

European Science Foundation Exchange Visit Grant 2980

Interdisciplinary Approaches to Functional Electronic and Biological Materials (INTELBIOMAT)

Scientific Report

SURFACE CHEMICAL PROCESSES FOR BIOMOLECULE IMMOBILIZATION IN DIAGNOSTIC APPLICATIONS

Synthesis and Surface Initiated - Controlled Radical Polymerization (SI-CRP) of 2-Vinyl-4,4-Dimethyl-2-Oxazolin-5-One (VDMO)

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Host Institution: Ecole Polytechnique Fédérale de Lausanne (EPFL), Suisse

Visit Period: November 2010 – February 2011 (18 weeks)

Purpose of the visit:

Aim of this exchange was the improvement of applicant's knowhow in functional surface building. The "Laboratory of Polymers" of prof. Harm-Anton Klok at EPFL- Lausanne, is a famous group for its research on the technology of polymer brushes, macromolecular systems useful in surface modification. Polymer brushes are ultrathin coatings made of polymer chains tethered with one end to an interface which at high density present a well ordered conformation. These coatings have found widespread usefulness, among others, in fields like biosensing and in biomedical applications.

For this specific work we chose the monomer 2-vinyl-4,4-dimethyl-2-oxazolin-5-one (VDMO) also called 2-vinyl-4,4-dimethyl-2-azlactone which represents a promising molecule in the field of functional polymeric surfaces. Its main feature is the ability to react with the common nucleophiles such amines, alcohols and thiols, at room temperature, without any activation or pretreatment and with no by-products.

In the first part of this work we synthesized and characterized the VDMO monomer. Subsequently the product was employed in polymer brush synthesis via surface initiated-controlled radical polymerization (SI-CRP). The utilized CRP techniques were "atom transfer radical polymerization" (ATRP) and "reversible-addition fragmentation chain transfer" (RAFT) polymerization.

Description of the work:

1. VDMO synthesis by dehydration of N-acryloyl-2-methylalanine

The synthesis of the monomer was carried on involving a two step reaction. In the first part we prepared N-acryloyl-2-methylalanine (NAM) starting from 2-methylalanine and acryloyl chloride as illustrated in figure 1.

Typically, to 40 ml of a 10 M water solution of sodium hydroxide, cooled to about 10 °C, 0.2 mol of solid 2-methylalanine were added stirring and sonicating until complete solubilization.

After addition of 0.9 mmol of 2,6-di-tert-butyl-p-cresol (BHT) as a polymerization inhibitor, 0.2 mol of acryloyl chloride were added dropwise after the activation by centrifugation on Al_2O_3 (10 min, 2500 rpm) stirring for 30 min. To this solution of NAM sodium salt we slowly added 0.25 mol of concentrated HCl, again keeping the temperature at 15 °C. The resulting solid was filtered by suction, washed well and recrystallized with a hot mixture of ethanol and water. After drying at 60 °C in Schlenk line until constant weight, the obtained white solid is usually of analytical purity.

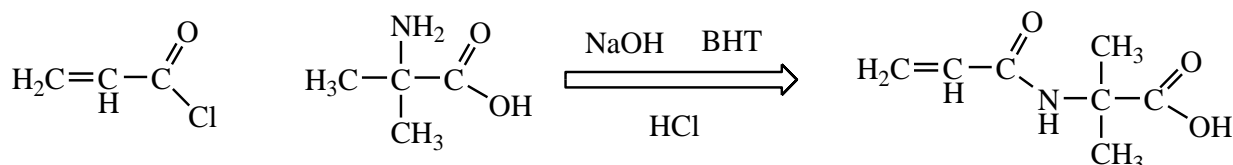


Fig. 1: *N*-acryloyl-2-methylalanine synthesis

In the following step, NAM was dehydrated to get VDMO. After tried different methods, the most effective was the one employing dicyclohexylcarbodiimide (DCC) as dehydrating agent (figure 2).

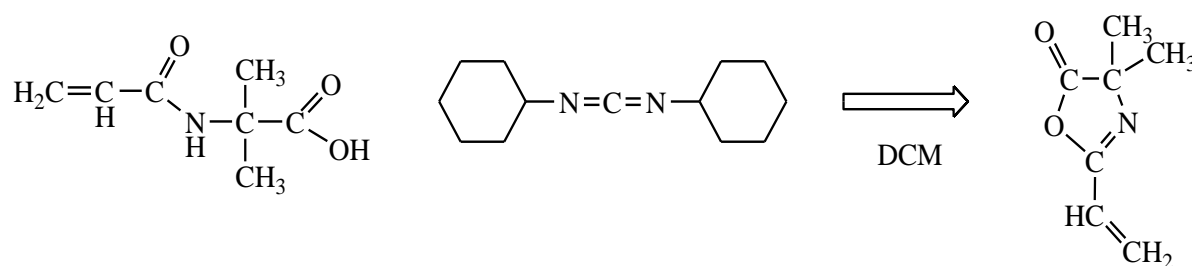


Fig. 2: *VDMO* synthesis by dehydration of *NAM* with *DCC*

Into a 250 ml round-bottomed flask fitted with stirrer and rubber cap 0.042 mol *NAM* were slurried in 100 ml of dichloromethane (DMC) containing 0.04 mol of *DCC*. The mixture was stirred at room temperature overnight. The dicyclohexylurea by-product was filtered on paper and thoroughly rinsed with *DCM*. The filtrate was concentrated by rotavapor and vacuum distilled (Schlenk line, 65 °C) in order to collect a colorless fraction, which was stored under N_2 at -20 °C.

2. *VDMO* polymer brushes

The initial considered procedure to realize *VDMO* brushes was atom transfer radical polymerization (ATRP). We prepared several Si chips with polymerization initiator but after a tenth of experiment, using different condition, without remarkable results we concluded that this method was not effective for *VDMO* polymerization.

In order to overcome the problems related to the previous method, we employed reversible addition-fragmentation chain transfer (RAFT) polymerization.

Typically, in a 25 mL air-free dry polymerization flask, we put anhydrous toluene and 1 ml of *VDMO* monomer in order to obtain a *VDMO* concentration between 1.2 M and 1.6 M. The solution was degassed with three freeze-purge-thaw cycles. Then we put AIBN (fig. 3), which is a radical polymerization initiator, in a molar ratio of about 1:5000 with the monomer. Finally we placed into the reactor a RAFT agent functionalized Si wafer, which was previously treated

with UV rays for 10 minutes using a TEM grid as a mask in order to have an immediate visible check of the polymerization. Polymerization was allowed to proceed at 80 - 95 °C overnight. At the end the Si-g-PVDMO substrate was removed and sequentially rinsed with toluene, tetrahydrofuran and acetone and dried under N₂.

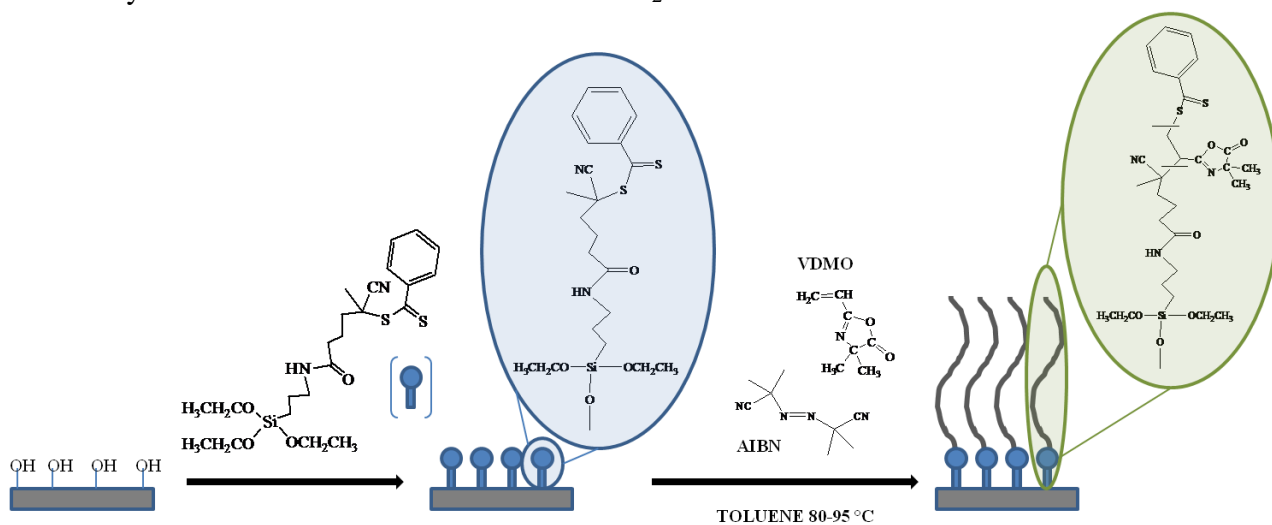


Fig. 3: Scheme of VDMO RAFT polymerization

Description of the main results obtained:

1. VDMO synthesis

The above described synthesis method for VDMO allowed to produce the monomer with a good purity, although the yield was not particularly high, because of the recrystallization step. Characterization data were in good agreement with literature. Concerning FTIR data (spectrum not shown) the C=O stretching peak at energy higher than 1800 cm⁻¹ and the signal at about 1670 cm⁻¹ related to the imide bond, reveal the presence of an azlactone ring. Also NMR data are coherent with that kind of structure and in agreement with literature (see figure 4).

2. VDMO polymer brushes

Atomic force microscopy (AFM) measurement allowed us to evaluate the presence and estimate the thickness of the brushes (fig. 5). Here are reported two examples of brush growth which are quite similar in thickness:

RAFT pVDMO 1

Thickness measurement in different zones (nm): 19.00, 18.65, 15.54, 18.49, 17.62
Average value: **(17.86 ± 1.4) nm**

RAFT pVDMO 5

Thickness measurement in different zones (nm): 13.21, 13.45, 17.18, 18.81, 15.79
Average value: **(15.69 ± 2.4) nm**

FTIR measurements also confirmed the presence of azlactone ring (typical of VDMO) after the polymerization (fig. 6).

Once the RAFT polymerization process will be optimized, the azlactone ring pending from brushes will allow the realization of surfaces extremely easy to modify with custom nucleophiles, therefore providing a useful platform for applications, among others, in biomedical field.

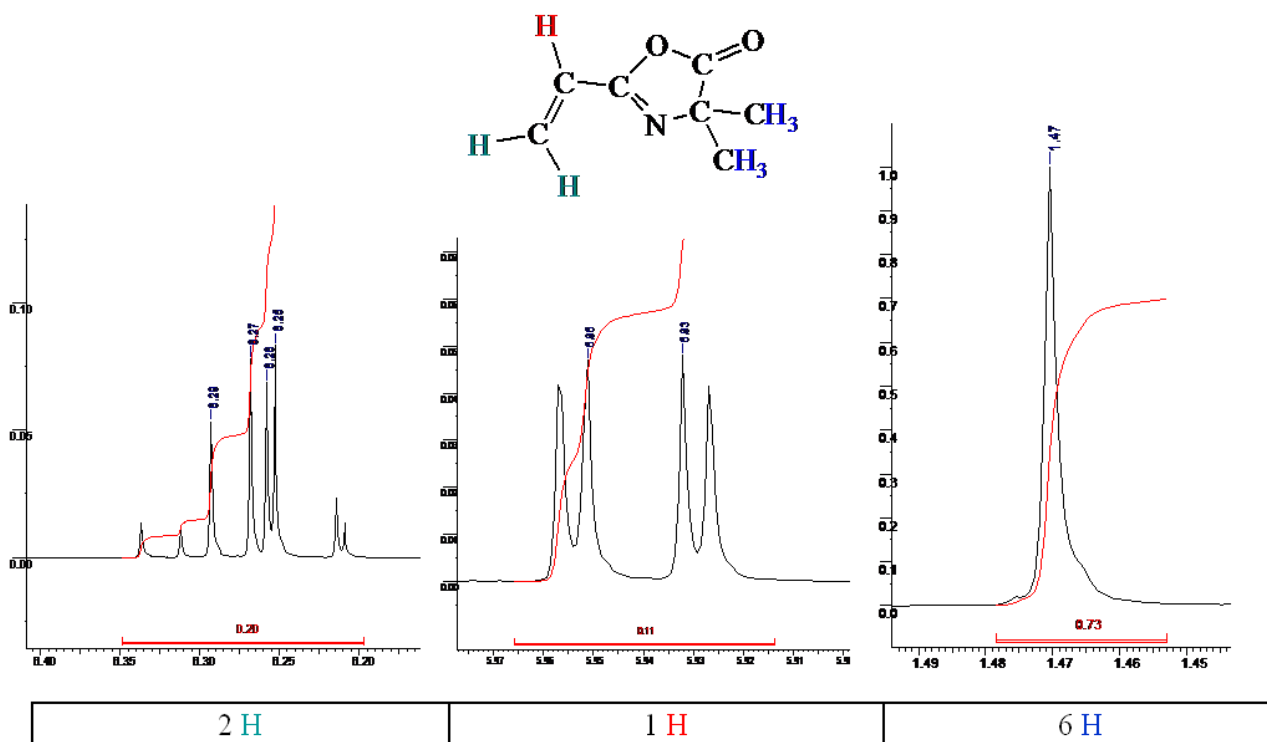


Fig. 4: Details of VDMO NMR spectrum and signal attributions

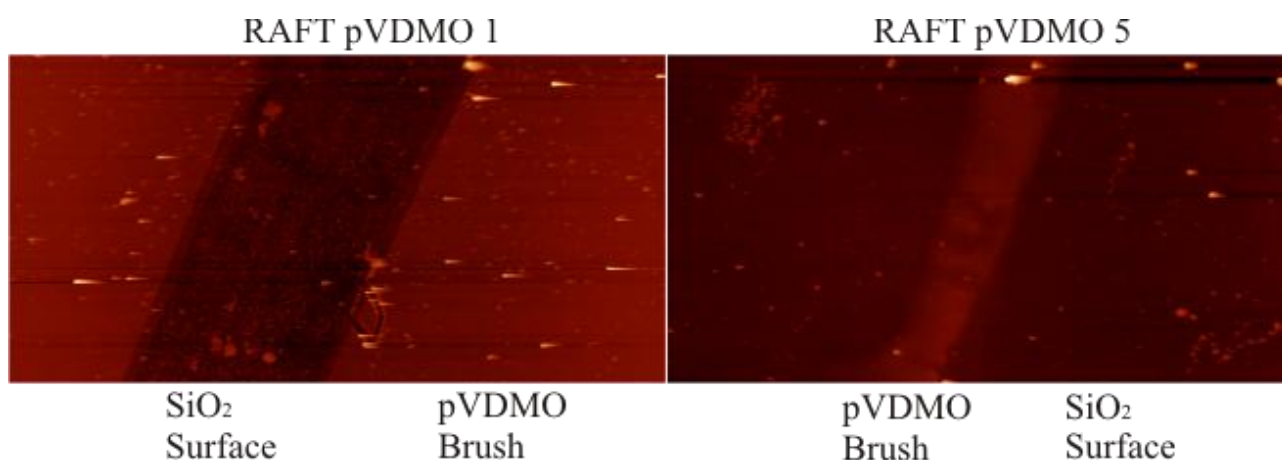


Fig. 5: AFM picture of two different synthesis batches

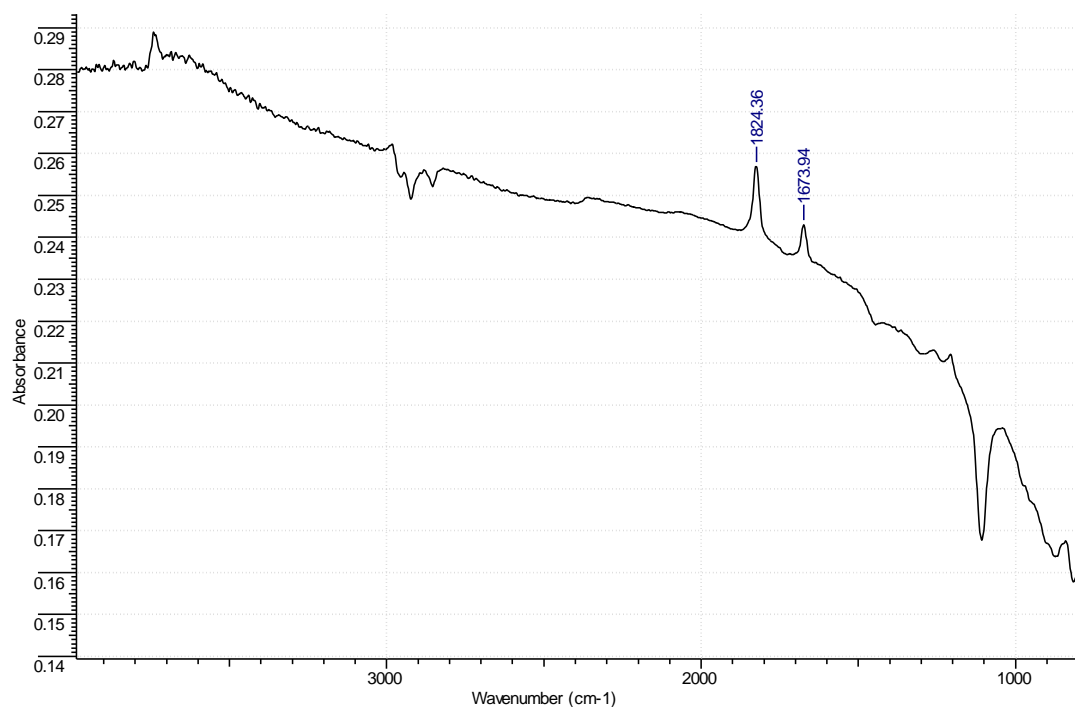


Fig. 6: ATR spectrum of sample RAFT-VDMO 1. Characteristic peaks of VDMO are present

Projected publications/articles resulting or to result from your grant:

A review paper concerning the application of surface initiated polymer brushes in genomic field was started during my visit period and is currently in progress.

Bibliography:

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