Pattern formation in the Belousov–Zhabotinsky-PAMAM dendrimer system

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The Belousov–Zhabotinsky reaction was studied under the influence of nanometric confinements induced by a complex polymer, the PAMAM-G4 dendrimers. They are well-defined in both molecular weight and architecture and are capable of molecular inclusion, making “unimolecular active micelles”. The effect of such nanocompartments in the BZ reaction is analyzed by changing both the excitability and the concentration of the dendrimer, obtaining a wide range of behaviours, ranging from stationary Turing-like patterns to time dependent structures, such as jumping waves or packet waves.

Introduction

Pattern formation in Nature constitutes a crucial problem for understanding life in general. In fact, several mechanisms have been proposed aiming to understand wave propagation in such systems as well as stationary patterns. Examples of these behaviors are almost ubiquitous in Nature; waves in heart tissue, waves in heart tissue, intracellular media,1 the retina,2 social amoeba,3 catalytic surface reactions4 or the Belousov–Zhabotinsky reaction.5–10 The Turing mechanism11,12 is believed to explain the problem of symmetry breaking in biological morphogenesis underlying cell differentiation in the first days of development of early embryos13 or the pigmentation of the skin of animals.14–16 Just to name a few examples.

Nevertheless, most of the living systems that exhibit pattern formation in a spontaneous way are not continuously distributed but rather the active part is confined to a discrete distribution of cells. Recently, Vanag and Epstein dispersed the reactants of the Belousov–Zhabotinsky (BZ) reaction in an aerosol OT (AOT) water-in-octane microemulsion17 producing nanoscale water droplets that can communicate both through the oil phase (via oil-soluble intermediates of the BZ reaction, like Br2) and by collisions between reverse micelles. A remarkable set of new patterns has been observed in this system pointing out the fact that the discrete nature of these systems plays a crucial role in the pattern formation mechanism.

In the present paper, we propose a new kind of confinement by using polyamidoamine (PAMAM) dendrimers18 on the Belousov–Zhabotinsky (BZ) reaction. These materials, so-called ‘cascade molecules’, have already found use as drug candidates for receptor–ligand interactions, drug carriers for conferring bio-survival, membrane permeability and targeting, and have found wide use as carriers for vaccine antigens as well. Furthermore, dendrimers have proven very useful as scaffolds in the design of biosensors, imprinting scaffolds and artificial receptors (specific host–guest interactions). This will add a new degree of sophistication to well-known drugs and reagents, as well as creating entirely new classes of drugs and bioactive substances based on these macromolecular, yet beautifully simple, structures. Dendritic structures are, despite their large molecular size, structurally well-defined, with a low polydispersity in comparison with traditional polymers. They are well-defined in both molecular weight and architecture and are capable of molecular inclusion, making “unimolecular micelles”.18–20 Molecules have been encapsulated in a non-covalent fashion within dendrimers but this does not mean that dendrimers have a permanent and rigid cavity. Most dendrimers are flexible enough to accommodate inclusion guests, indeed solvent molecules, but they are also capable of rearranging themselves with a significant volume collapse when the solvent is removed. This collapse may leave guest molecules trapped inside the dendrimer, especially if favorable interactions exist, as in some “dendritic micelles”,21–23 or if the dendrimer structure has been rigidified to prevent their escape as in the “dendritic box”.24,25

The presence of dendrimers within the Belousov–Zhabotinsky reaction is systematically analyzed and the different macroscopic structures observed are reported below. First, the materials and setup used for this experimental study are described. The different structures observed are related in the following section and the effect of the dendrimer on the reaction is described within the context of pattern formation mechanisms.
Experimental setup and methods

In this work, we show the behavior of the liquid BZ reaction in the presence of polyamidoamine (PAMAM) dendrimers generation 4 (named G4 throughout this paper). These polymers, extensively studied in the latest years, are commercially available and their properties are well known. These dendrimers are amphiphilic with 126 amine groups located as follows: 64 on the surface and an additional 62 inside the dendrimer structure. Its molecular weight is 14415 and 4.5 nm is the molecular diameter. The molecular diameter is increased up to 5.6 nm when the dendrimer is immersed in the low-pH and high-ionic-force BZ reaction. A scheme of the G4 structure is plotted in Fig. 1. Note that many anchoring locations are in the dendrimer structure that are actually used as containers to transport other substances within different organisms. It can also accumulate positive charges by protonation of the primary amines at the rim and the tertiary amines in the inside when immersed in the low pH BZ reaction.

The BZ reaction was carried out at constant temperature (21 °C) by mixing PAMAM G4 (previously evaporated at room temperature in order to completely eliminate methanol) with the following reactants, sulfuric acid, malonic acid and sodium bromate, in a beaker under stirring. Once the solution is well mixed, then ferroin is added and the reaction starts.

Malonic acid, sodium bromate, sulfuric acid, sodium bromide and ferroin (Fe(phen)SO₄) were commercial grade reactants (SIGMA) and used without further purification. The dendrimers PAMAM G4 (generation 4) were purchased from Aldrich Chemical Co. as a 10 wt% solution in methanol and evaporated under inert atmosphere prior to dissolving in other solvents. Experiments are done with distilled deionized water (Millipore filtration system). All dendrimer solutions prepared were kept at room temperature for 2 h and de-aerated prior to measurements, except for fluorescence measurements, where spectra were recorded immediately after mixing.

The control parameters for our research are the concentration of polyamidoamine (PAMAM) dendrimers (G4) and the excitability of the medium, basically controlled by the ratio [H₂SO₄][NaBrO₃]/[MA]. The concentrations of all the chemicals used are summarized in Table 1.

The BZ reactants, without the dendrimer, are such that the parameters lay within the oscillatory regime. This behavior is easier to find experimentally with this system.

![Fig. 1](image-url)  
**Fig. 1** Schematic structure of the dendrimers used, poly(amidoamide) (PAMAM), dendrimers generation 4 (G4). These dendrimers are amphiphilic with 64 amine surface groups and with additional 62 amine groups located inside the dendrimer structure.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Different concentrations used for the reactants in the BZ-G4 system</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Minimum concentration used</td>
</tr>
<tr>
<td>[PAMAM G4]</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>[H₂SO₄]</td>
<td>0.2 M</td>
</tr>
<tr>
<td>[NaBrO₃]</td>
<td>0.2 M</td>
</tr>
<tr>
<td>[MA]</td>
<td>0.1 M</td>
</tr>
<tr>
<td>[Ferroin]</td>
<td>0.5 mM</td>
</tr>
</tbody>
</table>

The BZ/PAMAM fluid system obtained as described is finally poured in a thin layer of 1 mm height into a 5 cm diameter Petri dish and allowed to evolve. Typical experiments last for up to 70 min. During this time the characteristics of the patterns observed remained almost unchanged. This BZ recipe was chosen because of its large duration and high contrast. In order to avoid contamination of the surface and minimize evaporation of reactants, the Petri dish was covered by a glass plate. As the patterns exhibited by the system are optically visible, BZ-G4 experiments were recorded from above with a CCD camera by a DVD for post processing.

It is important to note that there is no methanol in the PAMAM G4 prior to its use in the BZ reaction; this avoids pattern formation due to evaporation as reported elsewhere.

Thus, we consider diffusion as the only transport phenomena involved in our system. The reason convection is not present is that the geometry and dimensions prevent flows from appearing. In particular, the small vertical dimension, 1 mm, makes the boundary layer to cover the whole system. One can estimate the Reynolds number so the flow, if it existed, could be considered laminar. Thus, we can use the Blasius boundary layer model in order to calculate the boundary layer thickness. Rough estimates show that in order to have a boundary layer thickness smaller than 1 mm, flow velocities in the system should exceed 100 mm s⁻¹. Consequently, we believe the boundary layer covers the whole system and its field velocity is given by the boundary, i.e., it is at rest. Nevertheless, the reactor was in contact with a large air thermostated reservoir that kept the temperature constant in the reaction avoiding large gradients of temperature within the system.

Control experiments were also performed with inert particles (Black CromoSpheres, Brookhaven Instruments, mean diameter of 502 ± 24 μm with a density of $\rho \approx 1.06$ g cm⁻³) and no flow was observed for the parameters considered in this manuscript. Thus we believe that the only transport mechanism involved in this system is diffusion rather than convection.

Complementary measurements were done by fluorescence and visible spectroscopy. PAMAM G4 characterization experiments were made by uorescence spectra of a 0.7 mM solution in a sulfuric acid solution of pH = 1 with and without the BZ reactants (in the absence of ferroin). They were performed on a Perkin Elmer LS55 fluorescence spectrophotometer $\lambda_{\text{excitation}} = 390$ nm and $\lambda_{\text{emission}} = 450$ nm.

UV-visible spectrophotometric runs were performed as follows. Experiments on the dynamics of the BZ reaction, both in the absence and in the presence of PAMAM G4, were performed following the variation of ferroin concentration at a wavelength of 590 nm (maximum absorption wavelength...
for ferroin) with a PC-controlled Vernier spectrometer (Ocean Optics) equipped with a magnetic stirring apparatus. The reactor was a thermostated cuvette (1 x 1 x 5 cm) with a path length of 1 cm. Temperature was kept equal to 21.0 °C for all experiments. Samples were prepared directly in the cuvette by mixing under stirring all reactants except ferroin with the PAMAM G4 stock solution in desired proportions. Finally, oscillations were started by adding ferroin in the cuvette sealed with a Teflon plug. The stirring at a reduced rate was continued during the entire course of the experiments. The solutions volume was kept at 3 mL for all experiments. Concentrations of reactants used in the experiments on wave propagation led to a large production of CO2 bubbles that eventually caused serious problems in the spectrometric data acquisition.

**Results**

The first part of the research consisted of a detailed inspection of the different behaviors observed for the different chemical concentrations of our species. A summary of the observed spatio-temporal patterns is shown in Fig. 2. Here, we plot a phase diagram where the relevant parameters are the excitability in the x-axis while the vertical axis shows the concentration of the dendrimer in the solution. Each region in the diagram plots the typical structure observed for that range of parameters.

Low values of excitability produced patterns with spatial instabilities. For low concentrations of G4 stationary Turing-like patterns appear (case I in Fig. 2). Thus the BZ-G4 system becomes one of the few systems that can exhibit such a pattern especially in a closed reactor and at room temperature.

Increasing the concentration of G4 modifies dramatically the geometry of the patterns observed. Case II in Fig. 2 shows a spatiotemporal pattern that resembles jumping or leaping waves. Fig. 3 shows a sequence of snapshots that reveals the propagation nature of these waves. An ordinary circular wave propagates from a central white spot until a new wave or set of waves suddenly appears in front at a short distance ahead of the original wave front. This new wave expands slightly in all directions. Then, the parent wave vanishes, the new wave stops expanding, and generates another front a short distance ahead in the direction of propagation. The new wave fronts continue to jump along the direction of propagation of the original wave; dark separation curves mark the collision lines between successive waves for some time. These waves look somewhat like trigger waves propagating by discrete jumps, so they are named jumping waves. Fig. 4 contains a space–time plot that shows the dynamics of such waves.

Larger values of G4 concentration reveal the presence of new spatiotemporal behavior which is described in the...
Low values of [G4] correspond with normal oscillatory waves excitability correspond with wave propagation in the medium. Each jump corresponds with the generation of new sources of waves in front of the propagating front. Same concentrations as in Fig. 3. Frame size = 60 s × 1.5 cm.

Packet waves have two different space scales. From the large-scale point of view, they behave as normal autowaves propagating through the medium with constant velocity and with a characteristic wavelength of 5.8 mm. The fine structure of the packet waves is revealed at shorter distances, each of the previous waves is actually made of a pair of waves travelling together at a constant velocity but separated by a distance much shorter than the natural wavelength (1.4 mm). Fig. 5 shows a space–time plot of this experiment. Notice the two characteristic wavelengths for the packet waves. Waves are originated by oscillations in the central part of the Petri dish and immediately split into pairs of waves located very close to each other that propagate stationary in time.

The regions in the phase diagram (Fig. 2) with larger excitability correspond with wave propagation in the medium. Low values of [G4] correspond with normal oscillatory waves.
absorbance in a well stirred reactor as a function of time for two concentrations of G4 measured by visible spectroscopy as described in the methods section. Note that for large concentrations of G4 the period becomes larger. This is consistent with Fig. 2 where increasing G4 concentrations actually induce a Hopf transition in the system yielding to excitable behavior.

Fig. 8 Wave velocity, \( v \), normalized by the velocity in the absence of dendrimers (\( v_0 = 11 \text{ mm min}^{-1} \)) versus concentration of dendrimer, [G4], normalized by the initial concentration of activator, [NaBrO3]. Experimental data are plotted as circled independent points.

In order to understand the behaviors observed it is necessary to analyze the underlying mechanisms where the dendrimer plays a role. Two main effects can be considered. The inner structure of the dendrimer plotted in Fig. 1 shows many internal cavities that actually behave as reservoirs that can capture some chemicals and transport them. Due to the large acidity of the BZ reaction, the dendrimer captures protons from the external solution, thus reducing the effective acidity of the solution. Thus, the actual concentration of protons in the solution is decreased proportionally to the dendrimer concentration. On the other hand, this process endows the dendrimer with a global positive charge, thus only negatively charged ions will be allowed inside its structure. The inclusion of such ions should take place within the first instants of the reaction thus BrO3\(-\) and SO4\(^{2-}\) ions are good candidates to neutralize the global positive charge of PAMAM G4. This phenomenon results in an effective decrease of the initial concentration of such species. In particular, the effective concentration of BrO3\(^{-}\) becomes smaller.

The velocity of wave propagation in an active media is generically given by,\(^{33}\)

\[
v = 2\sqrt{k D[H^+][BrO_3^-]}
\]  

where \( k \) is the global kinetics constant and \( D \) is the diffusion coefficient of the activator. Note that both effects described before point in the right direction, i.e., diminishing of the wave...
velocity. These two effects by themselves are not enough to
completely account for the quantitative results shown in
Fig. 8. Both partially account for the effect although some
other mechanisms must be underlying. We believe this
difference is due to the kinetics of the reaction and that it is,
in fact, affected by the dendrimer. This effect may be included
into eqn (1) via the kinetics constant k, although a detailed
analysis of the reaction kinetics (exceeding the aims of this
paper) needs to be done in order to fully understand this part
of the mechanism.

The last issue that remains to be checked is the stability of
the dendrimer structure. Such a point was verified by fluo-
crescence measurements as described in the methods section.
Fig. 10a presents the results of fluorescence techniques. The
intensity of fluorescence emission is plotted versus the
wavelength. The curve at the bottom is the standard emission
spectrum for the free dendrimer in an acidic medium showing
a characteristic maximum at 450 nm. The other curve was
measured when the dendrimer was immersed in the BZ
reaction (without ferroin as its dark color may mask the
measurements). Note that the effect of the BZ reaction is just
to increase the value of the maximum intensity emitted but
without any change in the wavelength due to the presence of
ionic species in the medium as reported elsewhere. Fig. 10b
presents several spectra measured every 20 min during 2 hours.
Note that all spectra remain almost unchanged during this
period of time. These results, coherent with the reported
behaviors in the literature, demonstrate the stability of the
dendrimer structure during the recording periods.

Conclusions

Dendrimers are polymeric substances that recently achieved
importance in biochemistry as they are used as targeted
vehicles for drug delivery in living organisms. Many of the
potential application systems are also important from the
pattern formation point of view. We have demonstrated along
this paper that dendrimers play an important role in pattern
formation mechanisms. The BZ-G4 system demonstrated
that it is capable of reproducing the most typical waves in
active media, but it also produced, under the appropriate
parameters, different patterns as jumping waves or packet
waves endowed with very unusual properties. Turing-like
structures are also reported in the vicinity of the previously
mentioned waves, thus we believe Turing instability may play
a role in the deep mechanism for such structures.

This paper opens possibilities for future research as different
ranges in the parameter space remain unexplored and unexpected
dynamics may appear.

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Notes and references

1 J. D. Murray, Mathematical Biology, Springer-Verlag, Berlin,
1993.
2 J. M. Davidenko, P. Kent and J. Jalife, Physica D (Amsterdam),
1991, 49, 182; J. M. Davidenko, A. M. Pertsov, R. Salomontz,
3 J. Lechleiter, S. Girard, E. Peralta and D. Clapham, Science, 1991,
252, 123.
89, 6433.
343, 355.
7 A. T. Winfree, Science, 1972, 175, 634.
8 A. T. Winfree, in Lectures in Complex Systems, ed. L. Nadel and
9 M. Markus, Zs. Nagy-Ungvari and B. Hess, Science, 1992,
257, 225.
37-72.
15 K. J. Painter, in Mathematical Models for Biological Pattern
Formation, ed. P. K. Maini and H. G. Othmer, IMA Volumes in
Mathematics and its Applications, Springer-Verlag, Berlin, 2000,
vol. 121, p. 59.
228301.
18 J. L. Jackson, H. D. Chanzy, F. P. Booy, B. J. Drake, D. A. Tomalia,
T. Cagin, S. T. Lin and W. A. Goddard III, Macromolecules, 2005,
38, 979–991.
19 D. A. Tomalia, S. Uppuluri, D. R. Swanson and J. Li, Pure Appl.
20 L. L. Miller, R. G. Duan, D. C. Tully and D. A. Tomalia, J. Am.
4782–4787.
van Boxtel, E. M. M. de Brabander-van den Berg and E. W.
24 J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg and E.
25 J. F. G. A. Jansen, E. W. Meijer and E. M. M. de Brabander-van
27 D. Wang, T. Imae and M. Miki, J. Colloid Interface Sci., 2007, 306,
222–227.
2008, 128, 204508.
088303.
30 A. Kulkzynska, T. Frost and L. D. Margerum, Macromolecules,
2006, 39, 7372–7377; D. Caķara, J. Keimann and M. Borkovec,
32 A. D. Meltzer, D. A. Tilzer, A. A. Jones, P. T. Inglefield, D. M.
Hedstrand and D. A. Tomalia, Macromolecules, 1992, 25,
4551–4558.
34 M. J. Jasmine, M. Kavitha and E. Prasad, J. Lumin., 2009, 129,
506–513.
35 T. Frost and L. Margerum, Macromolecules, 2010, 43,
1218–1226.