JAP&T
TPA-Timişoara

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Journal of Agroalimentary Processes and Technologies 2011, *17*(4), 419-427

Journal of Agroalimentary Processes and Technologies

Water content of oxicam/cyclodextrin nanoparticles

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Received: 28 October 2011; Accepted: 8 December 2011

Abstract

This paper presents the evaluation of water content of oxicam/cyclodextrin complexes by classical Karl Fischer titration; the two-component technique on a Karl Fischer 841 Titrando apparatus (Metrohm) was used. The total water concentration was in the range of 7.2-9.2%, 9.9-11.1%, and 5.4-9% for the α-cyclodextrin complexes, β-cyclodextrin complexes and 2-hydroxypropyl-β-cyclodextrin complexes respectively. However, for β-cyclodextrin complexes two types of water molecules could be evaluated: "surface" water, which is extracted and reacts with a higher rate, and "strong-bonded" water molecules, which is extracted and reacts with a lower rate in the titration process. These results are in good agreement with the thermogravimetric analysis.

Keywords: oxicam/cyclodextrin complexes, nanoparticles, Karl Fischer water titration

1. Introduction

Oxicams - piroxicam (P), meloxicam (M) and tenoxicam (T) belong to the class of anti-inflammatory (AI) thiazines (Figure 1), and are characterized by low solubility in water. Oxicams are substances with acid reaction and quite strong acids. Possible explanations for this high acidity also take into account the involvement of hydrogen bonds that lead to the stabilisation of the enolate anion. Being ionized, the oxicams molecules are spread in plasma and in extracellular water, but they are also lipophilic due to their heteroaromatic nuclei and sulphonic group, making biological membranes permeable to them. These physicochemical properties are determined by the chemical, electronic and spatial structures (molecules are flat-shaped) [1-3].

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides composed of α-1,4-linked d-glucopyranose units (Figure 1). The most commonly used form of these ring-shaped molecules are α-, β-, and γ-CDs formed by six, seven, and eight glucose units, respectively. CDs are toroidal molecules with a truncated cone structure with a low polarity central void which is able to encapsulate either partially or entirely a wide variety of guest molecules of suitable size and shape, resulting in a stable association without the formation of covalent bonds, being the resultant entity known as host-guest complex [4,5].

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Although cyclodextrin molecules are capable of forming numerous hydrogen bonds with water molecules surrounding them, their solubility in water is limited, especially for β-cyclodextrin. This is believed to be due to the relatively strong bond of cyclodextrin molecules in crystal state (relatively high lattice energy). The random substitution of hydroxyl groups and, therefore, the formation of amorphous mixtures of isomeric derivatives will lead to considerable improvement in their solubility [6,7].

The quality of encapsulation depends on many factors such as the guest molecule structure and hydrophobicity, encapsulation parameters (temperature, pH, solvents), and finally the water content of the complex can vary according to these factors.

In this paper we try to evaluate the water concentration of oxicams (piroxicam, meloxicam and tenoxicam)/α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (2HP-β-CD) complexes by using the classical Karl Fischer titration and to compare the results with those from the thermogravimetric analysis.

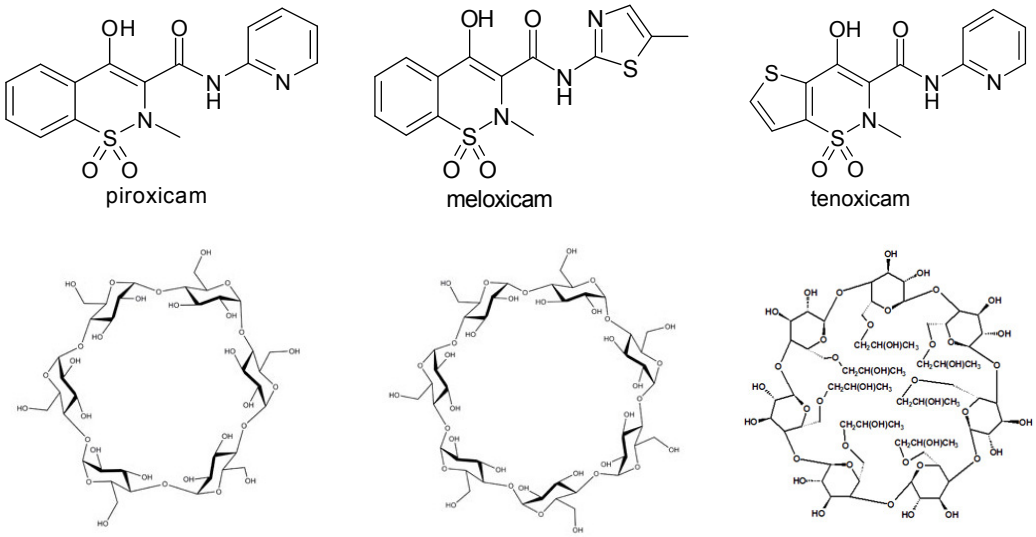


Figure 1. Chemical structure of piroxicam, meloxicam, tenoxicam – above and α-cyclodextrin (left), β-cyclodextrin (center), 2-hydroxypropyl-β-cyclodextrin (right) – down

2. Materials and methods

Materials. Oxicam/α-, β-, and 2-hydroxypropyl-β-cyclodextrin complexes (piroxicam, meloxicam şi tenoxicam were purchased from LaborMed Pharma, România; cyclodextrins – 99% purity, were obtained from Cyclolab-Hungary) were obtained according to our previous work [8-10] by using crystallization from ethanol-water solution method (the cyclodextrins were weighed on the analytical balance in a complexation minireactor, the installation was mounted on a mechanical stirring system with the possibility of thermostatation, and then 4 mL of distilled water were added. Immediately after the initiation of shaking and heating at 50°C, an ethanolic solution of oxicam containing 0.5 mmoles bioactive compounds was added dropwise to the cyclodextrin-water mixture. Dropwise addition was performed for 15 minutes, after which the system was kept under stirring for another 15 minutes at the same temperature. In order to obtain well formed crystals of the complex, the solution was slowly cooled to 25°C for 3 hours, and then the suspension was left in a refrigerator (5°C) overnight (~12 hours) in order to accomplish the crystallization. The suspension was then filtered by vacuum filtration, and the complex crystals were washed with 1 mL 93% ethanol and dried at a maximum of 40°C to constant mass) and kneading method (the cyclodextrin and oxicam in a 1:1 molar ratio were weighed, 0.5 mL of solvent

were added and the paste obtained was cold-triturated in a mortar for 30 minutes, after which it was dried in the oven at 50°C to a constant mass, while the obtained precipitate was dry-triturated in the mortar).

Oxicams/cyclodextrin complexes were initially analyzed by thermogravimetry (TG, on a TG-209 Netzsch apparatus, 20-550ºC, 4ºC//min., under nitrogen), differential scanning calorimetry (DSC, on a DSC-204 Netzsch apparatus, 20-550ºC, 4ºC/min.), and scanning electron microscopy (SEM, Inspect S the pictures were taken at an excitation voltage of 25 kV, a magnitude of 3000-12000) [8-10]. Karl Fischer water titration (KFT) was performed by using the two-component technique, with component 1: Hydranal® - Titrant 2 and component 2: Hydranal® - Solvent–which contains imidazole, sulfur dioxide, and methanol; both components were purchased from Fluka Analytical, Sigma-Aldrich. The titer of component 1 was performed by using Hydranal® - Water standard 10.0, standard for volumetric Karl Fisher titration (Fluka Analytical, Sigma-Aldrich).

Karl Fischer water titration. Classical Karl Fischer water titration (Figure 2) [11-14] of oxicams/cyclodextrin complexes were carried out by using a Karl Fischer 841 Titrando apparatus from Metrohm; a 800 Dosino dosing system and 703 Ti Stand mixing system were also used (both from Metrohm). For acquisition and data handling the Tiamo™, ver. 1.2 software (Metrohm AG, Switzerland) was used.

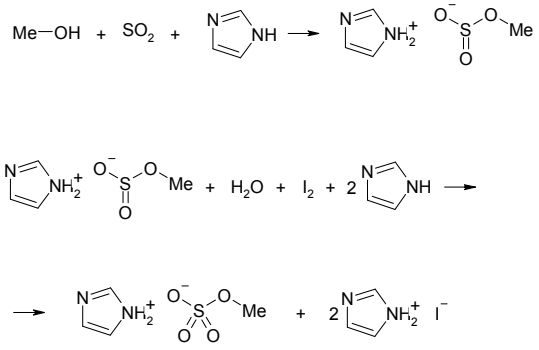


Figure 2. Karl Fischer reaction for two-component titration technique

The two-component technique was used for water determination. The temperature was set up to 40ºC by using a titration vessel with thermostatic jacket. The sample amount was 0.01-0.1 g for complexes and ~0.1 g commercial cyclodextrins.

The method parameters were: *I(pol)* of 50µA, end point and dynamics at 250 mV, maximum rate of 5 mL/min, drift was used as stop criterion, with a stop drift of 15 µL/min. For titration parameters, the extraction time was 300 s, time interval measuring point of 2 s. The conditioning was setup for start drift of 15 µL/min, spot volume of 20 mL, and delay after "*conditioning ok*" of 10 s.

3. Results and Discussion

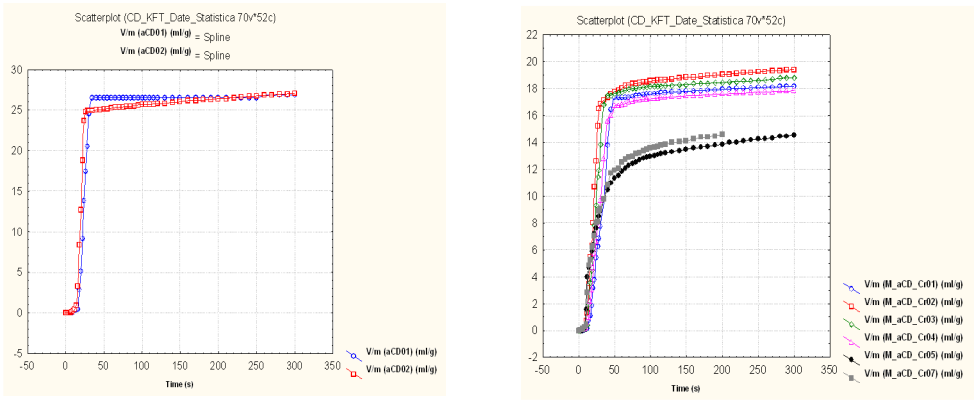
The complexation of oxicams with the commercial cyclodextrins was realized with yields in the range of 24-92%, the best results being obtained in the case of piroxicam / 2-hydroxypropyl-β-cyclodextrin complexes with ethanol 50% by kneading method (92%).

SEM analysis indicated that the crystals of α-cyclodextrin complexes have hexagonal shapes, while the corresponding β-cyclodextrin complexes have rhombohedral shapes and the case 2-hydroxypropyl-β-cyclodextrin complexes have rhombohedral crystals conglomerates, all with dimensions in the range of tens of nanometers to 40 µm.

Both TG and DSC analyses indicate the formation of complexes, the main peak corresponding to the dissociation of the complex being observed in the range of 100-300ºC.

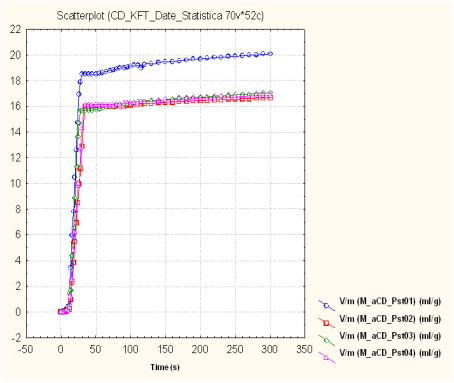
In the case of oxicam/α-cyclodextrin nanoparticles obtained by crystallization from solution method, the water concentrations, determined by KFT, were in the range of 7.2-9.2% (Figure 3 and Table 1), which are higher values comparatively with those from TG analysis.

In α-cyclodextrin complexes obtained by kneading method, the water concentration values were lower and similar in all three cases (7.2-7.6%), with titration curves similar to those for commercial α-cyclodextrin (Figure 4).

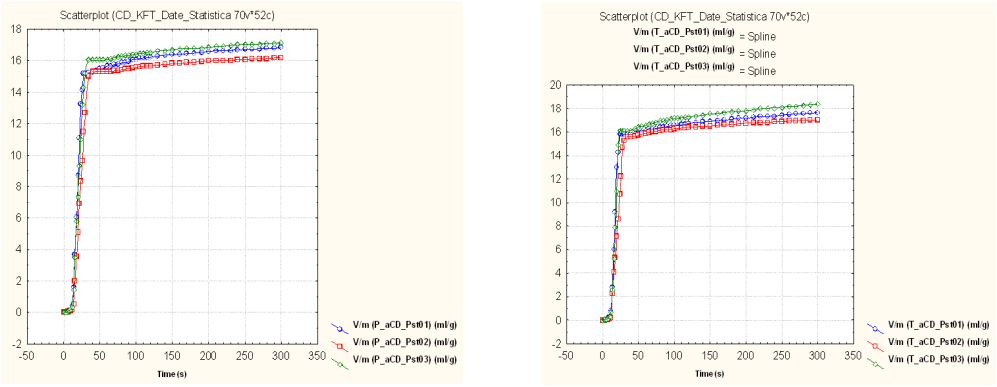


(a) (b)

Figure 3. KF water titration curves (*V/m vs. Time*) for commercial α-cyclodextrin (a) and M/α-CD complexes (b) – crystallization from solution method



(a)



(b) (c)

Figure 4. KF water titration curves (*V/m vs. Time*) for M/α-CD (a), P/α-CD (b), and T/α-CD (c) complexes – kneading method

The water concentration of β-cyclodextrin complexes was similar in both complexes obtained by crystallization and by kneading method, with lower values (3-4%) in comparison to the water concentration in commercial cyclodextrin. A differential titration speed can be noticed: high titration (extraction) speed in the first portion of the curve, followed by a relatively small portion at lower speed (probably strongly bonded "crystallization" water) (Figure 5).

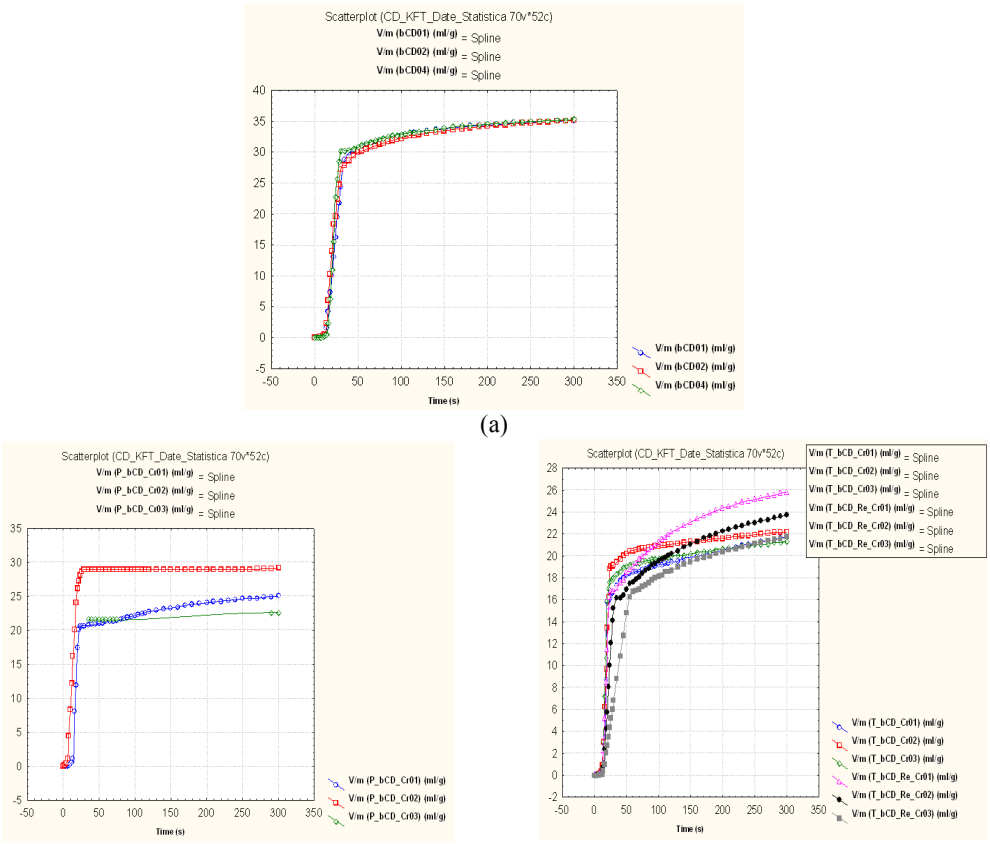


Figure 5. KF water titration curves (*V/m vs. Time*) for commercial β-cyclodextrin (a), P/β-CD (b), and T/β-CD (c) complexes – crystallization from solution method

The evolution of interaction energy between bioconjugates and cyclodextrins can be observed in the Figure 7. After the "accommodation" period of the pair molecules (up to 400 calculation cycles), the molecular encapsulation process are relatively rapid (up to 1000-1200 calculation cycles), followed by a "finishing" process which conduct to the final complex. The molecular encapsulation of biomolecule in α-cyclodextrin take a long time (great number of calculation cycles) and is relatively slower, compared with those corresponding to β- and γ-cyclodextrin, where the equilibrium is reached in a short time (Figure 7).

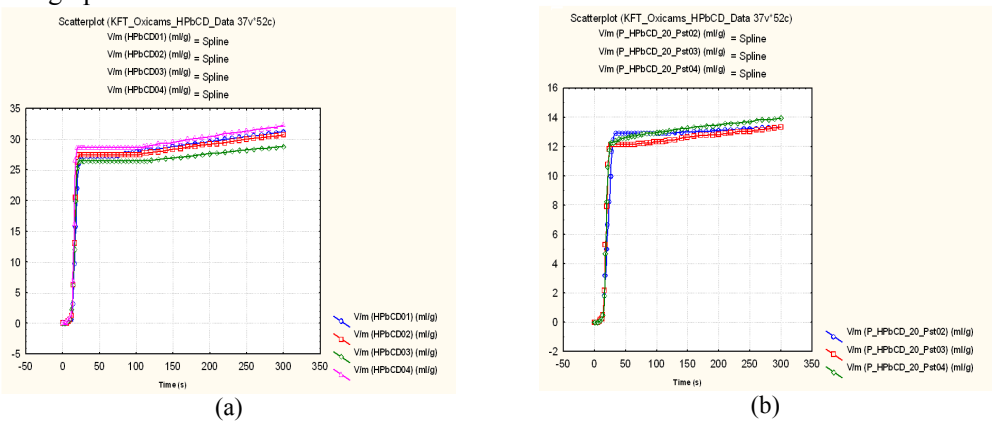
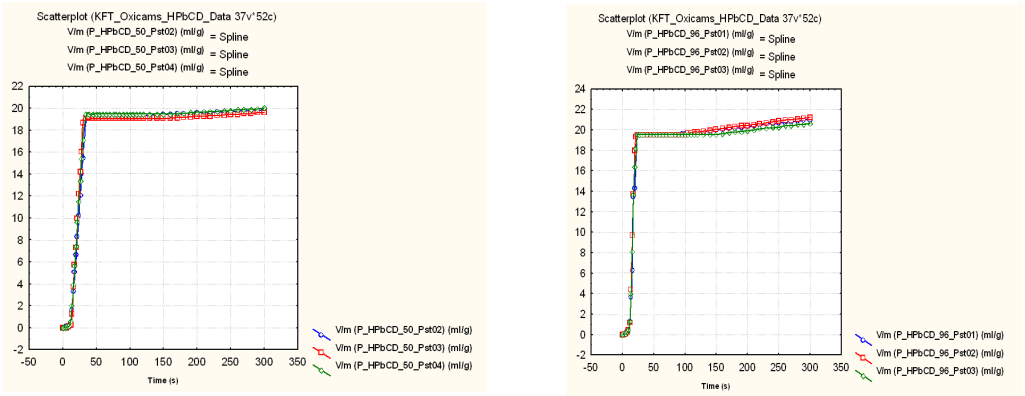


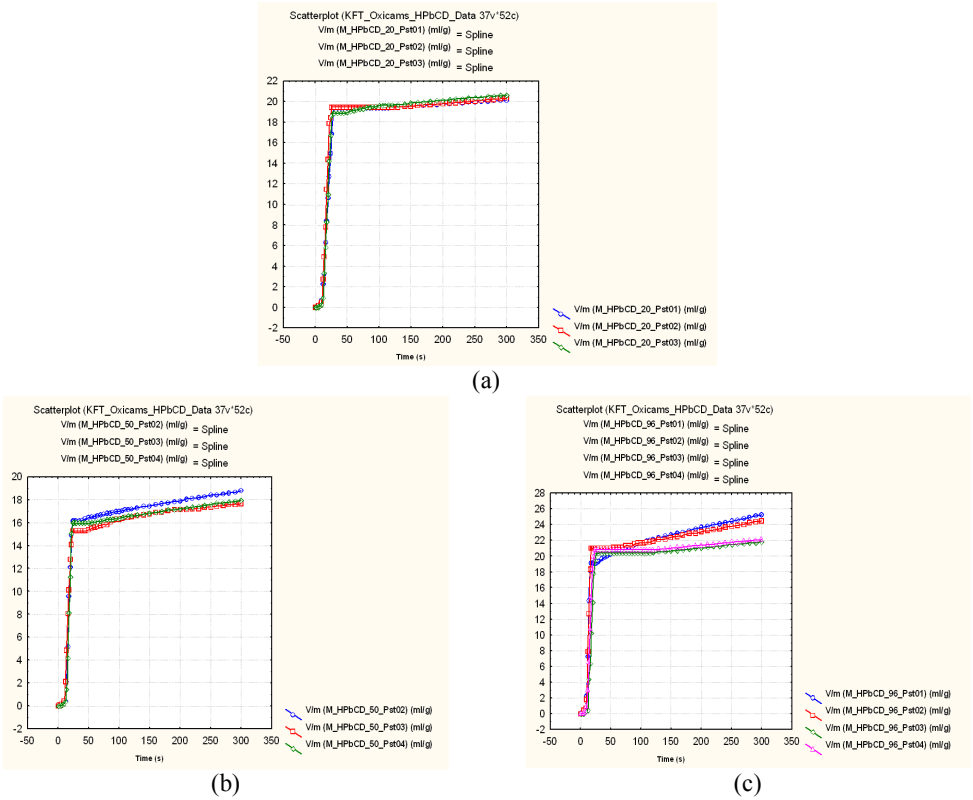
Figure 6. KF water titration curves (*V/m vs. Time*) for commercial 2-hydroxypropyl-β-cyclodextrin (a) and P/2HP-β-CD complexes with ethanol 20% (b) – kneading method



(a) (b)

Figure 7. KF water titration curves (*V/m vs. Time*) for P/2HP-β-CD complexes with ethanol 50% (a) and for P/2HP-β-CD with ethanol 96% (b) complexes – kneading method

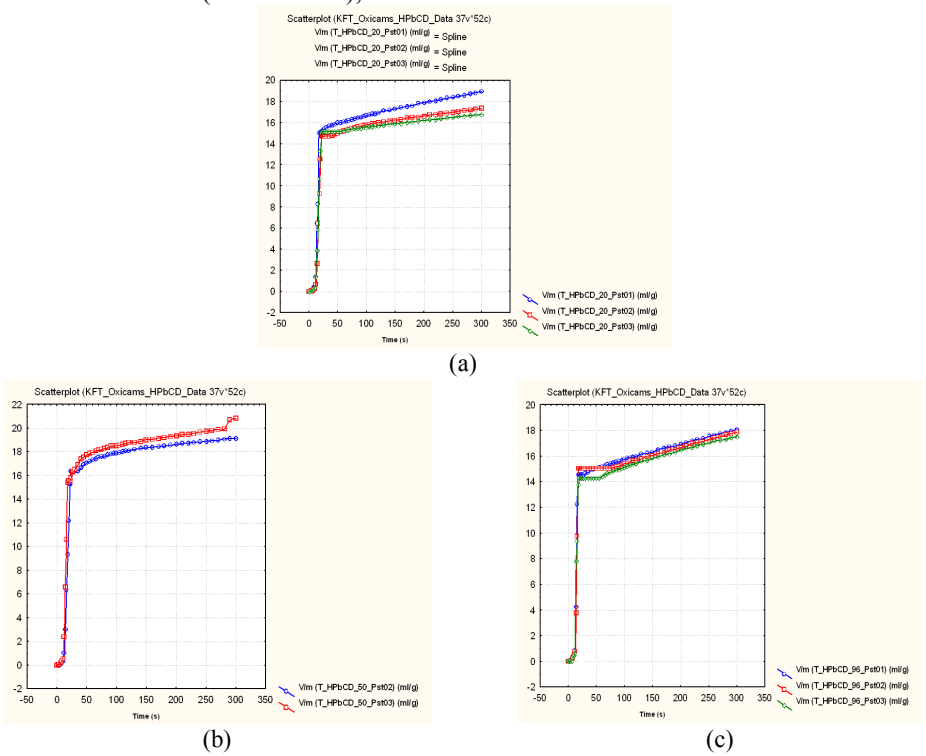
In inclusion complexes of meloxicam with 2-hydroxypropyl-β-cyclodextrin obtained by kneading method using ethanol of various concentrations, a differentiation almost similar to the first case was noticed, but water concentration values were closer when using lower concentration ethanol (20% and 50%) at 8.2% and 7.3% respectively, and approximately one percent higher (9%) for concentrated ethanol. In Figure 8 are presented the statistically significant titration curves.



(a) (b) (c)

Figure 8. KF water titration curves (*V/m vs. Time*) for M/2HP-β-CD complexes with ethanol 20% (a), for M/2HP-β-CD with ethanol 50% (b) complexes and for M/2HP-β-CD with ethanol 96% (c) complexes – kneading method

Similar behaviour is also observed in the case of inclusion complexes of tenoxicam and 2-hydroxypropyl-β-cyclodextrin. The lowest water concentration values were noticed in complexes obtained with 20% ethanol (6.7% water), while when using more concentrated ethanol the values were slightly higher (7.9% water for 50% ethanol and 6.8% water for 96% ethanol). The titration curves of tenoxicam complexes are shown in Figure 9.



(a) (b) (c)

Figure 9. KF water titration curves (*V/m vs. Time*) for T/2HP-β-CD complexes with ethanol 20% (a), for T/2HP-β-CD with ethanol 50% (b) complexes, and for T/2HP-β-CD with ethanol 96% (c) complexes

The water concentration in complexes of the three oxicams with 2-hydroxypropyl-β-cyclodextrin, was half as compared with commercial 2-hydroxypropyl-β-cyclodextrin. Thus, whereas for cyclodextrin this value was 8.8%, the piroxicam and tenoxicam complexes had water concentrations of 4.6% and 5.5% respectively. In this case we may also distinguish portions in the titration curve corresponding to strongly bonded "surface" water and "crystallization" water.

Ethanol concentration used in the encapsulation process in kneading method has a significant importance for the final water concentration in the complexes and further on for the quality of the encapsulation process: diluted ethanol (20%, v/v) is optimal for efficient encapsulation; with water use in the process, the oxicams will not be solubilized enough, and a more concentrated ethanol does not allow the proper solubilization of cyclodextrin.

4. Conclusion

Classical Karl Fischer water titration is a good tool for evaluation of water concentration in cyclodextrins and their inclusion complexes with oxicams. In the case of oxicam/2-hydroxypropyl-β-cyclodextrin complexes obtained by kneading method, the concentration of ethanol used in encapsulation process showed significance for the water final concentration of complexes and also for the quality of encapsulation. Therefore, the optimum concentration of ethanol for an efficiently encapsulation is 20%, v/v, while using more concentrated ethanol do not permit the dissolution of cyclodextrin and consequently, the oxicams will not be enough solubilized. The overall water content of oxicam/cyclodextrin inclusion complexes is low (suggesting a better hydrophobic interaction between host and guest molecules) and no significant difference among oxicams types is observed.

Table 1. Water content (%) of oxicam/cyclodextrin complexes by classical Karl Fischer titration

No	Code	Description	KFT water content (%)	n (no of determ.)
1	α-CD	α-Cyclodextrin (commercial)	11.5	2
2	P/α-CD_Cr	Piroxicam/α-cyclodextrin inclusion complex obtained by crystallization from solution	7.17	3
3	M/α-CD_Cr	Meloxicam/α-cyclodextrin inclusion complex obtained by crystallization from solution	7.96	6
4	T/α-CD_Cr	Tenoxicam/α-cyclodextrin inclusion complex obtained by crystallization from solution	9.25	3
5	P/α-CD_Pst	Piroxicam/α-cyclodextrin inclusion complex obtained by kneading method	7.21	3
6	M/α-CD_Pst	Meloxicam/α-cyclodextrin inclusion complex obtained by kneading method	7.58	4
7	T/α-CD_Pst	Tenoxicam/α-cyclodextrin inclusion complex obtained by kneading method	7.50	3
8	β-CD	β-Cyclodextrin (commercial)	15.27	3
9	P/β-CD_Cr	Piroxicam/β-cyclodextrin inclusion complex obtained by crystallization from solution	10.98	3
10	M/β-CD_Cr	Meloxicam/β-cyclodextrin inclusion complex obtained by crystallization from solution	10.25	7
11	T/β-CD_Cr	Tenoxicam/β-cyclodextrin inclusion complex obtained by crystallization from solution	9.91	6
12	P/β-CD_Pst	Piroxicam/β-cyclodextrin inclusion complex obtained by kneading method	11.14	4
13	M/β-CD_Pst	Meloxicam/β-cyclodextrin inclusion complex obtained by kneading method	10.14	3
14	T/β-CD_Pst	Tenoxicam/β-cyclodextrin inclusion complex obtained by kneading method	10.11	3
15	2HP-β-CD	2-Hydroxypropyl-β-cyclodextrin (commercial)	11.46	4
16	P/2HP-β-CD_20_Pst	Piroxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 20%	5.43	3
17	M/2HP-β-CD_20_Pst	Meloxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 20%	8.23	3
18	T/2HP-β-CD_20_Pst	Tenoxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 20%	6.67	3
19	P/2HP-β-CD_50_Pst	Piroxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 50%	8.23	3
20	M/2HP-β-CD_50_Pst	Meloxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 50%	7.34	3
21	T/2HP-β-CD_50_Pst	Tenoxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 50%	7.90	2
22	P/2HP-β-CD_96_Pst	Piroxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 96%	8.39	3
23	M/2HP-β-CD_96_Pst	Meloxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 96%	9.03	4
24	T/2HP-β-CD_96_Pst	Tenoxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex obtained by kneading method with ethanol 96%	6.77	3

Acknowledgements
Authors are grateful to Professor Heinz-Dieter Isengard (Hohenheim University, Germany) for the help in Karl Fischer water titration.

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