PAMAM dendrimers as a nanocarrier for Nalidixic acid delivery

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INTRODUCTION

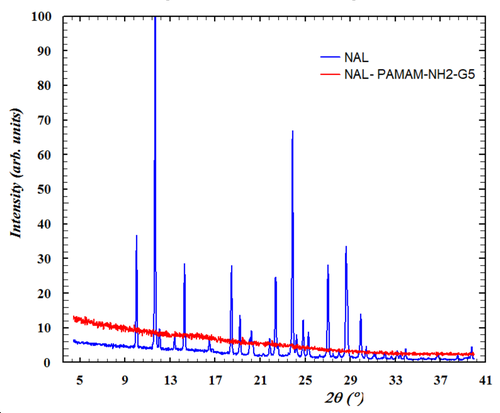
Nalidixic acid (NAL) was the first synthetic clinically applied quinolone derivative antibiotic, used in the treatment of urinary tract infections. It is the only FDA approved quinolone drug for pediatric formulation. According to BCS, Nalidixic acid belongs to the class II drugs with high permeability and low solubility (<0.1 mg/L), insufficient to dissolve the recommended dose under normal conditions. In order to improve its bioavailability, we performed a study regarding the incorporation into different types of PAMAM-dendrimers, as delivery agent by applying freeze-drying procedure. The dendritic structures were of different generations (G4 and G5) and possessed -OH and -NH_2 terminal functional groups. The loading of NAL and supramolecular complex formation with these nanostructures was confirmed by PXRD, DSC and FTIR techniques only in the case of PAMAM-NH_2-G5. These results sustain the improvement of NAL properties making the new supramolecular complex an interesting formulation for developing solid oral dosage form of enhanced bioavailability and therapeutic effect.

EXPERIMENTAL

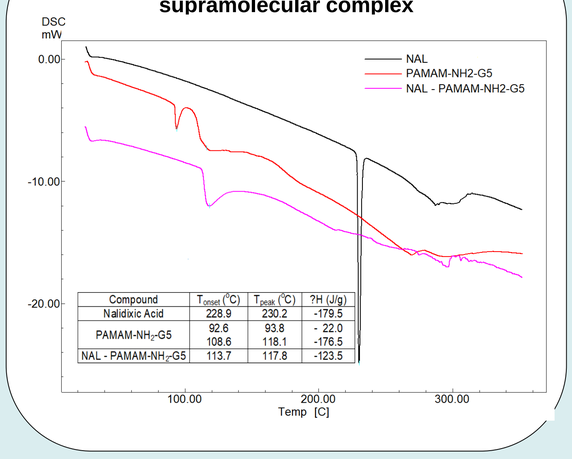
- The NAL - PAMAM-NH_2-G5 supramolecular complex was obtained by freeze drying (into an Alpha 1-2 LD type freeze dryer at -57ºC and 30 mbar for 24 hours) of the mixture prepared from NAL and PAMAM-NH_2-G5 in 20:1 molar ratio.
- X-ray powder diffraction (XRPD) measurements were made using the Bruker D8 Advance diffractometer, equipped with a Ge (1,1,1) monochromator placed in the incident beam and an ultra-fast LYNXEYE detector. The collection of diffraction patterns was performed in Bragg-Brentano geometry in reflection, in the angular range $5\ ^\circ \le 2\theta \le 35\ ^\circ$, using Cu $K\alpha_1$ radiation.
- DSC thermograms were registered with a DSC60 Shimadzu differential scanning calorimeter by heating of ~1.5 mg of the sample from room temperature up to 300ºC, in a crimped aluminum cell under flowing nitrogen flux, the heating rate being 10ºC/min, the used reference material being alumina. For data collection the Shimadzu TA-WS60 and TA60 2.1 software were employed.
- FT-IR spectra were recorded in the 4000–400 cm^{-1} spectral range with a JASCO 6100 Fourier transform-infrared spectrometer with 4 cm^{-1} spectral resolution, using the KBr pellet technique.
- 1H-NMR measurements were performed on a Bruker ADVANCE III spectrometer operating at 500.13 MHz and equipped with a broadband test head. 32 data scans with a spectral range of 10 ppm were used for data collection. In all experiments the temperature was maintained at 298 K and 5 mm diameter NMR tubes were used. The solutions were prepared in the deuterated solvent DMSO-d6.
- The 2D ^{1}H-^{1}H ROESY spectrum was obtained using 8 scans, a mixing time of 350 ms and the roesyphpr pulse sequence, having an 8K/4K dot matrix, covering a spectral range of 12 ppm to identify the interactions between NAL and PAMAM-NH_2-G5 in the formed supramolecular complex.
- The *in vitro* release of the drug from the supramolecular complex NAL - PAMAM-NH_2-G5-Nal was studied by the dialysis bag method. For this, two solutions were prepared: a control solution containing 2 mg NAL dissolved in 1 mL CH_3OH and a solution of the supramolecular complex obtained by mixing an amount of 2 mg NAL and 0.155 mL PAMAM-NH_2-G5 (ρ=0.797 g/cm3) (20: 1 molar ratio), which was made up to 1 mL with CH_3OH. The obtained solutions were introduced into cellulose dialysis bags (Sigma D9527, 14000 MCO). To determine the release of the free and complex drug, the dialysis bags were placed in 40 mL of deionized water, under continuous stirring at room temperature. The accumulation of NAL in the external solution by its diffusion from the dialysis bag was followed by measuring the absorbance at 325 nm of this solution at different time intervals (15, 30, 60 minutes for 24 hours) with a double beam JASCO V-550 spectrophotometer in the 190–400 nm spectral range, with a resolution of 0.2 nm. The absorption spectra were obtained using 1x1x4 cm^3 quartz cell.

RESULTS AND DISCUSSIONS

XRPD patterns of NAL and NAL - PAMAM-NH_2-G5 supramolecular complex

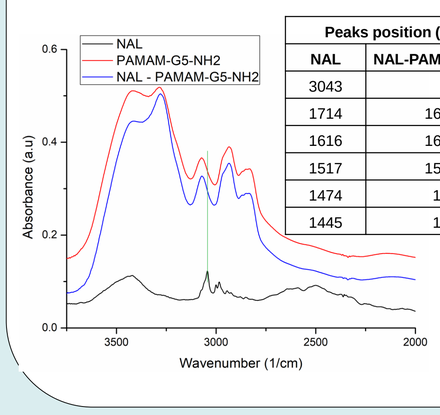


DSC traces of NAL and NAL - PAMAM-NH_2-G5 supramolecular complex

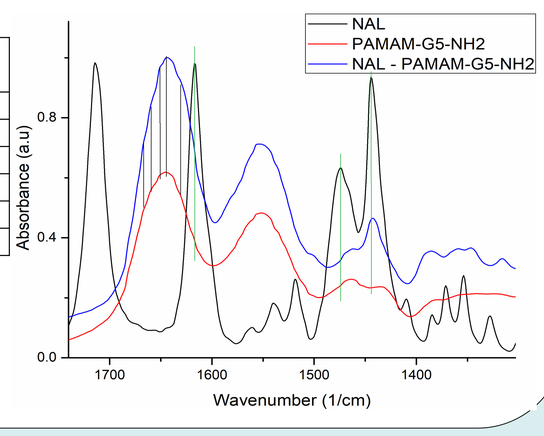


Compound	T_{onset} (°C)	T_{peak} (°C)	?H (J/g)
Nalidixic Acid	228.9	230.2	-179.5
PAMAM-NH_2-G5	92.6	93.8	- 22.0
	108.6	118.1	-176.5
NAL - PAMAM-NH_2-G5	113.7	117.8	-123.5

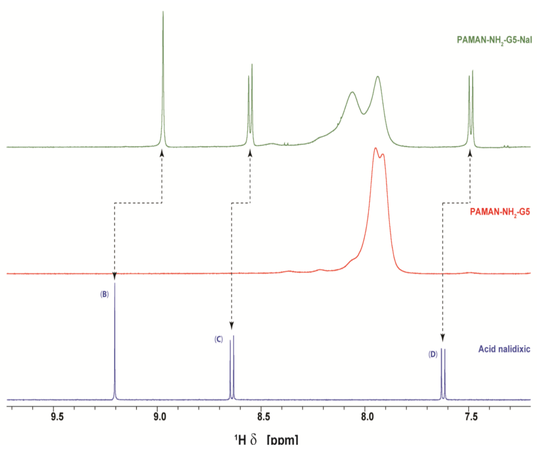
FT-IR spectra of NAL and NAL - PAMAM-NH_2-G5 supramolecular complex, spectral domains: 3750-2000 cm^{-1} and 1750-1000 cm^{-1}



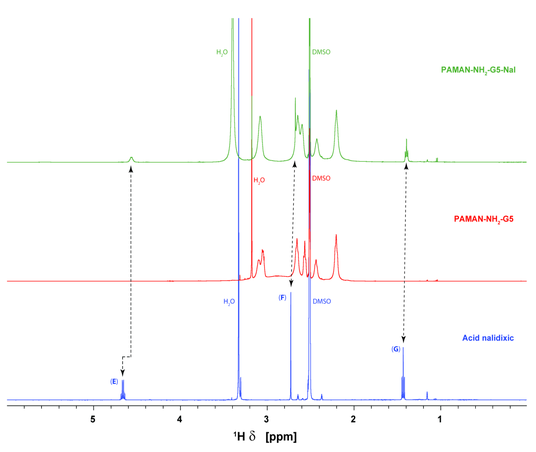
Peaks position (cm^{-1}) in		Assignments
NAL	NAL-PAMAM-NH2-G5	
3043	-	vC-H aromatic
1714	1666sh	vCOOH
1616	1619sh	vC=C ring
1517	1502sh	vC=N ring
1474	1463	vC=N ring
1445	1442	vC-O



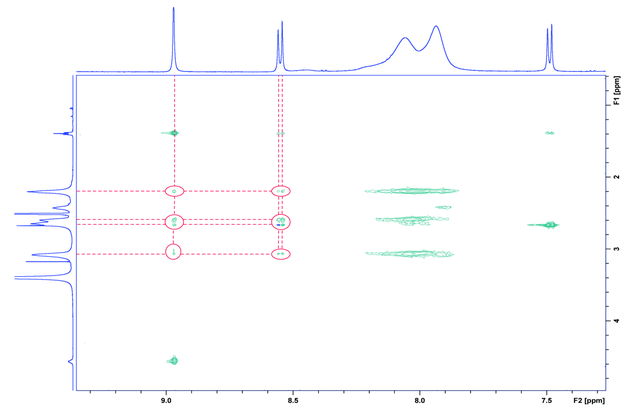
^{1}H-NMR spectra of NAL, PAMAM-NH_2-G5 and NAL – PAMAM-NH_2-G5 supramolecular complex spectral domains: 7-10 ppm and 0-6 ppm



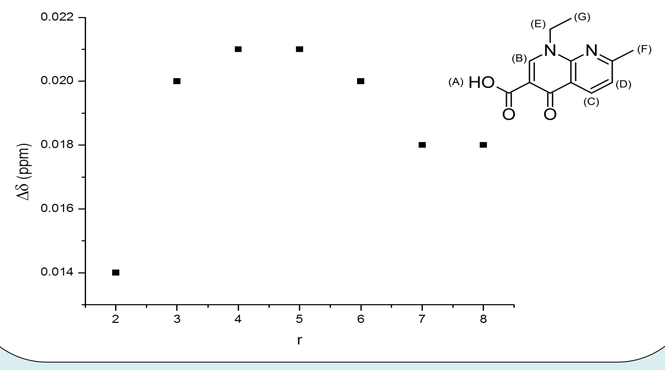
NAL δ ^{1}H (ppm)	PAMAM-NH_2-G5 δ ^{1}H (ppm)	NAL - PAMAM-NH_2-G5 δ ^{1}H (ppm)	Δδ (ppm)
9.204 (B)		8.972	0.232
8.648 (C)		8.559	0.089
8.631 (C)		8.544	0.087
	7.949	8.060	-0.111
	7.914	7.978	-0.064
7.630 (D)		7.497	0.133
7.614 (D)		7.480	0.134
4.683 (E)		4.585	0.098
4.669 (E)		4.571	0.098
4.655 (E)		4.559	0.096
4.641 (E)		4.545	0.096
	3.096	3.082	0.014
	3.052		-0.030
2.728 (F)		2.675	0.053
	2.658	2.648	0.010
	2.568	2.598	-0.030
	2.437	2.429	0.008
1.447 (G)		1.408	0.039
1.433 (G)		1.394	0.039
1.418 (G)		1.380	0.038



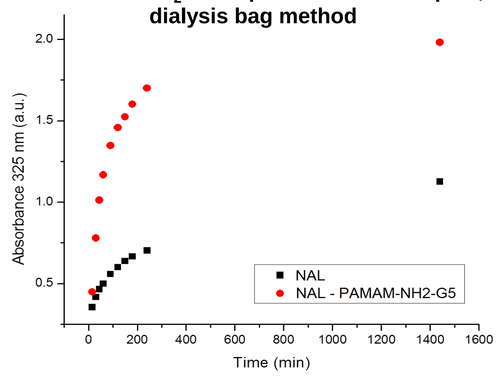
2D ^{1}H-^{1}H ROESY spectrum of the NAL - PAMAM-NH_2-G5 complex in the frequency ranges 0.5-5ppm [F1] and 7.3-9.3 ppm [F2], the interactions between NAL and PAMAM-NH_2-G5 in the complex are indicated by red circles



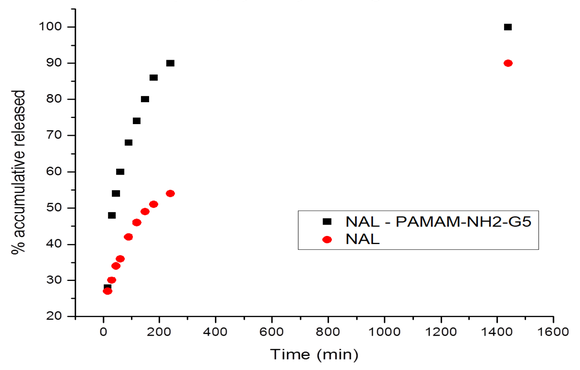
Job plot for the supramolecular complex NAL - PAMAM-NH_2-G5, built on the chemical shifts of the hydrogen atom C of Nalidixic acid



Variation of absorbance over time at the *in vitro* release of NAL from the free NAL solution and from the NAL - PAMAM-NH_2-G5 supramolecular complex, by dialysis bag method



In vitro release of NAL from the free NAL solution and from the NAL - PAMAM-NH_2-G5 supramolecular complex, by dialysis bag method



CONCLUSIONS

- The supramolecular complex NAL - PAMAM-NH_2-G5 was obtained by encapsulating the antibiotic Nalidixic Acid in a PAMAM type dendrimer of generation 5, with 128 peripheral amino groups (PAMAM-NH_2-G5) by non-covalent interactions.
- The supramolecular complex NAL - PAMAM-NH_2-G5 formation was confirmed by all three used analysis methods : XRPD, FTIR and DSC.
- Based on the 2D 1H-1H ROESY NMR analysis it was conclud that interactions appear between the hydrogen atoms B (9.204 ppm) and C (8.648 and 8,631 ppm) of NAL with the hydrogen atoms of PAMAM-NH_2-G5 from 2.437, 2.568, 2.658 and 3.052, 3.096 ppm.
- The supramolecular complex stoichiometry between NAL and PAMAM-NH_2-G5 was established by ^{1}H-NMR as 16:1.
- The *in vitro* release of NAL from the supramolecular complex was evaluated by UV-Vis spectroscopy using the dialysis bag method and it was observed that the release of NAL contained in the NAL – PAMAM-NH_2-G5 complex reached a percent of 90% compared to 54% of pure drug within 6 hours.
- These results sustain the improvement of NAL properties making the new supramolecular complex an interesting formulation for developing solid oral dosage form of enhanced bioavailability and therapeutic effect.

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