Development and characterization of novel fast-dissolving pentobarbital suppositories for pediatric procedural sedation and comparison with lipophilic formulations

Aurelien Freisz, Imen Dhifallah, Yoann Le Basle, Mireille Jouannet, Philip Chennell, Ghislain Garrait, Eric Beyssac, Yassine Bouattour, Valérie Sautou

European Journal of Pharmaceutics and Biopharmaceutics 204 (2024) 114532

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Development and characterization of novel fast-dissolving pentobarbital suppositories for pediatric procedural sedation and comparison with lipophilic formulations

Aurelien Freisz [a], Imen Dhifallah [b], Yoann Le Basle [c], Mireille Jouannet [a], Philip Chennell [c], Ghislain Garrait [b], Eric Beyssac [b], Yassine Bouattour [c,*], Valérie Sautou [c]

[a] CHU Clermont-Ferrand, Pôle Pharmacie, F-63003 Clermont-Ferrand, France
[b] Université Clermont-Auvergne, UFR Pharmacie, UMR MEDIS, F-63001 Clermont-Ferrand, France
[c] Université Clermont Auvergne, CHU Clermont Ferrand, Clermont Auvergne INP, CNRS, ICCF, F-63000 Clermont-Ferrand, France

ARTICLE INFO

Keywords:
Pentobarbital
Suppositories
Formulation
Excipients
Biopharmaceutics
Pediatric
Characterization
Dissolution
Sedation

ABSTRACT

For pediatric radiological procedures (RP), pentobarbital sodium (PNa) can be used orally or rectally to replace intravenous anesthesia. Since no commercial PNa suppositories exist, they must be prepared by compounding pharmacies. This study aims to develop fast-dissolving PNa suppositories for fast pharmacological activity during RP. We prepared gelatin (G), gelatin/polyethylene glycol 4000 (GP), and polyethylene glycol 4000 (P) suppositories, with and without pH adjustment, and assessed their dosage uniformity (DU), softening time, rupture resistance, and *in-vitro* dissolution. An optimal formulation was selected, and PNa release was compared to that of fat-based suppositories using dissolution tests. Additionally, the quality control process (analytical performance, safety/eco-friendliness and productivity/practical effectiveness) of these formulas were compared using a RGB method. All hydrophilic formulas (HF) met the DU requirement (AV < 8 %) except for P (AV 15.62 ± 4 %). pH adjustment enhanced G and GP suppositories resistance to 2.2 ± 0.2 kg and 2.0 ± 0.3 kg, respectively, and allowed **100 % release** of PNa in under 10 min. In contrast, lipophilic formulas released less than 80 % of PNa at best after 120 min. These results show the biopharmaceutical suitability of HF for RP compared to lipophilic ones, but a pharmacokinetic study is needed to confirm data.

1. Introduction

Children undergoing radiological examinations such as computed tomography scans, magnetic resonance imaging (MRI), and X-rays exams require special medical attention, as the quality of the data collected during the examination relies heavily on the child's cooperation. In fact, the failure of a radiological examination can be caused by movement artifacts secondary to the patient's movements. In a prospective observational study, sedation failures were mainly found to be due to the child waking up while being transferred from the stretcher to the MRI table [1]. Alongside creating a suitable environment, pharmacological methods are sometimes a necessary part of the sedation procedure. Sedation aims to reduce anxiety and discomfort while maintaining essential reflexes for airway protection, ventilation, and cardio-respiratory stability [2]. For easier procedures, sedation can serve as an alternative to more extensive treatments like general anesthesia, which requires additional resources such as an operating room and a specialist practitioner, most often an anesthesiologist [3]. In 1985, the National Institute of Health (NIH) and the American Academy of Pediatrics (AAP) issued the first guidelines for procedural sedation and analgesia in response to several cases of sedation-related deaths [4]. Since then, various societies and organizations, including the AAP and the American Society of Anesthesiologists (ASA) have released updated guidelines to enhance the safety and effectiveness of sedation procedures [5,6]. In recent years, the updated guidelines from AAP and ASA have emphasized the importance of understanding each drug's pharmacokinetics, specifically the time of onset, peak effect, and duration of action, as these factors are critical in tailoring pediatric procedural sedation to minimize risks and optimize efficacy [7,8]. Current literature describes various pharmacological methods using different classes of drugs such as benzodiazepines (e.g., midazolam), opioids, barbiturates (e.g., thiopental, pentobarbital), etomidate, propofol, and

* Corresponding author at: CHU Clermont Ferrand, Pôle Pharmacie, 58 Rue Montalembert, F-63000 Clermont-Ferrand, France.
E-mail address: ybouattour@chu-clermontferrand.fr (Y. Bouattour).

https://doi.org/10.1016/j.ejpb.2024.114532
Received 30 June 2024; Received in revised form 24 September 2024; Accepted 9 October 2024
Available online 11 October 2024

dexmedetomidine [9]. Choosing needle-free pharmacological sedation techniques in pediatric with a rapid onset and short duration of action is crucial to ensure optimal sedation and to avoid serious side effects, as documented in the literature [10–12].

For ambulatory imaging procedures, oral or rectal administrations are therefore generally the preferred route, avoiding the need for more logistically challenging and invasive routes such as intravenous administrations [13]. Until 2001, oral chloral hydrate was commonly used for pediatric sedation procedures and provided, according to AAP guidelines, what was considered at the time as a safe and effective sedation method for children [14]. However, ulterior studies showed that it has an unpredictable onset, long duration and high sedation failure [12,15]. Moreover, some clinical scenarios, such as palatability issues, medication noncompliance, nausea or vomiting, may preclude oral administrations, making the rectal route an interesting alternative for the delivery of drugs aiming for local or systemic effects. The rectal environment is characterized by its stability, with low enzymatic activity and reduced hepatic first-pass effect, and can overcome most of the issues associated with the oral route [16,17]. Advancements in rectal formulations to improve bioavailability and drug release kinetics have expanded the use of various drugs for sedation in medical imaging procedures, including midazolam, chloral hydrate, diazepam, or pentobarbital [16,18].

Pentobarbital acts as an allosteric modulator of gamma-aminobutyric acid (GABA) receptors. Its binding enhances the effect of GABA-A on its receptor, resulting in an increase in the opening amplitude of chloride channels. Additionally, it has been observed that barbiturates like pentobarbital block α-amino-3-hydroxy-5-methylisoxazol-4-propionate (AMPA) and kainate receptors, which are types of glutamate receptors [19]. These pharmacological properties mean it can be used as a depressant of the central nervous system, particularly during pediatric sedation procedures. The pediatric dosage for oral or rectal administration of pentobarbital typically ranges from 3 to 6 mg/kg, with a maximum dose of 100 mg per administration for patients under 4 years old, and 1.5 to 3 mg/kg with the same maximum dose for those over 4 years old [18]. However, the drug has an extremely unpleasant taste requiring cooperation during oral administration and can sometimes cause refusal of the drug by children [20,21]. Since pentobarbital is not currently marketed in any form allowing a rectal administration, compounding pharmacies have been developing new formulations, which need to ensure rapid release with minimal variability. In 1978, Doluisio et al [22] presented two suppository formulation of pentobarbital, one with cocoa butter base and the other with a synthetic base of undisclosed nature. They found that the serum peak concentration of pentobarbital in six subjects was reached in 4 h with the unidentified base versus 9 h for the cocoa butter base suppository, which could impact the drug onset [23]. The peak concentration differences were explained by the release kinetic found using *in-vitro* dissolution for both formulas (80 % of pentobarbital released in 30 min for the unidentified base versus 50.8 % in 90 min for the cocoa butter base) highlighting the importance of biopharmaceutical tests to predict formulation impact on pharmacological activity. Recently, a rectal hydrogel formulation of pentobarbital sodium [24] has been developed at a concentration of 25 mg/ml. However, besides the fact that this formulation contains sodium benzoate, a well-studied excipient with a notorious effect that can cause mucosal irritation [25], this formula was not subjected to a release kinetic study. More recently, two lipophilic formulations of pentobarbital in Witepsol® based suppositories have been proposed [26]. One of the major difficulties of these lipophilic matrix suppositories lies in ensuring accurate and consistent dosing, as lipophilic bases can lead to uneven drug distribution within the active substance – excipient mix, which can result in individual suppository dosing variability [27]. This is especially critical in pediatric applications, where even slight deviations in dose can significantly impact therapeutic outcomes and safety. Furthermore, the control process of those two formulations requires a solid–liquid extraction step using octanol in order to allow pentobarbital quantification, which complexifies the task for pharmacists and adds additional costs that could be difficult to bear, especially in low to middle income

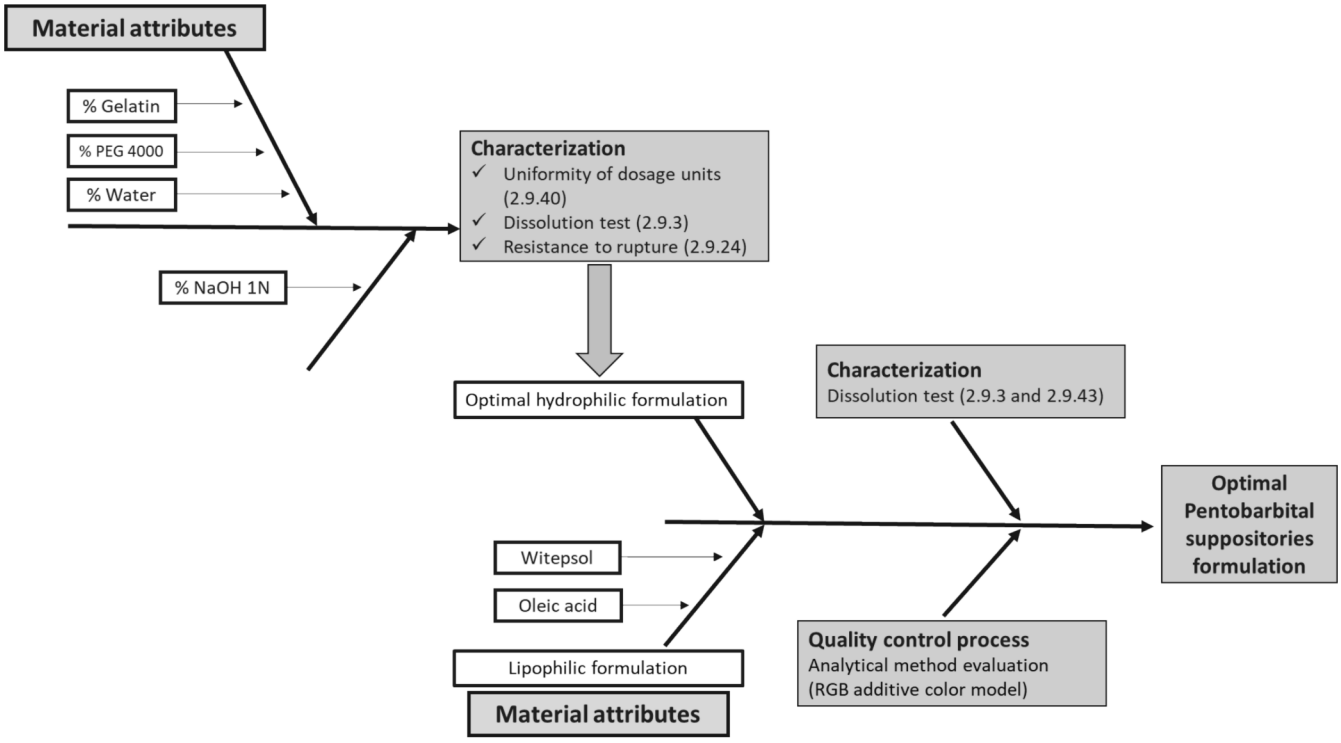


Fig. 1. Ishikawa diagram for the optimization of hydrophilic formulation. The upper segment corresponds to the optimization of the suppository formulation having a hydrophilic base consisting of a gelatin and gelatin-PEG 4000 with or without NaOH. The adequate hydrophilic base is then compared with the lipophilic base pentobarbital sodium formulation.

countries, as well as having an environmental impact. Besides, no dissolution studies were conducted on those formulations either. A fast-dissolving formulation ensures that the drug is quickly absorbed, allowing for timely sedation and reducing procedural delays [16,28]. This rapid drug release also ensures consistent bioavailability, leading to predictable sedation outcomes, which are essential in the controlled environment of diagnostic imaging procedures. The aim of this study was therefore to develop a novel and innovating formulation of hydrophilic sodium pentobarbital (PNa) suppositories which would:

- be easy to prepare.
- possess a control process of limited complexity.
- allow a fast release of the active substance and thus a rapid onset.

Additionally, the developed formulation would be compared with existing lipophilic formulations to assess their pharmacotechnical benefits

2. Materiel and methods

2.1. Chemicals

Pharmaceutical grade pentobarbital sodium (PNa) powder (ref: 57330–100), bovine gelatin 160 bloom (ref: 90007–250), oleic acid (ref: 11280–250) and sodium hydroxide (NaOH) (ref: 13107–100) were supplied by Inresa (Bartenheim, France). Witepsol W25 (ref: 1866400) was supplied by Cooper (Melun, France) and polyethylene glycol 4000 (PEG) (ref: 9714787) by Fagron (Colombes, France). Deionised water was purchased from Fresenius (Sèvres, France). **High performance Liquid Chromatography** (HPLC) grade methanol was obtained from Carlo Erba reagents (ref: 20864.320, Val de Reuil, France). Monopotassium dihydrogen phosphate (KH_2PO_4) (CAS 7778–77-0) was purchased from Sigma-Aldrich (St. Louis, 96 USA). Hydrochloric acid (HCl) (CAS 7647–01-0) and 1-octanol (ref: 112615–2.5L) were purchased from Honeywell through a local supplier (MC2, Clermont-Ferrand, France).

2.2. Study design

To establish hydrophilic formulations of PNa suppositories, instant PNa solubility in aqueous solution was firstly determined by saturating water with the compound, followed by centrifugation and quantification of PNa in the supernatant solution using a HPLC method. After that, gelatin-, gelatin/PEG4000 with or without NaOH 1 N (for pH adjustment), and PEG4000-based hydrophilic formulations were then prepared and characterized in order to determine the optimal formulation based on the uniformity of dosage units' test (2.9.40 monograph of the European Pharmacopoeia (EP)), dissolution test (2.9.3 monograph of the EP) and resistance to rupture test (2.9.24 monograph of the EP, 6th edition. It must be noted that this monograph was discontinued in later EP editions, but provides an assay framework that will be discussed further on) [29–31]. The dissolution kinetics of the optimal formulation (i.e. having the best homogeneity, fastest dissolution rate and highest resistance to rupture) was then compared with other developed PNa suppositories: those reported by Doluisio et al [22] (retrieved using Data Thief 3.0 software http://datathief.org/) where the release kinetic of PNa have already been described, and those developed by Lebrat et al [26] for which the release kinetic had not yet been studied. The release data from the *in-vitro* release study were analyzed using various kinetic models, including zero-order, first-order, Higuchi, and the Korsmeyer-Peppas models [32,33]**.** The quality control process of these suppositories (hydrophilic and fat-based) was also compared to establish the optimal formulation of pentobarbital suppositories. Fig. 1 shows the different stages of the study design.

Table 1
gradient used for the liquid chromatography mobile phase.

Time (minutes)	Mobile phase (in percentage)	
	A	**B**
0	40	60
12.5	40	60
15	5	95
16	5	95
16.5	40	60
20	40	60

2.3. Quantification method and pentobarbital sodium instant solubility determination

PNa quantification was carried out using a HPLC system (Prominence-I LC-2030C 3D (Shimadzu France 220 SAS, Marne La Vallée, France)) with a validated stability-indicating method allowing quantification from 60 to 180 µg/mL [34]. In brief, the stationary phase that was used was an EC 250/4.6 Nucleodur C18 HTec column (250 × 4.6 mm, 5 µm particle size; Macherey-Nagel, Düren, Germany) and the mobile phase was composed of 0.01 M phosphate buffer pH 3 adjusted with HCl (Phase A) and methanol (Phase B), 40:60, v/v. Table 1 describes the gradient method at a flow rate of 1.2 mL/min; injection volume was 15 µL. The column oven and rack temperature were set at 40 °C. Pentobarbital quantification was performed at 214 nm.

The calibration was validated following the Q2R1 guidelines of the international conference of harmonization [35] by the preparation of 3 calibration curves and 6 control points each day for three days. The matrix effect was evaluated by comparing three calibration curves (slopes and intercepts) obtained from sodium pentobarbital only (pharmaceutical quality) with those containing sodium pentobarbital in the presence of all the excipients. This method was then used to determine the instant solubility of PNa. First, a quantity of 250 mg of PNa was introduced into 1 mL of water in a test tube, then automatically stirred until completely solubilized. Additional quantities of 10 mg were then added successively with stirring until visual precipitation. Centrifugation was carried out (Universal 320, Hettichlab, France) and the supernatant was analyzed after dilution using the HPLC method. The test was performed 3 times in order to express the result as a mean instant solubility (in mg/ml).

2.4. Suppositories compositions and preparations

2.4.1. Compositions

Seven hydrophilic and two lipophilic suppositories formulations were prepared containing 60 mg of sodium pentobarbital (corresponding to 55 mg of pentobarbital base) following the composition mentioned in Table 2. Sodium hydroxide (in the form of a 1 N solution) was added to both gelatin and gelatin/PEG formulations to reach a measured pH between 9 and 10 in order to evaluate its impact on the suppository characteristics, since this pH range is compatible for rectal formulations [24,36].

2.4.2. Preparation

The suppositories were prepared following the steps described below (see Table 2 for quantities):

Gelatin and Gelatin/PEG-based suppositories without pH adjustment (Formulations G1, G2, GP1, and GP2) (Fig. 2A)

1- PNa was weighed and dissolved in the total volume of water.
2- When used, PEG 4000 was added directly to the PNa solution for the GP formulations. The mixture was stirred until completely dissolved.
3- Gelatin was added directly to the PNa solution and stirred.

Table 2
Composition of the hydrophilic (G: gelatin-base, GP: gelatin/PEG-base and P: PEG4000-base) and lipophilic formulations (F1 and F2) of the studied suppositories. Quantities shown correspond to the formulation of one unit of sodium pentobarbital suppository. % in w/w.

Components	G1	G2	G3	GP1	GP2	GP3	P	F1	F2
Gelatin (mg) *(% excipient base)*	300 *(25 %)*	240 *(20 %)*	300 *25 %*	300 *(25 %)*	240 *(20 %)*	300 *(25 %)*	–	–	–
Water (ml) *(% excipient base)*	0.900 *(75 %)*	0.960 *(80 %)*	0.850 *(71 %)*	0.852 *(71 %)*	0.912 *(76 %)*	0.802 *(67 %)*	0.089 *(7 %)*	–	–
PEG 4 000 (mg) *(% excipient base)*	–	–	–	48 *(4 %)*	48 *(4 %)*	48 *(4 %)*	1170 *(93 %)*	–	–
NaOH 1 N (ml) *(% excipient base)*	–	–	0.05 *(4 %)*	–	–	0.05 *(4 %)*	–	–	–
Witepsol W25 (mg) *(% excipient base)*	–	–	–	–	–	–	–	1040 *(100 %)*	775 *(71.8 %)*
Oleic acid (mg) *(% excipient base)*	–	–	–	–	–	–	–	–	304 *(28.2 %)*
Pentobarbital sodium (mg)	60	60	60	60	60	60	60	60	60

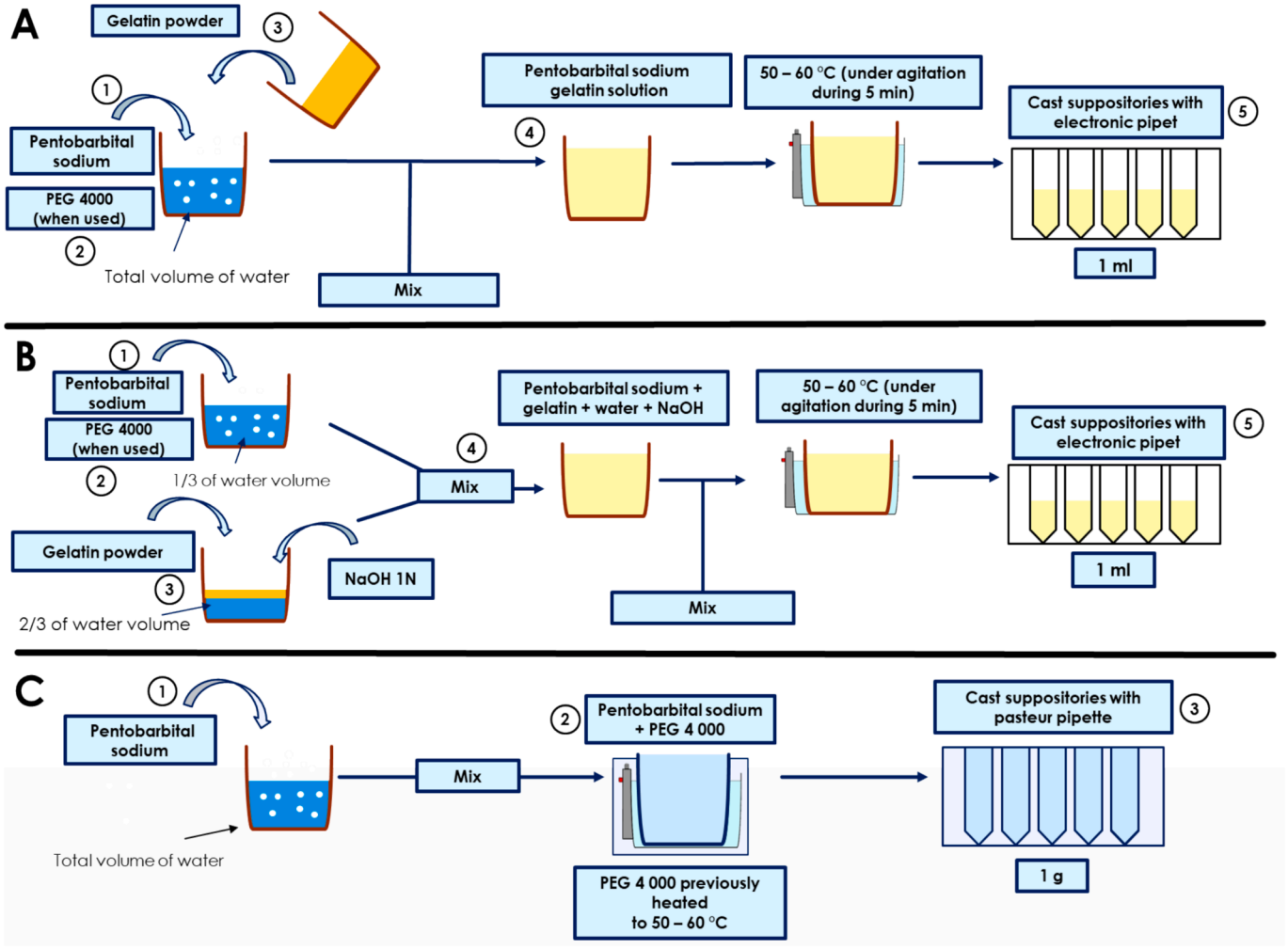


Fig. 2. Preparation steps for gelatin and gelatin/peg-based suppositories without (a), with (b) naoh solution as well as peg4000-based suppositories (c) (colored figure).

4- The solution was premixed at room temperature with a spatula to disperse any clumps, then stirred in a water bath at 50–60 °C for 5 min to obtain a homogenous mixture.
5- The mixture was distributed into 1 mL plastic molds of 2 g capacity (LGA S.A.S, La-Seyne-Sur-Mer, France) using a 5 mL electronic pipette (Eppendorf™ Xplorer™, l111530D). The molds were left to set at 5 ± 3°C for at least 24 h before characterization.

Gelatin and Gelatin/PEG-based suppositories with pH adjustment (Formulations G3 and GP3) (Fig. 2B)

1- PNa was dissolved in 1/3 of the total volume of water. The remaining 2/3 of water was reserved for gelatin solubilization.
2- When used, PEG 4000 was added directly to the PNa solution for the GP formulations. The mixture was stirred until completely dissolved.
3- Gelatin was first solubilized in the reserved 2/3 water, alkalinized with NaOH 1 N, then combined with the PNa solution. The pH of the final solutions was checked using a SevenMulti™ pH-meter with an InLabTM Micro Pro glass electrode (Mettler-Toledo, Viroflay, France).

4- The solution was premixed at room temperature with a spatula to disperse any clumps, then stirred in a water bath at 50–60 °C for 5 min to obtain a homogenous mixture.
5- The mixture was distributed into 1 mL plastic molds of 2 g capacity (LGA S.A.S, La-Seyne-Sur-Mer, France) using a 5 mL electronic pipette (Eppendorf™ Xplorer™, l11530D). The molds were left to set at 5 ± 3°C for at least 24 h before characterization.

Peg-only formulation (Fig. 2C)

1- PNa was dissolved in the total water volume.
2- PEG 4000 was weighed and melted at 55 °C in a stainless-steel container until a clear solution formed, to which added the PNa solution.
3- The mixture was stirred and distributed into 1 g plastic molds (LGA S.A.S, La-Seyne-Sur-Mer, France) using a Pasteur pipette, ensuring the molds were fully filled (Fig. 2C).

Lipophilic Formulations: The preparation followed previously published methods [26].

After preparation, all of the formulations were stored at 5 ± 3 °C for a minimum of 24 h before characterization.

2.5. Characterization

All the characterization procedures were realized on 3 units coming from 3 different batches per formulation, except for the uniformity of dosage units where 10 units per batch were analyzed. The results were expressed as mean ± standard deviation (SD).

2.5.1. Visual inspection

All the suppositories were unpacked from their molds and visually inspected in daylight to detect any change in appearance or visible inhomogeneity. Three units of each batch were inspected on days 1, 14 and 30 after refrigerated storage.

2.5.2. Quantification and uniformity of dosage units

The uniformity of dosage units assay was carried out in accordance with monograph 2.9.40 of the EP [31]. PNa quantification of the hydrophilic formulations was performed using the method described in paragraph 2.3. Sample preparation was performed as follows. Ten units from each batch were melted individually in 20 mL of water at 80 °C, then the solution was diluted to obtain the target concentration of 120 µg/mL and injected into the HPLC system. For the lipophilic formulations, each suppository was melted using a water bath at 50 °C. Then, 10 mL of 1-octanol was added to the Erlenmeyer flask and ultrasonicated for 15 min. A total of 20 mL of 0.1 N NaOH solution was then added, and the mixture was homogenized before transfer into a separating funnel, previously rinsed with the 0.1 N NaOH solution. The separating funnels were inverted 20 times and left to decant at room temperature. After 24 h, the lower aqueous phase was withdrawn for HPLC analysis after performing appropriate dilution in deionized water to obtain the target concentration of 150 µg/mL and injected into the HPLC system. The reference value corresponds to the acceptance value (AV), which must be lower than 15 % to establish a compliant dosage.

2.5.3. Resistance to rupture

This test was realized using an SBT apparatus (Erweka, Germany) at room temperature (22 ± 0.5 °C) following the recommendations of monograph 2.9.24 of the EP (6th edition) [25]. In order to determine the mass at which the suppository breaks or crushes at room temperature, an initial load of 600 g was applied to each suppository, and a load of 200 g was added to the rod attached to the upper jaw every minute on top of the initial load until the suppository broke or tore.

Table 3
thresholds for the RGB additive color method analysis (LAV: least acceptable value; LSV: least satisfactory value).

Color	Criterion	LAV	LSV
Red	Accuracy %	90 – 110 %	95 – 105 %
	Precision (RSD %)	10 %	5 %
Green	Analytical GREennEss Calculator score	0.33	0.66
	Liquids chemical consumption	648 mL / 10 units	324 mL / 10 units
	Chemical Hazard	5 hazards pictograms in total	3 hazards pictograms in total
Blue	Cost-effectiveness	22€ / 10 units	11€ / 10 units
	Human Time-effectiveness	2 h / 10 units	1 h / 10 units
	Process Time-effectiveness	28 h / 10 units	14 h / 10 units

2.5.4. Softening time

The softening time was measured according to monograph 2.9.22 of the EP using apparatus A [37], at 37 °C. Each suppository was inserted tip-first into a glass tube containing 10 mL of water, which was placed in a water bath maintained at 36.5 ± 0.5 °C. Immediately following the insertion of the suppository, a rod was introduced into the tube. The tube was then covered, marking the beginning of the timing process. The time recorded was the duration required for the rod to descend to the bottom of the glass tube and for the marked ring to align with the top of the plastic cover.

2.5.5. Dissolution

For the hydrophilic formulations, the USP basket method was employed for all the *in-vitro* dissolutions (USP 1, Sotax, ATS Xtend™) following the 2.9.3 EP monograph [29]. A volume of 500 ml of pH 6.8 USP buffer was used as the dissolution media at 37 °C and the stirring rate was of 50 rpm. The dissolution of the lipophilic suppositories were investigated using an automated flow-through cell dissolution apparatus (USP 4, Sotax, Aesch, Switzerland) as recommended by the 2.9.42 EP monograph [38], using 500 mL of pH 6.8 USP buffer at 37 °C in a closed loop setting with a flow rate set at 20 mL min^{-1}. At appropriate intervals, 5 ml of each sample was taken and PNa was quantified. The dissolution test results were analyzed according to the 2.9.3 and 2.9.42 monograph for immediate liberation forms, with a final concentration equal or higher than 100 % of the dosage.

2.5.6. Analytical process comparison between hydrophilic and lipophilic formulations

In order to compare the quantification process between lipophilic and hydrophilic formulations in an overall and non-subjective way, a RGB additive color evaluation was performed as described by Nowak and Kościelniak [39]. The red part evaluates the analytical performance of the methods used through the accuracy and precision values, as recommended by the ICH Q2R1. The green part focuses on safety and eco-friendliness. For that part, we used the AGREE calculator published by Pena-Pereira *et al* [40], along with the consumption (volume) of the liquid chemicals and chemical hazards. The blue section evaluates productivity and practical effectiveness through 3 items: cost-effectiveness, human time and process effectiveness (detailed in Table S1 in Supplementary data). For comparison purposes, the results obtained from the analytical control process of lipophilic suppositories were used as least acceptable values (LAV) and the least satisfactory value LSV corresponds to the double or half (depending on the parameter) of the LAV score (Table 3).

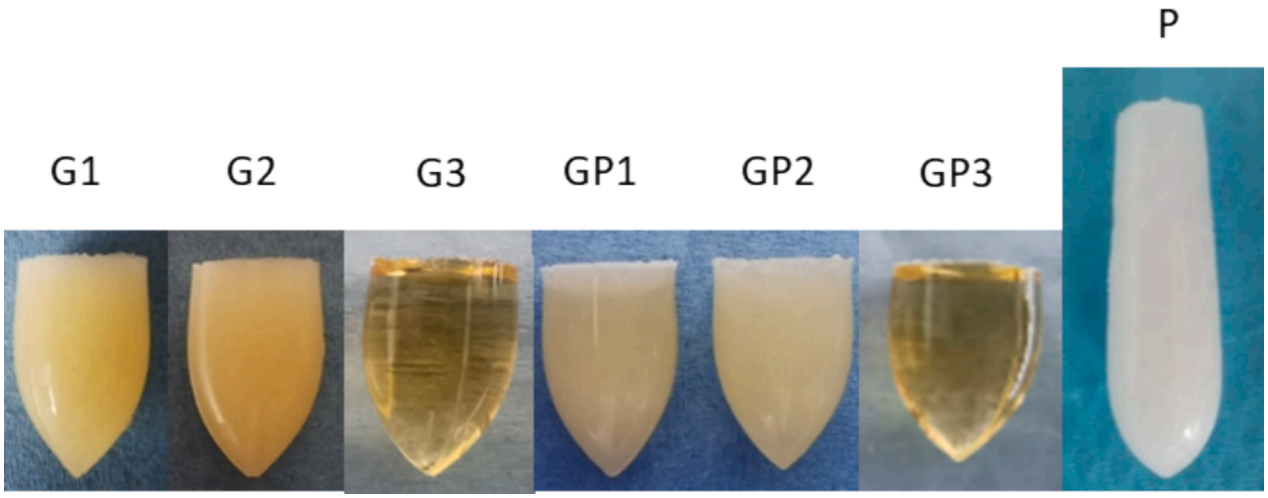


Fig. 3. Visual aspect of hydrophilic pentobarbital sodium suppositories one day after preparation. G: gelatin-based suppositories, GP: gelatin/PEG4000-based suppositories and P: PEG4000-based suppository (colored figure).

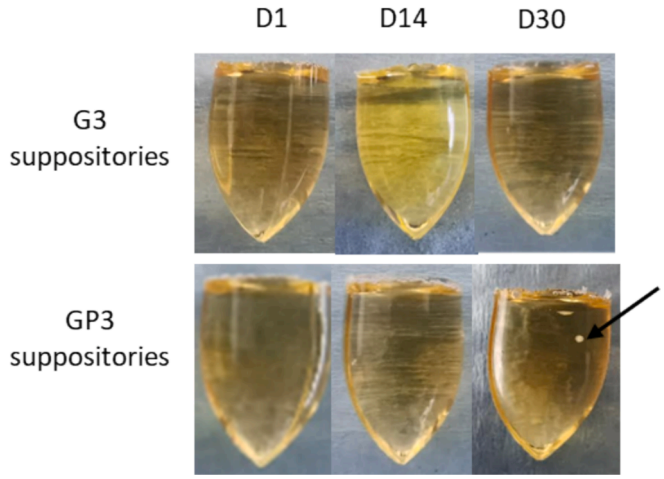


Fig. 4. Appearance of the Gelatin (G3) and Gelatin/PEG (GP3) hydrophilic formulations containing NaOH after 1, 14 and 30 days of storage (respectively D1, D14 and D30); the arrow indicates the presence of a precipitate (colored figure).

3. Results and discussion

3.1. Pentobarbital sodium quantification and instant solubility determination

The chromatographic method used was found to be linear for concentrations ranging from 60 to 180 µg/mL. The average weighted regression equation was $Y = 21336X + 11901$, where X is the pentobarbital concentration after dilution (in µg/mL), and Y is the surface area of the corresponding chromatographic peak. The average determination coefficient R^2 of the three calibration curves was 0.999. No matrix effect was detected, as the sodium pentobarbital retention time, the slope, and the intercept of the calibration curve did not change significantly with the matrix. The relative mean trueness bias coefficients were less than 1 %. The mean repeatability RSD coefficient was 1.50 %. Using this method of quantification, the instant solubility of PNa was determined at 450 mg/ml, which is more than a thousand times higher than the solubility of the un-ionized molecular form of pentobarbital, reported at 679 µg/mL [41].

Table 4
Uniformity of content of hydrophilic formulations (G1, G2, G3, GP1, GP2, GP3 and P). Values are expressed as mean ± standard deviation (SD), with n = 30 for dosage and n = 3 for acceptance value. AV = acceptance values.

	G1	G2	G3	GP1	GP2	GP3	P
Average dosage (mg) ± SD	59.61 ± 1.85	57.51 ± 0.97	57.31 ± 1.62	58.55 ± 1.53	57.48 ± 1.72	61.92 ± 1.31	59.83 ± 3.93
Mean AV (%) ± SD	5.75 ± 0.4	6.5 ± 1.0	6.0 ± 1.1	6.4 ± 1.2	7.9 ± 3.9	6.4 ± 2.4	15.62 ± 4

3.2. Characterization of the hydrophilic formulations

3.2.1. Visual inspection

The Gelatin and Gelatin/PEG formulations without NaOH (G1, G2, GP1 and GP2) presented a creamy, cloudy aspect one day (D1) after preparation, compared to the formulations containing NaOH (G3 and GP3) which remained translucent (Fig. 3). This aspect was not noticed during the actual preparation process, as all solutions presented a translucent aspect, but was only discovered after unwrapping the suppositories at D1. This is very probably due to the precipitation of PNa in the gelatin and gelatin-PEG solution since the pH of these solutions (before solidification during the cooling step) was found to be of 5.4 which is lower than the pKa of pentobarbital reported at 8.11, thus resulting in PNa reverting to its molecular low water-soluble form [41]. This phenomenon was possibly accentuated by the storage at refrigerated temperature [42]. The use of NaOH in gelatin and gelatin/PEG formulas allowed those solutions to reach a pH between 9 and 10, which is higher than the pKa of pentobarbital, allowing PNa to remain in its ionized and highly soluble form. The result was the production of a clear solution and translucent suppositories, with no visible precipitates in the formulations during 30 days of storage at 5 ± 3°C except for one unit of GP3 which showed the first signs of a precipitation at D30, (Fig. 4), possibly due to the presence of multiple acid functions in PEG4000 acidifying the media and the lower water proportion. It should be noted that instant precipitation occurred during the preparation of PEG-based suppositories (P), when the pentobarbital solutions were mixed with PEG4000 base before casting, resulting in white opaque suppositories.

3.2.2. Uniformity of dosage units

Content uniformity results for the hydrophilic formulations are presented in Table 4. All gelatin and gelatin/PEG formulations presented an AV which was lower than 8 %. This could be explained by the high

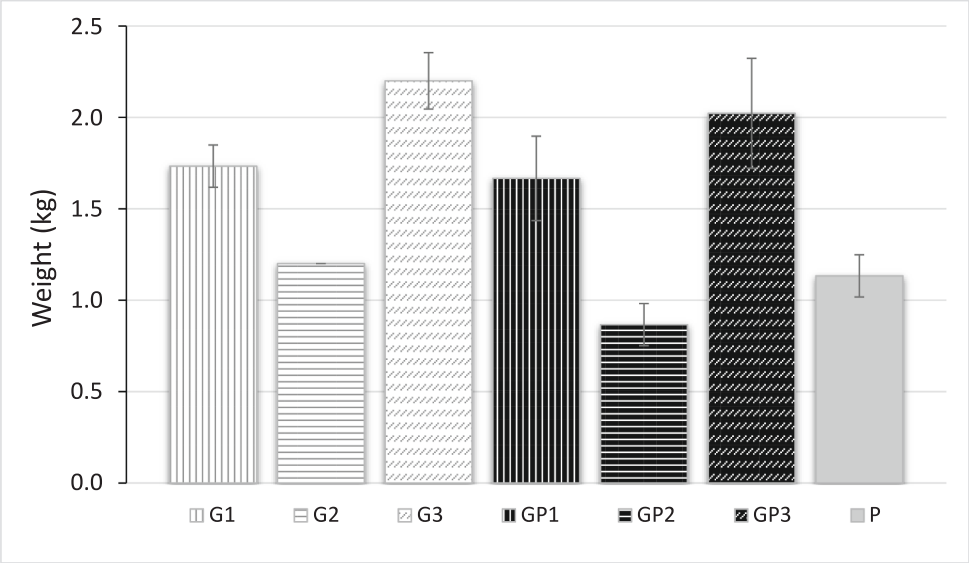


Fig. 5. Resistance to rupture of Gelatin (G1, G2, G3), Gelatin/PEG (GP1, GP2, GP3) and PEG4000-base (P) hydrophilic formulation. n = 3. Values are expressed as mean ± standard deviation (SD).

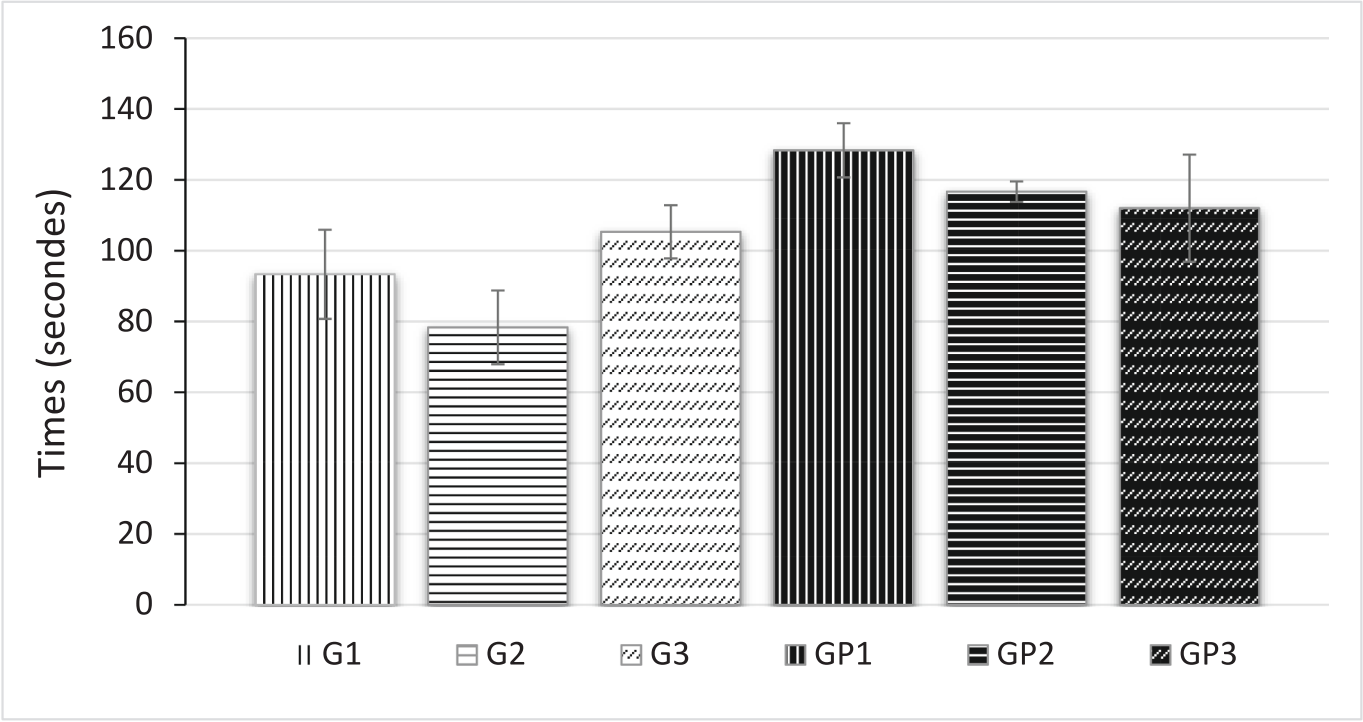


Fig. 6. Softening time of Gelatin (G1, G2, G3) and Gelatin/PEG (GP1, GP2, GP3) hydrophilic formulation. n = 3. Values are expressed as mean ± standard deviation (SD).

solubility of PNa in gelatin and gelatin/PEG solutions, therefore allowing a good homogeneity of the produced units. However, the high AV value found for PEG-only formula (higher than 15 %) is probably due to the precipitation of PNa during preparation in presence of PEG-4000 (theoretical concentration of PNa in water for this formula (660 mg/mL) exceeding the solubility threshold of 450 mg/mL), which decreased the homogeneity of the units during preparation and casting.

3.2.3. *Resistance to rupture and softening time*

The resistance to rupture of the suppositories was assessed in order to evaluate their fragility or brittleness, by determining their ability to withstand the stresses encountered during packaging, transportation, and routine handling, including rectal administration. The results of this assay are presented Fig. 5. The hardness of the hydrophilic formulations correlates well with the proportion of gelatin present in the formulation. In fact, formulations with a higher gelatin proportion had a higher breaking strength which would be explained by its elastic properties [43]. On the other hand, it can be noticed for both gelatin and gelatin/PEG formulation that the addition of NaOH (G3 and GP3) increased the resistance to rupture even further. This interesting observation would be due to the increased gel strength, such as described in other studies showing that alkalized gelatin (pH 9) had the highest gel strength and

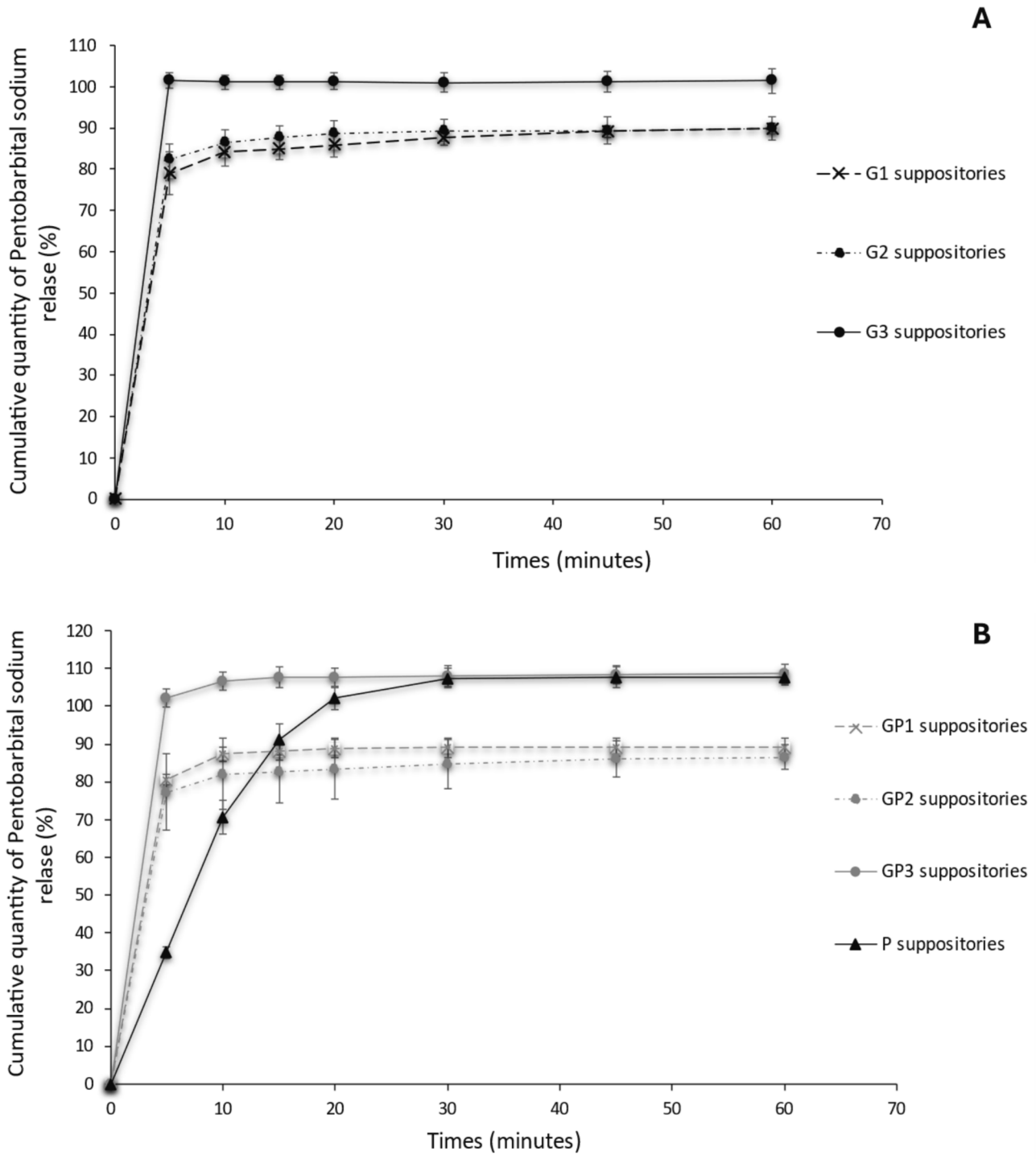


Fig. 7. Dissolution kinetics with basket apparatus representing the quantity of active ingredient (in %) released over time as a function of the different formulations in A: Gelatin (G1, G2, G3) and B: Gelatine/PEG (GP1, GP2, GP3) and PEG4000-base (P). Results are expressed as mean ± standard deviation (SD), n = 3.

exhibited a finer and more compact network structure with smaller pores, while acid addition weakened the gel strengths of the gelatin [44–46]. The PEG addition to the gelatin solution decreased the resistance to rupture of the suppositories, which is due to the low quantity of PEG4000 used in gelatin/PEG formulations. Nevertheless, softening times for almost all of the hydrophilic formulations were found to be of less than 2 min (Fig. 6), except for GP1 formulation. The results of the softening time of the P formulation is not shown as the average softening time was higher than 15 min, which coherent with the high melting point (reported as being higher than 50 °C) of PEG4000 [47].

3.2.4. *Dissolution tests*

The quantity of PNa released during the dissolution tests was above 80 % of the nominal quantity at 10 min for G1, G2, GP1 and GP2

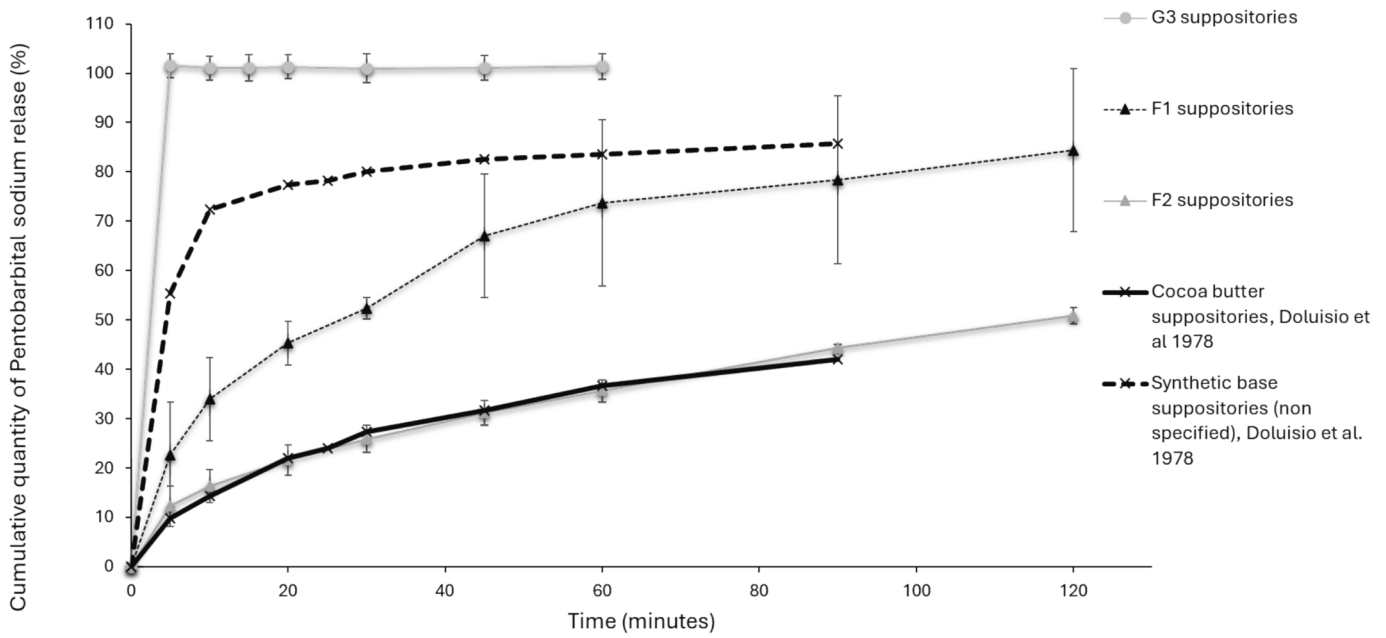


Fig. 8. Dissolution kinetics representing the cumulative quantity of active ingredient (in %) released over time and comparison between formulation gelatin suppositories (G3), lipophilic formulation (F1 and F2) and formulation reported by Doluisio et al. [22]. Results are expressed as mean ± standard deviation (SD), n = 3.

formulations (Fig. 7), however, the cumulative released amount of PNa amount didn't reach 100 % for these formulas devoid of NaOH. Since the pH of the gelatin solutions was acid, it led to the precipitation of pentobarbital by transforming its ionized salt form into its molecular unionized form. The precipitated pentobarbital was not able to dissolve into the dissolution media, which had a pH lower than the pka of pentobarbital (6.8 versus 8.1). Only formulas containing NaOH (G3 and GP3) released an amount of 100 % of PNa at 5 min, which can be also linked to the fast dissolution of gelatin as described in the softening time test results. Interestingly, the PEG4000 suppositories (P) showed a kinetic release of PNa reaching amounts of 100 % (Fig. 7-B). The release kinetic of PNa presented a less steep curve which reached 100 % of 20 min, This information, combined to the softening time results, could point to the main release mechanism being bulk diffusion and not erosion limited [48]. However, the interpretation of the kinetic release could be misleading due to the non-compliance of the produced PEG4000 batches with the uniformity of dosage units as they could contain amounts differing from the nominal quantity of 60 mg of PNa.

In view of the obtained results of the hydrophilic suppositories characterization, the optimal formulation appears to be G3 since it has the fastest PNa release, the highest resistance to rupture and a good homogeneity. Indeed, this formulation meets the European Pharmacopoeia recommendations for each test performed, with the absence of precipitation on visual examination over 30 days. This formulation was therefore chosen for comparison with lipophilic formulations, both in terms of dissolution kinetics and quantification process.

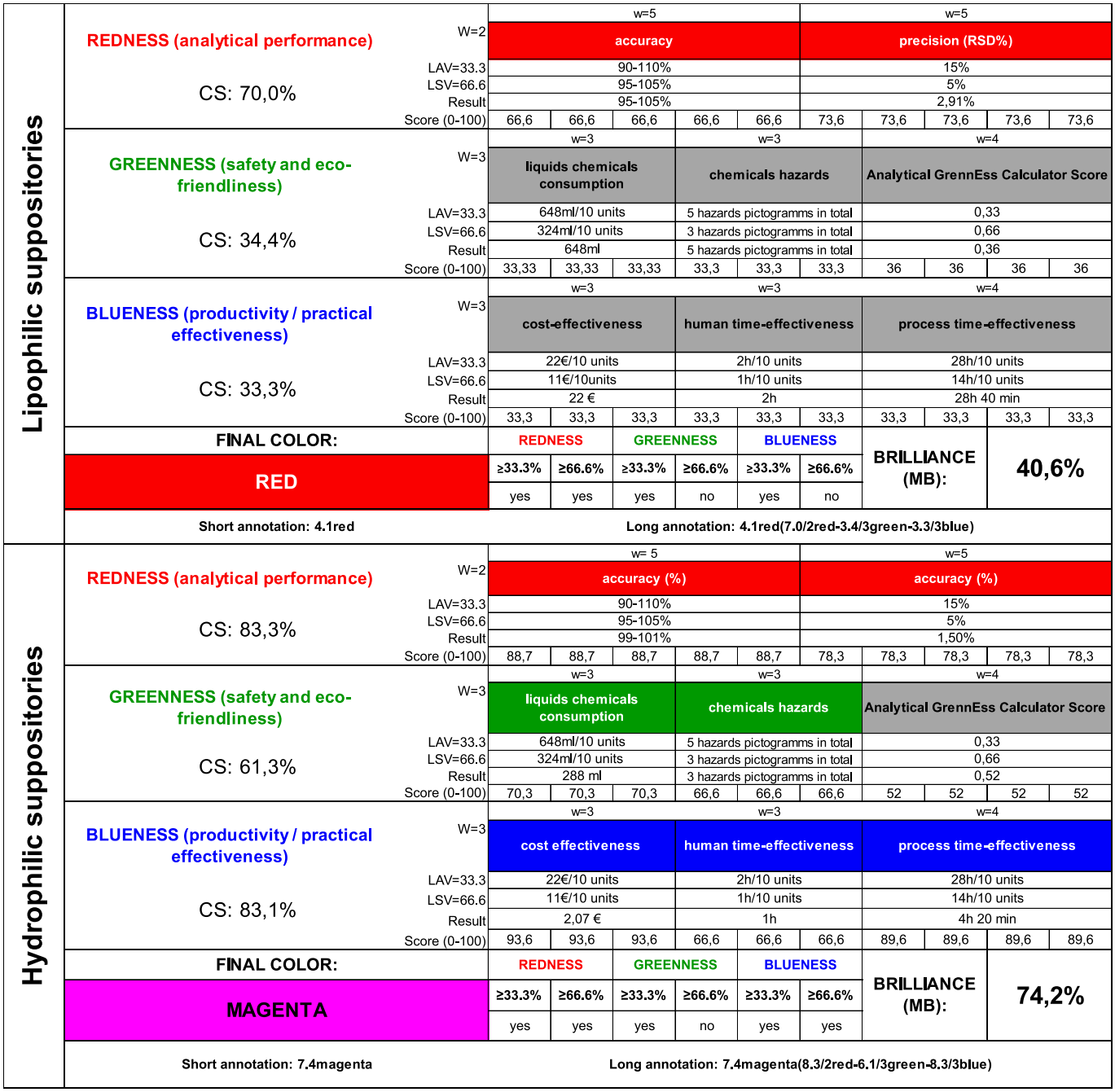
3.3. Comparison with lipophilic formulations

3.3.1. Dissolution tests

The kinetics of the release of PNa from the F1 formulas showed that 80 % of PNa was reached only after 90 min and that the 100 % release target was still not reached after 120 min of dissolution (Fig. 8). For the F2 formulations, the amount of pentobarbital released did not exceed 50.8 % after 120 min of dissolution. This is likely due to the transformation of PNa to its molecular unionized state by protonation with the oleic acid in F2 formula [26], which increased the affinity of pentobarbital for the glycerol esters of the fat-base [49], leading to a diffusion-controlled release of the drug (based on the Higuchi release model) [50] (Table 5). In fact, we found a highly similar kinetic release in cocoa butter-based formula reported by Doluisio et al [22], a base which also contains about 33 % of oleic acid [51]. They found that a peak concentration in serum was reached after 9 to 10 h in three subjects receiving this formulation. The other formula of PNa was in a synthetic media of undisclosed nature provided by Abbott®, but which is likely to be a hydrophilic formulation (based on the Korsmeyer-Peppas model implying a diffusion type release [52]). In this formula, the PNa amount didn't reach 100 % release either, however the time to reach a peak concentration in serum was found to be must shorter (4 h). As the pharmacological activity of PNa must take place quickly (so as for example to reduce waiting time), a fast release is therefore desired, especially for children. The enhanced release of pentobarbital from hydrophilic suppository bases, in comparison to lipophilic ones, might be attributed to the increased diffusibility of pentobarbital and its subsequent solubility in the dissolution medium. This phenomenon may be due to the low aqueous solubility of molecular pentobarbital, which results in a higher affinity of the drug for lipophilic bases over hydrophilic ones. Consequently, pentobarbital would tend to remain within the lipophilic matrix for a prolonged period compared to hydrophilic bases. Furthermore, the elevated release of pentobarbital from

Table 5
Release kintetic of hydrophilc (G3) and lipophilic (F1 and F2) formulations, Q: fraction of drug released per minute, t: time in minute.

Formulation	Release kinetic fit model	Release kinetic equations	Correlation coefficient (R^2)
G3	**Zero-Order Kinetics**	$Q = 20.304 \times t$	**0.9996**
F1	**First-Order Kinetics**	$1 - Q = e^{-0.0148 \times t}$	**0.9427**
F2	**Higuchi**	$Q = 4.6799 \times \sqrt{t}$	**0.9992**
Cocoa butter base (Doluisio et al. 1978)	**Higuchi**	$Q = 4.6657 \times \sqrt{t}$	**0.9983**
Unknown synthetic base (Doluisio et al. 1978)	**Korsmeyer-Peppas**	$Q = 0.515 \times t^{0.121}$	**0.9031**



Lipophilic suppositories											
REDNESS (analytical performance)	W=2	w=5					w=5				
		accuracy					precision (RSD%)				
CS: 70,0%	LAV=33.3	90-110%					15%				
	LSV=66.6	95-105%					5%				
	Result	95-105%					2,91%				
	Score (0-100)	66,6	66,6	66,6	66,6	66,6	73,6	73,6	73,6	73,6	73,6
GREENNESS (safety and eco-friendliness)	W=3	w=3			w=3		w=4				
		liquids chemicals consumption			chemicals hazards		Analytical GrennEss Calculator Score				
CS: 34,4%	LAV=33.3	648ml/10 units			5 hazards pictogramms in total		0,33				
	LSV=66.6	324ml/10 units			3 hazards pictogramms in total		0,66				
	Result	648ml			5 hazards pictogramms in total		0,36				
	Score (0-100)	33,33	33,33	33,33	33,3	33,3	33,3	36	36	36	36
BLUENESS (productivity / practical effectiveness)	W=3	w=3			w=3		w=4				
		cost-effectiveness			human time-effectiveness		process time-effectiveness				
CS: 33,3%	LAV=33.3	22€/10 units			2h/10 units		28h/10 units				
	LSV=66.6	11€/10units			1h/10 units		14h/10 units				
	Result	22 €			2h		28h 40 min				
	Score (0-100)	33,3	33,3	33,3	33,3	33,3	33,3	33,3	33,3	33,3	33,3
FINAL COLOR:		REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		40,6%	
RED		≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
		yes	yes	yes	no	yes	no				
Short annotation: 4.1red		**Long annotation: 4.1red(7.0/2red-3.4/3green-3.3/3blue)**									
Hydrophilic suppositories											
REDNESS (analytical performance)	W=2	w= 5					w=5				
		accuracy (%)					accuracy (%)				
CS: 83,3%	LAV=33.3	90-110%					15%				
	LSV=66.6	95-105%					5%				
	Result	99-101%					1,50%				
	Score (0-100)	88,7	88,7	88,7	88,7	88,7	78,3	78,3	78,3	78,3	78,3
GREENNESS (safety and eco-friendliness)	W=3	w=3			w=3		w=4				
		liquids chemicals consumption			chemicals hazards		Analytical GrennEss Calculator Score				
CS: 61,3%	LAV=33.3	648ml/10 units			5 hazards pictogramms in total		0,33				
	LSV=66.6	324ml/10 units			3 hazards pictogramms in total		0,66				
	Result	288 ml			3 hazards pictogramms in total		0,52				
	Score (0-100)	70,3	70,3	70,3	66,6	66,6	66,6	52	52	52	52
BLUENESS (productivity / practical effectiveness)	W=3	w=3			w=3		w=4				
		cost effectiveness			human time-effectiveness		process time-effectiveness				
CS: 83,1%	LAV=33.3	22€/10 units			2h/10 units		28h/10 units				
	LSV=66.6	11€/10 units			1h/10 units		14h/10 units				
	Result	2,07 €			1h		4h 20 min				
	Score (0-100)	93,6	93,6	93,6	66,6	66,6	66,6	89,6	89,6	89,6	89,6
FINAL COLOR:		REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		74,2%	
MAGENTA		≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
		yes	yes	yes	no	yes	yes				
Short annotation: 7.4magenta		**Long annotation: 7.4magenta(8.3/2red-6.1/3green-8.3/3blue)**									

Fig. 9. RGB method results of the quantification process for lipophilic and hydrophilic formulation (LAV: least acceptable value; LSV: least satisfactory value; W and w: weight of the color and criterion, respectively; CS: compound score) (colored figure).

hydrophilic bases can be associated with the rapid disintegration of gelatin-base suppositories and the solubility of the matrix in an aqueous medium [48]. Studying the *in-vitro* release profile of pentobarbital suppositories can bring extra information useful for the development of formulations with optimal absorption, potentially leading to predictable pharmacological effects in patients, but should be completed with pharmacokinetic studies to determine the safety of these medicines [16].

In this study, we aimed to conduct preliminary evaluations of formulations to assess their potential for pediatric procedural sedation. *In-vitro* testing is a crucial step in formulation development as it allows for the estimation of the influence of critical quality attributes and critical process parameters on the formulation. While *in-vitro* dissolution testing is an important quality control method, it does not fully replicate the complexities of the *in-vivo* rectal environment, which includes factors such as variable fluid volume, enzyme activity, and the presence of mucosal barriers [16]. Additionally, for fast-release formulations, dissolution may not be the limiting factor in drug absorption into the systemic circulation; permeability also needs to be considered [28]. This discrepancy is particularly relevant for drugs like pentobarbital, where factors such as rectal mucosal permeability and the local environment significantly impact bioavailability. Given that dissolution tests cannot fully predict actual drug absorption *in-vivo*, further *in-vivo* studies are required to confirm absorption kinetics and optimize the formulation. While the current available data provides valuable insights into the *in-vitro* characteristics of the formulations and their impact on PNa release (and thus on their potential pharmacological activity), *in-vivo* studies are necessary to fully determine their clinical efficacy and safety [53]. Future research will focus on conducting comprehensive *in-vivo*

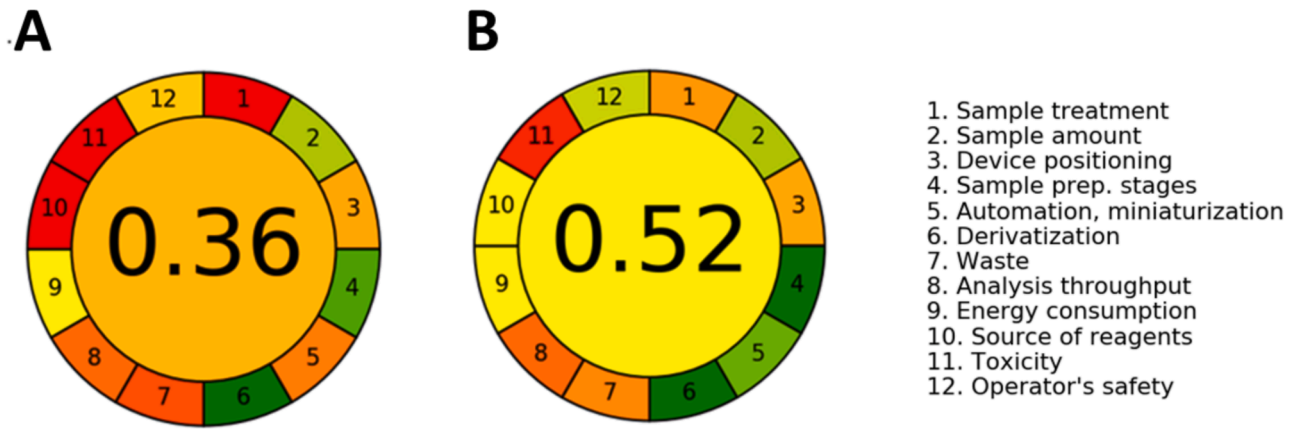


Fig. 10. Results of the AGREE analysis (on a scale of 0 to 1) for A: Lipophilic and B: hydrophilic suppositories. (colored figure).

evaluations in appropriate animal models or clinical trials, which will allow a more direct comparison with conventional sedation systems. These studies are essential to confirm the effectiveness of the formulations in real-world pediatric settings.

3.3.2. Comparative quantification process

The use of the RGB additive color evaluation method (Fig. 9) made it possible to compare the two quantification processes used according to the formulation (lipophilic (A) versus hydrophilic (B)), particularly from an ecological and safety aspect point of view, as well as in productivity terms.

The analytical parameters (red section) remain substantially identical for the two quantification processes, using the same analytical method and both meeting the ICH Q2R1 guidelines [35]. The ecological and safety parameters (green section) show that the quantification process used for the hydrophilic formulation is safer, more environmentally friendly, with a volume of toxic solvents used reduced by more than 50 % (648 mL/ 10 units for A versus 288 mL/10 units for B). This is explained by the use of water during the dilution step before analysis, as well as the absence of extraction steps requiring 1-octanol. This is visualized through the AGREE score ((Fig. 10) with a score of 0.52 for B compared to 0.36 for A, highlighting safer preparation stages (item number 1, 4, 5, 7, 10 and 12 in the AGREE calculator). The productivity parameters (blue section) results show a reduction in the time associated with the preparation of the hydrophilic suppository quantification process, with a reduction of 24 h on the process itself (28 h for A versus 4h20min for B for the uniformity of dosage unit) and a reduction by half of the human time mobilized for this task (2 h for A compared to 1 h for B per batch). The associated cost is reduced by a factor of 10 for the quantification of hydrophilic suppositories (22€/10 units for A compared to 2.07€/10units for B). The improvement in these productivity factors and practical efficiency are notably due to the absence of the extraction phase requiring time and expensive solvents. The RGB method expresses this result in color coding allowing the qualitative characteristics of a method, its coherence and its general predispositions to be annotated and recognized. For the lipophilic formulations, the result is red which means that only the analytical performance exceeds the LSV, indicating that the method may be "the method of choice if the number of analyzes planned is relatively low, and if there is no "greener" alternative". The results obtained for the hydrophilic formulation G3 are better; in fact, the color expressed is magenta with higher "brilliance" (indicating perfection or flawlessness of the method). Magenta is a mixture of red and blue color (meaning that the process present a good analytical performance with high productivity), which corresponds to "a method of choice if there is no "greener" alternative"[39]. This new formulation of gelatin-based hydrophilic suppository allows an increase of productivity, freeing up human time and allowing significant savings on the control process associated with the production of a batch.

4. Conclusion

The work detailed here presents the development of fast-dissolving pentobarbital suppositories that could represent a significant advancement in pediatric procedural sedation, offering a safe and efficient alternative for healthcare providers. By prioritizing patient comfort and procedural success, we aim to contribute to the improvement of pediatric healthcare practices and outcomes. Our research demonstrates that the fast-dissolving gelatin-based sodium pentobarbital suppositories showed promising preliminary results from a biopharmaceutical point of view in order to get an effective sedation for pediatric patients undergoing radiological examinations. The use of NaOH in hydrophilic suppositories allowed a total release of the pentobarbital from the matrix, and the comparison with traditional lipophilic formulations indicated faster liberation of pentobarbital, as well as a higher cost-effective quality control process. A physical, chemical, and microbiological stability study could now be conducted to establish the storage conditions and the shelf life of these suppositories.

CRediT authorship contribution statement

Aurelien Freisz: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Imen Dhifallah:** Writing – review & editing, Validation, Methodology, Investigation. **Yoann Le Basle:** Writing – review & editing, Validation, Methodology. **Mireille Jouannet:** Writing – review & editing, Visualization, Resources. **Philip Chennell:** Writing – review & editing, Validation. **Ghislain Garrait:** Writing – review & editing, Validation, Methodology. **Eric Beyssac:** Writing – review & editing, Validation, Resources, Methodology. **Yassine Bouattour:** Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Valérie Sautou:** Writing – review & editing, Validation, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2024.114532.

Data availability

Data will be made available on request.

References

[1] T. Voepel-Lewis, S. Malviya, G. Prochaska, A.R. Tait, Sedation failures in children undergoing MRI and CT: is temperament a factor? Paediatr. Anaesth. 10 (2000) 319–323, https://doi.org/10.1046/j.1460-9592.2000.00510.x.

[2] J. Stern, A. Pozun, Pediatric Procedural Sedation, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2024. http://www.ncbi.nlm.nih.gov/books/NBK572100/ (accessed June 5, 2024).

[3] C.J. Coté, S. Wilson, American academy of pediatrics, american academy of pediatric dentistry, Guidelines for monitoring and management of pediatric patients before, during, and after sedation for diagnostic and therapeutic procedures, Pediatrics 143 (2019) e20191000.

[4] Guidelines for the elective use of conscious sedation, deep sedation, and general anesthesia in pediatric patients. Committee on Drugs. Section on anesthesiology, Pediatrics 76 (1985) 317–321.

[5] American Society of Anesthesiologists Task Force on Sedation and Analgesia by Non-Anesthesiologists, Practice guidelines for sedation and analgesia by non-anesthesiologists, Anesthesiology 96 (2002) 1004–1017. Doi: 10.1097/00000542-200204000-00031.

[6] Committee on Drugs. American Academy of Pediatrics, Guidelines for monitoring and management of pediatric patients during and after sedation for diagnostic and therapeutic procedures: addendum, Pediatrics 110 (2002) 836–838. Doi: 10.1542/peds.110.4.836.

[7] C.J. Coté, S. Wilson, A.A.O. Pediatrics, Guidelines for monitoring and management of pediatric patients before, during, and after sedation for diagnostic and therapeutic procedures, Pediatrics 143 (2019), https://doi.org/10.1542/peds.2019-1000.

[8] Practice Guidelines for Moderate Procedural Sedation and Analgesia 2018: A Report by the American Society of Anesthesiologists Task Force on Moderate Procedural Sedation and Analgesia, the American Association of Oral and Maxillofacial Surgeons, American College of Radiology, American Dental Association, American Society of Dentist Anesthesiologists, and Society of Interventional Radiology*, Anesthesiology 128 (2018) 437–479. Doi: 10.1097/ALN.0000000000002043.

[9] B. Krauss, S.M. Green, Procedural sedation and analgesia in children, LancetLond. Engl. 367 (2006) 766–780, https://doi.org/10.1016/S0140-6736(06)68230-5.

[10] M.C. Mondardini, F. Sperotto, M. Daverio, A. Amigoni, Analgesia and sedation in critically ill pediatric patients: an update from the recent guidelines and point of view, Eur. J. Pediatr. 182 (2023) 2013–2026, https://doi.org/10.1007/s00431-023-04905-5.

[11] I. de Rover, J. Wylleman, J.J. Dogger, W.M. Bramer, S.E. Hoeks, J.C. de Graaff, Needle-free pharmacological sedation techniques in paediatric patients for imaging procedures: a systematic review and meta-analysis, Br. J. Anaesth. 130 (2023) 51–73, https://doi.org/10.1016/j.bja.2022.09.007.

[12] Y. Arlachov, R.H. Ganatra, Sedation/anaesthesia in paediatric radiology, Br. J. Radiol. 85 (2012) e1018–e1031, https://doi.org/10.1259/bjr/28871143.

[13] R. Fallah, F. Ferdosian, A. Shajari, Non-parenteral medications for procedural sedation in children- a narrative, Iranian Journal of Child Neurology 9 (2015).

[14] A. Vade, R. Sukhani, M. Dolenga, C. Habisohn-Schuck, Chloral hydrate sedation of children undergoing CT and MR imaging: safety as judged by American academy of pediatrics guidelines, Am. J. Roentgenol. 165 (1995) 905–909, https://doi.org/10.2214/ajr.165.4.7676990.

[15] J. Anderson, S. Dalabih, E. Birisi, A. Dalabih, Is orally administered pentobarbital a safe and effective alternative to chloral hydrate for pediatric procedural sedation? J. Pediatr. Pharmacol. Ther. JPPT 23 (2018) 460–465, https://doi.org/10.5863/1551-6776-23.6.460.

[16] S. Hua, Physiological and pharmaceutical considerations for rectal drug formulations, Front. Pharmacol. 10 (2019) 1196, https://doi.org/10.3389/fphar.2019.01196.

[17] V. Jannin, G. Lemagnen, P. Gueroult, D. Larrouture, C. Tuleu, Rectal route in the 21st century to treat children, Adv. Drug Deliv. Rev. 73 (2014) 34–49, https://doi.org/10.1016/j.addr.2014.05.012.

[18] S.H.F. Lam, D.R. Li, C.E. Hong, G.M. Vilke, Systematic review: rectal administration of medications for pediatric procedural sedation, J. Emerg. Med. 55 (2018) 51–63, https://doi.org/10.1016/j.jemermed.2018.04.025.

[19] W. Löscher, M.A. Rogawski, How theories evolved concerning the mechanism of action of barbiturates, Epilepsia 53 (2012) 12–25, https://doi.org/10.1111/epi.12025.

[20] J. Schlatter, S. Kabiche, N. Sellier, J.-E. Fontan, Oral pentobarbital suspension for children sedation during MR imaging, Ann. Pharm. Fr. 76 (2018) 286–290, https://doi.org/10.1016/j.pharma.2018.03.002.

[21] C. Taketomo, J. Hodding, D. Kraus, Pediatric & neonatal dosage handbook: a comprehensive resource for all clinicians treating pediatric and neonatal patients, 23rd ed., Lexi-Comp, Inc, Hudson, Ohio, n.d. https://www.decitre.fr/livres/pediatric-neonatal-dosage-handbook-9781591953654.html (accessed October 4, 2022).

[22] J.T. Doluisio, R.B. Smith, A.H.C. Chun, L.W. Dittert, Pentobarbital absorption from capsules and suppositories in humans, J. Pharm. Sci. 67 (1978) 1586–1588, https://doi.org/10.1002/jps.2600671122.

[23] A.B. Johnson, N.M. Sadiq, Pentobarbital, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2024. http://www.ncbi.nlm.nih.gov/books/NBK545288/ (accessed June 26, 2024).

[24] S. Belo, J. Touchard, P.-H. Secretan, F. Vidal, V. Boudy, S. Cisternino, J. Schlatter, Stability of pentobarbital hydrogel for rectal administration in pediatric procedural sedation, Hosp. Pharm. 56 (2021) 332–337, https://doi.org/10.1177/0018578719901276.

[25] Ł.J. Walczak-Nowicka, M. Herbet, Sodium benzoate—harmfulness and potential use in therapies for disorders related to the nervous system: a review, Nutrients 14 (2022) 1497, https://doi.org/10.3390/nu14071497.

[26] M. Lebrat, Y. Bouattour, C. Gaudet, M. Yessaad, M. Jouannet, M. Wasiak, I. Dhifallah, E. Beyssac, G. Garrait, P. Chennell, V. Sautou, Development and stability of a new formulation of pentobarbital suppositories for paediatric procedural sedation, Pharmaceutics 15 (2023) 755, https://doi.org/10.3390/pharmaceutics15030755.

[27] M.A. Hedaya, 2024 Routes of Drug Administration, in: Pharmaceutics, Academic Press: pp. 537–554. Doi: 10.1016/B978-0-323-99796-6.00006-0.

[28] R. Rathi, A. Sanshita, V. Kumar, K. Vishvakarma, I. Huanbutta, T.S. Singh, Advancements in rectal drug delivery systems, Clinical Trials, and Patents Perspective, Pharmaceutics 14 (2022) 2210, https://doi.org/10.3390/pharmaceutics14102210.

[29] European Pharmacopoeia 2.9.3. Dissolution test for solid dosage forms, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France ,2017, (p. 377), (n.d.).

[30] European Pharmacopoeia 2.9.24. Resistance to rupture of suppositories and pessaries, Council of Europe: Strasburg, France, 2005, (n.d.).

[31] European Pharmacopoeia 2.9.40. Uniformity of Dosage Units; Council of Europe: Strasburg, France, 2009; pp. 3117–3120., (n.d.).

[32] T. Higuchi, Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices, J. Pharm. Sci. 52 (1963) 1145–1149, https://doi.org/10.1002/jps.2600521210.

[33] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, Mechanisms of solute release from porous hydrophilic polymers, Int. J. Pharm. 15 (1983) 25–35, https://doi.org/10.1016/0378-5173(83)90064-9.

[34] M. Ajemni, I.-B. Balde, S. Kabiche, S. Carret, J.-E. Fontan, S. Cisternino, J. Schlatter, Stability-indicating assay for the determination of pentobarbital sodium in liquid formulations, Int. J. Anal. Chem. (2015) 697937, https://doi.org/10.1155/2015/697937.

[35] J. Abraham, 2010 International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use, in: C. Tietje, A. Brouder (Eds.), Handb. Transnatl. Econ. Gov. Regimes, Brill | Nijhoff: pp. 1041–1053. Doi: 10.1163/ej.9789004163300.i-1081.897.

[36] J.P. Jantzen, I. Tzanova, P.K. Witton, A.M. Klein, Rectal pH in children, Can. J. Anaesth. J. Can. Anesth. 36 (1989) 665–667, https://doi.org/10.1007/BF03005418.

[37] European Pharmacopoeia 2.9.22. Softening time determination of lipophilic suppositories, Council of Europe, Strasburg, France, 2008.

[38] European Pharmacopoeia, Dissolution test for lipophilic solid dosage forms, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France 2017 (2017) 377.

[39] P.M. Nowak, P. Kościelniak, What Color Is Your Method? Adaptation of the RGB Additive Color Model to Analytical Method Evaluation, Anal. Chem. 91 (2019) 10343–10352, https://doi.org/10.1021/acs.analchem.9b01872.

[40] AGREE Instrument Results Calculator - Resource Library, (n.d.). https://resourcelibrary.stfm.org/viewdocument/agree-instrument-results-calculator?CommunityKey=2751b51d-483f-45e2-81de-4faced0a290a&tab=librarydocuments (accessed June 7, 2024).

[41] PubChem, Pentobarbital, (2022). https://pubchem.ncbi.nlm.nih.gov/compound/4737 (accessed April 25, 2022).

[42] J.X. Lu, C. Tupper, A.V. Gutierrez, J. Murray, Biochemistry, Dissolution and Solubility, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2024. http://www.ncbi.nlm.nih.gov/books/NBK431100/ (accessed June 26, 2024).

[43] C. Nakatomi, C.-C. Hsu, K. Ono, Correlations of sensations of hardness and springiness of agar and gelatin gels with mechanical properties in human participants, J. Oral Biosci. 65 (2023) 316–323, https://doi.org/10.1016/j.job.2023.09.001.

[44] P. Kaewruang, S. Benjakul, T. Prodpran, Characteristics and gelling property of phosphorylated gelatin from the skin of unicorn leatherjacket, Food Chem. 146 (2014) 591–596, https://doi.org/10.1016/j.foodchem.2013.09.111.

[45] K.J. Goudie, S.J. McCreath, J.A. Parkinson, C.M. Davidson, J.J. Liggat, Investigation of the influence of pH on the properties and morphology of gelatin hydrogels, J. Polym. Sci. 61 (2023) 2316–2332, https://doi.org/10.1002/pol.20230141.

[46] Q. Zhou, Z. Zhang, Y. Huang, L. Niu, J. Miao, K. Lai, Effects of acidulants on the rheological properties of gelatin extracted from the skin of tilapia (Oreochromis mossambicus), Foods Basel Switz. 11 (2022) 2812, https://doi.org/10.3390/foods11182812.

[47] B. Perissutti, J.M. Newton, F. Podczeck, F. Rubessa, Preparation of extruded carbamazepine and PEG 4000 as a potential rapid release dosage form, Eur. J. Pharm. Biopharm. 53 (2002) 125–132, https://doi.org/10.1016/S0939-6411(01)00209-0.

[48] E.J. Al-Akkam, L.A. Alwan, Formulation and in vitro evaluation of piroxicam conventional and hollow suppositories, Int. J. Drug Deliv. Technol. 10 (2020) 200–209, https://doi.org/10.25258/ijddt.10.2.3.

[49] P.J. Sheskey, W.G. Cook, C.G. Cable, eds., 2017 Handbook of pharmaceutical excipients, Eighth edition, Pharmaceutical Press ; APhA, London, Washington, DC.

[50] P. Costa, J.M. Sousa Lobo, Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci. off. J. Eur. Fed. Pharm. Sci. 13 (2001) 123–133, https://doi.org/10.1016/s0928-0987(01)00095-1.
[51] E.L. Ding, S.M. Hutfless, X. Ding, S. Girotra, Chocolate and prevention of cardiovascular disease: a systematic review, Nutr. Metab. 3 (2006) 2, https://doi.org/10.1186/1743-7075-3-2.
[52] S. Dash, P.N. Murthy, L. Nath, P. Chowdhury, Kinetic modeling on drug release from controlled drug delivery systems, Acta Pol. Pharm. 67 (2010) 217–223.
[53] V. Kandi, S. Vadakedath, Clinical trials and clinical research: a comprehensive review, Cureus 15 (2023) e35077, https://doi.org/10.7759/cureus.35077.