Research Article

Inorganic-Organic Hybrids to Improve the Performances of Anti-Inflammatory Topical Formulations

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ABSTRACT

Purpose: Current available topical formulations containing non-steroidal anti-inflammatory drugs (NSAIDs) suffer from inadequate efficacy due to drug limited solubility (class II of Biopharmaceutics Classification System) responsible for the incomplete absorption of the administered dose in the application site. Another problem is represented by inadequate formulation, difficult to be applied by a massage on inflamed and injured skin. However often this practice results painful for the patient as the formulation used has been projected without taking account of this problem. The aim of this research was to develop a new topical formulation able to release and make available to absorption the complete administered dose and to make the application easy and pain free using the anti-inflammatory drug ketoprofen (KET) as model drug.
Methods: At first KET was intercalated in two lamellar anionic clays hydrotalcites (HTlcs), MgAl-HTlc-NO_3 and ZnAl-HTlc-NO_3, obtaining the inorganic-organic hybrids MgAl-HTlc-KET and ZnAl-HTlc-KET deeply characterized (XRPD, ICP, TGA, DSC). Then they were formulated in hydrogels submitted to rheological characterization and in vitro release studies using the commercially available formulation for comparison.
Results: Hydrogels containing the hybrids showed enhanced flow properties responsible for the enhanced their usability. Particularly, the presence of MgAl-HTlc meets the requirements for KET topical application best of all. Hydrogels showed a sustained release of KET useful to reduce the applications number.
Conclusions: KET intercalation into HTlc interlamellar spaces is a good strategy allowing the preparation of topical formulations stable during their shelf life and safe under administration, with enhanced performances in comparison to marketed formulations.

Keywords: NSAID, ketoprofen, topical formulation, inorganic-organic hybrids, drug delivery, rheology

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I. INTRODUCTION

Topical therapy based on non-steroidal anti-inflammatory drugs (NSAIDs) is the most common practice adopted for the treatment of muscle pains, sprains, strains, and arthritis. Among the numerous formulations containing NSAIDs, a significant percentage is represented by topical formulations realized with the aim to be applied on the pathological site in order to obtain a localized action. In fact, the topical administration offers the advantage of a local, enhanced drug delivery to the inflamed tissues reducing the systemic adverse effects associated to oral therapy [1]. Topical formulations containing NSAIDs currently available are represented by gels, creams, patches, sprays, medicated adhesive systems or foams [2]. Despite the high amount of topical products containing NSAIDs available on the market, some drawbacks are still associated to their use limiting their efficacy. The first aspect that must be taken into account is that many NSAIDs suffer from poor solubility, thus they are classified in class II of the biopharmaceutics classification system (BCS) [3]. In the case of topical formulations that greatly restrict their effectiveness as they are applied on a dry surface and if the active pharmaceutical ingredient (API) is poor soluble and not well formulated its absorption is very limited. Thus, a suitable NSAID topical formulation must contain the drug is solution thus, once applied on the skin, it will be able to enter the stratum corneum and to reach the action site. With this aim numerous topical formulations are prepared by using ethanol as cosolvent in order to enhance NSAID

solubility. However this approach has some limitations in fact, sometimes drug precipitation takes place giving rise to small crystals dispersed in the final formulation with consequent problems of low homogeneity, limited stability and bad spread ability.

The second aspect associated to the use of topical formulations containing NSAIDs it that they are intended to be applied by massage on inflamed, injured or sore skin. During this procedure care, the product should be used in order to avoid the general skin conditions alteration. However during the massage the formulation becomes less fluent with consequent development of the friction with the skin. The main consequence of this is the pain deriving from the application of the formulation on the pathological site provoking discomfort for the patient. During an inflammation process the integrity of inflamed skin is generally compromised, resulting in increased percutaneous migration and systemic absorption of most drugs [4]. The combination of such problems brings to an incomplete and ineffective local treatment of a confined disease with the consequent extension of the therapy for long periods. The abovementioned aspects highlight that that the limited efficacy of NSAIDs topical products is mainly associated to the inappropriate formulation approach. In this context a suitable strategy to overcome the exposed limitations and to furnish an improved topical formulation, in terms of effectiveness homogeneity, stability and proper flow properties can be realized by means of the realization of NSAIDs inorganic-organic hybrids with the inorganic lamellar matrices hydrotalcites (HTlc).

HTlcs are natural anionic lamellar solids with positively charged layers balanced by exchangeable anions [5,6], rare in nature, but quite easy to prepare in laboratory. They have the general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}[A^{n-}_{x/n}]^{x-}$m S where M(II) is a bivalent metal cation, usually Mg, M(III) is a trivalent metal cation, usually Al, A^{n-} is an exchangeable inorganic or organic anion that compensates the positive charge of the layer and m are the moles of solvent S, usually water, co-intercalated for mole of compound [6]. An intercalated anion can be replaced by another via ion-exchange, with consequent variation of the interlayer distance [7]. Consequently, the lamellar host in the interlayer region can be considered a microvessel in which anionic molecules (guests) may be stored. When a guest species is intercalated in the interlamellar space loses the crystalline structure and organizes in an ordered "liquid like" state [8-10]. In these conditions the re-crystallization of guest molecules does not occur thus, the API results easily and homogeneously dispersible in the formulation and able to reach the skin without difficulty.

The present paper deals with the intercalation of the NSAID ketoprofen (KET) in HTlcs to realize inorganic-organic hybrids then formulated as hydrogels with the aim to purpose an advanced technological formulation with improved performances than currently available formulations present on the market and containing the same API. In this work new KET intercalation conditions have been purposed and performed in comparison to a previous study [8].

MATERIALS AND METHODS

Materials

Ketoprofen in acidic form (KETH) was provided by Bidachem, Fornovo San Giovanni, Bergamo, ZnO (Caelo), Urea (Chemische Fabrik Lehrte), sodium carboxymethyl cellulose (NaCMC, Caelo), propylene glycol, liquid paraffin, silicon oil, macrogol 4000 and macrogol 400, were furnished from Comifar (Perugia, Italy).

$AlCl_3 \cdot 6H_2O$ (Carlo Erba), $MgCl_2 \cdot 6H_2O$ (Carlo Erba), K_2CO_3 (Carlo Erba), $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4 were furnished from DueM (Perugia, Italy).

Deionized water was obtained from reverse osmosis process with Milli Q System (Millipore, Roma, Italy). Other chemicals and solvents were of reagent grade and were used without further purification. Commercially available ketoprofen gels (Fastum® gel, Lasonil®c.m. gel) were purchased in pharmacy.

The fluids used in the release studies were: phosphate buffer solution pH 5.5 (1000 ml), prepared according to European Pharmacopoeia (Ph. Eur. VIII Ed.), mixing 964 ml of KH_2PO_4 water solution (13.61 g/L) to 36 ml of a Na_2HPO_4 water solution (35.81 g/L). K_2CO_3 solution 0.025 N.

HTlc Syntheses

Crystallized $[Mg_{0.66}Al_{0.34}(OH)_2]$ $(NO_3)_{0.34}$ $0.76 \cdot H_2O$ and $[Zn_{0.70}Al_{0.30}(OH)_2]$ $(NO_3)_{0.30}$

$0.4{\cdot}H_2O$ were obtained by titration of the carbonate forms by HNO_3 0.1 M [11]. Carbonate anions are strongly held and difficult to exchange, whereas nitrate anions are more suitable for anionic exchange reactions [12], thus carbonate form of HTlc was converted into nitrate form. MgAl-HTlc and ZnAl-HTlc in carbonate form were obtained by coprecipitation of Mg(II)/Zn(II)-Al(III) accomplished by the hydrolysis of urea [13]. The synthetic hydrotalcite MgAl-HTlc-CO_3 was obtained by adding solid urea to a 0.5 M metal chloride solution. Al^{3+}/ (Al^{3+} + Mg^{2+}) and urea/ (Mg^{2+} + Al^{3+}) were in the molar ratio of 0.33 and 3.3 respectively. The hydrolysis of urea, inducing slow pH increase, led to the precipitation of metals in a well-crystallized HTlc carbonate form. The mixture was heated at 100°C under stirring for 36 hrs. The final product (MgAl-HTlc-CO_3) was recovered, washed with water to eliminate chlorides and stored in desiccator over P_2O_5 at room temperature.

For ZnAl-HTlc-CO_3 [13] synthesis a weighed amount of ZnO was dissolved in a stoichiometric amount of 6 mol/dm^3 HCl solution. Solid urea was added to 0.5 mol/dm^3 metal chloride solutions ($AlCl_3{\cdot}6$ H_2O), having molar fraction Al^{3+}/ (Al^{3+} + Zn^{2+}) equal to 0.33, until the molar ratio urea/ (Al^{3+} + Zn^{2+}) reached the value 3.3. The clear solutions were heated, under stirring, at 100°C for 36 hrs. The final product (ZnAl-HTlc-CO_3) was recovered, washed with water to eliminate chlorides and stored in desiccator over P_2O_5 at room temperature.

KET intercalation in HTlcs

The preparation of the inorganic-organic hybrids MgAl-HTlc-KET and ZnAl-HTlc-KET was performed by KET intercalation into HTlc lamellae. Pristine hydrotalcites in carbonate form (HTlc-CO_3) were transformed in nitrate form (HTlc-NO_3) and then put in contact with drug as previous reported [14]. For both intercalation products the molar ratio HTlc/KET 1:2 was used [8]. Both MgAl-HTlc-KET and ZnAl-HTlc-KET were obtained starting from a suspension of drug in water (carbon dioxide free) treated with a stoichiometric amount of 0.1 NaOH to obtain the sodium salt that dissolves obtaining a clear solution to which HTlc-NO_3 was added. The resulting suspensions were stirred at room temperature under magnetic stirring for 24 hrs. The solids were recovered by centrifugation and washed alternatively three times with degassed water and ethanol and finally dried at room temperature over P_2O_5.

Inductively coupled plasma spectrometry (ICP)

Metal analyses were performed by Varian 700-ES series inductively coupled plasma-optical emission spectrometers (ICP-OES) using solutions prepared by dissolving the samples in some drops of concentrated HNO_3 solution and properly diluted.

X-ray powder diffraction (XRPD)

The X-ray powder diffraction (XRPD) patterns were performed with a diffractometer (PW 1710 Philips, Lelyweg, Netherlands), using the Ni-filtered Cu Kα radiation, that works at 40 KV, 30 mA with goniometer PW 1820 and graphite's monochromator for diffracted ray. Diffractograms were registered with step scanning method (step size 2θ = 0.03°) and were elaborated by PC-APD program.

Thermogravimetric analysis (TGA)

Coupled thermogravimetric and differential thermal analyses were performed with a Netzsch STA 449C apparatus, in air flow and heating rate of 10°C/min to determine the weight loss (water and drug) as a function of increasing temperature.

Differential scanning calorimetry (DSC)

DSC analyses were performed using an automatic thermal analyser (Mettler Toledo DSC821e) and indium standard for temperature calibrations. Holed aluminum pans were employed in the experiments for all samples and an empty pan, prepared in the same way, was used as a reference. Samples of 3-6 mg were weighted directly into the aluminum pans and the thermal analyses of samples were conducted, at a heating rate of 5°C/min, from 25-200°C.

HyperChem

HyperChem program (Hyperchem™, 2000) has been used to obtain the computer intercalation compound simulated model [15].

Hydrogels manufacturing

Ketoprofen (KET), in free form and intercalated into HTlcs (MgAl-HTlc and ZnAl-HTlc), has been formulated as hydrogels, prepared according to the procedures reported in the European Pharmacopoeia (VIII Ed.) for semisolid formulations intended for skin application.

Hydrogels had the following composition:

- NaCMC-5.0 g
- KET-2.5 g

(2.5 g of free KET corresponds to MgAl-HTlc-KET 4.8 g or ZnAl-HTlc-KET 5.9 g)

- Propylene glycol-10.0 g
- Deionized water until to 100.0 g.

In the case of hydrogels containing free KET the mixture constituted by ethanol and propylene glycol was warmed in a steam water bath in order to induce KET solubilization. In the case of the hybrid products, MgAl-HTlc-KET and ZnAl-HTlc-KET were previously well mixed to NaCMC, then propylene glycol and finally water were added under continuous and vigorous stirring

Rheological studies

Viscometry measurements (viscosity and yield stress 25°C and 32°C) of formulations were performed by a Stresstech HR (Reologica Instruments AB, Milano, Italy) rheometer with cone-plate geometry (diameter of 40 mm angle 1°). Samples were carefully applied to the lower plate using a spatula to avoid formulation shearing and air bubble formation.

***In vitro* release studies**

For this investigation, horizontal Franz diffusion cells was used (PermeGear, Inc., Bethlehem, PA, diameter 20 mm) constituted by a water jacketed receptor chamber (15 ml) and a donator chamber (2 ml). Two experiments have been performed using two types of receptor medium: phosphate buffer pH 5.5 (Ph. Eur. VIII Ed.) and a K_2CO_3 solution 0.025 M (simulating air CO_2). The receptor fluid was maintained at 32°C and magnetically stirred at 600 rpm. The two chambers were separated by a cellulose membrane (Filter paper Whatman 41, Whatman GmbH, Dassel, Germany) in which was placed the formulation (200 mg) was loaded into the upper donor chamber and was successively sealed with parafilm®.

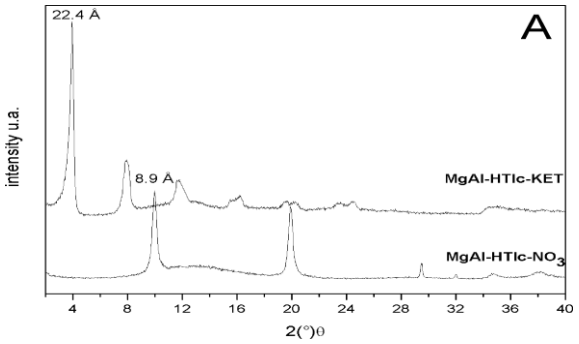
RESULTS AND DISCUSSION

Preparation of the inorganic-organic hybrids

In a previous work [8] KET was intercalated into MgAl-HTlc in chloride form. That intercalation procedure required the equilibration HTlc-Cl with a KET sodium salt aqueous solution (carbon dioxide free), obtained by ketoprofen in acidic form (KETH) salified using a stoichiometric amount of NaOH. The obtained suspension was kept under stirring and the intercalation reaction left for 7 days at 60°C [8]. The procedure purposed and carried out in the present work, was based on the use of a different precursor, MgAl-HTlc in nitrate form instead of chloride form, reducing the intercalation reaction time to 24 hrs and working at room temperature. Another important aspect is represented by the intercalation of KET in the lamellar solid ZnAl-HTlc in nitrate form. The use of two starting materials (MgAl-HTlc and ZnAl-HTlc), differing from the bivalent metal present in the lamellar composition, is useful in order to evaluate the properties and thus the potential application of the inorganic-organic hybrid products deriving from KET intercalation.

Hybrids characterization: X-ray diffraction analysis (XRPD)

The obtained products were firstly submitted to X-ray diffraction analysis (XRPD), the main analytical technique to assess drug intercalation. Obtained data demonstrated that the typical reflection corresponding to nitrate anion (8.9 Å) disappears in both intercalation products (hybrids) patterns, meaning that complete substitution of nitrate ions by KET anions took place. As KET dimension is higher than nitrate anion the intercalation produced an increase of HTlc interlayer distance, from 8.9 Å to 22.4 Å for MgAl-HTlc-KET and 22 Å for ZnAl-HTlc-KET (**Figures 1A** and **1B**).



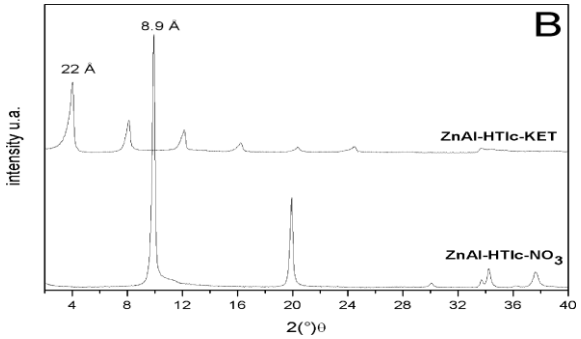


Figure 1: A) X-ray patterns of MgAl-HTlc-NO_3 and the corresponding hybrid MgAl-HTlc-KET; B) X-ray patterns of ZnAl-HTlc-NO_3 and the corresponding hybrid ZnAl-HTlc-KET.

Hybrids characterization: thermogravimetric analysis (TGA) and inductively coupled plasma spectrometry (ICP).

The exact formula of the products was determined by submitting the hybrids both to thermogravimetric analysis (TGA), in order to measure the percentage of organic fraction (KET) in each one, and to the inductively coupled plasma spectrometry (ICP), in order to measure the amount each metal, Mg, Zn and Al in both HTlcs. In regard to TGA analyses (**Figures 2 and 3**), in both cases similar thermal behaviour can be observed. The profiles show a exothermic weight loss about 210-215°C attributable to hydration water lost; increasing the temperature, up to 500°C in the case of MgAl-HTlc-KET and up to 450°C in the case of ZnAl-HTlc-KET, another exothermic process can be highlighted, due to the combustion of the organic part (KET decomposition). Additionally, at these values of temperature are observed also the condensation of the lamellar hydroxyls. After 500°C both thermograms show a plateaux due to the presence of stable oxides to high temperature.

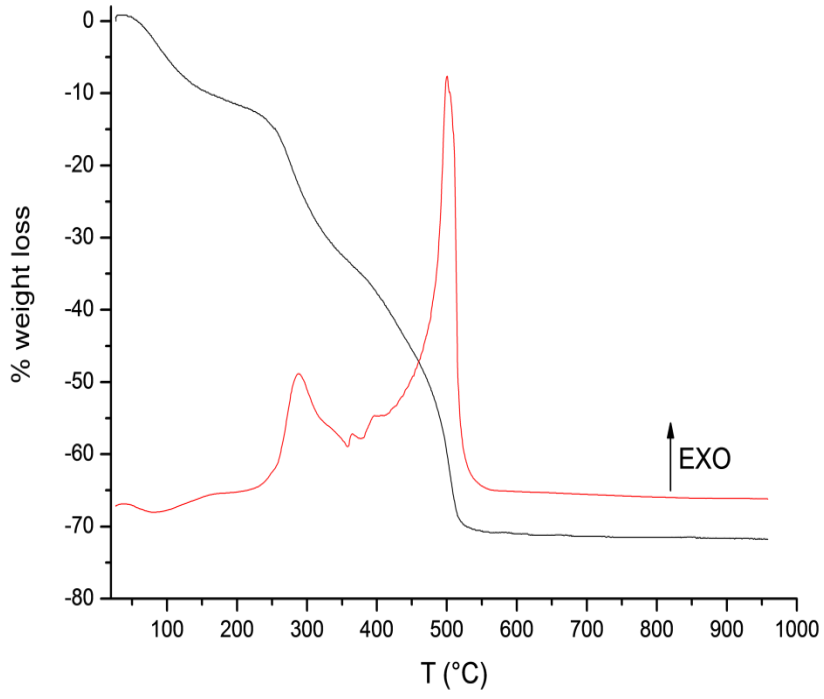


Figure 2: Thermogravimetric curve of MgAl-HTlc-KET.

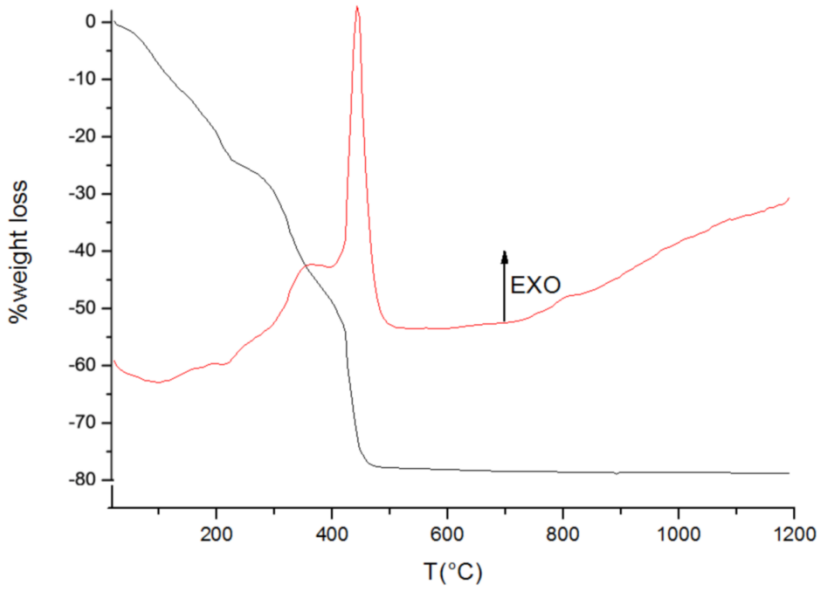


Figure 3: Thermogravimetric curve of ZnAl-HTlc-KET.

Combining data coming from ICP analysis and TGA, for KET content, the molecular formulas of the HTlcs (MgAl-HTlc-NO_3 and ZnAl-HTlc-NO_3) and of the corresponding hybrids were determined (**Table 1**). It is important to underline that the intercalation procedure allowed to obtain a better KET loading in MgAl-HTlc (52.28%) compared to that prepared in the previous work (50%) [8]

Precursor	hybrids product formula	KET loading % (wt./wt.)
$[Mg_{0.66}Al_{0.34}(OH)_2]$ $(NO_3)_{0.34}\cdot 0.4\cdot H_2O$	$[Mg_{0.66}Al_{0.34}(OH)_2]$ $(KET)_{0.32}(NO_3)_{0.02}\cdot 0.76\ H_2O$	52.28 %
$[Zn_{0.70}Al_{0.30}(OH)_2]$ $(NO_3)_{0.30}$ $0.4\cdot H_2O$	$[Zn_{0.70}Al_{0.30}(OH)_2]$ $(KET)_{0.30}\cdot 0.98\ H_2O$	41.89 %

Table 1: Hybrids compositions and KET loading percentages.

Hybrids characterization: HyperChem

On the basis of the chemical compositions and of the interlayer distances of both hybrids a computer simulated model was obtained by the HyperChem program [14] for MgAl-HTlc-KET and ZnAl-HTlc-KET (**Figure 4**)

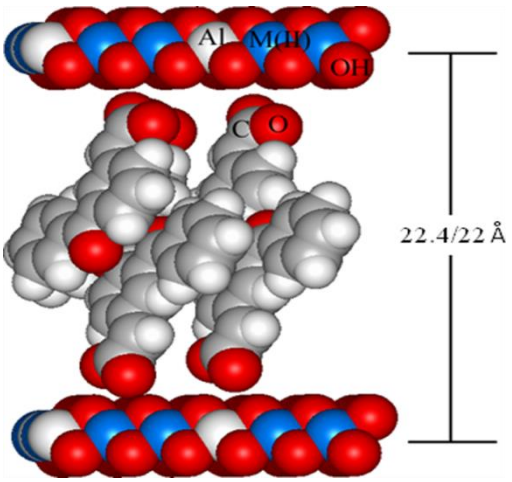


Figure 4: Computer-generated representation of HTlc-KET.

The presence in the interlayer region of KET anions, as double layers, was hypothesized. In this model, the intercalated anions show the carboxylic group oriented toward the aluminium positive charge on the lamellae so the π–π interactions between KET aromatic rings are encouraged. The bond formation between KET lipophilic moiety allows the drug to align in ordered manner forming a film of interdigitated drug molecules.

Hybrids characterization: differential scanning calorimetry analysis (DSC)

About DSC analysis, thermograms registered for both hybrids MgAl-HTlc-KET, ZnAl-HTlc-KET in comparison to crystalline KET (**Figures 5** and **6**) show the lack of the endothermic peak at 97°C, corresponding to the melting of pure crystalline substance [16]. This means that a new compound was produced by KET intercalation into HTlcs. In fact, the peak at 97°C is visible just in the case of crystalline KET and its physical mixture with both HTlcs types.

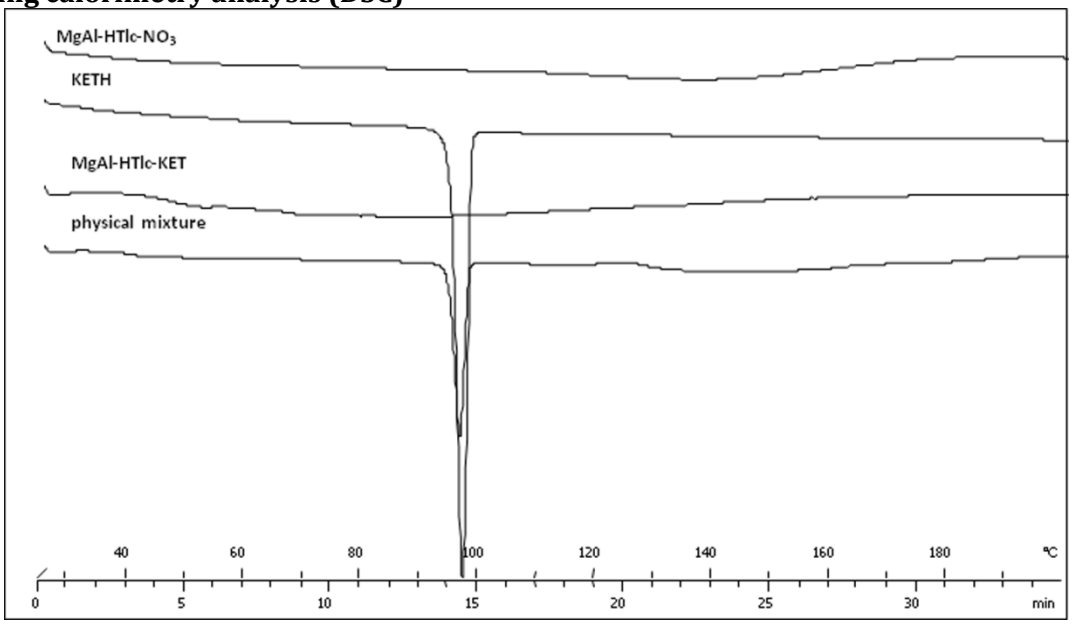


Figure 5: Thermal profiles of MgAl-HTlc-NO_3, crystalline acid KETH, MgAl-HTlc-KET and MgAl-HTlc-NO_3/crystalline acid KETH physical mixture.

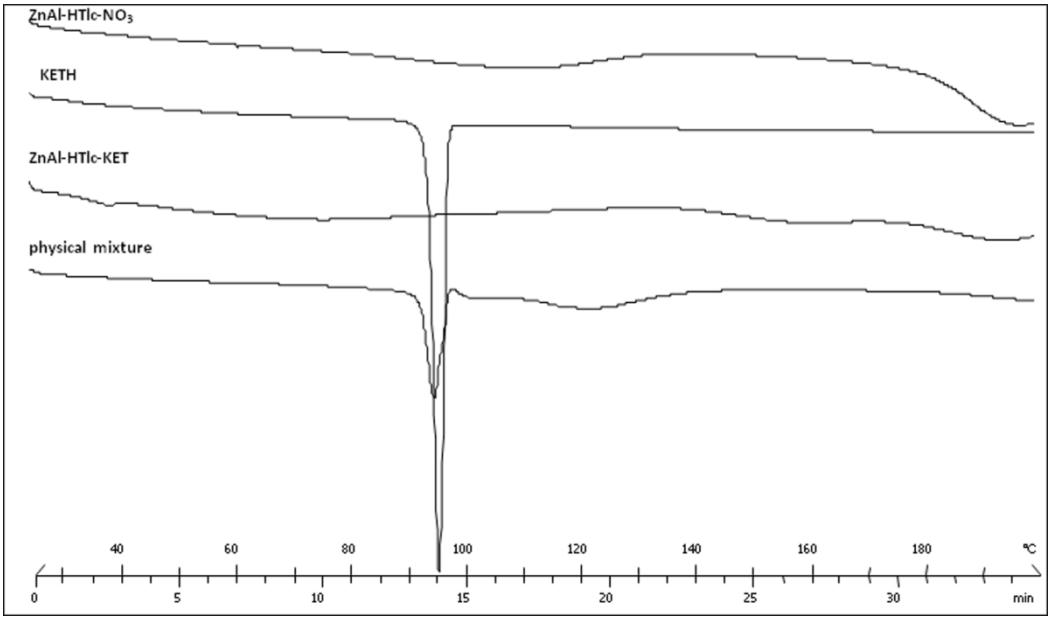


Figure 6: Thermal profiles of ZnAl-HTlc-NO_3, crystalline acid KETH, ZnAl-HTlc-KET and ZnAl-HTlc-NO_3/crystalline acid KETH physical mixture.

Hybrids characterization: scanning electron microscopy analysis (SEM)

In order to evaluate the morphology of the starting HTlcs and of the corresponding hybrids, all the samples have been submitted to SEM analysis. From the micrographs (**Figure 7A**) it can be observed that MgAl-HTlc-NO_3 shows microcrystals of regular shape, almost hexagonal and flat, quite homogeneous with very sharp edges and with an average diameter minor than 2 microns. The corresponding hybrid MgAl-HTlc-KET (**Figure 7B**) shows different morphology. KET intercalation process appears to produce changes on the crystals characteristics which appear are flat but not hexagonal, smoothed almost rounded. About the dimensional aspect, the main part of the crystals preserves the size of starting material (1.5-2 microns) but the crystal population results polydisperse as very small crystals can be observed. The last, always flat, do not have a regular shape and seem to be fragments of the larger ones. ZnAlHTlc-NO_3 (**Figure 7C**) micrograph shows that crystals are irregular both for shape and dimensions, also their edges are not regular. In regard to the dimension, two populations can be identified. The first one appears big and quite regular crystals with an average diameter of 5.8 microns, the second one show smaller crystals irregularly shaped probably come from biggest breaking.

ZnAl-HTlc-KET (**Figure 7D**) maintains the same characteristics of the precursor and also in this case two populations can be distinguished. Micrographs analysis offers many and interesting information as crystal characteristic can affect the usability of final formulation. The observed crystals, even if not uniform in terms of size, however can be considered suitable to be incorporated into a topical formulation to spread on the skin resulting not abrasive, because of their size.

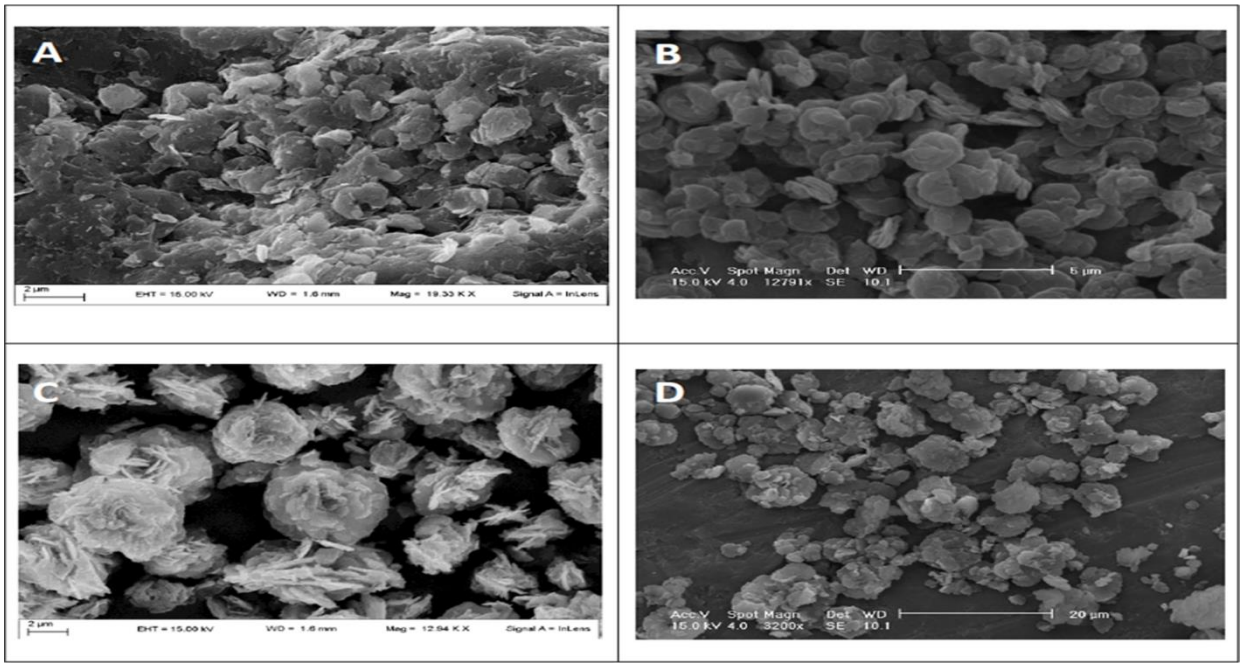


Figure 7: SEM micrographs of MgAl-HTlc-NO_3(A), MgAl-HTlc-KET (B), ZnAl-HTlc-NO_3 (C)and ZnAl-HTlc-KET (D).

Hydrogels manufacturing

MgAl-HTlc-KET and ZnAl-HTlc-KET were formulated as hydrogels, stable monophasic formulations having a simple composition and easy to prepare and for this reason scalable to industry.

Hydrogels were prepared by using the cellulose derivative NaCMC, a cheap water soluble polymer provided of different properties: thickener, suspending aid, stabilizer, binder, and film-former in a wide variety of uses [17]. The choice of a cellulose derivative comes from the necessity to use a polymer which swelling is driven just by water uptake, independently from pH value. After many attempts, the formulation was prepared with a KET final loading of 2.5% as the market available formulations. Initially NaCMC and crystalline KET (or the hybrid) was gently mixed to propylene glycol, then water was slowly added, under vigorous stirring, in order to promote gradual and uniform polymer swelling. By this procedure

were prepared three kind of gel which compositions is reported in (**Table 2**).

Table 2: Compositions of the hydrogels containing crystalline KET and hybrids.

	KETH hydrogel	MgAl-HTlc-KET hydrogel	ZnAl-HTlc-KET hydrogel
KETH	2.50 g	4.80 g of MgAl-HTlc-KET corresponding to 2.5g of KETH (loading 52.28%)	5.95 g of ZnAl-HTlc-KET corresponding to 2.5g of KETH (loading 41.89%)
propylene glycol	10.00 g	10.00 g	10.00 g
NaCMC	5.00 g	5.00 g	5.00 g
Deionized water	82.50 g	80.20 g	79.05 g

Rheological characterization of hydrogels: viscosity at 25°C and 32°C

The measurement of rheological characteristics (flow properties) provides essential information about different aspects concerning semisolid preparations as hydrogels. In fact, the flow characteristics of such formulations affect the manufacture stages (e.g. mixing, pumping, filling etc.), the physical stability as well as patient acceptability and compliance [18]. In regard to the latter, these aspects must be taken into account particularly in the case of hydrogels intended to be applied by massage on inflamed, injured or sore skin. As the application is performed by massage, it must be easy in order to do not cause pain and compromise the general skin conditions. Thus, it should be preferable to formulate gels provided for plastics behaviour because of their low resistance to flow when they are applied under high shear conditions [19].

The experiments were carried out by a Stresstech HR rheometer provided by cone-plate geometry (diameter of 40 mm angle 1°). Samples were applied to the lower stationary plate of the rheometer and allowed to equilibrate for 5 min before analysis.

Three different measurements were performed: i) viscosity at 25°C, to reproduce the storage temperature, ii) viscosity at 32°C, to reproduce skin surface temperature and iii) yield value at 25°C (the stress value necessary to make the system flow).

These experiments were performed on the prepared gels containing crystalline KETH, MgAl-HTlc-KET and ZnAl-HTlc-KET. As a control three other types of gel were introduced in the study namely: not medicated gel (having the same composition of KET based gels but free from KET), the market medicinal formulations Fastum® gel and Lasonil®c.m. gel.

About viscosity measurement, rheograms were registered in steady state conditions and produced by gradually increasing the shearing stress applied to the sample and the applied shear stress presented as a function of shear rate. Rheograms registered at 25°C are reported in three different plots because of the impossibility to use the same scale (**Figure 8**).

Not medicated gel (**Figure 8, panel A**) results the most viscous showing a plastic behaviour as high force (10^3 Pa) is required in order to induce the flow, almost limited. In fact, the shear field is not strong enough to appreciably untangle the polymer chains. The small flow observed for not medicated gel is ascribed to the high cross linking of the gel like structure. The lack of KET (in free form or intercalated), able to interact with polymeric chains and to promote their enlargement, prevents the network distension resulting highly tangled.

Both commercial gels (**Figure 8 panel B**) are the most viscous formulations displaying a plastic character with a yield value of about 10000 Pa and 20000 Pa for Fastum® gel and Lasonil®c.m. gel respectively. In this case the increase of shear stress led to a progressive breakdown of gel structure, with a decrease in the slope (apparent viscosity), this behaviour is mainly visible for Fastum® gel.
About gels containing the hybrids and KETH (**Figure 8 panel C**) also in this case a plastic behaviour can be observed. Among them, rheogram of gel containing KETH shows the lowest slope compared to those relative to MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel.

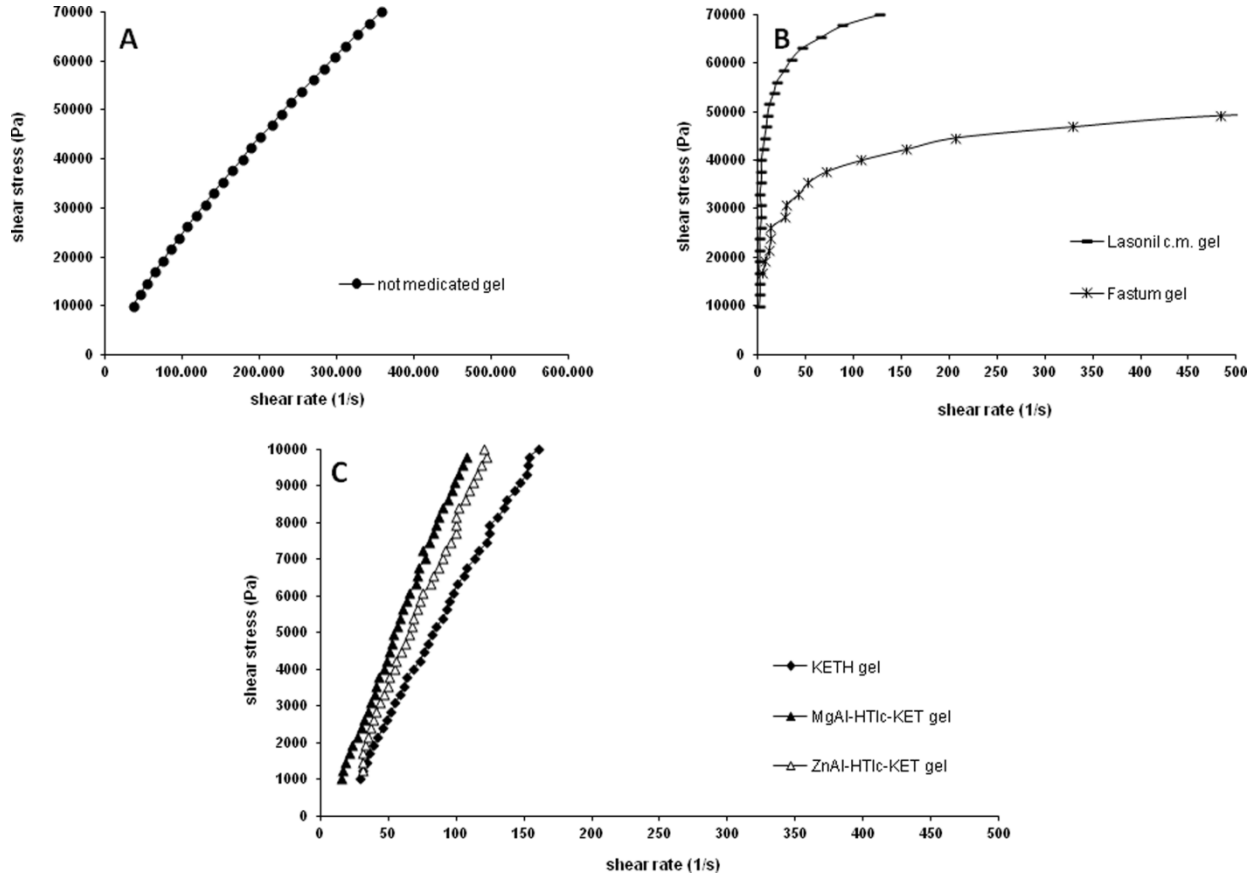


Figure 8: Rheograms, shear stress *vs* shear rate, registered at 25°C of A) not medicated gel, B) Fastum® gel and Lasonil®c.m. gel, C) KETH gel, MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel.

The observed differences can be ascribed to the different form in which KET is inserted in the gels. In fact, MgAl-HTlc-KET and ZnAl-HTlc-KET are dispersed as solid particles in the polymer network working as thickening agents [20]. This means that the NaCMC gel results more viscous and compact than that containing KETH. The high consistency of hydrogels containing the hybrids does not represent a limit for such formulations. In fact, the viscosity assures a high stability of gels during storage conditions, when a force (stress) is applied HTlc crystals dispersed in the gel orientates themselves parallel to the flow direction indicating better spreadability upon topical application of the formulations thus, making the application procedure easy and pleasant.
Rheograms registered at 32°C show that in all cases the temperature increase produces some modifications. Not medicated gel (**Figure 9 panel A**) maintains the high viscosity, showing a slight reduction of flow properties by temperature increase probably because of the evaporation of water responsible for more compact gel generation. Marketed formulations are the most viscous also at 32°C. While in the case of Fastum® gel no modifications can be highlighted in comparison to 25°C behaviour, for Lasonil® c.m gel viscosity increase is observed. This can be ascribable to the evaporation of

ethanol, present in the composition as cosolvent (for KET solubilisation enhancement), able to generate a thicker gel (**Figure 9 panel B**). On the other hand, the prepared hydrogels, especially those containing the inorganic-organic hybrids, showed a different and interesting behaviour. They, in fact, show a reduced viscosity as consequence of temperature increase. KETH gel is still less viscous that those containing the hybrids. At 32°C MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel show the same rheograms meaning that the temperature increase reduces the slight differences observed at 25°C (**Figure 9 panel C**).

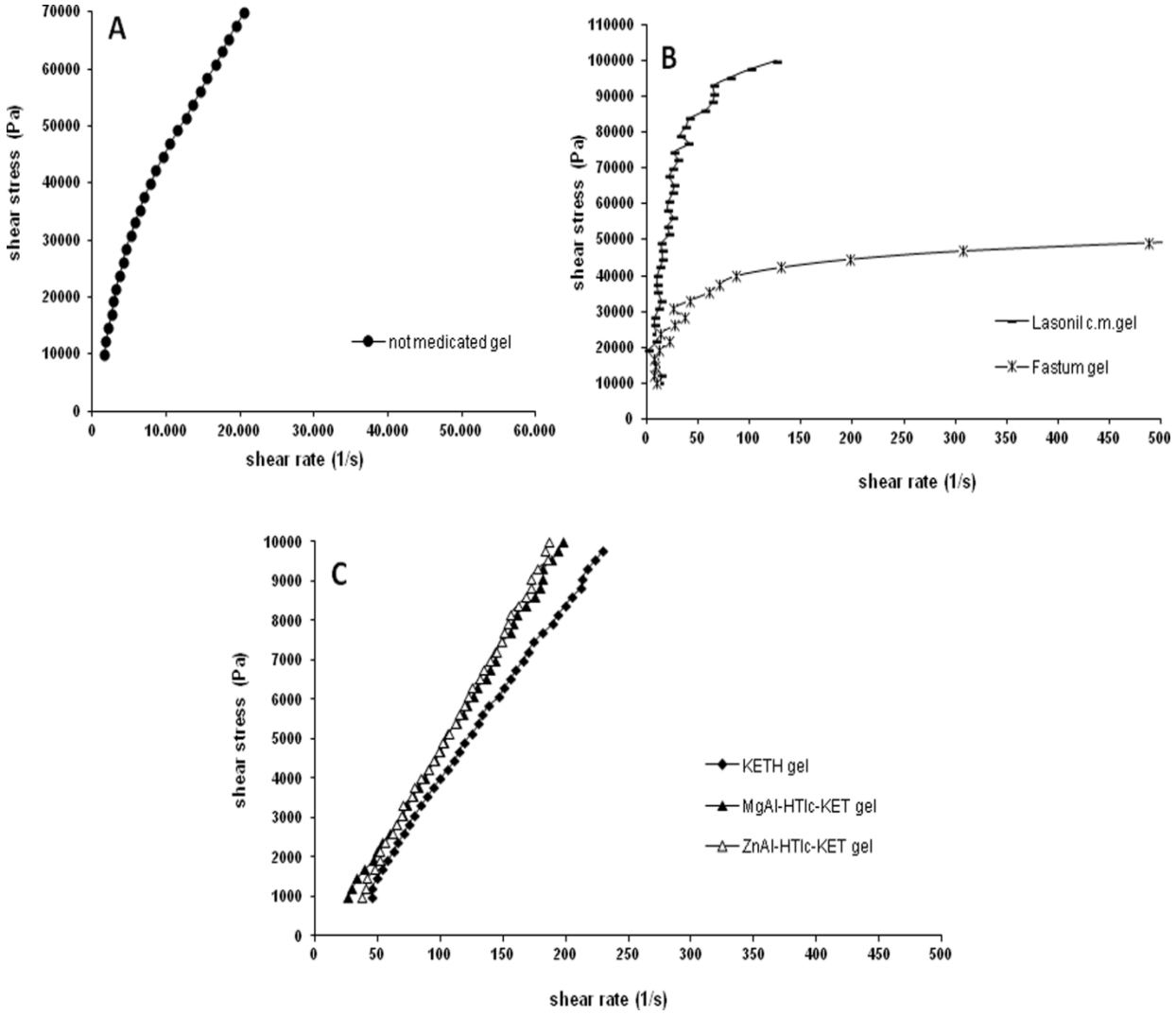


Figure 9: Rheograms, shear stress *vs* shear rate, registered at 32°C of A) not medicated gel, B) Fastum® gel and Lasonil®c.m. gel, C) KETH gel, MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel.

Rheological characterization of hydrogels: Yield stress

Yield stress rheograms were registered in steady state conditions and produced by gradually increasing the shearing stress applied to the sample, in this case the viscosity was presented as function of applied shear stress. The yield stress value measures the force required to extrude the material from a tube/package and for this reason measured at room temperature (25°C).

The formulations showing the highest yield value are Lasonil® c.m. gel > not medicated gel and > Fastum® gel (**Figure 10 panel A and panel B**). Hydrogel containing not intercalated KET shows a high yield stress value as consequence of the high viscosity. MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel show the lowest yield value (**Figure 10 panel C**) suggesting that a small stress is needed to initiate flow, suitable property making easy the formulation application on the skin.

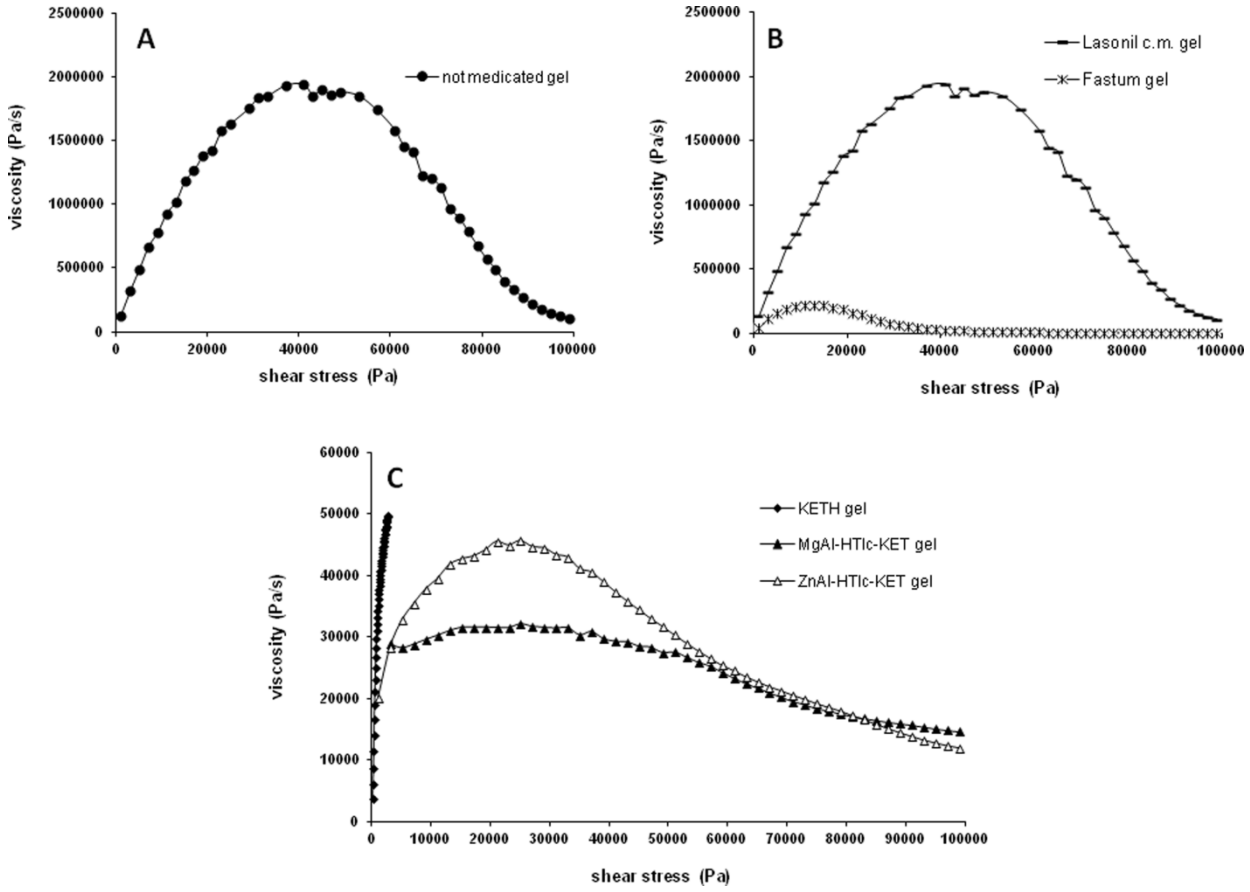


Figure 10: Rheograms, viscosity *vs* shear stress, registered at 25°C of A) not medicated gel, B) Fastum® gel and Lasonil®c.m. gel, C) KETH gel, MgAl-HTlc-KET gel and ZnAl-HTlc-KET gel.

In vitro release studies

KET release studies from topical formulations were performed by using the vertical Franz diffusion cell. Two kinds of acceptor media were employed and the experiments were carried out using phosphate buffer pH 5.5, in order to reproduce skin pH value, and a solution of K_2CO_3 0,025 M [21], in order to simulate air exposure influence. All the prepared formulations, KETH hydrogel, MgAl-HTlc-KET gel and ZnAl-HTlc-KET hydrogels were submitted to the study and the commercial product Fastum® gel was introduced as further control. About the experiment performed using phosphate buffer pH 5.5, the profiles are reported in (**Figure 11**). It is to underline that KET is gradually released from all formulation, mainly from those containing free KET (Fastum® gel > KETH gel).

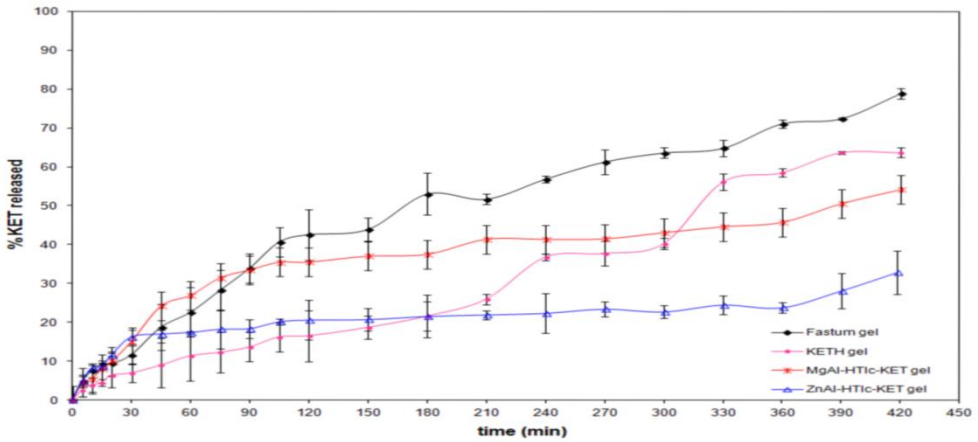


Figure 11: Release profiles in phosphate buffer pH 5.5 of KET released from KETH gel, MgAl-HTlc-KET gel, ZnAl-HTlc-KET gel and Fastum® gel.

After 30 min the lowest amount of KET was released from KETH gel (~6%) and the value maintained low until 120 min (16.6%). At the end of the experiment (420 min) the total amount of drug released was 77%. Observing the profile reported in Fig. 11 it is irregular and for this reason not predictable.

About the commercial product Fastum® gel after 30 min ~12% of KET was released then the value increases, resulting the highest of all gels, reaching 42% after 120 min and 79% after 420 min.

Hydrogels containing the hybrids show high values in the first 30 min, 15% and 16.3% for MgAl-HTlc-KET and ZnAl-HTlc-KET respectively. After this first period the release slows down reaching the values of 54% and 33% after 420 min for MgAl-HTlc-KET and ZnAl-HTlc-KET respectively. This behaviour can be ascribed to the mechanism involved in drug release from HTlc. In fact, this process is driven by an ion exchange mechanism established between KET anions stored in the interlayer space and the anions of the dissolution medium (as Cl^-, $H_2PO_4^-$, HPO_4^{2-}). In this context the ionic strength is responsible for the amount of drug released as it influences the ion exchange rate.

KET release data coming from the experiment performed K_2CO_3 solution are reported in (**Figure 12**).

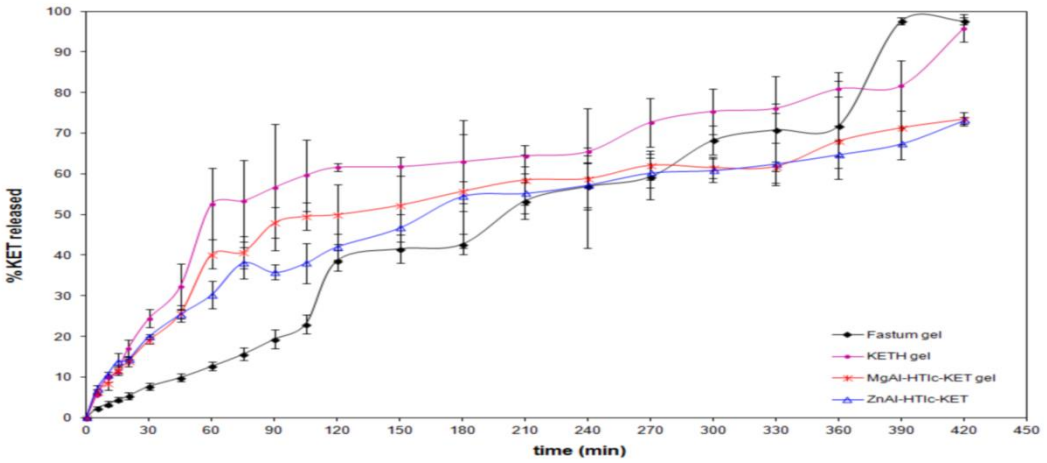


Figure 12: Release profiles in carbonate solution 0.025 N of KET released from KETH gel, MgAl-HTlc-KET gel, ZnAl-HTlc-KET gel and Fastum® gel.

These profiles are different from those obtained in phosphate buffer pH 5.5. Hydrogels containing the hybrids show an enhanced KET release reaching both after 30 min about 20%. Also in this case the release proceeds gradually but highest values were obtained in comparison to the previous experiment. After 120 min the release was 50% and 42% for MgAl-HTlc-KET and ZnAl-HTlc-KET respectively reaching in both cases 73% after 420 min. The incomplete release is ascribable to an established equilibrium between released KET molecules and those still intercalated in HTlc. At the beginning of the assay it is established a high concentration gradient between the intercalated KET (hybrids) and free KET (in solution). As the release proceeds, this driving force decreases as the experiment is performed in a close system (paddle apparatus), and for this reason the ion exchange rate is reduced.

KETH gel shows and enhanced release 24.4% after 30 min, 52.6% after 120 min and 96% after 420 min (**Figure 12**). This behaviour can be explained considering that the use of an alkaline medium as K_2CO_3 solution 0,025 N is able to improve KET solubility. In fact, because of its acidic nature it is better soluble in this medium than in phosphate buffer pH 5.5 obtaining an almost complete release after 420 min. Marketed product Fastum® gel shows a reduced release in the first 120 min (**Figure 12**) compared to the results obtained in the previous experiment, 7.7% after 30 min, 12.7% after 60 min and

38.6% after 120 min. However, at the end of the experiment, a complete release of KET was obtained (**Figure 12**).

Statistical and kinetic analysis

All gel release profiles were fitted to Ritger and Peppas's kinetics mathematical model $Mt/M\infty = Kt^n$ [22], applied to swellable matrices, in order to investigate the mechanism responsible for KET release by diffusional exponent evaluation. This release mechanism can be controlled by water penetration rate, responsible for hydration and drug diffusion, and polymeric chain relaxation time. If diffusional exponent value is 1, it means that drug release occurs as an apparent zero-order mechanism time dipendent while if the value is 0.5 it means that release is controlled by a pure Fickian diffusion mechanism. A value between 0.5-1 indicates an anomalous mechanism (not Fickian) meaning that both liquid penetration rate and polymeric chain relaxation rate control drug release. Because of HTlc presence, it must be considered that the release kinetic is affected by ion exchange process. For this reason it was necessary to take into account the kinetic model proposed by Bhaskar et al. [23] for ion exchangeable resins.

By analyzing the r coefficient (correlation coefficient of the linear regression lines obtained from the release values of each formulation) it was investigated in which cases was obtained the best fitting. In regard to the gels containing the hybrids, both in phosphate buffer pH 5.5 and in carbonate solution, r value increases from n = 1 to n = 0.5 (**Table 3**).

From the observation of the calculated r values (correlation coefficient of linear regression), it can be noted that, both for pH 5.5 and alkaline solution, hydrogels containing intercalated KET (hybrids) show progressive r increase from n = 1 to n = 0.5. The best data fitting is observed for n=0.5 meaning that KET release follows fickian diffusion and in particular Higuchi kinetic. The r values obtained applying Bhaskar kinetic (r = 0.940 for MgAl-HTlc-KET hydrogel, r = 0.926 for ZnAl-HTlc-KET hydrogel) are similar to those obtained from Higuchi, meaning that drug is able to cross easily the polymeric network and that its release is also controlled by HTlc. KETH hydrogel shows the best fitting for Higuchi kinetic, only in the case of carbonate solution, meaning that drug release follow fickian diffusion (**Table 3**).

In phosphate buffer pH 5.5 (**Table 3**) KET release follows a zero order kinetic (best fitting for n = 1, r = 0.983).

Table 3: Ritger and Peppas's kinetic mathematical model and first order kinetics model fitting for hydrogels in phosphate buffer pH 5.5 and carbonate solution.

	Phosphate buffer pH 5.5								
	$M_t/M_\infty=kt^n$							**$M_t/M_\infty=1-e^{-kt}$**	**$M_t/M_\infty= 1-e^{-kt0.65}$**
	N = 1 zero order	**N = 0.9**	**N = 0.8**	**N = 0.7**	**N = 0.6**	**N = 0.5**	**N = 0.5 Higuchi (release 0-60%)**	**First order**	**Ion exchange resins**
KETH gel	y = 0.1492 x + 0.742 r = 0.983	y = 0.2718 x – 0.751 r = 0.978	y = 0.4971x – 2.578 r = 0.971	y = 0.9151x – 4.883 r = 0.963	y = 1.7014x – 7.908 r = 0.952	Y = 3.2156x – 12.094 R = 0.939	y = 2.9963x – 10.439 r = 0.938	y = -0.001x + 0.016 r = 0.957	y = 0.0231 – 1.288 r = 0.946

MgAl-HTlc-KET gel	Y = 0.0985 x + 15.724 r = 0.892	y = 0.1845 x + 14.316 r = 0.906	y = 0.3474x + 12.585 r = 0.920	y = 0.6592x + 10.398 r = 0.934	y = 1.2651x + 7.529 r = 0.947	Y = 2.4711x + 3.5762 r = 0.959	Y = 2.4711x + 3.5762 r = 0.959	y = -0.0006x – 0.072 r = 0.927	y = 0.0222x – 1.273 r = 0.940
ZnAl-HTlc-KET gel	Y = 0.043x + 12.235 r = 0.884	y = 0.0802 x + 11.652 r = 0.894	y = 0.1504x + 10.931 r = 0.905	y = 0.2844x + 10.015 r = 0.915	y = 0.5442x + 8.808 r = 0.925	y = 1.0605x + 7.136 r= 0.935	y = 1.0605x + 7.136 r= 0.935	y = -0.0002x – 0.056 r = 0.896	y = -0.0021x – 0.042 r = 0.926
Fastum® gel	Y = 0.1709 x + 12.454 r = 0.968	y = 0.3177 x + 10.234 r = 0.976	y = 0.5934x + 7.521 r = 0.983	y = 1.1159x + 4.109 r = 0.988	y = 2.1209x – 0.348 r = 0.992	y = 4.0992x – 6.479 r = 0.994	y = 4.2781x – 7.703 r = 0.989	y = -0.0014x – 0.031 r = 0.992	y = -0.0124x + 0.047 r = 0.982

Carbonate solution									
	$M_t/M_\infty = kt^n$							**$M_t/M_\infty = 1-e^{-kt}$**	**$Mt/M_\infty = 1-e^{-kt^{0.65}}$**
	n = 1 zero order	**n = 0.9**	**n = 0.8**	**n = 0.7**	**n = 0.6**	**n = 0.5**	**n = 0.5 Higuchi (release 0-60%)**	**First order**	**Ion exchange resins**
KETH gel	y = 0.1715x + 25.439 r = 0.893	y = 0.3209x + 23.015 r = 0.906	y = 0.6037x + 20.032 r = 0.919	y = 1.1445x + 16.261 r = 0.932	y = 2.1949x + 11.313 r = 0.944	y = 4.2842x + 4.490 r =0.955	y = 7.576x – 14.733 r =0.983	y = -0.0017x – 0.114 r = 0.951	y = 0.0147x – 0.018 r = 0.975
MgAl-HTlc-KET gel	Y =0.1429x +21.343 r = 0.899	y = 0.2676x + 19.298 r = 0.913	y = 0.5038x + 16.788 r = 0.927	y = 0.9557x + 13.622 r = 0.940	y = 1.8335x + 9.476 r = 0.953	y= 3.5793x + 3.772 r=0.964	y = 4.4685x – 2.385 r=0.969	y = -0.0012x – 0.096 r = 0.953	y = -0.0106x – 0.024 r = 0.979
ZnAl-HTlc-KET gel	y =0.1435x + 18.386 r = 0.949	y = 0.2675x + 16.451 r = 0.959	y = 0.5010x + 14.083 r = 0.969	y = 0.9454x + 11.101 r = 0.978	y = 1.8032x + 7.199 r = 0.985	y= 3.4989x + 1.831 r=0.991	y = 3.963x – 10.406 r =0.994	y = -0.0012x – 0.074 r = 0.982	y = 0.0222 – 1.273 r = 0.941
Fastum® gel	y =0.2238x + 1.625 r = 0.987	y = 0.4101x – 0.825 r = 0.987	y = 0.7544x – 3.8143 r = 0.987	y = 1.3971x – 7.572 r = 0.984	y = 2.6137x – 12.484 r = 0.979	y = 4.9705x – 19.250 r = 0.972	y=4.3981x– 15.098 r = 0.969	y = -0.0024x + 0.0882 r = 0.837	y = -0.0132x + 0.093 r = 0.975

This means that the drug diffusion capability in the medium is the main force responsible for the release kinetic. This different behaviour is ascribable to the different

solubility of KET at two different pH values. In the case of carbonate solution (alkaline) drug solubilization and diffusion is easy while in phosphate buffer pH 5.5 the dissolution rate is slow due to the low solubility of KET in acidic environment.

Also in the case of Fastum® gel a difference in the two dissolution media can be observed (**Table 3**). In phosphate buffer the best fitting was obtained for n = 0.5 (r = 0.994), meaning that fickian diffusion is the main mechanism involved in drug release kinetic. Also r value of first order kinetic is high r = 0.992, meaning that also KET concentration in the formulation plays an important role in conditioning the kinetic.

In carbonate solution the best fitting was obtained for 0.8 < n < 1 (r = 0.987 for both values), meaning that the polymeric network controls drug diffusion.

A previous part of this work was presented at XXI Simposium ADRITELF [24].

CONCLUSIONS

At the end of this study positive conclusions can be drawn on hybrids and their influence on the final performances of anti-inflammatory topical formulations.

KET intercalation between HTlc-NO_3 lamellae produced an improvement of the drug loading in comparison to the previous method in which HTlc in chloride form was used as starting material. A new synthetic method has been successfully extended also to the preparation of inorganic-organic hybrid ZnAl-HTlc-KET, in which KET intercalation into HTlc occurs at room temperature in 24 hrs. In both hybrids, MgAl-HTlc-KET and ZnAl-HTlc-KET, this intercalation procedure allowed to obtain a high drug loading. These products have been characterized and used to prepare topical hydrogels which performances were compared to commercially available gels containing the anti-inflammatory KET. Particularly rheological characterization pointed out that:

1. The prepared hydrogels presented good flow properties, good viscosity and good yield stress, better than marketed formulations. HTlc presence gives a positive contribution to the formulations. In fact for gels containing inorganic-organic hybrids the viscosity decreased as temperature increases, especially in the case of MgAl-HTlc-KET. At 25°C the hydrogels containing hybrids resulted less viscous than unmedicated gel (empty gel free from KET) and marketed formulations, resulting the most viscous. Marketed gels are more viscous also than prepared KETH gel, containing not intercalated KET. It is possible to hypothesize that free KET is capable to bind the polymeric network of the gel making the structure less rigid and more flowing.
2. Increasing the temperature from 25 to 32°C the rheological behaviour of prepared hydrogels is almost comparable. All of them showed pseudoplastic behaviour and a viscosity decrease. The hydrogel containing MgAl-HTlc-KET was the most sensitive to temperature variation. This phenomenon is very positive for the topical formulation success because the higher viscosity at rest conditions (shelf life) will allow a good preservation (eg. no sedimentation or sedimentation rate reduction) while the flowing increase at 32°C is able to improve the formulation spreadability on the skin with consequent enhancement of patient compliance.
3. Hydrogels containing the hybrids (MgAl-HTlc-KET and ZnAl-HTlc-KET) appear to be easily extrudable, better than the marketed medicinal products used as control in the study. This may depend on both the presence of HTlc (especially in the case of the magnesium-aluminum) and the composition of hydrogels, prepared with a polymer gelling agent different from commercial ones.

KET *in vitro* release performed using acceptor media both mimic the carbon dioxide present in the air and the skin pH value showed that hydrogels containing intercalated KET (hybrids) released the drug slowly than those containing the free drug. Kinetic studies applied to the release profiles data reveal that KET release is controlled by a mixed mechanism, both by the polymer gelling agent (polymeric chains stretching and relaxation time) and the inorganic matrix (ionic exchange).

Results coming from the performed research revealed that it is possible to formulate a product having the characteristics that allow the proper execution of the therapy and the respect of the tissues with which it comes into contact. In particular for topical formulations intended to treat inflamed and painful tissues, the use of

lamellar inorganic clays hydrotalcites (HTlc) both as drug carrier and rheological agent was a winning choice. The intercalation of the poor soluble drug KET into HTlc galleries is able to create a new product resulting more workable than the free drug (KETH) due to the presence of the inorganic matrix. Thus, hydrogels containing hybrids were prepared easily than those containing KETH. Moreover, the presence of the inorganic matrix is able to enhance the flow properties of hydrogels as observed by rheological studies results.

From these observations it is possible conclude that KET intercalation into HTlc interlamellar spaces is a good strategy allowing the preparation of topical formulations stable during their shelf life and safe under administration, with enhanced performances in comparison to marketed formulations.

The flow characteristics depending on hybrids presence are another aspect worth mentioning. In fact the introduction of inorganic matrices in the formulations improved their rheological behaviour enhancing their usability. Particularly, the presence of MgAl-HTlc meets the requirements for KET topical application best of all.

The use of KET intercalated in inorganic matrices formulated as suitable hydrogel, results in a very considerable formulation in terms of stability, effectiveness, safety, and patient's compliance.

It is important to underline that in the study reported in this paper KET was used just as model drug. Anyway the proposed strategy can be easily transferred applied to other kind of molecules intended for topical therapy.

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