Journal of Engineering and Applied Sciences 2 (8): 1283-1289, 2007
ISSN: 1816-949X

Preparation of Hydrogel by Copolymerization of PMMA with a Polysaccharide (Fucan N1) in Presence of a Ceric Initiator in Acidic Environment

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Abstract: Graft polymerization of Polymethyl Methacrylate (PMMA) on polysaccharide in presence of ceric ammonium nitrate, used as initiator, has been conducted to obtain hydro gel under best conditions (Fucan N1) = 0.5g ; (CAN) = $3.6.10^{-3}$ Mol L^{-1}; (HNO_3) = 0.2 M, temperature = 40°C, reaction time = 40 min, initiator/ Monomer rate = 1/10. In addition to a PH analysis of reaction media, an Infrared spectroscopy (IR) has been carried out to establish the appropriate synthesis conditions. This analysis has revealed a characteristic peak of the carbonyl group situated at 1733 cm^{-1} for the obtained copolymer Fucan-PMMA, with respect to the individual spectra of PMMA and Fucan N1. Furthermore, a viscosity analysis has been conducted on two fractions of raw solutions of Fucan in aqueous medium (H_2O) and on the obtained copolymer as well. The outcome of this research has given us an insight to a suggestion of appropriate reaction mechanism in which the rate of polymerization (R_P) is first order dependant on the monomer concentration and on the square-root of the CAN and initiator concentrations.

Key words: Graft polymerization, poly-acrylic, polysaccharide, ceric initiator, hydrogel synthesis

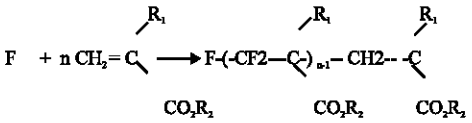
INTRODUCTION

The main goal of this research is to apply the pH-metric measuring method to study the kinetic of copolymerization of PMMA with polysaccharides Fucan N1 and Dextran T-70. Present experimental analysis by measuring the medium pH of polymerization has been proven to be more precise and easily reproductible to determine the ratio of polymerization as compared to the results of other authors, (Mansor and Haron, 1999). We have Polymerized a Poly Acrylic (PMMA) on a sulfated polysaccharide (Fucan N1 extracted from marine brown algae of Ascophyllum nodosum type). This polymerization was carried out in presence of ceric ions (Ce^{4+}) in a highly nitric acid medium PH = 1, at a temperature of 40°C during 40 min under Argon atmosphere (Chowdhury and Pal, 1999; Dattow *et al*., 2002; Masci *et al*., 2004; Costa and Vasconcelos, 2002). Iinitially, we were able to put in evidence a relative initiator/monomer ratio that has to be taken into account. The value of this ratio was found to be in the order of 1/10 while the proportion of polysaccharide was taken constant. Under these conditions, we got a rate of grafting that is equal to 95-97% and this gives evidence that our biomaterial has been synthesized in better conditions. The reaction was conducted in suspension and initiated by the ions (Ce^{4+}) (Hexanitratocerate of ammonium, $(NH_4)_2$ (Ce $(NO_3)_6$), Acros. Formation of a white milky complex is first produced and which disappears immediately after by giving a radical (F) to a proton H^+ and an ion Ce^{3+}, as shown by the following reaction equations.

Initiation:

$$F\text{-}H + Ce^{4+} \rightarrow (\text{Complexe}) \rightarrow F^{\bullet} + Ce^{3+} + H^+$$

Propagation:



$R_1 = R_2 = CH_3$- for the methacrylate of methyl
R_1 = H and $R_2 = CH_2$---CH_2---OH
for the 2-hydroxylethyle acrylate

It is required that present research should be performed in a highly acidic medium, exempt of any trace of polymerization inhibitor. For this reason, we are obliged to purify the reagents before starting any manipulation. The MMA was treated by a mixture of NaOH at 5% with NaCl at 20%, i.e., to 100 mL of MMA taken in a separator funnel, are added 10 mL of this mixture.

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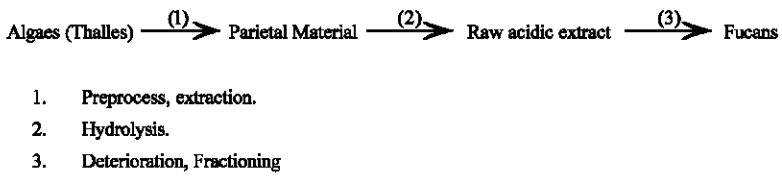

Fig. 1: Simplified flow chart of fucans synthesis

The solution is, then, well agitated and after 10 min both the aqueous phase and the inhibitor of the organic phase are eliminated by simple decantation (organic phase at the top).

This operation is repeated three times until a limpid aqueous phase which is difficult to distinguish form the organic phase is obtained.

In order to avoid any possible homo-polymerization, the purified MMA is let for a while to settle down in a hermetic small bottle coated with aluminium paper and conserved, under little argon, on a molecular sifter in a cold chamber at 6°C.

The initiator $(NH_4)_2$ (Ce $(NO_3)_6$), Acros and the nitric acid HNO_3, Fluka, have been used in pure form. Fucan N1 and BBP-2, having a mass of 95500 and 46600 mol L^{-1}, respectively, are obtained by HPLC chromatography on a 5300HR gel after an acidic extraction followed by an acid hydrolysis (Hoppe, 1979; Colliec *et al*., 1993) (Fig. 1).

These fractions are the main constituents of the sulphated brown algae. They are mainly contained in the Ascophyllum nodosum which represent 6 to 8% of pheophyceas (Mulloy *et al*., 2002).

Their chemical composition are generally composed of 36% of fucose bound by alternate links of alpha 1-3 and, alpha 1-4 types, 50.1% of neutral ose, 13.8% of uric acid, 6.8% of sulphur and a small rate of protein that is less than 2% the chemical composition of fucans is, in fact, very variable and depends on the species used, (Ascophyllum nodosum, Fucunes vesiculosus, Fucus spiralis, Pelvetia canaliculated…). As a matter of fact, the Fucan is water-soluble whereas its copolymer with the PMMA is soluble in water/ THF mixture (30/70) (v/v) and in DMSO.

The other polysaccharide used in this research is the Dextran T-70, a white powder having a mass of 73000 g mol^{-1}. It consists of α-D units of glucose essentially linked to each other by alpha 1-6 glucosidic link and sometimes by links of alpha 1-3 type. It is synthesized by bacteria of the lactobacillus family. Due to its biologic quasi-inertia, the Dextran is the subject of numerous pharmaceutical and medical applications (Hoppe, 1979). As for the Fucan, the Dextran is soluble in water, while its copolymer with the PMMA is soluble in the water /THF mixture (30/70) (v/v) and in DMSO.

EXPERIMENTAL WORK

Copolymerization reaction and synthesis: Once the reagents are purified and ready to be used, 0.5 to 1 g of the polysaccharide is dissolved in 500 mL of HNO_3 at 0.2M in a 1 L reactor equipped with five flasks. This solution is next, agitated for 10 min under an atmosphere of argon at a temperature of 40°C. At this stage, 3.6×10^{-3}M of cerium and 0.5 of MMA are added, simultaneously, to the content of the reactor. The time variation of the PH is measured during 40 min at a sampling rate of 5 min. The reaction is considered terminated when the PH does not change any more which is the case after, approximately, 30 min of reaction.

Subsequently, a centrifugation is conducted to eliminate the cerium Ce^{4+} that did not react and the excess of Ce^{3+} (to make measure out of it if necessary). In order to induce perception, the recovered cap is poured into 500 mL of methanol with the PH adjusted to a value of 8 and NaOH at 10M. A second centrifugation is applied to the obtained solution and the recovered cap, at this stage, is added to 100 mL of bi-distilled water and then submitted to an ultra filtration. This operation continues until the volume of solution resulting from ultra filtration should be about 20 mL. Next, this solution is freezed during one night before undergoing a lyophilization. Then, the reaction product is put in a vacuum dryer to be prepared for an Infrared analysis (IR) and also for rheologic and biological analysis. We started by monitoring the PH evolution of the reaction medium of the copolymerization. As we worked in a highly acidic environment, the PH variation of the copolymerization started weak in the beginning, ranging from 0 to 1 and then increased to become more or less constant at the end of 40 min. At this time, the consumption of hydrogen is almost total, as is shown on Fig. 2.

Copolymers analysis by infrared spectroscopy: This technique allows us to identify some chemical groupings that may be present in the polymers and the copolymers. Tablets of 150 mg of potassium bromide (KBr), with infrared quality (Fluka) at 5% of sample mass to be analyzed, is prepared for this matter. The sample is mixed in the KBr, ground and then vacuum dried at 45°C during

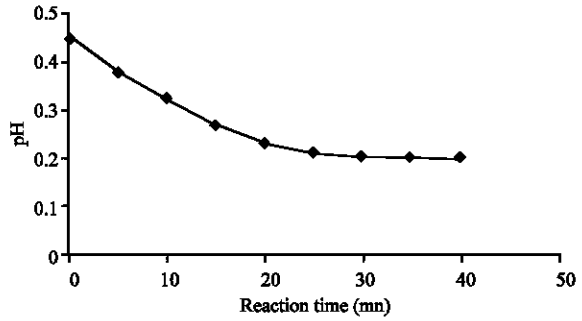

Fig. 2: pH evolution during synthesis reaction of copolymer fucan PMMA; temperature 40°C; solvation; HNO_3 at 0.2M

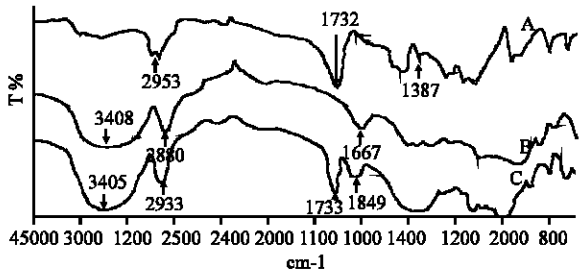

Fig. 3: Infrared spectrum of copolymer dextran-PMMA ; curve (A): PMMA alone; curve (B): dextran alone; curve (C): copolymer of dextran, 0.5 g; MMA 2%; $Ce^{4+}3.6.10^{-3}M$

6 h. It is, then, pressed under 10 tons for 2 min and stored at 45°C in a humidity-free environment. These tablets are analysed by means of a Fourier transform based infrared spectrophotometer (Perklin Elmer, 1600). In order to decrease the effect of background noise, every spectrum is taken to be an average of the cumul of 16 increments. Figure 3 shows the spectra of PMMA and dextran taken individually, in addition to that of the synthesized dextran-PMMA copolymer.

The curve (A) presents several strips characteristic of the PMMA: One at about 2953 cm^{-1} which is assigned to the (CH_2) group, another strip is situated at 1732 cm^{-1} which represents the carbonyl (C = 0) group and a third band is located at 1380 cm^{-1} specific for the (CH_3) methyl group.

The curve (B) shows strips that are specific for Dextran: The presence of radical (OH) is attested by the absorption band that appears at about 3400 cm^{-1}, the band at 2930 $cm^{-1}$is assigned to the methylene group (CH_2), the strip around 1650 cm^{-1} corresponds to the grouping (OH) of the Dextran.

The Curve (C) illustrates the spectrum of the product obtained by copolymerization of the Dextran and the MMA monomer after a ceric initiation by cerium (Ce^{4+}). This graph shows bands that are common to both Dextran and PMMA. One can notice an important intensity at 1733 cm^{-1} which corresponds to the grouping (C = 0).

Figure 4 is a good illustration of the effective formation time of Fucan-PMMA copolymer. In this case, the intensity of this peak increases with the cerium (Ce^{4+}) concentration when the latter changes from 1.8×10^{-5} M to 3.6×10^{-3} Mol L^{-1}, whilst the monomer concentration is kept constant, see curves (B) and (C).

Synthesis (characterized by a peak at 1725 cm^{-1} for PMMA, shown on Curve (C)) with respect to the spectrum of Fucan N1 alone, as depicted by curve (A).

Viscosity measurement: The molar mass of synthesized polymers is an important criterion for evaluating the polymerization efficiency on the polysaccharide. In order to determine the molar mass of the various produced substances whenever it was possible, the viscosity of copolymer solutions was measured for different cerium concentrations . To this end, a well adapted viscometer (the Ubbelohde Viscometer), with a capillary diameter of 0.7 mm, was used for tow different polysaccharide, Fucan N1 and Fucan BBP-2 having a mass of 95500 and 46600 g mol^{-1}, respectively. These 2 polysaccharides were dissolved in H_2O containing less concentration in univalent ions and in a solution of NaCl at 0.5 M, rich in univalent ions, that is necessary to decrease interactions between chains and to avoid the formation of aggregates (Hoppe, 1979). On the graph, we have simultaneously plotted the curves of $\eta_{sp}/c = (t-t_0)/c$ and $(\ln\eta_{sp})/c= (\ln (t/t_0))/c$ in function of polysaccharide concentration.

The intrinsic viscosity (η) is derived by extrapolation and intersections of these 2 curves at a zero-point concentration. On the other hand, this intrinsic viscosity is related to the molar mass through the formula of Mark-Houwink given by $(\eta)= K\times M^a$, where a and K are two constants and these are given for the Dextran, for a comparison purpose. As for the two fractions of Fucan N-1 and BBP-2 with known mass, the value of this constant is deduced from measures of the intrinsic viscosity, Fig. 5. For instance, in the case of the first fraction cited above, $(\eta_1) = 0.252$ and $(\eta_2) = 0.086$ which leads, therefore, to values of a = 1.49 and $k = 8.8\times10^{-9}$ dL g^{-1}. The value of a, thus found, is greater than that of a rigid rod macromolecule (a = 0.8) and lower than that of a branched macromolecule (a = 1.8). This means that our Fucan is presented in aqueous solution as chains with medium mobility. As a comparison, the constant values a and k of the Dextran (with a mass of 73000 g mol^{-1}) are respectively equal to 0.50 and 98.8×10^{-5} dL g^{-1}. This gives an explanation for the higher flexibility of the Dextran as compared to the Fucan.

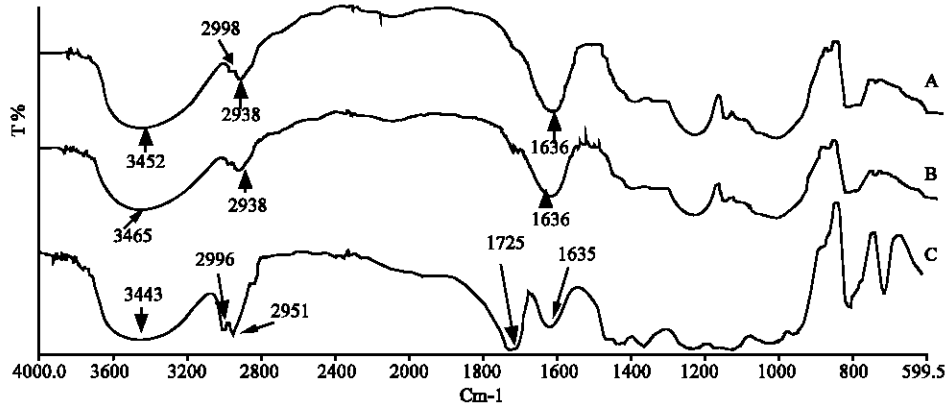

Fig. 4: Infrared spectrum of copolymer dextran-PMMA; curve (A): PMMA alone; curve (B): 0.5 g of fucanN1; 0.2% MMA; Ce^{4+} $1.8.10^{-5}$M; curve (C): 0.5 g of fucanN1 0.2% MMA; Ce^{4+} $3.6.10^{-3}$M

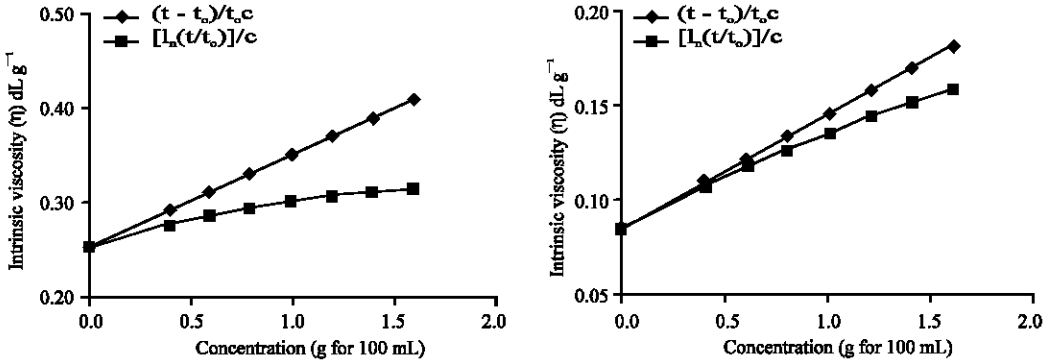

Fig. 5: (a) Viscosity of Fucan N1 (b) Viscosity of Fucan BBP-2; Solvent H2O, Temperature 40°C

Viscosity and mass measures of obtained copolymer: To measure the intrinsic viscosity of the copolymer Fucan-PMMA, 2 different experiments were performed: One experiment utilizes a monomer and the other is without monomer and both are conducted at equal concentration of the cerium (Ce^{4+}) (3.6×10^{-5} mol L^{-1}) and 0.5 g of Fucan N1. We notice that the intrinsic viscosity increases with the concentration in monomers (from 0.16 dL g^{-1} in absence of monomer MMA and with Fucan N1 of an estimated mass of 70500 g L^{-1}, to a viscosity of 0.244 dL g^{-1} at 0.4% of monomer).

This increase of viscosity in presence of the monomer MMA is, mainly, due to the increase of PMMA mass and consequently, it indicates the yield of copolymer Fucan-PMMA and confirms the PMMA grafting on the Fucan, Fig. 6.

As for the case for the Dextran, there is a decrease in Fucan mass as a result of an acid hydrolysis of macromolecular chains during different stages of synthesis. Indeed, the synthesis was carried out in a very acid environment, having a PH of nearly 1. Identical

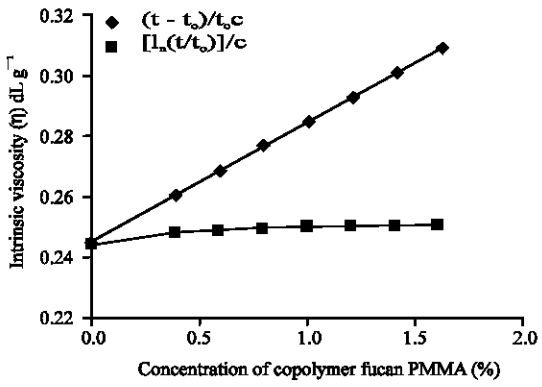

Fig. 6: Intrinsic viscosity of copolymer Fucan-PMMA Fucan N1: 0.5g; (MMA) = 0.4%, (CAN) = 3.6.10-5 M/L, (HNO3) = 0.2M; Temperature 40°C

remarks have been observed concerning the effects of cerium (Ce^{4+}) on the intrinsic viscosity of obtained copolymers.

RESULTS AND DISCUSSION

A main part of our research was concerned with the synthesis of hydro gel or biomaterial that should have biological properties in compatibility with biomedical application, namely, in the vascular domain. To achieve this goal, Fucan-PMM and Fucan-Dextran copolymers were synthesized so as to produce films onto which endothelial cell proliferation could be evaluated and monitored.

We were also able to establish evidence for best copolymerization conditions; these are expressed by a rate of active grafting ranging from 95-97% under a relative ratio of 1/10 between initiator and monomer for a constant concentration of polysaccharide.

On the other hand, the Infrared (IR) analysis performed on the synthesized copolymer showed that copolymerization depends, heavily, on the nature of each polysaccharide, the monomer used and the cerium concentration.

To conclude and under the light of the results found, we may establish that our copolymerization follows a less complex reactional mechanism whose stages are governed by the following reaction equations:

Initiation:

$$F{-}H + Ce^{4+} \underset{}{\overset{K}{\rightleftharpoons}} (Complex) \xrightarrow{K_0} F^{\bullet} + Ce^{3+} + H^{+} \quad (1)$$

$$F^{\bullet} + M \xrightarrow{K_a} FM^{\bullet} \quad (2)$$

$$Ce^{4+} + K \xrightarrow{K_1} M^{\bullet} + Ce^{3+} + H^{+} \quad (3)$$

$$Ce^{4+} + F^{\bullet} \xrightarrow{K_2} F\text{-}OX + Ce^{3+} + H^{+} \quad (4)$$

$$Ce^{4+} + F\text{-}OX \xrightarrow{K_3} F^{\bullet} + Ce^{3+} + H^{+} \quad (5)$$

Propagation:

$$FM^{\bullet} + M_n \xrightarrow{K_p} FM_{n+1} \quad (6)$$

$$M^{\bullet} + Mn \xrightarrow{K_p} M_{n+1}^{\bullet} \quad (7)$$

Termination:

$$FM^{\bullet} + FM^{\bullet} \xrightarrow{K_t} FM_{n+m}F \quad (8)$$

$$FM^{\bullet} + M_n^{\bullet} \xrightarrow{K_d} FM_{n+1} \quad (9)$$

$$M_n^{\bullet} + M_m^{\bullet} \xrightarrow{K_a} M_{n+m} \quad (10)$$

We suppose that the complex formed between the Fucan and the Cerium does not participate in the propagation process, as shown in Eq. 9 and that the termination reaction by recombination of the active centers FM° is the most likely. To bear evidence of such polymerization mechanism, we should, thus, use the assumption that the states of the following initiating species are quasi-stationary, that is to say:

$$d[F^{\bullet}]/dt = Kk_0[F][Ce^{4+}] + k_3[Ce^{4+}][F\text{-}OX] - k_a[F^{\bullet}][M] - k_2[F^{\bullet}][Ce^{4+}] = 0 \quad (11)$$

$$d[FM^{\bullet}]/dt = k_a[F^{\bullet}][M] - k_t[FM^{\bullet}]^2 - k_{t1}[FM^{\bullet}][M^{\bullet}] = 0 \quad (12)$$

$$d[M^{\bullet}]/dt = k_1[Ce^{4+}][M] - k_{t1}[FM^{\bullet}][M^{\bullet}] - k_{t2}[M^{\bullet}]^2 = 0 \quad (13)$$

$$d[F\text{-}OX]/dt = k_2[F^{\bullet}][Ce^{4+}] - k_3[Ce^{4+}][F\text{-}OX] = 0 \quad (14)$$

By summing together Eq. 11, 12 and 14, while ignoring Eq. 13 which is less likely to occur (in fact, we did not observe any appreciable attack by the Cerium (Ce^{4+}) on the monomer) and by assuming that the rate coefficients are identical, (i.e., $k_t = k_{t1} = k_{t2}$), we easily obtain that:

$$Kk_0[F][Ce^{4+}] - k_t[FM^{\bullet}]^2 - 2k_t[FM^{\bullet}][M^{\bullet}] - k_t[M^{\bullet}]^2 = 0 \quad (15)$$

or, we can also write:

$$Kk_0[F][Ce^{4+}] = k_t([FM^{\bullet}] + [M^{\bullet}])^2$$

Hence, we deduce that:

$$[FM^{\bullet}] + [M^{\bullet}] = (Kk_0[F][Ce^{4+}]/k_t)^{1/2} \quad (16)$$

The copolymerization rate (R_p) of the Fucan with poly acrylic in presence of a ceric initiator is given by the following expression:

$$R_P = k_p[FM^{\bullet}][M] + k_p[M^{\bullet}][M] = k_p([FM^{\bullet}] + [M^{\bullet}])[M] \quad (17)$$

By substituting the expression of Eq. 16 into 17, we obtain therefore that:

$$R_P = k_p(Kk_0/k_t)^{1/2}[F)]^{1/2}[Ce^{4+}]^{1/2}[M] \quad (18)$$

From Eq. 18, we see that the rate of polymerization (R_P) of Fucan N1 with poly-methacrylate of methyl, in presence of a ceric salt (Cerium Ce^{4+}) used as initiator and in acidic environment, shows a first-order dependence on Monomer concentration (M) and is related to the square-root of both the initiator Concentration (Ce^{4+}) and Fucan concentration (F).

The quantity $k_p (kk_0/k_t)^{1/2}$, as derived from Fig. 7-10, is equal to: 5.23; 10.22 and 13.71 for soluble starch (Mansor, and Haron, 1999), Fucan N1 and Dextran T-70, respectively. This quantity varies increasingly at a rate of 2.5 nearly, while going from the soluble starch to the Fucan N1 and this is in proportion with the mass of each polysaccharide used, namely: $M_n = (164.4)_n$ g mol^{-1} for the soluble starch, 73000 g mol^{-1} for the Dextran T-70 and finally 95500 g mol^{-1} for the Fucan N1.

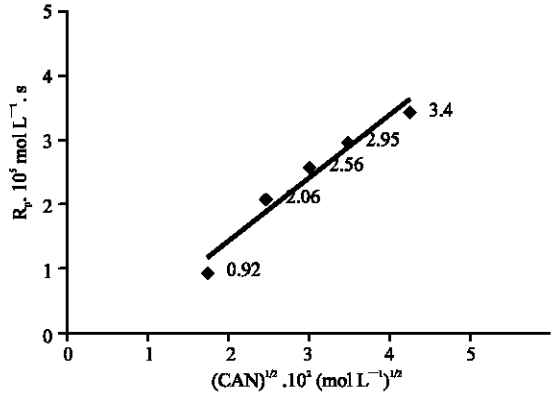

Fig. 7: Rate of Polymerization (RP) of Fucan N1 with MMA a function of square root of initiator concentration (CAN). (MMA) = 0.0742M; (Fu) = 0.0417 g; (HNO_3) = 0.2M Temperature = 40°C; Time = 40 min

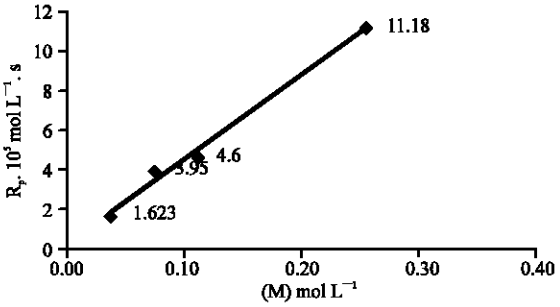

Fig. 8: Rate of Polymerization (RP) of fucan N1 with MMA as a function of monomer concentration. (M) (Fucan) = 0.0417 g; (CAN) = 0.503. 10^{-3}M; (HNO_3) = 0.2M; T = 40°C; Time = 40 min

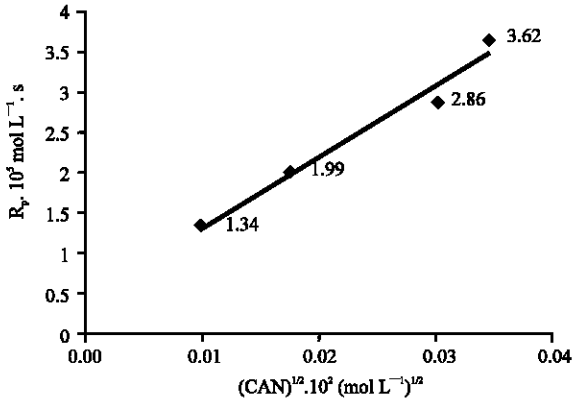

Fig. 9: Rate of Polymerization (RP) of Dextran with MMA as a function of square root of initiator Concentration. (CAN): (Dextran) = 0.0318 g; (MMA) = 0.0742 M; ([HNO_3) = 0.2M; T= 40°C; Time = 40 min

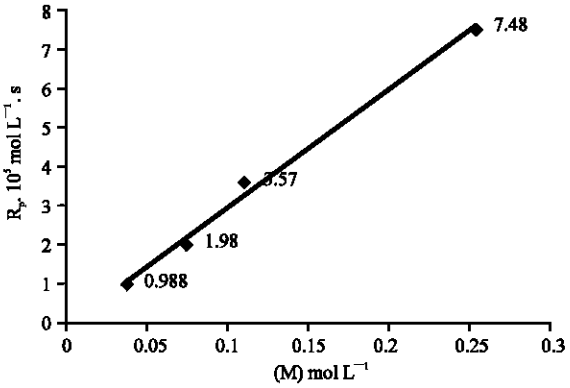

Fig. 10: Rate of Polymerization (RP) of dextran with MMA as a function of monomer concentration (M): (Dextran) = 0.0318 g; (CAN) = 0.503.10^{-3} M; (HNO3) = 0.2M; T = 40°C; Time = 40 min

CONCLUSION

The reaction mechanism, thus found, shows that the rate of polymerization (R_p), as established by the PH-measurement method, for Fucan N1 with a poly acrylic PMMA initiated by a ceric salt (Ce^{4+}) in acidic environment, is first-order proportional to the monomer concentration and depends on the square-root of both the initiator Concentration (CAN) and the polysaccharide concentration (F). Same remarks are observed for the polymerization of the polysaccharide Dextran T-70, under the same conditions as applied to.

ACKNOWLEDGEMENT

This research has been conducted in the Bio-engineering Polymer and Cardio-vascular laboratory (BPC) at University Paris Nord in Co-operation with University of Annaba in Algeria the Fucan N1 polymerization. Consequently, we arrive at a conclusion that the polymerization of all polysaccharides with poly acrylics initiated by Cerium salt (Ce^{4+}) in acidic medium shows a good accordance with the general kinetic model as described by the Eq. 18. In addition, many methods have been used to analyse polymerization reactions and it is to the best of present knowledge that the PH measurement method, is, herein, used for the first time in the study of polymerization rate in this context.

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