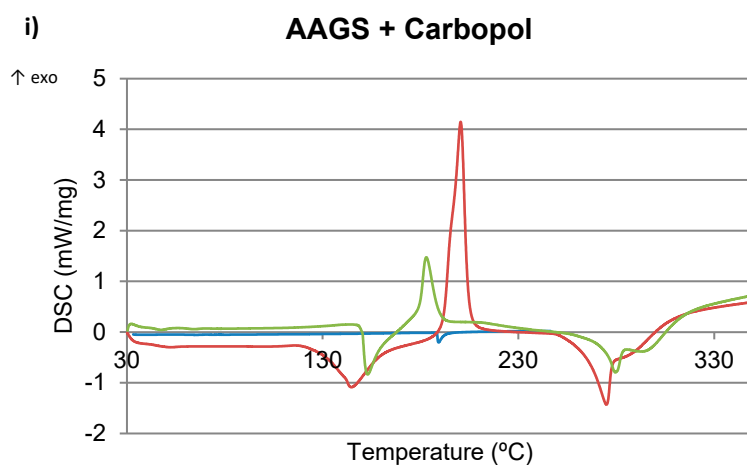
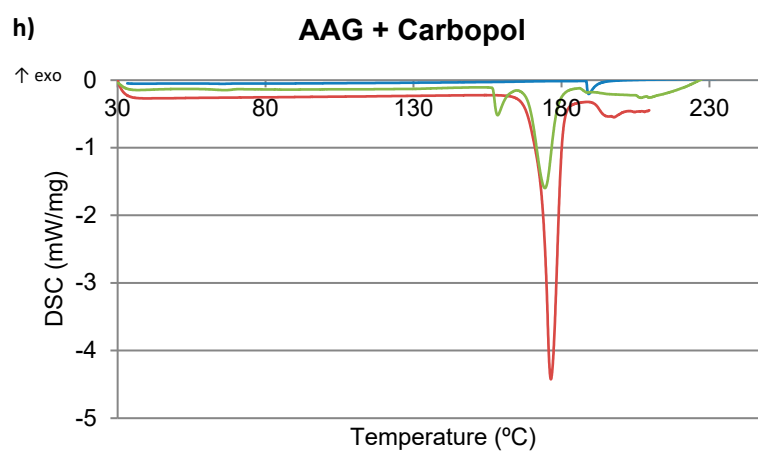
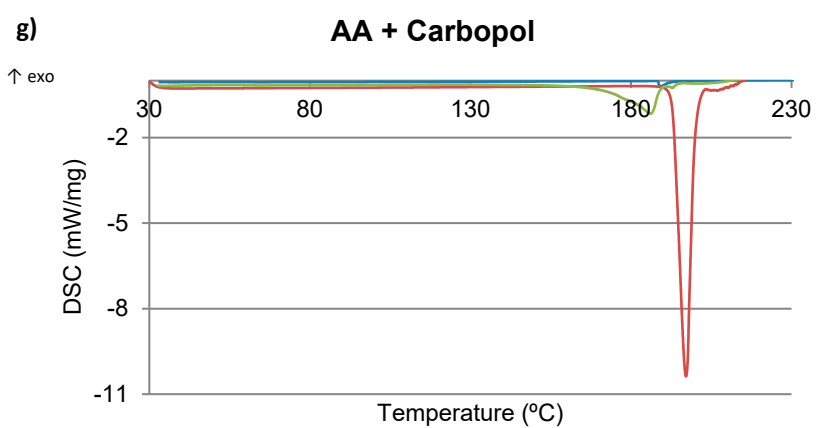
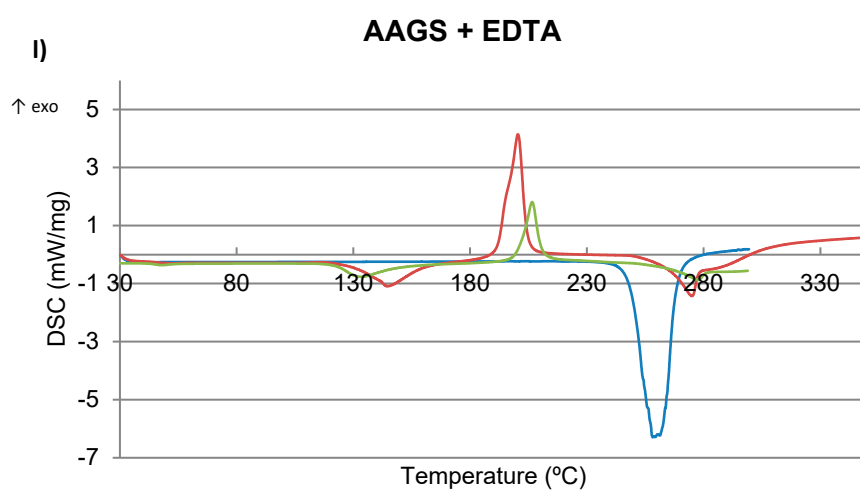
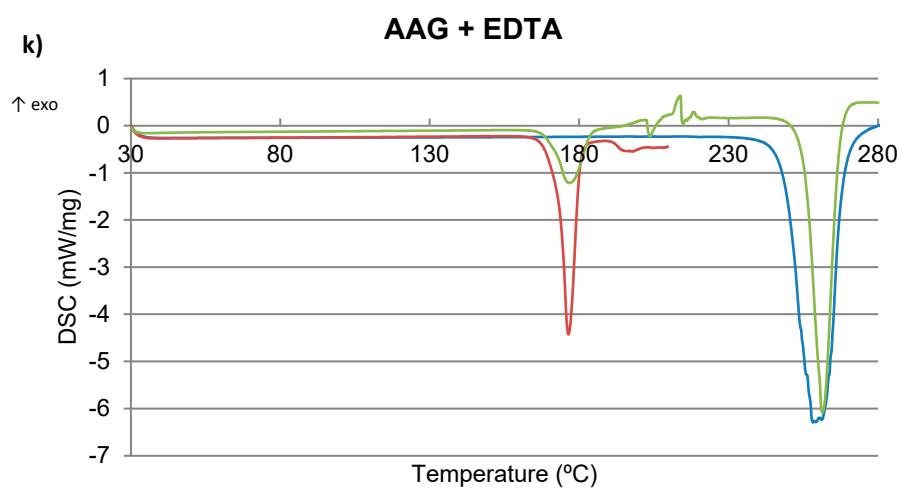
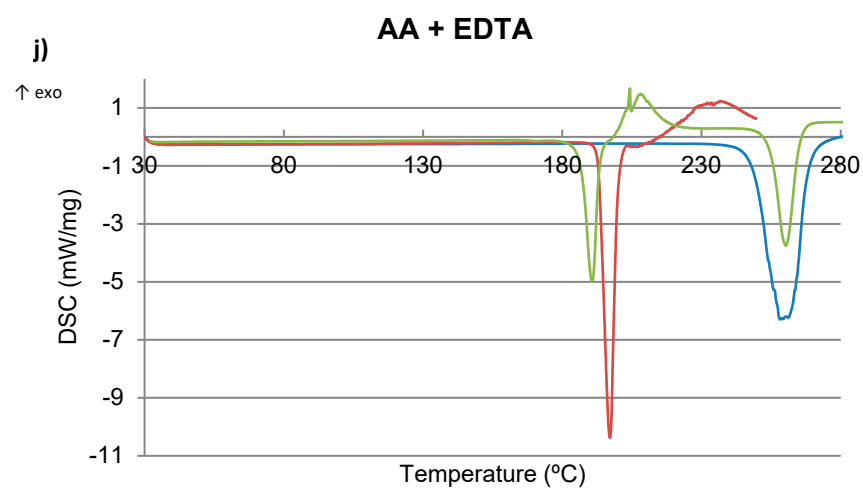


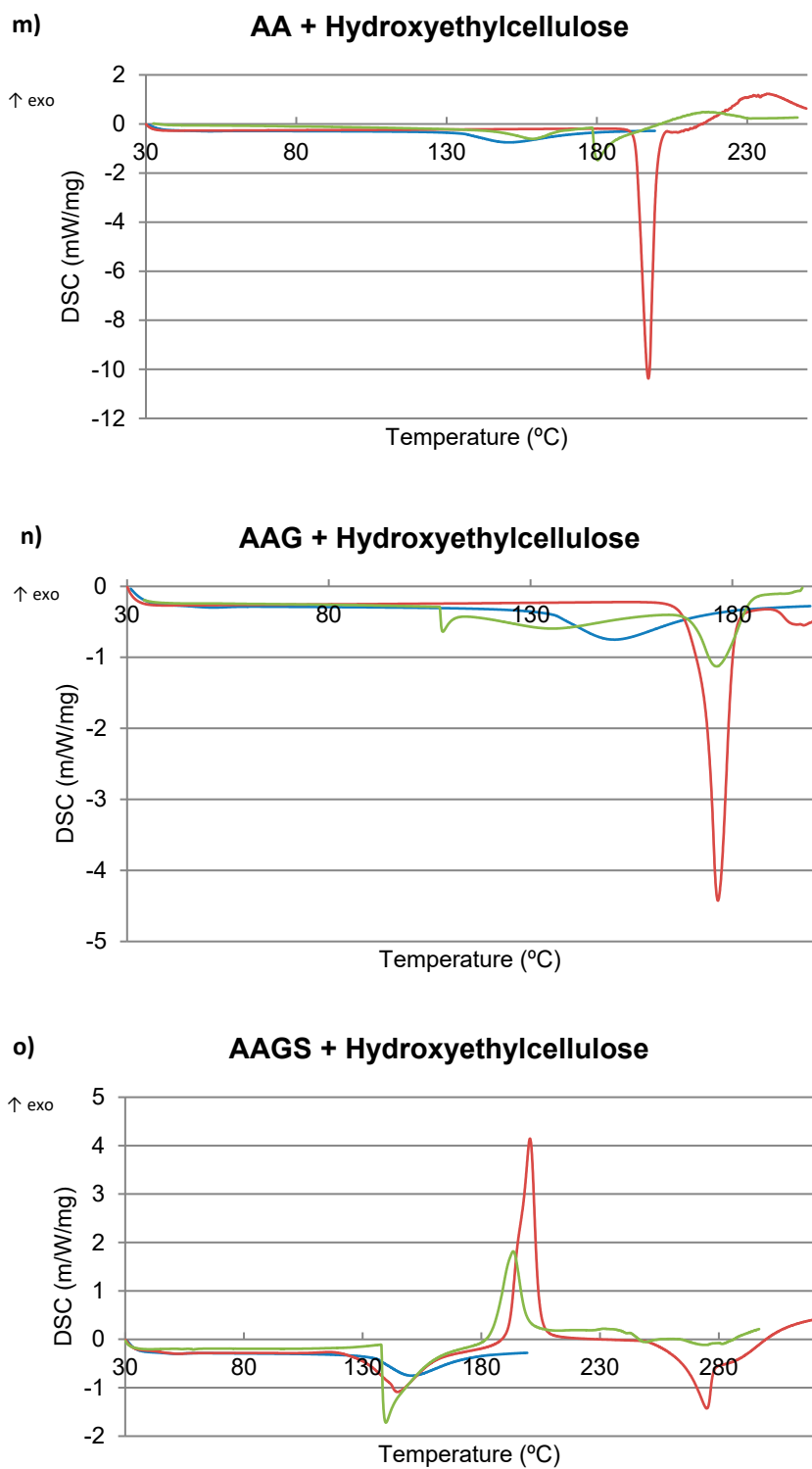
Figure S8 Stability studies for compounds AA; AAG and AAGS at pH 5, for different metals, a) Fe(III), b) Cu(II) and c) Ca(II). Results are expressed as % of absorbance normalized to the absorbance at the time t=0, in triplicate.

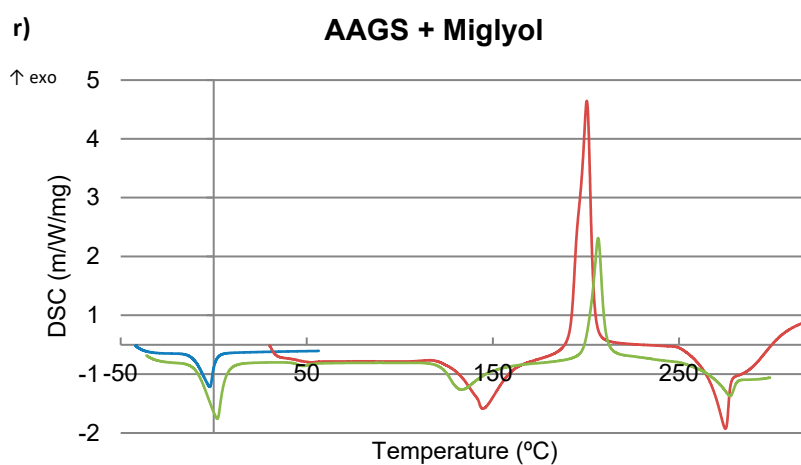
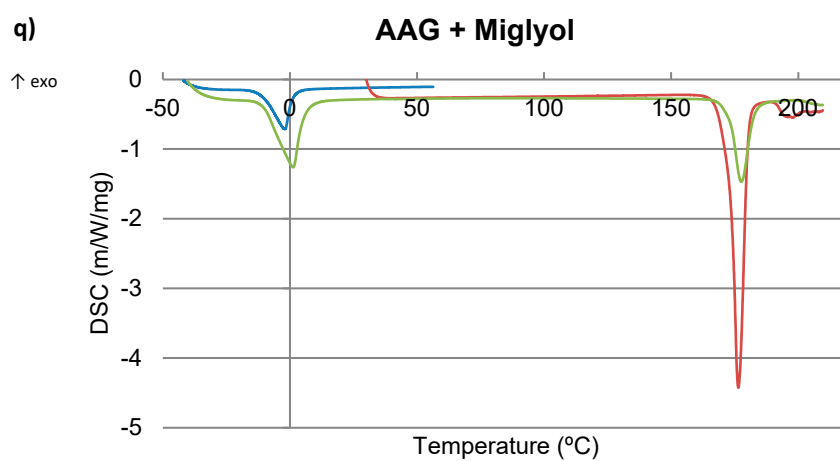
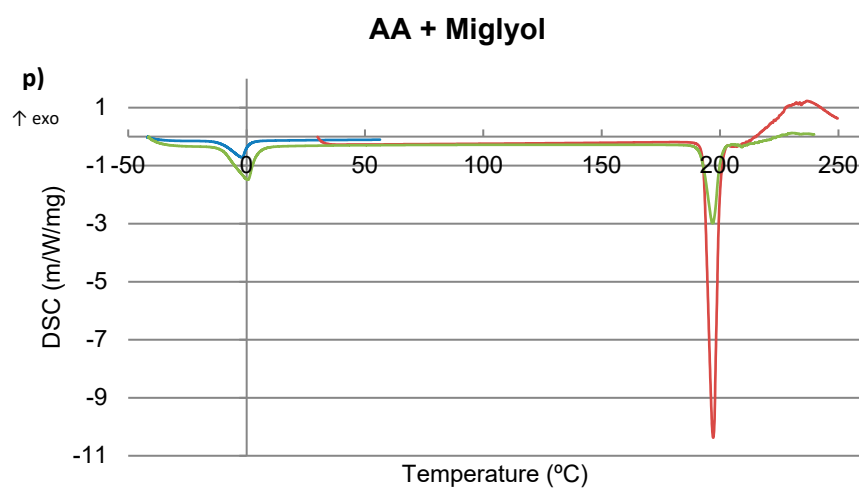


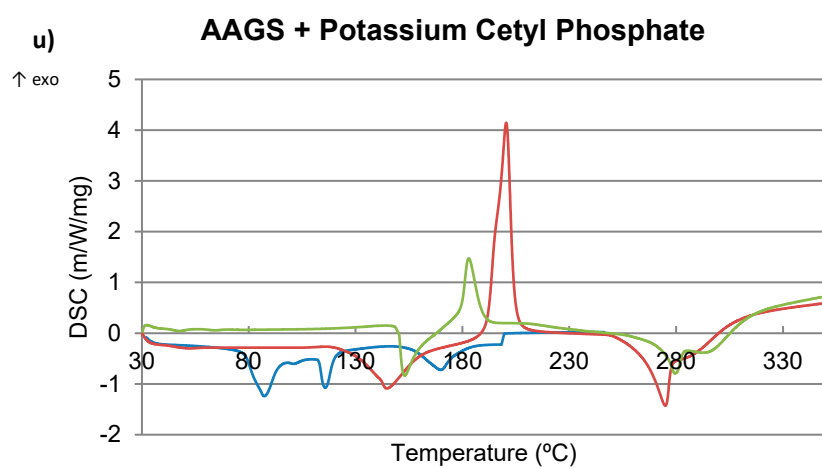
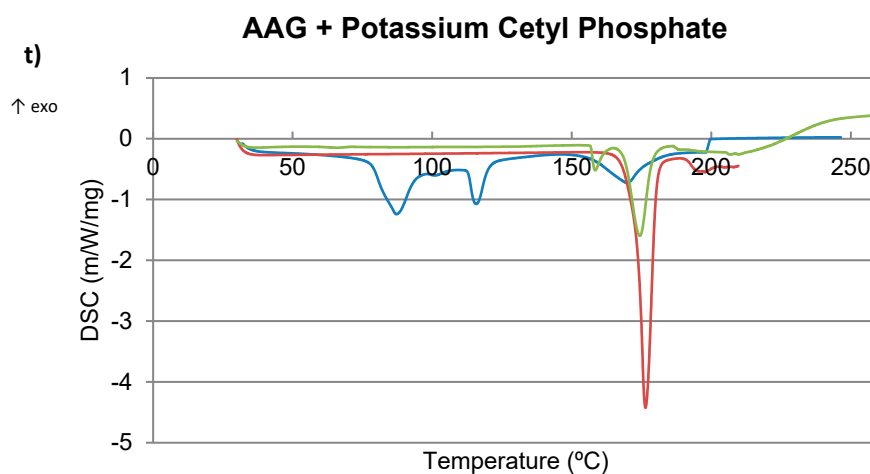
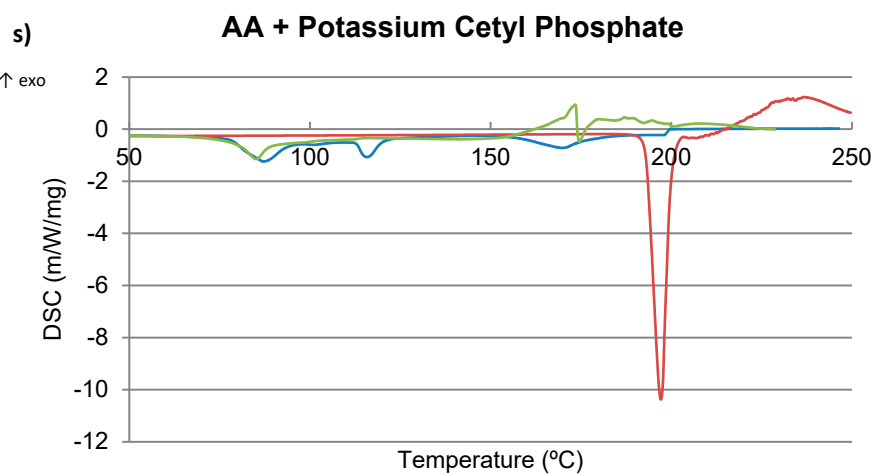












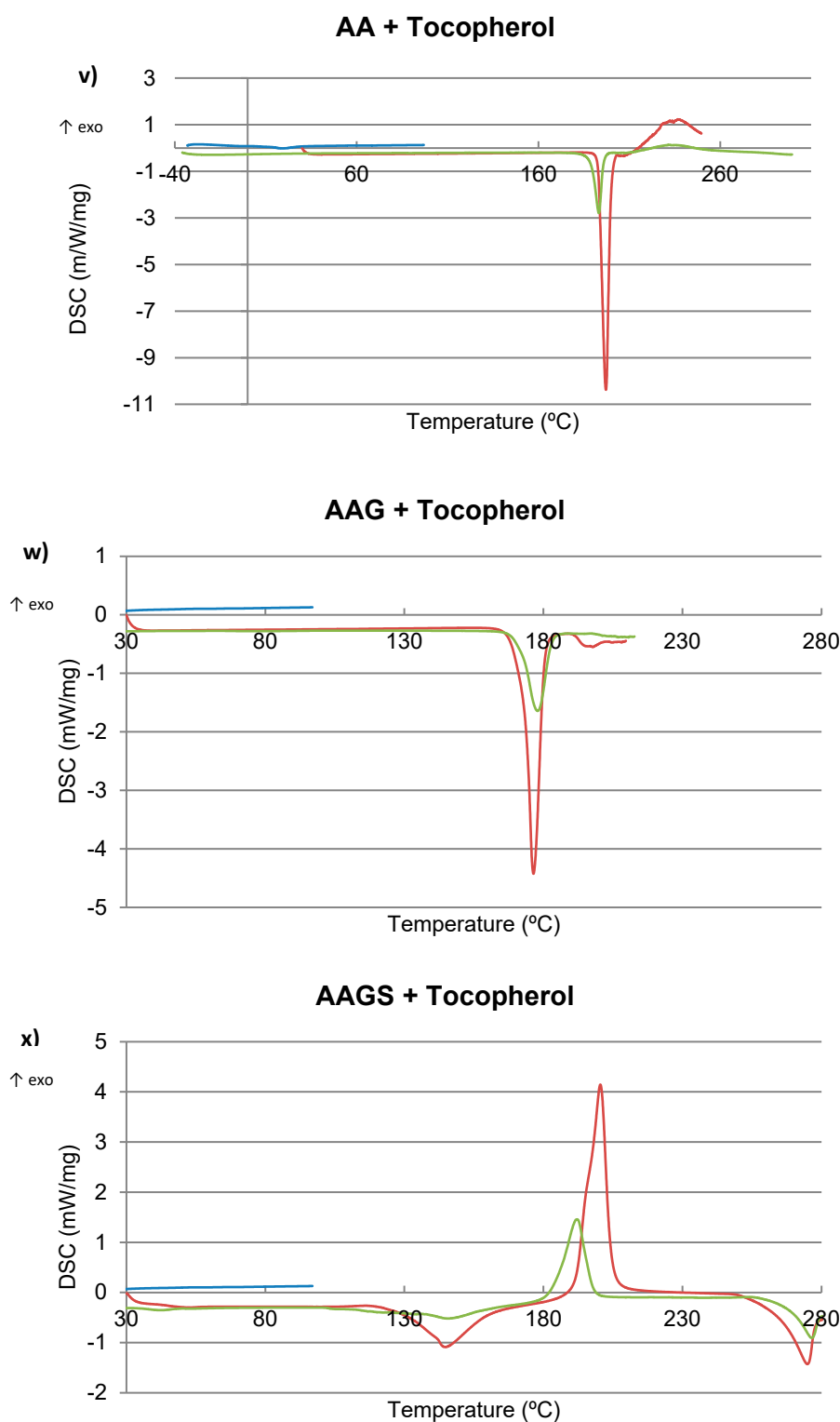


Figure S9 Thermograms of individual compounds and the respective equivalent (1:1) mixtures for the compounds a) AA, b) AAG, c) AAGS, d) AA + stearic acid, e) AAG + stearic acid, f) AAGS + stearic acid, g) AA + carbopol, h) AAG + carbopol, i) AAGS + carbopol, j) AA + EDTA, k) AAG + EDTA, l) AAGS + EDTA, m) AA + hydroxyethylcellulose, n) AAG + hydroxyethylcellulose, o) AAGS + hydroxyethylcellulose, p) AA + miglyol, q) AAG + miglyol, r) AAGS + miglyol, s) AA + potassium cetyl phosphate, t) AAG + potassium cetyl phosphate, u) AAGS + potassium cetyl phosphate, v) AA + tocopherol w) AAG + tocopherol, x) AAGS + tocopherol. Red line: antioxidant (AA, AAG or AAGS), blue line (excipients), and green line: 1:1 (w/w) mixtures.

References:

1. Correia-da-Silva M, Sousa E, Duarte B, Marques F, Cunha-Ribeiro LM, Pinto MM. Dual anticoagulant/antiplatelet persulfated small molecules. *Eur J Med Chem.* 2011;46(6):2347-58.
2. Afsar T, Razak S, Shabbir M, Khan MR. Antioxidant activity of polyphenolic compounds isolated from ethyl-acetate fraction of *Acacia hydaspica* R. Parker. (1752-153X (Print)).