Extemporaneous Indomethacin Oral Suspension Prepared from Injectable Ampules for Therapy in Premature Infants and Pediatric Patients

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Abstract

Indomethacin is used for off-label prescription for the treatment of patent ductus arteriosus in premature infants. In Argentina, indomethacin is only available as a suppository, dermic cream, injectable ampules, and delayed-release capsules. Aiming to improve pediatric treatment and minimize the risk associated with improper dosage, this work focused on the development of an extemporaneous 0.2% indomethacin oral suspension, starting from the commercially injectable formulation. Two 150-mL batches of suspension were prepared using Generally Recognized as Safe excipients. The suspensions were stored for 17 days at room temperature. Physical stability, morphological analysis of suspended particles, sedimentation volume, easy re-suspension, and dynamic viscosity were studied. The indomethacin content, dissolution studies, and microbiological attributes of nonsterile pharmaceutical products were also evaluated. After 17 days of storage, the suspension was easily re-dispersed after 15 seconds of the hand-shaking technique. There were no detectable changes in color, odor, and/or flavor. The suspension showed minimal changes in pH, viscosity, shape, and mean size of the suspended indomethacin particles. The content uniformity and drug dissolution remained within the acceptable range during storage. This oral liquid suspension is an interesting alternative to be prepared by hospital pharmacy services for optimizing the pediatric treatment of patent ductus arteriosus.

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Introduction

The ductus arteriosus plays a key role in the foetus. This is an important conduit that allows deoxygenated blood to bypass a collapsed lung and enter the placenta through the descending aorta and umbilical arteries. After birth, the ductus arteriosus normally closes within the first several days of life. A persistently patent ductus arteriosus (PDA) can cause significant problems, mainly in premature infants.[1] Ductal closure mechanisms are weak and immature in preterm neonates compared with their term counterparts.[2]

During the first 2 to 3 days of life, spontaneous closure of the ductus occurs in 55% of full-term newborn infants. By 2 to 6 months of age, closure occurs in more than 95% of healthy infants. PDA following birth is inversely related to gestational age.[1,3,4] This may be due to the smaller amount of muscular tissue in the media with lower intrinsic tone, and lower responsiveness to oxygen but higher sensitivity to the vasodilating effects of prostaglandin E2 and nitric oxide.[4] In clinical practice, most extremely-low-birth-weight infants who are less than 28 weeks gestation have a PDA during the postnatal period.[1,4]

It is well known that prescribing a drug outside its licensed recommendations with regards to age, indication, dose, route of

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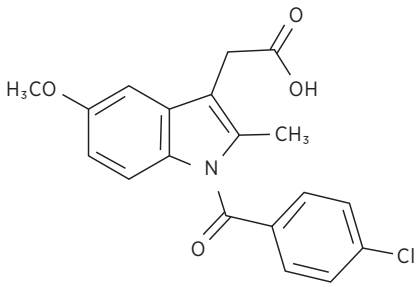
administration, and formulation, known as off-label prescribing, is a common prescribing trend particularly in children.[5] Indomethacin (IND, FIGURE 1), a nonsteroidal anti-inflammatory drug, is a potent stimulator of ductal closure, and it is the most commonly used agent for this medical purpose.[1] It blocks the enzyme cyclooxygenase, which inhibits prostaglandin synthesis, thereby facilitating ductal closure.[6] It also increases the thickness of the avascular zone by causing constriction of circumferential and longitudinal muscles, decreasing blood flow in the vasa vasorum, and causing vessel wall hypoxia with release of vascular endothelial cell growth factor.[1] Most studies have shown that the use of IND in closing PDA has reduced the need for subsequent surgical closure.[7]

FIGURE 1.

MOLECULAR STRUCTURE OF INDOMETHACIN.



In Argentina, IND is only commercially available as suppository, dermic cream or gel, capsules, and injectable pharmaceutical forms.[8] The last one is the dosage form currently used for the treatment of PDA in premature infants due to this drug not being available in a convenient, easy-to-take dosage form. Liquid formulations are the commonly compounded preparations by pharmacists for pediatric patients,[9,10] both at individual and batch scales.[9]

In this sense, it should be noted that the *United States Pharmacopoeia* encoded an IND oral suspension,[11] and this dosage form is commercially available in the U.S. under the brand name INDOCIN, which contains 25 mg of IND per 5 mL, 1% alcohol, and 0.1% sorbic acid as a preservative.[12] Even though the Ministry of Health from Argentina approved the commercialization of INDOCIN,[13] this medicine is not available for the treatment of infants. Thus, there is a need to develop a convenient suspension starting from the available products in order to supply safe and reliable pediatric dosages and minimize the risk associated with improper dosage.

In this context, this study focused on the development of an extemporaneous 0.2% IND oral suspension with adequate physicochemical and microbiological stabilities, starting from the commercial injectable. It is expected that the formulated suspension can be prepared and dispensed by the pharmacy services of hospitals for the treatment of pediatric patients using free alcohol and safe excipients, currently available in a galenic area of hospital pharmacy.

Materials and Methods

MATERIALS

IND injectable ampules (Batches DA034FU027-0 and DA040FU027-0I.M, 75 Montpellier, 50-mg IND/ampules; Buenos Aires, Argentina) and IND pharmaceutical-grade powder (Parafarm, Buenos Aires) were used as purchased. All Generally Recognized as Safe (GRAS) excipients, including wetting agents, suspending agents, sweeteners, flocculant agents, and antimicrobial preservatives, were selected:

- Methylcellulose (Batch 280435, 1500 cP; Parafarm, CABA, Argentina)
- Xanthan gum (Batch 201412A-N31; Parafarm)
- Sorbitol (Batch 1415P391; Parafarm)
- Polysorbate-80 (Batch 141002G9797; Pura Quimica, Córdoba, Argentina)
- Acesulfame-K (Batch 20070203; Parafarm)
- Citric acid (Batch 50520024; Parafarm)
- Sodium citrate (Batch 50722018; Parafarm)
- Methylparaben (Batch PR0117-0115; Parafarm)
- Propylparaben (Batch PR1940-0415; Parafarm)
- KH_2PO_4 (Batch 19312-88, Pro-analysis grade; Anedra, Research AG SA, Tigre, Argentina)
- 1.0N NaOH solution (Batch 24108-2; Anedra)
- Absolute ethanol Batch 68743; Cicarelli, Cordoba, Argentina)
- Distilled water

PREPARATION OF 0.2% INDOMETHASIN SUSPENSION

Two 150-mL batches of 0.2% IND suspension were prepared in accordance with the composition detailed in Table 1, following this procedure:

METHOD OF PROCEDURE

1. Calculated the required quantity of each ingredient for the total amount to be prepared.
2. Weighed and/or measured each ingredient accurately.
3. Dispersed methylcellulose 1500 cP with 30 mL of water and mechanically stirred for 24 hours until complete homogenization (reactor-1) had occurred.
4. Dispersed in parallel and under moderate heating at 55°C the sorbitol, polysorbate-80, and antimicrobial preservatives until homogenization (reactor-2) had occurred. *Note: Reactor-2 was also left overnight until complete homogenization had occurred.*
5. Dispersed the xanthan gum, separately, with 22 mL of water and mechanically stirred for 24 hours until homogenization (reactor-3) had occurred.
6. Transferred the content of reactor-2 to a mortar, and 6 ampules of IND (300 mg) were slowly incorporated and mixed manually with pylon.
7. Incorporated the dispersion in reactor-3 into the mortar and mixed until

uniform dispersion had occurred.
8. Added the content of reactor-1 and all the blend was mixed until complete homogenization had occurred.
9. Transferred the blend to a 250-mL glass graduated tube, and water was incorporated to achieve a final volume of 150 mL of suspension.
10. Bottled the suspension in 8 light-resistant containers of 7.5 mL and 2 with 45 mL of suspension.
11. Stored the samples for up to 17 days under room temperature (25°C).

PHYSICOCHEMICAL AND PHARMACOTECHNICAL CHARACTERIZATION

Physicochemical and pharmacotechnical quality attributes of the formulation were evaluated according to general pharmacopoeia specifications for oral suspensions and non-necessary sterile oral liquid formulations.

Physical Stability Evaluation

Physical stability was defined as the absence of color, odor, and/or flavor changes, and the evaluation of re-suspension of cake solid phase by a reasonable amount of 15 seconds of the hand-shaking technique.[14] Physical stability was assessed by visual examination, optical microscopy, measurement of the sedimentation volume of suspensions (V_s) and easy re-suspension, and determination of dynamic viscosity.

A morphological analysis of IND suspended particles was conducted by means of 10× optical microscopy (Olympus, BX41TF; Japan) and Infinity Analyze specific software (Release 5.0.2v; Lumera Corp, Ottawa, Canada]. Morphological analyses were performed after 5 days, 10 days, and 17 days of storage.

The V_s was calculated by the ratio between the measure of volume of the dispersion at different stored times (V_f) versus the original volume of suspension (V_0) bottled in the container, according to Equation 1:

$$V_s = \frac{V_f}{V_0} \qquad \text{Equation 1}$$

V_s was determined after 3 days, 7 days, 10 days, and 17 days of storage. In order to evaluate the easy re-suspension, at 17 days of storage the containers were subjected to 15 seconds of the hand-shaking technique to re-suspend the cake solid phase of the suspension. The experiments were carried out in triplicate at 25°C.

Dynamic viscosity (η) of 10 mL of suspension was assayed on day 3, day 7, day 10, and day 17, using a rotational viscometer (VT500; Haake, Germany) equipped with NV-cylinder and NV-cup sensors. The upward and downward flow curves were performed at the interval of 0 rpm to 60 rpm and 60 rpm to 0 rpm, during 60 seconds each segment. η was measured at a constant shear rate of 60 rpm during 60 seconds. The experiments were carried out in duplicate at 25°C for each batch.

pH Measurement

The pH values were recorded with a digital pH-meter (SevenMulti S40; Mettler-Toledo, CH) with an Ag/AgCl-reference electrode (DG-115-SG), calibrated with commercial reference buffer solutions of pH 4.01 and 7.00 (Anedra) in automatic temperature compensation mode. The experiments were carried out in triplicate at room temperature for each batch.

Content Uniformity and Chemical Stability Evaluation

Content uniformity as a function of time was assessed following IND concentrations in the suspension after re-dispersion of the stored formulation by 15 seconds of the hand-shaking technique. IND concentrations were determined by ultraviolet (UV) spectrophotometry (Evolution-300; Thermo-Scientific, CH). A sample of 0.55 mL of suspension was placed in a graduated glass flask of 50 mL in the presence of a phosphate buffer solution pH 7.2 ± 0.5 (PBS 7.2) and sonicated for 15 minutes at ~55°C. Then, the filtered solution was spectrophotometrically assayed at 320 nm. The experiments were carried out in quadruplicate for each batch.

Before performing the content uniformity evaluation, all components of the suspension in the absence of IND were assayed in order to ensure non-interference of them at the wavelength of maximum absorption of IND (320 nm). This assay was carried out as a measure of the selectivity of the method. A calibration curve was constructed by using seven standard solutions, which were prepared with different concentrations of IND pharmaceutical grade (concentration range used: $1.7 \times 10^{-5} - 15 \times 10^{-5}$ M). Linearity was found over the concentration range studied, and the regression equation and correlation coefficient (R^2) obtained were $y = 6879.4x$ and 0.998, respectively. The percentage variation coefficient (C_v%) of all values of precision (at lower: 1.7×10^{-5} M, mid: 6.9×10^{-5} M, and higher: 15×10^{-5} M concentrations of IND solutions) were 15.3%, 0.4%, and 4.2%, respectively. The before-mentioned demonstrated the suitability of the quantification method for IND analysis. In addition to that, to complete the chemical stability analysis of IND in the suspension high-performance liquid chromatography with UV-visible detection (HPLC-UV) was carried out based on the method proposed by Liu et al.[15] The HPLC chromatographic analyses was performed using a Waters HPLC system equipped with an isocratic Waters 1525 pump, an autosampler Waters 717 Plus, and a PDA-UV detector (PDA 2296 detector) at 320 nm, with data acquisition and processing being performed using Empower system software. Chromatographic separations were carried out using a Phenomenex C18 reverse phase column (250 × 4.6 mm, 5-µm particle size) and a Phenomenex guard column (C18 4 × 3 mm ID). Analysis was performed with acetonitrile: 0.2% phosphoric acid in water (pH 2.0) (60:40, v/v) as the mobile phase at a flow-rate of 1.5 mL/min in the isocratic mode. A sample of 0.55 mL of suspension was processed as stated above. Moreover, a sample of pure IND was prepared by dissolving 50 mg of IND pharmaceutical grade in a graduated glass flask of 25 mL in the presence of absolute ethanol (solvent selected considering the low water solubility of IND in water[16]), achieving a final concentration of IND similar to

that present in the suspension. From this solution, a sample of 0.55 mL was diluted in PBS 7.2 and processed using the same methodology applied for the suspension. In addition, a sample of IND subjected to forced degradation under basic conditions was prepared by adding 0.5 mL of IND solution (0.2% p/v) in a graduated glass flask of 5 mL in the presence of NaOH 0.1M solution. From this solution, a sample of 1 mL was diluted in PBS 7.2 for further analysis. For HPLC, 20 µL of each filtered sample was injected, and the run time was set at 10 minutes. Peak areas were used for quali-quantitative analysis. This assay was performed in duplicate.

DISSOLUTION TEST

Dissolution tests were performed with a Smart-AR7 dissolution tester (Sotax, CH) *USP*-Aparatus-2, applying a paddle stirring speed of 50 rpm. Volumes of 900 mL of PBS 7.2 at 37.0 ± 0.5°C were used as a dissolution medium. Samples of 10 mL of suspension, on day 0 and day 17, containing about 20 mg of IND, were subjected to dissolution testing. Aliquots of 4 mL of dissolution medium were taken out at predetermined times (i.e., 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes). The IND concentration dissolved was measured at 320 nm. The experiments were carried out six times, and the results were expressed as the average ± SD.

MICROBIOLOGICAL ASSAY

The samples of 0.2% IND suspension after 17 days of storage were subjected to microbiological assessment in order to determine whether they met the microbiological attributes of "nonsterile pharmaceutical products" according to the *Argentinean Pharmacopoeia*, which were set as a total aerobic microbial count below 10^3 cfu/mL and total combined yeasts/molds count below 10^2 cfu/mL.[17] Also, Enterobacteriaceae levels were analyzed. Both aerobic microbial count and Enterobacteriaceae levels were performed by using the pour plate technique, while the total combined yeasts/molds count was carried out by applying the spread plate technique.[18] The term "growth" has been used in a special sense herein (i.e., to designate the presence and presumed proliferation of viable microorganisms). These trials were outsourced to the Centro de Química Aplicada (CEQUIMAP-FCQ-UNC), and they were performed in duplicate.

Results

An extemporaneous, oral suspension containing 0.2% IND was prepared from commercially available sterile, lyophilized IND for

TABLE 1.

QUALITATIVE AND QUANTITATIVE FORMULA OF 0.2 % INDOMETHACIN SUSPENSION.

COMPONENT	PROPORTION (G)	FUNCTIONAL CATEGORY
IND (eq. 4 ampules)	0.20	Active pharmaceutical ingredient
Polysorbate-80 Xanthan gum Methylcellulose (1500 cP)	0.20 0.10 0.50	Wetting and suspending agents
Sorbitol	5.00	Co-solvent
Methylparaben Propylparaben	0.02 0.02	Antimicrobial preservatives
Acesulfame-K	0.05	Sweetening agent
Citric acid Sodium citrate	0.30 0.50	Buffering agents
Distilled water *s.q.t.*	100	Vehicle

s.q.t. = sufficient quantity to

TABLE 2.

STABILITY PARAMETERS OF 0.2% INDOMETHACIN SUSPENSIONS UP TO 17 DAYS OF STORAGE.

PARAMETERS	STORAGE TIME (DAYS)				
	0	3	7	10	17
IND (% ± SD)[a]	97 ± 4	105 ± 2	106 ± 1	101 ± 1	100 ± 6
pH ± SD[b]	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	5.0 ± 0.2
η (*mPa.s* ± SD)[c]	-	25.8 ± 0.2	25.5 ± 0.8	25.0 ± 0.5	24.4 ± 1.5
Microbial growth (cfu/mL)[d]					
Total aerobic microbial	-	-	-	-	11[e]
Total combined yeasts/molds	-	-	-	-	8[e]
Enterobacteria	-	-	-	-	negative [f]
Vs[b]	ND	ND	0.96 ± 0.04	0.78 ± 0.03	0.59 ± 0.05

Notes:
- *Data is expressed as averages with their respective standard deviations (SD).*
- *There are not official acceptable levels defined for Vs and η values. Minimal or no changes of Vs and η values along time is regarded as criteria of physical stability.*
- *ND = not detected (since no sedimentation was observed). It means that the Vs was equal to the total volume of suspension.*

[a]IND amount in the suspensions, determined by UV-vis spectrophotometry, and expressed as % of IND respect to the initial amount of drug added to the suspension. This determination was performed in quadruplicate. IND concentration of not less than 90% and not more than 110% is considered as criteria for acceptable stability levels.[11]
[b]pH and volume of sedimentation (Vs) were carried out in triplicate.
[c]viscosity (η) was determined in duplicate.
[d]microbial count assay was carried out in triplicate.
[e]Total yeasts and molds were 5 and 3, respectively. Values are below the limits required by the *Argentinean Pharmacopoeia* for nonsterile pharmaceutical products.[17]
[f]Negative microbial growth according to the criteria encoded in the *Argentinean Pharmacopoeia*.[17]

injection. GRAS excipients for pediatric formulations were used.[19]

In previous pre-formulation studies (data not shown), different proportions of wetting and suspending agents were assayed, using recommended concentration ranges,[20] in order to preliminarily evaluate the capacity of wetting the dispersed phase. The viscosity of suspensions and re-dispersion of particles during the storage by a reasonable amount of the hand-shaking technique were evaluated. The quali-quantitative formula composition of the optimized formulation is reported in TABLE 1.

PHYSICAL STABILITY

Physical stability parameters and results of pH measurements at different times of storage at room temperature are shown in TABLE 2. The suspension showed a pale-white color, with particles uniformly dispersed, and no changes of color, odor, or flavor were detected. The pH values of the suspension were in the range 4.7 to 5.0. Microscopic analysis revealed the presence of drug crystals (FIGURE 2A-F). The morphological characterization of the suspended IND particles showed that the sample displayed particle sizes in the range of 200 to 300 µm and the shapes and mean size of suspended particles remained stable during storage conditions (FIGURE 2A-C). The crystals of IND exhibited the phenomenon of birefringence under the application of polarized light (FIGURE 2D-F).

The determination of V_s showed a slight decrease of V_s during the first week of storage. However, a greater and progressive decrease of V_s after day 7 and up to day 17 was observed (TABLE 2).

FIGURE 3 shows the flow curves of shear stress as a function of rotational speed for suspensions at different times of storage. Under assayed conditions, both the upward and downward curves presented similar profiles, suggesting pseudoplastic flow with narrow or negligible thixotropy. Flow properties and η of the suspension at different times of storage did not show significant changes, which indicated that this attribute of quality was kept (TABLE 2). Moreover, the suspensions at 17 days of storage were easily re-dispersed.

DRUG CONTENT UNIFORMITY AND CHEMICAL STABILITY OF INDOMETHACIN

The drug content uniformity analysis demonstrated acceptable re-dispersion of IND, ensuring the pouring of a liquid volume containing the desired dose. At different times of storage, the suspensions were within the acceptable range of IND content with standard deviations of ≤6% (TABLE 2). Also, under assayed conditions, non-significant changes in IND concentration were detected and at least 97% of total IND could be quantified in a volume of ~0.5 mL of suspension after 17 days of storage.

By HPLC analysis, it was observed that IND was eluted within the retention windows of 7.2 to 7.3 min. Only one peak was observed in the chromatogram of pure IND as well as IND suspension. Moreover, degradation products of IND obtained under forced basic conditions were eluted with retention times of 2.5 minutes and 3.4 minutes (FIGURE 5). Considering the peak area of pure IND

FIGURE 2.

OPTICAL MICROSCOPY IMAGES (10×) OF INDOMETHACIN SUSPENSIONS PREPARED FROM COMMERCIALLY AVAILABLE STERILE, LYOPHILIZED INDOMETHACIN FOR INJECTION AT DIFFERENT TIMES OF STORAGE AT ROOM TEMPERATURE.

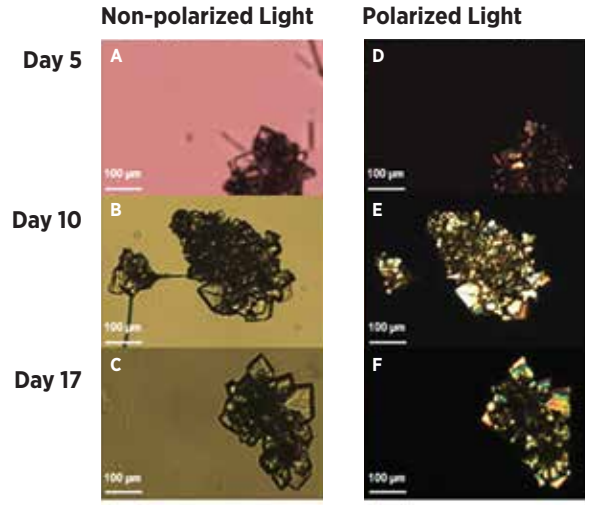
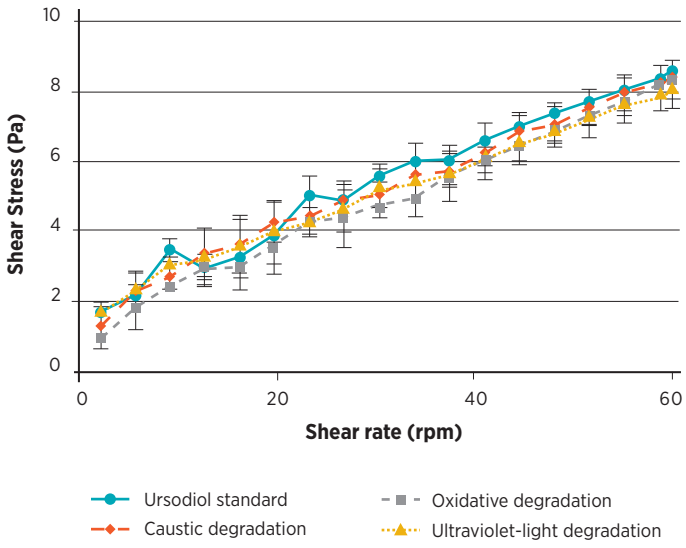


FIGURE 3.

UPWARD FLOW CURVES OF INDOMETHACIN SUSPENSION PREPARED FROM COMMERCIALLY AVAILABLE AMPULES AT DIFFERENT DAYS OF STORAGE AT ROOM TEMPERATURE.



as a reference, the concentration of IND in the suspension could be estimated, and the value obtained was similar to that quantified by UV-visible spectrophotometry (TABLE 2).

DISSOLUTION ASSAY

In vitro IND dissolution profiles are shown in FIGURE 4. Immediate and fast dissolution rate of IND was observed for suspensions at different times of storage, in which ~90% and ~85% of IND were dissolved at 10 minutes of assay for freshly prepared suspensions and after 17 days of storage, respectively, reaching up to 95% of IND dissolved at 30 minutes of assay.

MICROBIOLOGICAL ASSAY

The suspensions after 17 days of storage at room temperature, which could be an unfavorable condition, were subjected to microbiological evaluation in order to determine if they fulfilled the microbiological specifications of nonsterile pharmaceutical products and the absence of enterobacteria. The suspension showed a very low count of total aerobic microbial and total combined yeast/mold, and did not show microbiological growth of enterobacteria, which is expressed as "negative" in TABLE 2.

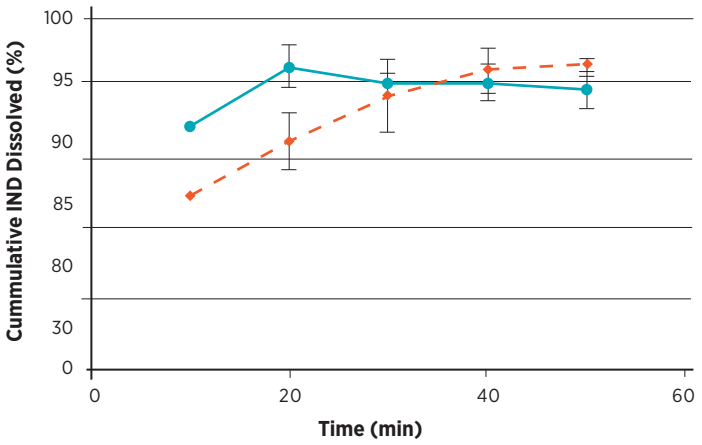
Discussion

In this study, we developed and characterized an extemporaneous, oral liquid formulation containing 0.2% of IND from commercial injectable ampules. Because of the intended application in premature infants and pediatric patients, specific attention was paid to child-friendly oral dosage forms and alcohol-free excipients (TABLE 1).[9,21] The suspension exhibited adequate physical and chemical stability, and a beyond-use date of at least 17 days, which is a storage time three-folds higher than required for the treatment of PDA in premature infants.[22] Moreover, this suspension can be expected to provide good dosing accuracy during the treatment. The suspension was prepared through an easy and reproducible process under current Good Manufacturing Practices for pharmaceutical compounding.[17] The selected excipients were not only part of the GRAS list, but were also adequate for pediatric formulations, alcohol free, and currently available from a hospital pharmaceutical service.[19]

As well known, suspensions are thermodynamically unstable pharmaceutical dosage forms and suspended particles will sediment at a certain sedimentation rate. That is the main reason why they have to be shaken before use in order to ensure content uniformity at each volume dose.[14,23] If the solid phase cannot be resuspended by a reasonable hand-shaking technique, an indication of instability in a suspension is observed, which could negatively impact its quality. Even though the official compendium does not specify values of V_s as a quality requirement, higher values of V_s and minimal variations of V_s (TABLE 2) over time indicate that a formulation can remain in a flocculated state, assuring drug uniformity at each dispensed volum.[24,25] Furthermore, the observation of rela-

FIGURE 4.

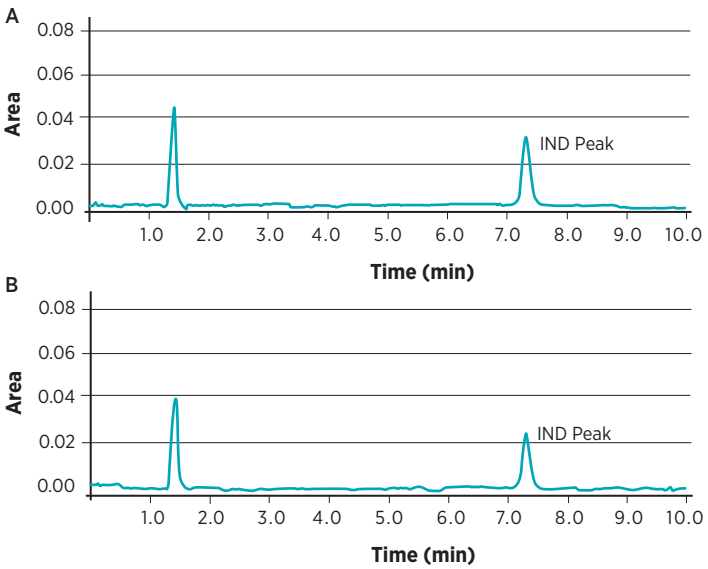
DISSOLUTION PROFILES OF INDOMETHACIN SUSPENSIONS.



The suspensions were freshly prepared (day 0) and stored for 17 days at room temperature (*United States Pharmacopeia*-apparatus 2, 50 rpm, 900-mL phosphate buffer solution pH 7.2 ± 0.5 at 37.0 ± 0.5°C). Results are expressed as the average ± standard deviations of six independent determinations.

FIGURE 5.

SUPPLEMENTARY MATERIAL: REPRESENTATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-ULTRAVIOLET CHROMATOGRAMS OF INDOMETHACIN.



Representative HPLC-UV chromatograms of IND. A) Suspension (after 17 days). B) Reference of pure IND. The first peak in both chromatograms correspond to the solvent front.

tively large particles would suggest that excessive crystal growth has taken place during storage (FIGURE 2).[14]

In addition, as detailed by Stoke's law,[26] an increase in the viscosity of an aqueous vehicle can reduce the sedimentation rate of the suspended particles, providing a higher physical stability of the suspension. Even when the official compendium does not define criteria for acceptable viscosity levels for liquid oral dosage forms, it is considered that viscosity should be as high as possible to minimize the particle sedimentation rate and prevent their agglomeration and irreversible solid-cake sedimentation. Also it should be low enough to ensure the pouring of a liquid volume containing the desired dose.[24,25] The results of rheology studies showed pseudoplastic flow with narrow or negligible thixotropy (FIGURE 3), which is the typical behavior observed in pharmaceutical suspensions. A minimal, time-dependent change was observed for the values of viscosity, which slightly decreased at 17 days of storage compared to the suspensions at 3 days of storage. This reduction in the viscosity for aged suspensions has also been previously observed by other authors.[14] Interestingly, easy re-dispersion after the hand-shaking technique was maintained for suspensions after 17 days of storage, which ensures the pouring of a liquid volume containing the desired dose for the treatment.

USP encoded, as general criteria, the acceptable levels of drug content uniformity should be not less than 90.0% and not more than 110.0% of the labelled amount of IND.[11] This assay showed an acceptable re-dispersion of IND suspension, and all samples were within the acceptable range of IND concentration with minimal standard deviations along time (TABLE 2), which indicates an adequate re-dispersion by simple shaking. Even though it is well known that IND is chemically stable in aqueous media, the pH values need to be in the range 2.5 to 5.0 to prevent alkaline hydrolysis to p-chlorobenzoate and 2-methyl-5-methoxy-indole-3-acetate.[28] Under assayed conditions, the suspension pH remained unchanged with slightly acidic values (~5), which were adequate for the chemical stability of IND and also in accordance with *USP–NF* specifications for IND oral suspension.[11,29] In addition, a non-significant change of IND concentration at different times of storage was observed. Furthermore, the chemical stability and no degradation of IND in the suspension were confirmed by HPLC analysis. The absence of degradation products of IND because of alkaline hydrolysis was confirmed by comparative analysis of the chromatograms of a sample of IND forced to be degraded under basic conditions and a sample of IND storage, since the retention times were 2.5 and 3.4, and 7.2, respectively. The chromatograms of the pure drug and the drug formulated in the suspension were comparable (TABLE 5) not only because of the similarity of retention times but also considering the peak areas of both samples. This is in agreement with the literature in which the chemical stability of IND as powder and formulated products, such as the pure drug and the suspension, respectively, has been reported.[16] In this sense, the drug could preserve its integrity at least 5 years at room temperature as powder or formulated in products with adequate pH value, as stated above. Besides, the IND formulations can also be stable after four months of storage at 50°C, which could be useful in some subtropical regions of Latin America.

It is important to stress that based on the results obtained by HPLC analysis and taking into consideration those obtained by UV-Vis spectrophotometry, the suitability of this last methodology to quantify the drug and to evaluate the chemical stability of IND suspension after 17 days of storage can be confirmed. This is highly important considering the well-known advantages of the method UV-Vis namely simplicity, low cost of implementation, and wide availability in laboratories for quality control.

On the other hand, the *in vitro* dissolution performance of IND from the freshly stored suspensions (i.e., 17 days of storage) exhibited rapid and complete dissolution of IND (FIGURE 4), which could ensure more uniform drug absorption through the gastrointestinal tract and could increase the bioavailability of IND, resulting in greater efficacy.

Finally, the microbial examination test of the suspensions after 17 days of storage at room temperature denoted minimal microbial growth for the total aerobic microbial and the total combined yeast/mold (TABLE 2), complying with official quality requirements encoded in the official compendium.[11,30]

In summary, the results of this work indicated that IND suspensions obtained by reformulation of commercially available ampules would ensure a correct and convenient IND dosage form, containing the drug dose in an appropriate volume of suspension (0.1 mL to 1.0 mL) to be administered to newborns and babies (0.1 mg/kg/dose to 0.25 mg/kg/dose).[31] Moreover, the presence of a sweetening agent in the formulation would improve pediatric formulation acceptance and treatment adherence (in cases that it is needed).

Conclusions

The pediatric oral liquid suspension containing 0.2% IND was easily prepared starting from commercially available lyophilized IND for injection. The formulation showed adequate chemical, physical, and microbiological stability for at least 17 days of storage and kept all quality attributes required during this time of storage. The developed formulation is an interesting alternative, in terms of efficacy, safety, and reliability, to be prepared in a hospital pharmaceutical service for optimizing the pediatric treatment of PDA.

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