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IN VITRO EVALUATION OF KETOPROFEN CONTROLLED RELEASE FROM VARIOUS FORMULATIONS

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IN VITRO EVALUATION OF KETOPROFEN CONTROLLED RELEASE FROM VARIOUS FORMULATIONS (Abstract): The aim of this study is to investigate the potential utility of some hydrogels, based on chitosan, chitosan modified with phthalic anhydride and 75/25 poly(N-isopropyl acrylamide)/alginate, for preparing drug release systems containing ketoprofen, as model drug. **Material and method**: The *in vitro* release profiles and swelling studies were done in ethanol medium, where the studied drug presents high solubility, at 25^0C (room temperature). The ketoprofen release was observed by monitoring the absorbance at λ_{max} = 254 nm as a function of time. **Results**: The experimental results indicated that the smallest amount of drug was released from chitosan matrices, appreciatively 31%. **Conclusions**: The composition of hydrogels had an important effect on ketoprofen release. **Key words**: ALGINATE, CHITOSAN, SWELLING, CONTROLLED RELEASE, KETOPROFEN

Among the temperature-sensitive hydrogels reported to date, poly (N-isopropyl acrylamide) (PNIPAAm) and its copolymers have been widely used for cell separation as well as for pharmaceutical and tissue engineering applications because of their unique thermo-responsive properties (1,2, 3). PNIPAAm undergoes a reversible temperature-sensitive coil-to-globule transition in aqueous solutions at approximately 32^0C (its lower critical solution temperature, LCST), which lies between room temperature and physiological temperature (37^0C). The LCST of PNIPAAm can be increased by copolymerization with hydrophilic monomers or decreased by using hydrophobic monomers (4,5,6).

Alginate, a polysaccharide extracted from brown algae has been used as a hydrophilic component of hydrogels for many biomedical applications. The ability of alginate sodium salt to pharmaceutical industry, being applied as a carrier (hydrophilic matrix) for controlled release of oral dosage forms. Matrices incorporating alginate salts or a combination of alginate with other polymers have been employed to successfully prolong release of many drugs (7,8).

Since chitosan itself is non-toxic (9), biodegradable (10) and biocompatible (11), several biological applications have been reported for chitosan, including chelation processes (12), a cholesterol trap (13) and a drug carrier (14,15). Recently, there has been a growing interest in chemical modification of chitosan to improve its solubility and widen its applications (16,17). The chemical modification is a powerful tool to control the interaction of the polymer with drugs, to enhance the drug loading efficiency as well as tailor the drug release period. Chemically modified chitosans have great utility in controlled release and targeting studies of almost all class of bioactive molecules.

Chitosan has been modified by ionic crosslinking (e.g. with tripolyphosphate, TPP) to prepare intelligent drug delivery systems

(18), and by carboxymethylation for repair and regeneration of bone tissues (19) or use as antioxidant agent (20).

The aim of the present investigation to compare the kinetics of swelling and ketoprofen release from different matrices as N-isopropyl acrylamide (NIP)/alginate (Alg) hydrogel (75/25), chitosan (C) and chitosan modified with phthalic anhydride (CF) matrices.

EXPERIMENTAL

Materials

The monomer N-isopropyl acrylamide (Aldrich) 97% (NIPAAm), the polysaccharide alginic acid extracted from brown algae (Fluka), M_w = 48 000 - 186 000 with reduced viscosity at 25^0C of 2.41 mL·g^{-1}, c = 0.2 g/dL (Alg) and the crosslinking agent N,N'-methylene bis-acrylamide (Fluka) were all used as received.

Chitosan – (Aldrich) with weight average molecular weight M_w = 400 000 and acetylating degree of 68% was used.

Phthalic anhydride-modified chitosan was synthesized as follows: the N- substituted chitosan derivative was obtained by treating chitosan with phthalic anhydride, an Aldrich product, in dimethylsulfoxide (DMSO) under nitrogen flow and mixing for 6 hours at 80°C according to Hirano's method (21). The obtained modified chitosan was further purified by reprecipitation in ice-distilled water and sodium carbonate (Na_2CO_3); then the precipitate was collected, centrifuged and washed with ethyl ether and then dyalized against acetone for 3 days in order to remove the traces of solvent. The structure of obtained product was confirmed by of FT-IR and H-NMR spectra (22).

Ketoprofen is a classical nonsteroidal anti-inflammatory (first generation), analgesic and antipyretic drug efficient in the treatment of the rheumatoid arthritis and osteoarthritis, spondylitis, as well as soft tissue injuries and also to reduce fever. It is

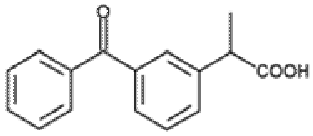

Fig. 1. Structure of ketoprofen (23)

a water insoluble substance but it dissolves in solvents like ethanol, methylene chloride, dimethylsulphoxide (DMSO) and dimethylformamide (DMF). The structure of ketoprofen is presented in fig. 1.

METHODS

Swelling kinetics

The kinetics of the swelling was carried out by weight measurements performed at room temperature in ethanol. The equilibrium swelling degree was calculated according to the Eq. (1).

$$Q_{max}\,(\%) = (W_t - W_d)/W_d \bullet 100 \qquad (1)$$

where W_t is the weight of the swell samples at time t and W_d is the dry weight of the sample.

To determine the kinetics of solvent diffusion into the matrices (swelling) the following equation was used (24):

$$F_t = \frac{W_t}{W_{eq}} = k_{sw} t^{n_{sw}} \qquad (2)$$

where W_t and W_{eq} represent the amount of ethanol solution, absorbed by the matrices at time t and at equilibrium, respectively, k_{sw} is the swelling constant characteristic of the system and n_{sw} is the power law diffusion exponent which takes into account the type of solvent transport. Eq. (2) applies to initial states of swelling (swelling degree less than 60%) and linearity is observed when log F_t as a function of log t is represented.

Drug loading and *in vitro* release studies

The drug loading of the hydrogel matrices was carried out by mixing ketoprofen with dried hydrogel in powder form. During the drug release study, at predetermined time intervals, 1 mL sample was withdrawn

from the release medium and concentration of ketoprofen at 254 nm in the release medium were determined using a UV-VIS spectrophotometer HP 8450A.

The concentrations of the drugs were calculated based on calibration curves determined for drug at specific maximum absorption wavelengths.

A simple, semi-empirical equation using Korsmeyer and Peppas model was used to kinetically analyze the data regarding the drug release from studied matrices system which is applied at the initial stages (approximately 60 % fractional release) (25, 26,27,28,29,30,31) :

$$M_t / M_\infty = k_r t^{n_r} \quad (3)$$

where $M_t/M_á$ represents the fraction of the drug released at time t, M_t and M_8 are the absolute cumulative amount of drug released at time t and at infinite time (in this case maximum release amount in the experimental conditions used, at the plateau of the release curves), respectively, k_r is a constant incorporating characteristics of the macromolecular matrix and the drug n_r is the diffusion exponent, which is indicative of the release mechanism. In the equation above a value of $n_r = 0.5$ indicates a Fickian diffusion mechanism of the drug from matrix, while a value $0.5 < n_r < 1$ indicates an anomalous or non-Fickian behaviour. When $n_r = 1$ a case II transport mechanism is involved while $n_r > 1$ indicates a special case II transport mechanism (32,33,34,35).

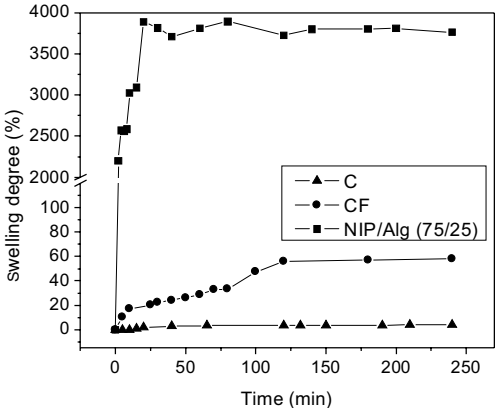

Fig. 2. Swelling profiles of studied hydrogels in ethanol medium, at 25 ^{0}C

The corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release versus time.

FT-IR spectroscopy

The hydrogels loaded with ketoprofen were analyzed by FT-IR spectroscopy, using the KBr pellet technique. The spectra were scanned on a Bruker VERTEX 70 (USA) device, over a 4000-500 cm^{-1} range, at a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSIONS

Swelling kinetic studies

The swelling behaviour of the tested matrices in ethanol medium at 25^{0}C is shown in fig. 2.

To determine the swelling behavior, the chitosan-based matrices were tested in saturated ethanol vapors atmosphere, at 25^{0}C. The swelling of chitosan in ethanol is very low of only 4%. It is evident that the character of swelling curves for both chitosan matrices is different, because of modifying process with phthalic anhydride, the swelling ratio in this case increased being of 58%.

For 75/25 NIP/Alg hydrogels, the swelling studies were done by immersing the samples in ethanol solution at 25^{0}C, reaching a stable equilibrium after 30 minutes with a swelling degree of 3800 %.

In tab. I, the kinetic parameters of swelling for studied hydrogel are given.

The values obtained for swelling parameter (n_{sw}) in ethanol medium at 25^{0}C varies in range between 0.01-0.15 indicating an anomalous mechanism of swelling.

In vitro release studies

In case of chitosan-based matrices, the modification process with phthalic anhydride, determines a difference between amounts of drug released, the highest percent, about 43%, being obtained for CF-

TABLE I
Kinetic parameters of swelling for studied hydrogels

Hydrogels	n_{sw}	k_{sw} (min^{-n})
C	0.01	0.94
CF	0.05	0.65
75/25 NIP/Alg	0.15	0.53

-based matrices and the lowest for chitosan matrix. Release profiles are similar with the swelling ones. To explain this behaviour FT-IR spectroscopy is used. Appreciatively 96% amount of drug released characterizes the 75/25 NIP/Alg hydrogels.

Infrared spectroscopy was used to study the interactions between the drug and the polymers. The spectra of the studied systems are given in fig. 4.

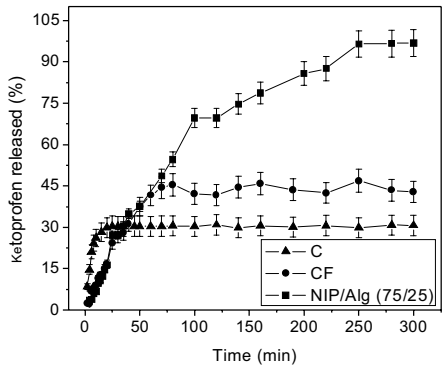

Fig. 3. The release profiles of ketoprofen in ethanol from studied hydrogels, at 25^{0}C

Ketoprofen has a carboxylic acid group, which can interact with the functional groups of the polymers. For ketoprofen, the cha-

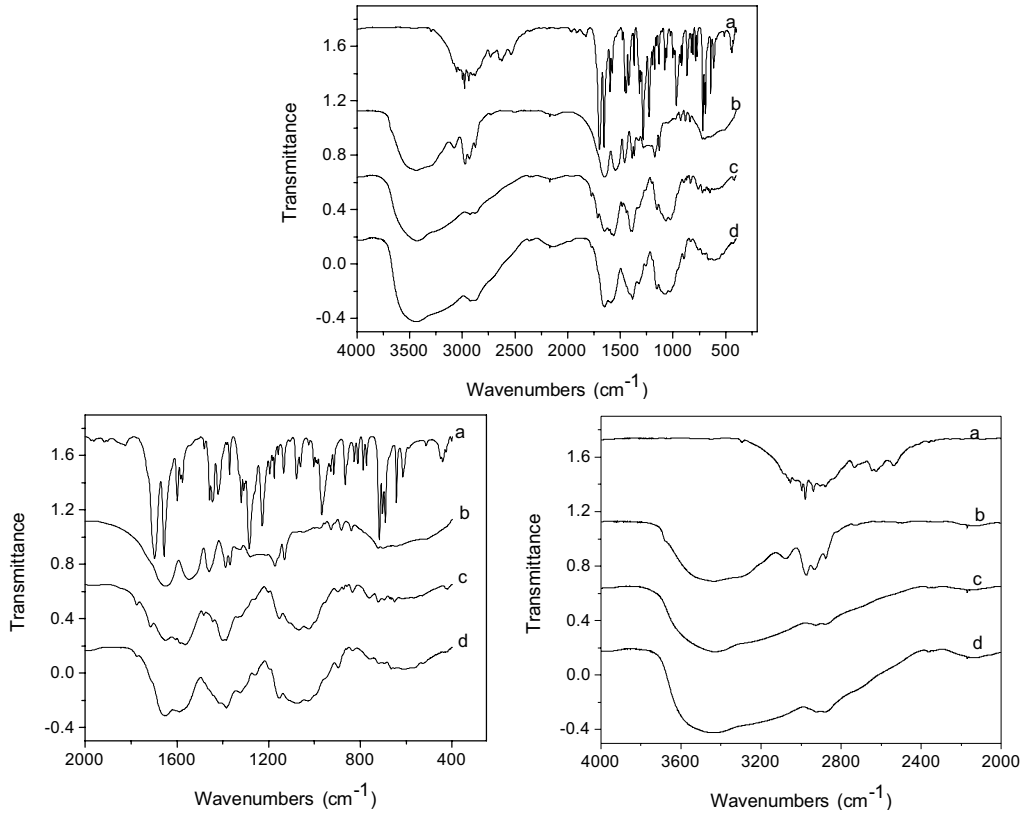

Fig. 4. FT-IR spectra in two representative spectral region of (a) ketoprofen, and studied matrices : (b) 75/25 NIP/Alg, (c) chitosan modified with phthalic anhydride, (d) chitosan, loaded with ketoprofen

TABLE II
The kinetic parameters of ketoprofen released from studied matrices

Hydrogels	Korsmeyer-Peppas equation			First order kinetic model	
	n_r	R	$k_r 10^3$ (min^{-n})	$k_r 10^3$ (min^{-1})	R
C	0.72	0.99	55.25	33.04	0.98
CF	0.83	0.99	14.14	8.16	0.99
75/25 NIP/Alg	1.18	0.99	4.43	7.52	0.98

racteristic bands appeared at 2876 cm^{-1} due to CH stretching of CH3 group masked by symmetric OH, 1697 cm^{-1} due to the C=O stretching vibration of the carboxylic group, a band observed at 1655 cm^{-1} represented by the carbonyl C==O stretching vibrations, and a wide band between 811 cm^{-1}-642 cm^{-1}, characteristic to C-H deformation of aromatic ring (fig. 4a).

In case of NIP/Alg (75/25) ketoprofen loaded hydrogels, the IR spectra showed characteristic peaks of ketoprofen at 2974 cm^{-1}-2876 cm^{-1}, 2169 cm^{-1}-2125 cm^{-1}, 1650 cm^{-1} regions and 839 cm^{-1}-1094 cm^{-1} band (fig. 4b).

For chitosan-phthalic anhydride ketoprofen loaded matrices, the IR spectra showed characteristic peaks of ketoprofen at 2978 cm^{-1}-2877 cm^{-1} band, 1384 cm^{-1}, 1651 cm^{-1}, and the region 834 cm^{-1}-615 cm^{-1}, respectively (fig. 4c).

The spectra of ketoprofen loaded hydrogels prepared with chitosan showed that the peaks at 1697 cm^{-1} and 1655 cm^{-1} were found at 1652 cm^{-1} and 1646 cm^{-1} respectively and the band 811 cm^{-1}- 642 cm^{-1} is replaced by 758 cm^{-1}-502 cm^{-1} bands, respectively (fig. 4d) which indicate major modifications in the structure after loading.

These bands correspond to amide groups indicating formation of a new compounds and possible drug-matrix interactions through covalent bonding and/or hydrogen bonding. This explains the slower release rate and smaller release amount of ketoprofen from the chitosan matrix.

Kinetic mechanism

The kinetic parameters for ketoprofen release in ethanol solution from tested hydrogels are presented in table II.

The values of the diffusion exponent (n_r) obtained for ketoprofen release from tested matrices indicates an anomalous transport mechanism for chitosan and chitosan modified with phthalic anhydride hydrogels, namely 0.72 and 0.83, respectively, which appeared by coupling Fickian diffusion with the relaxation of the hydrogel network. For 75/25 NIP/Alg hydrogel the n_r value is 1.18 sugesting a case II transport mechanism.

CONCLUSIONS

Potential applications of chitosan-based matrices and 75/25 NIP/Alg hydrogels in controlled drug delivery were tested. The composition of hydrogels had an important effect on ketoprofen release.

The swelling exponent values $n_{sw} < 0.5$ indicated a non-Fickian character of ethanol diffusion into the hydrogels.

The values of the diffusion exponent (n_r) obtained for ketoprofen release from chitosan-based matrices indicates an anomalous transport mechanism and a case II transport mechanism for 75/25 NIP/Alg.

REFERENCES

1. Kavanagh CA, Rochev YA, Gallagher WM, Dawson KA, Keenan AK. Local drug delivery in restenosis injury : thermoresponsive co-polymers as potential drug delivery systems. *Pharmacoll Ther* 2004 ; 102 : 1-15.

2. Piskin E, Dincer S, Turk M. Gene delivery-intelligent but just at the beginning. *J Biomater Sci Polym Edn* 2004 ; 15 : 1181-1202.
3. Hirose M, Yamato M, Kwon OH et al. Temperature surface for novel co-culture systems of hepatocytes with endothelial cells : 2-D patterned and double layered co-cultures. *Yonsei Med J* 2000 ; 41 : 803-813.
4. Schmaljohann D, Oswald J, Jorgensen B, Nitschke M, Beyerlein D, Werner C. Thermo-responsive PNiPAAm-g-PEG films for controlled cell detachment. *Biomacromolecules* 2003 ; 4 : 1733-1739.
5. Neradovic D, Soga O, Van Nostrum CF, Hennink WE. The effect of the processing and formulation parameters on the size of nanoparticles based on block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide) with and without hydrolytically sensitive groups. *Biomaterials* 2004 ; 25 : 2409-2418.
6. Ramanan RMK, Chellamuthu P, Tang L, Nguyen KT. Development of a temperature-sensitive composite hydrogel for drug delivery applications. *Biotechnol Prog* 2006 ; 22 : 118-125.
7. Efentakis M, Buckton G. The effect of erosion and swelling on the dissolution of theophylline from low and high viscosity sodium alginate matrices. *Pharm Dev Technol* 2002 ; 7 : 69–77.
8. Liew CV, Chan LW, Ching AL, Heng PWS. Evaluation of sodium alginate as drug release modifier in matrix tablets. *Int J Pharm* 2006 ; 309 : 25–37.
9. Hwang DC, Damodaran S. Selective precipitation and removal of lipids from cheese whey using chitosan. *J Agric Food Chem* 1995 ; 43 : 33.
10. Shigemasa Y, Saito K, Sashiwa H, Saimoto H, Enzymatic degradation of chitins and partially deacetylated chitins. *Int J Biol Macromol* 1994 ; 16 : 43.
11. Suh JK, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering : a review. *Biomaterials* 2000 ; 21 : 2589.
12. Bhatia SC, Ravi NA. A magnetic study of an Fe-chitosan complex and its relevance to other biomolecules. *Biomacromolecules* 2000 ; 1 : 413.
13. Chiang MT, Yao HT, Chen HC. Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. *Biosci Biotechnol Biochem* 2000 ; 64 : 965.
14. Koseva N, Manolova N, Markova N, Radoucheva T, Rashkov I. Chitosan gel beads as drug carriers. *Polym Bull* 1999 ; 43 : 101.
15. Prabaharan M, Mano JF. Chitosan-based particles for controlled drug delivery systems. *Drug Delivery* 2005 ; 12 : 41.
16. Jayakumar R, Prabaharan M, Reis RL, Mano JF. Graft Copolymerized Chitosan-Present Status and Applications. *Carbohydr Polym* 2005 ; 62 : 142.
17. Prabaharan M, Mano JF. Chitosan derivatives bearing cyclodextrin cavitiesas novel adsorbent matrices. *Carbohydr Polym* 2006 ; 63 : 153.
18. Mi FL, Shyu SS, Chen CT, Lai JY. Adsorption of indomethacin onto chemically modified chitosan beads. *Polymer* 2002 ; 43 : 757.
19. Tokura S, Tamura H. O-carboxymethyl-chitin concentration in granulocytes during bone repair. *Biomacromolecules* 2001 ; 2 : 417.
20. Xie W, Xu P, Liu Q. Antioxidant activity of water-soluble chitosan derivatives. *Bioorg Med Chem Lett* 2001 ; 11 : 1699.
21. Hirano S, Moriyasu T. N-(Carboxyacyl) chitosans. *Carbohydr Res* 1981 ; 92 : 323–327.
22. Duncianu CN, Vasile C. Hydrogels based on chitosan and its derivatives. poster presentation at "14th Rolduc Polymer Meeting 2008", May 18-21st 2008, Kerkrade, Netherland.
23. http : //www.aapspharmscitech.org/view
24. Berens AR, Hopfenberg HB. *Polymer* 1978 ; 19 (5) : 489.
25. Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm Sci* 1961 ; 50 : 874.
26. Gohel MC, Panchal MK, Jogani VV. Novel Mathematical Method for Quantitative Expression of Deviation from the Higuchi Model. *AAPS Pharm Sci Tech* 2000 ; article 31.

27. Ritger PL, Peppas NA. A simple equation for description of solute release. I. Fickian and non-fickian release from non-swellable devices in the form slabs, spheres, cylinder or discs. *J Controlled Release* 1987 ; 5 : 23.
28. Chen J, Sun J, Yang L et al. Preparation and characterization of a novel IPN hydrogel membrane of poly (Nisopropylacrylamide)/carboxymethyl chitosan (PNIPAAM/CMCS). *Radiation Physics Chemistry* 2007 ; 76 : 1425.
29. Peppas NA. Analysis of fickian and non-fickian drug release from polymers. *Pharm Acta Helv* 1985 ; 60 : 110.
30. Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int J Pharm* 1989 ; 57 : 169.
31. Peppas NA. *Hydrogels in Medicine and Pharmacy*. Boca Raton : CRCPress, 1986.
32. Katime I, Novoa R, Zuluaga F. Swelling kinetics and release studies of theopylline and aminophylline from acrylic acid/n-alkyl methacrylate hydrogels. *Eur Polym J* 2001 ; 37 : 1465.
33. Korsmeyer RW, Peppas, NA. Solute and penetrant dDiffusion in swellable polymers. III. Drug release from gGlassy P(HEMA-co-NVP) copolymers. *J Control Release* 1984 ; 1 : 89.
34. Serra L, Domenechc J, Peppas NA. Drug transport mechanisms and release kinetics from molecularly designed poly(acrylic acid-g-ethylene glycol) hydrogels. *Biomaterials* 2006 ; 27 : 5440.
35. Berg MC, Zhai L, Cohen RE, Rubner MF. Controlled drug release from porous polyelectrolyte multilayers. *Biomacromolecules* 2006 ; 7 : 357.

NOUTĂŢI

TERAPIE CU ^{131}I ÎN TUMORI MALIGNE NON-TIROIDIENE ?

Sistemul de transport Na/I (Na/I symporter, NIS) este o proteină existentă în mod normal în membrana celulelor tiroidiene, care mediază influxul de iod, atât stabil, utilizat, ulterior, fiziologic în sinteza hormonilor tiroidieni, cât şi radioactiv (^{131}I, ^{123}I, ^{124}I), permiţând vizualizarea scintigrafică tiroidiană şi mediind radioiodoterapia cancerului tiroidian. Captarea scintigrafică tiroidiană de $^{99m}TcO_4^-$ este, de asemenea, mediată de proteina NIS. Studii experimentale recente aduc în discuţie posibilitatea utilizării NIS pentru imagistica neinvazivă a expresiei genice şi, posibil, radioiodoterapia unor tumori maligne non-tiroidiene cărora li s-a indus genetic expresia membranară a acestei proteine (Baril P, Martin-Duque P, Vassaux G. Visualization of gene expression in the live subject using the Na/I symporter as a reporter gene : applications in biotherapy. *Br J Pharmacol* 2010 ; 159 (4) : 761-771) .

Cipriana Ştefănescu

ACTIVITATEA UNEI NOI FLUOROCHINOLONE IN CERCETARE, FINAFLOXACIN, ASUPRA UNOR IZOLATE DE *ACINETOBACTER BAUMANII*

Autorii studiului compară activitatea unei noi fluorochinolone, finafloxacin, cu cea a ciprofloxacinului, asupra unor izolate de *Acinetobacter baumanii*, în condiţii de pH diferite : 7,2 *versus* 5,8. Finafloxacinul a demonstrat o activitate superioară ciprofloxacinului, în condiţii de aciditate. Activitatea celor două flourochinolone a fost comparabilă, în condiţii de pH 7,2. Finafloxcin promite a fi un nou agent antimicrobian pentru tratamentul infecţiilor cu *Acinetobacter baumanii* în compartimentele acide ale organismului uman (Higgins PG, Stubbings W, Wisplinghoff H, Seifert H. Activity of the investigational fluoroquinolone Finafloxacin against Ciprofloxacin-sensitive and –resistant *Acinetobacter baumannii* isolates. *Antimicrob Agents Chemother* 2010 ; 54 (4) : 1613-1615).

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