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A LOOK BACK UPON SOME 'NON-CONVENTIONAL'
BIOCHEMICAL MECHANISMS OF SUPRAMOLECULAR
STRUCTURES ASSEMBLING DURING PEROXIDASE-OXYDASE
CATALYZED POLYMERIZATION OF LIGNINS AND THE RELATED
DEHYDROPOLYMERS (DHP) FORMATION

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*This paper is dedicated to Prof. Takayoshi Higuchi,
on the occasion of his 80th anniversary,
to congratulate his great stimulating contribution to lignin biosynthesis and biodegradation;
I shall never forget our visit and wishes for a closer cooperation,
in front of the Silver Phenix at Byodo-Hin, in May 1981*

The role of the 'peroxidase-oxidase' reaction (catalytic formation of H₂O₂ by O₂ activation), now well-known but seldom discussed, is recalled with respect to biochemical studies on lignin polymerisation and emphasis on the physico-chemical mechanisms of pattern formation and of molecular organisation during the synthesis of lignin model compounds (DHP). The corresponding processes of supramolecular assembly involving oligolignols interactions are related to more fundamental kinetic and thermodynamic mechanisms of cooperative inter- and intra-molecular binding reactions of polymer ligands, through interfacial- and template-constrained polymerisation. Finally, viewing synthetic and natural lignified structures as 'open systems far from equilibrium', typically self-organized by nucleation processes and by the formation of 'dissipative structures', as previously illustrated in this journal (*Cellulose Chem. Technol.*, 2005, **39**, pp. 341-367), the so-called 'random' process of lignification is tentatively described in terms of intra- and inter-molecular cooperative non-covalent and covalent 'binding' and of hydrophobic 'solvation'. Here, the somehow controversial word 'random' refers to the pre-existence of elementary (*i.e.* atomic, molecular and polymer) structured building-blocks and to the absence of pre-formed systemic (*i.e.* supramolecular assemblies) organization.

The formation and progressive evolution of diverse macroscopic patterns during the biosynthesis of lignified cell walls are currently well-established on the basis of unambiguous anatomical and cytochemical observations. During the first half of the XXth century, most of these observations, mainly founded on biochemical studies were, with rare and often neglected exceptions, seldom discussed in terms of the fundamental physico-chemical mechanisms

that subtend these macro- and supra-molecular morphogenetic organizations. The review-chapter entitled "Biosynthesis of lignin", written by T. Higuchi in 1985, is one of such exceptions in which, among many other topics, the author emphasized not only the structural differences evidenced in the formation of dehydrogenation polymers (DHP), involving diffusion across the cell membrane, for example in the dialysis tube, but also the possible regulation of lignin

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polymerization, involving the formation of H_2O_2 from O_2 by peroxidase-catalyzed reactions, cooperatively affected themselves by the number of reduced compounds, monolignols and ascorbic acid, for example.

Also, highly significantly, in 1985 this chapter was included in the book "Biosynthesis and biodegradation of wood components", viewed as "the first comprehensive book on wood biochemistry" intending, in the terms of its preface, "to seek to apply biotechnology to wood production and use".^{1a} With biochemistry as a necessary condition for some "new uses" of wood, the contents of both this chapter and the book still remain completely relevant.

The continuously increasing number of papers published in specialized "biochemical" journals clearly illustrates this fact, accounting, however, a correlated increasing need of complementary studies on the related physico-chemical mechanisms substratum. Such "non-biochemical" data and hypotheses, often briefly and/or only rarely discussed by biochemists, were thus "non-conventional", even now often "neglected" and finally "forgotten".

This is still currently the case of the two just cited topics; **first**: the peroxidase-oxidase reaction, when looking back at its reported complex, non-linear dynamics induced by naturally occurring aromatic substrates allowing chaotic oscillations and convective pattern formation, **secondly**: the molecular structure of DHP, when looking back at the diverse spatial patterns, observed after their enzyme-initiated and chemically-driven organization in the presence of mono- or/and multi-functional ligands, as the result of inter- and intra-molecular reactions, depending on cooperative binding and solvation ranking mechanisms in both solutions and at interfaces.

In this short note, the author supports the lasting and increasing interest for these non-conventional topics in a specific biochemical view. In this respect, the significance of some "neglected" old key-papers is eventually recalled, on emphasizing some very recent papers more directly concerned with these physical chemistry views, with the aim of inciting to experimental validation in lignification studies. As a "short note", the

paper does not provide extended critical views. It has to be considered as complementary to a previous review focussed on some macromolecular aspects of the biological variability of lignins.^{1b} This is a kind of part 2 of this review, providing more "fundamental" mechanistic views and references, in addition to those newly cited in the previous one, along with others,^{1c,f} which are thus only mentioned, without being included in the reference list.

The peroxidase-oxidase reaction and lignin- or dehydropolymer- polymerization

The involvement of the activated oxygen species (especially the superoxide radical) of monophenols (especially coniferyl alcohol) and of reduced metabolites (especially malate and NADPH or NADH), in the hydrogen peroxide formation and related polymerisation of lignols and monophenols by isolated plant cell walls, was unambiguously demonstrated by G. Groos and coworkers in 1977. Even if extensively commented by Higuchi (**1^a** pp. 148 and 159) and confirmed in 1978 by A.-M. Catesson and coworkers (**1^d** p. 8), through a direct histochemical probe based on *in situ* malate-NAD dependence, this enzymatic reaction mechanism seems to have been only rarely cited and exclusively in studies of H_2O_2 plant metabolism during a localized hypersensitive defence reaction,² xylem cell wall lignification³ and cellular redox signalling the involvement of NAD(P)H and plant oxidases and peroxidases as simple enzyme oscillators⁴ in the general and secondary lignification metabolism,^{1b,d} for example. To the best of our knowledge, based on citations and data base analysis, this reaction mechanism is apparently only seldom cited, "neglected", during most of the current model studies of oxidative polymerisation of simple oligolignols and DHP supramolecular assembly, either in the presence or absence of copolymers. With some very rare exceptions of enzymatic biosynthesis of H_2O_2 by glucose-oxidase in the reaction medium, as reported by M.-T. Tollier and by N. Terashima and coworkers (**1^b**, pp. 361 and 352), for example, H_2O_2 is almost invariably used as a solution of "commercial H_2O_2 " directly added reagent during the synthesis of

model lignin DHP. Equally, almost invariably, the effects of H_2O_2 local concentration and reaction kinetics, even if unambiguously demonstrated on the basis of Freudenberg's conventional "Zulauf" and "Zutropf" conditions, are very seldom discussed even when the validity of the extrapolations of the mechanisms and the results of such *in vitro* synthesis to the lignified plant cell walls biosynthesis and their H_2O_2 -related finely retro-regulated metabolism are involved. As evidenced in the introduction, it seems worth pointing out that the peroxidase-oxidase reaction supplemented with simple phenolics shows a typical oscillatory and complex dynamics,^{5a} depending on the occurrence of the non-linear, second order, intermediate autocatalytic reaction steps,^{5b,5c} as a potential source of enzyme-catalyzed formation of special, macroscopic patterns,^{5d} generators of characteristic instabilities or turbulences in homogeneous reactors or in a reaction-diffusion environment, depending, for example, on the differences in proton diffusion, pH,^{5e} or in substrate inhibition,⁶ being thus a function of the spatio-temporal organisation of the reaction-diffusion system. Viewed in 1994 by the authors as "a challenge for physiologists",⁷ the "nonlinear behaviour in the peroxidase-oxidase reaction" still remains a challenge for lignin biochemists but, rather surprisingly, for organic chemists as well! The metabolic stability of the related spatial patterns, described as "dissipative structures" (as later viewed here), was questioned by these authors "to stay on long-term *in vivo*" on the basis of model calculations,^{5e} even when, however, the combination of autocatalytic reactions with the diffusion of reagents in an open reactor, leading to the formation of these dissipative structures, was viewed by others as "conditions for the formation of functional order in the evolution of life".⁸ Inviting thus experimentalists to validation, these assertions also warrant the very common need of "improving the catalytic performance of peroxidases in organic synthesis", as recently illustrated, too, in the field of industrial chemistry, by Van de Welde and coworkers,⁹ who strongly emphasized the "crucial" mode of H_2O_2

addition, when comparing the results of *in situ* generation of H_2O_2 with the introduction of a concentrated solution at a single point, through pumping into the reaction vessel.

In the field of lignin biochemistry, whatever the experimental conditions of DHP synthesis and, similarly, of the biological synthesis of lignins, may be,^{1d,b} the systematic, well-established differences between the macromolecular structure of lignins and DHP unambiguously show that conditions other than peroxidase-catalyzed reaction mechanisms are, at least, crucial. As emphasized in the introduction, this seems to be specifically the case, during the initial steps, enzymatic or not, of molecular assembling leading, through non-covalent or covalent interactions, to macromolecular structures and to the so-called "macroscopic patterns".

Even abridged, a survey of these steps is well beyond the scope of such a short note. Thus, aiming here not at "neglecting" a possible contribution of the "dissipative structures" to such a spatial organization, the importance of a cooperative interaction mechanism is suggested as one of the crucial factors during lignin and DHP spatial organisation, as illustrated and identified here when the physico-chemical conditions of DHP synthesis are compared for example, in agitated "homogeneous" solution and at "heterogeneous" liquid-air or liquid-solid interface.

DHP spatial organisation and cooperative interaction mechanisms

Organic synthesis through dehydrogenation of phenols as lignin model polymers has been initiated by Freudenberg and coworkers^{10a} around 1950, with "the intention to duplicate as closely as possible lignin biosynthesis as it occurs in the plant" and again, around 1970, "the most significant result that lignin produced *in vivo* in nature and *in vitro* by biosynthesis in the laboratory are as closely identical, as might be reasonably expected of high-molecular mass amorphous materials".^{10b} Sometimes, although less and less frequently assimilated to the "natural" lignin biosynthesis, the chemical synthesis of DHP can and has to be currently perceived as the essential

complement of the *in situ* physico-chemical analysis contributing, at a microchemical level,^{11a} to the systematic identification and characterisation of the occurrence of *in vitro* interactions among the lignified cell wall layers biopolymers, namely cellulose, hemicellulose, pectin, lignin and proteins, systematically analyzed *in situ* by L. Salmen and coworkers.^{11a,b}

The same type of complementarity is found when comparing the physico-chemical conditions of synthesis in agitated solution and at liquid-solid or liquid interfaces.

In “solution”, according to Freudenberg’s bulk and end-wise conditions of polymerisation, the peroxidase activity has been recently demonstrated as “a crucial parameter for structural variations of DHP”,¹² which agrees with the results of Tanahashi and Higuchi,^{1a} for example, and with others papers, cited elsewhere.^{1a-d, 2}

Studies on DHP polymerisation under similar conditions, yet with the addition of macromolecular compounds, at “solid interfaces”, provided similar evidence on the variations – mainly – of the relative frequency of the inter-monomeric linkages in the presence of cyclodextrins,^{13a} cellulose,^{13b} macromolecular lignin fraction,^{13c,d} arabinoxylan,^{13e} pectin composites^{13f,g,h} and xylan.¹³ⁱ Even when differences in the molecular mass and relative variations in the size and composition of oligomeric and copolymer fractions were less frequently reported, such studies, unambiguously demonstrating the highly significant occurrence of the physico-chemical interactions at solid-liquid interfaces, when compared to the variations reported in aqueous phase. Very stimulating figures have been drawn,^{13a, 13b, 13d, 13i} to illustrate some so-called molecular “template”, “scaffold”, “aggregation” effects, when identifying some molecular mechanisms of the assembling steps which, however, remained of quality when only “pure” analytical biochemistry was involved.

At the “air-water interface”, a similar type of results was also reported when studying the kinetics and layer organisation during the peroxidase-polymerization of coniferyl alcohol, when the reaction occurs at and near the interface.^{14a,b} In the early stages of the

reaction, when only coniferyl alcohol dimers were analyzed, the relative proportions of dimers changed when compared to the polymerization in “solution”. Furthermore, by using combined methods of “pure” analytical biophysics for surface pressure and molecular organization, the formation of a 2D layer and of a 3D structure was observed. The modification, according to the mechanical surface constraints of the surface selected during the polymerisation study, leads to a process described as organized in the “subsurface”. Interestingly, the adsorption of the oxidation products, yet not the diffusion of the phenolic substrate, was identified as the rate-limiting step, allowing the idea of a kinetic model of DHP interphase organization at the “subsurface”, a model which quantitatively agrees with the surface and interfacial organization behaviour, as previously reported^{14c,d} in the case of DHP synthesized “in solution”.

Now, bringing together, at the molecular level, the similitude between the products of these three types of DHP synthesis and the reference at the macroscopic level, to the differences in the corresponding processes, perceived as “homogeneous” in agitated solution and as “heterogeneous” at liquid-air or at solid interface, it is thus suggested to look, at the elementary submolecular mechanisms level, on the cooperativity of the physico-chemical interaction steps as a crucial factor of DHP organisation. In this respect, one has to underline the essential difficulty to quantitatively conceptualize the notions of hetero- and homogeneity at least at two levels, not only in the case of interfacial assembly processes, in which the just cited macroscopic perceptions of “homogeneity” and “heterogeneity” could not be relevant at the macromolecular organisation level, as due to the submolecular interphase-subsurface-interface conceptual continuity, but also in the case of peroxidase-initiated and chemically-driven polymerisation of DHP which, under solid interface spatial constraints, unambiguously depends, too, on the relative proportions, often qualitatively perceived as “small” or “large”, of numerous reagents such as in H₂O₂, O₂-activated species, phenolic substrates, in effectors and, of course, in

“polymer templates” concentrations. This latter point has been demonstrated and illustrated by, for example, Sarkanen and coworkers,^{13c,d} who reported that “dehydrogenative polymerisation of coniferyl alcohol in homogenous solution has now been found to be strongly affected by small quantities of macromolecular lignin components”. Essential but more specific, difficult and sometimes even controversial,^{1e} such questions are however not commented here any more, for the sake of shortly recalling the more general and elementary, “universal” mechanisms of cooperativity, aiming at stimulating its account in the case of DHP and lignin formation studies.

Cooperative binding between “small” or “large” simple molecules to (bio)polymers is obviously “universal”, bearing more than a biochemical significance. According to the conventional polymer chemistry views, cooperativity can be conceptualized as a mechanism in which the interaction of a molecular structure with a binding site affects, in some way, the reactivity and, consequently, the binding capacity of its other sites. In this respect, the binding of an isolated ligand – “nucleation” –, the binding at the immediate neighbourhood of an already bound one – “aggregation” – and the intricate effect due to ligand-ligand binding – “dimerization” – were identified by G. Schwarz^{15a} as standard elementary steps, which allowed the formulation of the quantitative relations required, recalled as the most useful tools in the experimental analysis of (bio)polymer formation processes. Among the plethora of other current relevant publications, this interest is here illustrated by only two particularly clear series of papers, combining model parameter calculations with subsequent experimental applications. As to the static and dynamic properties of the cooperative binding between ligands and linear polymers, the respective association constants, determining the type and degree of cooperativity – in addition to some related equilibrium concentration of components – were first calculated on the basis of a pertinent kinetic model.^{15a} In the two following papers, this model was experimentally validated and extended to the dependence of cooperativity

on ionic strength, polymer ligand ratio, ligand dimerization and stacking tendency of the neighbouring ligands, dyes, molecules. As to the complementary cases of multiple inter- and/or intra-molecular binding with mono- and/or poly-valent ligands, similar calculations, applied to reversible disassembly and gelification,^{15d} have also provided quantitative criteria used to successfully assess the types of binding reported by other authors, positive- vs. negative- cooperativity or non-cooperativity, statistical or random binding, by the determination of the inter- and intra-molecular equilibrium constants.^{15e} By extending the general treatment of the macrocyclization control,^{15f} tentatively viewed here as a modular polymerization of oligomeric chains as networks cycles, these data could be relevant in the modular formation of DHP and lignins, by assembling the oligolignols suggested, among others, by Freudenberg and Wayman, as previously reviewed (1^b pp. 353; 1^f). Of course, concerning only the reversible cooperativity in self-assembly processes, this last model cannot be directly involved in DHP formation studies. However, it is suggested that such model could be tentatively applied at least during the initial steps of DHP synthesis, corresponding to the binding interactions between water solvated mono- and oligo-lignols and to their phase-transition from a “free” liquid phase into an “interphase” constrained at the liquid-solid interface. Such a transition step would involve a thermodynamically controlled rearrangement of free-, solvated- and structural-water, as reviewed (1^b pp. 351, eq.1; 1^d), thus occurring prior to the local decrease of water concentration and chemical activity, as experimentally evidenced and discussed by Cathala and coworkers^{13g,h,i} for the hydrophobic domains of the DHP nano-aggregates, for example. Specifying the concept of “concerted supramolecular organization” suggested in this respect, as well,^{1b} the possibility of an either reversible or not concerted cooperative folding and unfolding of oligolignols has to be considered, along with concurrent dehydration and hydration effects, as due to the so-called “nonpolar”, “hydrophobic

hydration”, as evidenced in both experimental^{16a} and model, theoretical^{16b,c} studies of “hydrophobic proteins”, elastin and elastin-like polymers,^{16a,b,c} but also in the often forgotten case of cellulosic fibres.^{16d,e} Ironically, it could even be added that such a model can be directly applied to the study the complex metachromatic effects reported during the cytochemical characterisation of *in situ* lignin,^{1b} prior to and after mechanical breaking,¹⁷ thus aiming at identifying some supramolecular intercalation reaction mechanisms and the related controversial occurrence of stereochemical effects due to the chiral spaces at the interfaces with solids, during DHP and even lignin (bio)synthesis!

A last, much more short-term ... reason of the interest for the molecular cooperativity effects in DHP lies in the recent report of Salmen and coworkers,¹⁸ who quantified the intermolecular coupling effects associated with the *in situ* lignin during glass transition changes, pointing out the possibility to enlarge the concepts and procedures of the global and segmental dynamics of polymer studies, in the case of *in situ* lignins. Accordingly, the large range of segmental behaviour in more or less smooth or constrained polymers systematically identified by Ngai and coworkers¹⁹ provides relevant incitation to elaborate models and to similarly quantify the role of submolecular differences and of the heterogeneity in DHP, as also suggested on the basis of “pure” biochemical analysis, when comparing the bulk – more “compact” Zutropf – and the end-wise – more “linear” Zulauf – types of DHP composites.

Opposite to such (bio)chemical analyses, performed at polymer, molecular and submolecular “elemental” levels, polymer segmental chain motion, molecular steric substituent constraints or atomic orbital interactions, steering – for example, the additional possibility of physico-chemical studies, based on global, “systemic”, thermodynamic bases – have to be finally recalled.

The possibility to analyse the very early steps of formation and assembly of macromolecules, and thus of lignin and DHP, according to the thermodynamic views initiated and developed mainly by Prigogine

and co-workers, has been recently discussed in this journal,^{1b} even if exclusively at the biochemical level. Complementary views on the principles of the thermodynamic control of the physico-chemical mechanisms of spatial self-organisation and of auto-catalyzed peroxidase polymerisation are thus presented here with reference mainly to cited, yet not listed papers.

Adapted from the previously mentioned paper (1^b, Fig. 2, p. 356 and ref. 55-56), Figure 1 summarizes the central suggestion that “the early stages of lignin and DHP depend on non-equilibrium stationary states allowing, under certain conditions, phenomena of macroscopic self-organization described as temporal rhythms and special patterns”, which depend on both external and internal constraints, expressed by a very limited number of thermodynamic parameters describing the system. According to both theoretical bases, expressed by Prigogine^{20a,b,c} and coworkers, and to the corresponding unambiguous experimental validations of De Keppers, Boissonnade^{20d,e} and Epstein groups,^{20f,g} the spatial organization processes of DHP and lignin are tentatively described as “chemical morphogenesis” processes initiated by a non-linear, “complex”, oxidasic enzyme system and driven by random chemical interactions. Adapted from a cartoon of De Keppers *et al.* (20^e, p. 86), this figure is only a draft. It tends to outline, as a function of time and of a generalized spacial-3D distance, the evolution of the concentrations of some molecular species possibly involved in the sub-molecular mechanisms of DHP-aggregate formation at a liquid-solid interface occurring in the diffusion cell reactor,^{13g,h} in which the peroxidase-polymerization of DHP is performed through diffusion into a cellulose-pectin composite mat, for example.

As previously discussed,^{1f,c} and also according to Wayman’s and Freudenberg’s earlier models, the formation of relatively short and thus soluble oligolignols (2 *f.*) would occur first, leading, as just mentioned, to their cooperative self-assembly, as insoluble lignin, like oligolignol networks (3 *f.*), finally appearing as 3D patterns, granular nano-structures, at macroscopic cyto-

chemical level (*4 f.*). Thus, DHP formation by cooperative interactions and self-organization is viewed, according to polymer chemistry, as a thermodynamically and stochastically (“statistically” or “randomly” would be equivalent terms!) “simple and natural” phenomenon. In this respect, the (bio)synthesis of DHP and lignin appears to be driven not only in the initiation (*1 f.*) but also in the aggregation (*4 f.*) steps, according to a balance mechanism between energy dissipative and conservative processes, again emphasized, through thermodynamically-constrained mechanisms very similar to those described during the “chaotic reactions of polymer string formation” and also during

the “bifurcation process”, as extensively discussed by Prigogine (20^d, 20^a pp. 246-250). According to Eigen’s authoritative contribution to the self-organization of the matter and to the evolution of biological macromolecules,²¹ the word “random” refers here to the pre-existence of elementary (*i.e.* atomic, molecular and polymer) structured building-blocks and to the absence of a pre-formed systemic (*i.e.* supramolecular assemblies) organization. For avoiding, as previously done,^{1c} any controversy on the question of the “random or not-random” nature of lignin origin, this point of view is not accounted here any more.

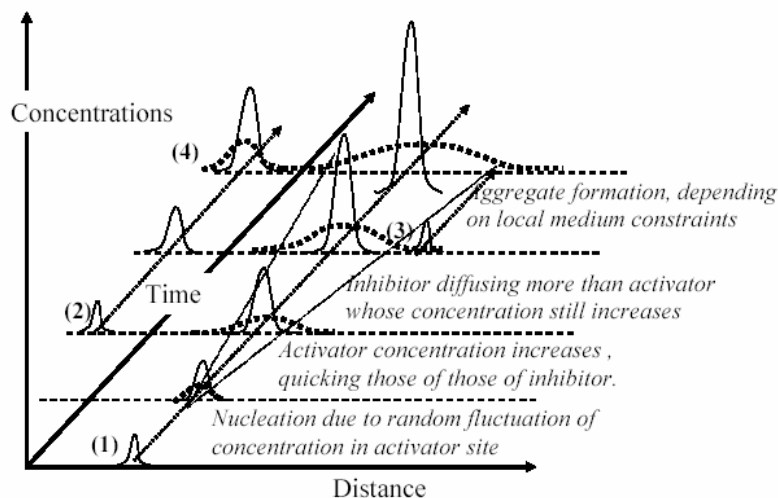


Figure 1: Formation of “clusters”, a granular structure of DHP, during “chemical morphogenesis”, involving the antagonistic effects of activation and inhibition of the polymerisation reactions of free radical monomers and of the oligomeric fractions of lignins. The formation of a first aggregate, (1), initiated by a random “local” fluctuation, induces, as a function of time, an autocatalyzed activation and polymerization of monomers (2), with the formation of oligomers, subsequently aggregated by a first localized and fast accumulation (continuous bell-shaped distribution curves), which becomes more extended and slower (discontinuous curves). The formation of other aggregates is possible (3) or not (4), depending on the “distance” types vs. the internal and external constraints of the system and on the cooperative molecular interactions (see the text)

Consequently, if considering first – in this respect – the peroxidase-oxidase mechanism, one has to emphasize, as also clearly stressed by Eigen,²¹ that Prigogine and his coworkers demonstrated that “a combination of autocatalytic reaction behaviour with transport process may lead to a peculiar spatial distribution of the reaction partners”,

which he called “dissipative structures”, *i.e.* structures resulting from a dissipation of energy rather than from molecular conservative forces. A very clear presentation of the corresponding thermodynamic balance mechanism between dissipative and conservative processes in, respectively, equilibrium and non-

equilibrium structures, is given by Prigogine in his seminal book,²² as illustrated in Figure 4 of Chapter 8, on the creation of order in open systems, shifted far from equilibrium by external constraints. Including the reaction loops and complex kinetics depending on the redox potential of the phenolic effectors not consumed during the reaction, as reported by Kummer and coworkers,^{5a-c,7} the peroxidase-oxidase system mechanism is consistent not only with the possibility of supporting pattern formation but also with the nucleation steps in the synthesis of DHP models and of *in situ* lignins.

As to the related dehydropolymer (DHP) formation, one can emphasize, as illustrated by Pacault,^{20d} Epstein,^{20f} and Kondepudy and coworkers,²³ that the spatial inhomogeneities can interact with and even amplify the temporal non-linearities in evolving complex systems brought about by autocatalytic mechanisms and by the competition between the internal and external constraints, which fully agrees with Prigogine's above-mentioned views. The importance of the "perfect" mixing conditions in either "small" reaction vials or "large" reactors, to the structure of the reaction products was experimentally demonstrated by these authors, who emphasized the practical importance of the instabilities in "thermokinetic phenomena".^{20d} The poor reproducibility observed during DHP synthesis according to the reaction conditions, "mixing", sometime deplored but seldom discussed when quantitatively ... analyzed, is fully consistent with this type of instability of the autocatalytic enzyme system. According to these observations, B. Cathala and coworkers²⁴ showed, again in agreement with other authors, that an "as careful as possible control" of the state of the reactor, of the preparation conditions and of the reagent mixing allows significant improvements of the reproducibility between DHP preparations. However, because of the lack, to the best of our knowledge, of more precise quantitative and specific data on the other autocatalytic process steps of nucleation and aggregation during the DHP synthesis plotted in Figure 1, no other comments are attempted here.

CONCLUSIONS

Trying to combine and to express as simply as possible, in a "foreign language", thus sometimes unavoidably inexact, a large number of data arising from biochemistry, physical chemistry and thermodynamics, this short note has to be viewed mainly as an illustration of the interest of the multidisciplinary approaches to the biosynthesis of lignin and synthesis of DHP models. It is thus seen as an invitation to reinforce and enlarge the cooperations; the idea is common and of course not new,²⁵ yet requiring some insistence to currently survive.^{1c} Furthermore, it strengthens the wish that such scientific cooperativity ... would contribute not only to breaking some "closed circles of conventional research", but also to opening accounts on not only old, "forgotten", but also new, "non-conventional" ideas as typical and ultimate illustrations in the case of lignin biochemistry, formation of nano clusters and related fractal properties of lignin and DHP, already pertinently explored, but not yet revealed here, by J. Gravitis and coworkers!²⁶

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