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Synthesis of some poly (N-2-vinyl pyrrolidone-co-metha crylamide)s as model carriers of anilines. Study of the release of anilines in aqueous heterogeneous medium of pH=1.2 at 37°C.

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Abstract: Four secondary amides have been prepared by the Schotten-Baumann reaction between model anilines (Pa_{1-4}: p-$XC_6H_4NH_2$: X_1: H; X_2:CH_3; X_3:$COCH_3$; X_4: CN) and methacryloyl chloride using aqueous THF/NaOH mixture at 0°C. MS_1, MS_2 and MS_4 liquid monomers are obtained whereas MS_3 is a solid monomer. Mass radical copolymerization of the different monomers (MS_{1-4}) with N-vinyl-2-pyrrolidone yields to the corresponding four copolymers. All the monomers have been characterized by IR, ^1H and ^{13}C NMR. The (CP_{1-4}) have been characterized by IR spectra, microanalysis, Tg ° and M_v. The kinetics of aniline delivery to give anilinium cations $(PaH^+)_{1-4}$ from solid MS_3 and CP_{1-4} dispersed in water (pH= 1.2, 37°C) showed that aniline delivery from the different supports is controlled by a diffusion process and not the rate of amide hydrolysis. The amount (%) of free anilinium cations is inversely proportional to the molecular weight of polymeric supports. Accordingly, the monomer MS_3 gave the largest amount of free anilinium cations $(PaH^+)_{1-4}$.

Keywords: monomer support, copolymer, hydrolysis, diffusion, delayed effect.

Introduction

In pharmaceutical industry, drug ligation onto polymeric supports has been largely studied in recent years. Such polymeric supports have been used to modulate the pharmacokinetic of the drug and thus, to control drug delivery for a well-defined time limit[1-5]. The main objective is to reduce systemic clearance and to extend circulation half-time in vivo, after oral absorption or intravenous injection. To fulfill this purpose, several methodologies have been reported. Thus, it is possible:

a) To introduce the drug in porous membrane allowing osmotic diffusion[6].
b) To spread the drug in polymer or copolymer and then prepare microspheres, sheets or capsules[7].
c) To bind the drug onto macromolecules through weak bonds[8].

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d) And finally, to prepare a monomer with a ligated drug followed by a polymerization or a copolymerization reaction.

In the last two possibilities, the release of the drug after oral absorption, involves hydrolysis of the organic group connecting the drug to the support. This hydrolysis may be associated with diffusion of the gastric liquid through the polymeric support. Whatever method it is chosen to ligate the drug, the proposed solutions are mainly related to the way to introduce the drug, injection or oral absorption. Actually, it is necessary that the drug is free from the support after few hours, the time corresponding to the stay of the drug-support system in the body. In the particular case of a drug grafted onto a polymer, the organic group must be easily hydrolyzed and the kinetic must be independent of the initial concentration. Moreover, it is very important to have non-toxic macromolecular systems.

Remarkably, Kopecek et al.[9-11] prepared N-(2-hydroxypropyl) methacrylamide comonomer as a macromolecule support using chymotrypsin for enzymatic hydrolysis[12]. Amines such as 1-phenyl-2-aminopropane (amphetamine)[13] and anti-vitamine K[14] have been ligated to methacrylic supports. Procaine[15], atropine[16] and aspirine have been grafted onto low-molecular weight polyethylene-glycols since these systems are generally not toxic[17-19]. Several toxicity studies on gnawing animals were undertaken since the beginning of the use of polymers in current life[20-22]. One of undesired side-effect of polymers is their ability to accumulate in tissues and organs. This accumulation increases as soon as the molecular weight of the polymer increases[23]. New systems, such as nanocapsules and nanospheres, used mainly encapsulation for drug release control[24-28]. In these systems, the active drug is localized inside the nanosphere which is surrounded by the polymer.

Polymeric biodegradable core-shell nanoparticles have been prepared by Chan et al.[27]. In this system, the authors encapsulated Doxyl/Caely and Genexol-Pm for clinically approved therapeutics. Biodegradable polyurethanes have also been prepared for the controlled release of dexamethasone acetate for a therapeutic effect on uveite[28].

Preparations of acrylic and methacrylic polymers ligated to a drug through an amide group have been reported with aniline, 2-aminothiazole, sulfanilamide and phenylethylamine. The effect of the molecular weight to the kinetic of the drug release has been reported[29-31].

We want to report herein, first the preparation and characterization of the monomers containing model anilines ligated by amide groups and then, their copolymerization with N-2-vinyl pyrrolidone.

Results and discussion

Acid hydrolysis (pH= 1.2, 37°C) of solid-dispersed (MS_3) and (CP_{1-4}) was examined versus time. Amide hydrolysis (Scheme 2) yields to the formation of methacrylic acid for (MS_3) and copolymers (CP_{1-4}) bearing carboxylic acids for(CP_{1-4}) and free anilinium cations $(PaH^+)_{1-4}$ in aqueous solution. Figure 1 shows the kinetic results obtained by hydrolysis of monomer (MS_3) and copolymers (CP_{1-4}). Because the monomer (MS_3) and polymers (CP_{1-4}) are not soluble in water, all the kinetic experiments are heterogeneous.

Thus, it could be underlined that the order of the reaction is different from 0, 1, 2 or n and aniline delivery needs three main steps:

- liquid diffusion through solid architectures of macromolecules (CP_{1-4}) and solid particles (MS_3) in suspension at pH 1.2.
- hydrolysis of amide near the surface and inside the solid particles.

- diffusion of the drug (Pa_{1-4}) outside (MS_3) and (CP_{1-4}) after hydrolysis.

Different aniline delivery systems cannot be described by a classical kinetic equation. This process is related to a phenomenon of mass transfer controlled by diffusion according to the curves shown in Figure 1 (amount of liberated aniline versus time). Thus a vertical tangent is observed at the beginning of the process. A linear effect is observed (at short time) if we plot free aniline versus the time square root according also with Fick equations[33] and diffusion process (Fig 2). Besides a diffusion process, it is well-known that hydrolysis of macromolecular functional groups varied with other parameters such as the molecular weight of the macromolecule, the nature and the length of the chains and the steric effect of the different functional groups.

As shown in Figure 1, monomer (MS_3) gave 70% of free p-amino-acetophenone from (Pa_3) whereas copolymers (CP_{1-4}) yield 33-47% of free anilines (Table 1).

Table1: Percentages of anilinium cations $(PaH^+)_{1-4}$ released after 200 min by copolymers (CP_{1-4}).

Pa_{1-4}	Pa_1: X_1=H	Pa_2 : X_2=CH_3	Pa_3: X_3=$COCH_3$	Pa_4: X_4=CN
M_v	52260	13980	21160	9500
Released(%PaH^+)$_{1-4}$ at 200min	33	38	38	47

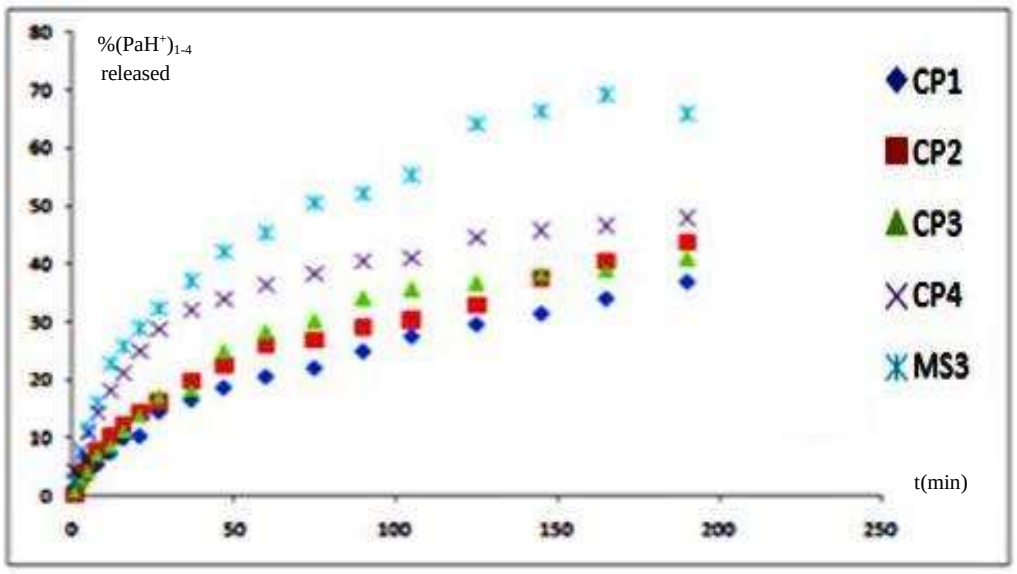


Figure 1: Percentages of anilinium cations $(PaH^+)_{1-4}$ released versus time (min) at pH = 1.2, T = 37 °C .

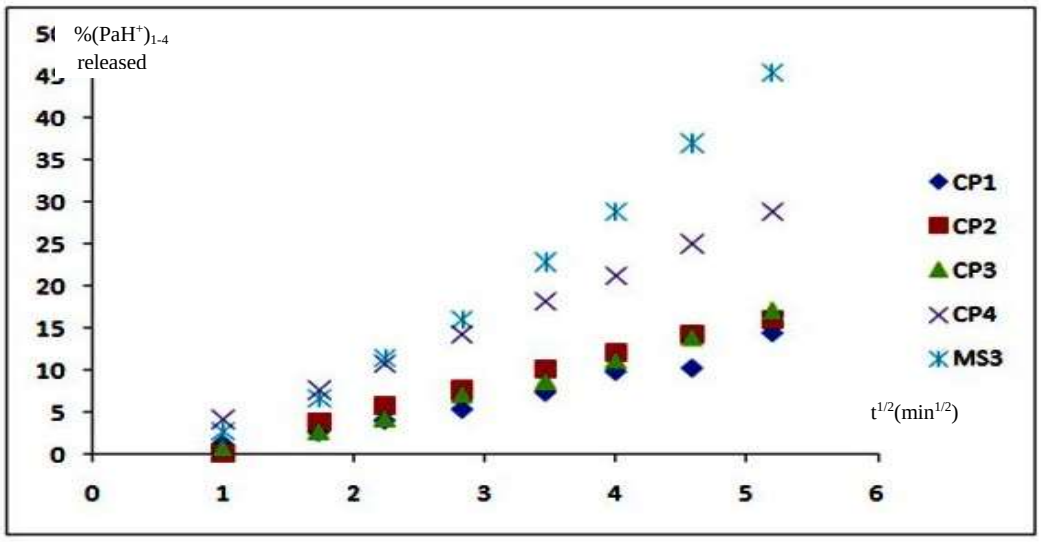


Figure 2: Percentages of anilinium cations $(PaH^+)_{1-4}$ released according to the square root of time at pH = 1.2 and T = 37 °C.

As expected, the drug delivery system is much better with (MS_3) than the polymeric systems (CP_{1-4}). Delivery of (Pa_3) is easier with (MS_3) because amide groups are easily accessible. In contrast, a more difficult access is observed with the copolymers. The amount (%) of free anilines delivered from solid copolymers (CP_{1-4}) varies conversely with the molecular weight determined by viscosity (M_v) according to less accessible amide groups.

It is also worth noting that the straight line which is observed for(CP_{1-4}) (aniline concentration versus time square root) confirms the diffusion control of anilinium cations first located inside the solid particle (Fick equation). For (MS_3), the straight line is detected at even shorter times.

Conclusion

In a first part, four secondary amides have been prepared by the Schotten-Baumann reaction between anilines models: (Pa_{1-4}: p-$XC_6H_4NH_2$: X_1: H; X_2:CH_3; X_3:$COCH_3$; X_4: CN) and methacryloyl chloride. Monomers MS_{1-4} characterized by IR and NMR ^1H, ^{13}C, were used to prepare four copolymers CP_{1-4} using radical way in the presence of AIBN and of the common co-monomer N-2-vinylpyrrolidone. The latter were characterized by IF spectra, microanalyses, DSC for Tg°C and finally viscosimetry for the average molecular masses M_V.

The release of the anilinium cations PaH^+ was studied by hydrolysis of the function amide of the solids MS_3 and CP_{1-4} at pH=1.2 and 37°C, directly dispersed in powder form, in the reconstituted gastric acid medium . The comparable shape of the curves giving the percentages of anilines released brought back the initial masses of p-$X_6H_4NH_2$ contained in the support considered MS_3 or CP_{1-4} indicates that the process which regulates the release is the same whatever the dispersed solid. The various curves approximately reach limiting values of balances beyond 150min. The order obtained being inversed of Mv confirming the influence of random coil structure of chains of polymers, which is minimal in the case of MS_3 that gives the highest limit values (70%) for PaH^+: p-aminoacetophenone released. The linearity of the same percentages of the released amines $(PaH^+)_{1-4}$ with the square root of time confirms the limiting diffusionnal character at short times inside the solid copolymers particles dispersed at pH=1.2

This work will be contained by studying the release of different aniline carried by polymers having the same weights and of polymers with different molar masses carrying the same structure aniline.

Acknowledgements

We wish to thank Professor Carpentier (UMR CNRS 6226), University of Rennes-1 for DSC graphs recorded in his laboratory.

Experimental Section

• The viscosimetric molecular masses M_V of soluble copolymers in chloroform were measured using an Ubbelhode viscosimeter plunged into a thermostat adjusted to 25°C.

• The glass transition temperatures Tg °C were recorded under argon using DSC Setaram (University of Rennes-1, France).The mass of the sample, between 5 and 10mg, undergoes a cycle of heating in 10°C/min and cooling in 20°C/min between 30 and 350°C. The Tg °C was confirmed on the two curves as showed in the Fig 3.

• The microanalyses were realized in the Center of Microanalyses of the University of Rennes-1 (France).

The rates α and β are calculated from the percentage of the elements found in microanalyses; for example a calculation from %N engaged in the repeat unit of MS_2 and VP in copolymer CP_2 gives α =0.11 and β =0.89. According to this formula:

$$N\% = \frac{\alpha_{exp} N + \beta_{exp} N}{MS2\,\alpha_{exp} + VP\,\beta_{exp}} = \frac{(\alpha_{exp} + \beta_{exp})\,14}{175\,\alpha_{exp} + 111\,\beta_{exp}} = 0.1186 \quad (1).$$

$$\alpha_{exp} + \beta_{exp} = 1$$

We find very close values by using %H (α =0.18, β =0.88) and %C (α =0.12, β= 0.88).

• The IR spectra of the monomers and copolymers were recorded on Schimadzu Ftir-8300 spectrometer of the Center of Physic-Chemical Measurements (CMFS) of the Faculty of Science of the University "Djilali LIABES" of Sidi-Bel- Abbes.

• The UV spectra of anilines were recorded on Schimadzu UV-2401PC spectrometer of (CMFS).

• The ^{1}H and ^{13}C NMR were recorded in the DMSO and TMS as internal reference, on the 500 MHz Brücker “AVANCE” of the University of Rennes-1 (France).

1. Synthesis and characterization of the monomers supports (MS_{1-4})

1.1. Synthesis of the monomers supports (MS_{1-4})

The monomer supports (MS_{1-4}) were prepared using the biphasic reaction of Schotten-Baumann[31-32]. To a solution of 0.035 mol of amine p-$XC_6H_4NH_2$ (Pa1: X_1: H, Pa2: X_2:CH_3, Pa3: X_3:$COCH_3$, Pa4: X_4: CN) in 25 mL of THF, we drop an organic solution of methacryloyl chloride/THF (0,035mol/ 25mL), then 25 mL of aqueous solution of NaOH (0.035 mol) were poured on the reaction mixture cooled to 0°C and shaken for 2 hours.

The organic layer is concentrated on rotavapor and the solid amide (MS_3) was isolated by precipitation with heptane. The liquid monomers MS_1, MS_2 and MS_4 were purified under vacuum distillation.

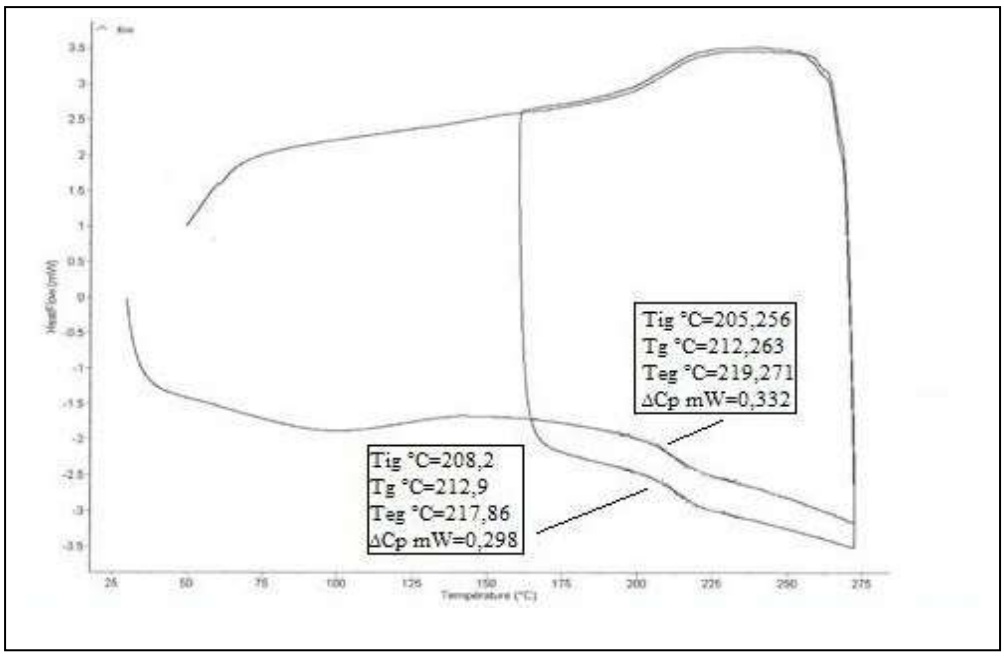


Fig 3: DSC spectra of $CP_3(X_3{:}COCH_3)$.

1.2. Characterization of (MS $_{1-4}$)

MS$_1$: IR: 2927 (=CH), 935, 756 (vibrations "out of plane" CH_2=C), 3315 (NH amide), 1664.6 (C=O carbonyl amide), 1624 (vinyl bond) 1599 (aromatic double bond) cm^{-1}. ^{1}H NMR (500MHz, DMSO/TMS) δ (ppm): 1.64 (C-CH_3), 5.53-5.83 (CH_2 = C), 7.1 (H aromatic, H_p), 7.3 (H aromatic, H_o), 7.75 (H aromatic, H_m), 9.8 (NH). ^{13}C NMR (500MHz, DMSO/TMS) δ (ppm): 120 (C of Phenyl, C_o), 124 (C of Phenyl, C_m), 121 (C of Phenyl, C_p), 129 (C linked to the amide function), 18 (H_2 C=C-$\underline{CH_3}$), 138 ($\underline{CH_2}$=C), 141 (CH_2 = $\underline{C}$), 167 (C=O amide).

MS$_2$: IR: 2864 (=CH); 933.3, 756 (vibrations "out of plane" CH_2 = C), 3354 (NH amide), 1678 (C=O Carbonyl amide), 1623 (vinyl bond), 1596 (aromatic double bond) cm^{-1}. ^{1}H NMR (500MHz, DMSO/TMS) δ (ppm): 1.67 (C-CH_3), 2.28 (Ø-CH_3), 5.5-5.82 (CH_2 = C), 7.1 (H aromatic, H_o), 7.64 (H aromatic, H_m), 9.8 (NH). ^{13}C NMR (500MHz, DMSO/TMS) δ (ppm): 119 (C of Phenyl, C_o), 121 (C of Phenyl, C_m), 129 (C of Phenyl, C_p), 132 (C linked to the amide function), 18 (H_2C=C-$\underline{CH_3}$), 22 (Ø-$\underline{CH_3}$), 136 ($\underline{CH_2}$= C), 140 (CH_2 = $\underline{C}$), 167 (C=O amide).

MS$_3$: mp110 °C. IR: 2933 (=CH), 995.2, 750.8 (vibrations "out of plane" CH_2 = C), 3357 (NH amide), 1700 (C=O acetone), 1674 (C=O Carbonyl amide), 1631 (vinyl bond) 1593 (aromatic double bond) cm^{-1}. ^{1}H NMR (500MHz, DMSO/TMS) δ (ppm): 1.64 (C-CH_3), 1.9 (CO-CH_3), 5.61-5.88 (CH_2= C), 7.83 (H aromatic, H_o) 7.89 (H aromatic, H_m), 10.1 (NH). ^{13}C NMR (500MHz, DMSO/TMS) δ (ppm): 119 (C of Phenyl, C_o), 121 (C of Phenyl, C_m), 129 (C of Phenyl, C_p), 132 (C linked to the amide function), 18 (H_2C=C-$\underline{CH_3}$), 26.5 (CO$\underline{CH_3}$), 140 ($\underline{CH_2}$ = C), 143 (CH_2 = $\underline{C}$), 197 (C=O acetone), 167 (C=O amide).

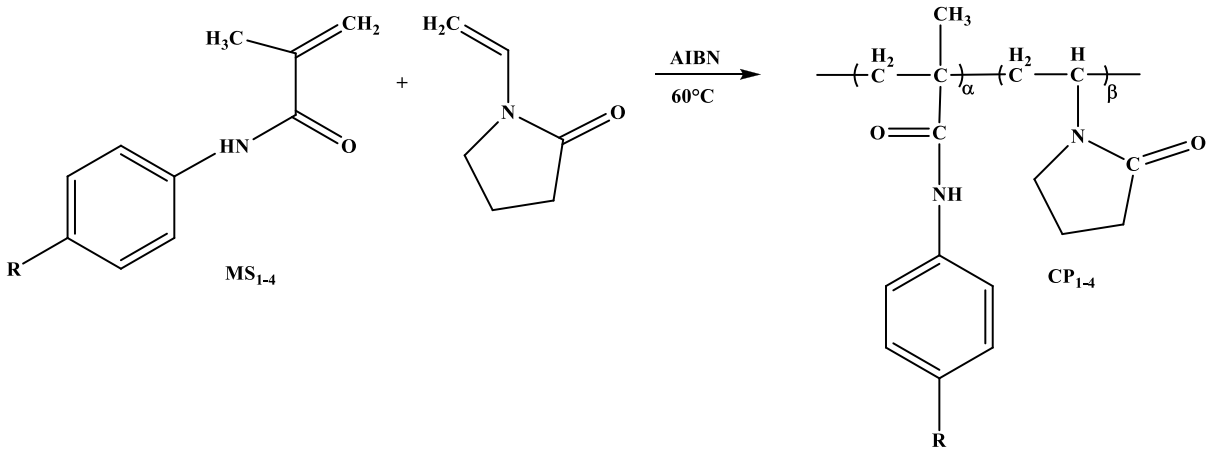
MS$_4$: IR: 2923.9 (=CH), 933.5, 746 (vibrations "out of plane" CH_2 = C), 3446 (NH amide), 1652 (C=O Carbonyl amide), 1622 (vinyl bond), 1604 (aromatic double bond), 2221.8 (C≡N) cm^{-1}. ^{1}H NMR (500MHz, DMSO/TMS) δ (ppm): 1.65 (C-CH_3), 5.5-5.91 (CH_2=C), 7.5 (H

aromatic, H_o), 7.93 (H aromatic, H_o), 10.3 (NH). ^{13}C NMR (500MHz, DMSO/TMS) δ (ppm): 121 (C of Phenyl, C_o), 129 (C of Phenyl, C_m), 120 (C of Phenyl, C_p), 132 (C linked to the amide function), 20 (H_2C=C- $\underline{CH_3}$), 117 (CN: 117), 138 ($\underline{CH_2}$= C), 141 (CH_2 = $\underline{C}$), 168 (C=O amide).

2. Synthesis and characterization of the copolymer supports (CP_{1-4}):

2.1. Synthesis of the copolymers (CP_{1-4}):

Each monomer (MS) was then copolymerized by radical way with the co-monomer N-vinyl 2-pyrrolidone (VP) used as reactant and solvent with an initial mass ratio $[(MS)/(VP)]_0$ = 1/2.4 in the presence of 0.5% in mass of AIBN as initiator (Schema 1).



Scheme 1: Synthesis of (CP_{1-4}).

After 10 min degassing with nitrogen of the solution, the tube of polymerization with a cork and folding skirt is closed and then plunged in an oil bath at 60°C. The polymerization reaction is stopped just before the total grip, occurring between 2hr and 6hr.

The copolymer (CP) obtained was solubilized in the chloroform and precipitated with cold heptane poured on the concentrated solution. The polymer, after washing with heptane, was dried in the oven (40°C) for 1 hour; we obtained 73% to 82% of yields.

2.2. Characterization of the copolymers (CP_{1-4}):

CP_1: IR (KBr): 3446.6 (NH), 1654.8 (C=O), 1592 (aromatic double bond) cm^{-1}. Tg (DSC)**:** 215 °C. Viscosimetric Molecular Masses M_v**:** 52260. Microanalyses (% C, H, N): and α, β were calculated from the percentage of N: C, 66.73; H, 07.88; N, 11.91, α = 0.13 and β= 0.87.

CP_2: IR (KBr): 3365.6 (NH), 1652.9 (C=O), 1607.3 (aromatic double bond) cm^{-1}. Tg (DSC)**:** 150 °C. Viscosimetric Molecular Masses M_v**:** 13980. Microanalyses (% C, H, N): and α, β were calculated from the percentage of N: C, 66.72; H, 07.79; N, 11.86, α= 0.11 and β = 0.89.

CP_3: IR (KBr): 3415.7 (NH), 1654.9 (C=O), 1605 (aromatic double bond) cm^{-1}. Tg (DSC)**:** 212 °C. Viscosimetric Molecular Masses M_v**:** 21160. Microanalyses (% C, H, N): and α, β were calculated from the percentage of N: C, 66.83; H, 07.92; N, 10.98, α = 0.18 and β= 0.82.

CP_4: IR (KBr): 3446 (NH), 2222.6 (C≡N), 1688.4 (C=O), 1588 (aromatic double bond) cm^{-1}. Tg (DSC)**:** 205 °C. Viscosimetric Molecular Masses M_v**:** 9500. Microanalyses (% C, H, N): and α, β were calculated from the percentage of N: C, 65.92; H, 07.67; N, 12.99, α = 0.1 and β = 0.9.

2.3. Kinetics of drug release $(PaH^+)_{1-4}$:

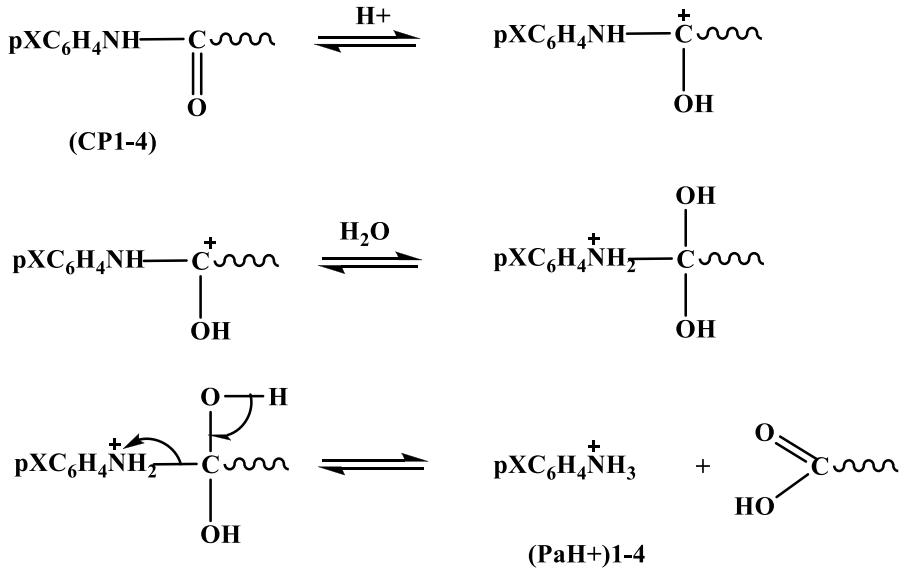
We examined the behavior of the solid monomer support (MS_3) and copolymers (CP_{1-4}) in acid aqueous medium at pH=1.2. The link graft between polymer and model anilines is of amide type. The acid hydrolysis led to the rupture of the amide bond with formation of protonated "R-NH3 $^+$ "groups and a copolymer support bearing acid groups. It is interesting to also consider the possible opening of the cycle of VP engaged in copolymers CP_{1-4} with the acid pH used. Ruptur of the cyclic bond amide led to the aminoacid function without releasing the material.

The linearity of the same percentages with the square root of the time for the four CP_{1-4} confirms that the release of $(PaH^+)_{1-4}$ is controlled by diffusion inside the solid copolymer particles dispersed in the solution of the study according to the simplified Fick's equation for the short times. The linearity of MS seems operating at times much shorter than those observed for copolymers.

2.4. Procedure

The kinetics of aniline release from supports were followed by using an UV-Vis spectrometer 2401PC SCHIMADZU.

A sample of the support, (200 mg) was shaken (600rpm separately) in 100mL of a prepared solution of pH=1.2 by adding 80mL of HCl 1N and 2g of NaCl in 920mL of distilled water. A constant temperature is insured in the media (37°C). The dosage of released anilines under the form of ammonium salts versus time is made on taking out of 1mL of acid solution containing the support (reading of the optical density). The UV apparatus is beforehand calibrated at the wavelength λ_{max} of protonated anilines $(PaH^+)_{1-4}$ at pH=1.2.



Scheme 2: Mechanism of hydrolysis of the amide (MS_3) and (CP_{1-4})

The UV spectra were released in the same operating conditions as those of the hydrolysis. The method "non-sink"[33] was used, and the percentage of release versus time was calculated with regard to the initial mass of model anilines (Pa_{1-4}) grafted in the 200 mg of the studied sample.

Table 2: UV Characterization and pK of released anilinium cations $(Pa\ H^+)_{1-4}$at pH=1.2.

Anilines : X	Pa1: H	Pa2 : CH_3	Pa3: $COCH_3$	Pa4: CN
λ_{max}(nm)	253	259.5	285	270

ε(L.cm^{-1}. mol^{-1})	840.54	1100	1592.4	1864.5
pK$^{25°C}$	4.62	5.10	2.19	1.74
[RNH_3^+]/ [RNH_2]	2630	7963	9.8	3.5

The pK (25°C) of anilinium cations[33] and the acid/base ratio (Pa H^+)$_{1-4}$ of the anilines were showed in Table 2. The wavelength λ_{max} corresponds to the charged particles RNH_3^+ of model anilines.
The released mass at the time t *(m_t)* was given by the relation:

$$m_t = DO \ .V_F. V_d. MM_{(PaH^+)1-4} \ / V_p . \varepsilon \quad (2)$$

DO: Optical density of every taking.
ε: Molecular coefficient of anilinium cations $(PaH^+)_{1-4}$ released previously.
V_F: Volume of the flask.
V_d: dilution volume
V_p: sample volume

$MM_{(PaH^+)1-4}$:Molar Mass of $(PaH^+)_{1-4}$ released.
The value of ε was calculated from 5 standard solutions of the pure active principle with known concentrations under the same conditions (pH, 600 rpm, 37°C).

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