Chapter 8

Hydrophilic Matrix Tablets Based on Carbopol for Improving the Oral Bioavailability of Sodium Alendronate — *In vitro* and *In vivo* Assessment

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Additional information is available at the end of the chapter

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1. Introduction

Sodium alendronate (hereinafter, AL-Na) is one of the most important drug substances of the bisphosphonate class administered orally for the treatment of all types of osteoporosis. Alendronate has a positive influence on all symptoms of osteoporosis by increasing bone mineral density and pacient mobility, reducing the risk of osteoporotic fractures in all vulnerabilities (vertebrae, hip, arm and femoral neck) and having an analgesic effect in bone pain. The anti-resorptive mechanism of action of AL-Na is complex and involves effects at both the molecular and the cellular level. Osteoclasts and their precursors are the target cells of AL-Na and, by internalization, they absorb the AL-Na molecule from the surface of bone. At the molecular level, AL-Na prevents the conversion of dimethyl-allyl pyrophosphate to geranyl - geranyl - pyrophosphate. Besides the biochemical effects described above, AL-Na also produces a number of effects at the cellular level, among which loss of brush border and fractures in the osteoclast cytoskeleton. All these actions of AL-Na ultimately lead to an increase in bone mineral density and to osteoclast-mediated inhibition of bone resorption. It is important to note that the anti-resorptive effect of AL-Na occurs the day after the first dose, the intensity of the therapeutical effect being directly dependent on the active substance dose administered [1, 2]. The results of the Fosamax Fracture Intervention Trial Long-term Extension (FLEX) showed that patients treated with AL-Na for 5 years presented an obvious increase in bone mineral density in the femoral neck, as well as in the lumbar spine. The results of this 10-year study showed a 17.7% decrease in the risk of vertebral fracture compared with placebo [3, 4]. Currently, AL-Na is used in daily (10 mg/tablet) or weekly (70 mg/tablet with or without

2 800 I.U. of vitamin D_3) conventional release tablets. Tablets with a higher concentration of vitamin D3 (70 mg AL-Na/5600 IU cholecalciferol/tablet) have also been introduced in therapy quite recently [5]. The exogenous intake of cholecalciferol contributes to the normalization of calcium homeostasis in the body and to the optimization of the anti-resorptive effect caused by AL-Na. Furthermore, it is known that a low level of vitamin D3 manifests in muscle weakness which can cause accidents with high risk of fracture [6].Once-weekly administered tablets (70 mg/tablet) have largely increased patient compliance with alendronate and led to a reduction in the incidence of gastrointestinal side effects. Pharmacokinetic studies have shown that oral doses of alendronate in the range 5-80 mg generate a bioavailability of 0.1-1%, with a fraction of 50% of the amount deposited in the bone. In addition, it was shown that oral administration of 10 mg alendronate daily and of 70 mg alendronate once-weekly generates the same level of increase in bone mineral density: 5-6% in the vertebrae and 3-9% in the femoral bone. Alendronate administered orally in the form of a solution (70 mg/vial) is intended for patients with deficiencies in swallowing. The latest alendronate formulation approved by Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMEA) is the association of 70 mg alendronate with 2800 I.U. vitamin D in immediate release tablets [7, 8]. According to FDA guidelines, there is no deadline or optimal duration for the treatment with AL-Na. The treatment with AL-Na is recommended for as long as necessary along with regular reassessment of patients (at intervals of no longer than five years) in order to determine bone mineral density and fracture risk [9].

However, low oral bioavailability (under 1%) is the most important disadvantage of AL-Na. It is caused by several factors such as: low permeability due to its negatively charged molecules (AL belongs to the 3rd class of biopharmaceutical classification system); short plasma half-time ($T_{½}$ = 0.5-2 h); its chelatation by Ca^{2+} ions resulting in non-absorbable complexes. Research in bisphosphonates in general aims *to increase the bioavailability of these substances, to decrease side effects, to increase adherence to treatment* especially for elderly patients. AL-Na has been in the attention of drug researchers and literature has presented a large number of studies on this topic. Investigations conducted in the formulation of alendronate sodium are directed to conventional pharmaceutical forms (solutions, emulsions, gels) as well as to modified-release forms (matrix tablets, microemulsions, micro– and nanoparticulate drug delivery systems) administered on various routes (Table 1).

In this study we have investigated the possibility of improving the oral bioavailability of AL-Na by including it in hydrophilic matrix tablets based on various sorts of Carbopol. The role of chitosan (CHT) and trimethyl chitosan (TMC) as AL-Na absorption enhancers was also researched.

Carbopol polymers form an important class of excipients used for the formulation and preparation of sustained release hydrophilic matrix tablets. Carbopols were synthesized and patented in 1957 [24]. Since then, a variety of therapeutic agents (such as the agents in the 3rd group of the biopharmaceutical system of drug classification: atenolol, verapamil, theophylline, metoprolol, ranitidine, etc.) have been formulated and prepared in different Carbopol-based formulations with controlled release [25-30].

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Administration route	Pharmaceutical dosage form	References
Oral route	*Modified-release tablets:* *1. HPMC-based matrix tablets* with different degrees of viscosity; *2. Gastro-resistant tablets* using various sorts of ethyl cellulose and acrylic derivatives as coating agents(Aquacoat ECD, Eudragit L30, Eudragit L-30-D55)	[10] [11]
	Effervescent tablets	[12, 13]
	Solutions	[14]
	H/L Microemulsions with Captex 200 ® and lecithin as emulsifier	[15]
	Microparticulate therapeutic systems based on Eudragit S100/HPMC PLGA	[16-18]
Gingival-dental mucosa	*Carbopol-based gels*	[19]
Vaginal mucosa	*HPMC-based gels*	[20]
Nasal mucosa	*Microparticulate therapeutic systems based on* HPMC as unique excipient or associated with PVP	[21]
Transcutaneous	*Reservoir-type transdermal therapeutic systems* based on acrylic polymers in combination with various percutaneous absorption promoters: lauric acid, oleic acid, linoleic acid, myristic acid	[22]
	Gels prepared with different sorts of Duro-Tak® (acrylic acid copolymers) using propylene glycol and fatty acids as absorption promoters (lauric acid, oleic acid, linoleic acid and myristic) at various concentrations	[23]

Table 1. Examples of studies focused on increasing AL-Na bioavailability

From the chemical point of view, Carbopols are crosslinked polymers of acrylic acid (Figure 1), insoluble in water, characterized by a high degree of hydration.

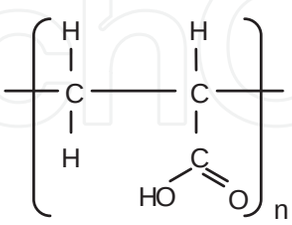

Figure 1. General chemical structure of Carbopol polymers

According to the literature, Carbopol polymers generally exhibit good compressibility characteristics, and this achieves compression at low pressures; are compatible with other matrix forming hydrophilic agents and many excipients; may help mask taste and have bioadhesive properties [31].

In the formulations studied, we used three Carbopol sorts with different crosslinking degrees: Carbopol 971 C (C 971), Carbopol 71 C (C 71) and Carbopol 974 C (C 974). These polymers are obtained by the crosslinking reaction of acrylic acid with allyl penta erythrol, followed by a polymerization reaction in ethyl acetate and neutralization with aqueous potassium hydroxide 1-3%. Although C 971 and C 974 are obtained by a similar technological process, the difference is that sort C 971 has a lower level of crosslinking agent compared to C 974. C 71 is the granular form of C 971 recommended to be used in direct compression [32-34]. Unlike the hydrophilic linear polymers soluble in polar solvents, whose absorption capacity is dependent on molecular weight, crosslinked Carbopol polymers are insoluble in water, since crosslinking degree is the determinant parameter of he matrix absorption capacity [35-37].

2. Investigations on the influence of carbopol sort on the release of alendronate sodium from modified release matrix tablets

Prior to this research, in our department of Pharmaceutical Technology we have also studied the influence of the concentration of Carbopol on the hydration and erosion characteristics of matrices in order to establish the optimal conditions for the development of these formulations [38-40]. Based on these results, in the present study we introduced 15% hydrophilic matrix forming polymer in the formulation. We used three sorts of Carbopol with different crosslinking degrees as matrix generating polymers: C 71 to C 971, polymers with a low of crosslinking degree, and C 974, a Carbopol with a high crosslinking degree. The research conducted has been focused on investigating the influence of the Carbopol sort on the release of alendronate from modified release matrix tablets.

2.1. Materials and methods

2.1.1. Materials

Alendronate sodium trihydrate (Apotex Pharmaceutics INC, USA), Carbopol 974 P NF, 971 P NF, 71 G NF (Noveon Inc.), Ludipress LCE (BASF), Aerosil 200 (Degussa), Magnesium stearate (Union Derlivan S.A. Spain).

2.1.2. Methods

2.1.2.1. Preparation of matrix tablets

Three formulations of alendronate sodium matrix tablets were prepared by the method of direct compression in the Korsh EK0 tablet machine (punch diameter of 9 mm, 8 to 10 kN compression force). As matrix forming polymer, formulations denoted F1, F2, F3 contain, as follows: 15% C 71 in F1, 15% C 971 in F2, and a mixture of 15:2% C 971:C974 – 15:2% in F3 (Table 2).

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Ingredients (mg %)	Formulation		
	F1	F2	F3
C 71	15	–	–
C 971	–	15	15
C 974	–	–	2
AL-Na	13.05	13.05	13.05
Mg Stearate	0.5	0.5	0.8
Aerosil	–	0.5	1.5
Ludipress LCE	71.45	70.95	67.65

Table 2. Formulations of matrix tablets with AL modified release based on Carbopol

2.1.2.2. "In vitro" dissolution investigations

"In vitro" dissolution tests were carried out on a *SR 8 Plus Series* (AB & L Jasco) device, under the following experimental protocol: *dissolution medium*: pH 1.2 solution (0.1 N HCl) for the first 2 hours (a medium simulating gastric fluids) and solution pH 6.8 (phosphate buffer solution) for the next 10 hours (a medium simulating intestinal fluids); *Apparatus 2* (*paddle*); *bath temperature*: 37ºC±0.5 ºC; *rotation speed*: 50 rpm; sampling interval was set to every hour during the 12 hours of the test (at every collection, 7 ml of sample were replaced with the same volume of medium).

One sample from the aliquot was subjected to the derivation and dosage procedure described in the USP monography for the HPLC analysis of sodium alendronate. The quantitative determination equipment included the following modules: HPLC type HP 1090 series II provided with a diode array detector, UV-VIS spectrophotometer Agilent technologies 8453 and Zorbax C18 column. The mobile phase was a mixture of methanol, acetonitrile and water in the following proportions: 17.5:17.5:65. Mobile phase flow was set at 0.3 mL/min., the injection volume was 20μL and the detection was performed at 266 nm [41]. All the experiments were performed in triplicate.

Quantitative data were presented as mean ± standard deviation and statistical analysis was performed using a one-way analysis of variance (one-way ANOVA). A comparison between two means was made using Tukey's test, with statistical significance set at $p < 0.05$.

2.1.2.3. The analysis of the difference factor (f_1) and the similarity factor (f_2)

The two factors assessing the AL release profile in the investigated formulations were calculated according to the following equation:

$$f_1 = \left\{ \frac{\sum_{f-1}^{n} \left| R_t - T_t \right|}{\sum_{t-1}^{n} R_t} \right\} \times 100 \quad (1)$$

$$f_2 = 50 \log_{10} \left\{ \left[1 + \frac{1}{n} \sum_{t-1}^{n} (R_t - T_t) 2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

where: n = the number of sampling time points, R_t = released AL percentage of the reference formula at time point t, T_t = released AL percentage of the test at time point t and $\log_{10} x$ represents the logarithm of x to the base 10 [42].

Difference factor f_1 is a measure for quantifying the differentiation degree of the release profile followed by a drug substance in the formulation. The value of this factor is in the interval 0 - 50. The analogy degree varies inversely with f_1 value. Thus, a value closer to 0 indicates a high similarity between two or more compared formulations. When the dissolution profile between the tested and the reference formula is identical, f_1 is 0 and increases with the dissimilarity between the formulas analyzed. In general, the values of the f_1 factor in the range 0-15 correspond to very small differences between the formulations tested, while values greater than 15 indicate major differences in the release profiles of the drug substances in the compared formulations.

Similarity factor f_2 is a parameter commonly used to compare the dissolution profiles of the solid oral pharmaceutical forms.

When two dissolution profiles of the drug substance are similar, f_2 is in the range of 50-100 [43, 44]. According to the FDA guidelines in force, the values of the f_2 similarity factor included between 50 and 100 show a high degree of similarity (≥ 90%) between the release profiles of the tested formulation against the reference formulation. Theoretically, values of the similarity factor f_2 smaller than 50 are accepted in the interpretation of the *in vitro* dissolution tests. Mention should be made that these values show a similarity <90% between the formulas analyzed.

Note that these two parameters are accepted and recommended by international quality guidelines for the comparative evaluation of the release profile of two or more formulations compared to a reference formulation, as can be seen in equations 1 and 2. While EMEA has not issued any regulations on the determination and analysis of difference factor f_1 and similarity factor f_2, FDA and USP regulate the applicability of these factors for the comparison of the release profile of two or more pharmaceutical formulations by the following clarifications: 1. release profiles between two or more formulas can be compared only when the dissolution test includes a minimum of 12 sampling points; 2 the conditions of the *in vitro* dissolution test should be identical for the reference formulation and for the tested formulations [45-47].

2.2. Results and discussions

Figure 2 presents the results obtained from the *in vitro* dissolution test for the studied formulations.

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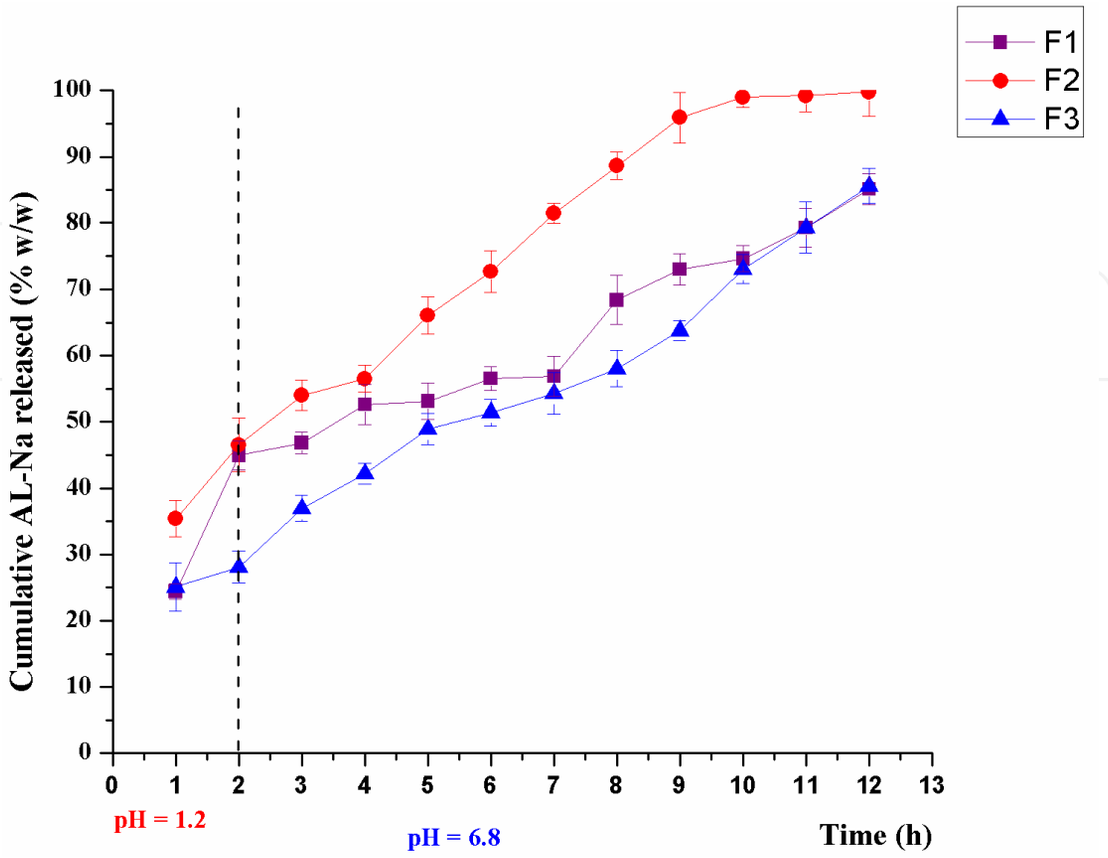

Figure 2. *In vitro* dissolution profile for AL in hydrophilic matrix tablets based on Carbopol

We can see from the results obtained that the dissolution profile of alendronate sodium in formulation F2 based on C 971 is superior to other formulations (99.7889% AL-Na released; t = 12 hours). In theory this result was not foreseeable because C 71 and C 971, the polymers in formulas F1 and F2, have the same degree of crosslinking, sort C71 actually having flow properties optimized by the granular particle form.

The influence of the crosslinking degree of the polymer in the formulation was also highlighted by the pH value of the dissolution medium. Thus, at acidic pH 1.2 simulating gastric fluids, C 71 and C 971, which are polymers with a low degree of crosslinking, showed a uniform hydration resulting in a uniform release of alendronate sodium in the matrix gel layers.

C 974, a polymer with a high degree of crosslinking, optimizes the retardation effect at gastric level through the closely crosslinked gel structure which acts as an element that reduces the diffusion of the drug substance in the hydrated matrix layers during the first hours of the test and accelerates this process after the total hydration of the matrix.

Finally, after performing the dissolution test within 12 hours, we found that the dissolution profile of alendronate sodium is identical in F1, the formula based on C 71, and in F3, the formula in which we associated C 974 as well (F1 = 85.1128% AL-Na released; F3 = 85.5984% AL-Na released), specifying that sort C 974 generates a slow dissolution profile within the first

two hours of the test, at pH 1.2 (F1 = 44.9356% Na released; F2 = 46.5233% AL-Na released, F3 = 28.0566% AL-Na released, t = 2 hours).

The different behavior as regards the dissolution profile of alendronate sodium in modified release matrix tablets is also confirmed by the results obtained in the determination of f_1, the difference factor, and f_2, the similarity factor (Table 3).

Reference formula (R_t)	Test formula (T_t)	f_1	f_2
F1	**F2**	25.149	37.041
F1	**F3**	12.608	***50.940***
F2	**F3**	26.9546	34.1921

Table 3. Values of f_1 and f_2 factors obtained in the comparative analysis F1-F3

In the comparative analysis of the three formulations studied, the difference factor f_1 has values > 0. The similarity factor f_2, which is the most representative for the comparison of the dissolution profile of solid dosage forms also has values that indicate a different dissolution profile for the three formulations investigated (f_2 <50), with the exception of the comparison F1, reference, F3, test, when f_2 = 50, 940. We estimate that this value is determined by the evolution of the release pattern of AL-Na towards the end of the *in vitro* dissolution test, in 6.8 buffer system because there were no significant differences between the two formulas during the first two hours of the test, under conditions of simulated gastric fluid.

2.3. Conclusions

The results of the *in vitro* dissolution test reveal that every sort of Carbopol influences the release profile of alendronate sodium in matrix tablets with modified release. The crosslinking degree of Carbopol is the defining element of the release properties of hydrophilic matrix tablets. Sorts C 71 and C 971, with a low degree of crosslinking, hydrate much better at pH 1.2, a phenomenon which facilitates the dissolution of alendronate sodium, its diffusion through the gel layers of the matrix, and therefore, the release of a greater amount of drug substance compared to formula F3, in which we associated C 974, a high crosslinking polymer, which causes a slowing of the hydration process. The influence of the crosslinking degree of Carbopol also manifests at pH 6.8; F2, the formula based on C 971, generated a release profile of AL-Na superior to formula F3, in which we associated C 974 and C 971, and to formula F1 based on C 71, respectively.

The different dissolution profile of alendronate sodium in modified release matrix tablets based on Carbopol is further confirmed by the values of the two control parameters f_1 and f_2. The similarity factor f_2 is set to 50.940, little over 50, the lower limit of the acceptance criteria, when comparing F1 as reference formulation and F3 as test formulation, but this value is determined by the percentage of sodium alendronate dissolved in the second half of the period of the dissolution test, after t = 6 hours. In the first part of the test, especially in the first two hours, at pH 1.2, the two formulations have very different dissolution profiles.

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3. Investigation of the influence of some absorption promoters on the release of alendronate sodium in modified release matrix tablets

Chitosan is a natural polysaccharide produced by the partial deacetylation of chitin which includes in its structure two copolymers: N-acetylglucosamine and glucosamine (Figure 3). This biodegradable and biocompatible polymer was investigated in various pharmaceutical formulations as absorption promoter, excipient for controlled release tablets or bio-mucoadhesive preparations [48].

Chitosan is soluble in acidic media, pH < 6.5, when the amino groups in its structure are protonated. At pH values > 7, the solubility of chitosan is lower, the occurrence of the transition from the solution to the gel state, which gives chitosan unique properties as an absorption promoter for the oral administration of hydrophilic macromolecular substances including peptides and proteins [49-52]. Some quaternary chitosan derivatives, for example, trimethyl chitosan (TMC), are soluble in a higher pH range (pH=1-9) in a concentration of up to 10% m/m, which results in widening the scope and applicability in this field.

The main action mechanism of CHT and TMC as absorption promoters is to open the intercellular junctions at membrane level, as a result of the interaction between CHT or its positively charged derivative and the negative charges of the sialic acid present in the intestinal mucosa. The absorption of the drug substance is therefore facilitated by para- and transcellular mechanisms [53-58].

In this study we aimed to analyze the influence of the two main absorption promoters, CHT and TMC, on the release of alendronate sodium in modified release matrix tablets based on Carbopols.

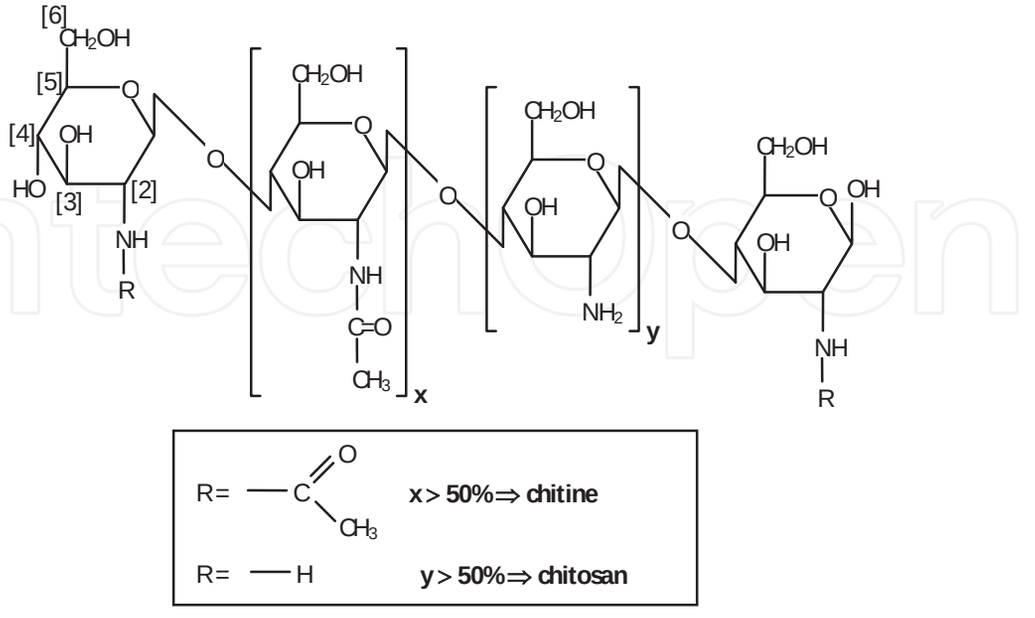

Figure 3. Chitosan - chemical structure

3.1. Materials and methods

3.1.1. Materials

Sodium alendronate trihydrate (Apotex Pharmaceutics INC, USA), *Carbopol 974 P NF, 971 P NF, 71 G NF* (Noveon Inc.), *Ludipress LCE* (BASF), *Aerosil* 200 (Degussa), *Magnesium stearate* (Union Derlivan S.A. Spain), high molecular weight *Chitosan* (CHTh) (degree of deacetylation > 85%, Aldrich), N - *Trimethyl chitosan* (G.L.S. Chemicals & Materials, India).

3.1.2. Methods

3.1.2.1. Preparation of matrix tablets

Nine alendronate sodium matrix tablets formulas were formulated and subjected to direct compression in a tablet machine Korsh EK0 (punch diameter 9 mm, 8.10 kN compression force). Table 4 presents the raw materials used in the preparation of studied formulations.

Ingredients (mg %)	Formulation								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
C 71	15	–	–	15	–	–	15	–	–
C 971	–	15	15	–	15	15	–	15	15
C 974	–	–	2	–	–	2	–	–	2
CHT	–	–	–	6	6	6	–	–	–
TMC	–	–	–	–	–	–	6	6	6
AL	13.05	13.05	13.05	13.05	13.05	13.05	13.05	13.05	13.05
Mg stearate	0.5	0.5	0.8	0.5	0.5	0.8	0.5	5	0.8
Aerosil	–	0.5	1.5	–	0.5	1.5	–	5	1.5
Ludipress LCE	71.45	70.95	67.65	65.45	64.95	61.65	65.45	64.95	61.65

Table 4. Pharmaceutical formulations of modified release matrix tablets with AL-Na based on Carbopols, with absorption promoters

3.1.2.2. "In vitro" dissolution studies

"*In vitro*" dissolution tests were carried out on a *SR 8 Plus Series* (AB & L Jasco) device, under the following experimental protocol described in section I. The tests were carried out on a total of six tablets and the results shown are the average of the six determinations.

The analysis of the difference factor $f_1$and similarity factor f_2 was performed according to the equations described in Section I.

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3.2. Results and discussions

The results of the *in vitro* dissolution test shown in Figure 4 reveal that the AL-Na release profile of the Carbopol-based matrix tablets is maintained even in the presence of CHT. It is worth noting that the released AL-Na amount for formula F5 is approximately 5% lower than for formula F2 without CHT. This result can be explained by the different behavior of CHT at the two pH values.

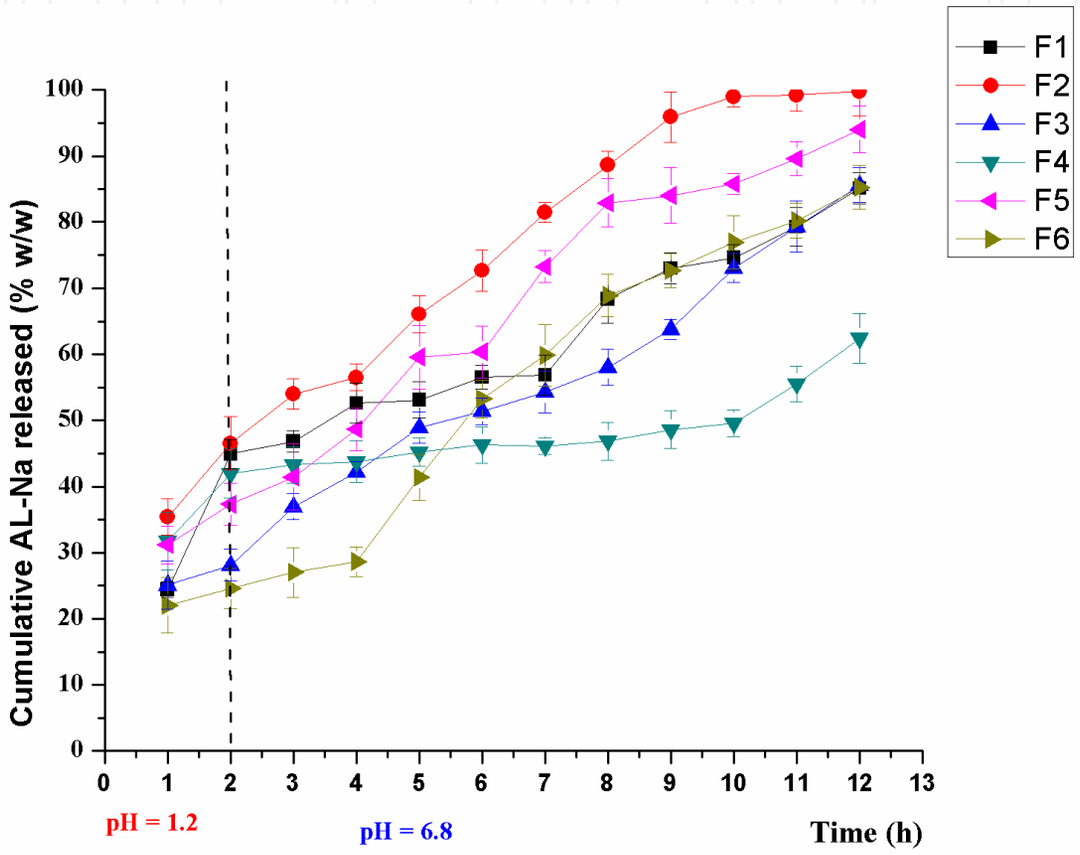

Figure 4. *In vitro* AL dissolution profile in F1-F6 formulas

To reveal the influence of CHT on the alendronate sodium release profile in modified release matrix tablets, we calculated the two control factors f_1 and f_2. The results obtained are presented in Table 5 and show that the dissolution profile is different in formulations F4 and F5 compared to formulas F1 and F2. The comparative analysis of formula F6 as test and formula F3 as reference led to a similarity factor value f_2 greater than 50 (f_2 = 57.187), which means that CHT does not alter the dissolution profile for this formulation. We assume that the mechanism by which CHT influences AL-Na dissolution is based on the low solubility of CHT at pH 6.8. In the case of F6, there is also the influence of C 974, which, as we noted in the previous section, due to its high crosslinking degree, causes a slower hydration of the matrix. In this context we argue that between CHT and C 974 there is a synergism of action on the release profile of alendronate sodium in modified release matrix tablets.

Reference formula (R_t)	Test formula (T_t)	f_1	f_2
F1	**F4**	28.991	31.947
F2	**F5**	12.422	46.748
F3	**F6**	10.799	***57.187***

Table 5. Values of f_1 and f_2 factors obtained in the comparative analysis F1-F3 versus F4-F6

The comparative results of TMC-containing formulations F7-F9 to formulations F1-F3 are shown in Figure 5.

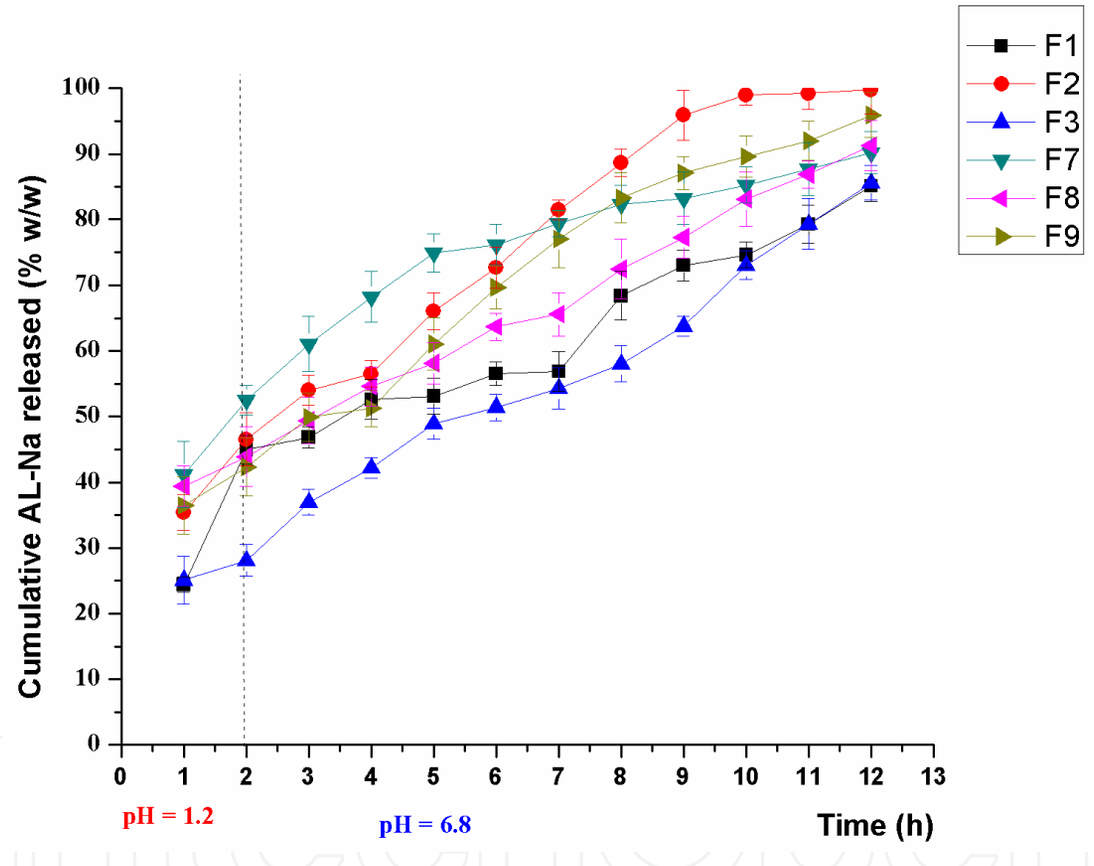

Figure 5. *In vitro* AL dissolution profile in F1-F3-F7-F9 formulas

From the analysis of the data obtained we find that all formulas in which we introduced TMC provide an alendronate sodium release in percentages above 90% during the 12 hours of the dissolution test. For this absorption promoter as well we assume that the influence on the dissolution profile of AL-Na is based on the TMC optimal solubility in the two dissolution media, a property which determined the proper hydration of the matrix tablet, thus facilitating the diffusion and release of alendronate sodium in the matrix. Difference factor f_1 and similarity factor f_2 have values that reveal for formulations F7-F9 a different release profile compared to reference formulations F1-F3 (Table 6).

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Reference formula (R_t)	Test formula (T_t)	f_1	f_2
F1	**F7**	23.247	39.942
F2	**F8**	13.119	47.307
F3	**F9**	27.969	39.230

Table 6. Values of f_1 and f_2 factors obtained in the comparative analysis F1-F3 versus F7-F9

3.3. Conclusions

The comparative analysis of the results obtained from the *in vitro* dissolution test showed that formulas containing absorption promoters have an alendronate sodium dissolution profile that differs from the formulas of the modified release matrix tablets based on Carbopol without absorption promoters, test formulas (F1-F3). This conclusion is confirmed by the values of the two parameters assessing the dissolution profile: difference factor f_1 and similarity factor f_2. The influence of absorption promoters on the AL-Na dissolution profile in the studied modified release matrix tablets can be assigned to the different solubility of CHT and TMC in the two dissolution media.

4. Assessment of the release kinetics of alendronate sodium in modified-release matrix tablets

The analysis of the release kinetics of a drug substance from a dosage form is particularly important in characterizing and defining the pharmacotechnical and biopharmaceutical properties of that particular preparation. In the case of the modified release tablets based on matrix forming hydrophilic polymers, in order to determine the mechanism by which the drug is released and its evolution over time, the understanding of its kinetic release profile is a prerequisite.

The objective of this study is to identify a representative pattern for each formulation studied; taking into account the multiple formulation factors that may influence drug substance release from the modified-release matrix tablet.

4.1. Materials and methods

4.1.1. Materials

Sodium alendronate trihydrate (Apotex Pharmaceutics INC, USA), *Carbopol 974 P NF, 971 P NF, 71 G NF* (Noveon Inc.), *Ludipress LCE* (BASF), *Aerosil* 200 (Degussa), *Magnesium stearate* (Union Derlivan SA Spain), high molecular weight *chitosan* (CHTh) (degree of deacetylation > 85%, Aldrich), *N - Trimethyl chitosan* (G.L.S. Chemicals & Materials, India).

4.1.2. Methods

4.1.2.1. Preparation of matrix tablets

Nine formulations of matrix tablets with alendronate sodium were prepared by the direct compression method according to data presented in Table 4, section II.1.

4.1.2.2. "In vitro" dissolution studies

"*In vitro*" dissolution tests were carried out on a *SR 8 Plus Series* device, under the following experimental protocol described in section I. The tests were carried out on a total of six tablets and the results shown are the average of the six determinations.

4.1.2.3. Data fitting and kinetics of drug release

The kinetics of AL-Na release from the formulations studied was analyzed by fitting on five mathematical models according to the following equations:

The equations corresponding to the models applied in the study are:

$$\text{The zero-order kinetics:} M_t = K_0 \cdot t \tag{3}$$

$$\text{The first-order kinetics:} M_t = 100 \cdot (1 - e^{-k \cdot t}) \tag{4}$$

$$\text{Higuchi release model:} M_t = K_H \cdot t^{0.5} \tag{5}$$

$$\text{The Korsmeyer-Peppas releasemodel:} M_t = K_p \cdot t^n \quad (n = 0.45) \tag{6}$$

$$\text{The Hopfenberg release model:} M_t = 100 \cdot [1 - (1 - K_{HF} \cdot t)^n] \ \ (n = 2; n = 3) \tag{7}$$

where:

$\mathbf{M_t}$ = the amount of the drug dissolved at time t;

$\mathbf{K_0}$ = zero order rate constant;

K = first order rate constant;

$\mathbf{K_H}$ = Higuchi rate constant;

$\mathbf{K_P}$ = Korsmeyer-Peppas rate constant;

$\mathbf{K_{HF}}$ = Hopfenberg rate constant;

n = the release exponent which characterizes the mechanism of drug release;

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t = time

Data fitting was performed by linear and nonlinear regression, using Matlab 7.1 software.

The Akaike index (AIC) and the correlation coefficient R^2 were the criteria for the selection of the model which describes with the highest fidelity the release profile for each formulation studied. The best model prediction requires that the value of R^2 is as close as possible to 1, and the Akaike index has the smallest values [59-61].

The two model prediction parameters were calculated according to the following equations:

$$AIC = n \cdot \ln(SSR / n) + 2 \cdot p \tag{8}$$

where:

n = number of data points;

SSR = sum of squared errors;

p = number of estimated parameters.

$$R^2 = 1 - \frac{\sum_{i-1}^{n} \left(yi - y\hat{}i \right)^2}{\sum_{i-1}^{n} (yi - \bar{y})^2} \tag{9}$$

where:

y_i = data obtained experimentally;

$y\hat{}i$ = values approximated by the model;

$\bar{y}$ = average of experimental values

4.2. Results and discussions

The results obtained from fitting data on the five experimental models are shown in Table 7 and Figures 6-11.

Analyzing these data we can see that the zero-order release kinetic model is not representative for the formulations studied (Figure 6); this conclusion was predictable considering the data in the literature according to which this model is representative for the description of the release profile of drug substances in osmotic systems with a constant speed independent of the concentration (62-65). Mention should be made that the inclusion in the formula of C 74, a polymer with a high degree of crosslinking which causes a reduction in the rate of matrix hydration in an acidic environment, has led to good values of parameter R^2. However, the values of the Akaike index are not representative as to validate the zero-order kinetic release model for the formulations containing this polymer (Table 7).

The fitting of data from the nine formulas analyzed on the Higuchi and Korsmeyer-Peppas models confirms the release of alendronate sodium in Carbopol-based matrix tablets by a process of diffusion. The values of the parameters of the equation corresponding to the Higuchi model are representative enough to suggest that the release of the active substance in the studied matrices occurs through a diffusion process based on Fick's law according to which the release of the active substance is directly proportional to the square root of time. The Korsmeyer-Peppas model, where n is equal to 0.5 defines the active substance release through a phenomenon of Fickian diffusion from plane shapes. Fitting on this model is further substantiated by the data obtained in previous studies in which we have investigated the hydration behavior of matrix tablets, when we found an increase in direct proportion between the diameter and the thickness of the matrix, leading to the preservation of the plane geometric shape [39, 40].

Kinetic model	Parameters of model	Formulation								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero-order	K_0	8.3238	10.2973	7.6325	7.9905	9.4400	7.8339	9.6863	8.8962	9.6104
	R^2	0.8401	0.9073	***0.9511***	0.8678	0.8990	***0.9678***	0.7391	0.8765	0.9036
	AIC	73.12	72.36	***59.09***	70.98	70.50	***50.12***	82.49	73.56	72.38
First-order	K	0.1900	0.2500	0.1200	0.1700	0.2100	0.1500	0.2600	0.1800	0.2100
	R^2	0.9371	***0.9758***	0.9572	0.9138	0.9677	0.9460	0.9514	0.9308	0.9624
	AIC	57.95	***43.41***	48.59	59.70	44.21	52.35	55.10	56.43	48.44
Higuchi	K_H	24.7646	30.3197	22.2784	23.7095	27.7771	22.5600	29.1919	26.4044	28.3790
	R^2	***0.9652***	***0.9885***	***0.9720***	***0.9646***	***0.9728***	***0.9391***	***0.9364***	***0.9763***	***0.9871***
	AIC	***42.33***	***32.38***	***37.67***	***41.67***	***41.41***	***54.16***	***56.61***	***40.81***	***32.29***
Korsmeyer-Peppas	K_P	27.4199	33.5240	24.6139	26.2487	30.7108	24.8812	32.3696	29.2325	31.3944
	R^2	***0.9726***	***0.9862***	***0.9631***	***0.9697***	***0.9697***	***0.9214***	***0.9565***	***0.9814***	***0.9872***
	AIC	***37.12***	***36.96***	***42.47***	***37.41***	***43.82***	***57.80***	***50.42***	***34.65***	***30.73***
Hopfenberg (n=2)	K_{HF}	0.0548	0.0844	0.0495	0.0521	0.0679	0.0517	0.0686	0.0612	0.0703
	R^2	0.9160	0.9835	0.9631	0.9228	0.9575	0.9806	0.08664	0.9499	0.9771
	AIC	65.57	47.84	50.97	64.66	54.60	41.43	73.27	65.06	58.29
Hopfenberg (n=3)	K_{HF}	0.0405	0.0706	0.0363	0.0383	0.0521	0.0382	0.0522	0.0461	0.0545
	R^2	0.9324	*0.9732*	*0.9562*	0.9290	*0.9672*	*0.9794*	0.9022	0.9578	0.9840
	AIC	62.95	*44.36*	*48.73*	62.55	*49.26*	*42.77*	70.05	62.21	53.58

Table 7. Parameter values of the mathematical equations corresponding to the evaluation models of release kinetics and their predictability indicators

Data fitting on the Hopfenberg model revealed the presence and involvement of the erosion phenomenon in the process of release of alendronate sodium. The prediction parameter R^2 has acceptable values (> 0.90) for all formulations studied, but the Akaike index does not allow the validation of this model, regardless of the value of n. According to data obtained from the analysis of the hydration behavior of matrices, erosion increases after 7-8 hours after the introduction of the matrix in the dissolution medium. Figures 10-11 show a good fitting of the

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model particularly for formulations based on C 971, C 971 associated with C 974, as well as in similar formulas in which we introduced CHT and TMC after moment 6-7 of the study. Therefore, we consider that the Akaike index values are determined by the nonlinearity of the erosion process.

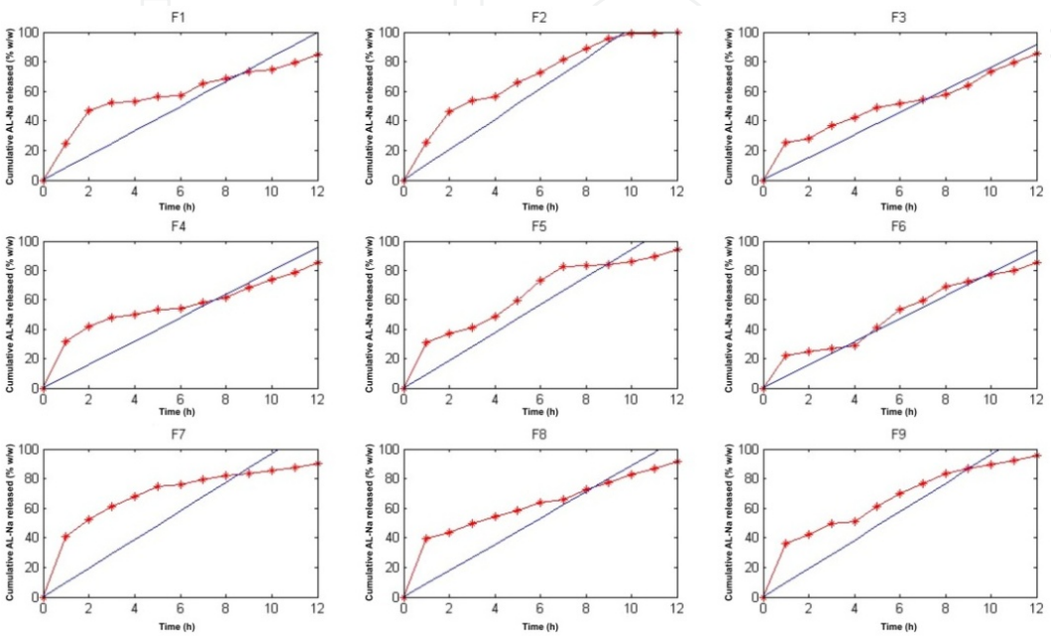

Figure 6. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the zero-order kinetics model

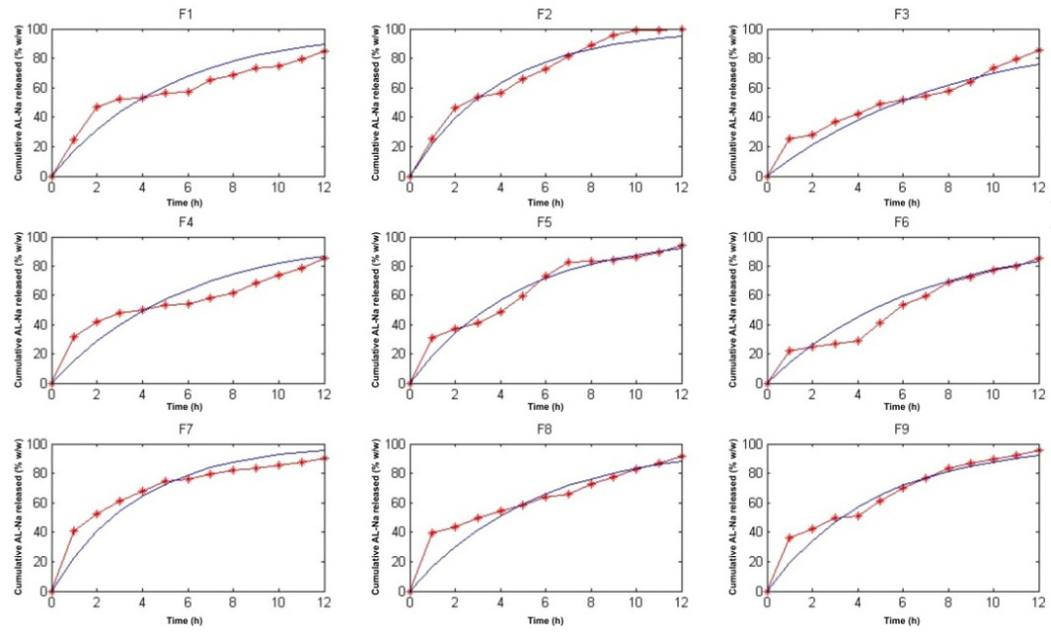

Figure 7. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the first-order kinetics model

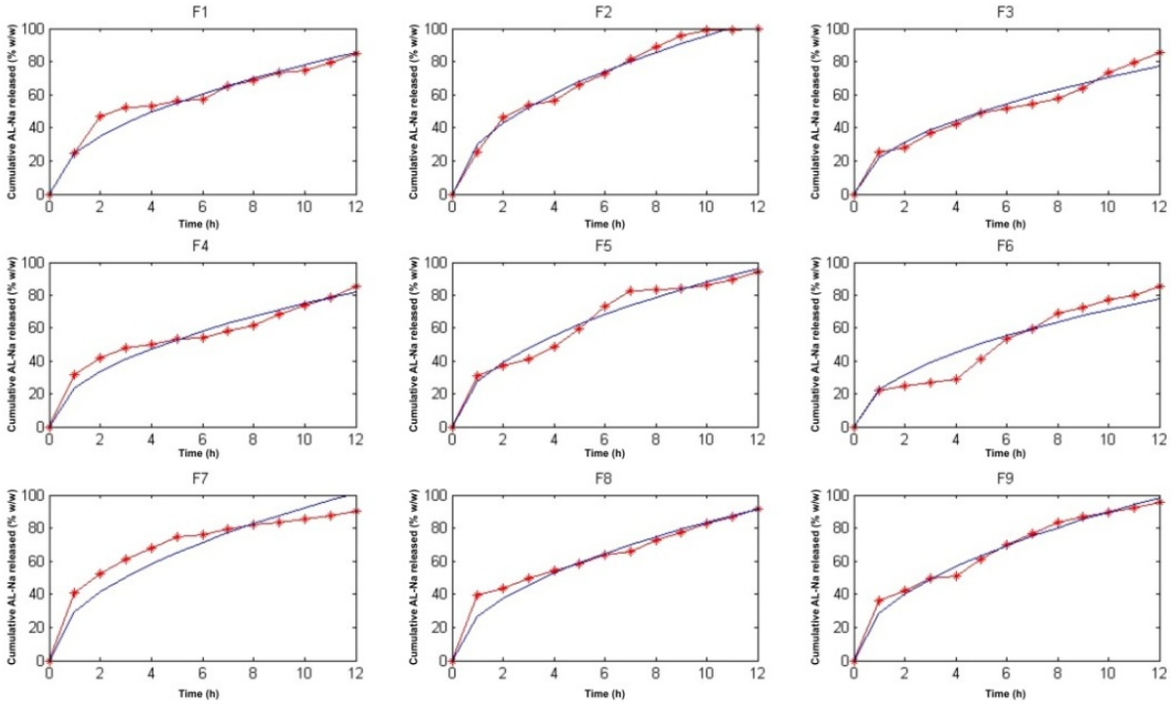

Figure 8. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the Higuchi model

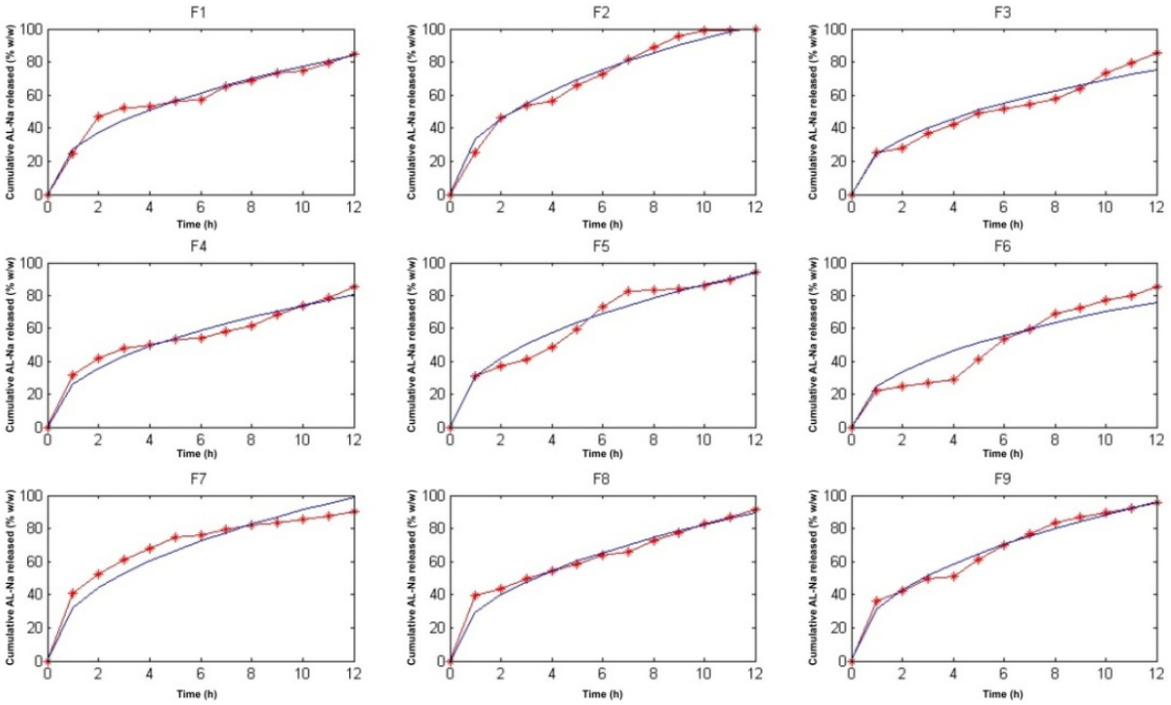

Figure 9. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the Korsmeyer-Peppas model

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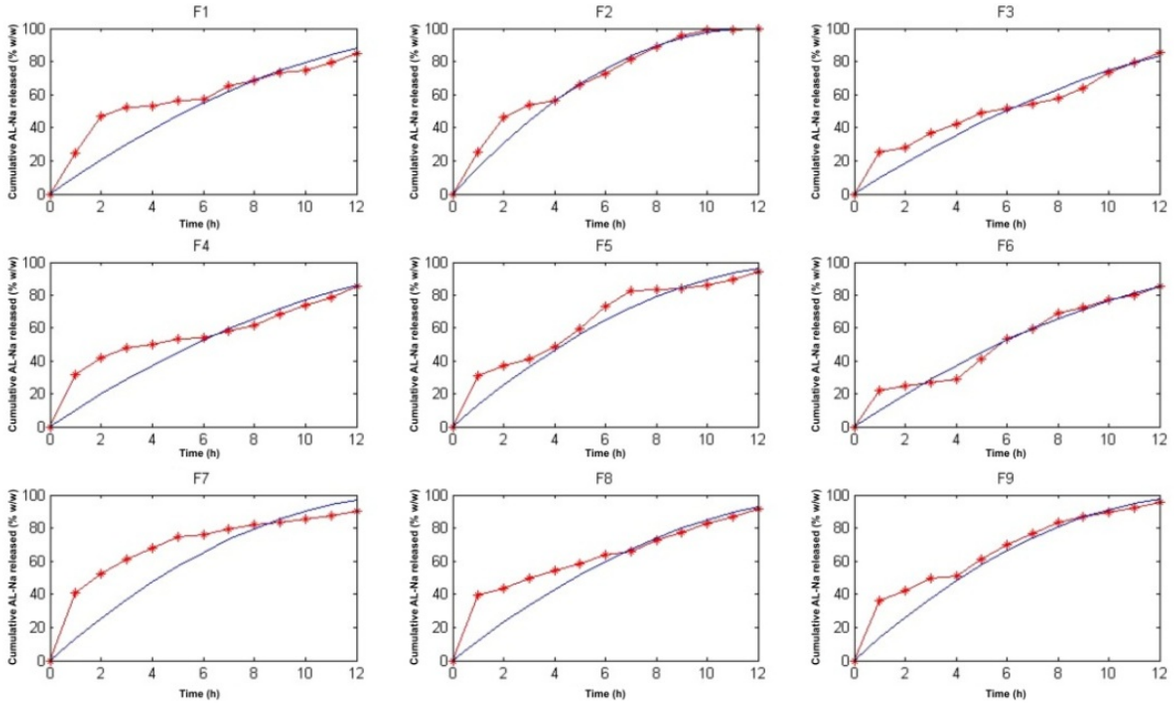

Figure 10. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the Hopfenberg (n=2) model

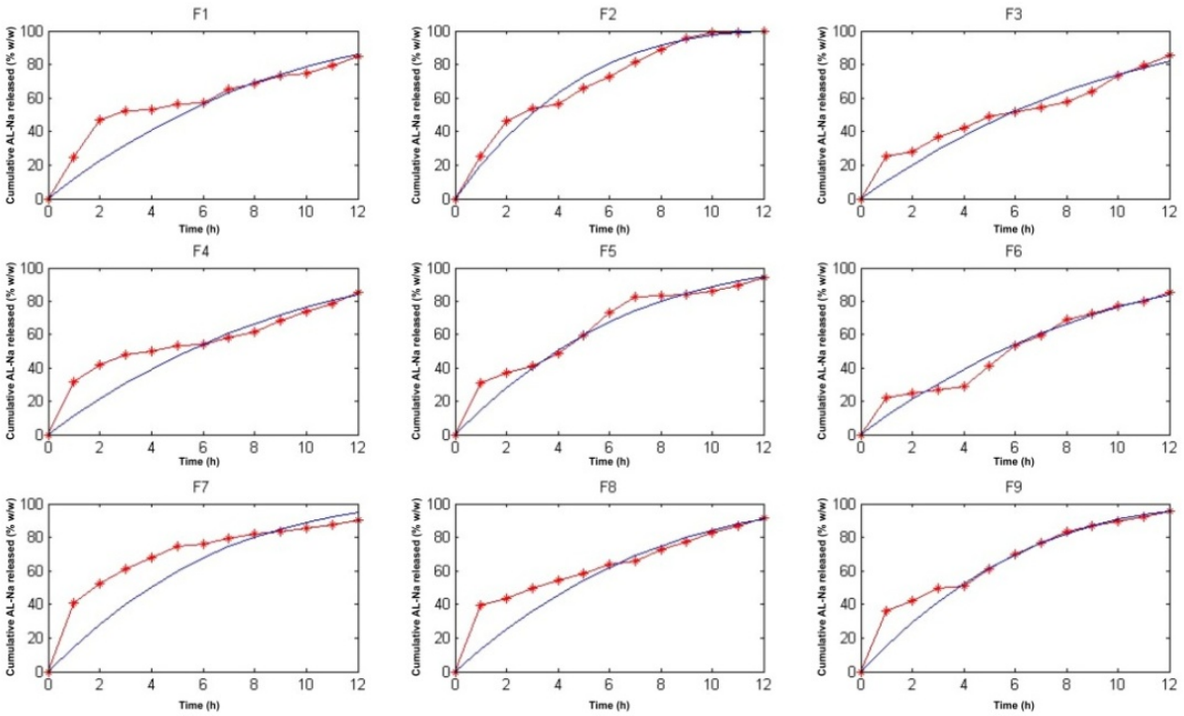

Figure 11. The fitting plot of the AL-Na dissolution profiles from formulations F1-F9 with the Hopfenberg model (n=3)

4.3. Conclusions

The results obtained showed good data fitting on the Higuchi model for all formulations studied, which leads to the conclusion that AL-Na release from hydrophilic matrix tablets occurs through a diffusion process based on Fick's law. In addition, the results obtained when fitting data on the Korsmeyer-Peppas mathematical model is an additional argument for considering diffusion as the main mechanism of AL-Na release from flat modified-release hydrophilic matrix tablets. The Hopfenberg model applied to modified-release matrix tablets with a complex formulation based on combinations of matrix-forming polymers in order to highlight the involvement of erosion in the process of active substance release generated a good fitting of the data in the analysis of the correlation coefficient R^2, but the Akaike index has high values, which does not allow the validation of the model. Data analysis shows that in the second half of the duration of the dissolution test, the data obtained experimentally generate a better fitting for the Hopfenberg model both for n = 2 and for n = 3. In conclusion, the data obtained from fitting on the Hopfenberg model confirms the involvement of erosion as a mechanism of alendronate sodium release from modified-release hydrophilic matrix tablets, but the predictive parameter values of the model do not allow the validation of this model due to the nonlinearity of erosion.

In conclusion, Fickian diffusion is the main mechanism of alendronate sodium release from modified-release hydrophilic matrix tablets and erosion is the secondary mechanism.

5. Assessment of the pharmacokinetic parameters of alendronate sodium in modified-release matrix tablets administered on the oral route

Alendronate absorption occurs through passive diffusion, predominantly in the upper small intestine (duodenum, jejunum, pH = 6-6.5), only a very small amount being also absorbed at the gastric level [66].

Alendronate has a low oral bioavailability (0.6 to 0.9%) for doses ranging from 5 to 70 mg, in conventional release formulations administered in the morning on an empty stomach or two hours after breakfast. The bioavailability of the 10 mg tablet is 0.59, while the weekly dose administration of 70 mg in tablets or solution leads to a bioavailability of 0.64% [67, 68]. These values are determined by the following general characteristics of bisphosphonates:

- low lipophilicity, which restricts intra-and intercellular transport;
- high polarity given by the negative charge, which prevents paracellular transport [69].

Plasma *distribution* of alendronate exhibits very low concentrations (<5 ng/ml) at therapeutic doses. Plasmatic $t_{½}$ of alendronate is short 0.5 to 2 h compared to $t_{½}$ in deposits at the site of bone resorption that have values expressed in years for alendronate being > 10 years. Alendronate binds to plasma proteins in the ratio of 78% and also forms complexes with some divalent cations, eg calcium, magnesium, iron [70].

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Excretion of alendronate is in the ratio of 99% in the feces and only 0.4% of the amount absorbed is eliminated through the kidneys by glomerular filtration and tubular excretion.

Storage of alendronate in the bone tissue depends on the affinity for hydroxyapatite, the *in vitro* affinity constant $K_{L\ alendronat}$ 2.9 x 10^6 mol/l [71].

Pharmacokinetically, alendronate does not present any significant differences between the main groups of patients. The oral bioavailability of this active substance is similar in both children and adults [66, 72]. The main objective of these studies is to evaluate the pharmacokinetic parameters of alendronate administered as modified-release matrix tablets.

5.1. Materials and methods

5.1.1. Materials

According to the results obtained in the research presented in subsections I-III, in order to achieve the pharmacokinetic studies we selected matrix tablets formulas that exhibited optimal characteristics of release (Table 8).

Ingredients (mg %)	Formulation			
	F2	F5	F8	Fm
C 971	15	15	15	-
CHTh	-	6	-	-
TMC	-	-	6	-
AL	13.05	13.05	13.05	13.05
Mg Stearate	0.5	0.5	0.5	0.5
Aerosil	0.5	0.5	0.5	0.5
Ludipress LCE	70.95	64.95	64.95	85.95

Table 8. The formulations of modified-release matrix tablets containing alendronate sodium

The tablets used in pharmacological research were prepared by direct compression, in accordance with the technology described in Section I.

5.1.2. Methods

5.1.2.1. Experimental protocol

The research was performed on adult male dogs weighing 30-40 kg, divided into four groups of 2 animals. Samples containing 20 mg alendronate sodium/tablet corresponding to the three formulations studied were denoted F2, F5, F8. Fm, the control sample, comprised immediate-release tablets with Alendronate sodium. The samples were administered to the four groups as follows:

- F2 → group I;
- F5 → group II;
- F8 → group III
- Fm → group IV.

The experimental study was conducted over a period of 48 hours and the tablets were administered orally after the animals had fasted for 12 hours. During the experiment, the animals had free access to water and food, except for 4 hours of food deprivation following the administration of tablets.

Mention should be made that in the literature we did not identify the lethal dose 50 (DL 50) of alendronate sodium administered to dogs, being conducted research by administering 200 mg / kg, a dose for which a mild renal toxicity was recorded [8, 73].

Preparation of the animals: 7 days prior to the experiment, each animal had free access to food and water and was kept under normal conditions of light and temperature.

Blood sampling was performed using the following procedure:

- in order to collect the blood samples a catheter was placed in the femoral vein;
- to characterize the pharmacokinetic parameters, samples of 4 ml of blood were collected at each moment in heparin vacutainers;
- sampling times were as follows: M 0 - prior to administration; M 1 - 30 min. after administration; M 2 - 1 h after administration; M 3 - 1 h and 30 min. after administration; M 4 - 2 hours after administration; M 5 - 2 h and 30 min. after administration; M 6 - 3 hours after administration; M 7 - 3 h and 30 min. after administration; M 8 - 4 hours after administration; M 9 - 6 hours after administration; M 10 - 8 h after administration; M 11 - 10 h after administration; M 12 - 12 h after administration; M 13 - 24 hours after administration;

Processing of the blood samples consisted in:

- centrifugation after 30 minutes from collecting, at a speed of 3000 rpm, for 10 min.;
- serum was transferred into Ependorff test tubes and stored in a freezer, at – 20 °C, until the HPLC quantitative analysis, according to the method described in section I.

All animals used in this study were clinically healthy and haematological and biochemical analyses had been performed prior to the experiment.

5.1.2.2. Calculation of pharmacokinetic parameters

The main pharmacokinetic parameters that were evaluated directly, without a pharmacokinetic modeling, based on the experimental data, are: *the coordinates of the peak (C_{max} and T_{max}), the areas under the curve (AUC_{0-t}, $AUC_{0-\infty}$, $AUC_{t-\infty}$), the total clearance (Cl) and the average residual time (MRT).* These parameters are independent of the representation model of drug kinetics in the body, and are very useful in bioavailability studies. A series of other parameters depend on the pharmacokinetic model, so that it should be taken into account in calculating them.

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In the case of extravascular administration, the following parameters dependent on the model can be calculated: *elimination rate constant* (k_e), *biological half-life* ($t_{1/2}$), *absorption rate constant* (k_a) and *volume of distribution* (V_d).

The main pharmacokinetic parameters calculated were:

- *coordinates of the peak* (C_{max} and T_{max}) – individual C_{max} values expressed in μg/ml and T_{max}, expressed in hours, were obtained from the examination of experimental data;
- *areas under the curve* (AUC_{0-t}, $AUC_{0-\infty}$, $AUC_{t-\infty}$) - the area under the plasma concentration curve, noted AUC, is a parameter that reflects the extent of absorption in the administered dose and that is proportional to the amount of drug that reaches the blood after oral administration. As a result, this parameter is essential for the determination of bioavailability.
- the *area under the experimental plasmatic concentration curve* AUC_{0-t} was calculated by using the trapezoidal rule, which is the most common method. We calculated the area from time zero (initial time) to the time when the last blood sample was collected to determine the concentration of the drug substance. The area under the curve is equal to the sum of the areas of the trapezoids realized between two concentrations corresponding to two successive collections of blood samples. The area of each trapezoid was calculated according to the equation:

$$S_i = \frac{c_i + c_{i+1}}{2}(t_{i+1} - t_i) \tag{10}$$

Consequently,

$$AUC_0^t = \sum_{i-0}^{n-1} S_i = \sum_{i-0}^{n-1} \frac{c_i + c_{i+1}}{2}(t_{i+1} - t_i) \tag{11}$$

where, n = number of trapezoids in which we divide the area under the curve (n is the number of collections of biological samples);

- the *total area under the plasmatic concentration curve* $AUC_{0-\infty}$ is equal to the sum of the area under the experimental concentration curve and the area extrapolated from the time of the last sample collection to infinity.

$AUC_{0-\infty}$ was calculated using the equation:

$$AUC_0^\infty = AUC_0^t + AUC_t^\infty = \sum_{i-0}^{n-1} \frac{c_i + c_{i+1}}{2}(t_{i+1} - t_i) + \frac{c_n}{k_e}, \tag{12}$$

where ke is calculated based on the end points of the plasma concentration curve in time.

- the *extrapolated area* is the area from the last sampling time extrapolated to infinity and it was calculated by the equation:

$$AUC_t^{\infty} = \frac{c_n}{k_e} \tag{13}$$

where cn is plasma concentration of the last determination.

Totalclearance (Cl) – for oral administration, the absorbed dose and the total clearance cannot be calculated exactly, so we determined the ratio:

$$\frac{Cl}{F} = \frac{dose}{AUC} \tag{14}$$

where F – correction factor

- the *elimination rate constant* (k_e) was determined by the method of residuals, based on plasmatic concentrations of the drug substance determined at different time intervals. This method is also called the "least squares method" or the "method of the least sums of the squares errors" and is used to estimate the true values with the help of the experimentally determined values.
- *biological half-life* ($t_{1/2}$) was calculated by the mathematical method. Starting from the equation of the plasma concentration dependent on time in the disposal phase

$$c(t) = C_0 e^{-k_e t} \tag{15}$$

and knowing that the initial concentration is Co, and at the time t1/2 concentration is reduced by half, the equation becomes

$$C_0 = \frac{C_0}{2} e^{-k_e t_{1/2}} \tag{16}$$

from which we obtain:

$$t_{1/2} = \frac{\ln C_0 - \ln \frac{C_0}{2}}{k_e} = \frac{\ln 2}{k_e} = \frac{0.693}{k_e} \tag{17}$$

where: k_e was obtained by linear regression as a slope of the terminal phase of the logarithmically transformed plasma concentration curve versus time

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- *volume of distribution* (V_d) – similar to total clearance, V_d cannot be evaluated exactly for extravascular administration. Therefore the ratio is calculated:

$$\frac{V}{F} = \frac{dose}{k_e \cdot AUC_0^{\infty}} \tag{18}$$

Statistical analysis of data was performed using the PHARM-STAT program for the investigation of bioavailability.

5.2. Results and discussion

5.2.1. Bioavailability of alendronate sodium in modified-release hydrophilic matrix tablets based on Carbopol

Results from the comparative analysis of serum concentrations of alendronate administered as immediate-release tablets to the animals in (control) group IV and as modified-release matrix tablets to group I, show a higher absorption of alendronate sodium from the modified-release tablets.

We can distinguish two major phases in the evolution of the absorption of alendronate, in close correlation with the swelling characteristics of matrix tablets. Modified-release matrix tablets with sodium alendronate administered to the animals in group I generate an absorption maximum at 2.5 to 3 hours, after which there is a plateau condition in the concentration range of 0.25 to 0.30 μg/mL alendronate until time 12, i.e. 10 hours after administration (Figure 12).

Initially, in the first 1.5 hours after administration, the animals in control group IV exhibit an alendronate serum concentration that is higher compared to group I. Subsequently, after about 2 hours from the administration, when the matrix tablet has hydrated and the phenomenon of alendronate diffusion in the inner matrix layers occurs, the serum concentration of alendronate in the animals in group I increases significantly (Figure 13)

By analyzing the data obtained, we consider that the formulation of alendronate in matrix tablets based on Carbopol C 971 resulted in a considerable increase in oral bioavailability.

5.2.2. Bioavailability of alendronate sodium in modified-release hydrophilic matrix tablets in the presence of absorption promoters

In the first hour after administration, the animals in the control group had a higher serum concentration of alendronate compared to the modified-release formulations (Figure 14). Subsequently, at collection time 4, that is 1.5 hours after administration, the formulation corresponding to the modified-release matrix tablets containing trimethil chitosan as an absorption promoter exhibits the highest concentration. This superiority lasts until time 5, that is 3 hours after administration (Figure 15). According to these results, we consider that over this time the absorption enhancer action of trimethilchitosan manifests itself.

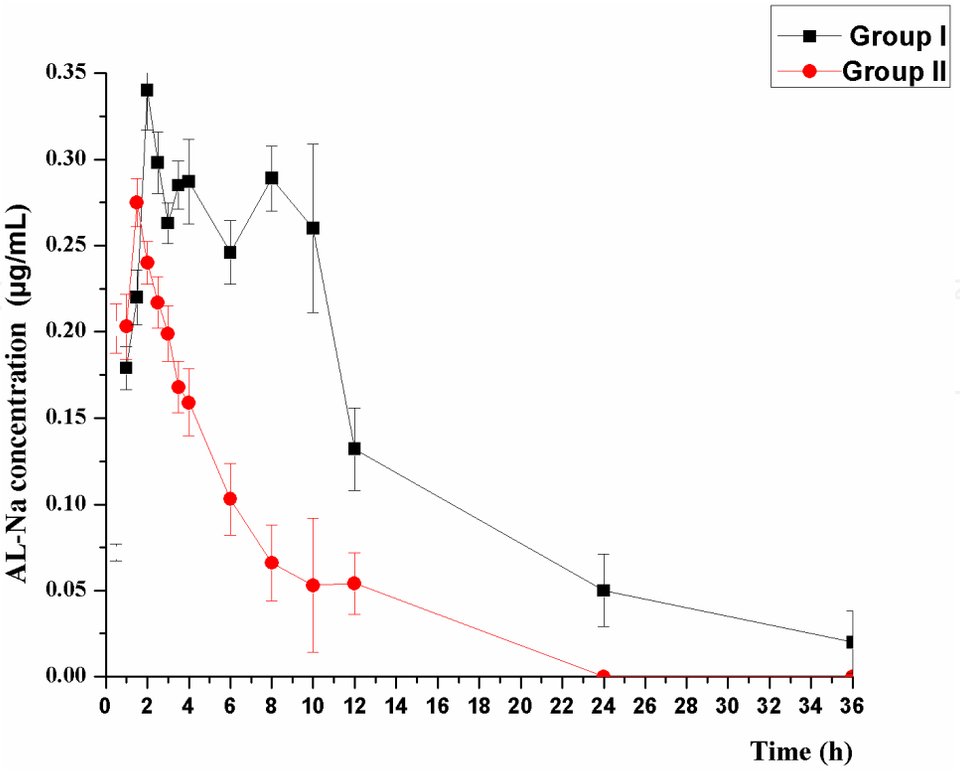

Figure 12. *In vivo* release of AL-Na from hydrophilic matrix tablets with modified release (0-12 hours)

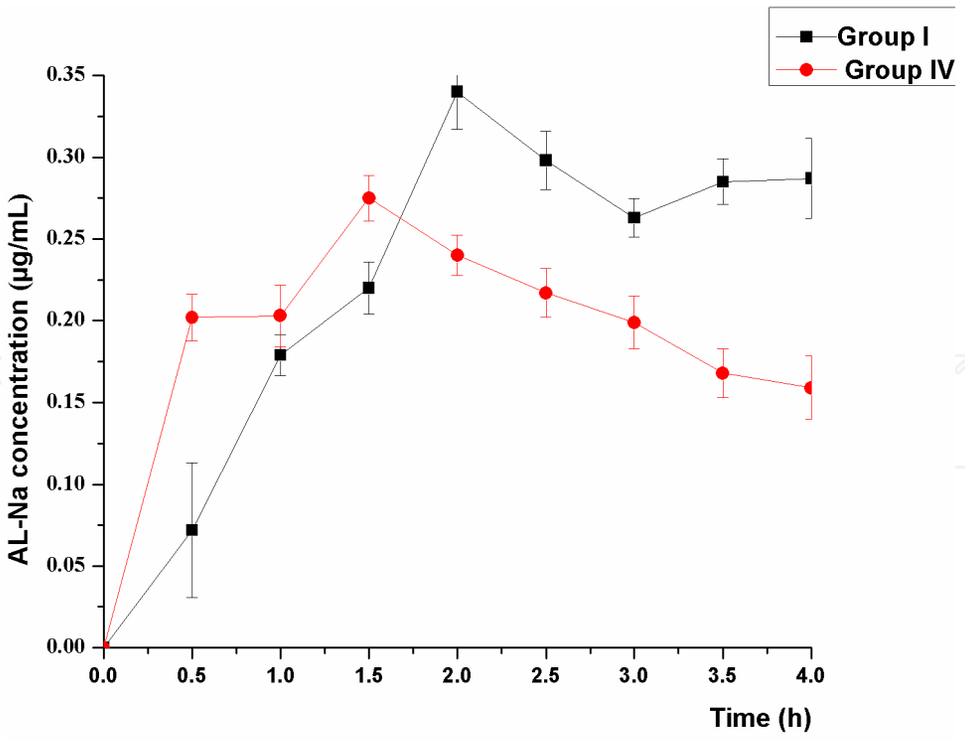

Figure 13. *In vivo* release of AL-Na from hydrophilic matrix tablets with modified release (0-4 hours)

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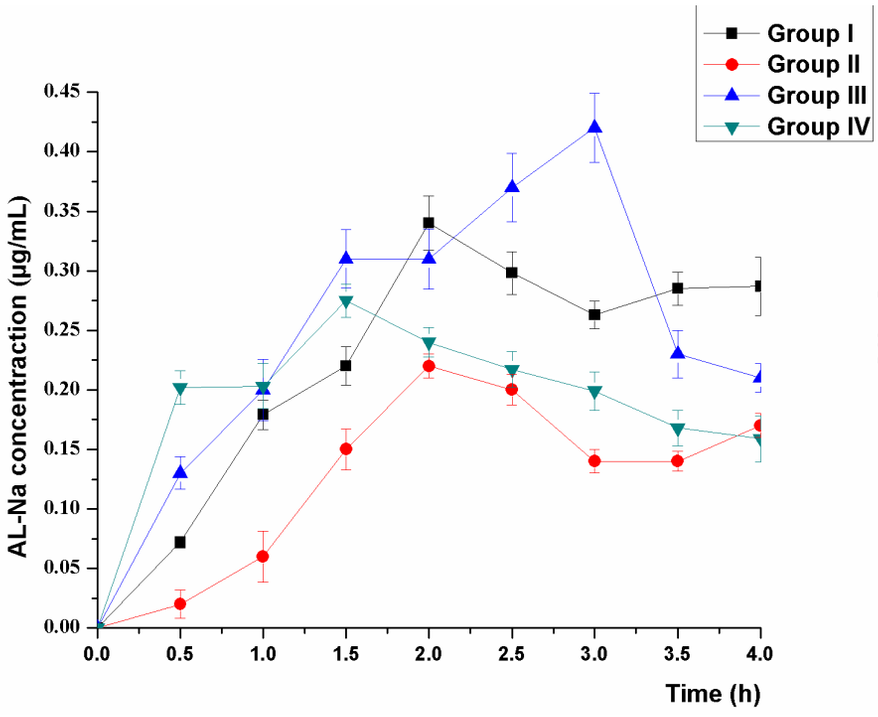

Figure 14. In vivo release of AL-Na from hydrophilic matrix tablets with modified release in the presence of absorption promotors (0-4 hours)

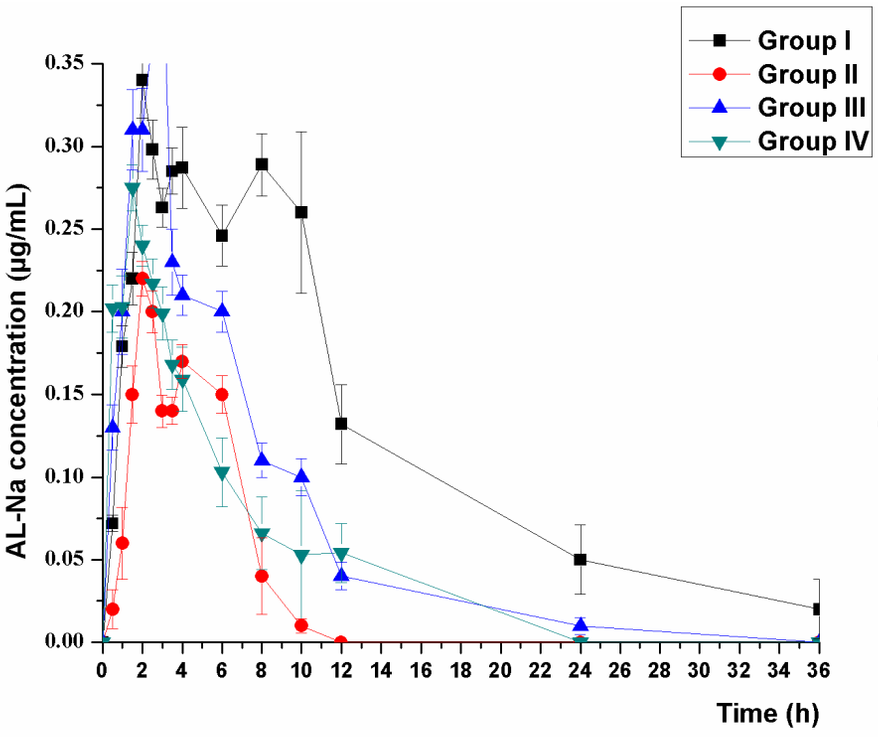

Figure 15. In vivo release of alendronate sodium from hydrophilic matrix tablets with modified release (0-12 hours)

An unexpected development was recorded for animals in group II, which were administered modified-release matrix tablets in which we had introduced chitosan as an absorption promoter. The serum concentration of alendronate in this group was lower than in the control formula throughout the experiment. There are two surprising aspects related to this: on the one hand, the fact that chitosan does not act as absorption promoter and, on the other, that there occurs a cancellation of the retardation effect and of the release optimization resulting from the administration of the modified-release of matrix tablets based on Carbopol.

5.2.3. Pharmacokinetic parameters: Cmax, Tmax, $t_{1/2}$, areas under the curve

The comprehensive review of the individual values of plasma concentrations over time and of the corresponding individual graphs allows for a primary pharmacokinetic analysis of results. Based on plasma concentration values over time the pharmacokinetic parameters (T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $AUC_{t-\infty}$, Cl, k_e, $t_{½}$, V_d) were determined. Subsequently, the data were analyzed statistically to determine the possible existence of significant differences.

Mean C_{max} peak plasma concentrations (Table 9) of the modified-release matrix tablets and of the formula related to the control group, respectively, in a single dose study, reveal differences between the formulations studied. Thus, for formula F2 given to group I, C_{max} = 0,34μg/ml, formula F5 associated with group II provides a C_{max} = 0.24 μg/ml, formula F8, group III, has C_{max} = 0.48 μg/ml, and the control formula given to group IV, C_{max} = 0.28 μg/ml. Result processing through the ANOVA test shows that the differences between peak plasma concentrations after administration of formulation F2, compared to control formulation and after administration of F8 compared to the control, are not statistically significant. For parameter Cmax only the differences between group I and III are statistically significant.

Mean value	GROUP			
	I	II	III	IV
C_{max}	0.34	0.24	0.48	0.28
T_{max}	2.00	2.25	3.00	1.50
AUC_{0-t}	2.93	1.28	2.65	1.94
$AUC_{0-\infty}$	3.17	1.40	2.72	1.94
$AUC_{t-\infty}$	0.24	0.12	0.07	0.00
% AUC_{extra}	7.57	8.56	2.37	0.00
Cl	0.03	0.05	0.04	0.05
k_e	0.08	0.08	0.13	0.08
$t_{1/2}$	8.31	8.26	5.79	8.63
V_d	0.37	0.62	0.32	0.63

Table 9. Pharmacokinetic parameters of AL-Na

The mean values of time taken to reach peak concentration, T_{max} (Table 9) exhibits equal values for groups I and II compared to control group IV. The highest value was determined for group

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III, i.e. T_{max} = 3 h. By applying a non-parametric test it was found that the differences are not statistically significant.

The mean of the values of the extrapolated area $AUC_{t-\infty}$ is below 20% in all situations, which proves the right choice for the last sample collection time. Analysis of the mean total area under the plasma concentration-time ($AUC_{0-\infty}$) reveals differences between products. The highest value was recorded for group I. Analysis of the mean values of Cl and V_d does not reveal statistically significant differences between products.

5.3. Conclusions

The results obtained reveal an optimization of the bioavailability of alendronate administered in modified-release matrix tablets from collection moment 5, i.e. two hours after administration, as the animals in group I, corresponding to this formula, had a serum concentration of alendronate superior to the control group.

The two absorption promoters used, trimethyl chitosan and chitosan, had completely different influences on the absorption of alendronate in the modified-release matrix tablets. Thus, trimethyl chitosan acted as an absorption promoter in the range 1.5 to 3 hours after administration, and caused the highest serum concentrations of alendronate, sometimes double compared to the control. After that moment, concentrations are above the values of the control group, but slightly lower than the values of group I, coresponding to the modified-release matrix tablets based on Carbopol.

The administration of modified release tablets with alendronate sodium in which we associated chitosan as absorption promoter has led to surprising results, because over the whole experiment, the serum concentration of alendronate sodium was lower than of group IV (control group). Chitosan exhibited virtually no absorption promoting action; it additionally canceled the release retardation and optimization effect proved on group I after the administration of alendronate in modified-release matrix tablets based on carbopol.

6. Conclusions

The crosslinking degree of Carbopol has a major influence on the release properties of the hydrophilic matrix tablets. The C71 and C971 Carbopol types, having a low crosslinking degree, hydrate much better in pH 1.2, and this facilitates the AL-Na dissolution, its diffusion through the gel layers of the matrix and, consequently, an optimized release of AL-Na. On the contrary, C 971 – a polymer with a high crosslinking degree, slows the hydration process of the matrix. Sort Carbopol 71 in a concentration of 15% results in hydrophilic matrix tablets with optimal release of AL-Na. The different dissolution profile of Al-Na in the Carbopol-based modified release matrix tablets is also confirmed by the values of the two control parameters f_1 and f_2. Fitting the release data to the kinetic analysis with the Higuchi and the Korsmeyer-Peppas model confirms the release of the Al-Na in the Carbopol-based matrix tablets by a diffusion process. The Hopfenberg model applied to modified-release matrix

tablets with a complex formulation based on associations of matrix-forming polymers to highlight the involvement of erosion in the release of the active substance generated a good fitting of the data ($R^2 > 0.90$), but the high values of the Akaike index do not allow the validation of the model. *In vivo* studies have shown a higher bioavailability of AL-Na in modified release matrix tablets at 1.5 hours after dosing. The results of the evaluation of the *in vivo* AL-Na release indicate the optimization of the bioavailability of AL-Na in the Carbopol 71-based matrix tablets, the optimal concentration being reached after 2 hours from the administration of the tablet. The two absorption promoters used - chitosan and trimethyl chitosan - had a totally different effect on alendronate absorption. Trimethyl chitosan showed a strong absorption promoting effect by doubling AL-Na serum concentration while CHT did not show this effect. According to the results obtained, Carbopol 71 is a good forming agent for modified-release tablets to improve the oral bioavailability of AL-Na.

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