A new approach of 7-hexahydro-aza-indolerelease study from dosage forms in heterogeneous media and calcul of diffusivities

Nabila Oukli [a*]**, Noureddine Mehnane** [b]**, Abderrezzak Mesli** [a]

[a] *Laboratory of Macromolecular and Physical Organic Chemistry, Faculty of Sciences, Djillali Liabès University, 22000 Sidi Bel Abbès, Algeria.*
[b] *A.M.L, Applied Materials Laboratory, Université de SidiBelAbbès, 22000 Sidi Bel Abbès, Algeria.*

Abstract

Several alkaloids have proved to be either too toxic or not enough biologically available to be used directly as treatments for diseases. Nevertheless they remain a source of inspiration for the design of new drugs mediators and can lead after chemical modifications to new interesting therapeutic candidates. In order to evaluate the biological interest of a product miming a natural product, 7-hexahydro-aza-indole as medical agent was synthesized and characterized recently. A spherical dosage forms composed from Eudragit RL100 and the medical agent were prepared and tested to control the drug release, these formulations was followed in heterogeneous media at different pH simulating the human digestive rout at 37°C. The release mechanism of the medical active agent is investigated according to Fick's laws. So, the diffusivities were calculated and the results demonstrated that the drug release can be modified using these formulations. The data on drug release provide the opportunity for predicting quantitatively the rate of medical agent release from dosage forms, directly into the organism and the gradual transfer of active molecules is well-known in the pharmaceutical field.

*Key words:*7-hexahydro-aza-indoles, Alkaloids, drug release, galenic form.

1. Introduction

The interest of the current study, 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].The 7-hexahydro-aza-indole as medical active agent, which could be considered as bioisosteres of indoles, occupy a central position in modern heterocyclic chemistry due to their physicochemical and therapeutic properties; it's a very interesting class of pharmaceuticals and investigational drugs. In this context, these derivatives have been reported to possess a range of biological activities and have the potential to act as kinas inhibitors, cardio-vascular components, antitumor agents and agonists or antagonists of other various pathologies [2; 3].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 physiological gastric and intestinal liquid through the polymeric support. Whatever method it is chosen to legate the drug, the proposed solutions are mainly related to the way to introduce the drug, injection or oral absorption [4].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 [5].The obtained medical active agent (M.A) was synthesized and characterized by FTIR, NMR (^1H, ^{13}C, 2D). In the second part, spherical dosage forms composed from the medical active agent synthesized and Eudragit RL were elaborated in order to modify the drug release. Eudragit matrixes were largely used in galenic industryto prepare controlled release formulations (%/%, Eudragit RL/7-hexahydro-aza-indole). In the other of the hand, the drug release was followed using UV-Vis spectrophotometer in gastro intestinal media at different pH (pH=1.2, 6.0 and 8.0) at 37°C with various initial masses (100, 200, and 300mg). In this case, the results demonstrated that the drug release is governed by transient diffusion. So, the diffusion coefficients were calculated from the approached analytical solutions of Fick's law [6-12].The drug release data showed that the system can provide drug release for a long time. Also, the device behavior is fully

predictable, according to our theoretical model of structure design, and gives many opportunities for model investigations of drug release and its kinetics.

2. Materials and methods

2.1. Synthesis of products

All the reactions were carried out under atmosphere of argon excepted for those carried out in aqueous solution. Thin layer chromatographies were carried out onplatessilicagelMerck-Kiesegel60F$_{254}$.Theseplateswere observedinlightUVandwere revealedbyKMnO$_4$. The spectra of NMR 1H and NMR 13C were recorded respectively to 200 MHz and 50 MHz on a device BRUKER AC 200 MHz in the deutered chloroform (CDC1$_3$) unless otherwise specified.

The THF and the ethylic ether were distilled on sodium in the presence of benzophenone, the methylene chloride and the acetonitrile were distilled on the calcium hydride. The chromatographies flashes were carried out on silica gel(230-400mesh) with eluants mixtures in variable quantity of acetate of ethyl(AcOEt) and cyclohexane(CH).The melting points were taken in capillary tubes on a device at melting point stuart SMP10. Infra-redspectrometry was carried out ona spectrometer Perkin.The solid compounds were put in the form of pastilles KBr (1mg of composed for 50 Mg of KBr) and the principal absorption bands are given of many waves (cm^{-1}). Classifications of the molecules for attributions NMR do not follow the nomenclature.

2.1.1. Procedure of synthesis of polysubstitutedimides (±) - a

Acryloyl chloride (500 μl, 6,0 mmol) was added at 0 °C to a solution of the (±)-2-Amino-1-benzyl-5-oxo-4,5-dihydro1*H*-pyrrol-3-carbonitrile (4.0 mmol) in CH$_2$Cl$_2$ (40 mL). After 12 h atrt, the reaction mixture was cooled to 0 °C and quenched carefully by addition of an aqueous saturated solution of NaHCO$_3$ (50 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (3×30 mL) and the organic layers were combined, dried over MgSO4 and evaporated. The residue was then purified by chromatography on silica gel to furnish (±)-a(AcOEt/cyclohexane).C$_{15}$H$_{15}$N$_3$O$_3$; Mp = 163-165 °C; Yield=60% (AcOEt/cyclohexane, 30/70). This product was isolated as a pink solid.

2.1.2. Procedure of synthesis (the 7-hexahydro-aza-indole(±) - c

In a balloon of 20ml, we put 1 eq of (a) more 5ml of CH$_2$Cl$_2$ under argon, to let well agitate during 30min. By follows to add drop by drop in the balloon 2eq of BF$_3$.OEt$_2$, to let the reaction react under agitation during 12h.Under agitation to wash with 5ml of NaHCO$_3$ solution, to extract the organic phase with 3*5ml from CH$_2$Cl$_2$, to dry under MgSO$_4$, to filter and recristallize the product obtained with Et$_2$O ether.We could obtain the final product(c) by using an acid of Bronsted (CF$_3$CO$_2$H) instead of the Lewis acid (BF$_3$.OEt$_2$).The strategy is simple to realize with identical outputs.The analytical sample was obtained by recrystallisation from dry ethanol and the product was isolated as a white solid: C$_{17}$H$_{19}$N$_3$ O$_3$; Mp = 163-165 °C; Yield=65% (AcOEt/cyclohexane, 20/80).

2.2. The buffered solutions for kinetics

The buffered solutions were prepared according to the acid/base couples proposed by Michaelis and Mizutani (pH=1.2: HCl solution, pH=4:CH$_3$COOH/CH$_3$COO-, pH=6: CH$_3$CH$_2$COOH/CH$_3$CH$_2$COO- and pH=8:NH$_4$Cl/NH$_3$).

2.3. Drug release kinetics from dosage forms

Various compositions and sizes have been prepared and the release of the drug in synthetic liquids was determined in vitro tests by using the galenic forms described above. The 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 gastro intestinal liquid (pH=1.2) for a period of 3 hours, then the bead was replaced successively in another buffered solutions: at pH=6.0 for 1h 30 min and finally for 3hours in buffered solution at pH=8.0, 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 7-hexahydro-aza-indol (pH=1.2: λ_{max}= 251.2 nm and ε = 17.600 L.cm^{-1}.mol^{-1}, in pH= 6.0: λ_{max} = 251 nm and ε= 16.500 L.cm^{-1}.mol^{-1} and in pH=8: λ_{max} = 253 nm and ε= 17.700 L.cm^{-1}.mol^{-1}). The release media are composed from: HCl and NaCl for pH=1.2, CH$_3$COOH/CH$_3$COO- and NaOH for pH=6.0, HCl and Borax for pH=8.0, in accordance with US pharmacopeia.

3. Results and discussion

3.1. Characterization results

The imides (±)-asee (Scheme1) was prepared particularly by examining their behaviour by using *N*-acyliminim chemistry [13-16], thereduction of the amide-imidepolysubstituted(±)-aprovideeasily the obtained of the αcorresponding-lactamshydroxy(±)-b,its interest since the transformation could target the racemates 7-hexahydro-aza-indole product (±)-c(Azindole poly unsaturated)**.** The structural assignments of the new compounds were based on their elemental analysis and spectral (IR, 1H NMR, 13C NMR and 2D NMR) data. The preparation data of all the new compounds is presented in experimental part.

3.1.1. Characterization of the (±)-3-(1-Benzyl-3-cyano-2, 5-dioxopyrrolidin-3-yl) propanamide (±)-a:

In the IR (KBr) spectra of this compoundshows the important absorption bands in the range of 2247 cm^{-1}, which are attributed to the CN group and the most important bands were observed are: 3525, 3015, 2400, 1396 cm^{-1}.
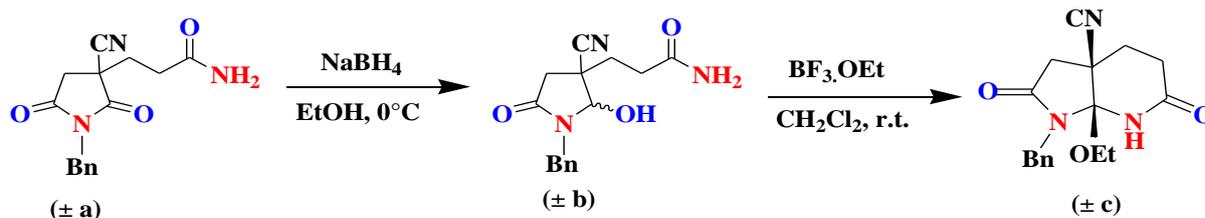
Scheme1. Scheme leading to7-hexahydro-aza-indole.

The structure of the compound is confirmed by[1]H NMR (300 MHz, CDCl$_3$), the spectrum gives the following proton signals δ(ppm): 7.36 (s, 5H), 5.65 (bs, 2H), 4.70 (s, 2H), 3.20 (d, *J* = 18.2 Hz, 1H) *J* = 18.2 Hz, 1H), 2.94 (d, *J* = 18.2 Hz, 1H), 2.60-2.48 (m, 1H), 2.46-2.19 (m, 3H).

In the ^{13}C NMR (300 MHz, CDCl$_3$) we not practically in the spectrum the following proton signals δ (ppm): 172.8, 172, 171.7, 134.6, 129.3, 128.5, 117.3, 43.6, 42.4, 39.4, 32, 30.7. The NMR 2D (cosy) of (±)-3-(1-Benzyl-3-cyano-2, 5-dioxopyrrolidin-3-yl) propanamide(±)–a is presented in Fig.1.
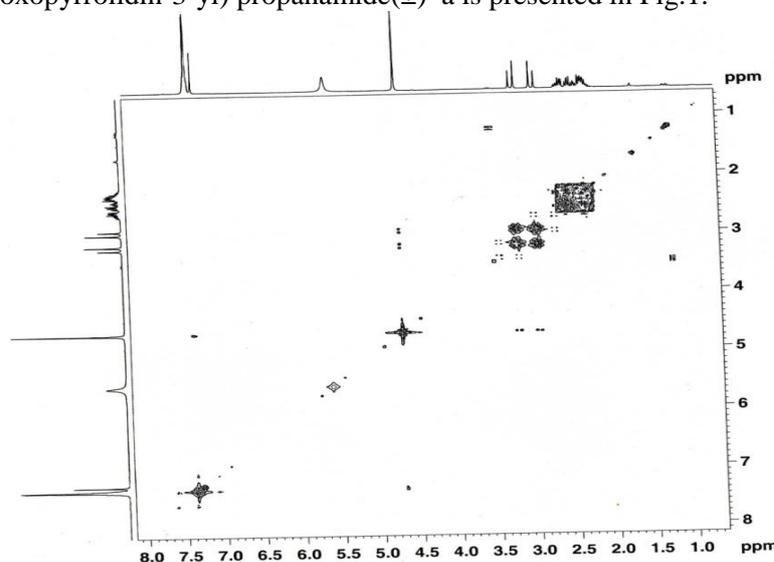
Figure1. Spectrum NMR 2D (cosy) of (±)-3-(1-Benzyl-3-cyano-2,5-dioxopyrrolidin-3-yl) propanamide(±)- a.

3.1.2.Regioselectivity of the reduction of the imide.
The Hydride H -must attack carbonyl according to an angle 109° with carbonyl, it is what one calls the angle of Burgi-Dunits. Thus if there are substituent's in α of the imide there are a constraint due to the steric obstruction what disadvantages this attack. This explains why one of the approaches is less under privileged (Scheme2). The expected obtains an equimolaire mixture of both hydroxylactames (±) -b that does not pose a problem because the center stereogenic created at the time of this stage will be lost in the following stage by consequent we could obtain the finished product (±)-c.
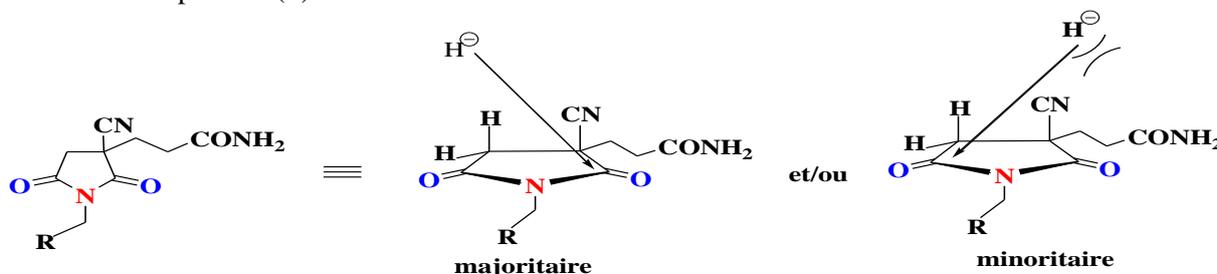
Scheme2. Regioselectivity of the reduction of the imide.

3.1.3. Characterization of the (±)-1-Benzyl-7a-ethoxy-2, 6-dioxooctahydro-1H-pyrrolo [2, 3-b] pyridine-3a-carbonitrile (±)-(c):
In the IR (KBr) spectra of this compound,the CN group was observed at value absorption band in the range of 2241cm-1 and the most important bands are: 3192, 3098, 2939, 2241, 1314,1207, 1064cm^{-1}. The structure of

this compound is confirmed by ^{1}H NMR (300 MHz, CDCl$_3$), the spectrum gives the following proton signals δ(ppm): 8.33 (s, 1H), 7.27 (s, 5H), 4.62 (d, J = 15.5 Hz, 1H), 4.31 (d, J = 15.5 Hz, 1H), 3.42-3.55 (m, 2H), 3.19 (d, J = 17.0 Hz, 1H), 2.66 (td, J = 18.0 and 5.0 Hz, 1H), 2.64 (d, J = 17.0 Hz, 1H), 2.40 (dt, J = 18.0 and 3.8 Hz, 1H), 2.19 (dt, J = 13.5 and 3.8 Hz, 1H), 1.96 (td, J = 13.5 and 4.4 Hz, 1H), 1.10 (t, J = 6.9 Hz, 3H).In the ^{13}C NMR (300 MHz, CDCl$_3$) we not practically in the spectrum the following proton signals δ (ppm): 170.3, 168.7, 136.4, 128.7, 128.0, 127.8, 117.9, 99.7, 60.9, 42.6, 40.6, 40.2, 30.5, 28.0, 14.8.

The Analysis calculated for C$_{17}$H$_{19}$N$_3$O$_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, 65.02; H, 5.98; N, 13.31.The NMR 2D (cosy) of the (±)-1-Benzyl-7a-ethoxy-2, 6-dioxooctahydro-1H-pyrrolo [2, 3-b] pyridine-3a-carbonitrile (±)-c is shown in Fig.2.
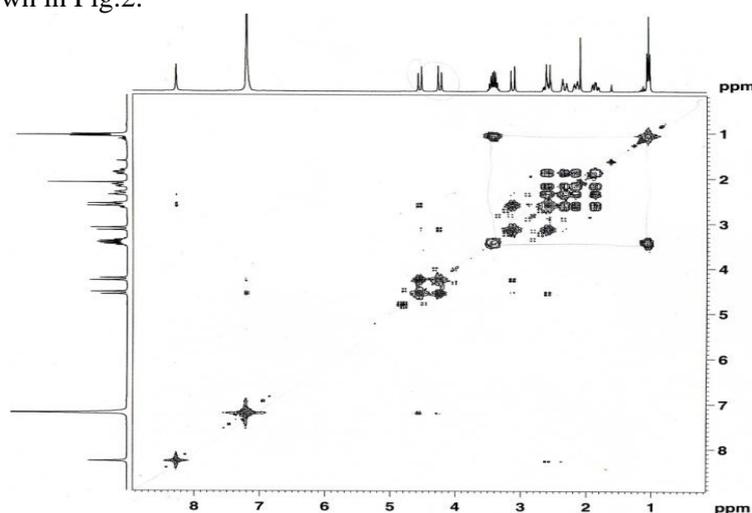
Figure2. Spectrum NMR 2D (cosy) of the (±)-1-Benzyl-7a-ethoxy-2, 6-dioxooctahydro-1H-pyrrolo [2, 3-b] pyridine-3a-carbonitrile (±)-c.

3.2. Kinetics results

3.2.1. Mechanism of hydrolysis

We have recently explored detailed (scheme 3) one of the mechanismof hydrolysis. However, the formation was proved by isolating an ethoxy equivalent (±)-c when the reaction was quenched with ethanol. The latter after acidic hydrolysis led to polysubstituted imides (±)-a.
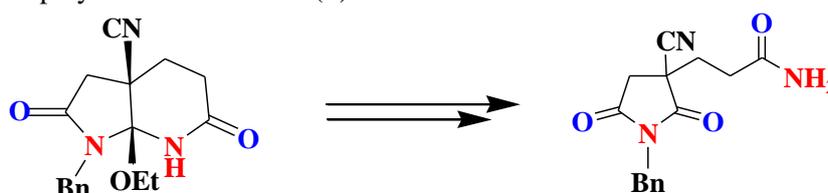
Scheme3. Transfer of the Bi cyclic product (closed) to the cyclic product (open).

The succinimide framework results from the second *N*-acyliminium ion after departure of HCl. The migration of grouping NH$_2$with this position showed that one obtained the product bicycle well, the bicycle (Scheme 3) was unstable in acid medium because it was hydrolyzed to give the opened product, the mechanism of hydrolysis at summer tested with a treatment with water in acid medium with some drops of THF (tetra hydro furane).The cascade process can be explained by a sequence tandem ring closure/ring opening of the γ-lactam skeleton. This produces unexpected nitrogen atom transfer by the way of the *N*-acyliminium ion hydrolyzed to lead to the hydroxyl bislactam (Scheme 4).

3.2.2. Preparation of oral forms

The7-hexahydro-aza-indole was chosen as a medical agent (M.A) and the matrix used in the preparation of the different oral forms was the Eudragit RL100, copolymer of dimethylaminoethylacrylate and ethylmethacrylate with Mn = 150.000 g/mole from Allemande RöhmPharma.

The medical agent (M.A)and Eudragit RL, in powder form were well dispersed (using Perkin-Elmer vibrator), and were intimately mixed in mortar and transformed into a thick paste with a small amount of absolute ethanol (2 or 3 pulverizations) which is a solvent of the drug and Eudragit RL matrix. Spherical beads were prepared

from this paste and dried at room temperature for 5 or 7 days. Several dosage forms were prepared with various values of drug percentage [17-21].All the dosage forms have approximately the same weight close to 100 - 300 mg for the same size. beads with 50% / 50% (W / W, Eudragit RL / M.A), diameter close to 0.301-0.305cm), indexed a*,b*,c* and beads with 80% / 20% (W/W, Eudragit RL / M.A, diameter close to 0.297-0.301cm, indexed a, b, c) were prepared and tested using synthetic liquids of pH= 1.2, pH=6.0 and pH= 8.When the dosage forms were soaked into simulated gastro intestinal liquid, liberation of the M.A was observed with typical kinetics.
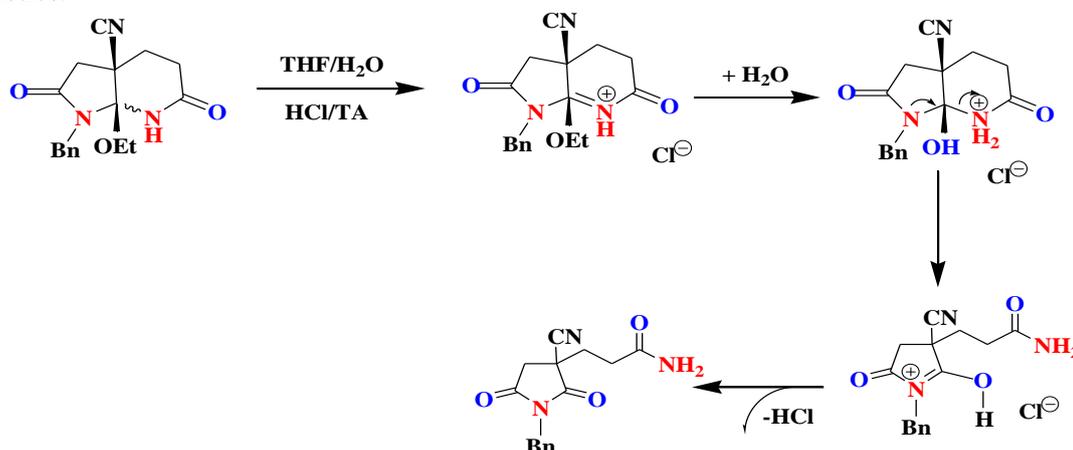
Scheme4. Transfer mechanism of hydrolysis of the (±) 1-Benzyl-7a-ethoxy-2, 6-dioxooctahydro-1H-pyrrolo [2, 3-b] pyridine-3a-carbonitrile.

3.2.3. Drug release study

The drug release and the absorption of the liquid by the dosage forms were studied from spherical dosage forms a, b, c and a*, b*, c* in tree media at different pH: 1.2, 6.0 and 8.0.The kinetic results demonstrate that the hydrolysis rate of the drug release is inferior to that the absorption of the liquid by the corresponding dosage forms in all pH. In fact, the hydrolysis of the drug release includes a preliminary stage which is the penetration of ionic solution (H_3O^+ or OH^-) through the tangled structure of macromolecule. Both, the hydrolysis rate from the dug release and the absorption of liquid by the dosage forms increases under the acidic (HCl/H_2O) hydrolysis. An example of the drug release and the absorption of the liquid were studied from spherical dosage form 'a*' with a composition (50/50 (Eud/M.A), weight 100 mg) consecutively at pH=1.2, pH=6.0 and pH=8.0. The release of drug and the absorption of liquid profiles are illustrated in fig.3.The results show (Fig.3) that the drug release is slower than the absorption of the liquid by the dosage form. In fact, after two hours (the time corresponding to the drug stay in human stomach from the dosage form), the percentages of drug released and the absorption of the liquid by dosage form are respectively 53% and 59%. At the end of 6 hours, 100% of drug released from 50/50 dosage form is reached.This is confirmed by the experimental results for the water levels absorbed by the dosage form after 36 h.
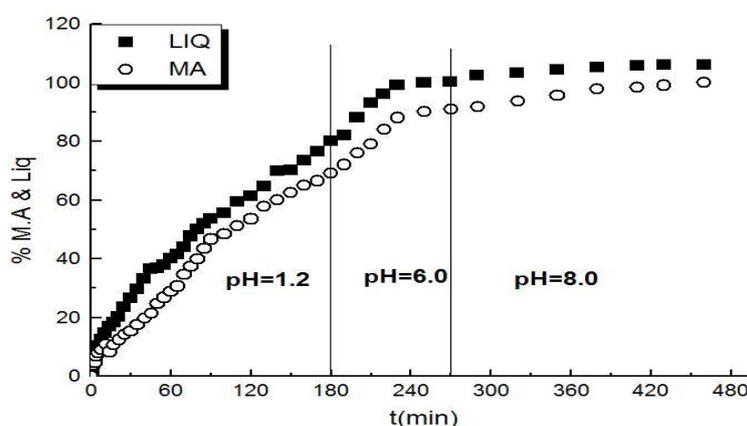
Figure3. Profiles of absorbed liquid and drug released from the dosage form 'a*' (100 mg) as a function of time, in pH= 1.2, pH=6.0 and pH=8.0.

In addition, the drug release and the absorption of the liquid were studied consecutively in tree media at different pH from spherical dosage forms at a composition of 80/20 and 50/50 (w/w): Eud/M.A. An example of the drug release and the absorption of the liquid were studied from spherical dosage forms from c and c* with a composition (80/20 and 50/50, weight 200 mg) at a pH= 6.0. The profiles are illustrated in fig.4.

We have observed (fig.4), that the absorption of the liquid by the dosage forms 50 / 50 and 80/20 was more significant than drug release from dosage forms corresponding, as well the absorption of the liquid by the dosage form 50/50 is more important than form 80/20 and this in spite of the existence of a strong rate in Eudragit thus in hydrophilic, it is due to the molecular composition of the medium of study. Consequently a larger molecule will diffuse less.
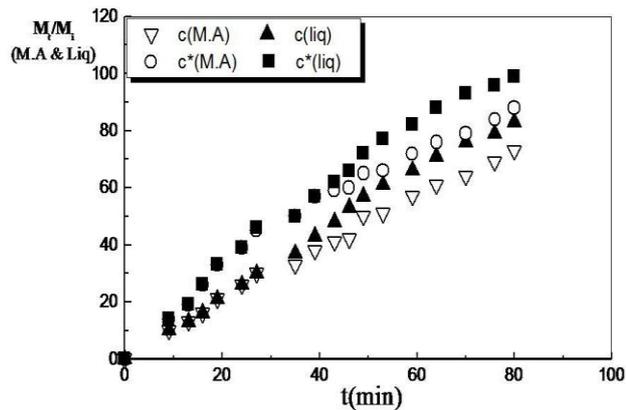
Figure4. Profiles of absorbed liquid and drug released from the dosage form 'c' and 'c*'(200 mg) as a function of time, in pH=6.0.
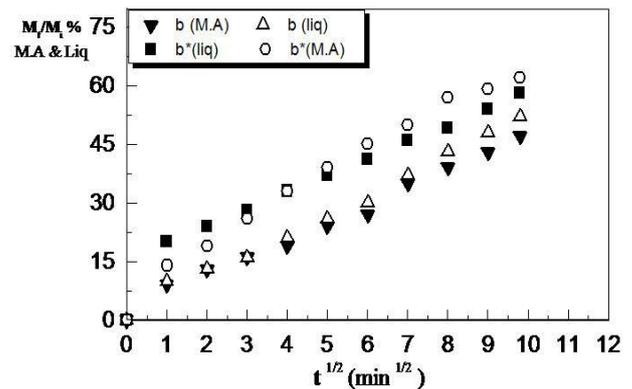
Figure5. Profiles of absorbed liquid and drug released from the dosage form 'b' and 'b*' (300mg) as a function of square root of time in pH= 8.0.

The process is not simple; two matter transfers take place when the form is in contact with liquid: the liquid enters the matrix-copolymer and initiates the drug dissolution.

At this stage, the drug can be released from the dosage form, a larger volume of liquid which facilitates drug dissolution and diffusion through the matrix structure.We have noted that the fractional release of 7-hexahydro-aza-indoleis proportional to the square root of time during the short time as shown in Fig.5, a linear relationship being observed for mainly short periods as in the case of a process controlled by diffusion. An example of the drug release and the absorption of the liquid were studied from spherical dosage forms from b and b* with a composition (80/20 and 50/50, weight 300 mg) at a pH= 8.0.

So, in line to draw a release model, we have tested the approached analytical solution derived from Fick's law for diffusion in a sphere. The following assumptions are thus made in order to simplify the problem: (*i*) The spherical dosage forms are homogeneous (the medicinal agent or copolymer being well dispersed into the Eudragit matrix), (*ii*) 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,(*iii*) 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 $0 \leq r < R$ $C = C_{in}$

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

On the surface

t>0   r= R $C = C_\infty$

$C\infty$= Concentration at equilibrium. In the earlier stages of the process [22], the following analytical solution is given by:

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

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

$$1 - \frac{M_t}{M_\infty} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} . \exp\left[-\frac{n^2.\pi^2.D}{R^2} .t \right] \qquad (2)$$

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

$$\frac{M_t}{M_\infty} = \frac{6}{R} \cdot \left(\frac{D;t}{\pi} \right)^{\frac{1}{2}} \qquad (3)$$

For long times, another analytical solution is deduced from (Eq. (2)), which is also of interest for calculating the diffusivity for long periods.

$$\ln(1 - \frac{M_t}{M_\infty}) = -\frac{\pi^2 . D.t}{R^2} + \ln \frac{6}{\pi^2} \qquad (4)$$

The table 1 and 2 gives the results of the diffusion coefficient Ds,t in short periods and Dl,t in long periods consecutively in tree media at different pH: 1.2, 6.0 and 8.0 . The diffusivities of 7-hexahydro-aza-indoleare in the range of 10^{-7} cm^2 sec^{-1} in Eud/M.A dosage forms. Of these different values from "D" we can say that in the heterogeneous media; the release mechanism includes the hydrolysis reaction of amide- imide 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. When equilibrium is attained, the increase of the process is visible, it reaches approximately the 90.12% for the dosage forms a, b, c and about 94.52% for the dosage forms a*, b*, c* at pH=1.2. We noted that the mass at equilibrium are function of the characteristics of the dosage forms and the medium of study influencing the coefficients of diffusion see (Table 2: mass % liquid/drug).

Table1: Values of diffusivities of the M.A released at pH= 1.2, 6.0 and 8.0.(s,t : short time, l,t : long time).

Composition	80/20	80/20	80/20	50/50	50/50	50/50
Indexation	a	b	c	a*	b*	c*
Initial mass of dosage form (mg)	100	200	300	100	200	300
pH=1.2						
Diffusivities Ds,t (cm^{2}min^{-1}).10^7	2.35	1.49	1.62	1.83	1.80	1.38
Diffusivities Dl,t (cm^{2}min^{-1}).10^7	0.34	0.47	0.50	0.31	0.52	0.68
pH=6.0						
Diffusivities Ds,t (cm^{2}min^{-1}).10^7	0.70	1.24	1.17	0.78	0.60	0.71
Diffusivities Dl,t (cm^{2}min^{-1}).10^7	0.56	0.52	0.50	0.52	0.61	0.50
pH=8.0						
Diffusivities Ds,t (cm^{2}min^{-1}).10^7	2.45	2.36	2.16	2.38	2.34	2.26
Diffusivities Dl,t (cm^{2}min^{-1}).10^7	0.45	0.50	0.52	0.61	0.70	0.71

Table2: Values of percentage of masses at equilibrium at pH=1.2, 6.0 and 8.0.

Oral forms	a	b	c	a*	b*	c*
% $M_{M.A}\infty$ pH=1.2	98.67	91.23	80.47	98.60	92.55	92.35
% $M_{M.A}\infty$ pH=6.0	93.54	91.56	90.12	95.69	69.15	70.10
% $M_{M.A}\infty$ pH=8.0	98.12	97.54	96.58	99.89	98.17	96.84:

Such hydrolyses were not able to be described by traditional kinetic equations, the equilibrium masses obtained is a function of characteristics of the dosage forms and of the nature of the medium and the amount of drug released at the end of the process. Finally it is very important to know the plasmatic concentrationofmedicinal agent which will determine the optimal quantity to obtain in therapeutic zone, in order to avoid all the harmful side-effects [23-26].

Conclusion

This study paves the way to a new technique of develop controlled release formulations (copolymer, dosage form) based on 7-hexahydro-aza-indole as a medical agent using physicochemical methods. In homogeneous media, the kinetic constants depend strongly on the type of support (polymer /copolymer) and also on the pH medium .The synthesis of this kind of molecules miming natural products allows opening new ways for the pharmacology with various significant biological potentialities to in vivo treatment. The studyand thecomprehensionof the complexbiologicalsystems, suchphenomenawill intraorextracellular,is a scientificreal challengewhichpassesineluctablyby the experimentation.

References

1. Bernik D.L., Zubiri D., Monge M.E., Negri R.M.A*., Physicochemical Engineering.Aspects.*273 (2006) 165-173.
2. Fernández D.,Ahaidar A.,Danelón G.,Cironi P., Marfil M., Pérez O., Cuevas C., Albericio F., Joule J.A., Alvarez M.*,Monatsh. Chem*. 135(2004) 615–627.
3. Popowycz F., Routier S., Joseph B.,Mérour J.-Y.,*Tetrahedron* 63 (2007) 1031–1064.
4. Arab A., Zacarin G.G., Fontanetti C.S., Camargo-Mathias M.I., Dos Santos M.G., Cabrera A., *Entomotropica.* 18 (2003) 79–82.
5. Abdelmalek I., Mesli A., Boudouaia N., Chafi N.,Simonneaux G., *Mediterranean J. Chemistry* 1(2) (2011) 88-97.
6. Boudier A., Aubert-Pouëssel N., Mebarek A., Chavanieu J., Quentin D., Boukhaddaoui H.M., Gérardin C., Jorgensen C., Devoisselle J.M., Louis-Plence P., Bégu S., *Journal of Controlled Release* .154 (2011)156–163.
7. Siepmann J., Karrout Y., Gehrke M.,Penz F.K., Siepmann F., *Inter. J. Pharmaceutics.*441 (2013) 826–834.
8. Rick Finch A., Liu M.C., Grill S.P., RoseW.C., LoomisR., Vasquez K.M., Cheng Y.-C.,Sartorelli A.C*., Biochemical Pharmacology*. 59 *(*2000) 983-991.
9. Li J., Chen S.H., Li X., Niu C., Doyle T.W*., Tetrahedron*. 54 (1998) 393-400.
10. Hadjitheodorou A., KalosakasG., *Materials Science and Engineering C.* 33 (2013) 763–768
11. Glaessl B., Siepmann F., Tucker I., Rades T., Siepmann J., *Inter. J. Pharmaceutics* 389 (2010) 139–146
12- P. Ahlin, J. Kristl, A. Kristl, F. Vrečer, *Int. J. Pharm.*239 (2002) 113-120.
13. Barbosa Y.A.O.,Hart D.J.,MagomedovN.A.*Tetrahedron*. 62 (2006) 8748–8754.
14. Sun H., Martin C., Kesselring D., Keller R., MoellerK.D. *J. Am. Chem. Soc*. 128 (2006)13761–13771.
15. Sannigrahi M., Pinto P., Chan T.M., Shih N.Y.,Njoroge F.G.,*Tetrahedron Lett*. 47 (2006) 4877–4880.
16. Luker T., Koot W.J., Hiemstra H., SpeckampW.N., *J. Org. Chem.* 63 (1998)220–221.
17. Sehil H., Bengharez Z., El Bahri Z., Merine H., Guemra K., *Mediterranean Journal of Chemistry.* 1(3) (2011) 125-134.
18. Elbahri Z., Chafi N., Mesli, A. *Journal-Société Chimique de Tunisie*. 6 (2004) 77-84.
19. Huang J., Kao H., Wu X.Y., *J Controlled Release*. 67(1) (2000) 45-54.
20. Siepmann J., Kranz H., Bodmeier R. and Peppas N.A., *Pharmaceutical Research.* 16(11) (1999) 1748-1756.
21. Mollo A. R., Corrigan O.I., *Inter. J. pharmaceutics* 268 (2003) 71-79.
22. Crank J., The Mathematics of Diffusion, 2nd Ed*, Clarendon Press, Oxford* (1975) 1–7.
23. Kalck, P.; Urrutigoïty, M.; Dechy-Cabaret, O. In Catalytic Carbonylation Ed. *Springer: Berlin Heidelberg*. 18 (2006)97-123.
24. Rochon P., Jourdain M., Mangalaboyi J., Fourrier F., Bégu S., Buys B., Dehlin G., Lesage J. Cl., Chambrin M.C., and MordonS., *Journal of BiomedicalOptics*. 12 (6) (2007)**.**
25. Bégu S.**,** Aubert-Pouëssel A., Polexe R., Leitmanova E., Lerner D. A., Devoisselle J.M., Tichit D., *Chemistry of Materials*. 21 (13) (2009)2679-87.
26. Aubert B.A., Louis-PlenceP., Quentin, J., Gerardin C., Jorgensen, C., Devoisselle J.M., Bégu S.**,** *Drug Development and Industrial Pharmacy*. 9 (2009) 1-9.

(2014) http://www.jmaterenvironsci.com