Saudi Pharmaceutical Journal 25 (2017) 1158–1168

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Development, stability and *in vitro* delivery profile of new loratadine-loaded nanoparticles

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ARTICLE INFO

Article history:
Received 31 March 2017
Accepted 16 July 2017
Available online 19 July 2017

Keywords:
Loratadine
Nanoparticles
Nanosuspension
Zeta potential
DLS
Simulated gastric fluids

ABSTRACT

Purpose: Loratadine is used as antihistaminic without side effects in nervous systems. This drug is a weak base and it is absorbed from the intestine. The nitrogen of the pyridine ring is protonated in the stomach affecting the oral bioavailability. The aim of this paper was obtaining, characterize and evaluate the release profiles and the stability of a gastroresistant loratadine nanosuspension. *Methods*: The nanosuspension was prepared by the solvent displacement evaporation method, using three different polymers (Eudragit® L 100 55, Kollicoat® MAE 100P and PEG 4000) and Polysorbate 80. Dynamic Light Scattering was used for evaluating the particle size (PS), zeta potential, and conductivity of the nanosuspension. Loratadine release profiles were evaluated in simulated gastrointestinal fluids. The shelf and accelerated stability were assessed during three months. *Results*: Nanosuspension particle size was 45.94 ± 0.50 nm, with a low polydispersion index (PdI, 0.300). Kollicoat® MAE 100P produced a hard and flexible coating layer. In simulated intestinal fluids, the 100 percent of loratadine was released in 40 min, while in simulated stomach fluids the release was lesser than 5%. Nanosuspension presented a good physicochemical stability showing a reduction in PS and PdI after three months (43.29 ± 0.16 and 0.250; respectively). *Conclusions*: A promissory loratadine nanosuspension for loratadine intestinal delivery was obtained, by using a low energy method, which is an advantage for a possible scale up for practical purpose.

1. Introduction

The allergy is an exaggerated immune response of the body to foreign substances. Allergic reactions promote the histamine mobilization. Histamine is a nitrogenous organic compound involved in local immune response, regulate the gut physiological function and as well as acting as a neurotransmitter (Kiningham, 2007). Histamine is involved in the inflammatory response and has an essential role as a pruritus mediator. Basophil and mast cells, located in the connective tissues, producing histamine as part of the immune response to foreign allergens (Andersen et al., 2015). Loratadine (Fig. 1) is a histamine H1 receptor antagonist. It is widely used, without side effects, in the central and autonomic nervous systems. This is, probably, the antihistamine most used to prevent and suppress the responses to allergens (Simons and Simons, 2008; Hadzijusufovic et al., 2010).

The loratadine solubility depends on the pH, if it increases the loratadine solubility decreases exponentially. This drug belongs to the class II of the BCS because of its low solubility and high permeability (Barbosa et al., 2015). In an acid medium, as the stomach, the N of the pyridine ring is protonated, forming salts of good solubility but suffering a decrease in the membrane permeability (Brunton et al., 2006). Because of this, when this drug is used by the oral route its bioavailability exhibits a high variability, reducing the therapeutic efficacy (Khan et al., 2004). Nonetheless, loratadine is a weak base that is well absorbed from the intestine (Kiningham, 2007). By the oral route, loratadine can produce side effects, including hepatotoxicity, allergic reactions as rash, hives, itching, difficulty in breathing, and chest tightness, swelling of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.07.008
1319-0164/

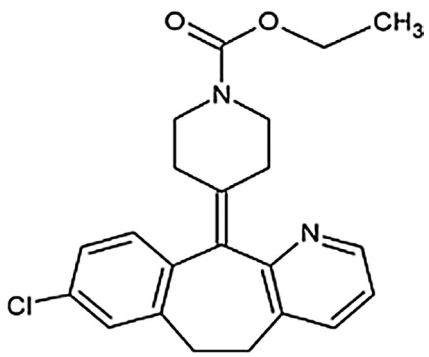

Fig. 1. Loratadine, an antihistaminic H1 receptor-blocking agent with non-sedating effects.

the mouth or face and dizziness (Üner et al., 2014). These limitations can be overcome protecting it inside nanoparticles as nanocapsules, by using a gastro-protective coating layer.

Several methods have been tested for improving the loratadine oral bioavailability. Thus, solid lipid microparticles containing loratadine were prepared in a solid dosage form (Milak et al., 2006). With the same purpose, inclusion complexes of loratadine-β-cyclodextrin were tested (Nacsa et al., 2008; Pooja et al., 2011). Frizon et al. (2013) prepared a polyvinylpyrrolidone solid dispersion for improving the loratadine dissolution by the oral route. In the same way, a self-microemulsifying delivery systems were prepared to protect loratadine from the action of the stomach acid medium (Patil and Paradkar, 2006; Li et al., 2014). In all cases, the dissolution occurred in the stomach, where the loratadine bioavailability is affected. Consequently, the aim of this work was to prepare a novel gastroresistant loratadine-loaded nanoparticle and evaluate the loratadine delivery profiles in physiological conditions, using simulated gastrointestinal fluids for testing the effectiveness of the polymeric coating layer. Additionally, a stability study in the shelf and stress conditions during three months was conducted.

2. Materials and methods

2.1. Chemicals

Loratadine and polyoxyethylene Sorbitan (Polysorbate 80) were obtained from Sigma, USA. Kollicoat® MAE® 100P [poly (methacrylic acid, ethyl methacrylate) 1:1], and Eudragit® L100 55 [poly (methacrylic acid, methyl methacrylate) 1:1], and Polyethylene glycol (PEG 4000) were purchased from Basf (Germany). The other chemicals were pure for analysis provided by Alphatec, Brazil.

2.2. Preparation of nanosuspensions

Nanoparticles were prepared by the solvent displacement evaporation method (Fessi et al., 1989) with some modifications as Lafourcade et al. (2016). First, the polymer was dissolved in 35 ml of ethanol under magnetic stirring at 800 rpm (Fisatom, SP, Brazil) for 20 min. After that, the exact amount of loratadine was dissolved in absolute ethanol (1:10 m/v). The organic phase was performed mixing the polymer and loratadine solutions under stirring for 10 min. The aqueous phase was prepared by diluting polysorbate 80 (Table 1) in 75 ml of water for injection (Conductivity < 4μS) under constant stirring (800 rpm) for 20 min. The organic phase was added to the aqueous phase at a rate of 1 ml/min, under constant stirring along 20 min. At the end, nanosuspension was concentrated under reduced pressure (IKA 06.05 HB CN, Switzerland) to a final volume of 50 ml.

2.3. Experimental design

In order to select the best polymer for the coating layer, 3 different polymers (Eudragit® L 100 55, Kollicoat® MAE 100 P and PEG 4000) were used under the same experimental conditions. Table 1 shows the composition of each tested formulation.

2.4. Dynamic Light Scattering (DLS)

Particle size and polydispersion index, were measured by DLS, in a Zetasizer (Malvern, UK). Nanosuspensions were diluted 1:9 (v/v) in water for injection and filtered by a Millipore® membrane (0.45 μm), before the measuring. The diluted samples were transferred to a 10 mm path length glass square cuvette and covered until the measure was made, to prevent dust contamination. The readings were performed at a laser wavelength of 633 nm, 173° of scattering angle, at 25 °C. The laser wavelength was equilibrated for 30 min before the use. Three measurements were performed for each sample and the mean ± standard deviation was reported (ISO 22412, 2008; McNeil, 2011).

2.5. Zeta potential and conductivity

Zeta potential and conductivity were measured in a Zetasizer using disposable plastic cells. In order to obtain a uniform medium ionic strength, samples were diluted with 10 mMol NaCl solution (1:9). The final solution was filtered by Millipore® membrane (0.45 um). Samples were measured at 25 °C, using a voltage of 150 V. The equipment was equilibrated for 2 min before measures. Three replicate were made and the mean ± standard deviation was reported (ISO 22412, 2008; McNeil, 2011).

2.6. pH- evaluation

The nanosuspensions pH was determined by using a pH-meter (mPA210, MS TECNOPON, Brazil). The instrument was calibrated by using buffer solutions (pH 7.0 ± 0.02 and 4.0 ± 0.02; Alphatec, Brazil). Measurements were performed in triplicate and results were expressed as the mean ± standard deviation.

2.7. Isothermal and thermal effect on particle size

The nanosuspension was poured into a screw transparent glass tube and heated, for two hours at 80 °C in a water bath (Quimis, Brazil). Particle size and the polydispersion index were determined every 10 min. The aspect of the solution was checked for detecting instability signs as phase separation, turbidity, or precipitate. Measurements were performed in triplicate and results were expressed as the mean ± standard deviation (Lafourcade et al., 2016; McNeil, 2011).

The effect of a range of temperatures on particle size and the polydispersion index was also evaluated. The temperature raised up from 20 to 80 °C at a constant rate (5 °C/min) and measures were made at interval of 10 °C. Measurements were made in triplicate and results were expressed as the mean ± standard deviation.

2.8. Effect of pH on the particle size and zeta potential

The titration technique was conducted using an MPT-2 titrator (Malvern, UK) coupled to Zetasizer, in order to evaluate possible changes in particle size, zeta potential and conductivity, induced by pH variation. The nanosuspension was diluted with water for

Table 1
Composition of loratadine nanosuspensions using different amount of polymers, and surfactant for selecting the best nanoparticle coating.

Code	Polymers	Quantity (g)	Polysorbate 80 (g)	Ethanol (ml)	Water (ml)
L1	Kollicoat® MAE 100P	0.25	0.50	22.50	75.00
L2	Kollicoat® MAE 100P	0.35	0.75	22.50	75.00
L3	Eudragit® L 100 55	0.25	0.50	22.50	75.00
L4	Eudragit® L 100 55	0.35	0.75	22.50	75.00
L5	PEG 4000	0.25	0.50	22.50	75.00
L6	PEG 4000	0.35	0.75	22.50	75.00

injection (1:9) at a final volume of 10 ml. As titrating solutions NaOH 0.10 mol/l and HCl 0.10 mol/l were used. The apparatus was calibrated with buffer solutions (pH 4, 7, and 10, Alphatec, Brazil). As zeta potential depends on pH and the conductivity of the dispersing medium (Malvern, 2015), the titration from pH 1 to 6 was made first. After that, a new fresh nanosuspension solution was used for titration from pH 7 to 10. In both cases, the titrator was calibrated for the addition of 1–3 μL of the titrating solutions, in order to avoid drastic changes in the solution. Measurements were performed in triplicate, at 25 °C, with an accuracy of 0.20, and an applied voltage of 150 V (Malvern, 2015).

2.9. Combined effect of pH and temperature

The combined effect of temperature and pH on nanosuspension main properties was evaluated. Kollicoat® MAE 100P dissolves at pH over 5.5 (Bühler, 2007; BASF, 2010). Nonetheless, it is important to know the effect of both factors acting together, as an approaching for evaluating possible process conditions for the preparation of final pharmaceutical forms. For this, 200 μl of nanosuspension was mixed with 1000 μL of buffer solutions (pH 1.0, 3.0, and 5.5). The cuvette was covered to avoid evaporation. Particle size, polydispersion index, and zeta potential were measured between 40, 50, and 60 °C using a constant heating rate (5 °C/min). After each measure, the appearance of the solution in the cuvettes was checked for detecting phase separation, turbidity, and/or precipitate. Measurements were performed in triplicate and results were expressed as the mean ± standard deviation.

2.10. Scanning Electron Microscopy

Scanning Electron Microscopy (Hitachi TM3030Plus, Japan) was used for observing the particle morphology, using an accelerating voltage of 5.0 kV, and a magnification of 4000x. One drop of nanosuspension was directly spread on a carbon tape and led evaporate before the analysis.

2.10.1. Encapsulation efficiency using HPLC

For this, 1 ml of nanosuspension was centrifuged 30 min at 5000 rpm (Alfa, Brazil), at 25 °C. After that, the supernatant liquid was filtered by a 0.45 μm Millipore® membrane and injected in the HPLC equipment. Loratadine entrapped (EE) in nanoparticles was evaluated, as the difference between the total amount of loratadine used in the preparation process (250mg) and the content of loratadine in the supernatant liquid (SNL). Loratadine content was determined in an HPLC-system (Agilent 1100, Shimadzu, Japan), using an automatic injector coupled to a Thermo Hypercarb column (150 × 4.6 mm id., 5 μm of particle size), adjusted at 25 °C. The elution was made 60 min with a gradient program using as mobile phases 0.3% sodium di-hydrogen phosphate solution (A) and acetonitrile (B) at a flow rate of 1.0 ml/min. The elution start with 100% of A and finish with 100% of B, changing the composition in 5% every 3 min, from 100/0 to 0/100 (A:B). The detection was made at 247 nm. The percent of loratadine was determined by the standard addition method, using a loratadine standard (Sigma, USA). The assay was made at 1, 7, 15, 30, 60, and 90 days, after the preparation. Three replicates were made and the mean ± standard deviation was reported. The encapsulation efficiency (EE) was calculated as:

$$EE(\%) = [(250 - SNL)/250] \times 100 \quad (1)$$

2.10.2. Long-term stability

After the preparation, nanosuspension samples were kept under laboratory conditions (25 °C) along 90 days, in screw glass tube protected from light. Along the time, the nanosuspension particle size, polydispersion index, zeta potential, conductivity, pH, and LC were determined. The loratadine content (LC) along the time was determined using the same procedure described in 2.2.11. Samples were withdrawn at 1, 7, 14, 21, 30, 60, and 90 days. Measurements were performed in triplicate and results were expressed as the mean ± standard deviation.

2.10.3. Effect of constant temperature (30 and 45°C) throughout three months

Samples of L2 nanosuspension were kept for three months in the stoves (Quimis, Brazil) at 30 and 45 °C. The particle size, polydispersion index and zeta potential were evaluated at 1, 7, 14, 21, 30, 60 and, 90 days. The procedure followed was the same described before for each individual property. Measurements were performed in triplicate and results were expressed as the mean ± standard deviation.

2.10.4. Effect of the temperature after three months

The nanosuspension particle size, polydispersion index, and zeta potential were evaluated from 10 to 90 °C. The procedure was made after three months stored in shelf conditions. A constant heating ratio (5 °C/min) was employed and measurements were performed at intervals of 15 °C. The temperature was equilibrated for two minutes before the measures. After the measures, the aspect of the solution in the cuvette was checked. Measurements were performed in triplicate and results were expressed as the mean ± standard deviation.

2.10.5. In vitro dissolution profile

The dissolution profiles were assessed *in vitro*, by using biorelevant gastric fluids, simulating the environment of the human body. The simulated gastric fluid in fast state (FaSSGF, pH 1.6) was prepared as Vertzoni et al. (2005); while in the fed state (FeSSGF, pH 5.0) was prepared as Jantratid et al. (2008). On the other hand, the loratadine release profile was also tested in simulated intestinal fluid in fast state (FaSSIF, pH 6.5) (Jantratid et al., 2008) and simulated intestinal fluid (SIF) without pancreatin (pH 7.4) (USP, 2012). All the solutions were filtered through a 0.45 um membrane (Milipore, USA). All the reagents were purchased from Sigma (USA) and Alphatec (Brazil). The dissolution study was performed at 37 ± 1 °C in 900 ml of dissolution medium, using a paddle dissolution apparatus (Nova Etica, Brazil) at 75 rpm. Aliquots (2 mL) were withdrawn every 10 min for two hours and

replaced by fresh dissolution medium. The amount of loratadine released was determined by HPLC as was described before.

2.10.6. Data analysis

For the statistical analysis, StatGraphics Centurion v.XV.I (Stat Ease, USA) was used. The One Way ANOVA test was applied for evaluating possible statistical differences among three or more groups of data. It was considered statistical differences for $p < 0.05$. Some graphs were obtained by using Origin 7.0 software (OriginLab Corporation, USA). Zeta potential graph and particle size distribution were obtained directed from the Zetasizer software (Malvern, USA).

3. Results

3.1. Characterizing nanosuspensions

Values of the particle size, polydispersion index, zeta potential, conductivity, pH, and LC of the six nanosuspensions obtained using different amount and type of polymers, are shown in table 2. The properties behavior along the time is also showed.

After 24 h, nanoformulations L5 and L6 presented a white milky color, with a small phase separation on the surface and a visible solid deposition at the bottom. The particle size of these nanosuspensions was high and the polydispersion index was close to 1 (Table 2). On the contrary, L3 and L4 showed a white color, with bluish reflections. Both nanosuspensions showed particle sizes between 240–270 nm and a polydispersion index below of 0.190 (Table 2). Nanosuspensions L1 and L2 showed a transparent aspect with small values of particle size (less than 46 nm) and polydispersion index around 0.300 (Table 2).

Fig. 2 shows the particle size distribution of loratadine nanosuspensions, which showed stability 24 h after the preparation (L1, L2, L3, and L4).

L1 nanosuspension (Fig. 2a) showed two distributions of particle size. The major peak (59.58 nm) accounted for 93.00% and the second peak (6.40 nm) accounted for the remaining 7.00%. L2 nanosuspension (Fig. 2b) presented two particle size distributions. The major peak (59.58 nm) represented about 97.00% and the second peak (4184.00 nm) accounted for the remaining 3.00%, however, in the graph obtained by DLS a small distribution was observed in size values around 10000 nm. Meanwhile, L3 (Fig. 2c) and L4 (Fig. 2d) had a monomodal size distribution with a single peak at 264.10 and 320.40 nm, respectively. Zeta potential values of nanosuspensions L1-L4 were lower (higher in modulus) than −13.5 mV, and the conductivity remained below 0.01 ms cm^{-1}.

Seven days after, a diminution in particle size of the four nanosuspensions was observed (Table 2), while the polydispersion index decreased on L1 and L2. After 15 days, the particle size in L3 continued increasing (Table 2) showing a bimodal distribution, with the major peak (89.90%) at 327.40 nm and a minor peak (10.10%) at 2024.00 nm (Graphs not showed). In addition, L3 presented a significant increase in the polydispersion index (above 0.260). In the same way, L4 showed a single monomodal size distribution, but with a wide base and misleading, showed a peak with a value of 703.50 nm and polydispersion index higher than 0.330 (Graphs not showed). Additionally, the conductivity as well as the zeta potential, increased in both nanosuspensions, which are evidences of instability (Table 2).

Contrasting L3 and L4, the particle size and the polydispersion index of L1 nanosuspension 15 days after the preparation (Fig. 3a and b) remained unaffected. However, a decrease (in modular value) of the zeta potential and an increase in the conductivity were observed. These, are signs of instability. Fig. 3 shows the particle size distribution of L1 and L2 nanoformulations at 7 and 15 days after the preparation.

After 15 days, L1 nanosuspension presented a precipitate, that was separated by filtration through Whatman No.1 filter paper. The crystals were collected, dried, and analyzed by FTIR. On the contrary, the particle size of L2 nanosuspension remained constant with a diminution in the polydispersion index (A narrower size distribution). At the same time, the zeta potential decreased (Increased modular value, Table 2), and a slight increase in conductivity was observed. Fig. 3c and d showed that those small peaks observed at 24 h, disappeared in the DLS graphic analysis, displaying a monomodal distribution at the day 15.

3.2. Encapsulation efficiency and pH

The encapsulation efficiency of nanosuspensions L1-L4 was higher to 83%, being superior in L2 with 93.65%. The LC in L1, L3, and L4, decreased 8.36, 8.01, and 10.98% respectively, along 15 days. At the same time, the pH of these nanosuspensions was modified (Table 2). Onwards this time, nanosuspensions L1, L3, and L4 were discarded for posteriors studies, because of an evident instability. On the contrary, LC of L2 (93–94%) and pH (5.02–5.05) remained practically constant along three months (Table 2). There

Table 2
Nanosuspensions properties of different loratadine-loaded nanoparticles prepared using different type of polymers.

Time (days)	Code	Droplet size (nm)	Polydispersion index	Z-potential (mV)	Conductivity ($mScm^{-1}$)	pH	LC (%)
1	L1	40.41 ± 0.50	0.300 ± 0.005	−13.6 ± 2.33	0.081 ± 0.004	5.05 ± 0.33	89.23 ± 0.55
	L2	45.94 ± 0.22	0.303 ± 0.005	−17.7 ± 1.55	0.097 ± 0.002	5.02 ± 0.15	93.65 ± 0.55
	L3	240.4 ± 4.53	0.078 ± 0.018	−16.6 ± 0.58	0.070 ± 0.003	5.23 ± 0.27	86.45 ± 1.47
	L4	269.6 ± 0.56	0.188 ± 0.012	−18.9 ± 0.16	0.031 ± 0.001	5.43 ± 0.21	83.25 ± 2.06
	L5	2421. 45 ± 18.56	0.967 ± 0.213	2.92 ± 0.34	0.787 ± 0.045	–	–
	L6	1891. 76 ± 58.97	0.998 ± 0.127	8.35 ± 0.54	0.986 ± 0.099	–	–
7	L1	42.84 ± 0.16	0.283 ± 0.011	−15.84 ± 1.44	0.080 ± 0.002	5.15 ± 1.27	85.47 ± 0.48
	L2	44.94 ± 0.27	0.280 ± 0.040	−14.40 ± 3.87	0.090 ± 0.002	5.24 ± 1.21	93.51 ± 0.84
	L3	220.50 ± 4.01	0.092 ± 0.017	−21.10 ± 0.76	0.030 ± 0.004	5.55 ± 1.27	86.38 ± 3.37
	L4	247.90 ± 3.17	0.187 ± 0.013	−17.3 ± 1.39	0.030 ± 0.007	5.64 ± 1.21	80.72 ± 2.36
15	L1	42.76 ± 0.19	0.284 ± 0.002	−9.15 ± 2.08	0.091 ± 0.005	5.35 ± 0.85	80.87 ± 2.01
	L2	43.88 ± 0.23	0.276 ± 0.005	−16.30 ± 0.52	0.102 ± 0.002	5.03 ± 0.08	93.28 ± 0.65
	L3	259.70 ± 4.62	0.263 ± 0.009	−13.80 ± 0.67	0.092 ± 0.003	5.42 ± 1.16	78.44 ± 3.12
	L4	344.50 ± 4.65	0.346 ± 0.007	−10.20 ± 0.56	0.095 ± 0.002	5.33 ± 1.42	72.27 ± 1.33
30	L2	43.88 ± 0.23	0.276 ± 0.005	−16.30 ± 0.52	0.102 ± 0.003	5.05 ± 0.05	93.42 ± 0.47
60	L2	44.35 ± 1.14	0.257 ± 0.009	−21.30 ± 1.31	0.104 ± 0.005	5.03 ± 0.05	93.57 ± 0.25
90	L2	43.29 ± 0.16	0.250 ± 0.040	−19.30 ± 1.50	0.106 ± 0.008	5.02 ± 0.08	93.76 ± 0.72

L1 and L2, Kollicoat® Mae 100 P; L3 and L4, Eudragit® 100 55 P; L5 and L6, polyethylene glycol 4000. LC, loratadine content in nanoparticles, n = 3.

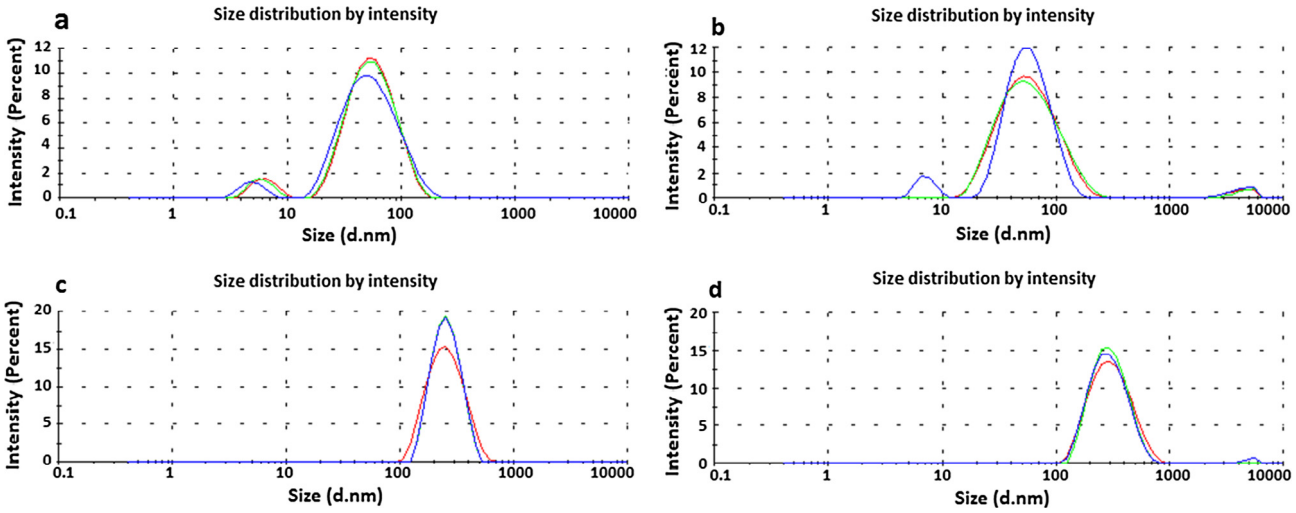

Fig. 2. Particle size distribution of loratadine nanosuspensions 24 h after the preparation. a: L1 (42.84 ± 0.16 nm); b: L2 (44.94 ± 0.27 nm); c: L3 (220.50 ± 4.01 nm) and d: L4 (247.90 ± 3.17 nm).

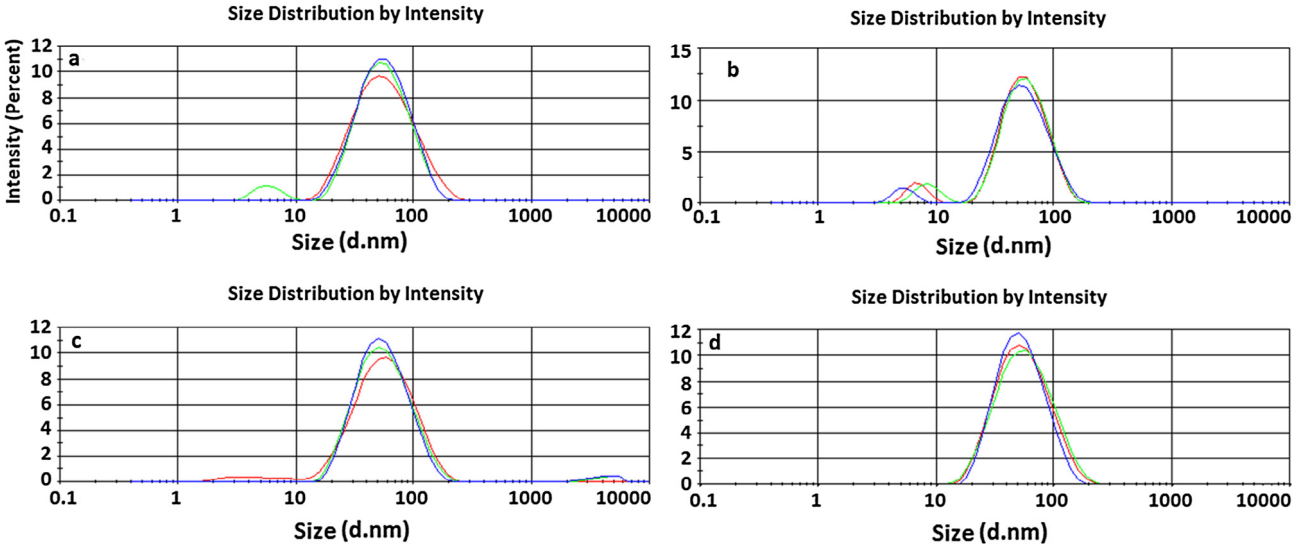

Fig. 3. Particle size distribution of L1 (a, and b) and L2 (c and d) nanosuspensions at 7 and 15 days, after the preparation. L1: a: 42.84 ± 0.16 nm at 7 days, b: 42.76 ± 0.19 nm at 15 days. L2: c: 44.94 ± 0.27 nm at 7 days; d: 43.88 ± 0.23 nm at 15 days.

was no significant difference in LC along three months ($F = 0.06$; $p = 0.9450$). Onwards this time, L2 nanosuspension was selected for a complete characterization and the stability studies.

3.3. *Effect of pH and temperature*

Fig. 4 shows the thermal effect (between 20–80 °C) on particle size and the polydispersion index of L2 nanosuspension. At all the temperatures, the particle size kept between 31.5 and 34.5 nm, while the polydispersion index remained between 0.210 and 0.290.

Fig. 5 shows the isothermal effect at 80 °C on particle size and the polydispersion index of L2 nanosuspension, along two hours. During the first 40 min, particle size remained constant. After that time, the size increased to 765.10 nm at 120 min. At the same time, the polydispersion index decreased from 0.296 to 0.233 from time zero to 60 min, onwards that time, increased reaching a value of 1.0 at 120 min.

Fig. 6 shows the effect of pH on L2 nanosuspension properties. Changes in pH did not produce any modifications in particle size up to pH 5. Onwards pH 5, a rapid increase occurred, reaching 873.70 nm at pH 10 (Fig. 6A). Polydispersion index increased from 0.093 at pH 1 to 0.161 at pH 5. From pH 5, the polydispersity index increased rapidly to reach 0.525 at pH 10. On the other hand, the zeta potential decreased slowly from 3.41 mV at pH 1 to −3.26 mV at pH 3 (Fig. 6B). From pH 3 to 5, the zeta potential remained practically constant. Onward pH 5, zeta potential decreased rapidly up to −36.3 mV at pH 9. The conductivity decreased from 5.90 at pH 1 to 1.38 ms cm^{-1} at pH 9, after that increased to 3.70mScm^{-1} at pH 10. The isoelectric point of L2 nanosuspension (Zeta potential = 0) was detected at pH 1.98.

Fig. 7 shows the combined effect of the temperature and pH on particle size, polydispersion index and zeta potential of L2 nanosuspension.

The particle size of L2 nanosuspension (Fig. 7A) showed a little variation from pH 1 to 3 at the three temperatures. At 40 °C, the particle size increased from 62.67 at pH 3 to 88.13 nm at pH 5.5. At 60 °C, the particle size increased from 74.17 at pH 3 to 402.37 nm at pH 5.5. At 40 °C, the polydispersion index (Fig. 7B) decreased from 0.134 at pH 1 to 0.119 at pH 3, up to 0.234 at pH

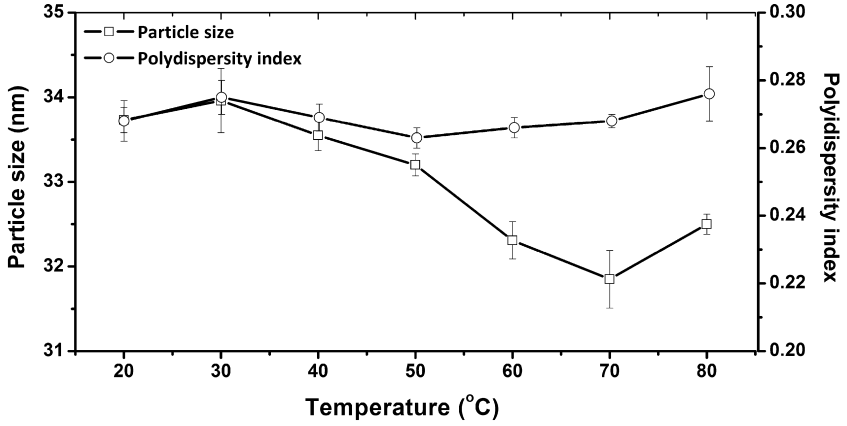

Fig. 4. Thermal effect (from 20 to 80 °C) on particle size and the polydispersion index of L2 nanosuspension.

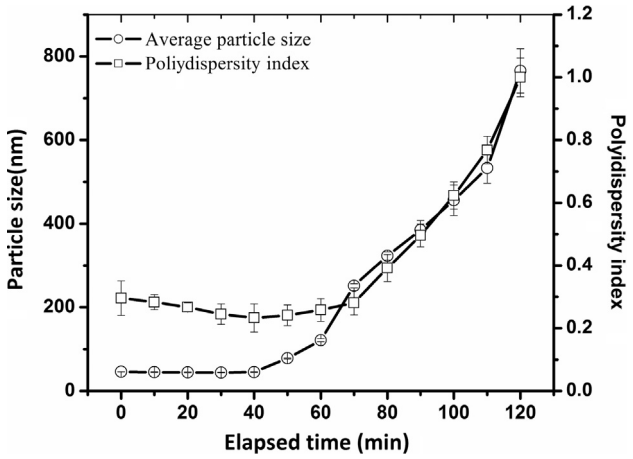

Fig. 5. Isothermal effect on particle size and the polydispersion index of L2 nanosuspension, heated at 80 °C for two hours.

5.5. At 50 °C, a progressive increase in polydispersion index was observed from 0.101 at pH 1 to 0.232 at pH 5.5. A similar behavior was observed at 60 °C, where polydispersion index increased from 0.110 at pH 1 to 0.239 at pH 5.5. On the other side, the zeta potential (Fig. 7C) showed a uniform behavior. At 40 °C remained practically constant in the three-pH values. From 50 to 60 °C, the zeta potential values did not show significant differences ($F = 3.44$, $p = 0.4251$).

3.4. Particle morphology

Fig. 8 shows a microphotography of L2 nanosuspension obtained by SEM. It was observed smooth and spherical white particles, uniformly dispersed in the whole image and others higher agglomerated particles as well.

3.5. Stability study

Fig. 9 shows the particle size distribution and zeta potential of L2 nanosuspension, kept at room temperature (25 ± 2 °C); in a screw glass tube, protected from light, during 30, 60 and 90 days. Particle size remained practically constant along the time (Fig. 9A, C, and E), while the size distribution was narrower, with a reduction in the polydispersion index. On the other side, the value of zeta potential decreased along the time (modular value increased, Fig. 9B, D, and F).

Fig. 10 shows the behavior of particle size, zeta potential and the polydispersion index of L2 nanosuspension, preserved in a screw glass tube, protected from light, at 30 and 45 °C along three months.

The nanosuspension particle size remained between 45.00 and 47.50 nm all the time at both temperatures (Fig. 10A). Similarly, the polydispersion index, at both temperatures, decreased slightly throughout the first 30 days, remained almost unchanged with values between 0.275 and 0.250 (Fig. 10B). Additionally, the zeta potential decreased during the first 30 days (increased in modulus) from −18 to −22 mV at 30 °C with no further changes. It was observed the same behavior at 45 °C, decreasing from −18 mV to

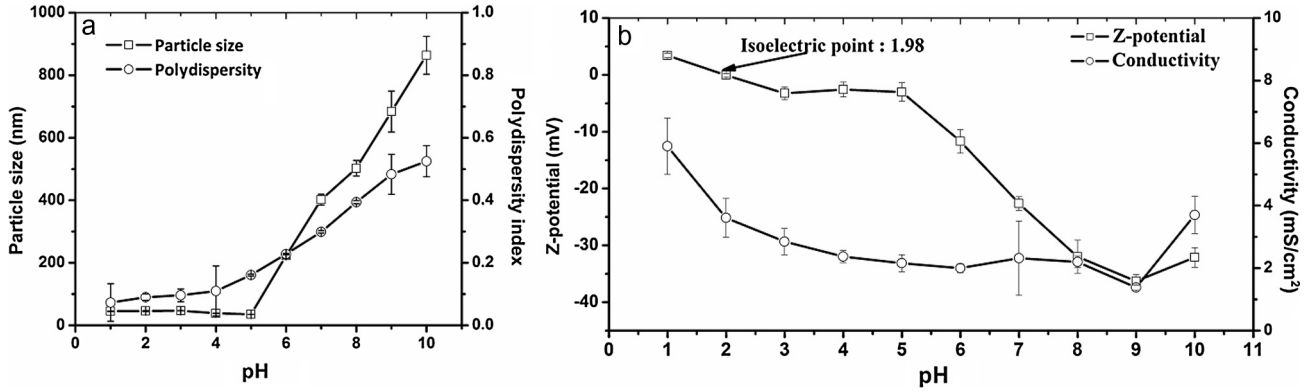

Fig. 6. Effect of pH on particle size and polydispersion index (a), and in zeta potential and conductivity (b) of L2 nanosuspension, after 15 days of preparation.

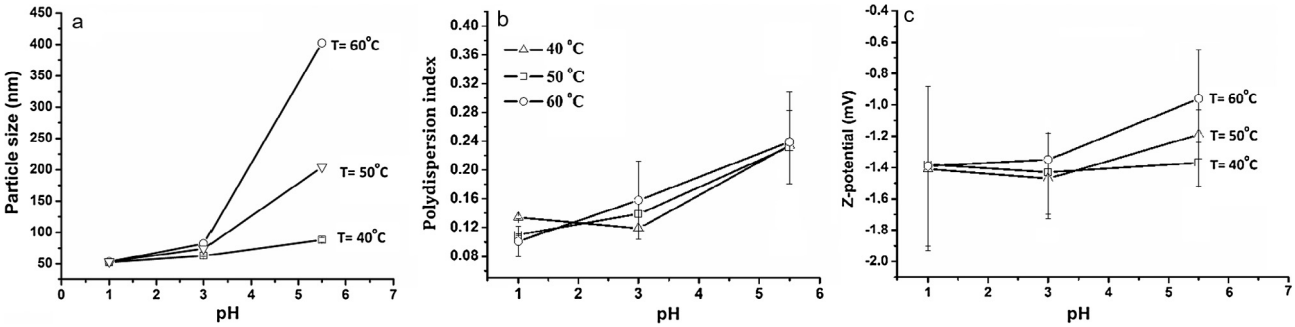

Fig. 7. Combined effect of the temperat. (40, 50 and 60 °C) and pH (1.0, 3.0 and 5.5) on particle size (a), polydispersion index (b) and zeta potential (c) of L2 nanosuspension.

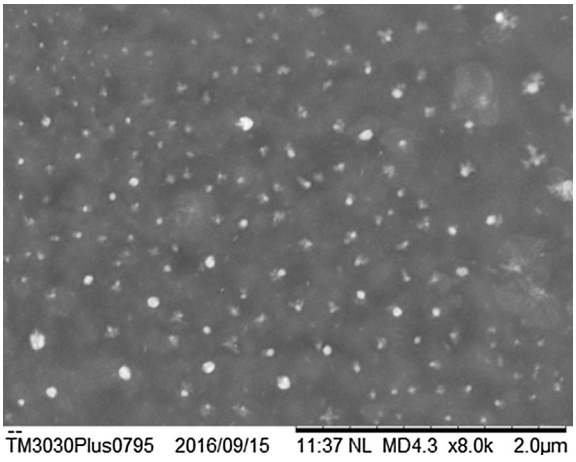

Fig. 8. SEM microphotography of L2 nanosuspension.

−25 mV the first 24 h, after that remained constant until the end of the study (Fig. 10C).

Fig. 11 shows the effect of temperature (From 10 to 90 °C) on particle size and zeta potential of L2 nanosuspension after 90 days of preparation. The particle size decreased while the temperature rising from 10 to 70 °C, after that temperature, the particle size remained practically constant. The polydispersion index decreased moderately for all the temperatures (data in parentheses, Fig. 11). At the same time, the zeta potential showed a tendency to increase (decrease in modular value) from 10 to 70 °C. After that, it decreased to −22.3 mV at 90 °C. Statistical analysis of zeta potential values at each temperature showed no significant differences ($F = 3.86$, $p = 0.0950$).

3.6. Delivery profiles of L2 nanosuspension

In Fig. 12 are shown the delivery profiles, *in vitro*, of loratadine-loaded nanoparticles of L2 nanosuspension, using FaSSGF, FeSSGF,

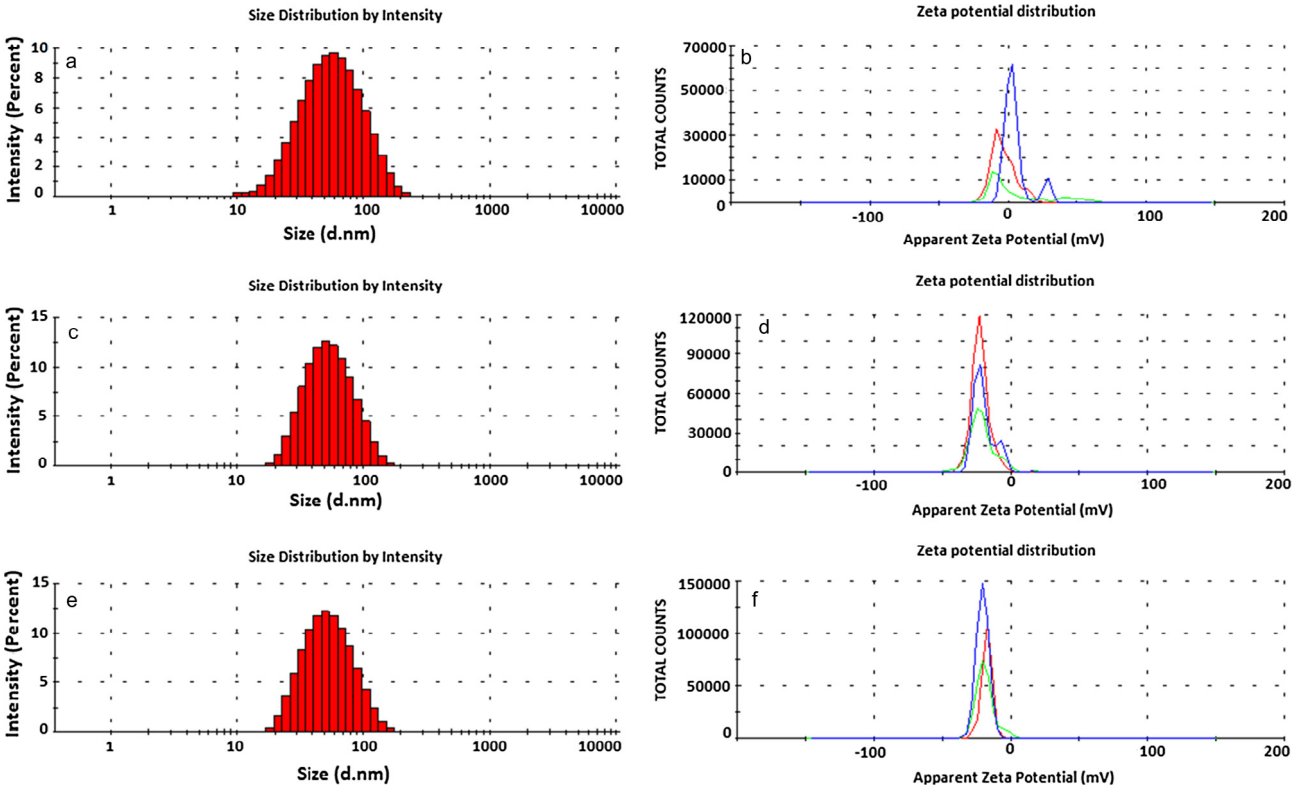

Fig. 9. Behavior of the particle size distribution and zeta potential of L2 nanosuspension at 30, 60 and 90 days. Mean particle size: A: 30 days (43.88 ± 0.23 nm); C: 60 days (44.35 ± 1.14 nm); E: 90 days (43.29 ± 0.16 nm) Zeta potential: B: 30 days (−16.30 ± 0.52 mV); D: 60 days (−21.30 ± 1.31 mV); F: 90 days (−19.30 ± 1.50 mV).

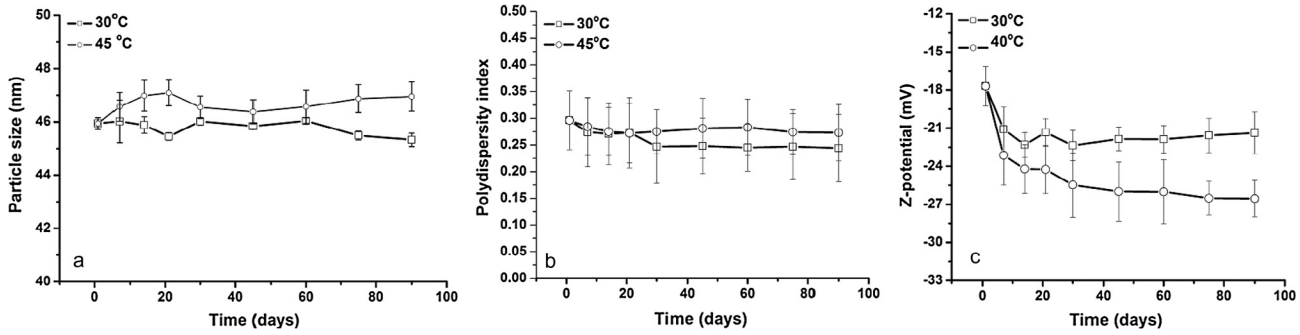

Fig. 10. Stress stability study of L2 nanosuspension at 30 and 40 °C, along three months.

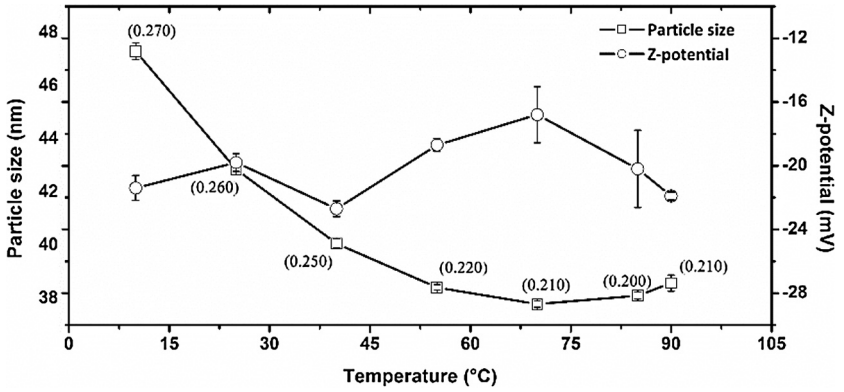

Fig. 11. Effect of temperature on particle size, zeta potential, and polydispersion index (data in parentheses) of L2 nanosuspension, after 90 days, stored in normal conditions.

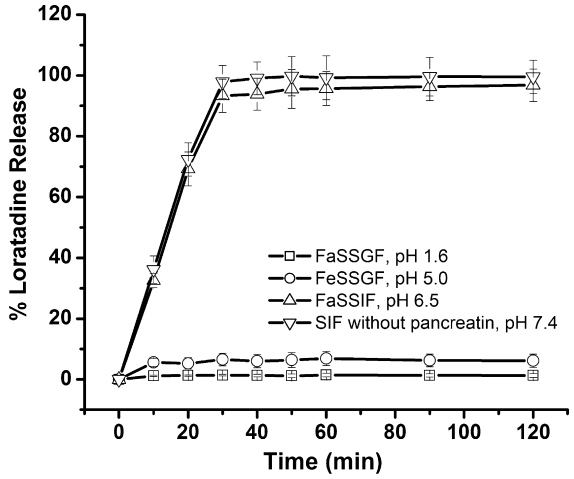

Fig. 12. Delivery profiles of loratadine from L2 nanosuspension in different dissolution media. FaSSGF, simulated gastric fluid in fast state; FeSSGF, simulated gastric fluid in fed state; FaSSIF, simulated intestinal fluid in fast state; SIF, simulated intestinal fluid.

FaSSIF and SIF without pancreatin. In both simulated gastric fluids, nanoparticles showed a low loratadine release, less than 2% in FaSSGF and minor than 5% in FeSSGF. On the contrary, in both simulated intestinal fluids (FaSSIF and SIF without pancreatin), 100% of loratadine was released in 40 min. There was not significant differences in the loratadine released in both simulated intestinal fluids after 30 min ($F = 12.37$, $p = 0.0650$).

4. Discussion

Nanosuspensions have a wide application in various industrial fields, among them, drug delivery systems of poorly soluble drugs, because of the particle size in nanoscale dimensions; enhance drug solubility and/or bioavailability (Fessi, 1989), enhancing the drug uptake, deposition, and clearance. Relatively few techniques allow determining the nanoparticles size in solution (McNeil, 2011). DLS is a powerful technique for determining, accurately, the hydrodynamic size in nanoparticle solutions. By using PEG 4000, it was not possible to obtain a good coating layer. Nanosuspensions L5 and L6, recently prepared; presented evidences of instability and were discarded. On the contrary, after 24 h, nanosuspensions L1, L2, L3, and L4 showed a clear appearance, with small particle sizes and low polydispersion index values (Fig. 2). Nanosuspensions L1 and L2, presented a translucent aspect, usually obtained in nanosystems with particle size less than 50 nm (MacNeil, 2011).

After 15 days, nanosuspensions L3 and L4 showed signs of instability. In both the particle size increased, probably because of the particles aggregation. Thus, new size distributions were detected by the presence of additional peaks in the particle size distribution graphs (Augment the polydispersion index). In addition, in both nanosuspensions, the modular value of zeta potential decreased. The DLS analysis allowed the rapid and simple detection, of the nanosuspensions instability. In Fig. 3, can be seen the changes in the size distribution of both formulations (L1 and L2) from the 7th to 15th day, after the preparation. Contrasting L3 and L4, nanosuspensions L1 and L2 exhibited a reduction in polydispersion index. The DLS analysis of L2 showed a transition from three-size distribution to a monomodal distribution. It was observed, an increase in the modular value of the zeta potential

of both nanosuspensions, which was superior in L2. Probably, the size distribution of nanoparticles in L2 reached a dynamic equilibrium (Lafourcade et al., 2016). The day 21, L1 showed instability, presenting a thin supernatant and an abundant precipitate, characteristic of the phenomenon known as Ostwald Ripening (Tadros, 2013). Probably, the amounts of polymer and surfactant used were not enough to produce a more complete coating layer. The precipitate formed was confirmed as loratadine, by using FTIR (data not shown). As a result, only the more stable nanosuspension (L2) was further monitored.

A good polymer for nanoparticle coating is one that allows obtaining robust physicochemical properties, guaranteeing the drug stability and keeping the drug content intact, until the release moment. A good coating layer and loratadine dispersion were not achieved using PEG 4000, probably because this polymer solubilize in the water, affecting the nanoparticle integrity (Tadros, 2013). On the contrary, methacrylic acid-derived as Kollicoat® Mae 100 P (methacrylic acid-ethyl methacrylate) and Eudragit® 100 55 P (methacrylic acid-methyl methacrylate) are water insoluble. Apparently, using Eudragit® (shorter ester chains) an incomplete covering was obtained, leaving bare zones and thin areas, which were broken along 21 days, releasing the loratadine content to dispersing medium, affecting the nanosuspension stability. It seems to be that, the longest chains of Kollicoat® (Basf, 2010), in the amount used on L2 nanosuspension, allows obtaining robust nanoparticles with a complete polymeric covering, without bare and/or thin zones, keeping the loratadine content protected inside nanoparticles, until the release moment.

Formulation processes require technological operations involving prolonged movement of materials. Some of these operations involve heat transfer, thus is important to know the effect of temperature in drugs physicochemical properties and materials involved in the preparations. Temperatures ranged from 20 to 80 °C (thermal effect) practically no affects the behavior of particle size and polydispersion index of L2 nanosuspension (Fig. 4). This suggests that, the coating layer of L2 nanoparticles is temperature-resistant. This can be associated, probably, to the presence of polysorbate 80. This nonionic surfactant improves the plasticity of nanoparticle coating layer (BASF, 2010). Polysorbate 80, also prevents the nanoparticles aggregation by a steric effect (Sauer and Meier, 2001). The hydrophobic alkyl group of Polysorbate 80 becomes adsorbed onto the hydrophobic particle or droplet surface, leaving the strongly hydrated polyethylene oxide chain dangling in solution. This, provide an effective repulsive barrier, avoiding aggregation (Tadros, 2013), protecting the loratadine content and preserving the nanosuspension stability. For this reason, manufacturers add 0.7% polysorbate 80 during the preparation of Kollicoat® Mae 100 P (BASF, 2010).

The prolonged heating (isothermal effect at 80 °C) for two hours allowed confirming that nanoparticles maintained their stability for up to one hour (Fig. 5). The particle size increased up to 121.25 nm and polydispersion index was practically unaffected along the time. The increase in particle size was probably related to the increased heat, which causes an expansion of the polymeric coating layer. Possibly, there was a loss of thickness of the electrical double layer because of an increase in particles thermal motion. Thus, the zeta potential tended to decrease, allowing aggregation, increasing the number of particles with bigger sizes, with an exaggerates enlargement of the polydispersion index until the system is destroyed (completely polydisperse system) (polydispersion index = standard deviation of the mean particle size/the mean particle size) (McNeil, 2011). The completely turbid aspect of the nanosuspension in the cuvette, at the end of the experiment, confirms the last statement.

The pH had a strong effect on nanosuspension particle size and the polydispersion index (Fig. 6a). Above pH 5, a sharp increase in both properties was observed, reaching a size of 1000 nm at pH 9–10. The increase was probably associated to the polymer solubilizing. Kollicoat® Mae 100 P is a methacrylic acid derivative (Bühler, 2007), insoluble at a pH from 1 to 5, resistant to the stomach acid action (Lafourcade et al., 2016; Bühler, 2007). However, at pH above 5.5, this polymer begins to swell and dissolve. Thus, nanoparticles Kollicoat®-coated begins to swell and become softer at a pH over 5.5, followed by a coating breakdown and releasing the loratadine content (Pandya et al., 2011). As the Kollicoat® properties remain intact at pH below 5.0, this polymer is an effective protector for drugs suffering stomach degradation, became it in an excellent polymer for formulating delivery systems of drugs that are absorbed in intestinal pH. As was expected, from pH 1 to 5, the nanoparticles integrity of L2 remained intact, which made this nanosuspension a promissory intestinal loratadine delivery system.

Particle size and zeta potential can be directly measured, depending on the nanoparticle system turbidity. In turbid solutions, a dilution is required, and the way in which the dilution is made is critical for determining the final value of the zeta potential because of the pH changes (Malvern, 2015). For this reason, in this work, the same dilution was used for all the samples analyzed. The increase in pH caused a significant decrease in L2 zeta potential (increase the modular value) to −37.90 mV at pH 9 (Fig. 6b). The conductivity also decreased, reaching values close to 1.0. Probably, the increase in particle size, resulting from the pH increase, induced a greater amount of negative counterions joining to the double layer in solution (Pandya et al., 2011). This might cause a significant decrease of zeta potential and a conductivity reduction, because of a diminution in the ions mobility in the solution. The isoelectric point of these nanoparticles was at pH 1.98, where the conductivity was 3.80 mS/cm-1. Thus, a further increase in the medium basicity would result in the coating layer breakdown losing the nanoparticles integrity.

Scanning Electron Microscopy is a suitable technique for nanomaterial observation. However, is difficult to observe the nanoparticles integrity due to the aggregation in the solvent evaporating process, during the sample preparation for this analysis (Gibson, 2006). In Fig. 7 can be observed a uniform distribution of the particles with different sizes, and a shape approximately spheroid. It seems to be that, as the solvent evaporation occurred in normal conditions, over a carbon tape, the nanoparticle mobility diminished as the amount of solvent is reduced. Thus, particles begin to agglomerate, and aggregates of superior sizes and different shapes were formed. A similar result was reported, in which the nanoparticle size obtained by SEM did not match with those sizes obtained by Dynamic Light Scattering, because of the particle aggregation in a solvent reduced medium (Thadkala et al., 2014).

In pharmaceutical preparations, is important to know all the variables that could affect the drugs and its possible interactions with the formulation components. Moreover, is very important to evaluate the effect of external factors such as the temperature, the presence of oxygen, pH, and other factors that may affect drugs in production phases, storage, and transportation (Bajdik and Pintye, 2006). In colloidal systems, is also important to know the particle behavior nearby the isoelectric point (IEP). It is known that in those values of pH = IEP ± 2, colloidal systems become unstable (Tadros, 2013). Therefore, in this study it was evaluated the combined effect of the temperature (40, 50 and 60 °C) and pH at values below (pH 1) and above (pH 3) of the IEP, and at the pH in which the polymeric coating begins to swell and dissolve (pH 5.5). The particle size and zeta potential showed no major changes at pH between 1 and 3 in the range of temperatures evaluated (Fig. 7A, B, and C). L2 nanosuspension, under these conditions, remained monomodal with polydispersion indexes below 0.160. This fact supports the resistance of the polymeric layer in an acid

pH (1 −3) and temperatures up to 60 °C. On the other hand, the increase in particle size at pH 5.5 (Fig. 7A), could be the result of the coating layer enlargement because of temperature, and an early aggregation process (Lafourcade et al., 2016), resulting a higher polydispersion index (Fig. 7B) and a lower modular value of zeta potential (Fig. 7C), which was more noticeable at 50 and 60 °C. These findings suggest that loratadine loaded nanoparticles (L2) coated with Kollicoat® MAE 100 P, were resistant to the combined action of acid pH (1 −3) up to 60 °C, because of in these conditions, the physical stability of nanosuspension remained intact.

Alterations of pH in pharmaceuticals solution is an instability indicator. Redox reactions, precipitation of a component, phase separation and other changes in a solution medium, are reflected as pH modifications (Tadros, 2013). During the shelf stability study, the pH of L2 nanosuspension remained around 5.02 ± 0.02 (Table 2), suggesting that no changes occurred in the nanosuspension. This statement is supported by the fact that the conductivity remained almost constant at 0.100 ± 0.005 $mS.cm^{-1}$ (Table 2), which is an indicator of the ionic composition stability of the nanosuspension. Particle size and zeta potential remained almost constant along the time (Table 2), while the polydispersion index decreased, suggesting that a dynamic equilibrium within the nanosuspension range of particle size was achieved. The DLS analysis (Fig. 9A, C, and E) showed a more homogeneous particle size distribution (less dispersion) at 90 days. Similarly, zeta potential at 30, 60 and 90 days (Fig. 9B, D, and F, respectively) showed a narrower and uniform base. These data suggest the good stability achieved in this nanosuspension along the first 90 days.

The stability studies in stress conditions allow the prediction, with some accuracy, the behavior of pharmaceutical formulations when preserved for a long-time under normal conditions. Additionally, they allow evaluating the effects of stress factors on pharmaceuticals and especially on the active substances, allowing the selection of formulation components as well as the packaging and storage conditions of the final dosage form (Bajdik and Pintye, 2006). In both temperatures evaluated (30 and 45 °C) the particle size remained below 48 nm throughout the study (Fig. 10A). The polydispersion index decreased during the first 30 days, but remained constant afterwards (Fig. 10B). In the same way, the modular value of zeta potential increased and remained constant after 30 days (Fig. 10C). These results demonstrate that the polymeric coating layer of L2 nanosuspension, obtained using Kollicoat® MAE 100 P was strong and stable along the time under temperatures up to 45 °C. The particle size and polydispersion index showed a tendency to decrease with the increase of temperature (Fig. 11), while there was no significant difference in zeta potential at each temperature value ($F = 3.46$, $p = 0.1098$). This result suggests that, under normal conditions, the nanosuspension will preserve the stability for a longer time. This statement seems to be true, if it considers that, the behavior of the nanosuspension properties versus the temperature is the same in the freshly prepared nanosuspension that, after three months stored under standard laboratory conditions.

Gastrointestinal juices can be very aggressive for drugs. In the stomach juice, the presence of enzymes and ions, especially the high content of H^+, made the stomach environment a critical barrier for drugs stability. Currently, biorelevant dissolution media, with a composition similar to those of aspirates collected from the human gastrointestinal tract, are used for simulating the stomach and proximal small intestine juices of humans (Jantratid et al., 2008). Under fasting, the stomach pH ranging from 1 to 3. All the marketed loratadine preparation for the oral route dissolves in the stomach (Law et al., 2014). However, the erratic behavior of loratadine dissolved in the stomach has been reported (Khan et al., 2004; Patil and Paradkar, 2006). They also reported that, loratadine release was slow in FaSSIF reaching 100% after 12 h. In our work, a nanosuspension of loratadine-loaded nanoparticles, resistant to the degradative action of the FaSSGF and FeSSGF media was prepared (Fig. 7). Loratadine release in FaSSIF (pH 6.5) and in SIF without pancreatin (pH 7.4) was superior to 90% after 30 min, while in FaSSGF (pH 1.6) and FaSSGF (pH 5.0) the release was minor than 2% in 2 h. Until the authors know, in all the others loratadine carrier systems (Nacsa et al., 2008; Pooja et al., 2011; Frizon et al., 2013; Patil and Paradkar, 2006; Li et al., 2014), the *in vitro* releasing occurs at the stomach pH. As a weak base, loratadine suffers a chemical transformation in the stomach, losing potency, and reducing the activity and therapeutic efficacy (Low et al., 2014). In our work, a loratadine-loaded nanoparticle protecting the drug from the stomach action was obtained. It seems to be that, nanosuspension overcomes the bioavailability problems above-mentioned releasing 100% of drug at intestinal pH, the loratadine's absorption site. As was expected, a complete release of the loratadine occurred at pH 6.5 and 7.4, because the polymeric coating layer, produced with Kollicoat®, begins to swell and dissolve at pH above 5.5. (BASF, 2010). The used of Kollicoat® MAE 100 P as coating polymer allowed obtaining a new resistant and flexible nanoparticles, able to avoid the strong degradative action of the stomach in fasted and fed conditions, which was not achieved in any other formulations reported until now.

5. Conclusions

In this work, different from all the loratadine preparations reported in the literature, in which the release occurs in the stomach, a new gastro resistant nanoformulation for the loratadine release at intestinal pH was obtained. The nanosuspension particle size was 45.94 ± 0.50 nm. Kollicoat® MAE 100 P produced a hard and flexible coating layer, releasing 100% of loratadine in 40 min, in simulated intestinal fluids. Nanoparticles were resistant in stress condition of pH (from 1 to 3) and temperature (from 40 to 60°) for a short time and isothermally stable at 80 °C up to one hour. Loratadine content in nanoparticles remained unalterable (over 93%) along three months as well as all the physical and chemical properties, in shelf conditions. Contrasting the high-energy input methods, the solvent displacement evaporation method (a low energy method) used here, does not need complex equipment, as a high pressure homogenizer i.e., which could be advantageous for the scale up and production of this nanosuspension for practical purposes. For the first time, as far as the authors know, a gastro resistant loratadine nanoparticle was obtained, which could be a promising alternative for the use of this drug in therapeutics.

Acknowledgment

The authors want to thanks, National Council for Scientific and Technological Development (CNPq), grant process 402332/2013-0 and CAPES/FAPEAP Grant Process N° 23038.000516/2013-01.

Conflict of interest

The authors declare that there are no conflicts of interest.

JRRA, made the experimental design coordinating all work of the team. ALP and JLD, made the experimental part of the formulation and evaluation of stability. HK and HRS, developed the release profiles. AMF, performed the study by Scanning Electron Microscopy. EHS, translated and made a critical proofreading of the manuscript. JCTC, made the design the release profiles study and the proofreading of the manuscript. All authors have read and approved the final version of the article.

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