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Stability of high concentrated triple intrathecal therapy for pediatrics and mitigation strategies

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ABSTRACT

Stringent formulation requirements are defined to intrathecally administer drug substances, avoiding neurological complications. In case of pediatric patients, these are further complicated due to the limited volumes of the celebrospinal fluid and, therefore, high concentrated solutions of methotrexate (MTX), cytarabine and corticosteroids (*i.e.*, methylprednisolone or hydrocortisone) are prepared based on the patient's age. This work aims to assess the chemical and physical stability of triple intrathecal mixtures differing in volume and composition by a bracketing approach and to identify possible stress causes and mitigation strategies.

Low solubility of MTX was the main factor limiting the physical stability of triple mixtures. Regarding solutions containing methylprednisolone, the amount of MTX remaining was about 95% in the solution at highest concentrations with the concomitant formation of a visible particulate sizing bigger than 1 μm after 24 h of storage at 25 °C. This behavior was mainly driven by the pH reduction due to the pH value of the cytarabine solution used; the shear stress also induced drug precipitation. In the case of the hydrocortisone based mixtures, the precipitate formation occurred at a slow rate. To improve the physical stability, a better control of the mixture pH (optimal value ≈ 7) is required or, as an alternative, the addition of the cytarabine solution to a pre-mixed binary mixture containing MTX and a corticosteroid should be preferred.

1. Introduction

Acute leukemia is the most frequent childhood malignancy, constituting one-third of all childhood cancer diagnosis (Parkin et al., 1988). Despite brought about treatment improvements and application of intensive chemotherapy protocols in the last decades, central nervous system (CNS) involvement remains one of the most severe complications and the primary cause of mortality (Johnston et al., 2017). Administration of substances directly into the cerebrospinal fluid (CSF), bypassing the problem of limited permeability of the blood-brain barrier, is a potentially powerful drug delivery approach. This route of administration allows achieving a high concentration of therapeutic agents within the CNS while minimizing systemic exposure and associated multi-organ toxicity (Calias et al., 2014). On the other hand, molecules by intrathecal (IT) injection encounter new barriers which include relatively high rates of CSF turnover and potentially inadequate delivery to tissue or cellular targets. Lymphatics, the primary outflow conduit for CSF, constitute a wide functional unidirectional vascular system that removes excess of fluid, waste products and therapeutic substances, (Fowler et al., 2020). Standard IT therapeutic approaches to CNS prophylaxis include IT methotrexate (MTX) (Gaynon et al., 2010; Möricke et al., 2010) and triple IT therapy with MTX, corticosteroids, and cytarabine (ARA-C) (Felix et al., 2018; Pui et al., 2010; Salzer et al., 2010). Direct delivery of a drug substance to the CNS requires stringent formulation due to the delicate balance of ions, proteins, and osmolality existing in the CSF. One fundamental approach is designing formulations with no buffering species or preservatives, adequate volume, osmolality and pH in order to avoid neurologic complications (de Lemos et al., 2009; Geiser et al., 1975; Thomas and Le, 2008). Consequently, most of the drug substances to be delivered in the IT space are formulated in a preservative-free saline solution and need an adequate concentration to ensure that a limited volume is introduced. Elliott's B solution may be an alternative to saline solution, being comparable to CSF in terms of pH, electrolyte composition, glucose content, and osmolarity; however, it is not commercially available in Europe (Cradock et al., 1978; Elliott and Jasper, 1949; Olmos-Jiménez et al., 2017, 2016).

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The design space is further constrained in case of infants younger than 3 years: given the small volumes of CSF (Jang et al., 2019), high concentrated solutions should be administered in order to shorten the time needed to complete intralumbar injections, hence reducing pain and stress associated with the procedure. Nowadays, routine therapeutic use of IT chemotherapy in children is restricted to few antineoplastic agents (*i.e.,* MTX and ARA-C) in combination with corticosteroids.

Tables 1 and **2** summarize the dose of each drug contained in the standardized solutions used for the prevention of CNS involvement in pediatric patients. The volume of each formulation has been adjusted according to the age of the patient. As the CSF volume is age-related, but it increases more rapidly than the body superficial area, reaching adult levels within 3 years (Grossman et al., 1982), age-related dosing is generally used in clinical practice (Ruggiero et al., 2001).

Noteworthy, the summary of product characteristics of ARA-C recommends not to mix with MTX, nor methylprednisolone (MP) sodium succinate or hydrocortisone (HC) sodium succinate (Aracytin polvere e solvente per soluzione iniettabile (SPC), 2021; Citarabina Accord soluzione iniettabile o per infusione (SPC), 2021; Citarabina Hikma soluzione iniettabile (SPC), 2021). These indications are in line also with the literature data (Dorr, 1979; Griffin and D'arcy, 1984; Olin, 1992; Trissel, 2001).

The most accepted protocol for preparing the triple IT therapy is to mix the three drug products in one single syringe in order to facilitate IT administration and prevent excessive handling (i.e., connecting and disconnecting the catheter), thus reducing the risk of accidental contamination during administration (Olmos-Jiménez et al., 2017). However, the great variability in composition, strength, volumes and diluents implies that stability data of such formulations are not always available (de Lemos et al., 2009), therefore clinicians may extemporaneously compound these solutions directly at the patient's bedside. Obviously, this situation is not optimal from the point of view of safety, neither of the patient nor of those who handle these hazardous substances, as this procedure may affect sterility and lead to errors in drug dosing.

Based on these considerations, this study aimed to assess the chemical and physical stability of triple IT solutions of high concentrated MTX, ARA-C, and MP or HC in saline solution (0.9% NaCl). Volumes and doses were referred to infants younger than 1 year and children up to 3 years.

Among the solutions containing MP (**Table 1**), the formula at highest concentration (thereinafter ITM-1) was chosen for the stability study. Given the broader strength range of the HC formulations (**Table 2**), stability was assessed by a bracketing approach, considering the highest and lowest concentrations, namely the mixture intended for patients below 1 year of age (thereinafter ITH-1) and the one adapted for patients over 3 years (thereinafter ITH-2).

In order to overcome the limited stability of triple mixtures, a different approach was proposed, namely the preparation of binary mixtures of MTX/MP and MTX/HC to be combined with ARA-C just before administration. To support this idea, the stability was assessed by a bracketing criterion.

2. Material and methods

2.1. Materials

MTX 50 mg/2 ml (Accord Healthcare Italia Srl, Italy), ARA-C 100 mg/5 ml (Aracytin®, Pfizer Srl, Italy), MP 21-sodium succinate 40 mg/1 ml (Urbason®, Sanofi Srl, Italy), HC 21-sodium succinate 1 g/10 ml (Flebocortid®, Sanofi Srl, Italy) and sodium chloride 0.9% for infusion (Baxter Spa, Italy) were used for the preparation of IT solutions. Solvents were of analytic grade, unless specified.

2.2. Preparation of IT solutions

After reconstitution of lyo-powders (*i.e.*, ARA-C, MP and HC) using saline solution, aliquots of each solution were withdrawn, diluted, and subsequently mixed to obtain the final concentrations. The composition of both binary and triple IT mixtures are reported in **Tables 1** and **2**.

Samples were stored in polypropylene syringes or in polystyrene cuvettes (for dynamic light scattering and turbidity analysis) at 25 ± 1 °C, protected from light, over 24 h. All formulations were prepared in triplicate.

2.3. Chemical stability study

The initial concentrations of MTX, ARA-C, MP and HC were assumed to be 100% of the nominal value, and subsequent sample concentrations were expressed as percentage of the initial concentration. After 2, 6 and 24 h, 50 µL samples were withdrawn from each test solution and diluted with mobile phase before analysis. Drugs were considered stable when their mean concentration was more than 95% of the initial value, with a 95% confidence interval around that mean.

Table 1
Composition of IT mixtures containing methylprednisolone (MP) sodium succinate, methotrexate (MTX) and, eventually, cytarabine (ARA-C). Doses and volumes are adjusted based on the patient's age.

Age	< 1 Y		1–2 Y *(ITM-1)*		1–2 Y *(ITM-1B)*		2–3 Y		> 3 Y *(ITM-2)*		> 3 Y *(ITM-2B)*	
Volume (mL)	3.0		3.5		1.5		5.0		6.0		4.0	
	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose(mg)	C(mg/mL)
MP	0.8	0.3	1.2	0.3	1.2	0.8	1.6	0.3	2.0	0.3	2.0	0.5
MTX	6.0	2.0	8.0	2.3	8.0	5.3	10.0	2.0	12.0	2.0	12.0	3.0
ARA-C	16.0	5.3	20.0	5.7	–	–	26.0	5.2	30.0	5.0	–	–

Table 2
Composition of IT mixtures containing hydrocortisone (HC) sodium succinate, methotrexate (MTX) and, eventually, cytarabine (ARA-C). Doses and volumes are adjusted based on the patient's age.

Age	< 1 Y *(ITH-1)*		< 1 Y *(ITH-1B)*		1–2 Y		2–3 Y		> 3 Y (ITH-2)		> 3 Y (ITH-2B)	
Volume(mL)	2.0		1.0		3.5		5.0		6.0		4.0	
	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)	Dose (mg)	C(mg/mL)
HC	4.0	2.0	4.0	4.0	6.0	1.7	8.0	1.6	10.0	1.7	10.0	2.5
MTX	6.0	3.0	6.0	6.0	8.0	2.3	10.0	2.0	12.0	2.0	12.0	3.0
ARA-C	16.0	8.0	–	–	20.0	5.7	26.0	5.2	30.0	5.0	–	–

MP and HC concentrations were calculated considering the sum of the 21-hemisuccinate and 17-hemisuccinate isomers peak areas, in accordance with the United States Pharmacopoeia (USP) monographs (Hydrocortisone Sodium Succinate for Injection 2020; Methylprednisolone Sodium Succinate for Injection, 2020). The amount of free corticosteroid was also determined, assuming a relative response factor of 1, and subsequently the USP acceptance criteria – no more than 6.6% and 6.7%, respectively, of the labeled amount of MP and HC – were also verified (Hydrocortisone Sodium Succinate for Injection 2020; Methylprednisolone Sodium Succinate for Injection, 2020).

Drug content was assessed adapting the gradient high performance liquid chromatography (HPLC) method proposed by D'Hondt et al. (2012). The HPLC system consisted of an HP 1100 ChemStation (Agilent Technologies Inc, USA) equipped with a diode array detector. The separation was achieved using a reverse-phase C18 column (Gemini, 5 μm, 250 × 4.6 mm, Phenomenex Inc, USA) and an optimized mobile phase consisting of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). After injecting a 10 μl sample, the column was eluted using a linear gradient form 90% A to 90% B over 15 min (total run time 30 min) at 25 °C. The flow rate was 1.0 ml/min. The detection wavelength was set at 280 nm for MTX and 240 nm for the other compounds.

A five-point calibration curve was constructed for each molecule and linearity ($R^2 > 0.999$) was demonstrated for MTX (25–500 μg/ml), ARA-C (50–1000 μg/ml), MP (1–50 μg/ml) and HC (25–250 μg/ml). Repeatability was evaluated by triplicate injection of samples with an intermediate concentration, coefficient of variation was below 1% in all cases.

To force the degradation, single-drug sample solutions were stored at 80 °C for 1 h. Degradation products of ARA-C and MTX were detected at 280 nm, while corticosteroids' byproducts were monitored at 240 nm. Relative retention times (RRT) are summarized in **Table S1**. pH and osmolality were measured immediately after preparation of the mixtures and after 24 h of storage using an InLab Expert Pro-ISM (Mettler-Toledo Spa, Italy) and a K-7400S (Knauer GmbH, Germany), respectively. pH and osmolality values outside of range ± 0.5 and ± 5%, respectively, compared to the initial values, were considered an indication of instability.

2.4. Physical stability study

Physical instability was defined as change in color, appearance, or precipitation after compounding and then after storage at 25 ± 1 °C for 2, 3, 4, 5, 6 and 24 h. Signs of precipitate formation were detected by combining the results of spectrophotometric turbidimetry (Lambda 25, PerkinElmer Spa, Italy) and dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Panalytical, UK). In particular, an absorbance greater than 0.01 AU at 650 nm was considered a sign of turbidity (Dasta et al., 1988). Regarding DLS analysis, the attenuator position, which corrects the intensity of the laser to reach an ideal count of 200–500 kilo-counts per second (kcps) – *i.e.*, the average scattering intensity during the measurement – was qualitatively related to the formation of a precipitate. The maximum value of 11 is typical of samples that do not scatter much light (*i.e.*, particle size below the limit of detection or low concentration). In this set of experiments, the presence of a precipitate was considered significant when the attenuator automatic setting was equal to 10 or lower. The particle size was expressed as Z-average, namely intensity-weighted mean hydrodynamic size of the ensemble collection of particles. Samples were considered physically unstable when both turbidimetric and DLS threshold values were exceeded.

The effect of shear stress on precipitation was assessed by pumping 10 times through a disposable single-use syringe equipped with a 22 $G \times 3.5''$ spinal needle immediately after the preparation of the solution.

2.5. Precipitate isolation and characterization

When a precipitate was formed, consecutive centrifugations were carried out at 5000 rpm for 10 min. The solid was washed twice with ultrapure water and dried until constant weight. To determine their composition, samples were dissolved in methanol, diluted in mobile phase, and analyzed by HPLC.

Moreover, samples physically unstable at the end of the study were gently shaken, diluted 1:1 with Elliott's B solution (in 1 L: dextrose 0.8 g; NaCl 5.9 g; Na_2CO_3 2.3 g; KCl 0.3 g; $Na_2HPO_4 \bullet 12H_2O$ 0.2 g; $CaCl_2$ 0.16 g; $MgCl_2 \bullet 6H_2O$ 0.08 g; 37% HCl ≈ 2 ml, q.s. to pH 7.3–7.4) (Elliott and Jasper, 1949) and, subsequently, the physical stability was monitored until complete solubilization of the precipitate. Z-average and derived count rate (*i.e.*, the average scattering intensity normalized according to the attenuator setting) were the main values taken into consideration. Suspended particles were considered completely dissolved when the automatic attenuation setting of DLS was 11.

3. Results

3.1. Stability of triple IT mixtures

ITM-1 was found to be chemically stable for at least 6 h after compounding (**Table 3**). Over the entire observation period, the pH values of all solutions remained between 5.9 and 6.1, osmolality values also were stable (≈ 328 mOsm/kg).

Regarding the physical stability, ITM-1 resulted to be stable for 2 h. All mixtures were yellow and clear immediately after compounding, but they began to cloud after a few hour and a precipitate was visually observed at the end of the study. This corresponded to a dramatic decrease in MTX content at 24 h (**Table 3**). The HPLC analysis confirmed that the sediment was constituted by MTX.

This result agreed with the turbidity data since the spectrophotometric measurements were about 0.06 AU in all replicates after 3 h. Similarly, particles sizing up to 0.5 μm were detected with an erratic behavior at 2 h and in all samples at 3 h. Z-average value was greater than 0.5 μm at 4 h. Precipitate particle size remained sub-micrometric during the first 6 h (**Fig. 1**).

Table 3
Chemical and physical stability of triple IT solutions. Chemical stability is expressed as % remaining of methotrexate (MTX), cytarabine (ARA-C), and methylprednisolone (MP)/hydrocortisone (HC) sodium succinate (mean ± SD, $n = 3$). Physical instability is qualitatively expressed as absorbance at 650 nm greater than 0.01 AU (turbidity) and attenuator automatic setting of DLS equal to 10 or lower.

IT solution	Drug	% remaining		
		2 h	6 h	24 h
ITM-1	MTX	99.4 ± 1.4	99.5 ± 1.2	95.7 ± 2.5*
	ARA-C	101.0 ± 1.1	101.0 ± 1.3	100.0 ± 1.3
	MP	99.6 ± 0.9	99.5 ± 1.4	98.3 ± 0.8
	Turbidity	–	> 0.01	> 0.01
	DLS	≤ 10	≤ 10	≤ 10
ITH-1	MTX	99.8 ± 1.0	100.1 ± 1.0	99.1 ± 1.8
	ARA-C	99.7 ± 1.2	99.7 ± 1.0	99.8 ± 1.6
	HC	99.3 ± 1.5	99.3 ± 1.0	99.4 ± 1.7
	Turbidity	–	> 0.01	> 0.01
	DLS	11	≤ 10	≤ 10
ITH-2	MTX	99.8 ± 1.8	101.3 ± 1.6	100.8 ± 2.5
	ARA-C	99.1 ± 1.6	100.8 ± 1.6	100.9 ± 4.8
	HC	99.5 ± 1.8	100.6 ± 1.6	101.8 ± 2.9
	Turbidity	–	–	> 0.01**
	DLS	11	11	≤ 10**

* Beyond threshold (95% confidence limit).
** Signs of turbidity were evident only in one sample.

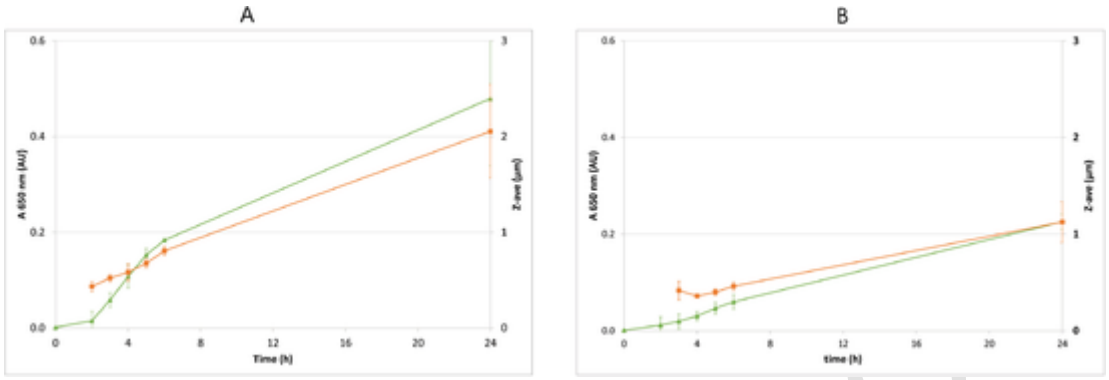

Fig. 1. Spectrophotometric turbidity (green, ▲) and Z-average values (orange, ■) of ITM-1 (panel A) and ITH-1 (panel B) samples over 24 h.

ITH-1 and ITH-2 were found to be chemically stable for at least 24 h after compounding (Table 3). Over 24 h, the pH values of all solutions remained between 5.9 and 6.1, osmolality values were stable as well (ITH-1 ≈ 353 mOsm/kg; ITH-2 ≈ 334 mOsm/kg).

Similarly to ITM-1, all mixtures were yellow and transparent immediately after compounding. ITH-1 resulted to be physically stable for 2 h, as solutions began to cloud after about 3 h, with an erratic behavior. This corresponded to the formation of particles with a Z-average below 0.5 µm. Particles sizing greater than 0.5 µm were observed only at 24 h (Fig. 1). Also in this case, the sediment was constituted by MTX. ITH-2 was physically stable over 6 h; afterwards, presence of particles and turbidity were evidenced in only one sample at 24 h.

3.2. Stability of binary IT mixtures and ARA-C

ITM-1B and ITM-2B were found to be chemically stable for at least 6 h. After 24 h, the concentration of MP underwent a significant decrease, exceeding the acceptability limit (Table 4). Over the entire observation period, the pH values of all solutions remained between 7.1 and 7.3.

All mixtures were yellow and clear, and resulted physically stable over 24 h.

ITH-1B and ITH-2B were found to be chemically stable for 24 h (Table 4). Over the duration of the experiment, the pH values of all solutions remained between 7.2 and 7.5.

All mixtures were yellow and clear, and resulted physically stable over the observation period.

ARA-C was found to be chemically stable at both 10 and 20 mg/ml for at least 24 h (the remaining drug quantities were 100.2 ± 0.5% and 100.4 ± 0.8%, respectively), the pH was 5.0 and remained constant. All solutions were clear and colorless, and remained so over the duration of the experiment.

Table 4
Chemical stability of binary mixtures containing methotrexate (MTX) and methylprednisolone (MP)/hydrocortisone (HC) sodium succinate (mean ± SD, $n = 3$).

IT solution	Drug	% remaining		
		2 h	6 h	24 h
ITM-1B	MTX	99.4 ± 0.6	98.7 ± 1.0	99.3 ± 1.1
	MP	98.4 ± 0.4	97.6 ± 0.9	95.7 ± 1.0*
ITM-2B	MTX	100.4 ± 1.7	101.1 ± 1.7	100.2 ± 2.2
	MP	99.2 ± 1.5	98.9 ± 1.6	95.9 ± 2.0*
ITH-1B	MTX	97.9 ± 0.3	99.3 ± 0.8	99.6 ± 0.1
	HC	97.3 ± 0.2	98.0 ± 0.8	95.8 ± 0.1
ITH-2B	MTX	100.3 ± 1.0	101.3 ± 0.6	101.5 ± 0.7
	HC	99.6 ± 1.1	99.6 ± 1.4	96.5 ± 0.8

* Beyond threshold (95% confidence limit).

4. Discussion

4.1. Stability of triple IT mixtures

The availability of stability data regarding extemporaneous preparations is essential, as it allows to manage compounding and administration of the therapy in a more easy and safe way. It has been observed that all the triple IT solutions are chemically stable for at least 6 h, yet the limiting factor is physical stability.

The degradation byproducts of MP 21-hemisuccinate were identified by D'Hondt et al. as free MP (RRT 0.88) and MP 17-hemisuccinate isomer (RRT 0.91) (D'Hondt et al., 2012). Given the substantial similarity of the two molecules, it can be assumed that HC 21-hemisuccinate related degradants correspond to free HC (RRT 0.86) and HC 17-hemisuccinate isomer (RRT 0.90). Coherently with previous works, 21-hemisuccinates of HC and MP presented the lowest chemical stability among the components of the triple IT solutions, as their strength undergoes a noticeable decrease over time, while a progressive increase in peak areas of related degradants occurs (Cheung et al., 1984; D'Hondt et al., 2012; Trissel et al., 2002).

In our set of experiments, the degradation byproducts ranged 0.7–2.5% at 6 h and 1.2–3.2% at 24 h. According to the USP, medicinal products can be considered suitable for administration despite the conversion to 17-hemisuccinate form and only an upper limit for the free corticosteroid fraction is set, as it is less soluble and could lead to precipitation (Hydrocortisone Sodium Succinate for Injection 2020; Methylprednisolone Sodium Succinate for Injection, 2020). The free corticosteroid content complied the USP acceptance criteria and, therefore, MP and HC were confirmed to be chemically stable in all conditions.

As far as MTX is concerned, precipitation is clearly the result of its pH-dependent solubility. Indeed, MTX presents the highest solubility at alkaline (≈ 37 mg/ml) and neutral pH (≈ 9 mg/ml), where the dianionic species (pK_{a3} 5.6) prevails; it is slightly soluble at very acidic pH, where the cationic form (pK_{a1} 3.2) is most abundant; the solubility, however, drastically decreases at pH 6 (≈ 1 mg/ml) and lower, since the monoanionic (pK_{a2} 4.5) and zwitterionic (pH(I) 3.9) species prevail (dos Santos et al., 2017; Mazák and Noszál, 2020; Messman and Allegra, 2001; Ouyang et al., 2010; Szakács and Noszál, 2006).

When these mixtures are prepared, the pH value equal to 8.5 of the commercially available MTX solution is drastically reduced to about 6.0 in the IT solution (MP and HC solutions pH≈7; ARA-C Aracytin® pH≈5). The pH-dependent formation of a precipitate was further confirmed after increasing the pH, by adding a small amount of sodium hydroxide (NaOH), as the sediment was completely dissolved following agitation. Similarly, it was observed that the addition of NaOH *q.s.* to bring pH to 7.0 (*i.e.*, 1–2 µl/ml of a 2 M solution), immediately after preparation, leads to physically stable formulas.

Based on these considerations, the possible fate of MTX particles was also investigated using Elliott's B solution, as it is comparable to CSF in pH, buffer capacity, electrolyte composition, glucose content, and osmolality (Cradock et al., 1978; Elliott and Jasper, 1949). In other words, suspensions with particle size greater than 3 μm obtained from of ITM-1 and ITH-1 were diluted 1:1 and consecutive DLS analyses were carried out until the attenuator value was at 11. The solubilization of precipitates from ITM-1 and ITH-1 required about 15 and 30 min, respectively (**Fig. 2**). These data allow to hypothesize that accidentally present sub-micrometric particles, due to MTX precipitation, would rapidly dissolve after IT administration.

Moreover, shear stress, which may occur during manipulation, caused increased physical instability. Indeed, the repeated aspiration-ejection through spinal needle induced the formation of particles sizing 2–4 μm at 24 h, this corresponded to turbidity values three times higher than those observed in non-stressed samples. Moreover, clouding and precipitate formation were observed in a stressed ITH-2 sample after a 6 h storage period. Hence, this evidence suggests that all formulations are in a metastable condition.

In light of the above considerations, IT solutions containing MP should be administered within 2 h after compounding due to the limited stability. As regards HC, it is suggested to administer the solutions within 3 h after compounding, as in this timeframe possibly present MTX particles would be few and small and would reasonably dissolve in a short time.

Based on these results, a series of recommendations can be also formulated to optimize the preparation protocol. The pH of IT solutions should be carefully evaluated since its variation can trigger MTX precipitation. As an example, ARA-C (Aracytin®), the most acidic solution, should be the last component to be added. Alternatively, the pH of the compounded IT solution could be increased by using sodium hydroxide or sodium bicarbonate (Olmos-Jiménez et al., 2016). Finally, factors that could promote MTX precipitation (*i.e.*, shear stress) and/or reduce its solubility (*i.e.*, low temperature) should be avoided.

4.2. Stability of binary IT mixtures and ARA-C

Due to the erratic behavior of IT triple solutions, binary mixtures of MTX/MP and MTX/HC were prepared in advance to be combined with ARA-C just before administration. In other words, two syringes containing the binary mixtures and ARA-C, respectively, connected by a simple syringe coupler, or Y infusion connector, may be transferred to the pediatric ward where the mixing occurs. This procedure would allow increasing the time available between compounding and administration.

This idea was supported by the pH value of the binary mixtures (> 7.0) which is high enough not to compromise solubilization of MTX. As a matter of fact, no clouding nor precipitate formation were detected over the 24 h observation period by DLS and naked eye. Spectrophotometric determination of turbidity did not provide reliable data since erratic measurements were obtained, probably due to the ability of MTX to absorb even at high wavelengths when the pH of the solution is neutral or basic.

As far as the chemical stability is concerned, MP and HC underwent a faster transition – compared to the triple solutions – to the 17-hemisuccinate and free forms: the degradation byproducts ranged 1.6–3.5% at 6 h and 4.1–6.5% at 24 h. Nevertheless, the MP and HC solutions met the stability criteria up to 6 h and 24 h, respectively.

Regarding ARA-C solutions, the concentrations to be combined with the binary mixtures range from 10 to 16 mg/ml, based on patient's age. This study confirmed that ARA-C solutions ranging 10–20 mg/ml can be stored for at least 24 h in polypropylene syringes before being mixed with the binary mixtures. These results are in agreement with a recently published work which demonstrated that the strength at 10 mg/ml is stable up to 5 days under the same conditions used in this study; lower concentrations have a longer stability (Ayed et al., 2021).

5. Conclusion

In this work we described the physico-chemical stability of triple IT mixtures containing MTX, ARA-C and corticosteroids (*i.e.*, MP or HC) at high concentration. The stability of triple IT mixtures was very limited due to the low solubility of MTX resulting from mixing of several different medicinal products; the main cause of precipitation was identified in the pH decrease induced by the addition of ARA-C (Aracytin®). On the basis of these observations, triple IT therapy should be administered within 2 h after compounding in the case of solutions containing MP and within 3 h in the case of solutions containing HC. It has been shown that it is alternatively possible, let alone preferable, to prepare a binary mixture – containing MTX and the corticosteroid drug – which is meant to be combined with a second solution containing ARA-C just before administration. Under such circumstances, the binary solutions containing MP are stable up to 6 h, while the ones containing HC, as well as the ARA-C solutions, are stable for at least 24 h.

CRediT authorship contribution statement

Davide Zanon: Conceptualization, Resources, Supervision. **Francesca Selmin:** Conceptualization, Data curation, Methodology, Validation, Writing – review & editing. **Giorgio Centin:** Conceptualization, Validation. **Natalia Maximova:** Methodology, Formal analysis, Data curation, Investigation, Visualization, Writing – original draft. **Antonella Casiraghi:** Validation, Writing – review & editing. **Paola Minghetti:** Conceptualization, Methodology, Writing – review & editing, Supervision.

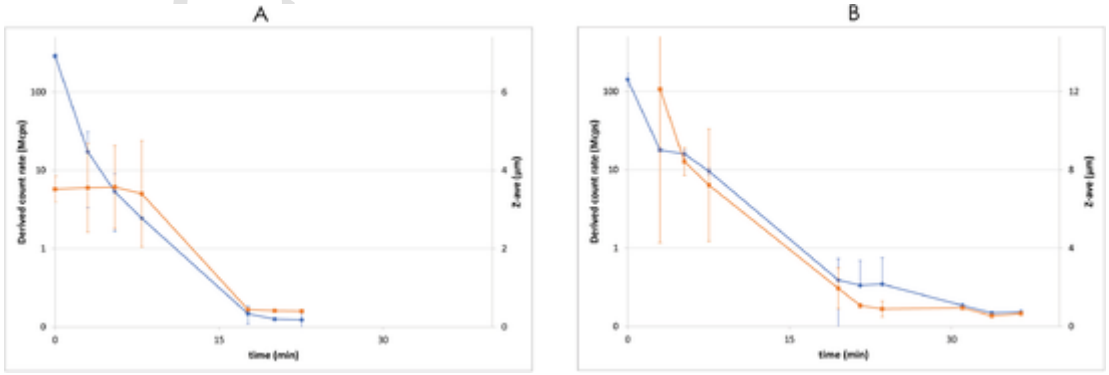

Fig. 2. Derived count rate (Mega counts per second, logarithmic scale; blue, ●) and Z-average values (orange, ■) of ITM-1 and ITH-1 samples over time before dilution (t0) and after dilution (1:1) with Elliott's B solution.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2021.106039.

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