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3-aminopyridine release study from polymeric supports in homogeneous and heterogeneous media

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Abstract: In order to control and modify the drug release, in one hand 3-aminopyridine as active agent was grafted via chemical azomethine bond on monomer then copolymerized with N-vinylpyrrolidone. The obtained supports were characterized and the drug release from these formulations was followed using UV-Vis spectrophotometer in homogeneous media at four different pH (pH=1.2, 4.0, 6.0 and 8.0) simulating the human digestive rout. The results showed that the hydrolysis of monomer and copolymer obeyed to the first order and the apparent kinetic constants were calculated. In the other hand, spherical dosage forms composed from Eudragit RL100 and the active agent or the copolymer support were prepared and tested to control the drug release. The heterogeneous kinetics were established successively in the four cited media. The drug release seemed that be governed by diffusion model according to the Fick's laws. So, the diffusivities were calculated and the results demonstrated that the drug release can be modified using these formulations.

Keywords: 3-aminopyridine, diffusion, Fick's law, galenic form.

Introduction

The gradual transfer of active molecules is well-known in the pharmaceutical field. It permits to control a drug release over a defined period of time and represents a significant pathway for optimizing drug effects through dosage forms. Several techniques have been developed in this way and different formulations based on polymers were conceived [1-4]. One of the most formulation technologies consists of a chemical drug grafting onto polymers like for example sulfanilamide, 5-aminosalicylic and benzoic acids which were grafted on acrylic or styrenic supports [5-8]. In this domain, the methacrylic supports were widely used for amine drugs such as amphetamine [9] and anti-vitamin K [10]. Also, copolymer of acrylamide and acrylic acid was employed as carrier of theophylline by Katime and al.[11]. In these cases, the drug release depends on the hydrolysis of the function group of linkage. Other researches developed by kopecek and al. studied the enzymatic hydrolysis of the pro-drug N-(2-hydroxy-propyl)-methacrylamide using chymotripsyne as catalyst [12-15]. Moreover, the monolithic dosage forms were largely used such as co-precipitates [16], cylindrical [17] or spherical [18-20] dosage forms, disks or tablets [18, 21-22] and micropsheres [23-25].

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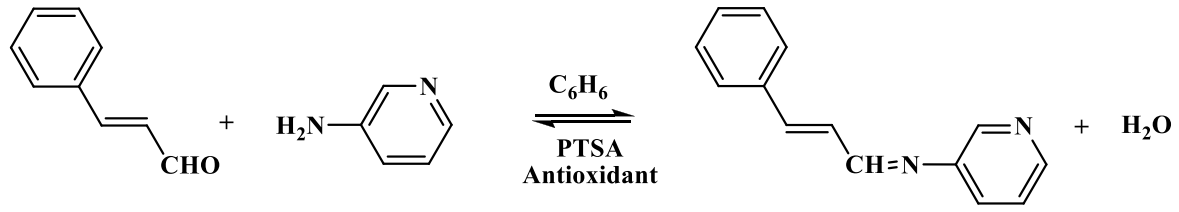
In this paper, different formulations based on 3-aminopyridine as active agent were developed. This intermediate active molecule constitutes the principal part in triapine (3-aminopyridin-2-carboxaldehyd thiosemicarbazone) which has a broad anti-cancer activity [26-27]. In our first part, 3-aminopyridine was grafted via azomethine bond on cinnamaldehyde as monomer support. Then, the last was copolymerized with N-2-vinylpyrrolidone. The drug release was studied in homogeneous (hydro-alcoholic) media at different pH (1.2, 4.0, 6.0 and 8.0) and the apparent kinetic constants were calculated.

In the second part, spherical dosage forms composed from the active agent or a synthesized support i.e. copolymer and eudragit RL as additional matrix were elaborated in order to modify the drug release. Eudragit matrixes were largely used in galenic industry [28] to prepare controlled release formulations [29-30]. Also, the drug release was followed in different media at various pH (1.2, 4.0, 6.0 and 8.0). In this case, the results demonstrated that the drug release is governed by diffusion. So, the diffusion coefficients were calculated from the approached analytical solutions of Fick's law [31].

Results and Discussion

Characterization of monomer and copolymer

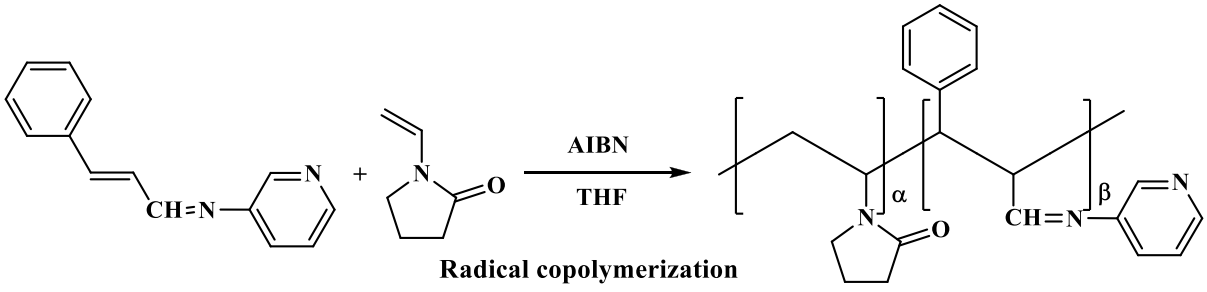
The scheme 1 gives the synthesis reaction of the monomer support of 3-aminopyridine.



Scheme 1

In the FTIR Spectrum of the monomer, the principal IR bands are: 750 cm^{-1} for C-H (aromatic), 990 cm^{-1} for C-H (vinyle), 1423 cm^{-1} for aromatic C-C stretching, 1604 cm^{-1} and 1672 for C-N and C-C stretching frequencies in pyridine, 1627 cm^{-1} for C=N (imine), 3000-3010 cm^{-1} C-H stretch (aromatic and pyridine).

The obtained monomer is then copolymerized with N-2-vinylpyrrolidone as shown in scheme 2.



Scheme 2

The FTIR spectrum of copolymer shows the absence of C-H vinylic band at 990cm^{-1} and gives the following characteristic bands: 1662 cm^{-1} for C=O; 1461 cm^{-1} for $-CH_2-$; 1170 cm^{-1}

for C-C (alkane); 2925 cm^{-1} for C-H (alkane).

The incorporation ratios α and β are 88.49% and 11.51% respectively, they are calculated from the microanalysis results of %N: 10.80 and %H: 7.65.

The average viscosimetric molecular mass of copolymer is Mv=12700, the glass transition temperature is Tg=135°C and the rate of copolymerization is 91%.

Drug release study

Homogeneous kinetics of a monomer and a copolymer hydrolysis

Examples of complete UV spectra of slow kinetics established at pH= 6.0 for monomer are given in figure 1. While, a rapid hydrolysis of the monomer at pH = 4.0 is represented in figure 2.

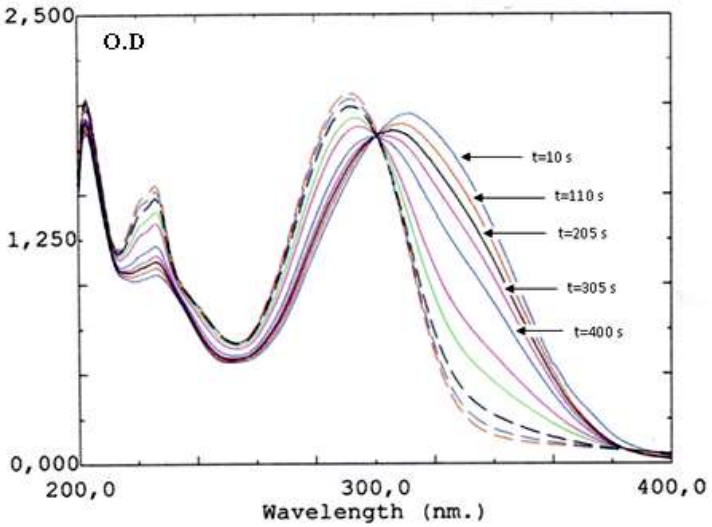


Figure 1. UV Kinetic curves of monomer hydrolysis in pH = 6.0

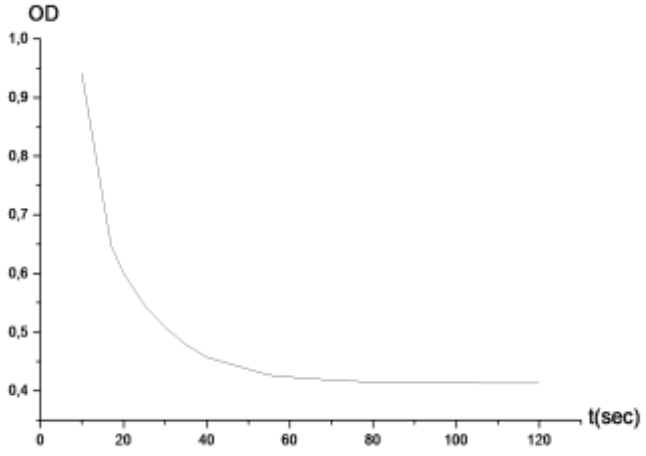


Figure 2. UV rapid Kinetic curve of monomer hydrolysis in pH =4.0 (analytical λ_a = 335nm).

All kinetics obeyed to the first order, so the apparent kinetic constants k_{exp} (s^{-1}) were calculated by the linear plot of $\log(O.D_t)=f(t)$ at the corresponding analytical wave length. An example of plot is given in figure 3 and the obtained K_{exp} are resumed in table 1.

The theoretical kinetic equation of the classical first order is given by:

$$\ln(O.D_t)=\ln(O.D°)-K_{exp}.t$$

where:

$O.D_t$ is the correct optical density at time t which is calculated from:

$O.D_t=O.D_{read} - O.D_\infty$.

$O.D_{read}$: optical density read from the UV spectrum at a time t.

$O.D_\infty$: optical density obtained at infinite time.

O.D° is the initial optical density (t=0).

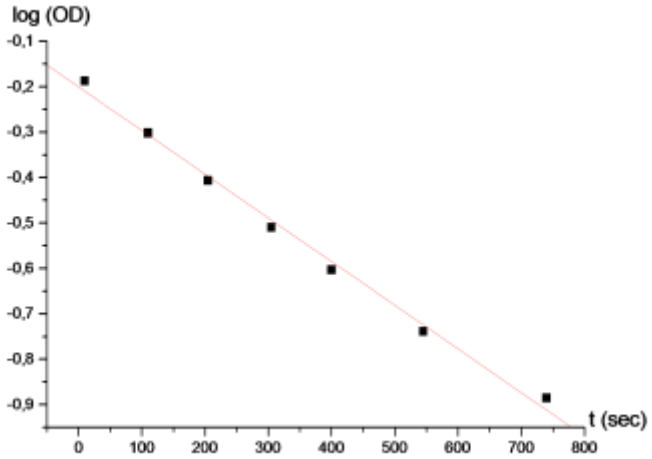


Figure 3. A plot of log (O.D) as a function of time for monomer hydrolysis at pH = 6.0 (analytical λ_a = 335nm).

Table 1: Homogeneous kinetic results of 3-aminopyridin from the monomer and the copolymer supports.

	Monomer			Copolymer		
pH	log(O.D)=f(t)	R^2	$K_{exp}(s^{-1})$	log(O.D)=f(t)	R^2	$K_{exp}(s^{-1})$
1,2	-	-	-	$-2{,}92.10^{-4}$ t-1,23	0,993	$6.71* 10^{-4}$
4,0	-0,026 t -0,142	0,993	$6.13 * 10^{-2}$	$-7{,}50.10^{-5}$ t-1,22	0,994	$1.77* 10^{-4}$
6,0	$-8{,}74.10^{-4}$ t -0,22	0,997	$2.01* 10^{-3}$	$-1{,}26.10^{-6}$ t-0,91	0,993	$0{,}28* 10^{-5}$
8,0	$-1{,}35.10^{-5}$ t+0,13	0,999	$3{,}11* 10^{-5}$	$-3{,}10.10^{-7}$ t-0,59	0,997	$0{,}07* 10^{-5}$

R^2: coefficient of correlation

The kinetic results demonstrate that the hydrolysis rate of the copolymer is inferior to that the corresponding monomer in all pH. In fact, the hydrolysis of copolymer includes a preliminary stage which is the penetration of ionic solution (H_3O^+ or OH^-) through the tangled structure of macromolecule.

Both for monomer and copolymer, the hydrolysis rate increases with the medium acidity, this note indicates that the mechanism is catalyzed by the oxonium ion and the protonated imine is hydrolyzed via a nucleophilic addition of water[32,33].

3-aminopyridin (AP) release from dosage forms

The drug release was studied from spherical dosage forms at a composition of 80/20 (w/w): Eud/AP or Eud/C consecutively in four media at different pH: 1.2, 4.0, 6.0 and 8.0. The release profiles are illustrated in figure 4.

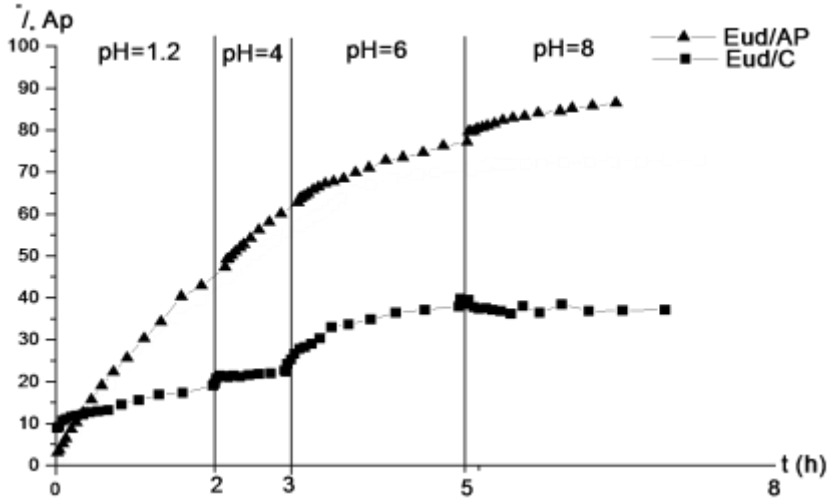


Figure 4. Release profiles of 3-aminopyridine from dosage forms

The results show that the drug release from dosage form Eud/C is slower than that Eud/AP dosage form. In fact, after two hours (the time corresponding to the drug stay in human stomach), the percentages of drug released from Eud/AP and Eud/C dosage forms are respectively 45% and 20%. At the end of 5 hours, 80% of drug released from Eud/AP dosage form is reached.

We have noted that the fractional release of 3-aminopyridine is proportional to the square root of time during the short time as shown in Figure 5.

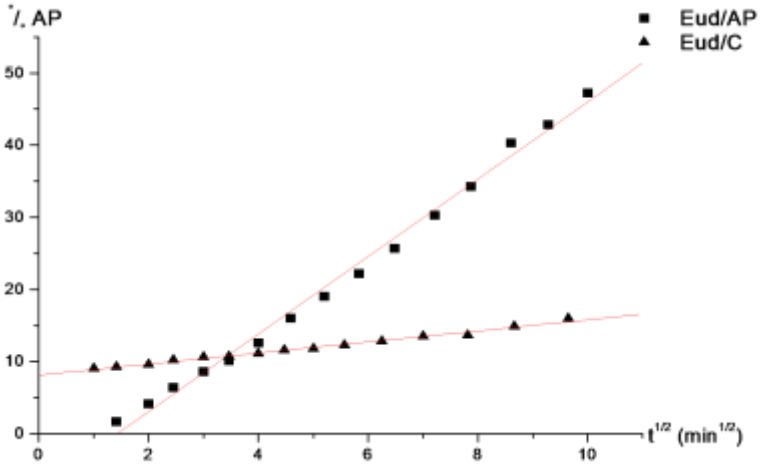


Figure 5. %3-aminopyridine released from dosage forms as a function of a square root of time at pH=1.2.

So, in order to draw a release model, we have tested the approached analytical solution derived from Fick's law [31] for diffusion in a sphere. The following assumptions are thus made in order to simplify the problem:

1) The spherical dosage forms are homogeneous (the medicinal agent or copolymer being well dispersed into the Eudragit matrix).
2) Two matter transfers take place: the liquid entering the dosage form, and the drug leaving the galenic form. They are studied successively but not simultaneously.
3) Both these transfers are controlled by transient diffusion throughout the galenic form.

If we suppose that drug diffuse through dosage form with radius R and constant diffusivity and taking into account the following initial and boundary conditions:
Within the sample:

t = 0 $0 \leq r < R$ $C = C_0$ C_0 = initial concentration
t>0 $0 \leq r < R$ C=f(t, r)

On the surface:

t > 0 r = R $C = C_\infty$ C_∞= Concentration at equilibrium

In the earlier stages of the process [31], the following analytical solution is given by:

$$\frac{C-C_0}{C_\infty-C_0}=\frac{R}{r}\sum_{n=1}^{\infty}\left\{erfc\frac{(2n+1)R-r}{2\sqrt{Dt}}-erfc\frac{(2n+1)R+r}{2\sqrt{Dt}}\right\} \quad (1)$$

This equation is written as a function of the amount of diffusing substance at time t (M_t):

$$\frac{M_t}{M_\infty}=6\left(\frac{Dt}{R^2}\right)^{1/2}.\left\{\pi^{-1/2}+2\sum_{n=1}^{\infty}ierfc\frac{nR}{\sqrt{Dt}}\right\}-\frac{3Dt}{R^2} \quad (2)$$

where M_∞ is the amount of diffusing substance at infinite time (equilibrium) and n is an integer.
For the very short times, the equation (2) is more simplified and the diffusivity can be calculated by applying the following equation:

$$\frac{M_t}{M_\infty}=6\sqrt{\frac{Dt}{\pi R^2}}=k.t^{0,5} \quad (3)$$

The table 2 gives the release constant (k) i.e. the slope value of the last equation, and the results of the diffusion coefficient $D_{s,t}$ in short periods. The diffusivities of 3-aminopyridine are in the range of $10^{-4}cm^2sec^{-1}$ in Eud/AP dosage forms and in the range of $10^{-7}cm^2sec^{-1}$ in Eud/C dosage forms, so the diffusion in the last dosage forms is lower than the others because it includes additional step which is a diffusion through the copolymer backbone. Contrariwise, the effect of pH on the diffusion coefficients is not significant.

Table 2 : 3-aminopyridine release results from spherical dosage forms

	Eud/AP : 80/20			Eud/C: 80/20		
pH	$\%M_t/M_\infty=f(t^{1/2})$	R^2	$D_{s,t}*10^4$ (cm^2sec^{-1})	$\%M_t/M_\infty=f(t^{1/2})$	R^2	$D_{s,t}*10^7$ (cm^2sec^{-1})
1,2	$0.081\ t^{1/2}$-0,025	0.994	2.68	$0.003\ t^{1/2}$ +0,292	0.997	3.92
4,0	$0.078\ t^{1/2}$-0,149	0.993	2.49	$0.002\ t^{1/2}$ +0,375	0.999	2.94
6,0	$0.081\ t^{1/2}$-0,317	0.998	2.68	$0.003\ t^{1/2}$ +0,361	0.998	5.96
8,0	$0.079\ t^{1/2}$-0,447	0.993	2.53	$0.003\ t^{1/2}$ +0,510	0.992	3.92

Finally, if we compare the drug release in the tow media i.e. homogeneous and heterogeneous as shown in figure 6, the difference is important. In the homogeneous medium, the drug release mechanism is only based on the hydrolysis reaction however in the

heterogeneous medium; the release mechanism includes the hydrolysis reaction of imine function and the diffusion phenomenon which is the determinant. Consequently, we can choose the appropriate medium as a function of the desired medicinal application and pharmacokinetic.

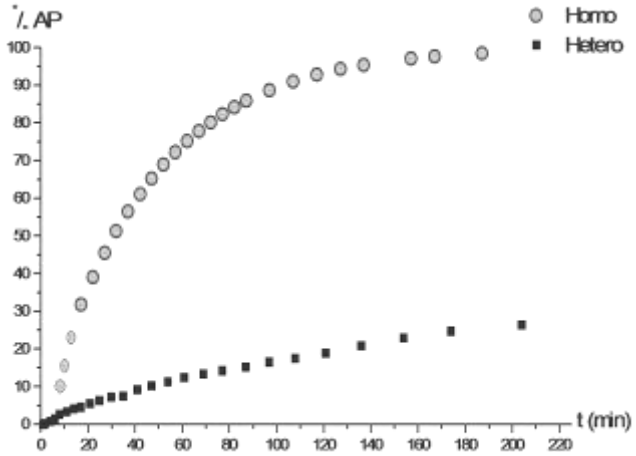


Figure 6. % active agent released from copolymer in homogeneous (homo) and heterogeneous (hetero) media at pH=6.0

Conclusion

The objective of this paper is to develop controlled release formulations (monomer, copolymer, dosage form) based on 3-aminopyridine as an active agent using physicochemical methods. The homogeneous and heterogeneous drug release kinetics were established at four different pH media and the results demonstrate that these formulations permit to modify and to control the drug release. In homogeneous media, the kinetic constants depend strongly on the type of support (monomer/copolymer) and also on the pH medium. As well, in heterogeneous media, the diffusion coefficients were calculated according to Fick's laws and depend especially on the dosage form composition.

Experimental part

Chemicals

3-aminopyridine (AP) from Flucka, Eudragit RL100 (Eud) (copolymer of dimethylaminoethylacrylate and ethylmethacrylate) Mn=150,000g/mol from Röhm Pharma, N-vinylpyrrolidone and cinnamaldehyde from Aldrich, analytical solvents (benzene, tetrahydrofuran, petrol ether, absolute ethanol) used as received.

Synthesis of monomer and copolymer Support

The monomer was prepared according to the following procedure [33] (scheme 1):
A mixture of cinnamaldehyde and 3-aminopyridine in equimolar ratio was dissolved in anhydrous benzene in the presence of 2,4-ditertiobutylcathecol and paratoluensulfonic acid (PTSA) as an antioxidant and a catalyst successively , in a flask equipped with Dean-Stark apparatus. The mixture was heated until reflux at the azeotrope temperature (experimental T°:79.5°C) in order to eliminate the produced water. After filtration, the organic phase was concentrated by rotavapor and the Schiff base was recovered.

Copolymer (C) was obtained via radical mechanism of polymerization (scheme 2). In tetrahydrofuran (THF) solution, using 2,2-azo-bis-(isobutyronitrile) (AIBN, 5% on mass) as initiator and under nitrogen atmosphere, the Schiff base and N-2-vinylpyrrolidone (VP) on a molar ratio of 4:96 (monomer:VP) were heated at 65°C during 24 hours. The obtained copolymer was purified by re-crystallization in THF/Petrol ether couple.

Characterization of monomer and copolymer Supports

FTIR spectroscopy: The FTIR spectra of monomer and copolymer were recorded on dried KBr disks using SCHIMADZU FTIR-8300 apparatus.

Microanalysis of copolymer was established in the microanalysis center of university of Rennes-1 (France).

By thermo gravimetric analysis, the temperature of transition of a copolymer was measured using DSC SETARAM apparatus at the University of Rennes-1 (Service Pr J.F Carpentier-UMR 6226 CNRS).

The average mass M_v of copolymer was obtained by viscosimetry method using a Ubbelhode viscometer and by applying Mark-Houwink law : $[\eta] = KM^a$ where [η] is intrinsic viscosity and the coefficients K and a are : a = 0.74, $K.10^{-3}$(mL/g) = 8.86 for poly (1-vinyl-2-pyrrolidone) as reference polymer backbone in sodium acetate (0.1M) as solvent[34].

Dosage forms preparation

200mg of mixture of active principal (3-aminopyridine)(A.P) pure or copolymer support (C) and Eudragit RL (Eud) at a composition of 80/20: w/w (A.P or C/Eud), in powder form was well dispersed, and intimately mixed in mortar and transformed into a thick paste with a small amount of absolute ethanol (2 or 3 pulverizations). Spherical beads were prepared from this paste and dried at room temperature for 4 or 5 days in desiccator. All the beads had approximately the same weight (~200mg) for the same size (mean diameter d=0.696±0.004 cm).We have not prepared dosage forms based on monomer because it is liquid.

Drug dissolution tests

a- Homogeneous hydrolysis of monomer and copolymer

The hydrolysis kinetics were performed at 37°C in hydro-alcoholic media (67/33% v/v: water-ethanol) prepared at different pH (1.2, 4.0, 6.0 et 8.0), where the ionic force was constant (μ = 0.15). The percentage of ethanol (33%) was selected on the base of copolymer and monomer solubility's in the different media. First the monomer or copolymer was dissolved in ethanol and poured in UV cell, and then an appropriate volume of buffered solution at the desired pH was added. To follow the hydrolysis kinetics, complete UV spectra of solution were registered as a function of time using UV-Vis spectrophotometer (UV-Vis-2401PC-SHIMADZU). When the kinetic was rapid, only the optical density was measured at fixed analytical wave length as a function of time.

The buffered solutions were prepared according to the acid/base couples proposed by Michaelis and Mizutani[35] (pH=1.2:HCl solution, pH=4:CH_3COOH/CH_3COO^-, pH=6: $CH_3CH_2COOH/CH_3CH_2COO^-$, pH=8:$NH_4Cl/NH_3$).

b- Drug release kinetics from dosage forms

Experiments were carried out in closed flask, kept at 37C° with a controlled rate of stirring (500 rpm). The bead, inserted in permeable fiber glass basket was soaked into 100 ml of

simulated gastric liquid (pH=1.2) for a period of 2 hours, then the bead was replaced successively in another buffered solutions: at pH=4 for 1 hour, for 2 hours in pH=6 and finally for 3hours in buffered solution at pH=8, in order to simulate the in vivo rout of the dosage form.

Samples (1 ml) of liquid were taken at different intervals for analysis after appropriate dilutions using UV-Vis-2401PC-SHIMADZU spectrophotometer at the corresponding λ_{max} of 3-aminopyridine(pH=1.2: λ_{max}=315nm and ε=3400L.mol^{-1}cm^{-1}, pH=4: λ_{max}=315nm and ε=3460 L.mol^{-1}cm^{-1}, pH=6: λ_{max}=297nm and ε=3481 L.mol^{-1}cm^{-1}, pH=8: λ_{max}=288nm and ε=3420L.mol^{-1}cm^{-1}).

The release media are composed from: HCl and NaCl for pH=1.2, CH_3COOH/CH_3COO^- for pH=4.0, KH_2PO_4 and NaOH for pH=6.0, HCl and Borax for pH=8.0, in accordance with US pharmacopeia.

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