Photopolymerization as a potential method for sensing biorecognition events

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The present review summarizes the main topics related to the photopolymerization process focusing on its applications in biosensing chemistry. Among the variety of known polymeric substances, the wide spread usage of ethylene glycol derivatives in medical applications justifies their selection for this purpose. Fundamental aspects of photopolymerization are commented together with the nature and
reactivity of the molecules involved, considering both a semi-empirical thermodynamic approach and the development of a kinetic model leaning on experimental values. An effort is made to sum up the most referred characterization techniques applied to figure out the composition of the formed products and to follow the reaction advance. Finally, initial and more recent photopolymerization examples and applications are presented as visiting card of this promising biosensing methodology.

Keywords: hydrogel, initiator, photopolymerization, immunoassay, microarray

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I. INTRODUCTION

1. Methods for detection of biointeractions

Immunosensing is a broad interdisciplinary research field[1] connecting analytical, biological and superficial chemistry to medical, agricultural, electronical, environmental or food sciences[2-9].

The aim of this area, as being part of chemical analysis, is to determine the nature and quantity of a target analyte present in a problem sample, but taking into consideration that some of the reactants added to interact with that substance belong to the immunological system of a concrete animal i.e. antibodies. Thus, a specific interaction takes place between the appropriate antibody molecule and the objective substance, when present.

Subsequently, a crucial step in the analysis procedure, involves the generation of a sufficiently intense signal to be scanned by any detection technique (e.g. colorimetric, fluorescent or UV-VIS spectroscopies). The goal of any analyst is to develop a biorecognition system (antibody to analyte complex), that generates the highest signal with the lowest analyte concentration present in the target sample. This can be achieved by using biological substances with highest affinity for the substrate. These primary complexes may be formed in a competitive or non competitive methodology as well as in a sandwich format assay. The last molecule to bind the complex is commonly referred as the *label* as it may carry a pending molecule (enzyme or light excitable substance) attached to one of its ends, far away from the recognition region. When these floating groups are given the appropriate excitation source (specific substrate for the enzyme or adequate light wavelength for the radiation excitable substance), the final readable signal is generated. In the case of light excitation pending group, a high quantum yield is desired to ensure a mayor signal intensity, while in the case of enzymatic pending group the appropriate reacting substrate is required to catalyze a series of processes that leads to the signal observation. These catalytic reactions are also known as amplification reactions because they generate a high response signal from a single (or few) initial interaction.

The development of diagnostic tools targeting human diseases through the analysis of pathogens or genetic biomarkers have motivated much of the efforts undertaken. Food, environmental, agricultural or even sports, are other fields of interest for scientists implied. Up to date, some concrete experimental procedures are used to obtain an amplification response due to biorecognition

[1] M. A. G. Martínez, R. Puchades, A. Maquieira, Trends. Anal. Chem. 18 **1999** 204
[2] E. Mallat, D. Barcelo, C. Barzen, G. Gauglitz, R. Abuknesha, Trends. Anal. Chem. 20 **2001** 124
[3] B. Hock, Acta Hydrochim. Hydrobiol. 29 **2002** 375
[4] M. C. E. Alberola, M. P. Marco, Anal. Bioanal. Chem. 378 **2004** 563
[5] S. R. Moraz, M. Farré, D. Barcelo, Trends. Anal. Chem. 24 **2005** 165
[6] S. R. Moraz, M. J. L. Alda, M. P. Marco, D. Barcelo, Talanta 65 **2005** 291
[7] K. R. Rogers, Anal. Chim. Acta 563 **2006** 222
[8] A. J. Baeumner, Anal. Bioanal. Chem. 377 **2003** 434
[9] H. Nakamura, I. Karube, Anal. Bioanal. Chem. 377 **2003** 446

events between DNA strands or proteic moieties. The determination of the amplified DNA is commonly assayed by anchoring the oligos on a solid surface (DNA microarray of biochip), on top of which a reaction takes place with appropriate sustrates generating a readable signal.

When DNA oligos specific sequence interactions are to be determined, several polymerase chain reactions (PCR, from now on) may be considered as amplification strategies. For an enzyme is required to generate multiple copies of the target DNA sequence, this family of methods is refered as enzymatic bioamplifications. Some of the most outstanding procedures embrace rolling circle amplification[10],[11],[12] reverse transcription-PCR[13] or real time-PCR[14],[15], all of them sprung up from the original polymerase methodology[16]. Microfabricated DNA sensors based on electrochemical detection of electroactive products formed by enzymatic action have been described[17]. When protein or antibodies are to be determined, catalyzed deposition methods employing alkaline phosphatase or horseradish peroxidase enzymes are mostly used, as visible coloured products form when appropriate sustrates are incorporated[18].

On the other hand, enzyme free amplification procedures are based on the use of gold or silver nanoparticles[19],[20],[21], fluorophores[22] or radioactive labels[23] bound to the protein or DNA labelling molecules. Branched[24], dendrimeric DNA[25],[26], detection have been reported with this approach. In a similar way, the formation of avidin to biotin complexes[27] provide a wide spread anchoring strategy example for binding target and recognition entities. A last example of enzyme free methods is the amplification based on polymer formation. It is a rapid procedure capable of offering visual discrimination of the presence of appropriately labeled molecules on a biochip surface. Different approaches have been made to determine the presence of targets by polymer deposition, some of the most remarkable considering the atom transfer radical chemistry[28],[29], others exploring the use of macroinitiator substances to

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[10] J. Baner, M. Nilsson, M. M. Hartvig, U. Landegren, Nucleic Acids Res. 26 1998 5073
[11] G. Nallur, C. Luo, L. Fang, S. Cooley, V. Dave, J. Lambert, K. Kukanskis, S. Kingsmore, R. Lasken, B. Schweitzer, Nucl. Acids Res. 29 2001 e118
[12] J. Jarvius, J. Melin, J. Göransson, J. Stenberg, S. Fredriksson, C. Gonzalez-Rey, S. Bertilsson, M. Nilsson, Nature Methods 3 2006 725
[13] J. Li, S. Chen, D. H. Evans, J. Clin. Microbiol. 39 2001 696
[14] M. Kubista, J. M. Andrade, M. Bengtsson, A. Forrotan, J. Jonak, K. Lind, R. Sindelka, R. Sjoback, B. Sjogreen, L. Strombom, A. Stahlberg, N. Zoric, Mol. Aspects Med. 27 2006 85
[15] C. A.Heid, J. Stevens, K. J. Livak, P. M. Williams, Genome Res. 6 1996 986
[16] S. Sengupta, K. Onodera, A. Lai, U. Melcher, J. Clin. Microbiol. 41 2003 4542
[17] D. Xu, K. Huang, Z. Liu, Y. Liu, L. Ma, Electroanalysis 13 2001 882
[18] J. J. Chen, R. Wu, P. C. Yang, J. Y. Huang, Y. P. Sher, M. H. Han, W. C. Kao, P. J. Lee, Genomics 51 1998 313
[19] S. J. Park, T. A. Taton, C. A. Mirkin, Science 295 2002 1503
[20] R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, Science 277 1997 1078
[21] I. S. Alexandre, S. Hamels, S. Dufour, J. Collet, N. Zammatteo, F. Longueville, J. L. Gala, J. Remacle, Anal. Biochem. 295 2001 1
[22] M. Beier, J. D. Hoheisel, Nucleic. Acids Res. 27 1999 1970
[23] A. Biljana, M. E. Cavic, r. N. Mcgovern, R. N. Thompson, Analyst 126 2001 485
[24] D. Kern, M. Collins, T. Fultz, J. Detmer, S. Hamren, S. Peterkin, P. Sheridan, M. Urdea, R. White, T. Yeghiazarian, J. Todd, J. Clinical Microbiol. 34 1996 3196
[25] M. L. Collins, B. Ivine, N. Tyner, E. Fine, C. Zayati, C. A. Chang, T. Horn, D. Ahle, J. Detmer, P. L. Shen, J. Kolberg, S. Bushnell, M. S. Urdea, D. D. Ho, Nucleic Acids Res. 25 1997 2979
[26] R. Stears, R. C. Getts, S. R. Gullans, Physiol. Genomics 3 2000 93
[27] C. J. Adams, Histochem. Cytochem. 40 1992 1457
[28] Lou, Langmuir 22 2006 2640; Shah, Macromol. 33 2000 597

accentuate the polymer growth[30] or self-assembled monolayers of the labeled molecules[31]. More recently, polymerization activated by light has attracted attention. The so called photopolymerization method (PMR), provides the formation of polymeric deposits exclusively on the regions where the reactive mixture (incorporing one or more types of monomer) contacts with the light sensitive labels anchored somehow to the surface REFS de Bowman, pero cuidado porque están ya más adelante. Enzyme free amplification procedures are not affected by the delicacy and instability of enzymes to temperature and preservation conditions, providing more robust experimental methologies.

A third family of detection methods includes those which do not require the presence of any labeled moiety for a readable signal to be generated. Traditionally, the most outstanding label free techniques have considered the analysis of the evanescent wave appeared when confined light gets to the interface between two different media. The evanescent field is generated from a total internal reflection phenomenon and penetrates perpendicular to the surface device into the surrounding medium for 100 to 200 nm. Molecules adsorbed at the surface of a sensor chip are meant to intereact with the evanescent field resulting in a phase shift, which will be detected when compared to a reference beam[32],[33]. By analizing the interference pattern in the exiting light beam, information regarding thickness, refractive index or quantity of the adsorbed molecule film can be obtained[34] together with the kinetics of probe immobilization and hybridization (in case of DNA studies)[35],[36]. Related examples are classical interferometers (Mach-Zehnder[37], Young[38], Hartman[39] or Backscattering[40] instruments), surface plasmon resonance (SPR) technologies[4142],[43],[44],[45], with improvements enabling to detect changes in protein conformation (by coupled plasmon waveguide resonance CPWR[46]) and enhanced sensitivity (by resonant microcavities –sphere, cylinder, ring or toroid shaped- that create a whispering gallery mode phenomenon,[47],[48],[49],[50],[51],[52],[53],

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[29] Lou, Anal. Chem. 77 2005 4698
[30] D. Bontempo, H. D. Maynard, J. Am. Chem. Soc. 127 2005 6508
[31] M. Husemann, D. Mecerreyes, C. J. Hawker, J. L. Hedrick, R. Shah, N. L. Abbott, Angew. Chem. Int. Ed. 38 1999 647
[32] M. J. Swann, L. L. Peel, S. Carrington, N. J. Freeman, Anal. Biochem. 329 2004 190
[33] G. H. Cross, A. A. Reeves, S. Brand, J. F. Popplewell, L. L. Peel, M. J. Swann, N. J. Freeman, Biosens. Biolectron. 19 2003 383
[34] A. W. Sonesson, T. H. Callisen, H. Brismar, U. M. Elofsson, Colloids Surf. B Biointerfaces 54 2007 236
[35] R. Georgiadis, K. P. Peterlinz, A. W. Peterson, J. Am. Chem. Soc. 122 2000 3166
[36] A. W. Peterson, R. J. Heaton, R. M. Georgiadis, Nucleic Acids Res. 29 2001 5163
[37] R. G. Heideman, R. P. H. Kooyman, J. Greve, Sens. Actuators B chem. 10 1993 580
[38] A. Brandenburg, R. Henninger, Appl. Opt. 33 1994 5941
[39] B. H. Schneider, J. G. Edwards, N. F. Hartman, Clin. Chem. 43 1997 1757
[40] V. S. Y. Lin, K. Motesharei, K. P. S. Dancil, M. J. Sailor, M. R. Ghadiri, Science 278 1997 840
[41] B. Liedberg, C. Nylander, I. Lunström, Sens. Actuators 4 1983 299
[42] J. Homola, S. S. Yee, G. Gauglitz, Sens. Actuators B Chem. 54 1999 3
[43] J. Homola, Chem. Rev. 108 2008 462
[44] J. Homola, Anal. Bioanal. Chem. 377 2003 528
[45] X. D. Hoa, A. G. Kirk, M. Tabrizian, Biosens. Bioelectron. 23 2007 151
[46] Z. Salamon, H. A. MacLeod, G. Tollin, Biochim. Biophys. Acta 1331 1997 131
[47] F. Vollmer, S. Arnold, Nature Methods 5 2008 591
[48] A. Ksendzov, Y. Lin, Opt. Letters 30 2005 3344
[49] A. Schweinsberg, S. Hocdé, N. N. Lepeshkin, R. W. Boyd, C. Chase, J. E. Fajardo, Sens. Actuators B 123 2007 727

(WGM), dual polarization interferometry[54],[55] (DPI) and optical fiber based biosensors[56],[57].

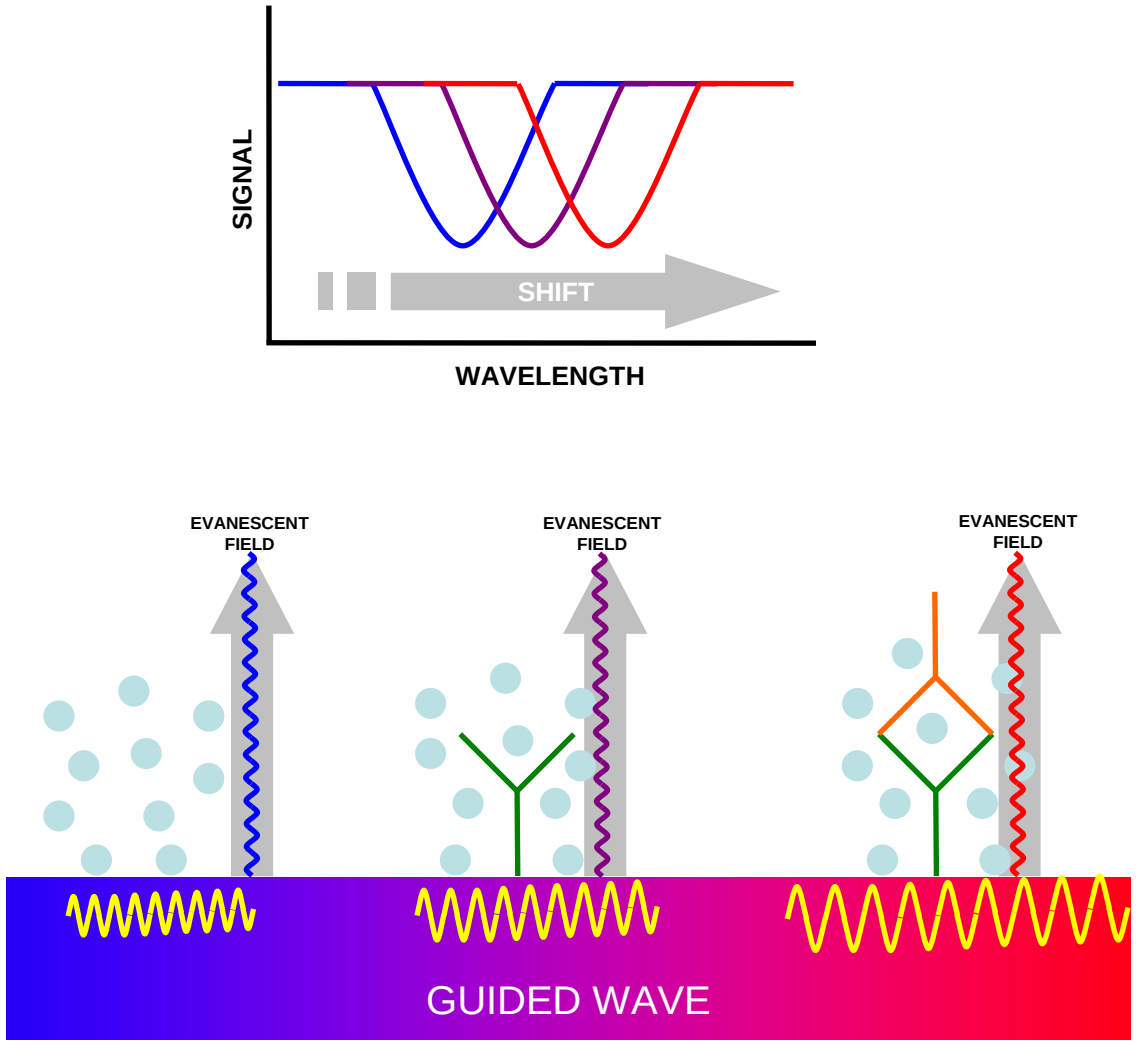

Figure XX. Evanescent field concept related to sensing on surface sensing.

Other label free techniques not based on evanescent field measures are ellipsometry[58],[59], atomic force microscopy[60] or photonic crystals[61],[62]. Photonic

[50] C. A. Barrios, K. B. Gylfason, B. Sánchez, A Griol, H. Sohlström, M. Holgado, R. Casquel, Opt. Letters 32 2007 3080
[51] C. A. Barrios, M. J. Bañuls, V. G. Pedro, K. B. Gylfason, B. Sánchez, A. Griol, A. Maquieira, H. Sohlström, M. Holgado, R. Casquel, Opt. Letters 33 2008 708
[52] H. Zhu, I. M. White, J. D. Suter, X. Fan, Biosens. Biolectron. 24 2008 461
[53] F. Vollmer, S. Arnold, D. Keng, PNAS 105 2008 20701
[54] S. J. Biehle, J. Carrozzella, R. Shukla, J. Poplewell, M. Swann, M. J. Freeman, J. F. Clark, Biochim. Biophys. Acta 1689 2004 244
[55] J. R. Lu, M. J. Swann, L. L. Peel, N. J. Freeman, Langmuir 20 2004 1827
[56] X. Chen, K. Zhou, L. Zhang, I. Bennion, Appl. Opt. 46 2007 451
[57] M. P. DeLisa, Z. Zhang, M. Shiloach, S. Pilevar, C. C. Davis, J. S. Sirkis, W. E. Bentley, Anal. Chem. 72 2000 2895
[58] S. Elhadj, G. Singh, R. F. Saraf, Langmuir 20 2004 5539
[59] D. E. Gray, S. C. Green, T. S. Fell, P. J. Dobson, E. M. Southern, Langmuir 13 1997 2833
[60] S. L. Shlyakhtenko, A. A. Gall, J. W. Jeffrey, D. D. Hawn, Y. L. Lyubchenko, J. Biophys. 77 1999 568

crystals posses a periodic dielectric structure within wavelength scale that enables them to be used as biosensors as any event disturbing the periodic structure (for instance, a biomolecule attached to its surface) will lead to a detectable defect mode. More recently, bio-photonic sensing cells (BICELLS) have been reported as a promising technology for quantification of biointeractions[63].

2. *Photopolymerization as a potential sensing strategy*

Among polymerization reactions, those initiated by wavelength irradiation are typified as *photopolymerization*[64-66]. There, the monomer or an additive in the mixture, presenting a structure sensitive to a concrete wavelength, result sufficiently excited to generate a radical entity upon being irradiated with a convenient light source thus converting the absorbed light energy into chemical energy (more detailed information in section 5.1). Afterwards, propagation and termination steps proceed in a general trend. In the end, the formation of stable gel deposits over the labeled molecules is to be observed.

PMR fall within free radical polymerization chemistry and so is characterized by a generic mechanism (see Figure X) covering a initiation step (when the active radical entities are formed), the propagation steps (when the radical species are transferred from one initial entity to a second one, thus providing a longer chain that, at the same time, posses a final radical group ready to continue reacting) and the termination steps (when two radical species get close enough to react producing two neutral molecules which are no more reactive). Propagation together with termination steps are considered bimolecular reactions as two molecules are required to accomplish such steps[67]. During the process the initial material properties change dramatically due to the polymer network formation, resulting in a crosslinked glassy or gelatinous substance, named as polymer.

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[61] L. Rindorf, J. B. Jensen, M. Dufva, L. H. Pedersen, P. E. Hoiby, O. Bang, Opt. Express 14 2006 8224

[62] J. Topolancik, P. Bhattacharya, J. Sabarinathan, P. C. Yu, Appl. Phys. Lett. 82 2003 1143

[63] M. Holgado, C.A. Barrios, F.J. Ortega, F.J. Sanza, R. Casquel, M.F. Laguna, M.J. Bañuls, D. López-Romero, R. Puchades, A. Maquieira, Biosens. Biolectron. 25 2010 2553

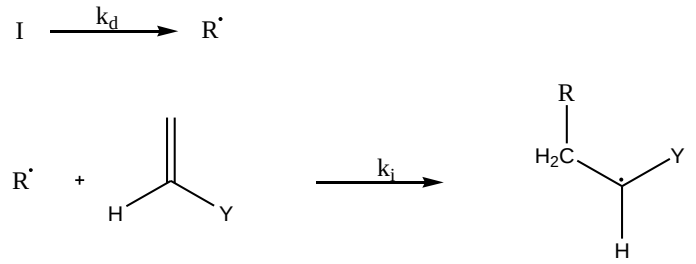
[64] A. Ledwith, Photoinitiation, photopolymerization and photochemical processes in polymers, C. E. H. Bawn ed., Int. Rev. Sci: Phys. Chem. Ser. Two 8 **1975** 253

[65] S. S. Labana, J. Macromol. Sci. Rev. Macromol. Chem. C11 **1974** 299

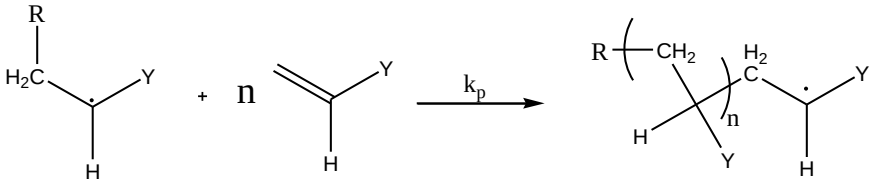
[66] D. Philips, Photochem. 5 **1974** 691

[67] G. Moad, D. H. Solomon, The chemistry of free radical polymerization, Pergamon Elsevier science eds. Oxford **1995**

Initiation

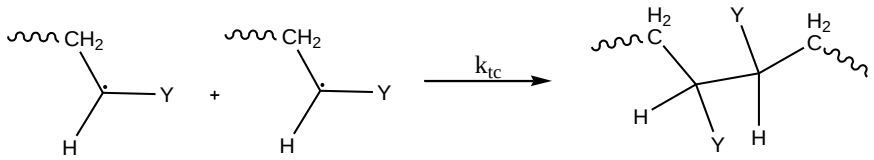


Propagation

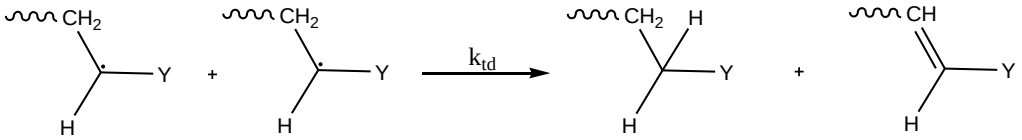


Termination

by combination



by disproportion



by chain transfer

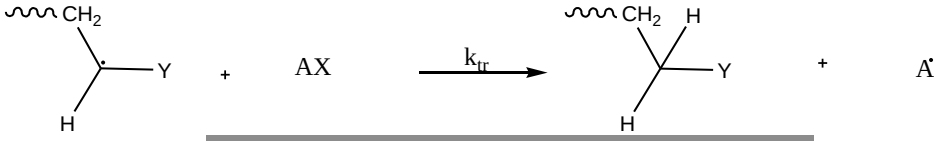


Figure. Generic polymerization processes scheme.

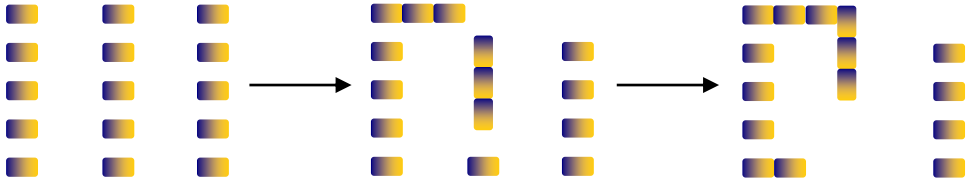
There are two main polymerization strategies depending on the nature of the reactivity that takes to the polymer formation[68-70]: Basically, step and chain growth polymerizations (see Table XX for a brief overview). Most photopolymerizations run on chain growth mecanisms.

[68] K. J. Saunders, Organic polymer chemistry, Chapman and Hall eds., London **1988**
[69] G. Odian, Principles of polymerization, Wiley eds., NY **1991**
[70] P. Rempp, E. W. Merrill, Polymer synthesis, Huthig and Wepf eds., Basilea **1991**

Step growth polymerization

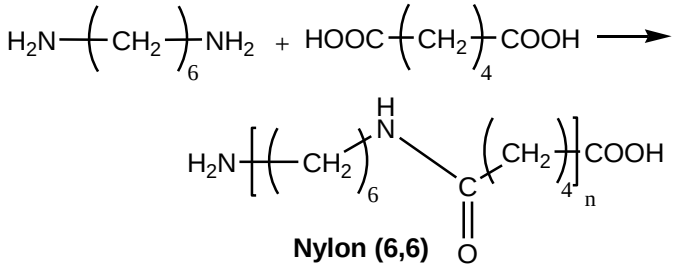
Fundamentals:
Consecutive condensation reactions between two bifunctional molecules.
Elimination of a small molecule from each interaction is expected.
Reaction may happen between oligomers: growth > 1monomer/addition.
Slow chain growth (minutes to days).

Pool monomer evolution:



Functional groups typically used:
Carboxylic acids (to produce poliethylene tereftalathe (PET)
Polyimida (to produce Nylons))
Isocianates (to produce urea resins)
Aldehydes (to produce melamine of fenolic resins)
Epoxydes (to produce epoxy resins)
Aromatic ring containing molecules (very interesting for ring opening polymerization).

Example:

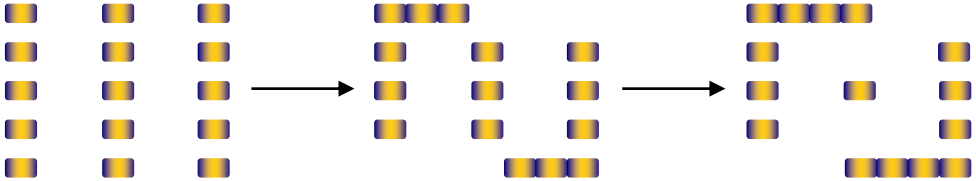


Nylon (6,6)

Chain growth polymerization

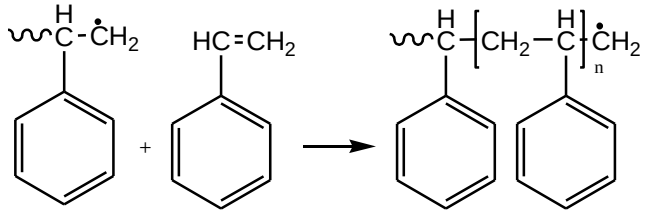
Fundamentals:
Reaction of monomer double bonds activated by an initiator substance.
No elimination takes place.
Reaction happens between monomers: growth = 1monomer/addition.
Rapid chain growth (seconds or below).

Pool monomer evolution:



Different types of processes:
Ionic polymerization: anionic and cationic forms.
Coordinative polymerization
Photopolymerization (the initiator is a photo sensitive molecule)
Reversible addition-fragmentation chain transfer (reversible transfer of a dithioesther moiety occurs between active and dormant chains)
Atom transfer radical polymerization (reversible transference of halogen atoms happens between a dormant chain and a transition metal catalyst).

Example:



Polyethylene

Photopolymerization has become a relevant field of investigation as its applications spread over a wide range of practical uses. The growing interest on this topic is made clear in Figure 1, where the increasing number of related publications that each year arises is shown.

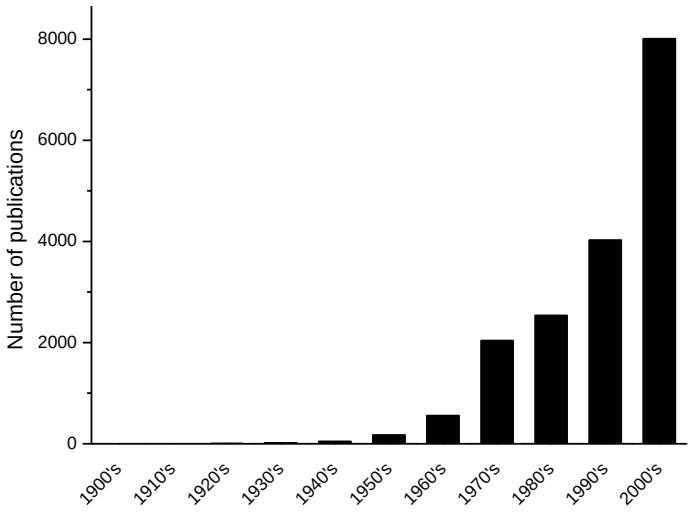


Figure 1. Time evolution for "photopolymerization" topic scientific publications[71].

Here, some of the principal characteristics that make the photopolymerization reaction a very attractive procedure in biorecognition chemistry are reported.

As mentioned above, the non-enzymatic dependence provided by this kind of reactions, makes them more robust and resistant to experimental fluctuations that may affect, on the contrary, to the sensitive enzyme solutions. Secondly, major appeal is found in the economic aspects relating to this family of procedures, as they reveal to be inexpensive compared to routine UV-VIS and colorimetric techniques or more specialized equipments required in most label free methods. For one to get an approximate idea, while a routine laboratory procedure based on spectroscopic detection should cost above 10 € per analysis, a photopolymerization procedure is to cost 5 times lower. Thirdly, the rapidity in getting the signal points again in favor of the photopolymerization processes, as they may generate a readable signal in the order of minutes while other methodologies will take longer times, even hours.

Besides, another highlight establishes that photopolymerization reactions can take place on solid surfaces, which behave as supports on which the immobilization and reactive stages are to occur. Once bound to the surface, it is very easy to handle the reactants and also to separate the non-reacted liquid mixture from the selectively surface adsorbed products. Thus, PMR on surface is useful because allows both spatial and temporal control of the surface modifications aimed to supply a desired superficial density of photoinitiators. Such kind of processes lay in the heterogeneous area of analytical chemistry, in view of the necessary primary solid surface for biomolecules interactions.

[71] Source: SciFinder Scholar data base, available through the UPV library service.

So far, photopolymerization reaction for sensing and detection of biointeractions is able to amplify the signal due to each single molecular recognition event, rather than amplify the total number of molecular recognition events. With this regard, every molecular recognition is to be amplified by the polymerization reaction up to 10^6 monomers, or even more[72], for the technique to be analytically attractive. Up to now, PMR is been used in DNA analysis by hybridization techniques allowing flu virus detection[73] or single nucleotide polymorphisms testing (REF). Protein to protein[74] or protein to chemicals interactions[7578]. (for example, avidin to biotin) are again susceptible of being detected by PMR techniques.

3. *Hydrogel importance*

Most of the photopolymerization reactions use one substance from the following list as monomer: acrylic acid, acrylamide, cyclic lactams, ethyleneglycol acrylates, methacrylamides, methacrylates, polysaccharides (alginate, chitosan or cellulose), oligopeptides, proteins (albumin or gelatin) and styrene compounds. Among them, ethylenglycol acrylate and acrylamide derivatives are the preferred. In all cases a hydrogel is formed during the polymerization process.

The term *hydrogel* refers to a sort of water insoluble polymer chain networks that behaves as highly absorbent natural or synthetic polymers. They are prepared by polymerization of a hydrophilic monomer under conditions where the polymer becomes cross-linked in a 3-dimensional matrix sufficient to gel the solution. Due to their significant water content (they can contain up to 99% water), hydrogels also possess a degree of flexibility very similar to natural tissue.

Two major families of hydrogels are based on ethylene glycol acrylate or acrylamide derivatives, as follows.

Firstly, poly (ethylene glycol) acrylates and diacrylate derivatives (PEGA and PEGDA, respectively) are exceptional polymers with relevant properties that make them very attractive for bio-sciences[79]. Due to their oxygen and carbon central skeleton, they are biocompatible, little toxic or immunogenic, hydrophilic and as mentioned, may retain high water content when chemically cross-linked showing tissue-like physical and mechanical properties[80]. Moreover, PEG derivatives can be prepared with different average molecular weights what enhances their manipulation by technicians. Briefly, here we report different uses of biocompatible ethylene glycol hydrogels:

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[72] K. L. Rowlen, J. W. Birks, C. N. Bowman, H. Sikes, R. Hansen, R. Kuchta, US Patent 7354706B2 **2008**
[73] L. R. Kuck, US Pat 60/773532 **2007**
[74] L. R. Kuck, A. W. Taylor, Biotechniques 45 **2008** 179
[75] K. L Rowlen, J. W. Birks, C. Bowman, H. Sikes, R. Hansen, L. M. Johnson, R. Jenison, Nature materials 7 **2008** 52
[76] R. R. Hansen, H. D. Sikes, C. N. Bowman, Biomacromolecules 9 **2008** 355
[77] R. R. Hansen, H. J. Avens, R. Shenoy, C. N. Bowman, Anal. Bioanal. Chem. 392 **2008** 167
[78] H. J. Avens, T. J. Randle, C. N. Bowman, Polymer 49 **2008** 4762
[79] J. M. Harris, S. Zalipsky, Eds. ACS Symposium Series 680 ACS, Washington **1997**
[80] N. B. Graham, Poly(ethylene glycol) chemistry, pp263-280, J. M. Harris ed., Plenum press, NY **1992**

- Drug carriers to release protein and other low molecular weight compounds[81-82].
- Wound covering applications to accelerate injuries healing[83].
- Immunoprotective barriers for therapeutic cell transplantation protect the rejection of the transplanted cell by the host's immune system[84].
- Type I diabetes treatment using hydrogel encapsulated pancreatic islets. The insulin is able to permeate through the gel membrane in a controlled manner to the blood current[85-87].
- Tissue engineering progress on transdermal cartilage and other hydrogel bioactive scaffolds reduces the number of invasive surgical interventions[88-89].
- Sensing molecules encapsulated within hydrogel layers allow for biochip substances detection[90-91].
- PEG hydrogels can encapsulate bone marrow derived mesenchymal stem cells (MSC) inducing chondrogenesis[92].
- High-throughput drug or pathogen screening by immobilization of different cell phenotypes in microstructured hydrogel arrays sensitive to UV initiated free radical polymerization[93].

Secondly, acrylamide hydrogels and their derivatives have undesirable features, such as the toxicity of their components and their low environmental compatibility. The environmental compatibility and life cycle are important aspects that must always be considered when developing new materials[94]. From the health point of view, acrylamide is a substance thought to be carcinogenic from long time ago, as recent investigations have confirmed[95-96]. Nevertheless, acrylamide shows smaller inhibition time before initiation of polymerization and it polymerizes faster, reaching higher conversions than polyethyleneglycol acrylate derivatives with films 4-8 folds ticker[78]. The lower acrylamide molecular weight together with the lower crosslinking extent and higher content of water allows amide radical species to diffuse more readly throughout the growing regions inducing an enhanced polymerization on the substrate surface. Hydrophylic photopolymers based on acrylamide as the polymerizable monomer are versatile materials with industrial applications in the

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[81] X. Zhao, J. M. Harris, Division of polymer chemistry, 213th national ACS meeting, San Diego CA, **1997**
[82] G. Gozzelino, G. A. Dellaquila, D. R. Tobar, J. Appl. Polym. Sci. 112 **2009** 2334
[83] A. S. Sawhney, C. P. Pathak, J. A. Hubbell, Macromolecules 26 **1993** 581
[84] G. M. Cruise, O. D. Hegre, F. V. Lamberti, S. R. Hager, R. Hill, D. S. Scharp, J. A. Hubbell, Cell transplant. 8 **1999** 293
[85] G. M. Cruise, O. D. Hegre, D. S. Scharp, J. A. Hubbell, Biotechnol. Bioeng. 57 **1998** 655
[86] G. M. Cruise, D. S. Scharp, J. A. Hubbell, Biomaterials 19 **1998** 1287
[87] A. S. Sawhney, C. P. Pathak, J. A. Hubbell, J. Am. Chem. Soc. 114 **1992** 8311
[88] J. Elisseeff, K. Anseth, D. Sims, W. McIntonsh, M. Randolph, R. Langer, Proc. Natl. Acad. Sci. USA 96 **1999** 3104
[89] J. Zhu, Biomaterials 31 **2010** 4639
[90] K. Sirkar, M. Pishko, Anal. Chem. 70 1**998** 2888
[91] A. Revzin, J. R. Russell, V. K. Yadavalli, W. G. Koh, C. Deister, D. D. Hile, M. B. Mellott, M. V. Pishko, Langmuir 17 **2001** 5440
[92] C. G. Williams, T.K. Kim, A. Taboas, A. Malik, P. Manson, J. Elisseeff, Tisue Eng. 9 **2003** 679
[93] W. Koh, L. J. Itle, M. V. Pishko, Anal. Chem. 75 **2003** 5783
[94] F. Pellaschiar, Eur. Coat. J. 81 **2005** 22
[95] K. Hashimoto, W. N. Aldrige, Biochem Pharmacol. 19 **1970** 2591
[96] F. Mendel Chemistry, J. Agric. Food Chem. 51 **2003** 4504

field of holographic recording materials or holographic memories[97-98] but also in sensing development[78].

II. FOCUSING ON PMR PROCESS

4. First concepts to approach the PMR process

4.1. Induction period and plateau behaviors

Irradiation with the appropriate wavelength prompts the polymer growth in the illuminated regions. Nevertheless, a short induction period of virtually no growth is observed at the beginning fo the reaction[99]. During this time, consumption of the inhibitors (present in the commercial monomer solutions as growth retardants) and dissolved O_2, takes place[100] by the photogenerated initial radicals. The induction period is normally in the order of ms time. The polymerization will begin once the concentrations of the inhibiting substances are sufficiently reduced and the radical moieties are able to compete with the scavenger species[101]. In this sense, the equilibrium dissolved oxygen concentration in acrylate typical monomer mixtures is reported to be around 0,6-2,0x10^{-3} M (in water it is expected to be 0,3x10^{-3}M aprox.[102]), yet the polymerization reaction will only proceed if this value is reduced down to 4x10^{-6} M[103]. The time necessary for the inhibitor concentration to be sufficiently reduced is kwnon as inhibition period and can be solved approximately as follows[104],

$$t_{inhib} = \frac{[O_2]_{cons}}{R_i}$$

where, $[O_2]_{cons}$ is the amount of oxygen that needs to be consumed and R_i is the initiation rate of the polymerization reaction.

When the induction period is overcome, an approximately linear relationship can be established between monomer conversion (or similarly, hydrogel thickness evolution) and illumination time, after which a plateau behavior is reached; presumably due to the progressive exhaustion of the

[97] E. Fernández, C. García, I. Pascual, M. Ortuño, S. Gallego, A. Beléndez, Appl. Opt. 45 **2006** 7661
[98] A. Marquez, C. Neipp, A. Beléndez, S. Gallego, M. Ortuño, I. Pascual, Opt. Lett. 28 **2003** 1510
[99] P. Tigulla, U. Vuruputuri, J. Chem. Sci. 116 **2004** 115
[100] P. Cheben, M. L. Calvo, Appl. Phys. Lett. 78 **2001** 1490
[101] L. Gou, N. Corestsopoulous, A. B. Scranton, J. Polym. Sci. Part A Polym. Chem. 42 **2003** 1285
[102] D. W. Green, R. H. Perry, Perry's chemical engineers' handbook, table 2-123, 8th ed. McGraw-Hill, NY 2008
[103] C. Decker,A. D. Jenkin, Macromolecules 18 **1985** 1241
[104] A. K. OBrien, C. N. Bowman, Macromolecules 39 **2006** 2501

source of free radicals by bleaching of available initiating moieties[105]. The thickness the hydrogel will achieve depends on the reactants concentrations and on the irradiation conditions. In one hand, a thicker hydrogel layer is obtained when more the N-vynil pyrrolidinone (NVP) additive is incorporated in the blend at any illumination time before the plateau is reached. In the same way, a linear relationship between thickness and monomer percentage is detected in a PEG-co-poly(alfa-hydroxy acid) polymerization for a 30s illumination time. Concerning the illumination intensity, the time to reach the plateau results accelerated with higher intensity, but once arrived at this state no further effect is registered[105]. The duration of these two stages (induction period and plateau) depends mainly on the monomer reactivity, which is related to its molecular structure[106], ranging from few seconds to minute order. On the other hand, the light intensity influences the reaction rate and the degree of polymerization, both increasing with greater intensity values. At the initial steps of the reaction, radicals may link with other monomers to form aggregates with pendant double bonds which can cycle through intramolecular interaction to form microgel regions. In a second stage, intermolecular links may attach different microgel regions forming a network structure. These two processes are directly affected by the irradiation intensity. The higher light intensity, the smaller localized microgel regions will be detected due to the greater reaction rate which prompts reaching the gelation point. Thus, mixtures cured with high light intensity present more open structures, while those cured with low light intensity show more compact structures and an increased viscosity[107].

[105] M. D. Lyman, D. Melanson, A. S. Sawhney, Biomaterials 17 **1996** 359
[106] C. N. Bowman, C. J. Kloxin, AIChE J. 54 **2008** 2775
[107] H. He, L. Li, L. J. Lee, *Reactive and Functional Polymers* 68 **2008** 103

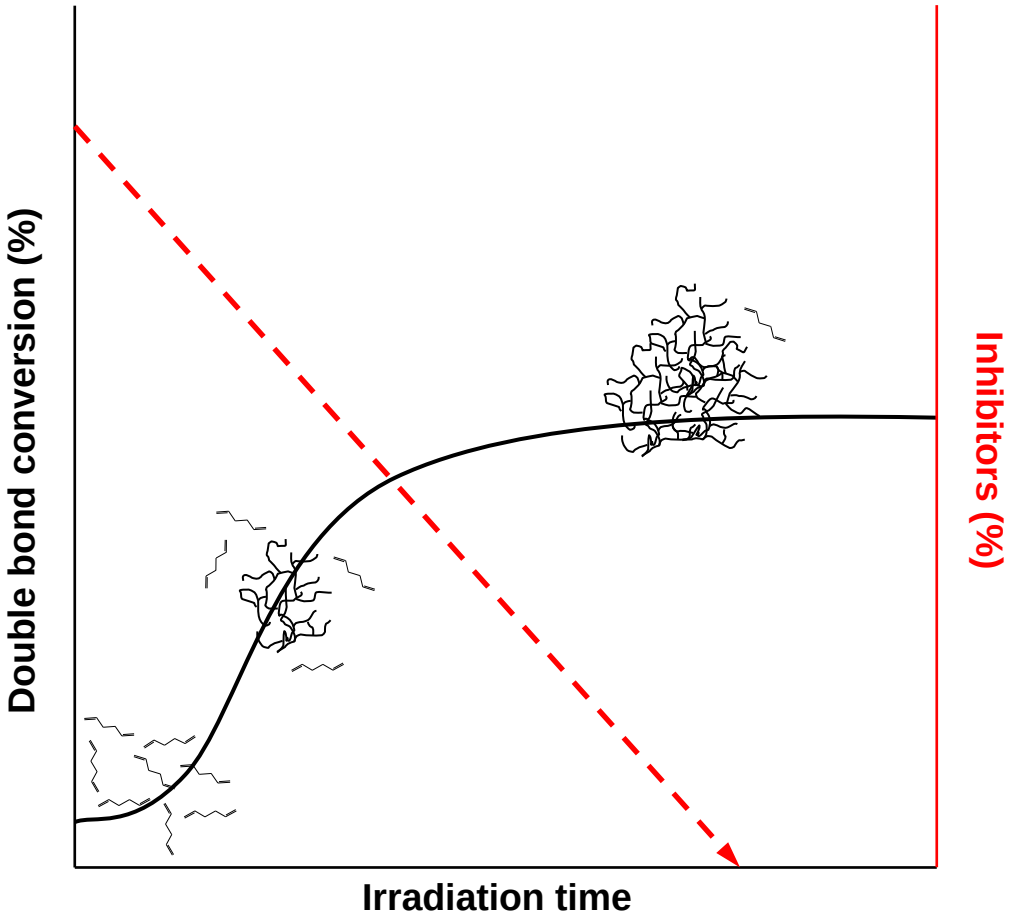

Figure XX. Polymer evolution as inhibitiors are consumed.

Variations in the molecular structure have also noticeable effects on the reactivity of the monomers because of changes in the ability to form hydrogen bonds, molecular dipole interactions or even affecting the propagating active centers[108-111]. Understanding the influence of the monomer atomic structure in its future performance is a relevant topic to bear in mind when facing any serious polymerization study.

4.2. Autoacceleration and autodeceleration stages

A typical photopolymerization kinetic curve is shown in Figure X:X by plotting the polymerization rate (R_p) front the double bond conversion. Two regions are unambiguously identified in the picture and reflect the typical kinetics behavior of a crosslinking photopolymerization reaction:

- Autoacceleration regime, at the beginning of the reaction, is characterized by the polymerization rate increase due to increased macroradical concentration. In this regime, the termination reaction (a bimolecular process) becomes diffusion controlled and the reduction in mobility,

[108] C. Decker, J. Coat. Technol. 59 **1987** 97
[109] J. Jansen, A. A. Dias, M. Dorschu, B. Coussens, Macromolecules 36 **2003** 3861
[110] H. Kilambi, J. W. Stansbury, C. N. Bowman, Macromolecules 40 **2007** 47
[111] H. Kilambi, J. W. Stansbury, C. N. Bowman, J. Polym. Sci. A Polym. Chem. 46 **2008** 3452

owing to the constant crosslinking elemental steps, reduces the termination constant because of the more hindered mixture, thus leading to a rapid increase of radicals concentration, what drives the observed rate increase. Specifically, in reactive systems where crosslinking reactions occur, the radicals mobility may be more probable by propagation than by spatial diffusion[112-115].

- Autodeceleration regine, at the later stages of polymerization is characterized by a polymerization rate decrease ending in a complete inertness, although radical and monomer species might be still present. This tendency is provoked by the progressive vitrification of the reactive mixture. When this stage is achieved, the double bonds migration towards the radical groups becomes rate limiting, thus diffusion controlled propagation. Further reactions favor the species mobility reduction, minimizing the polymerization process despite the continue radicals formation[116-119]. The formation of crosslinked acrylate polymer becomes possible when the macromolecular diffusion is dominant due to restricted steric constrains. Termination in this regime was clearly demonstrated to proceed through diffusion controlled reactions, that happen when radicals in the polymer mixture propagate through local unreacted double bonds till they terminate[120-123]. This termination mechanism depends on the crosslink density, the viscoelastic behavior and the polymerization temperature[114, 124].

[112] K. S. Anseth, C. M. Wang, C. N. Bowman, polymer 35 **1994** 3243
[113] K. S. Anseth, C. M. Wang, C. N. Bowman, Macromolecules 27 **1994** 650
[114] J. S. Young, C. N. Bowman, Macromolecules 32 **1999** 6073
[115] K. S. Anseth, C. N. Bowman, N. A. Peppas, Polym. Bull. 31 **1993** 229
[116] J. G. Kloosterboer, Adv. Polym. Sci. 84 **1988** 1
[117] K. S. Anseth, K. J. Anderson, C. N. Bowman, Macromol. Chem. Phys. 197 **1996** 833
[118] K. A. Berchtold, T. W. Randolph, c. N. Bowman, Macromolecules 38 **2005** 6954
[119] E. Andrzejewska, Prog. Polym. Sci. 26 **2001** 605
[120] K. S. Anseth, C. M. Wang, C. N. Bowman, Macromolecules 27 **1994** 650
[121] K. S. Anseth, C. N. Bowman, C. Decker, Macromolecules 28 **1995** 4040
[122] K. S. Anseth, C. N. Bowman, L. N. Kline, T. A. Walker, K. J. Anderson, Macromolecules 28 **1995** 2491
[123] K. A. Berchtold, T. M. Lovestead, C. N. Bowman, Macromolecules 35 **2002** 7968
[124] K. S. Anseth, C. N. Bowman, Polym. React. Eng. 1 **1993** 499

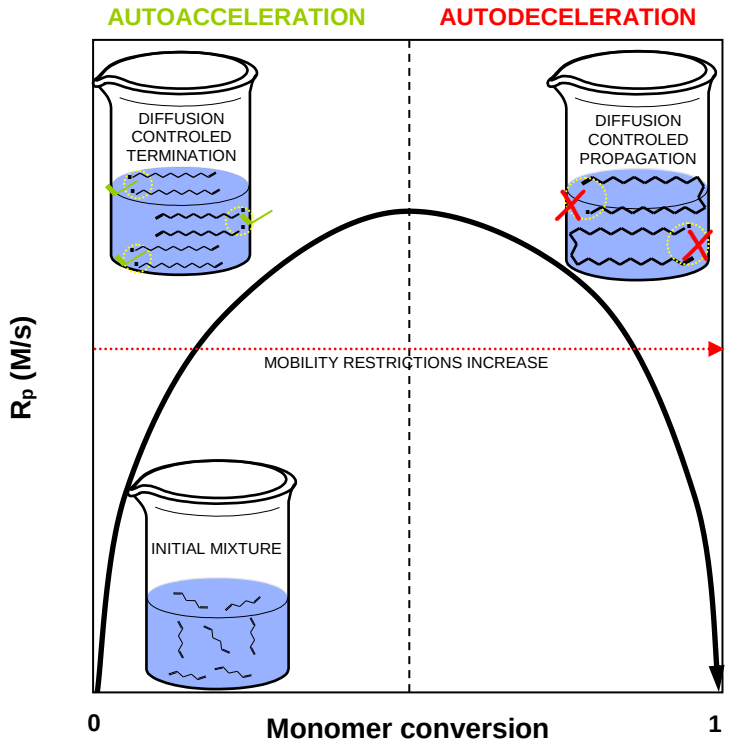

Figure. Autoacceleration and autodeceleration concepts.

4.3. Shrinkage phenomenon

The monomer polymerization takes place preferentially in the illuminated regions. The consumption of monomer species starts off a diffusion tendency of these moieties flowing from the dark to the illuminated regions. Before crosslinking, the unreacted molecules remain separated by van der Walls distances, which become shortened when covalent bonds are formed between them. The flow of monomer species in addition to their bonding, create voids in the mixture known as the shrinkage phenomenon[100, 125].

The trend to shrink of highly crosslinked networks is the reason for these materials noticeable internal stress. Depending on the irradiation pattern both high and low crosslinking density regions might be visible in the final material, resulting in a remarkable heterogeneity that will lead to physical fragility of the product. The introduction of the step-growth thiol-ene or the reversible covalent bond photolopymerization strategies, mitigate undesired heterogeneity and stress. Briefly, the first strategy is based on thiol compounds introduction in the reactive mixture which enhances control of polymer weight network structure, thus obtaining average shorter chain lengths and more homogeneous materials with less shrinkage stress related to delayed gelation[126-128]. The second strategy is based on the incorporation in the blend of an allylic sulfide functionality with the ability to undergo addition of a thiol radical species and a

[125] B. M. Monroe, W. K. Smothers, Technology and applications, Edited L. A. Hornak, Charpter 5, Marcel Dekker NY, **1992**
[126] C. E. Hoyle, T. Y. Lee, T. Roper, J. Polym. Sci. A Polym. Chem. 42 **2004** 5301
[127] C. R. Morgan, F. Magnotta, A. D. Ketley, J. Polym. Sci. A Polym. Chem. 15 **1977** 627
[128] A. F. Jacobine, Radiation curing in polymer science and technology III, London Elsevier **1993**

subsequent intramolecular fragmentation (S_H2' reaction), resulting in a bond rearrangement procedure within the matrix referred as post-gelation stress relief[106]. This methodology may release up to 90% of the initial product stress[129-130]. S_H2's are elemental steps in many chemical reactions involving various types of alkyl-oxides, amino, peroxy or alkyl R_1· radicals. An R_2 compound containing a multivalent saturated atom, as Si, Sn, S, Se, Te, Mg, Hg, B, P, Zr, or Ti is required. Depending on the transition state, S_H2 can proceed via a concerted or a stepwise mechanism, consisting in a radical R_1· addition to a second compound R_2, consuming inactive peroxyl R1· radicals and forming active radicals R2·, which allow to overcome the detrimental effect of oxygen[131-132].

4.4. PMR main advantages and drawbacks

Polymer properties are highly dependent on the preparing conditions. There are intimate correspondences affecting the material structure along with its physical properties and the details of its formation (Fig. XX).

Figure. Intrinsic hydrogels parameters interrelation.

In this section, brief considerations are made on the major factors affecting photopolymerization reactions:

- In the earliest stage of a polymerization reaction the system can be understood as an infinite pool of unreacted monomer, so the mixture behaves most ideally and is least influenced by diffusional constrictions. However, as the propagation and termination stages are reached, diffusional limitations due to crosslinking become more pronounced. When highly crosslinked hydrogel systems get to autodeceleration regime, radicals are very often forced to cease propagating and remain trapped inside the network; sometimes isolated from vinyl reactive bonds for months or even years[117, 118, 133].
- Delay in the attainment of equilibrium properties might be related to the kinetic evolution of the monomer blending[113, 116].

[129] D. N. Hall, A. A. Oswald, K. Griesbau, J. Org. Chem. 30 **1965** 3829
[130] D. N. Hall, J. Org. Chem. 32 **1967** 2082
[131] K. U. Ingold, B. P. Roberts, Free Radical Substitution Reactions, ed. Wiley Interscience, NY **1971**.
[132] M. E. Roz, M. A. Tehfe, J. Lalevee, B. Graff, X. Allonas, J. P. Fouassier, Macromolecules 43 **2010** 2219
[133] S. Zhu, Y. Tian, A. E. Hamielec, D. R. Eaton, Macromolecules 23 **1990** 1144

• Heat and mass gradients may appear in the curing step and affect the physical characteristics of the final material[134-135] creating a heterogeneous shrank product with stressed and fragile structure[136-139]. Such heterogeneities can cause different glass transition temperature varying up to 100ºC inside the very same sample[140-141]. Furthermore, when strongly absorbing initiators or thick polymer layers are expected to be obtained, a light intensity gradient should be taken into account from the surface to the inner core of the forming gel[142].

• Molecular oxygen dissolved in the reactive blend acts as a very efficient terminator agent because it reacts rapidly with the propagating radicals to form peroxy-based radicalary species quite unreactive from then on[108-105,143-145]. O_2 diffusion in the air-sample interface inhibits mainly the polymerization on the film top surroundings. On the contrary, in thick-film systems, the bottom of the layer reaches a higher conversion degree due to insulation.

As for the advantages[146] of photopolymerization processes compared to other forms of polymerization it could be said that:

• From the operation point of view, PMR requires no or small amount of solvent for the curing process. All components are copolymerized in the cured gel, and so the amount of volatile organic compounds is sometimes negligible.

• The curing process is triggered by light irradiation allowing it to occur on materials that would not withstand a thermal treatment.

• In the absence of light photoinitiators are stable compounds, what permits a good control of the curing time. Many formulations can be prepared and stored for long periods, maintaining the polymerization capacity until a suitable light source is applied.

• Light is easily applied under restricted spatial conditions by using a photomask. This manner, formulations can be polymerized in a controlled area, while unexposed zones remain liquid (unreacted).

• Radiaton curing use to proceed faster than thermal treatments, thus no or limited cooling zones are required and the devices can be immediately manipulated.

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[134] M. D. Goodner, C. N. Bowman, Macromolecules 32 **1999** 6552
[135] M. D. Goodner, C. N. Bowman, Chem. Eng. Sci. 57 **2002** 887
[136] P. E. M. Allen, G. P. Simon, D. R. G. Williams, E. H. Williams, Macromolecules 22 **1989** 809
[137] A. R. Kannurpatti, K. J. Anderson, J. W. Anseth, C. N. Bowman, J. Polym. Sci. Part B Polym. Phys. 35 **1997** 2297
[138] A. R. Kannurpatti, C. N. Bowman, Macromolecules 31 **1998** 3311
[139] A. R. Kannurpatti, J. W. Anseth, C. N. Bowman, Polymer 39 **1998** 2507
[140] H. Lu, L. g. Lowell, C. N. bowman, Macromolecules 34 **2001** 8021
[141] L. G. Lowell, H Lu, J. e. Elliot, J. W. Stansbury, C. N. Bowman, Dent. Mater. 17 **2001** 504
[142] G. A. Miller, L. Gou, V. Narayanan, A. B. Scranton, J. Polym. Sci. A Polym. Chem. 40 **2002** 793
[143] A. K. OBrien, C. N. Bowman, N. B. Cramer, J. Polym. Sci. A Polym. Chem. 44 **2006** 2007
[144] A. K. OBrien, C. N. Bowman, Macromol. Theory Simul. 15 **2006** 176
[145] L. J. Gou, B. Opheim, C. N. Coretsopoulos, A. B. Scranton, Chem. Eng. Commun. 193 **2006** 620
[146] www.basf.com

5. *Reactive substances taking part in the PMR process Mechanism proposal*

5.1. Photoinitiator

Photopolymerization processes can be classified in two groups depending on the reactivity nature of the photoinitiator substance, the first reactant taking part in a PMR process. In photopolymerization Type I reactions, the radicals are generated by direct homolytic cleavage or internal rearrangement of the light excitable molecule obtaining two neutral radicals. On the other side, photopolymerization Type II reactions are characterized by the need of an initiator or sensitizer molecule able to act as electron and hydrogen donor when interacting with the excited state of a radiation excitable substance, thereby producing two initiating radical moieties[147-148], at least one of them with ability to activate the monomer towards polymerization (see Figure X:X). Usually, the rate constants of the reactions between monomers and triplet excited initiator states are low and are commonly neglected[149], being necessary the presence of a co-initiator for the monomer radicals to be produced. However, some authors reported rate constats for the interaction between acrylates and excited dyes (eosin and riboflavin) around 10^5, which are assumed to have little influence in the process if compared to the rate constants involving a co-initiator (i.e. amine), with values around > 10^6 (see reference 178).

Because the Type II initiations are based on bimolecular reaction, they are generally slower than Type I reactions, which are based on unimolecular formation of radicals. Those systems are, therefore, expected to be more sensitive to the quenching of the excited triplet states. Either Type I or II approach, the photoinitiator is the key factor to convert luminic energy into chemical energy.

[147] A. Ledwith, M.D. Purbrick, *Polymer* 14 **1973**, p. 521
[148] R.S. Davidson In: D. Bethel and V. Gold, Editors, *Advances in Physical Chemistry*, Academic Pres, London **1983**
[149] J. P. Fouassier, E. Chesneau, Makromol. Chem. 192 1991 245

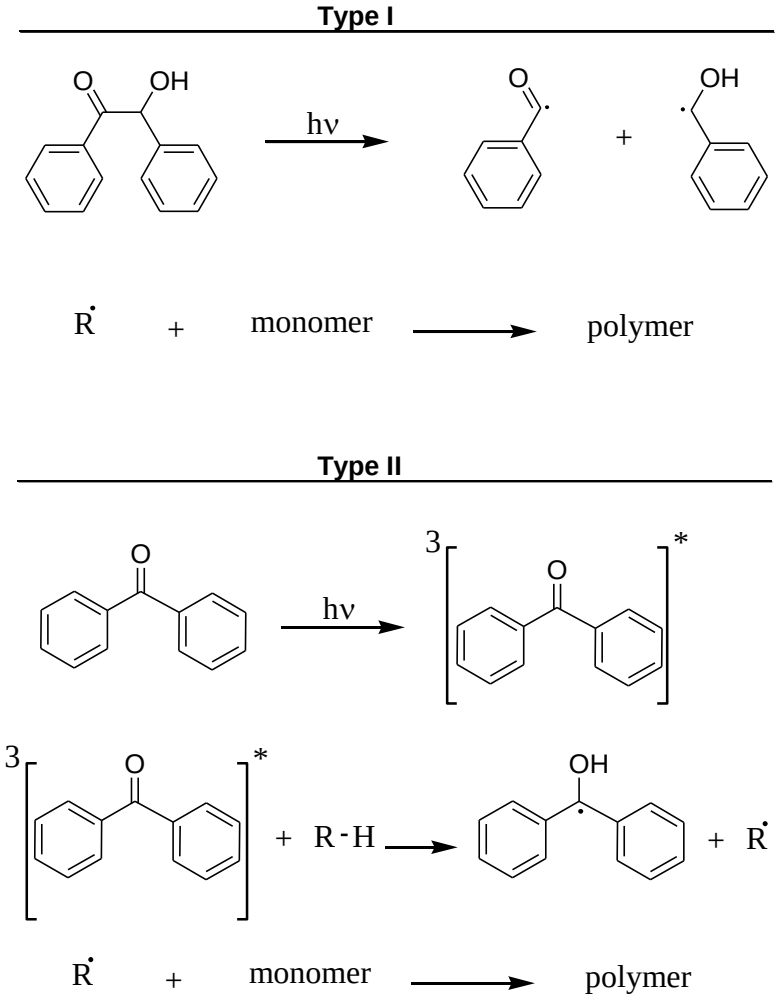

Figura. TypeI and II photopolymerization scheme using a benzoin and aryl ketone derivative as photoinitiators.

All Type I photoinitiators are commonly used for UV irradiation polymerizations whilst some Type II photoinitiators are used for UV irradiation and other for visible irradiation polymerization reactions. A few examples are depicted in Table X:X.

Table. Some examples of type I and type II photoinitiator compounds[150]. Sacado de la págida de Aldrich www.sigmaaldrich.com de abajo y Camphorquinone/benzylquinone de J polym sci A polym chem 40 2002 3171.

UV		VIS
I	II	II
Benzoin ethers	Benzophenones/sensitizer	Titanocenes/sensitizer
Acyl phospine oxides	Thioxanthones/sensitizer	Xanthines/sensitizer
Benzylketals	Anthraquinones/sensitizer	Camphorquinones/sensitizer
α-hydroxy-alkyl-phenone	Ketocoumarins/sensitizer	Benzylquinones/sensitizer

Several advantages predispose the analyst to use visible light source for photoinitiation, when possible:

- Low power and mild excitation source required to activate the initiation step

[150] Source: Sigma Aldrich database at www.sigmaaldrich.com.

• Low cost experimental hardware needed (normally a conventional lamp to irradiate)
• Important reduction of the undesired bulk polymerization (often observed when using UV light) is achieved.

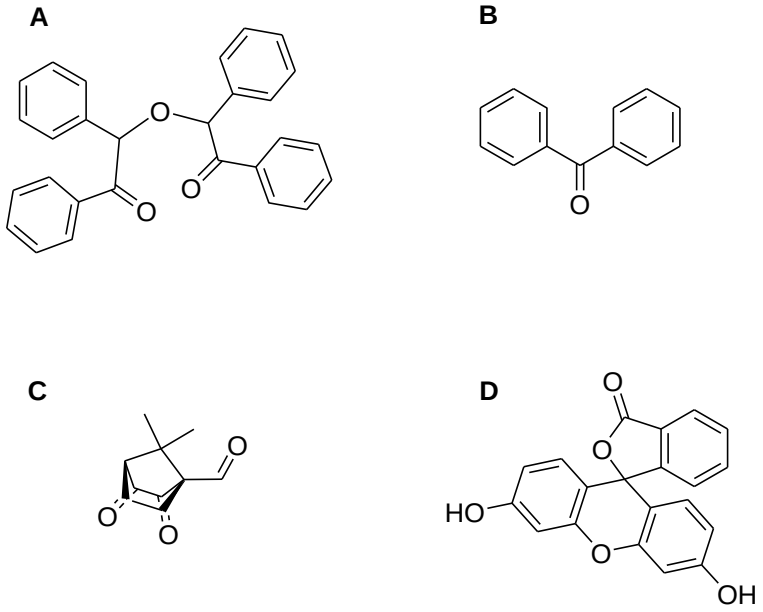

Figure. Some photoinitiator structures: Benzoin ether (A), benzophenone (B), camphorquinone (C), fluorescein (D).

Studies realized on oligonucleotide dilution chips show a critical dependence on the amount of polymer growth with the superficial biomolecules density achieved during the photoinitiator coating step. Manipulation of this parameter by changing the binding conditions brings the chance to tune the amount of amplification generated product[77]. For example, the concentration of a photoinitiator streptavidin-eosin conjugate solution must be optimized as high concentration values might lead to nonspecific adsorption of the initiator species on the support surface, raising the background signal, thus reducing sensitivity[76].

From the environmental point of view, the use of some dyes as photoinitiators involves potential risks. This is the case of the xanthine family of compounds (eosin, Bengal rose, erytrosin B and others) that posses halogen atoms in their formula. The decomposition by-products of these substances are no more photoactive but can continue degrading to release halogenated molecules which are toxic to the environment, what turned them potential pesticides[151]. Cleaner initiators, as Flavin derivatives (mainly riboflavin, a component of B2 vitamin), were proposed as xanthine substitutes because they contain no halogen groups but still absorb in the visible region (≈ 500 nm) turning excited to a triplet state ready to interact with an electron and proton donor. Besides, in this concrete case, after photodecomposition the by-products are still colored and can initiate photopolymerization with similar efficiency to the initial molecule[152].

[151] J. R. Heitz, Insect Mode Action, Academic NY USA **1982**
[152] M. Ortuño, E. Fernández, S. Gallego, A. Beléndez, I. Pascual, Opt. Express 15 **2007** 12425

5.2. Sensitizer

As mentioned above, Type II photopolymerizations require the presence of a co-initiator substance, referred as sensitizer. Typical sensitizer molecules belong to alcohol, ether, amine or thiol families; being tertiary amines the mostly used because of their high efficiency and relative low price. To become a potencial photopolymerization sensitizer a molecule must fulfill two requirements: to allow electron transfer to the photoinitiator and to have at least one labile hydrogen atom in its structure[153-155]. In this sense, carbonyl initiator excited triplet states are usually two to three orders of magnitude more reactive towards tertiary amines than towards alcohols or ethers[156].

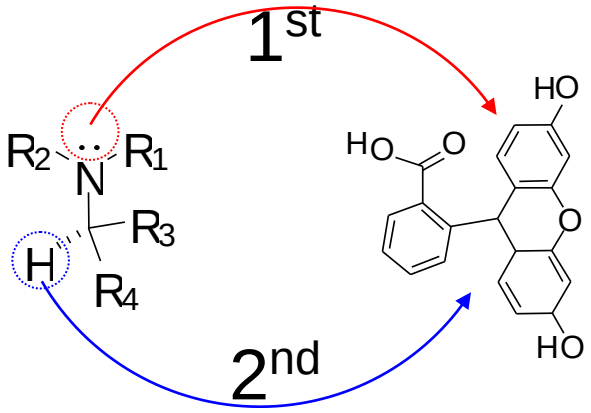

Figure x.x. Tertiary amine with a labile hidrogen.

The quantity of amine in the reactive mixture has to be optimized because an excess may act as a scavenger for radicals derived from the dye[152-157] and will also favor excessive chain transfer, making termination steps easier to occur[78]. In this sense, it has been found that the concentration of excited xanthene dyes in the presence of amine excess reduced around 20%. However, when the experiment is developed in the presence of an adequate monomer, the reported photobleaching is inhibited[158].

Very recently, a novel highly efficient Type II UV-photoinitiators, carrying in the same molecule both the irradiation sensitive (benzophenone) and the co-initiator amine (N,N-dimethylaminoethyl methacrylate or other alkyl amines) moieties, have been communicated[159-160].

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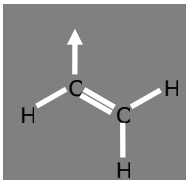
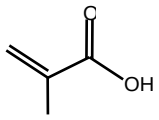
[153] R.S. Davidson, Exploring the Science, Technology and Applications of UV and EB Curing, SITA Technology Ltd., London **1999**
[154] H.J. Hageman, *Prog. Org. Coat.* 13 **1985**, p. 123
[155] R.S. Davidson and J.W. Goodin, *Eur. Polym. J.* 18 **1982**, p. 597
[156] Nergis Arsu, Meral Aydin , Yusuf Yagci, Steffen Jockusch and Nicholas J. Turro Photochemistry and UV Curing: New Trends, **2006**: Editor: J.P. Fouassier
[157] M. Ortuño, S. Gallego, A. Beléndez, I. Pascual, C. García, C. Neipp, Appl. Phys. B 76 **2003** 851
[158] M. V. Encinas, A. M. Rufs, S. G. Bertoloti, C. M. Previtali, Polymer 50 **2009** 2762
[159] J. Wei, R. Lu, F. Liu, Polym. Adv. Technol. 21 **2010** 656
[160] Y. Wang, X. Jiang, J. Yin, Eur. Polym. Journal 45 **2009** 437

5.3. Monomer

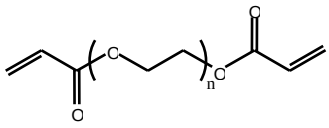
The monomer, whichever it is, acts as the growing brick of the forming polymer chains. Most common used monomers for PMR processes contain a vinyl group which is a well known reactive functional group for free radical reactions (Table XX). Precisely, molecules with an acrylate residue are widespread employed in photopolymerization processes, though styrene and cyclic lactam derivatives are mayor industrial polymer sources. When the monomer presents more than one reactive group (that is the case of diacrylate molecules), the growing polymer chain may have multiple reactive pendant double bonds that will contribute to make termination steps less effective, achieving thicker and more crosslinked hydrogels compared to linear polymer formation[161].



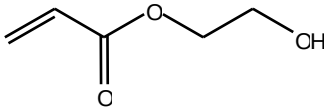
Vinyl functional group



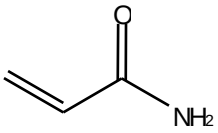
Methacrylate



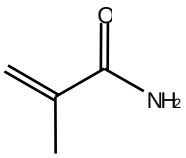
PEGDA



2-Hydroxyethyl acrylate



Acrylamide



Methacrylamide

Figura. Principal acrylate and acrylamide monomers used in photopolymerization reactions.

Biological derivatives as proteins (like albumin or gelatine derived from collagen hydrolysis) together with some polysaccharides (chitin, cellulose) should act as monomers to produce hydrogels, as well[72] as classical reactives. Recently, the preparation of a crosslinked photopolymer from allyl-modified sucrose[162] or the use of hyperbranched acrylate derivatives[163] (substances that can undergo photopolymerization without the addition of any initiators, just by irradiating with appropriate UV wavelength) have been reported. Other monomers like aniline[164], alginate[165] or chitosan[166] have been studied.

[161] S. Kizilel, V. H. P. Luna, F. Teymour, Langmuir 20 **2004** 8652
[162] R. A. Ortiz, A. Martínez, A. E. G. Valdez, M. L. B. Duarte, Carbohydrate polymers 82 **2010** 822
[163] L. Huang, Y. Li, J. Yang, Z. Zeng, Y. Chen, *Polymer* 50 **2009** 4325
[164] R. A. Barros, M. C. C. Areias, W. M. Azevedo, Synthetic metals 160 **2010** 61

The structure of the monomer affects in a critical manner the reactivity of the substance. Not only the number of vinyl residues but also the quantity of other functional groups are vital. In this sense, the presence of ether or hydroxyl groups in the monomer skeleton favour the polymerization by cosuming dissolved molecular oxygen through an hydrogen abstraction mechanism (Fig. XX). This competitive undesired reaction is expressed to a major extent in acrylates than in methacrylates, the later becoming a more stable radical[167].

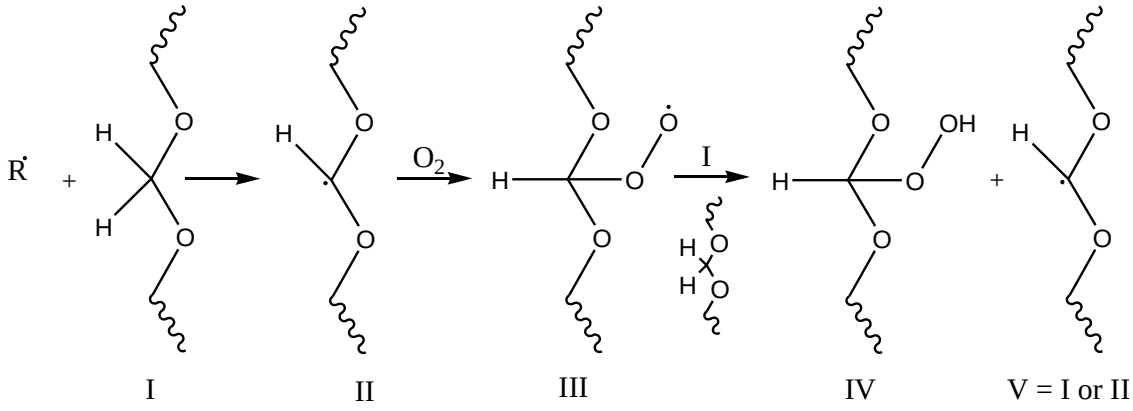


Figure. Ether functionalities as oxygen scavengers.

5.4. Additives

A diversity of substances can be used as additives in the photopolymerization reactions, each of them improving concrete aspects of the process. For instance, we could mention the huge efforts made to reduce the impact that molecular oxygen has on the degree of monomer conversion. In free radical polymerizations, the oxygen quenching rate constants for an initiating and propagating radical species are in the order of 10^8 and 10^9, respectively; so fast reactions are based on the triplet state biradical nature of oxygen[168]. Considering that the oxygen concentration in acrylate solution is approximately 10^{-3} M and that the oxygen concentration which allows the polymerization of a monomer mixture to occur is arround 10^{-6} M, the initial oxygen concentration must be reduced at least 100 fold before polymerization starts off[103,169]. Thus, the capacity to reduce oxygen detrimental behaviour on the reaction strongly depends on how fast it is consumed. Here, some strategies to slow down the inhibitory tendencies are shown, together with other ways to enhance monomer conversion.

5.4.1. N-vinyl pirrolidone.

In 1977 a first report using N-vinyl pirrolidone (NVP) as additive in an acrylate polymerization reaction, pointed a significant improvement in polymerization because of a reduction of the molecular oxygen role as radical

[165] C. Mu, S. Sakai, H. Ijima, K. Kawakami, J. Biosci. Bioengin. 109 **2010** 618
[166] Q. Li, D. Yang, G. Ma, Q. Xi, X. Chen, F. Lu, J. Nie, Int. J. Biol. Macromol. 44 **2009** 121
[167] T. Y. Lee, C. A. Guymon, E. S. Jonsson, C. E. Hoyle, *Polymer* 45 **2004** 6155
[168] N. J. Turro, *Modern molecular chemistry*, Mill Valley : University science books **1991**
[169] L. Gou, C. N. Coretsopoulos, A. B. Scranton, *J. Polym. Sci. A Polym. Chem*. 42 **2004** 1285

scavenger. Their conclusion hypothesized that NVP might consume O_2, thus reducing the negative effect on the polymer formation[170-171].

More recently, the work on polymerization reactions addressed to study holographic devices has confirmed that the use of NVP reduces the viscosity of the initial mixture and enhances the polymerization rate as well as monomer conversion values, presenting a maximum rate of propagation when the NVP to double bond ratio approaches 40%. These kind of studies also showed that upon complete conversion of the NVP present in the initial reaction solution, no more monomer continued reactioning. The accepted interpretation to this behavior states that the PMR process enhancement is due to the polymerization taking place between the "principal monomer" (acrylate or acrylamide) and the "co-reactant" (NVP), thus forming a *copolymer*[172]. When limitations on molecular diffusion appear, the only route for two chain radicals to terminate is by propagation through the local double bonds. However, the reduced dimensions and the low tendency to homopolimerize[78] of the NVP molecule allow it to diffuse through the low mobility polymer network until pendant macroradicals, facilitating additional reaction diffusion controlled bimolecular terminations[172]. Because of its behavior, NVP is commonly called an *accelerator*[78] as its presence ultimately favors the growth of the polymer and so the hydrogel thickness[105]. NVP could be replaced by ethylene glycol diacrylate or other reactive monomers with similar reactivity[73] but clearly less toxicity.

5.4.2. Acorbic acid.

Ascorbic acid has been widely used in the past for photopolymerization reaction studies[173-176]. It is considered a powerful reducing agent due to its electron donor capability, resembling that of metallic copper. A simple but surprising observation fixed the interest onto ascorbic acid: the propagation rate of a photopolymerization process (involving an acrylamide derivative as monomer and eosin as photoinitiator) increased proportional to the acid concentration beyond a given value, but below this value no reaction was observed[¡Error! Marcador no definido.]. The highest interest arose on the aerobic conditions required for the process to happen, while it did not progress under argon atmosphere. A mechanism proposal was delivered where the oxygen mediated H_2O_2 formation was pointed out as the key factor in the monomer radical formation. One should bear in mind the puzzling discovery this was, since aqueous dissolved O_2 was commonly assumed to scavenge any radicals formed in the reactive blend. Subsequently, H_2O_2 was said to react with the ascorbic acid derivative AH^- to produce hydroxyl radicals $OH^{\cdot}$, which were the ultimate responsible to initiate the monomer polymerization[177-178]. A schematic

[170] Lorenz, D. H.; Azorlosa, J. L.; Tu, R. S. Radiat Phys Chem 9 **1977** 843
[171] Miller, C. W.; Hoyle, C. E.; Jonsson, S.; Nason, C.; Lee, T. Y.; Kuang, W. F.; Viswanathan, K. ACS Symposium Series; **2003**, 847 (Photoinitiated Polymerization)
[172] T. J. White, W. B. Lietchy, C. A. Guymon, J. Polym. Sci. A Polym. Chem. 45 **2007** 4062
[173] S. Lenka, I. B. Mohanty, Polymer Photochemistry, 7 **1986** 447
[174] S. Lenka, I. B. Mohanty, Polymer Science A, 24 **1986** 2729
[175] S. Lenka, P. L. Nayak, B. Mohanty, Polymer Photochemistry, 7 **1986** 49
[176] S. Lenka, P. L. Nayak, M. K. Mishra, J Polymer Science, 19 **1981** 2671
[177] D. R. Pemberton, A. F. Johnson, Polymer 25 **1984** 529
[178] D. R. Pemberton, A. F. Johnson, Polymer 25 **1984** 536

mechanism proposal based on ascorbic acid role in aerobic reaction environment considers at least the following primary steps:

$$D \xrightarrow{h\upsilon} {}^1D \xrightarrow{ISC} {}^3D$$

$$AH_2 + H_2O \leftrightarrow AH^- + H_3O^+$$

$${}^3D + AH^- \rightarrow DH^{\cdot} + A^{\cdot -}$$

Further H_2O_2 production reactions:

$$DH^{\cdot} + O_2 \rightarrow D + HO_2^{\cdot}$$

$$DH^{\cdot} + HO_2^{\cdot} \rightarrow D + H_2O_2$$

$$HO_2^{\cdot} + AH^- \rightarrow A^{\cdot -} + H_2O_2$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2$$

When all O_2 is been consumed, the initiator is reduced to its inactive form (*leuco* form in the case of organic dyes):

$$DH^{\cdot} + AH^- \rightarrow A^{\cdot -} + DH_2$$

$$2DH^{\cdot} \rightarrow D + DH_2$$

Any ascorbic acid that remains after the initial reactions may produce hydroxyl free radicals:

$$AH^- + H_2O_2 \rightarrow A^{\cdot -} + HO^{\cdot} + H_2O$$

$$HO^{\cdot} + AH^- \rightarrow A^{\cdot -} + H_2O$$

Finally, vinyl monomers polymerization starts by reacting with $OH^{\cdot}$ species:

$$HO^{\cdot} + M \rightarrow M^{\cdot}$$

$$M^{\cdot} + M \rightarrow M_2^{\cdot}$$

$$M_n^{\cdot} + M \rightarrow M_{n+1}^{\cdot}$$

$$M_n^{\cdot} + M_m^{\cdot} \rightarrow M_{n+m}TERMINATED$$

$$M_n^{\cdot} + A^{\cdot -} \rightarrow M_nTERMINATED$$

When comparing the reactive mixture acrylamide/ascorbic acid to the typical acrylate/amine (monomer/coinitiator), the first system is found less sensitive, needing a higher dye (eosin) concentration, namely even 1000-fold[172]. On array assays with immobilized dye photoinitiators, no polymer is obtained in ascorbic acid systems. Leaning on the dye low sensitivity detected, the authors suggest that a higher superficial dye concentration will be needed for PMR to be observed.

5.4.3. Metallic copper.

Another electron donor additive is metal copper (0), which added as powder acts as a reducing agent and consumes dissolved O_2 in the aqueous polymerization reaction blend, thus eliminating the traditional need for an inert environment. The usage of this additive is exemplified on an atom transfer polymerization reaction (ATPR, a kind of free radical polymerization) where the probe molecules are attached to a gold surface through a thiol covalent bond with a pendant group including a halide atom which is readily transferred to a Cu(I)-Ligand catalyst leaving a superficial radical chain[179]. In this case the catalytic mixture to activate the polymerization of 2-hydroxyethyl methacrylate (HEMA) with triethylamine (TEA) is formed by the combination of copper powder, copper (I) chloride (CuCl), copper (II) bromide ($CuBr_2$) and 2,2-dipyridyl (bpy) salts dissolved in water[180]. Here we show the general mechanism highlighting the oxygen and copper (0) scavenging routes[181-182].

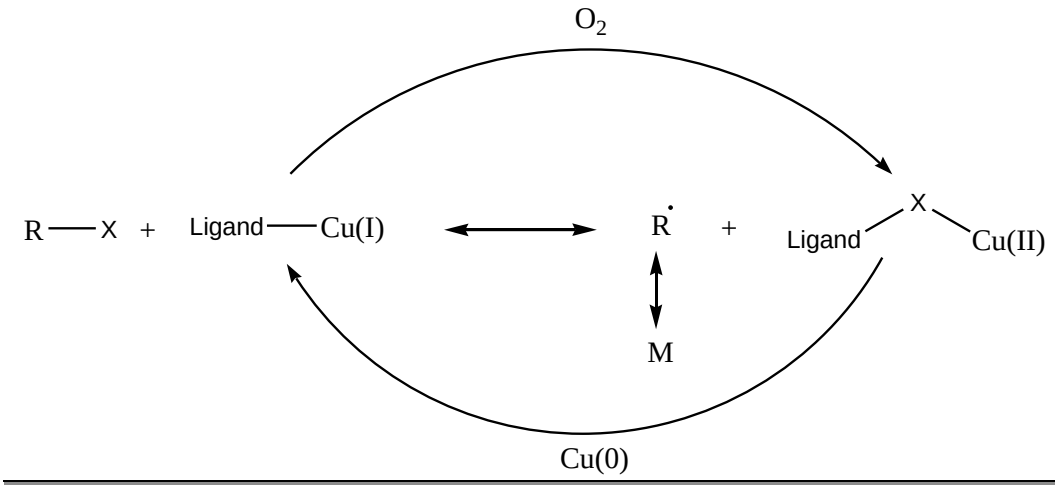


Figure. Cu (0) additive activity cycle.

5.4.4. Borane complexes.

A recent report communicated the use of boryl radical chemistry to improve the photopolymerization reaction in aerobic atmosphere conditions. Radicals with a boron-centered structure $L_{1\text{-}4}$-B· and a strong complexation coordination sphere present a high chemical stability, more than two weeks in some cases. These radicals are formed by interaction of the neutral borane complexes (for example, amine→BH_3 or phospine→BH_3) and an excited initiator (for instance, the triplet state of a benzophenone derivative generated by UV irradiation),

$$^3I + B \rightarrow I + B^{\cdot}$$

During polymerization under aerated conditions, low reactive peroxyl radicals may be formed,

[179] K. Matyjaszewski, J. Pyun, S. G. Gaynor, Macromol. Rapid Comm. 19 **1998** 665
[180] G. O. Okelo, L. He, Biosensors Biolect. 23 **2007** 588
[181] K. Matyjaszewski, J. Xia, Chem. Rev. 101 **2001** 2921
[182] K. Matyjaszewski, K. L. Beers, B. Woodworth, Z. Metzner, J. Chem. Edu. 78 **2001** 547

$$M_n - M^{\cdot} \xrightarrow{O_2} M_n - M - O - O^{\cdot}$$

these oxygenated radicals are known to reduce the extent of the polymerization formation as they are ineffective to continue the propagation reactions due to a conjugation phenomenum. The main advantage in using borane complexes relies in their ability to convert any alkyl peroxyl radical created on the polymer chain into a boryl radical, which is ultimately reactive to activate new monomer species[183]. Precisely, the use of 1% w/w of borane complexes have been reported as additives in photopolymerization systems under aerated environment because of their ability in scavenging the detrimental effect of O_2 in the process[184] and increasing the propagation rate.

$$R - O - O^{\cdot} + B \rightarrow R - O - O - H + B^{\cdot}$$

$$B^{\cdot} + M \rightarrow B - M^{\cdot}$$

In a similar way, triphenylphosphine and related compounds, have been reported as useful additives for reducing the concentration of peroxy radicals in UV-curable resins by a similar mechanism as described for boron derivatives[185].

5.4.5. Triazine derivatives.

At the beginning of the 2000 decade, triazine derivative compounds were introduced into the photopolymerization reaction mixtures in order to boost the efficiency of the process. A clear difference was observed in the behaviour of the system when a triazine derivative was incorporated in a typical Type II photopolymerization initiator/amine reactive solution: the conversion extent increased more pronouncedly and reached higher values. In light of the experimental results, triazine derivatives were assumed to act as removers of some inhibitor localized in the blend. Quenching and electrochemistry studies confirmed the role of triazine derivatives as electron acceptors towards the dye radical entities[186]. This statement, took the molecular oxygen out of the searching range; some species ready to be oxidized were in target now.

Investigators proposed the reduced initiator moieties $DH^{\cdot}$ (generated by the reaction of the initiator with the sensitizer substance, usually an amine) as the searched inhibitor; since $DH^{\cdot}$ species were assumed to probably bind the growing chains terminating their expansion. The triazine derivatives would oxidize that radical species restoring the initial neutral initiator molecules, which could re-enter the photopolymerization process[187], and a negative charged triazine compound that might generate new radicals involved in chain growth[186,188,189].

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[183] J. Lalevée, M. A. Tehfe, X. Allonas, J. P. Fouassier, K. U. Ingold, J. Org. Chem. 73 **2008** 6489

[184] J. Lalevée, M. A. Tehfe, X. Allonas, J. P. Fouassier, Macromolecules 41 **2008** 9057

[185] C. Belon, X. Allonas, C. C. Barghorn, J. Lalevee, J. Polym. Sci. A Polym. Chem. 48 **2010** 2462

[186] C. Grotzinger, D. Burget, P. Jacques, J. P. Fouassier, Macromol. Chem. Phys. 202 **2001** 3513

[187] C. Grotzinger, D. Burget, P. Jacques, J. P. Fouassier, Polymer 44 **2003** 3671

[188] J. Kabatc, M. Zasada, J. Paczkowski, J. Polym. Sci. A Polym. Chem. 45 **2007** 3626

[189] K. Kawamura, J. Photochem. Photobiol. A Chem.. 162 **2004** 329

$$^3D \xrightarrow{AMINE} DH^{\cdot} + AMINE^{\cdot}$$

$$DH^{\cdot} + Tz \rightarrow D + T^{\cdot -} + H^{+}$$

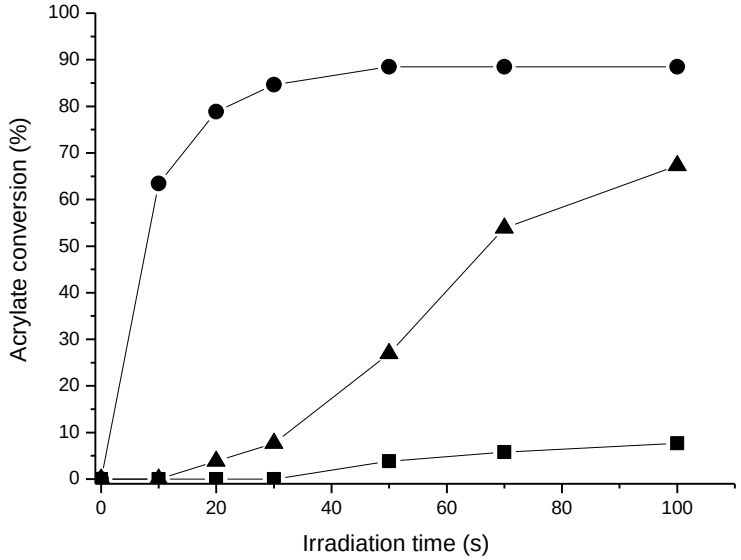

Figura. Influence of the photoinitiation blend used in the polymerization of acrylate monomers mixed with acridine orange photoinitiator (0,07%wt), MDEA (2,5%wt when used) and 2-(4'-methoxyphenyl)-4,6-bis(trichloromethyl)-1,3,5-triazine (0,3%wt when used)[186,187]: ■ dye/triazine, ▲ dye/amine, ● dye/amine/triazine. Adapted from .....

5.4.6. Thiol derivatives.

Since the 1970's, mercaptoethanol and other thiol compounds[72] have been employed in photopolymerization studies where the monomer possesses a terminal double bond: the so called *thiol-ene* photopolymerization reactions. This is a very attractive procedure to photopolymerization as it proceeds rapidly in air conditions, in contrast to many other free radical processes which malfunction in the presence of oxygen[127,190-191].

One of the most attractive goals of this kind of derivatives is that initiators are not needed to start the reaction off[126,192], what allows to cure very thick films, to reduce the volatile colored by-products generated when the initiator species are decomposed, to eliminate the residual initiator light absorption and to develop polymer films with higher stability. Here, the mechanism proceeds via consecutive propagation and chain transfer reactions to a step-growth hydrogel evolvement. Thiol compounds may themselves absorb an UV photon, or react with a photoinitiator, and suffer an intramolecular cleavage into a thiyl and a hydrogen radicals, any of them able to initiate the monomer

[190] D. P. Gush, A. D. Ketley, *Modern paint coat* 11 **1978** 58
[191] C. R. Morgan, A. D. Ketley, *J. Radiant Curing* 4 **1980** 10
[192] N. B. Cramer, J. P. Scott, C. N. bowman, Macromolecules 35 **2002** 5361

polymerization[193]. The thiyl radical attacks double pending bonds forming a carbon centered radical which may abstract a thiol hydrogen regenerating the thiyl radical, so called two-step propagation sequence.

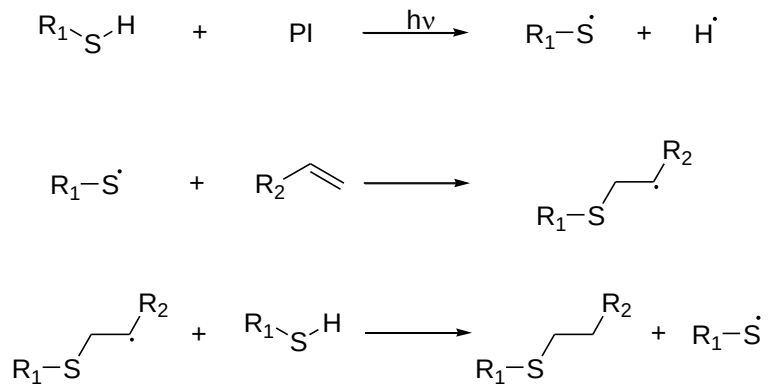

Figure. Two-step thiol-ene propagation scheme.

Besides, thiol compounds react readily with alkyl peroxy radicals formed in typically aerated mixtures, terminating this ineffective substances and producing a thiyl radical able to restore the neutral monomer activation towards polymerization:

$$M_N^{\cdot} \xrightarrow{O_2} M_n - O - O^{\cdot}$$

$$M_n - O - O^{\cdot} + R - SH \rightarrow M_n - O - O - H + R - S^{\cdot}$$

$$M + R - S^{\cdot} \rightarrow M^{\cdot} + R - SH$$

Despite the commented advantages, the step-growth nature of the thiol-ene photopolymerizations restricts the crosslinking density that can be achieved due to the chain transfer activity of the additives, producing polymer film thickness not detectable by visual inspection at low conversion experimental conditions[72]. Furthermore, the alkene structure and functionality are to be considered because they can significantly affect the physical and mechanical properties of the final thiol-ene network[194].

As mentioned in the introduction of this report, thiyl radicals involved in a mixture with sulphide atoms near a double pending bond (for example in β-position) undergo an addition-fragmentation reaction producing a plastic deformation in the network that releases part of the structural stress developed during the gelation process. Thus, shrinkage reduction is another interesting feature of thiol derivatives usage in PMR studies[128, 195-196].

Otherwise, the photopolymerization of cyclic allylic sulphide monomers showed that they can also react with oxygen to produce peroxy radicals. Then, these radicals may terminate by ring-opening from a methyl carbon at α position

[193] N. B. Cramer, S. K. Reddy, M. Cole, C. Hoyle, C. N. bowman, J. Polym. Sci. A Polym. Chem. 42 **2004** 5817
[194] Q. Li, H. Zhou, E. Charles, Polymer 50 **2009** 2237
[195] C. R. Morgan, F. Magnotta, A. D. Ketley, J. Polym. Sci. A Polym. Chem. 15 **1977** 627
[196] A. F. Jacobine, D. M. Glaser, P. J. Grabek, D. Mancini, M. Masterson, S. T. Nakos, M. A. Rakas, J. G. Woods, J. Appl. Polym. Sci. 45 **1992** 471

of the sulphur atom. A subsequent series of chain transfer reactions are stated to reduce the oxygen inhibition effect[197].

5.4.7. Crosslinkers and binders

Some substances added to the polymerization mixture serve as a sort of adhesive that sticks the components of the blending together. These crosslinking molecules contain at least two pending reactive groups which, when being activated by temperature, pressure or irradiation become reactive towards primary amine, sulfhydryl, vinyl or carboxylic functionalities forming covalent bonds that anchor one growing chain to the nearest one. The extent of crosslinking achieved affects the mechanical properties of the final polymer. In this sense, low crosslinked materials present high viscosity degree, while intermediate crosslinked materials become elastic gums and the high crosslinked polymers turn into rigid and glassy solids. Further, the nature of the crosslinkers (diacrylates, dimethacrylates or urethane diacrylates) can help tuning the hydrophobicity of the forming polymer[198-199].

There are two approaches to crosslink a mixture in a photopolymerization system. The first one is to prepare an aqueous solution rich in the crossbinding agent (for instance, 13% w/v of polyvinyl alcohol) that will form a primary matrix where the polymerization mixture (monomer, coinitiator and initiator) is added before the irradiation step[157]. Depending on the thickness and density of the product desired, one has to choose the adequate average molecular weight of the polyvinyl alcohol, which varies in the range 20000 to 200000 Da in commercial samples. The second one is to add the crosslinker as an additive in low percentage into the polymerization mixture (for example N,N'-methylenebisacrylamide, N,N'-(1,2-didhydroxyethylene, bisacrylamide[152] or ethyleneglycol dimethacrylate[72, 75]). There, it improves the connectivities among growing polymer chains but to a limited extent conferring a slight stabilization to the formed product.

5.4.8. Macrophotoinitiators.

The use of macrophotoinitiators, instead of the simpler photoinitiators, is addressed to increase the sensitivity of biodetections and at the same time to minimize the false positives that can arise if non-specific interactions occur between the initiator and the coated surface[200]. Macrophotoinitiators allow both to selectively bind to the functionalized surface and to subsequently initiate the photo induced polymerization. Macrophotoinitiators are synthesized by reacting a high molecular weight polymer, that will act as a carrier chain, with the photoinitiator and the specific receptors to target substances (avidin proteins are commonly employed as receptors for biotinilated samples). Thus, a macrochain is obtained containing proper average quantities of initiator molecules and recognition groups[75]. The number of initiator residues incorporated in the final macrophotoinitiator must be controlled since too many

[197] S. Yamada, Y. Goto, J. Photopolym. Sci. Technol. 23 **2010** 109
[198] A. B. Sidi, A. Srinivasan, J. A. Sheehan, L. M. Walker, C. Gayathri, R. Gil, J. O. Hollinger, K. Matyjaszewski, N. R. Washburn, Acta Biomater. 5 **2009** 1872
[199] A. B. Sidi, J. A. Sheehan, J. O. Hollinger, L. M. Walker, K. Matyjaszewski, N. R. Washburn, J. Biomedic. Mater. Res. A 90 **2009** 142
[200] H. D. Sikes, R. Jenison, C. N. Bowman, Lab Chip 9 **2009** 653

of them can lead to non-specific interactions between the macroinitiator and the surface[72].

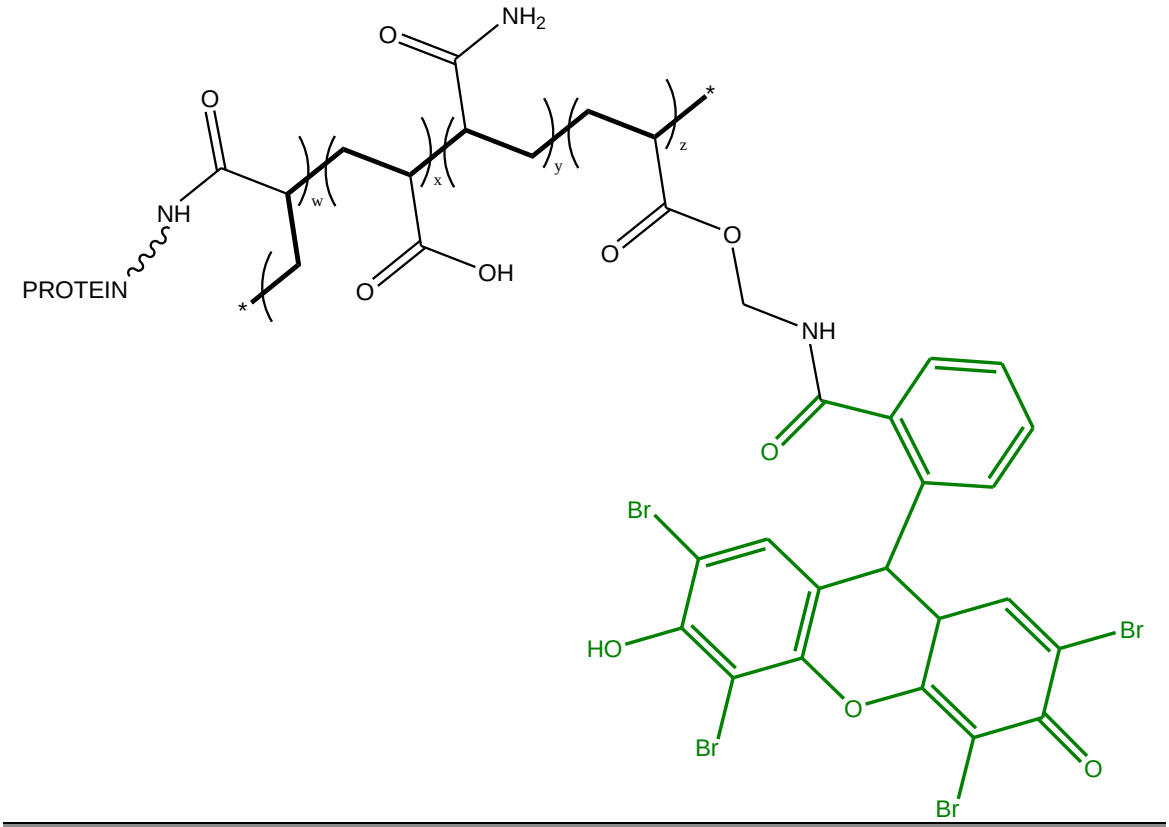

Figure. Example of macrophotoinitiator polymeric structure .

5.4.9. Initiators bearing amine side chain

An innovative approach in Type II photopolymerization processes considered the combination of both initiator and amine in the same molecule[201]. As any polymerization reaction proceeds, the initial fluid solution becomes more restricted due to gelation. When this stage is reached, reactants are submitted to higher diffusional restrictions before a positive interaction occurs. The synthesis of molecules including both initiator and co-initiator aims to overcome the gel formation drawback effect by favouring intramolecular electron cession and hydrogen transfer between both reactants. Polymeric photoinitiators display similar UV-VIS spectra to the low counterparts, but notably red-shifted.

The improvement of this kind of complex molecules has been successfully applied to polymerize mixtures by irradiation, with increased monomer conversion and higher polymerization rates. At this point, photo-DSC data reveal the potential of thioxanthone and benzophenone derivatives as initiators linked to different families of amines. So far, PMR efficiency is shown to be mainly affected by the amine structure and its topologic environment (secundary versus tertiary amine, cyclic versus linear chain, methyl versus ethyl pendant groups)[159]. Furthermore, if initiator and amine are multi-incorporated to a polymeric backbone the resultant co-polymeric molecule is more active

[201] X. Jiang, H. Xu, J. Yiu, Polymer 45 2004 133

towards polymerization than the combination of the corresponding of homo-polymers, the non-polymeric but linked reactants or the classical initiator and amine combined in solution[202,203].

When thio functionalities are present in the chemical structure of any of the members of the combined initiator (polymeric or not), the photo-induced cleavage of the C-S-C bond can be responsible for the initiaton of the reaction when appropriate wavelength is irradiated[204].

5.5. Mechanism proposal

On the basis of the above discussion, here we present the accepted principal mechanism lines involved in photopolymerization reactions. The central position of the diagram is occupied by the desired reactions that conduce to the formation of the polymeric hydrogel. The outskirts of the same diagram are reserved for the side routes taking place over the intermediate $DH^{\cdot}$ and $A^{\cdot}$ species: direct quenching of the excited initiator species or their ionic forms, quenching of the $DH^{\cdot}$ entities, reactions progressing over the oxidized peroxyl polymer forms and the copper route that applies when the polymer chain contains labile halogen atoms. The reader must consider this diagram as an approximate guide to the process, as only the main reactions of every route are gazed.

To summarize, the Type II polymerization activated by irradiation starts off when the reactive mixture is submitted to the appropriate wavelength. At this stage, the initiator species are excited to their singlet state which by internal section conversion generates the triplet state; the latter living long enough to continue the process[186]. Subsequently, electron transfer from the sensitizer to the excited triplet state produces the initiator anion radical and the sensitizer cation radical. A fast proton exchange from the same sensitizer to the initiator, transforms both in neutral radicals, with the first being assumed to be responsible for the free-radical polymerization initiation[161,205]. So, the sensitizers belonging to the alcohol, ether, amine or thiol families (basicaly, good reducing agents with labile protons) may be used, being the tertiary amine compounds the preferred (as exposed in 5.2).

[202] Y. Wang, X. Jiang, J. Yiu, Eur. Polym. J. 45 2009 437
[203] G. Temel, B. Euginol, M. Aydin, D. K. Balta, N. Arsu, J. Photochem. Photobiol. A Chem. 219 2011 26
[204] H. Wang, J. Wei, X. Jiang, J. Yiu, J. Photochem. Photobiol. A Chem. 186 2007 106
[205] D. C. Neckers, S. Hassoon, E. Klimtchuk, J. Photochem. Photobiol. A 95 **1996** 33

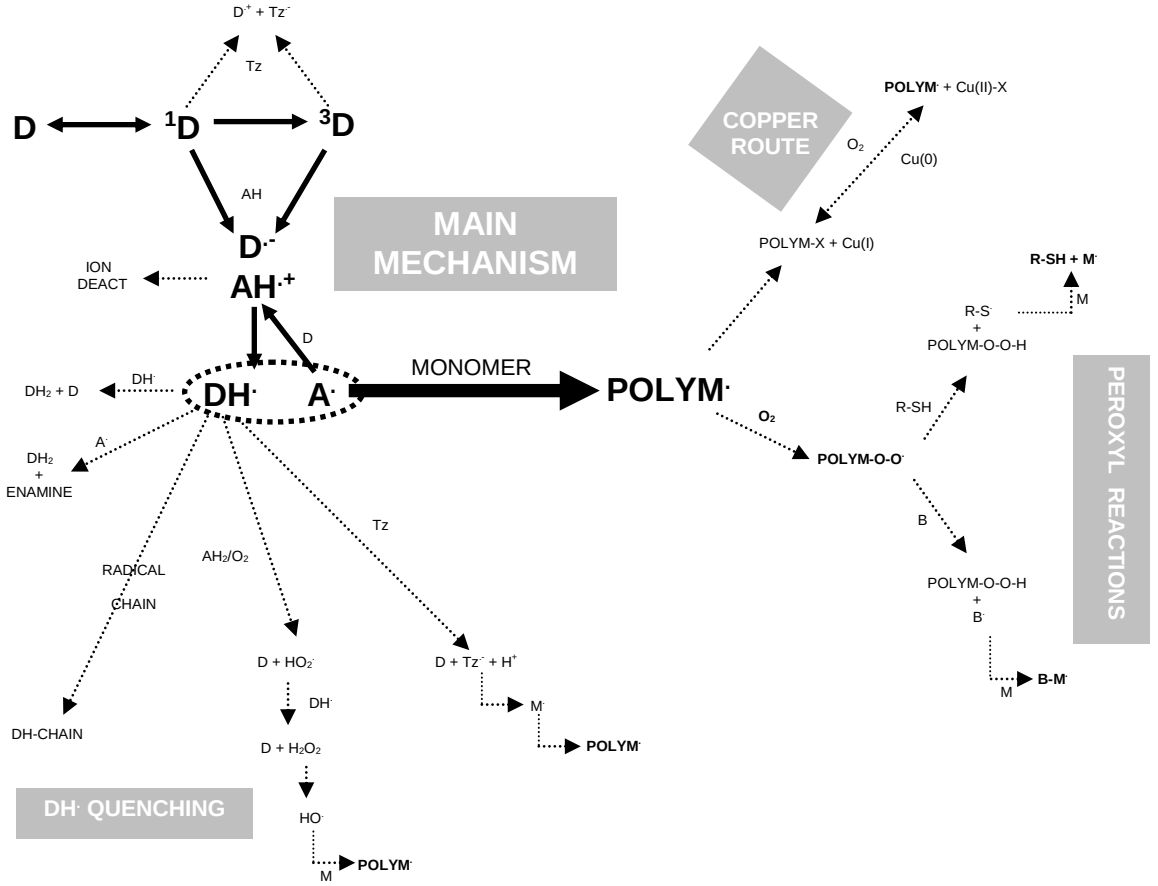

Figure. General photopolymerization mechanism main lines.

6. Kinetic model for photopolymerization reactions

6.1. General approach[206]

Here, a simple scheme for radical polymerization with any type of initiator is presented (see Fig. XX for more details):

First Initiation step $I \xrightarrow{k_d} 2R^{\cdot}$

Second Initiation step $R^{\cdot} + M \xrightarrow{k_i} RM^{\cdot}$

Polymerization step $RM_n^{\cdot} + M \xrightarrow{k_p} M_{n+1}^{\cdot}$

Termination step* $M_n^{\cdot} + M_n^{\cdot} \xrightarrow{k_t} Polymer$

Considering that the monomer disappears in both the second and third steps, the polymerization rate is expressed in differential form as,

$$R_p = -\frac{d[M]}{dt} = k_i \cdot [R^{\cdot}] \cdot [M] + k_p \cdot [M^{\cdot}] \cdot [M] \qquad \textbf{e1}$$

where [M·] represents the whole concentration of propagating species in the medium, independently of their length. That is to say, it is assumed that the different radicals react in a very same way independently of their length and, even not directly, it is suposed that each macroradical reactive center is found in the edge of the chain.

If it is accepted that the forming chains are all large enough, it can be considered that almost every monomer is consumed during the propagation step,

$$R_p = -\frac{d[M]}{dt} \cong k_p \cdot [M^{\cdot}] \cdot [M] \qquad \textbf{e2}$$

The radical concentration in a concrete moment is not easy to determine (modified electron spin resonance techniques can be employed for this task), so the steady state aproximation is called. Briefly, the radical concentration in the reaction medium is supposed constant at any time and so the forming and disappearing rates are the same,

$$R_i = R_t \qquad \textbf{e3}$$

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[206] J. Areizaga, M. M. Cortázar, J. M. Elorza, J. J. Iruin, "Polímeros" Ed. Síntesis **2002**

* Termination step may happen in three different ways: by direct combination of two radicals, by disproportion and by chain transfer from one radical to a substance in the medium (monomer, initiator, polymer, solvent, impurities or other agents). All these processes are included in one single constant rate, k_t, in order to simplify the model.

$$R_i \propto [I] \qquad \textbf{e4}$$

$$R_t = 2 \cdot k_t \cdot [M^{\cdot}_n]^2 \qquad \textbf{e5}$$

Making equal e3 and e5,

$$[M^{\cdot}_n] = \left(\frac{R_i}{2 \cdot k_t} \right)^{0.5} \qquad \textbf{e6}$$

To solve [$M_n^{\cdot}$] value, it is necessary to evaluate the initiation process rate (e4 in the simplified form). The nature of its formula depends on the initiation nature thermic, redox or photochemic:

1) Thermic: $R_i = 2 \cdot f \cdot k_d \cdot [I]$ **e7**
2) Redox: $R_i = k_d \cdot [Ox] \cdot [\mathrm{Re}\, d]$ **e8**
3) Photochemic: $R_i = 2 \cdot \Phi \cdot f' \cdot I_{abs}$ **e9**

where,

- "2" factors are refered to the formation of two radicals in the thermic or photochemic scission stages. Their use is stablished by convenium and depends on the text or authors.
- "f" is the efficiency factor as not every formed radicals get to polymerize. Some of them might ordenate, fragmentate or react among themselves (cage effect) before they diffuse to the reaction medium. Typical values 0.6 ≤ f ≤ 1 ensure a high polymerization degree.
- "Φ" is the quantum yield, i.e. the ratio of radical formation by absorbed photon.
- "f'" is the fraction of radicals that finally take part in the polymerization (not cage effect scavenged).
- "I_{abs}" represents the light intensity absorbed by the photoinitiator species.

Thus, in the case of a photochemical polymerization process,

$$[M^{\cdot}_n] = \left(\frac{\Phi \cdot f' \cdot I_{abs}}{k_t} \right)^{0.5} \qquad \textbf{e10}$$

With this in mind, the photopolymerization rate can be expressed in the form,

$$R_p = -\frac{d[M]}{dt} \cong k_p \cdot [M^{\cdot}] \cdot [M] = \frac{k_p}{k_t^{0.5}} \cdot (\Phi \cdot f' \cdot I_{abs})^{0.5} \cdot [M] \qquad \textbf{e11}$$

6.2. Application to the PMR reactive system: initiator-amine-monomer[207-208]

As refered before, a typical Type II photopolymerization mixture contains an initiator, an electron/proton supplier and a monomer pool. As mentioned, the amine co-initiator is able to interact with the initiator excited state becoming the primary radical responsible of the monomer molecules activation. Let's see how a photopolymerization reaction is initiated:

1) The initiator species absorbs the incident radiation and promotes to the corresponding singlet state. By fast internal system crossing process (ca. 100 ps for some xanthene dyes)[158], it gets to the triplet excited state,

$$I \xrightarrow{h\nu} {}^1I \xrightarrow{ISC} {}^3I$$

The triplet decay of excited dye has been measured in the absence of amine both in the presence of water and monomer. The results have showed that the triplet state is not quenched by the monomer, but only by the amine[158].

2) The collision complex is formed by the spatial combination of the triplet excited species and the co-initiator,

$${}^3I + NR_2H \xleftrightarrow{k_{dif}} \left[{}^3I.....NR_2H\right] \rightarrow \left[I.....NR_2H\right]^*$$

where k_{dif} referes to the reactants diffusion rate until they collide.

3) In a next stage, the excited charge-transfer complex is formed. Here, the co-initiator species ceases an electron followed by a labile proton to the excited initiator moieties (note the possible back electron transfer).

$$\left[I.....NR_2H\right]^* \xleftrightarrow{k_{ET}} \left[I^{\cdot -}.....NR_2H^{\cdot +}\right] \xrightarrow{k_H} HI^{\cdot} + NR_2^{\cdot}$$

When the photoinitiator moieties are attached to a solid surface, the formed radical dyes may bind covalently to growing polymer chains, anchoring the hydrogel to the surface[161] (see detail in Figure x.x above).

[207] Z. Kucybala, M. Pietrzak, J. Paçzkowski, L. Linden, J. F. Rabek, Polymer 37 **1996** 4585
[208] Z. Kucybala, J. Paçzkowski, Macromolecules 28 **1995** 269

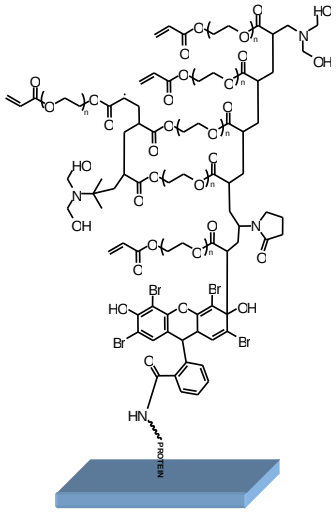


Figure 2. Acrylate photopolymer-eosin complex bound to a surface through protein physical adsorption.

Regarding amine radical formation, hydrogen abstraction is commonly assumed to occur on α-carbon to nitrogen atom[209,210]. ^{1}H-NMR data show that the amine actually binds an ethylene glycol diacrylate monomer through α-residue[211]. Beyond, ^{1}H NMR data related to the rose bengal (RB) – methyldiethanolamine (MDEA) – butyl acrylate (BA) system, indicated that the MDEA molecules that react with the monomers are ultimately incorporated as the polymer chain ends[211]. This is figured out by analizing the well resolved peak at 3.6 ppm attributed to the methylene group next to the MDEA alcohol functionality.

4) If the typical radical polymerization reactive scheme presented in 6.1 is considered, **e2** applied to the system dye-amine-monomer takes the form,

$$R_p = k_p \cdot [PEGDA] \cdot [NR_2H^{\cdot}] \qquad \textbf{e12}$$

As previous, to evaluate [$NR_2H^{\cdot}$] one must assume,

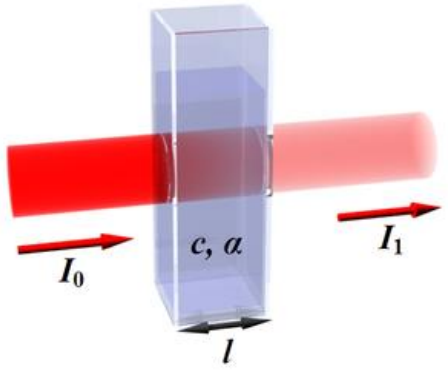
- Each excited dye molecule is able to activate one co-initiator amine molecule to generate, at least, one radical capable of initiating the chain polymerization reaction.
- For amine concentrations high enough the kinetic model is first order.
- In the electron-proton transfer mechanism, the dominant role is played by the electron ceasing step, thus $k_{ET} >> k_H$.

By assuming these statements it is possible to obtain (in a similar way as **e10**),

[209] Y. L. Chow, W. C. Danen, S. F. Nelsen, D. H. Rosenblatt, Chem. Rev. 78 1978 243
[210] S. G. Cohen, N. M. Stein, J. A. Chem. Soc. 93 1971 6542
[211] K. Jain, J. Klier, A. B. Scranton, Polymer 46 **2005** 11273

$$\left[NR_2H^{\cdot}\right]=\left(\frac{k_{ET}\cdot\Phi\cdot f'\cdot I_{abs}}{k_t}\right)^{0.5} \qquad \textbf{e13}$$

If the contact region between the reaction mixture and solid surface that anchores the initiator moieties is considered as a fluid system, the absortion of radiation by the initiator can be expressed by the Lambert-Beer law:



On one side,

$$I_{abs}=I_0-I_1 \qquad \textbf{e14}$$

By definition,

$$A=-\log T=-\log {I_1}/{I_0} \qquad \textbf{e15}$$

and,

$$A=l\cdot\varepsilon\cdot\left[I\right] \qquad \text{e16}$$

Making equal and removing the logarithm,

$$\frac{I_1}{I_0}=10^{-(l\cdot\varepsilon\cdot[I])} \qquad \textbf{e17}$$

so,

$$I_1=I_0\cdot10^{-(l\cdot\varepsilon\cdot[I])} \qquad \textbf{e18}$$

If **e18** is substituted in **e14**,

$$I_{abs}=I_0-I_1=I_0-I_0\cdot10^{-(l\cdot\varepsilon\cdot[I])}=I_0\left(1-10^{-(l\cdot\varepsilon\cdot[I])}\right) \qquad \textbf{e19}$$

where

- I_{abs}, I_0 and I_1 represent the intensity of radiation absorbed, incident and transmited, respectively. The energy absorbed by a molecule submited to the action of any radiation is relationed with its wavelength by the expression,

$$E={h\cdot c}/{\lambda}$$

- ε is the absorbtivity or extintion molar coefficient.
- l is the average length travelled by light in the medium.

Including **e19** in **e13**,

$$\left[NR_2H^{\cdot}\right]=\left(\frac{k_{ET}\cdot\Phi\cdot f'\cdot I_0\cdot\left(1-10^{-(l\cdot\varepsilon\cdot[I])}\right)}{k_t}\right)^{0.5} \qquad \textbf{e20}$$

Finally, the reaction rate takes the form,

$$R_p=\frac{k_p\cdot k_{ET}^{\ 0.5}}{k_t^{\ 0.5}}\cdot\left(\Phi\cdot f'\cdot I_0\cdot\left(1-10^{-(l\cdot\varepsilon\cdot[I])}\right)\right)^{0.5}\cdot[M] \qquad \textbf{e21}$$

If constants are reagruped,

$$R_p=k_{global}\cdot\left(\Phi\cdot f'\cdot I_0\cdot\left(1-10^{-(l\cdot\varepsilon\cdot[I])}\right)\right)^{0.5}\cdot[M] \qquad \textbf{e22}$$

Using SPSS licenced software it is possible to fit this expression with some experimental data reported in the literature[158] (as shown in Figure xx), obtaining the non-linear adjustment:

$$R_p=(0.483\pm0.018)\cdot\left[1-10^{-((2.356\pm0.598)\cdot[I])}\right]^{0.5} \qquad \textbf{e23}$$

And its corresponding regression coefficient:

$$r^2=0.943 \qquad \textbf{e24}$$

To compare those values, we assume the following statements:

- Irradiating time is long enough for any quenched triplet states be n-times re-excited.
- Amine concentration is much greater than dye concentration so that the chances for a triplet excited initiator to activate an amine molecule are high.
- Singlet excited state lifetime is shorter than triplet excited one.

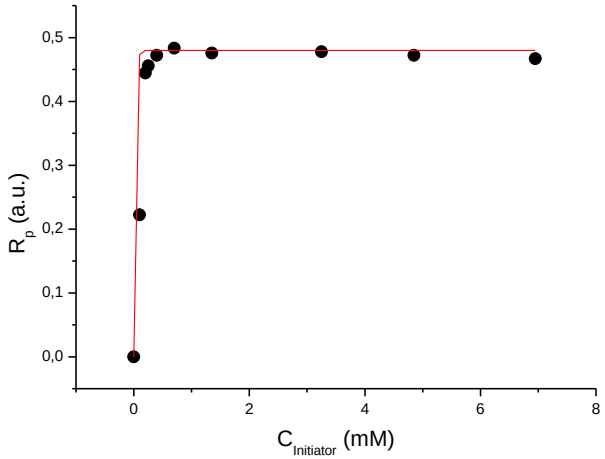


Figure x.x. Polymerization rate photoinitiated by erythrosine B as reported (spots) and our correponding kinetic adjustment to data (red line).

7. *Theoretical estimation of the PMR processes espontaneity*

The driving force dependence of bimolecular photo-induced electron transfer reactions has attracted the interest of many scientists[212-221] , from the very first studies related to the photosynthesis nature[222-223] to the conversion of solar energy into chemical potential[224-226]. In biological systems, the couple formed by the donor and the acceptor are so well evolutively synchronized that the electron transfer is accomplished almost instantanealy and with high quantum yields[227]. In the lab non-covalent, hydrogen bonding, π-stacking, metal-ligand coordination and other types of molecular interactions have been studied towards the control of artificial photoinduced electron transfer. Rehm's and Weller's investigacions pioneered this field[228-229]. It should be noticed that their equation is an empirical approach, thus not based on first principles. However it has been widely used to reasonably determine the free energy of a photo-induced electron transfer reaction:

$$\Delta G_{ET} = E_{D^{+\cdot}/D} - E_{A/A^{-\cdot}} - E_{\infty} - E_C$$

where,

- $E_{D^{+\cdot}/D}$ represents the oxidation potencial of the electron donor (amine co-initiator, in the case of interest).
- $E_{A/A^{-\cdot}}$ represents the reduction potential of the electron acceptor initiator.
- E_{∞} represents the excitation energy of the excitable component initiator and is estimated by the formula,

$$E_{\infty} = 12398/\lambda_{emission}$$

If the wavelength is expressed in Å, the excitation energy has eV units.

- E_c represents the interionic coulombic interaction (C-term), usually considered neglegible in polar solvents media.

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[212] G. J. Kavarnos, N. J. Turro, Chem. Rev. 86 **1986** 401
[213] M. R. Wasielewski, Chem. Rev. 92 **1992** 435
[214] B. Paulson, K. Pramod, P. Eaton, G. Closs, J. R. Miller, J. Phys. Chem. 97 **1993** 13042
[215] P. F. Barbara, T. J. Meyer, M. A. Ratner, J. Phys. Chem. 100 **1996** 13248
[216] I. R. Gould, D. Ege, J. E. Moser, S. Farid, J. Am. Chem. Soc. 112 **1990** 4290
[217] P. Finckh, H. Heitele, M. Volk, M. E. M. Beyerle, J. Phys. Chem. 92 **1988** 6585
[218] S. J. Formosinho, L. G. Arnaut, R. Fausto, Prog. React. Kinet. 23 **1998** 1
[219] G. P. Zanini, H. A. Montejano, C. M. Previtali, J. Photochem. Photobiol. A 132 **2000** 161
[220] S. M. Hubig, J. K. Kochi, J. Am. Chem. Soc. 121 **1999** 1688
[221] P. Jacques, X. Allonas, D. Burget, E. Haselbach, P. A. Muller, A. C. Sergenton, H. Galliker, Phys. Chem. Chem. Phys. 1 **1999** 1867
[222] G. J. Kavarnos, Fundamentals of Photoinduced Electron Transfer, VCH, NY **1993**
[223] N. J. Turro, Modern Molecular Photochemistry, Charpter 9, Benjamin-Cumming, CA **1978**
[224] S. Speciser, Chem. Rev. 96 **1996** 1953
[225] L. G. Arnaut, S. J. Formosinho, Photochem. Photobiol. A Chem. 100 **1996** 15
[226] E. David, R. Born, E. Kaganer, E. Joselevich, H. Durr, I. Willner, J. Am. Chem. Soc. 119 **1997** 7778
[227] C. A. Hunter, R. K. Hyde, Angew. Chem. Int. Ed. Engl. 35 **1996** 1936
[228] D. Rehm, A. Weller, Ber. Bunsen-Ges. Phys. Chem. 73 **1969** 834
[229] D. Rehm, A. Weller, Irs. J. Chem. 8 **1970** 259

The redox parameters can be obtained in two ways. Most authors apply cyclic voltammetry measurements to the reagents solutions (initiator and amine, respectively)[230-236,237], while others employ an approximation related to adiabatic ionization potentials obtained as extrapolation in a photoelectron spectra measurement[221].

The application of this knowledge to the free radical polymerization initiated by photoinduced intermolecular electron transfer was developed by, mainly, two groups of investigators in the late nineties. Paczkowski and collaborators showed how experimental results properly described the rate of polymerization as a function of the thermodynamic driving forces of the photoredox reaction between the organic donor and acceptor pairs; being the driving forces calculated as ΔG^{o} by the Rehm-Weller's equation (see above), a classical Marcus parabolic relationship is always presented with high correlation factors[207-208,238-239]. On the other hand, french investigators began using the Rehm-Weller's equation as an approach to estimate the espontaneity of a variety of electron donor-acceptor couples obtaining good reliability on the model[184-187, 205, 240-241].

8. *Solid substrates and related coatings used as supports for PMR studies*

A great variety of modified and unmodified supports are used as surfaces in photopolymerization reactions. The related literature presents the concrete examples of materials employed for this purpose. Two families of materials capture the main attention: polymer and glass-based solids.

Regarding polymer-based materials, polystyrene and polymethacrylate molds have been reported as appropriate substrates for the production of holographic memory devices prepared by polymerization under light activation[157]. Also polypropylene based materials can be used to accomplish the reaction between a sensitizer dye and a coinitiator amine in presence of a triazine derivative. In some cases when atmospheric oxygen is to be avoided, laminate experiments are developed between two polypropylene films that confine the reaction mixture[187].

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[230] B. Przyjazna, Z. Kucybala, J. Paczkowski, Polymer 45 **2004** 2559
[231] K. Kikuchi, T. Niwa, Y. Takahashi, Hiroshi Ikeda, t. Miyashi, J. Phys. Chem. 97 **1993** 5070
[232] Y. H. Wang, H. M. Zhang, L. Liu, Z. X. Liang, Q. X. Guo, C. H. Tung, Y. Inoue, Y. C. Liu, J. Org. Chem. 67 **2002** 2429
[233] Y. H. Wang, L. Liu, Q. X. Guo, M. Z. Zhu, X, Y. Ding, J. P. Yie, J. Phys. Chem. B 107 **2003** 14087
[234] H. Y. Hu, M. Z. Zhu, Z. P. Zhang, G. T. Wen, Q. X. Guo, Chin. Chem. Letters 17 **2006** 333
[235] A. Rosspeintner, D. R. Kattnig, G. Angulo, S. Landgraf, G. Grampp, Chem. Eur. J. 14 **2008** 6213
[236] B. Jedrzejewska, S. Urbanski, J. Appl. Polym. Sci. 118 **2010** 1395
[237] J. Narewska, R. Strzelczyk, R. Podsiadly, J. Photochem. Photobiol. A Chem. 212 **2010** 68
[238] J. Paczkowski, Z. Kucybala, M. Pietrzak, Macromolecules, 29 **1996** 5057
[239] J. Kabatc, Z. Kucybala, M. Pietrzak, F. Scigalski, J. Paczkowski, Polymer 40 **1999** 735
[240] D. Burget, J. P. Fouassier, F. A. Guerri, R. Mallavia, R. Sastre, Acta Polym. 50 **1999** 337
[241] D. C. Neckers, Y. Bi, Patent US5639802 **1997**

On the other hand, the glass-based materials cover a range of procedures that comprise different superficial treatments required to obtain the final organo-chemical modified silica surfaces. The main procedures are based on amine[77,161,242-243] and aldehyde[74,76,244] pending groups introduced by chemical reaction with appropriate precursors. Moreover, epoxy-functionalized glass slides may be used in array design for protein detection[78].

1000 Å gold layer deposited on float glass has been used as surface to attach 3'-terminally thiol modified DNA oligomers. Once anchored to the surface, the oligomers act as capture probes to immobilize the target DNA sequences[245].

9. Following the PMR advance and polymer characterization.

Several techniques are commonly used to characterize the polymer produced during the photopolymerization reactions. Here we report the most widely refered in the literature to this purpose. Furthermore, some of this techniques may be useful to determine the advance of the polymerization process, thus offering valuable kinetic data related to precise measurements.

9.1. Infrared spectroscopy (IR)

Infrared spectroscopy is usually applied to determine the nature of the bonds present in the polymeric molecules generated during the reaction. In this sense, FTIR equipments are required (sometimes adapted to an infrared microscope) to obtain the spectra of surface-bond moieties[76].

[242] R. R. Hansen, L. M. Johnson, C. N. Bowman, Anal. Biochem. 386 **2009** 285
[243] S. Kizilel, E. Sawardecker, F. Teymour, V. H. P. Luna, Biomaterials 27 **2006** 1209
[244] H. D. Sikes, R. R. Hansen, L. M. Johnson, R. Jenison, J. R. Birks, K. L. Rowlen, C. N. Boeman, Nature materials 7 **2008** 52
[245] P. He, W. Zheng, E. Z. Tucker, C. G. Borman, L. He, Anal. Chem. 80 (**2008**) 3633

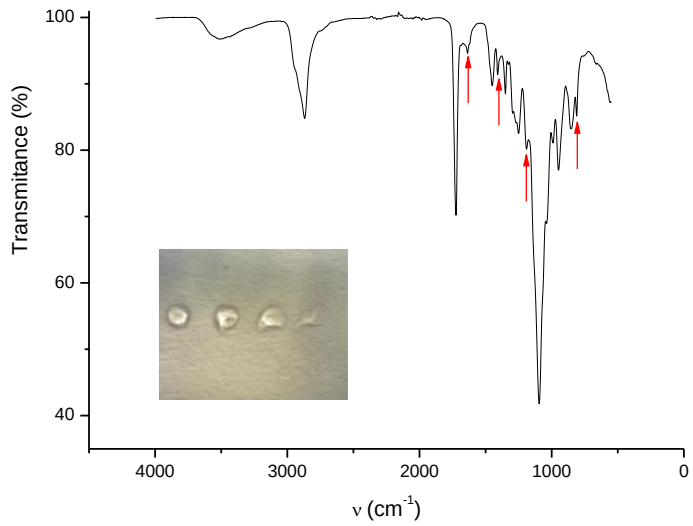

Figure XX. Detail of the photopolymer deposits formed by irradiation (525 nm, 260 mW/cm^2) of a mixture composed of polyethylene glycol diacrylate, methyl diethanol amine. Eosin immobilization on isocyanate modified glass was used as initiator. IR spectrum of the formed polymer shows intensity reduction of the signals attributed to double bond when compared to the non-polymerized commercial monomer. Espectrum obtained with a Tensor 27 ATR-FTIR Bruker aparatus.

On the other hand, this technique may also be useful to determine the degree of extension of the polymeric reaction by following the progressive disappearance of the IR absorption band of the acrylate double bond with the time (real time IR)[246]. The spectrometer is set in the transmission mode and the detection wavelength is fixed at a value where the monomer double bond exhibits a discrete and intense adsorption: some authors measure the twisting of –CH=CH$_2$ at 810 cm^{-1} [187, 239, 247-248]. Others track the peak centered approximately in the close IR at 6175 cm^{-1} to monitor the polymerization in real time[77-78, 249]. The evolution of the peak assigned to NVP reactant, centered at 1334 cm^{-1} may also be followed[172]. The degree of conversion can be evaluated from the equation[186]:

$$X(\%) = 100 \cdot \left[(A_{810})_0 - (A_{810})_t \right] / (A_{810})_0$$

Where $(A_{810})_o$ and $(A_{810})_t$ stand for the area of IR vinyl absorption peak of the sample before and after exposure at time t. This value should be corrected if shrinkage percentage was relevant.

When the system of interest consists on DNA single strands hybridization, IR techniques allows to confirm the immobilization of the strands by measuring the N-H amide bending at 1670 cm^{-1} characteristic peak. If poly-

[246] C. Decker, K. Moussa, Macromolecules 22 **1989** 4455
[247] J. Paczkowski, M. Pietrzak, Z. Kucybala, Macromolecules 29 **1996** 5057
[248] R. P. Sebra, K. S. Masters, C. N. Bowman, K. Anseth, Langmuir 21 **2005** 10907
[249] H. J. Avens, C. N. Bowman, J. Polym. Sci. A Polym. Chem. 47 **2009** 6083

oligo(ethyleneglycol) methacrylate is the formed product, the increase of the C=O peak centered at 1720 cm^{-1} may be apreciable[245, 250].

9.2. Calorimetry

Microcalorimetric measurements[239,247] or photo-differential scanning calorimetry (PDSC)[172] may render reliable kinetic data of polymerization processes. These techniques are based on measurement of the heat evolution during the reaction. Comercial or even home-made equipments are used for this task. As temperature sensor a semiconducting diode is immersed in a few mm thick sample solution. Irradiation of the polymerization mixture is carried out, at the same time, by an adapted ligth source (UV-VIS lamp or laser) with controlled intensity. If two monomers participate in the reaction, the rate of co-polymerization is obtained as an average of reiterative measurements:

$$R_p = Q/(\Delta H_1 + \Delta H_2)$$

$$\Delta H_i = x \cdot m \cdot f \cdot \Delta H_r / M$$

Where

- Q is the heat flow (J/s) measured by the calorimeter.
- ΔH_i is calculated for each monomer from the weight fraction (x), mass (m), functionality (f), reference values for the heat of reaction (ΔH_r) and molecular weight of the monomer (M).

9.3. Nuclear magnetic resonance spectroscopy (NMR)

Analysis of the ^1H and ^{13}C nuclear magnetic resonance spectra, before and after reaction, is a major aid in discovering the polymer stoichemistry as well as the extention of the reaction. When rose bengal, MDEA and butyl acrylate are mixed and irradiated at a proper wavelength, conversion and polymer yield around 85% and 70%, respectively, are observed. However, tracking the signals is not always easy as polymers show broadened characteristic NMR peaks[211,244]. The formation of the polymer is assumed as the relative intensity of the proton signals in the acrylate backbone is increased with time[245, 250, 251].

9.4. Electron spin resonance spectroscopy (ESR)

ESR trapping technique is a powerful recognized tool for the identification of radical centers[252-254]. Photopolymerization of a epoxyacrylate/tripropyleneglycoldiacrylate mixture in presence of borane amine complexes as co-initiators is followed by spin trapping of the boryl radicals

[250] X. Lou, L. He, Langmuir 22 **2006** 2640
[251] J. Chiefari, Y. K. Chong, F. Ercole, J. Kristina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Mejis, C. L. Moad, G. Moad, E. Rizzardo, S. H. Thang, Macromolecules 31 **1998** 5559
[252] P. Tordo, Spin trapping: recent developments and applications. In Electron spin resonance, N. M. Atherton, M. J. Davies, B. C. Gilbert, Eds. Royal society of chemistry, Cambridge **1998** vol. 16
[253] C. F. Chignell, Pure Appl. Chem. 62 **1990** 301
[254] Y. Kotake, K. Kuwata, Bull. Chem. Soc. Jpn. 54 **1981** 394

generated after irradiation. The whole mixture is placed in a quartz cylindrical ESR tube, where it is irradiated. The radicals are trapped in phenyl-N-t-butylnitrone and measurements are accomplished[184].

9.5. Gel permeation chromatography (GPC)

GPC is a routine technique used in polymer characterization. The gel polymer chromatograph instruments are equiped with a solvent delivery pump, an appropiate separating column (tipycal operanting molecular weight range 200-20000) and a differential refractive index detector and a system controller software. Tetrahydrofuran is commonly used as the mobile fase to eluate the polymer mixture. Commercial polystyrene standards are requiered to generate a calibration curve[245].

9.6. Ellipsometry

The thickness of fully dehydrated polymer films grown from microarray spots may be measured by a profilometer[72, 77, 161, 245, 255]. Measurements are made before and after polymer growth and the thickness differences reflect the amount of polymer formed. The force of the stylus tip is set at its minimum to reduce the mechanical deformation of the hydrogel layers under its preassure[76]. Operanting wavelength and angle of incidence on the films are to be set before measurements are done[180]. For each sample, the average of three measurements is recommended[250].

Plots of film thickness versus initiator surface density might fit the equation type

$$th = cte \cdot (density - a)^b$$

by least squares estimation. Here, it is assumed that the polymerization rate is related to the initiator density (i.e. initiation rate) to a given radiation power[78, 256].

[255] X. Lou, M. S. Lewis, C. B. Gorman, L. He, Anal. Chem. 77 **2005** 4698

[256] G. Odian, Principles of polymerization (charpter 3), 4th ed. New Jersey, John-willey and sons **2004**

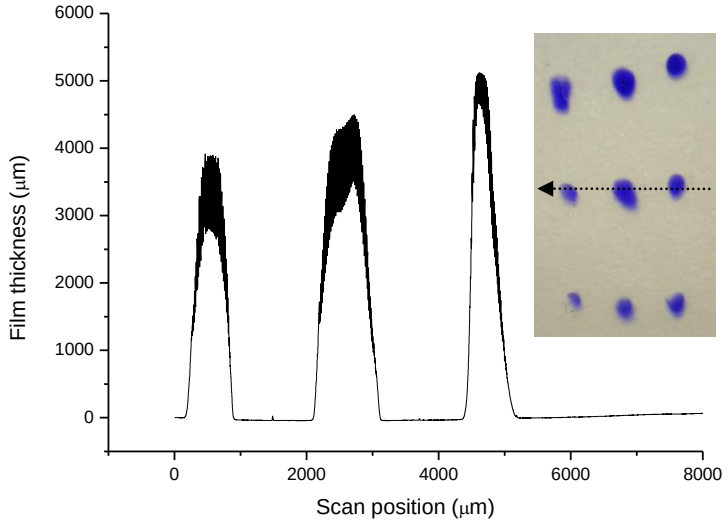

Figure XX. Ellipsometry profile corresponding to a polymerized mixture on isocyanate-modified glass. 8000 μm length profile with tip stylus force 1mg. Average values: 2900 μm wide and 4500 μm height. Obtained with a Dektak 6M Veeco aparatus. Arrow inside the image points tip movement on chip surface.

9.7. UV-VIS spectroscopy

This technique is not very common in polymer characterization due to the broadness of the typical bands and the little and not always clear information rendered. However some authors used it to analyse the nature of the obtained polymer[244].

9.8. Atomic force microscopy (AFM)

The topology and roughness of polymer films formed on a substrate surface may be measured by atomic force microscope in tapping mode. Resolution of the captured images, scan rate, scan area and z-range are tipycal parameters to be set. Concretely, z-range values should be adjusted depending on the estimated surface roughness. Root mean square heigh values are calculated with commercial software[106, 245, 257].

9.9. X-ray photoelectron spectroscopy (XPS)

XSP is a wide spread and sophisticated technique to obtain information concerning the composition of a surface. Wide scans (0 to 1000 eV binding energy) are run first to determine the elemental composition of said surface, using a monochromatized X-ray source to stimulate photoelectron emission. Subsequently, high resolution scans may be developed in order to get dipper detail in the elements of interest (C1s, O1s or N1s, for instance). The whole system is submitted to high vacuum conditions during the spectral acquisition.

[257] V. S. Khire, Y. Yi, N. A. Clark, C. N. Bowman, Adv. Mater. 20 **2008** 3308

In the case of eosin modified surfaces, the XPS spectrum will show the peaks adscribable to the Br atoms on the dye (Br3p and Br3d peaks centered around 200 and 60 eV, respectively). The formation of a hydrogel polymer on a surface may be revailed because the surface composition (C/O ratio, for example) fits, in good agreement, with the molecular structure of the expected polymer. Furthermore, if the experiment is carried out on silica supports, after the formation of the polymer film, the signals corresponding to silicon (Si2s and Si2p, localized around 160 and 100 eV) might have disappeared due to the blocking of the polymer film thickness (when greater than 100 Å) to the X-ray[161].

9.11. Contact angle titrations

Contact angle titration provides a method to examine the degree of ionization (wetability) of the functional groups existing on a surface[258]. Usually, it is determined at room conditions with an optical contact angle meter or goniometer. The contact angle is obtained by depositing a ≈ 1 μL drop of a concrete buffer or water on the top of the surface. Contact angles at both sides of the drop are recorded, repeating the procedure to obtain an average value at a given pH[161, 255].

9.12. Optical and fluorescence microscopy

Optical microscopy is a common and quick method to explore the morphology of the polymer films or spots generated during a photoinduced polymerization[161]. When the hydrogel contains a fluorescent label, fluorescence microscopy may help to visualize the product[243]. Usually, both type of microscopes host CCD cameras.

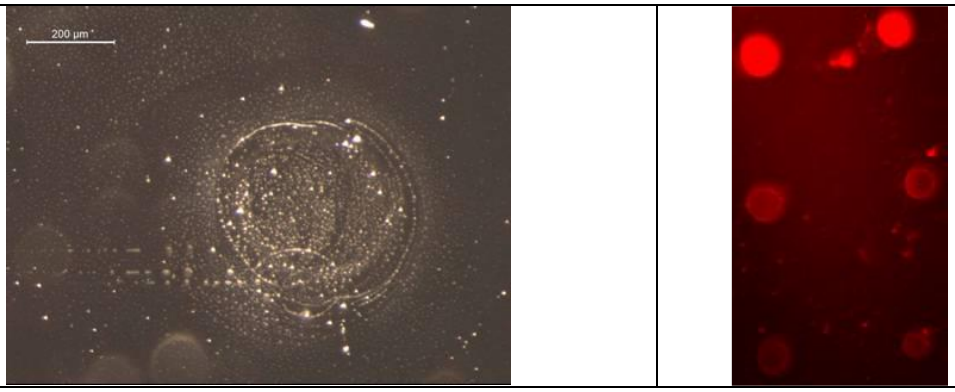

Figure XX. Optic microscopy detail of polymeric deposit (Leica MZAPO; Leica Microsystems) and downwards fluorescence gradient on assayed chip (homemade CCD device).

9.14. Mass spectroscopy (MS)

In the case of interest, MS may be used during the preparative steps to ensure that initiator moieties are properly coupled to the label molecules. Some

[258] S. R. H. Farley, C. D. Bain, G. M. Whitesides, Langmuir 4 **1988** 921

work has been made on this topic when bromoisobutyryl species are attached to DNA single strands in atom transfer radical polymerization studies[245, 250,255].

9.15 Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Up to now, few references have investigated the nature of the polymer formed after photo-induced polymerization under the optics of these two techniques.

III. PMR EXAMPLES AND APPLICATIONS

10. Initial approaches to photopolymerization

First interest in photopolymerization arose from the studies in holographic data storage devices[157, 259-262] and those related to cure faster and less toxic dental polymer implants[263-265]. Later on, biomedical interests emerged[105, 266-267]. Initially, photopolymerization was performed by UV light excitation but the search of technicians and target consumers safety, energy saving and superseding of harmful mercury based UV lamps by environmentally friendly ones were subjects of much interest[268-274]. During the first 2000s, there appeared innovative works on visible photopolymerization that would, finally, focus the target on biosensing. Some of them reported the behavior of mixtures incorporating triazine derivatives[275-280]: Higher efficiency of polymerization under

[259] D. H. Close, A. D. Jacobson, J. D. Margerum, R. G. Brault, F. J. McClung, Appl. Phys. Lett. 14 **1969** 159
[260] D. Psaltis, F. Mok, Sci. Am. 273 **1995** 70
[261] G. T. Sincerbox, Current Trends in Optics, charpter 4, J. C. Dainty ed., Academic London **1994**
[262] R. A. Lessard, G. Manivannan, Proc. SPIE 2405 **1995** 2
[263] W. D. Cook, Biomaterials 7 **1986** 449
[264] W. D. Cook, J. Dental Res. 61 **1982** 1436
[265] Y. Omata, J. B. Lewis, S. Rotemberg, P. E. Lockwook, R. L. W. Messer, M. Noda, S. D. Hsu, H. Sano, J. C. Wataha, J. Biomed. Mat. Res. A 77 **2006** 470
[266] H. Brondsted, J. Kopecek, Biomaterials 12 **1991** 584
[267] B. M. Oshea, M. F. A. Goosen, A. M. Sun, Biochim. Biophys. Acta 804 **1984** 133
[268] S. P. Pappas, UV curing: science and technology, Technology marketing corp. Stamford **1985**
[269] R. Holman, P. Oldring, UV and EB curing formulations for printing inks, coatings and paints, vol. I-VIII, eds. Sita Techn. Ltd. London **1997**
[270] J. P. Fouassier, Photoinitiation, Photolopymerization and photocuring, Hanser pub. **1995**
[271] S. Davidson, Exploring the science, technology and applications of UV and EB curing, ed. Sita Techn. Ltd. London **1999**
[272] D. C. Neckers, UV and EB at the millenium, ed. Sita Techn. Ltd. London **1999**
[273] V. Krongauz, A. Trifunac, Photoresponsive polymers, ed. Chapman and Hall NY **1994**
[274] A. B. Scanton, A. Bowman, R. W. Peiffer, Photopolymerization: fundamentals and applications, ACS symp. Ser. ACS Washington **1997** 673
[275] A. Reiser, Photoreactive polymers: the science and technology of resists, Wiley NY **1989**
[276] A. G. Hoechst. EP364735 **1990**
[277] K. Dietliker, P. Oldring eds., UV and EB curing formulations for printing inks, coatings and paints, vol. III, Sel. Ind. Training associates London **1991**

visible light irradiation were obtained when three component mixtures conformed by dye, amine and triazine derivatives were employed (as envisioned in 5.4.5).

Photographting of polymers onto carbon black[281-282], $Mo(CO)_6$, other transition metal-modified[283] and eosin-modified nanosized silica surfaces was accomplished. The surfaces were previously treated with appropriate alkyl metoxysilanes before anchoring the eosin molecules by reactions of their carboxilate groups with the superficial pending ones. This manner, styrene, acrylamide, acrylic acid or acrylonitrile were properly polymerized on the mentioned silica surfaces in the presence of ascorbic acid and atmospheric oxygen by visible light irradiation at room conditions[284].

Similar to these studies, photopolymerization of poly (ethylene glycol) diacrylate was performed on properly modified glass and silicon surfaces, extending the knowledge to the fabrication of patterned surfaces by microcontact printing and future sensing chip technology. Amino silinized surfaces were derivatized with eosin by Woodward's reagent K (WRK) strategy under N_2 enriched ambient to minimize the deactivation of superficial $–NH_2$ groups by CO_2 or moisture present in air[285], thus allowing amide bonds between eosin and amino superficial groups to be formed. PEGDA polymer film was obtained in presence of triethanolamine and NVP as co-initiators upon 514 nm argon ion laser irradiation was switched on. The hydrogel film formed remained firmly attached on the tested surface for more than one year immersed in aqueous solution. XPS measurements seemed to confirm, though not unambiguously, that the forming polymer and superficial eosin radicals ($M^{\cdot}$ and $Dye^{\cdot}$) may bind by covalent interactions[161]. In this line, the term *smart hydrogel* was introduced to refer to hydrogel membranes of miscellaneous composition increasing the number of applications a single membrane is able to achieve. So, a method to create multi-membranes formed of several single layers, each one with a desired specific property, was recently developed by varying the composition of the reactive mixture in each layer[243]. In this sense, a membrane constituted by a three layer system sensitive to pH, temperature and solvent composition could be prepared[286-288]. These complex hydrogel structures are generated when adding a portion of NH_2-acrylate derivative to the usual amine/ketone reactive mixture. Thus, each layer will posses pending amino groups (as those present in the initial aminosililanized glass support surfaces) able to interact with eosin molecules (WRK chemistry) when preparing the subsequent ontop layer, achieving a strong attachment between layers that keeps high multi-membrane unity. The implementation of photo masks allows

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[278] M. Kawabata, K. Kimoto, Y. Takimoto, EP211615 **1987**
[279] C. Decker, B. Elzaouk, J. Appl. Polym. Sic. 65 **1997** 833
[280] G. Pohlers, J. C. Scaiano, E. Step, R. Sinta, J. Am. Chem. Soc. 121 **1999** 6167
[281] N. Tsubokawa, Prog. Polym. Sci. 17 **1992** 417
[282] N. Tsubokawa, Fundamental and applied aspects of chemically modified surfaces, p36, J. P. Bilz, C. B. Lottle, Eds., RSC London **1999**
[283] N. Tsubokawa, Y. Shirai, K. Shirai, J. Polym. Sci. A Polym. Chem. 39 **2001** 2157
[284] M. Satoh, K. Hiroshi, H. Saitoh, T. Yamauchi, N. Tsubokawa, J. Polym. Sci. A Polym. Chem. 43 **2005** 600
[285] M. Wirde, U. Gelius, L. Nyholm, Langmuir 15 **1999** 6370
[286] T. Tanaka, Phys. Rev. Lett. 40 **1978** 820
[287] Y. Hirokawa, T. J. Tanaka, J. Chem. Phys. 81 **1984** 6379
[288] S. J. Lee, K. J. Park, J. Mol. Recognit. 9 **1996** 549

the formation of controlled spatial 3D-hydrogel structures with potential application in high sensing density biochips, tissue engineering, membrane separations or other type of multifunctional devices.

Some results obtained in our lab are shown in Figure XX as examples of polymer deposits formation, their microspe identification and fluorescent quantification. The strategies depicted are direct eosin covalent attachment, physical adsorption of eosin protein labelled and covalent attachment of eosin labelled DNA.

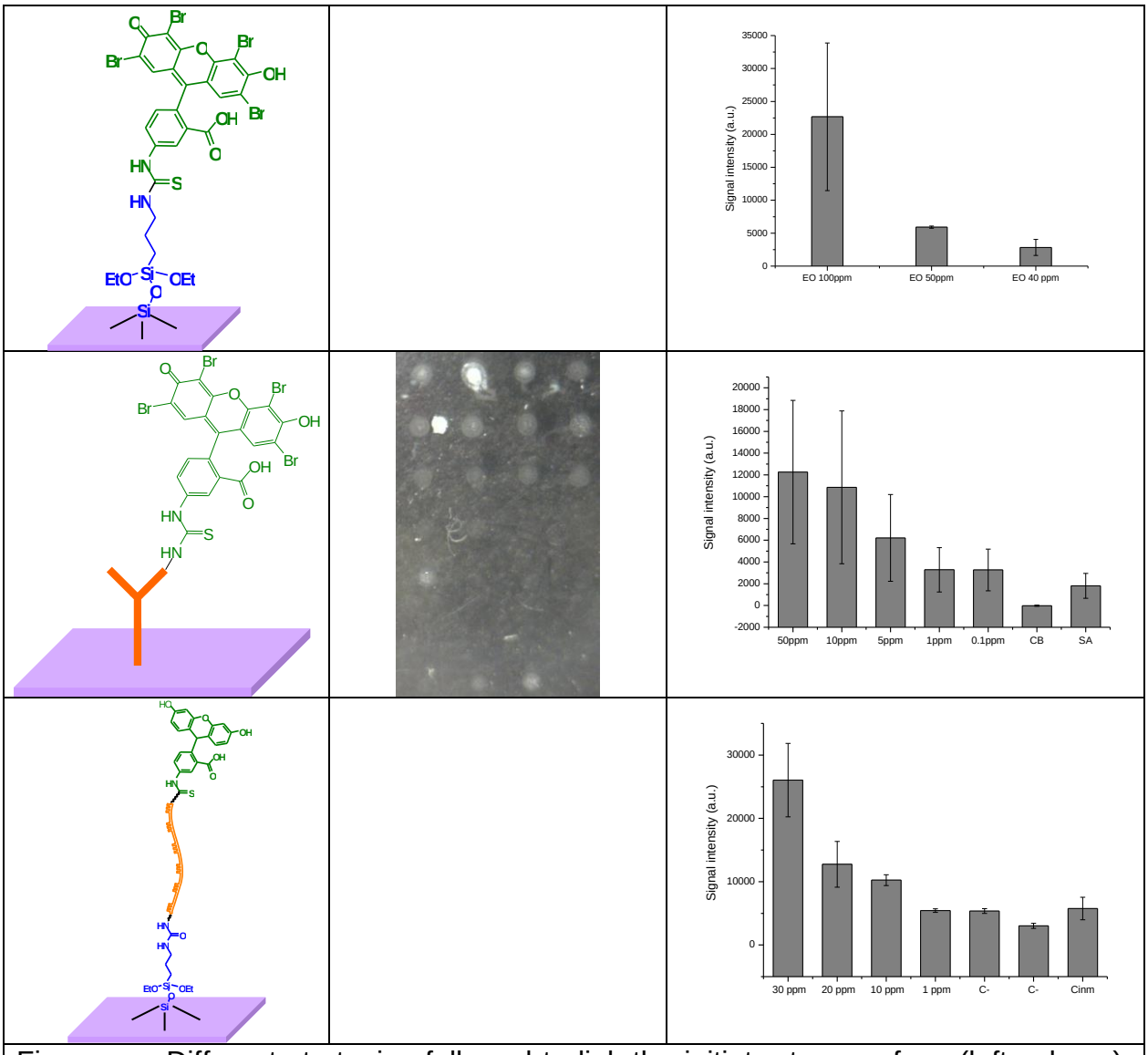

Figure x.x. Different strategies followed to link the initiator to a surface (left column). Optic microscope images (central column) and corresponding fluorescent signal intensities (right column).

11. Classical biotin-avidin strategy

The modification of a protein with a small molecule able to initiate a polymerization process allows generating the polymer on the same protein complex surface, what simplifies the residual monomer removal. Many health related disciplines have been attracted to protein-polymer conjugates[289-292]. In a first attempt, redox reactions could produce radicals on amino acid side chains, though in uncontrolled manner, heterogeneous conjugates being obtained[293-297]. Biotin-streptavidin is a well known biorecognition model system[298]: streptavidin is a protein with four subunits able to bind one biotin molecule each with a very high affinity constant, K_d = 10^{-15} M[299]. This strategy aids the formation of polymeric chains bound exactly to the sites of modification onto the protein. Briefly, biotin molecules labeled with a photoinitiator moiety are mixed with a streptavidin solution, resulting in the formation of the above mentioned conjugate. The addition of monomer solution, together with the initiating conditions, results in the formation of four polymeric chains bound to each of the biotin-initiatior entities. Proton NMR, surface plasmon resonance, gel permeation and size exclusion chromatography techniques conclude that after the formation of the polymer chains, streptavidin retains its affinity for biotin, thus it still binds biotin and so no monomeric biotin released subunits are expected[300].

Streptavidin contains 28 lysine residues able to be functionalized by the isothiocyanate photoinitiator derivative through the formation of a thiourea bond in appropiate buffered medium[301]. UV-VIS spectroscopy is useful to calculate the photoinitiator/protein molar ratio (commonly set between the values 1 to 4) by comparing both absorption characteristic peaks at 280 nm for the protein and the corresponding one for the initiator (590 as example), through the equations[76]:

$$[dye] = A_{590}{}^{dye} \Big/ Mw_{dye}$$

$$[protein] = \left(A_{280}{}^{prot} - X \cdot A_{280}{}^{dye}\right) \Big/ Mw_{prot}$$

The degree of labeling is determined rationing,

$$DOL = \frac{A_{590}{}^{dye}}{A_{280}{}^{prot} - X \cdot A_{280}{}^{dye}} \cdot \frac{Mw_{prot}}{Mw_{dye}}$$

Where,

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289 R. Duncan, Nat. Rev. Drug Discovery 2 **2003** 347
290 K. Velonia, A. E. Rowan, R. J. Nolte, J. Am. Chem. Soc. 124 **2002** 4224
291 J. M. Hannink, J. J. Cornelissen, J. A. Farrera, P. Foubert, F. C. Schryver, N. A. Sommerdijk, R. J. Nolte, Angew. Chem. Int. Ed. 40 **2001** 4732
292 Z. Ding, R. B. Fong, C. J. Long, P. S. Stayton, A. S. Hoffman, Nature 411 **2001** 59
293 J. Zhu, P. Li, J. Polym. Sci. A Polym. Chem. 41 **2003** 3346
294 A. George, G. Radhakrishnan, K. T. Joseph, Polymer 26 **1985** 2064
295 P. R. Chatterji, Appl. Polym. Sic. 37 **1989** 2203
296 Q. Dong, Y. L Hsieh, J. Polym. Sic. 77 **2000** 2543
297 Y. Imai, Y. Iwakura, J. Appl. Polym. 11 **1967** 1529
298 M. Wilcheck, E. A. Bayer, O. Livnah, Immunol. Lett. 103 **2006** 27
299 P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, Science 243 **1989** 85
300 D. Bontempo, H. D. Maynard, J. Am. Chem. Soc. 127 **2005** 6508
301 G. T. Hermanson, Bioconjugate Techniques, Academic Press, San Diego CA, **1996**

- A_{num} refers to the absorbance of the protein or dye respectively.
- X is a percentage value for correction due to the absorbance of the dye at the protein absorption wavelength.
- Mw is the molecular weight of the protein and dye used.

An example of this strategy is the evaluation of oligonucleotide surface concentration. Biotinylated oligonucleotide targets (which are preferably immobilized onto aminosililated-modified glass sensor surfaces, rather than aldehyde-modified ones, because a larger range of oligonucleotide densities is accomplished with the first approach)[76] are covalently coupled with a streptavidin-photoinitiator conjugate solution. Subsequently, monomer and co-reactants (if necessary) are added to the medium, followed by the surface light assisted polymerization. This would survey a positive/negative method for the determination of 3'-biotin labeled capture oligonucleotides.

In order to get quantitative results some approaches can be considered:

- Incorporation of fluorescent molecules to the monomer mixture produces a fluorescent signal that can be related with the polymer growth by the simple assessment: the most polymer formed onto a surface spot, the most fluorescent moieties will be captured inside the polymer formed[77]. With this in mind, a recognition assay of a biotin labelled antibody with eosin labelled streptavidin was developed in our lab with a reactive mixture containing Cy5 fluorescent moieties. Figure XX shows the schematic approach, an image of the resultant polymeric dots and the corresponding fluorescent quantitation.

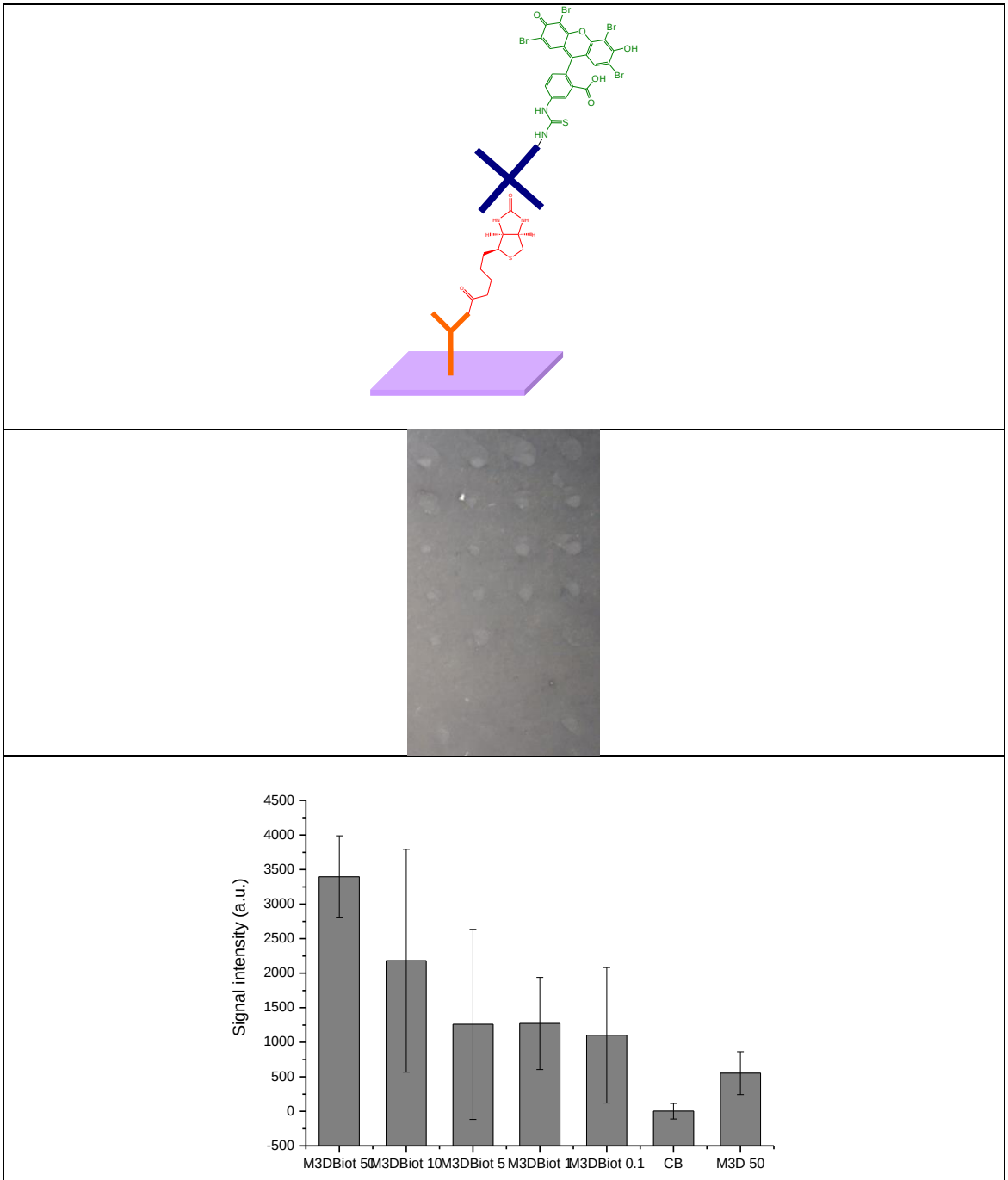

Figure XX. Biotin/antibody recognition by eosin labelled streptavidin and subsequent polymer formation quantified by CCD imaging. Reconocimiento 1/10.

- Direct hybridization of 3'-biotin labeled DNA probes to their surface complementary immobilized targets followed by streptavidin-photoinitiator conjugate addition which will bind exclusively to the hybridized sites. Afterwards, monomer solution and irradiation produce the polymer only onto the hybridized sites. When the 3'-biotin probe and the immobilized target oligonucleotide DNA single strands are complementary, high affinity sticks them together with a typical $K_{hybr} = 10^9\ M^{-1}$ value[302-303].

[302] W. Michel, T. Mai, T. Naiser, A. Ott, J. Biophys. 92 **2007** 999

• Primer extension methodology is another way to obtain quantitative information on the presence of a target oligonucleotide[304]. This procedure may provide single nucleotide polymorphism typing and mutation detection. If some of the deoxy-nucleotide triphosphates are biotinylated, when the PEX is accomplished by the retrotranscriptase enzymes, they will be bound exclusively to the properly hybridized DNA chains[242]. Already mentioned streptavidin-photoinitiator, monomer and irradiation will follow. The discrimination forced by the use of enzymes tandemed with the nature of photo-amplification permits great improvements in specificity of single base mutations determination (up to 0,5 nM target concentrations). However, this methodology requieres the polymerase chain technology, which is expensive and needs precaution in enzymes handling and storing.

12. Protein and DNA recognition events

Over more than a 30 years time is taken behind completing the human genome project, foreseen by the International Human Genome Sequencing Consortium, which contains approximately 3,08 billion nucleotides[305]. DNA diagnostic possibilities have been developed since the early 1970s, when specific restriction endonucleases[306-308] and reverse transcriptases[309-310] were discovered. But it was the finding of the polymerase chain reaction (PCR) in the mid 1980s[311,312] what spread out the use of nucleic acid tests in routine laboratory. Lately, in the 1990s, the development of oligonucleotide microarrays or DNA chips turned out to be one of the most relevant technologies to be evolved from the genome sequencing[313-315]. The first commercial DNA chips took more than 4 h to perform pathogen identification[316-317]. Variations and improvements on the PCR technique have been reported, as reverse transcriptase and real time PCR[318-320]. Nevertheless, all these methodologies are exposed to sensitive reactants (principally enzymes but also other biological compounds) and expensive equipments. As exposed above, photopolymerization appeared to be a very useful tool for protein to protein and

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[303] S. C. Andras, J. B. Power, E. C. Cocking, M. R. Davey, Mol. Biotech. 19 **2001** 29
[304] T. Pastinen, M. Raitio, K. Lindroos, P. Tainola, L. Peltonen, A. C. Syvanen, Genome Res. 10 2000 **1031**
[305] IHGSC, Nature 431 **2004** 931
[306] W. Arber, S. Linn, Annu. Rev. Biochem. 38 **1969** 467
[307] H. O. Smith, K. W. Wilcox, J. Mol. Biol. 51 **1970** 379
[308] K. Danna, D. Nathans, Proc. Natl. Acad. Sci. USA 68 **1971** 2913
[309] D. Baltimore, Nature 226 **1970** 1209
[310] H. M. Temin, S. Mizutani, Nature 226 **1970** 1211
[311] R. K. Saiki, S. Scharf, F. Faloona, Science 230 **1985** 1350
[312] K. B. Mullis, F. A. Faloona, Methods Enzymol. 155 **1987** 335
[313] K. M. Kurian, C. J. Watson, A. H. Wyllie, J. Pathol. 187 1**999** 267
[314] D. Gerhold, T. Rushmore, C. T. Caskey, Trends. Biochem. Sci. 24 **1999** 168
[315] M. J. Heller, Annu. Rev. Biomed. Eng. 4 **2002** 129
[316] G. Vernet, Virus Res. 82 **2002** 65
[317] T. R. Gingeras, R. Higuchi, L. J. Kricka, Y. M. Lo, C. T. Wittwer, Clin. Chem. 51 **2005** 661
[318] E. M. Elnifro, A. M. Ashshi, R. J. Cooper, P. E. Klapper, Clin. Microbiol. Rev. 13 **2000** 559
[319] C. T. wittwer, M. G. Herrmann, C. N. Gundry, K. S. E. Johnson, Methods 25 **2001** 430
[320] J. wihelm, A. Pingoud, Chembiochem. 4 **2003** 1120

DNA hybrization assays due to the relatively inexpensive labeling and signal amplification methods (no enzymes are required and hardware is commonly affordable).

To the present day, several examples of photopolymerization with sensing objective have been reported in the literature. Generally, epoxy, aminosililated or aldehyde modified glass surfaces serve as support for the low density array examples communicated. The basic structure of a DNA chip is a defined array of spots deposited on a flat surface, each of them containing a number of single DNA strands. Regarding protein chips, their structure is similar to the mentioned DNA ones, except that the array spots contain antibodies, antigens or related substances. For accurate measurement of binding events surface bound molecules must be correctly folded and functional[321], what is ensured using stabilizing buffers or high protein concentrations[322]. Such a way, 13 ng/mL of influenza A/H3 hemagglutinin protein have been reported in a proof-of-concept proteic microarray and amplification factors around 10^6 are achieved when analyzing a highly conserved influenza A DNA sequence of 23 nucleotides in DNA chips[73]. False positives can be minimized by the use of macroinitiators in the reactive mixture[72,75]. Even antibody-antigen sandwich assays with the secondary antibody attached to a macrophotointiator have been recently contributed to the community[200]. To end, just a brief commentary on the first report with protein and DNA influenza clinical samples performed on InDevR Intellichip equipment, which obtained amplification factors around 10^5, resemble to HRP strategy[74].

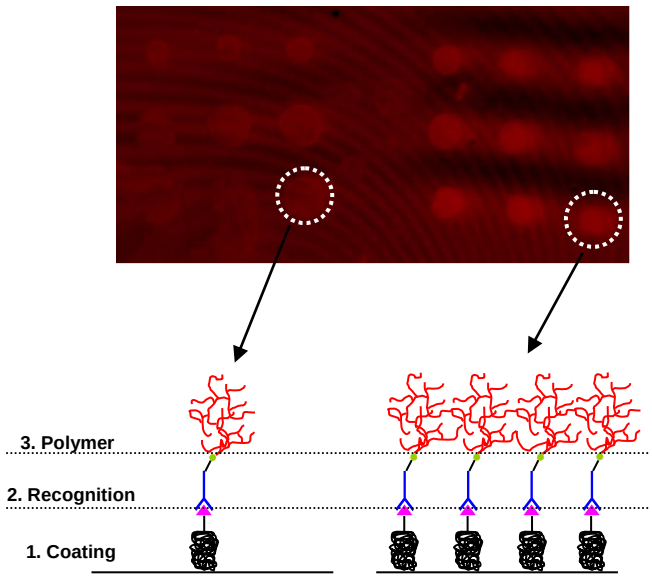

Figure. Example of mediated protein to protein photopolymerization process onto polycarbonate chip at two different coating concentration.

[321] A. Constans, The Scientist 18 **2004** 42
[322] M. Schena, Microarray analysis, p 154, John-Wiley and sons eds., NJ **2003**

IV. FUTURE TRENDS

From now on, the photopolymerization process as biointeraction events signaling methodology will face several topics in order to improve its applications and reliability. Concerning the literature and our knowledge, the hot spots this subject is promptly targeting are:

1) Search of environmental friendly and less toxic reactants. Habitually, volatile monomers have been widely used to obtain photopolymers; some of them defined carcinogenic (as acrylamide). The search of less aggressive monomers, together with sustainable and environmental compatible amines and dyes are major items. For instance, the use of glycine derivatives instead of alkyl amines, the substitution of toxic xanthine dyes for natural substances as riboflavin[323] (as seen in section 5.1) or others, and the search of biocompatible and degradable monomers (as phosphorous containing vinyl esters[324-326] and carbamates[327]) will receive future attention.
2) As for the mechanistic and primary steps of the reaction, the paper of molecular oxygen in the reactive mixture should be clarified. Some authors have sustained the detrimental activity of O_2 in the process, what makes necessary to purge the reaction vessel with N_2 or Ar[328]. On the other hand, recent studies apparently suggested the beneficial effect of O_2 in the reactive mixture, informing its participation in the regeneration of the very same semi reduced dye entities[249]. Thus, getting the role of oxygen straight is a second topic to consider.
3) Methods related to nucleic acid and protein testing need to optimize the automation and miniaturization towards microfluidic "lab-on-a-chip" devices[329-337] able to accomplish total chemical analysis[338], even high-throughput genotyping[317, 339-340]. The time of the whole process should be reduced by optimizing the PCR step. Furthermore, conducting more tests at each reaction site, i. e. multiplexing, would help maximizing the

[323] J. Khadem, T. Truong, J. T. Ernest, Cornea 13 **1994** 406
[324] J. Z. Du, T. M. Sun, S. Q. Weing, X. S. Chen, J. Wang, Biomacromolecules 8 **2007** 3375
[325] Q. Li, J. Wang, S. Shahani, D. D. N. Sun, B. Sharma, J. H. Elisseeff, K. W. Leona, Biomaterials 27 **2006** 1027
[326] D. Wang, C. G. Williams, Q. Li, B. Sharma, J. H. Elisseeff, Biomaterials 24 **2003** 3969
[327] C. Dworak, T. Koch, F. Varga, R. Liska, J. Polym. Sci. A Polym. Chem. 48 **2010** 2916
[328] V. A. Bhanu, K. Kishore, Chem. Rev. 91 **1991** 99
[329] P. R. Selvaganapathy, E. T. Carlen, C. H. Mastrangelo, Procds. IEEE 91 **2003** 954
[330] D. Choi, E. Jang, J. Park, W. G. Koh, Microfluid. Nanofluid. 5 **2008** 703
[331] U. Bilitewski, M. Genrich, S. Kadow, G. Mersal, Anal. Bioanal. Chem. 377 **2003** 556
[332] T. Chovan, A. Guttman, Trends Biotechnol. 20 **2002** 116
[333] D. Figeys, D. Pinto, Anal. Chem. 72 **2000** 330A
[334] S. C. Jakeway, A. J. Mello, E. L. Russell, Fresenius J. Anal. Chem. 366 **2000** 525
[335] N. Lion, T. C. Rohner, L. Dayon, I. L. Arnaud, E. Damoc, N. Youhnovski, Z. Y. Wu, C. Roussel, J. Josserand, H. Jensen, J. S. Rossier, M. Przybylski, H. H. Girault, Electrophoresis 24 **2003** 3533
[336] G. H. W. Sanders, A. Manz, Fresenius J. Anal. Chem. 19 **2000** 364
[337] S. K. Sia, G. M. Whitesides, Electrophoresis 24 **2003** 3563
[338] A. N. Manz, N. Graber, H. M. Widmer, Sens. Actuactors B Chem. 1 **1990** 244
[339] S. Jenkins, N. Gibson, Compar. Funct. Genom. 3 **2002** 57
[340] R. H. Liu, J. Yang, R. Lenigk, J. Bonanno, P. Grodzinski, Anal. Chem. 76 **2004** 1824

throughput[341]. Future efforts should enhance sensitivity and avoid PCR procedure by raising the number of eosin initiators to a hybridization event (specialized macroinitiators containing higher ratios of eosin molecules to SA proteins) or using directely eosing-labeled dNTPs in the PEX reaction.

4) In brief, other potential targets are the reduction of the detection limits and the overall cost of the assays, but at the same time enlarging the accuracy, the portability and onsite availability of the assay chips, designed as high density microarrays would turn this promising methodology into real routine clinic procedures.

[341] P. Moore, Nature 435 **2005** 235