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Starburst PAMAM dendrimers (-NH₂, -OH) G4 effects on *E. coli* growth monitored by microcalorimetry.

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Palabras clave: PAMAM, dendrímeros, microcalorimetría, *E.coli*, efecto inhibitorio y estimulante

Key words: PAMAM, dendrimers, microcalorimetry, *E.coli*, inhibitory and stimulatory effect.

Dendrimers are symmetrical and spherical macromolecules, comprising a relatively dense shell composed of a core, branching sites, and terminal groups that usually form a well defined surface.¹ A great active surface tribute to these structures function as special carriers, able to transport high concentrations of drugs and substrates. In the biomedical field dendrimers had been used for drug delivery,^{2,3} gene therapy,^{4,5} antigen conjugates,^{6,7} NMR contrast agents⁸ and synthetic vaccines.⁹

Poly (amido amine) PAMAM dendrimers are obtained by the iterative branching of β -alanine repeat units. Poly (amido amine) dendrimer based silver complexes and nanocomposites proved to be effective antimicrobial agents *in vitro*,¹⁰ this activity was comparable or better to those of silver nitrate solutions. This effect was attributed to the very high local concentration of nanoscopic size composite particles that are accessible to microorganisms.

Microcalorimetry¹¹ provides a means for revealing obscure metabolic phenomena of growing bacterial cultures. In general biological processes are accompanied by heat effect, the precise magnitude of which may be of considerable practical and theoretical importance. Such measurements may give valuable information concerning the stability and structure of molecules. Thus by monitoring the heat effects with sufficiently sensitive calorimeters, it is possible to study the metabolic process of living cells.¹²

In the present work an LKB-2277 Bioactivity Monitor was used to determine the effect of PAMAM dendrimers (-NH₂, -OH) G4 on thermogenic curves of the growth metabolism of *E. coli* (25922).

Microcalorimetric experiments were performed in a LKB-2277 Bioactivity Monitor (LKB, Sweden) in ampoules measuring mode at 37 °C, the amplifier was setup at 1 000 μ W. The performance of this instruments and the details of its construction have been described elsewhere.^{13,14}

Eighteen – twenty-four hours cultures of standard *Escherichia coli* ATCC 25922 grown in Müller-Hinton Agar were used in the experiments. DETID-Ec culture medium (DIRAMIC, Cuba) was used. (Figure 1) It contains Müller-Hinton Broth, 4-methylumbelliferyl- β -D-glucuronide, L-tryptophan and a linear polysaccharide polymer. Dendrimers PAMAM-NH₂ G4 y PAMAM-OH G4, were faintly added. The culture medium is sterilized in autoclave at 121 °C for 15 min, the pH of the medium was 7.3.

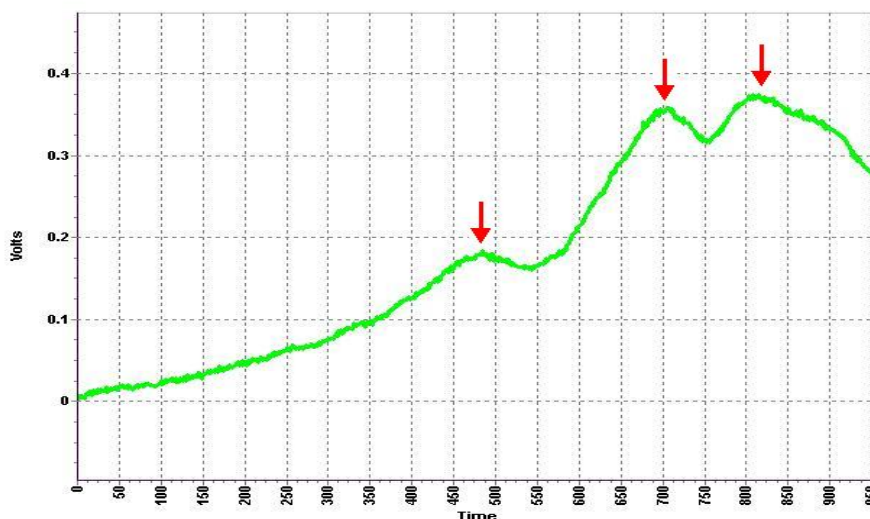


Fig. 1. Thermogram of *E. coli* growth during 4 h in DETIDEC medium (Control). There are three peaks (∇), probably related to culture medium complexity.

One hundred fifty microliters of 0.5 McFarland *E. coli* suspension were inoculated in vials with 4.5 mL of DETID-Ec and dendrimers, and then 500 μ L transferred to microcalorimetric test ampoules. The reference ampoules were charged with 500 μ L of sterile broth. Ampoules were held in the equilibrating position for 20 min and then were inserted into the ampoule holder in the thermophile zone. The calorimetric measurements were registered in an acquisition program design for this purpose and the calorimetric curves (thermograms) analyzed after 24 h of acquisition.

A commercial data acquisition card PCL- 711B for the communication of the microcalorimeter with a personal computer has been used. This card has 8 multiplexed A/D conversion channels with a resolution of 12 bits. Software for the data acquisition of the four channels was designed. The system allows setting the acquisition time, to save in independent files the data corresponding to each channel, to show graphically the results and to calculate the slope, the area under the curve and the maximum amplitude.

It had been established that continuous thermal monitoring of biological system provide a means for detection of subtle changes in metabolism.¹⁴ Most organisms typically produce maximum outputs and return to base line in 5 to 7 h thereafter; some profiles are identifiable within 3 h of onset of heat production. These results shows a different behavior of *E. coli* 4 h thermogram, depending of the dendrimer included (Table 1). In relation with control reference, PAMAM (NH₂) G4 dendrimer, decreased the slope (heat rate) by 23.58 % and also the maximum output by 48.8 %, the time for maximum output was similar to reference, evidencing a clear growth inhibition for *E. coli*. On the contrary, PAMAM (OH) G4 dendrimer, increase the slope (heat rate) by 78.3 %, and also the maximum output by 17.4 %, the time for maximum output decreased by 11.2 %, all these results evidence a clear growth stimulation for *E. coli*. (Figure 2)

Table 1. Result of main components of thermogram of *E. coli* in different conditions.

Control	Slope (heat rate)	Maximum output (MO) (V)	MO time (h)	Thermogram area
PAMAM (NH ₂) G4	1.06 (0.23)	0.4504 (0.11)	3.48 (0.2)	186.35 (21.3)
	0.8 (0.03)	0.2306 (0.06)	3.31 (0.05)	81.62 (2.39)
PAMAM (OH) G4	1.89 (0.14)	0.5289 (0.66)	3.09 (0.005)	154.85 (0.68)

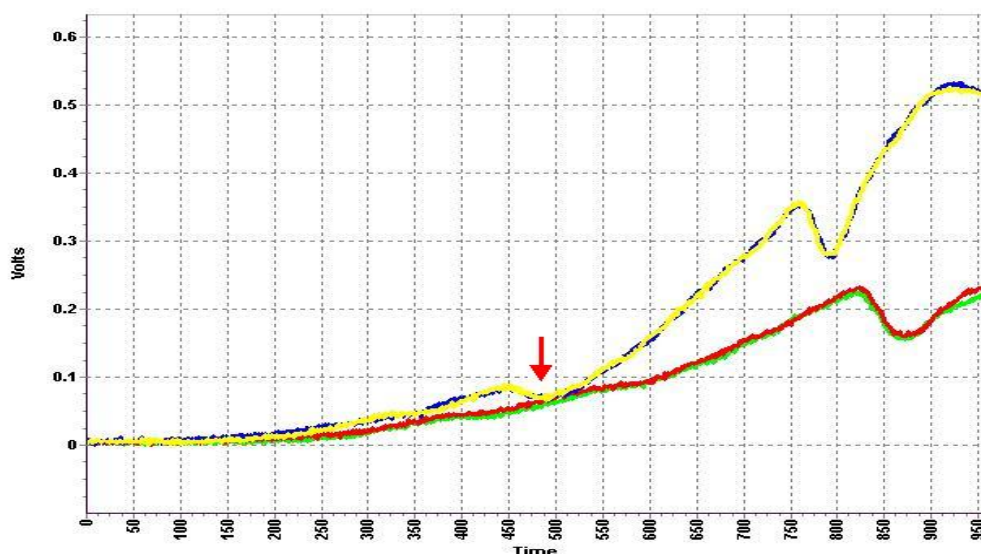


Fig. 2. Thermogram of *E. coli* growth during 4 h in the presence of starburst PAMAM dendrimers (NH₂, OH) G4, [green and red channel, (NH₂)], [yellow and blue channel, (OH)].

After 2 h of incubation (↓), the heat rate of channels with PAMAM dendrimer (OH) G4 is greater than channels with PAMAM dendrimer (NH₂) G4.

The inhibitory effect of PAMAM (NH₂) G4 dendrimer could be explained by a decrease of microbial cell viability, or by a metabolic inhibition of microbial cells, according to the similarity of the time for expression of maximum output with control reference, the explanation by a metabolic inhibition looks more reliable.

The stimulatory effect of PAMAM (OH) G4 dendrimer could be explained mainly by the predominance of both, de-repression effect, or by a more efficient use of substrates. The significant increment of slope and decrease of time for maximal output, observed in relation with control reference, makes better the explanation by a more efficient use of substrates. (Figure 3)

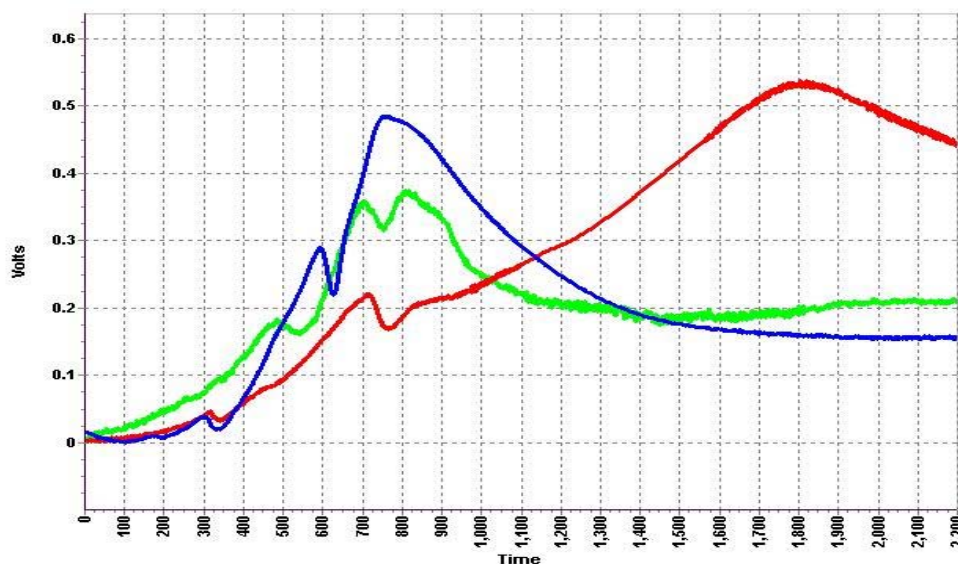


Fig. 3. Thermogram of *E. coli* growth during 9 h in the presence of starburst PAMAM dendrimers (NH_2 , OH) G4, [red channel, (NH_2)], [blue channel, (OH)].

At this time it's possible to see the difference in the three conditions tested, specially the tendency of PAMAM dendrimer (NH_2) G4 to compensate the maximum output seen in PAMAM dendrimer (OH) G4.

Still little is known about biological properties of dendrimers, in this study the macromolecules hold promise as potential tools for studying multivalent interactions with the growth media used for *E. coli*. The main components, for example inorganic ions, small molecules and macromolecules, could change dramatically by this interaction. It is possible that cationic species, that usually play an important role for growth, could be in front of a competitive scenario in the presence of polycations, like PAMAM (NH_2) G4 dendrimer, been possible to affect the electron transfer, explained some results obtained by microcalorimetry. Evidently, a great deal of work is needed in future for demonstration of some effects.

Results evidence in all components the clear stimulant effect of PAMAM (OH) G4 and the inhibitory effect of PAMAM (NH_2) G4.

CONCLUSIONS

It had been demonstrated that microcalorimetry could be used for evidence the effect of dendrimers on microbial growth.

Small changes in same macromolecules could produce significant different effects on microbial growth.

Results strongly suggest that inhibitory or stimulatory effects of PAMAM (NH_2 , OH) G4 dendrimers, relies more on metabolic effects than on the decrease of cell viability.

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