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RESEARCH AND DEVELOPMENT OF DRUG DELIVERY SYSTEMS FOR PAEDIATRIC THERAPEUTIC NEEDS

PhD Dissertation

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ABSTRACT

Manipulation of medicinal products is a common practice in paediatric patients due to the lack of formulations in this heterogeneous group. Paediatric patients are frequently treated with unlicensed medicinal products like galenic preparations, and off-label use is quite widespread. Medication administration to the paediatric population often involves the manipulation of adult dosage forms. However, using extemporaneous preparation may result in treatment failure, toxicity in children dosing, or unknown and/or variable bioavailability. Likewise, the lack of documented information about the physical, chemical and microbial stability of the preparation is also common. Consequently, the Paediatric Committee at the European Medicines Agency (PDCO) has identified the needs in the different therapeutic areas where research and development of medications for paediatric population should be conducted. According to the requirements of various pathologies and formulations of medicines in different paediatric age groups, the research activity was carried out during my PhD programme. This thesis collects all the research developed over 3 years and consists of three chapters. Chapter 1 provides a general introduction to state of the art of paediatric medicine development and the ongoing efforts in paediatric research. On the basis of a literature review, published in 2021 and performed as the initial step of this project, polymeric and lipid nanoparticles were identified to approach paediatric disease problems and needs. Therefore, Chapter 2 focuses on the development and characterisation of two drug delivery systems (DDSs) for medium-long term opportunities for paediatric therapeutic needs. DDSs were proposed as proof of concept for effective drug delivery. Firstly (section 2.3), nanoparticles (NPs) based only on chitosan and cellulose acetate phthalate containing captopril was developed for preparing solid/liquid dosage forms for the treatment of hypertension, heart failure and diabetic nephropathy in paediatric patients. NPs were prepared without using surfactants, which are used frequently to ensure the good stability of NPs in dispersion and their use can be associated with toxicity in children. Chitosan and cellulose acetate phthalate interact due to negative charges of cellulose acetate phthalate and cationic amino groups of chitosan, leading to a polymeric matrix that entraps and protects captopril from chemical degradation in water. Furthermore, a thermosensitive

chitosan/glycerophosphate (GP) nasal hydrogel containing dimethyl fumarate (DMF) was studied as a new approach to treating paediatric multiple sclerosis (section 2.4). Paediatric patients may benefit from this nose-to-brain formulation since it could reduce the adverse effects of DMF gelatin capsules. DMF was complexed with hydroxypropyl-β-cyclodextrin (HP-β-CD) to increase the drug solubility in water. Indeed, chitosan was selected for its promising potential for nasal drug delivery based on its high biocompatibility, mucoadhesive and penetration enhancement properties. For this study, GP was used as an agent for forming a thermosensitive gel. The technological properties (thermogelling properties, pH, osmolality, viscosity, water uptake and mucoadhesion) were investigated, and *in vitro* permeation studies were performed on vertical diffusion cells.

Finally, Chapter 3 provides an overview of the oral medicinal product considerations for administration via different enteral feeding tubes (EFTs) in paediatric patients. The first part is focused on a systematic review performed according to the Preferred Reporting Items for SR and Meta-Analyses (PRISMA). It was registered in the International prospective register of systematic reviews (PROSPERO) CRD42022299418. Characteristics/factors affecting the performance of the oral medicinal product when administered via EFT were identified and grouped into three categories 1) formulation characteristics for different dosage forms, 2) enteral feeding tube characteristics and, 3) administration characteristics. A total of 41 studies linked variation in medication performance or administration of medication via EFTs to the formulation characteristics. 90% of the formulation factors, 69% of enteral feeding tube factors and 47% of administration factors had data available to demonstrate the effect on tube administration to paediatric population. Feeding tube blockage can be caused by a variety of factors, including tube design, insufficient flushes, and off-label usage of medicinal products. Indeed, formulation characteristics for different dosage forms, administration characteristics, and enteral feeding tube characteristics must be considered to administer medical products via EFTs. On the basis of the systematic review, a feasibility study (*in vitro* study) was conducted to assess and streamline the key factors that impacted the administration of medication via nasogastric (NG) tubes (Section 3.5). Nurofen for Children (NfC) suspension was characterised. The present study applied the

utilisation of a factorial design to investigate the effect of dose strength, NG tube size and NG tube length on the product performance (dose recovery) when administered via paediatric NG tubes. Additionally, administration factors (flush volume and dose dilution volume) were evaluated individually. NfC suspension showed suitable physical characteristics.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Paediatric medicine development

In 2007, the Paediatric Regulation took effect in the European Union (EU) to enhance the promotion of medication development for the paediatric population. Paediatric legislation comprises of Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use and its amendment, Regulation (EC) No 1902/2006. Furthermore, Paediatric investigation plans (PIPs) to support the medicine's authorisation in children are supported by The Paediatric Committee (PDCO) that was established with the Regulation. **Figure 1** reveals the results from the ten-year report on the effect of the Paediatric Regulation implemented by the European Commission. Medicinal products for paediatric patients increased in different therapeutic areas, as well as completed PIPs, new paediatric medicines, and new paediatric indications [1]. However, new paediatric forms showed little progress. Likewise, The European Paediatric Formulation Initiative (EuPFI) was founded in 2007 with the objective of improving the paediatric medicines landscape. This consortium includes 5 workstreams on Taste Assessment and Taste Masking, Administration Devices, Age Appropriateness of Formulations, Biopharmaceutics and Pharmaceutical Excipients [2].

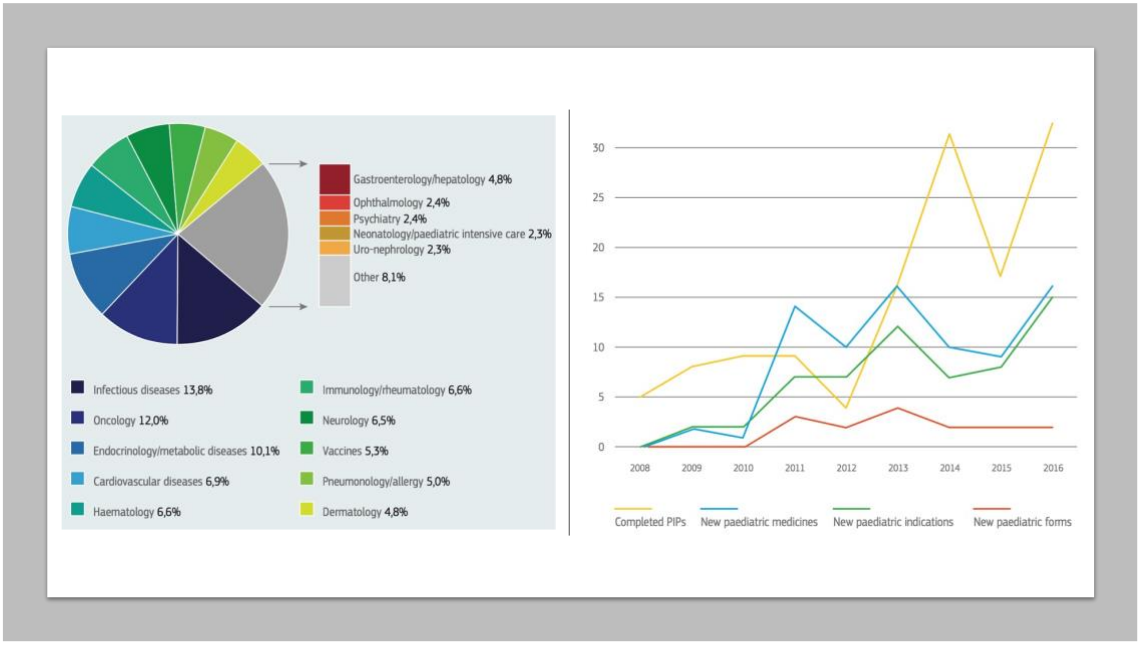

Figure 1. Analysis of the impact on the development of paediatric medicines in the EU. Adapted from EMA 2017 10-year report.

Many medicinal products are currently unavailable in formulations suitable for administering to the paediatric population [3]. Licensed drugs are often prescribed outside the terms of the product license (off-label) in paediatrics. Many drugs are not studied on children; thus, they are unlicensed for use in this population. Manipulation of adult dosage forms is a widespread practice in hospitals to facilitate administration to paediatric patients [4,5]. Nevertheless, the use of extemporaneous preparation may result in an increased risk of toxicity or sub-therapeutic treatment, as well as an unknown and variable bioavailability and lack of documented information about the physical, chemical and microbial stability of the preparation [6].

Children are a heterogeneous population, hence developmental stages are classified by European Medicines Agency (EMA) into preterm newborn infants, term newborn infants (0-27 days), infants and toddlers (1 month to 23 months), children (2 – 11 years), adolescents (12 – 16 or 18 years) [7]. The Food and Drug Administration (FDA) classifies instead this group into neonates (birth to 1 month), infants (1 month to 2 year), children (2 years to 12 years), and adolescents (12 to < 16 years) [8]. These age categories are to some extent arbitrary, wide in terms of body weight, and may include different maturation levels. Therefore, the study of new paediatric medicine must be based on knowledge of paediatric pharmacology and developmental biology [8]. In fact, each age group has different requirements considering the type of formulation and the administration route due to physiological, anatomical, and biochemical changes [3].

Pharmacokinetics and pharmacodynamics, and subsequently the bioavailability of drugs, are impacted by changes that start to take place at birth (**Figure 2**). Drug bioavailability in the paediatric population is influenced by organ volume, absorptive surface area, permeability, enzyme activity, pH, intestinal fluid content and transit time [9,10]. Likewise, the physicochemical properties of medicinal products (e.g., solubility, particle size, polymorphism, salt form, or complexation) and administration factors (e.g., food intake or enteral feeding tubes) may affect drug absorption [9,11].

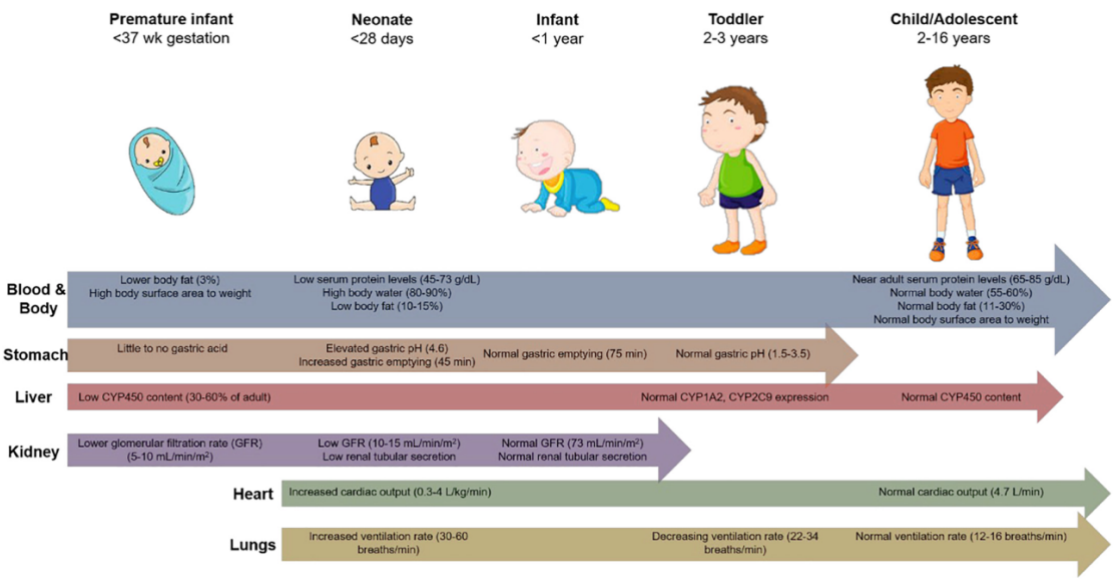

Figure 2. Growth and maturation across the paediatric age categories [10].

The Paediatric Committee at the European Medicines Agency (PDCO) has identified the needs in the different therapeutic areas where research and development of medications for paediatric population should be conducted. This inventory is based on different requirements for various pathologies like data implementation on pharmacokinetics, safety and efficacy and development of appropriate formulations for the different paediatric age groups as well [12].

Therefore, child age-appropriate dosage form is one of the main issues in the development of medicinal products for paediatric patients. Acceptability is defined by the EMA as the ability and willingness of a patient to use and its caregiver to administer the medicine as intended [13]. Pharmaceutical characteristics to consider for the development of an age-appropriate paediatric formulation include a) palatability, swallowability (e.g. size, shape, texture), b) appearance (colour), c) the required dose, d) dosing frequency and duration of treatment, e) administration device, f) packaging and g) the actual mode of administration to the child and any related pain or discomfort [13]. Thus, the acceptability of medicinal products is crucial to guaranteeing adherence and effective treatment [14]. Recently, a survey revealed that conventional dosage forms, i.e., liquid (35%), tablets (19%), and capsules (14%), were the most preferred options [15]. Due to essential constituents of the formulations, the excipients must be carefully chosen in the development of medicinal products for paediatric use. In fact,

excipients safely and commonly used in adult therapy could be harmful to children. Ongoing efforts in paediatric research have succeeded in a Safety and Toxicity of Excipients for Paediatrics ("STEP") database. This innovative tool provides safety and toxicity information on excipients and is a useful resource for paediatric formulation development [16]. Therefore, if palatability is crucial for paediatric formulation, the excipients chosen should follow a benefit-risk strategy. Additionally, the administration device also affects acceptability [17]. Every route of administration (including oral, pulmonary, parenteral, nasal, and ocular routes) requires an appropriate drug delivery system and a delivery device. Paediatric medicines should be administered in an appropriate device, such as an oral syringe, enteral feeding tube, inhaler, or a pen delivery system. However, delivery devices for the administration of paediatric formulation are associated with some challenges [18,19]. For instance, the use of enteral feeding tubes (EFTs) in neonates and children with chronic illnesses and neurodisability unable to safely swallow or take liquids via the oral route [20].

Therefore, developing paediatric-specific dosage forms is particularly challenging due to several factors, including the differences between the paediatric population and adult populations. There is still a need for paediatric-specific formulations and dosage forms in the field of medication study for children [12].

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1.2. Aims

New perspectives for conventional drugs or the application of alternative formulation technology may significantly improve an existing therapy, enhancing efficacy and reducing side effects. Considering also the needs in the different therapeutic areas identified by the PDCO, a research on the development of medications for paediatric population was conducted. The first aim of the research activity, carried out during the PhD programme, was to develop and characterize two drug delivery systems for medium-long term opportunities for paediatric therapeutic needs. Different strategies and technologies (polymeric nanoparticles and thermosensitive hydrogel) were investigated to improve the delivery of drugs.

The second aim of the PhD project, developed during the mobility abroad, was to carry out a systematic review to identify factors associated with the formulation and enteral feeding tubes (EFTs), their effect on the performance of drug products, and the studies required to demonstrate the feasibility of administering medication via EFTs to the paediatric population. Many oral dosage forms must be indeed administered via EFTs in paediatric patients with severe illness or disability that results in reduced swallowing capacity and limited gastrointestinal access. The determination of factors and their evaluations could support the development of evidence-based recommendations or guidance for pharmaceutical companies to evaluate the administration of new oral paediatric drug products via EFTs. In addition, a feasibility study (*in vitro* study) was conducted to assess and streamline the key quality/critical attributes of oral suspension (i.e. dose strength), NG (nasogastric) tubes (i.e. bore size and tube length), and administration methods (i.e. flush volume and dose dilution volume) affecting the product performance (dose recovery) when administered via paediatric NG tubes.

CHAPTER 2. DRUG DELIVERY SYSTEMS FOR PAEDIATRIC PATIENTS

2.1. Introduction

Drug delivery systems (DDSs) are an approach that enables active pharmaceutical ingredients (APIs) to be delivered at a predetermined rate to their target site in the body in order to produce the desired therapeutic response [1]. DDSs could influence the absorption, distribution, metabolism, and elimination (ADME) of APIs to improve their bioavailability. Furthermore, they show the potential to address the limited drug solubility, permeability, chemical stability and decrease toxic effects. Likewise, DDSs allow for modifying the release of the drug, enhancing its short half-life, and adjusting dose frequency [2]. Different DDSs have been studied to enhance drug delivery by modifying the physicochemical or pharmacokinetics drug characteristics. Liposomes, micelles, lipid and polymeric nanoparticles, microparticles or hydrogel are a versatile DDS platforms [3].

A literature review was conducted to present the state of the art in polymeric and lipid nanoparticles used or suggested to approach problems and needs associated with paediatric diseases and inspire further research in this field (Section 2.1) [4]. The United States Pediatric Formulation Initiative suggested applying new technologies to paediatric drug formulations, for instance, nanotechnology. The literature analysis showed that polymeric and lipid nanoparticles (NPs) have been widely studied to treat paediatric diseases, and albumin nanoparticles and liposomes are already used in clinical practice. Nevertheless, these studies are focused almost exclusively on paediatric cancer treatment. Although nanomedicine may solve many needs of paediatric diseases and medicines, the unavailability of data on pharmacokinetics, safety and efficacy of both drugs and NPs in paediatric patients limits the development of new paediatric medicines based on NPs. Therefore, this published literature review revealed that nanomedicine applied in paediatrics remains a significant challenge in the near future [4]. Nevertheless, nanosystems could show the potential for medium-long term opportunities for paediatric development to enhance drug delivery [5]. In fact, since 2021, it has been seen that lipid-based nanoparticles have played a key role as the mRNA encapsulation technology selected for developing the Pfizer/BioNTech and Moderna SARS-CoV-2 (COVID-19) vaccines [6]. Both are already approved for children from 5 and 6 years. Studies

indicate that the mRNA vaccine can offer strong protection against COVID-19 infection in paediatric age groups, and the safety is acceptable [7].

On the basis of this framework, surfactant-free chitosan/cellulose acetate phthalate nanoparticles containing captopril were developed for the treatment of hypertension, heart failure and diabetic nephropathy in paediatric patients (Section 2.3). The results obtained have been recently published [8]. Furthermore, NPs and microparticles were prepared to reduce the adverse effects of dimethyl fumarate (DMF) frequently reported in children with paediatric multiple sclerosis. Also, these DDSs were developed to increase the bioavailability of the drug into the brain. However, NPs and microparticles were inappropriate for DMF delivery because of drug instability like sublimation and hydrolysis (data no reported). Therefore, a second experimental study was conducted to develop a thermosensitive chitosan/glycerophosphate nasal hydrogel containing DMF as a new approach to treating multiple sclerosis (Section 2.4). Paediatric patients may benefit from this new approach since it could reduce the adverse effects reported in paediatric clinical trials where DMF is administered in gelatin capsules [9,10].

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2.2. Polymeric and Lipid Nanoparticles: Which Applications in Paediatrics?

Adapted from:

Polymeric and Lipid Nanoparticles: Which Applications in Paediatrics?

Noelia Nieto González, Antonella Obinu, Giovanna Rassu, Paolo Giunchedi, Elisabetta Gavini

Pharmaceutics **2021**, Volume *13, Issue* 5, 670.

1. Introduction

The European Union's (EU) Paediatric Regulation took effect in 2007. It aimed to encourage research in age-appropriate formulations and to raise high-quality information on paediatric medicines. This Regulation was inspired by United States (US) Pediatric Legislation, whose legislative approaches have been developed since 1997 [1]. Since the 1980s, it has been argued that paediatric patients deserve the same level of health care as the rest of the population [2,3] (**Figure 1**). The report published in 2017 by the European Commission (EC), on the 10th anniversary of the EU Regulation showed an increase in children's medicines. The most significant advances have been made in the treatment of diseases common in adult and paediatric patients.

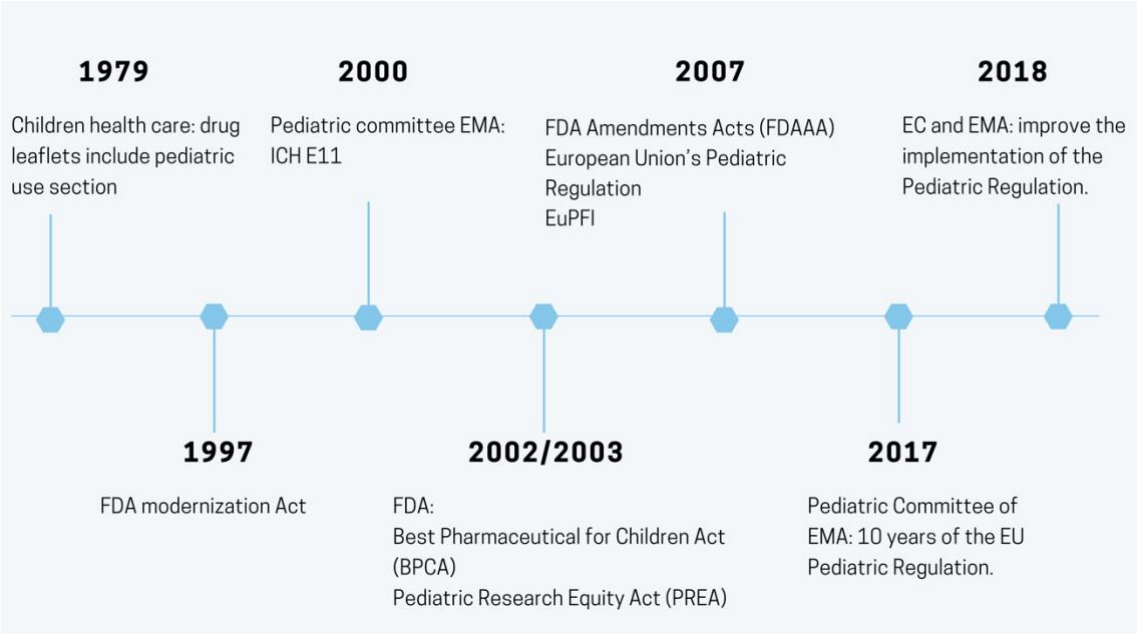

Figure 1. Schematic representation of the key milestones in the Regulation of paediatric medicines.

Nevertheless, in the context of rare or exclusive children's diseases (e.g., some form of tumours), the impact was minor [4]. However, since the Regulation's implementation, from 2007 to 2015, 238 new medicines for paediatric patients and 39 new formulations appropriate for this population were authorized in the EU [2]. The Paediatric Committee at the European Medicines Agency (PDCO) has established an inventory that defines the requirements for various pathologies and drug formulations in different age groups in the paediatric population to promote their investigation [5]. The European Paediatric Formulation Initiative (EuPFI) was founded in 2007 to study the different needs related to paediatric formulation development in order to formulate better drugs for children [6]. On 20 March 2018, in a workshop organized by the EC and European Medicines Agency (EMA), one of the topics discussed was again identifying paediatric medical needs [4].

Several drugs are currently not formulated for administration to children. In hospitals, they are frequently formulated in galenic medicines, using adult dosage forms or off-label use. Surveys suggested that in many therapeutic areas, off-label use was higher than 50% [3]. Moreover, the use of extemporaneous preparation may result in an elevated risk of toxicity or low therapeutic dose, an uncertain bioavailability and an absence of documented information about the stability [7].

The development of paediatric medicines is complex because children cannot be considered small adults or a homogeneous group. The EMA classifies them into five groups: (i) preterm newborn infants, (ii) term newborn infants (0–27 days), (iii) infants and toddlers (1 month to 23 months), (iv) children (2–11 years), (v) adolescents (12–16 or 18 years). Each age group has different requirements considering the type of formulation and the administration route due to physiological, anatomical and pharmacokinetic changes [7]. The optimal considerations for paediatric formulations are (i) age-appropriate dosage forms, (ii) facility of preparations and instructions for use for careers, (iii) acceptability, (iv) choice and quantity of excipients, (v) use of alternative delivery systems, and vi) appropriate packaging to improve efficiency and avoid the risk of dosage errors [8]. Also, excipient choice for enhancing drugs is one of the main issues in the paediatric area. Excipients safely and commonly used in formulations for grown-ups could be harmful to children [7]. For example, regarding preservatives,

the use of benzyl alcohol should be considered carefully, and it is better to avoid it in young paediatric patients due to their immature metabolism [9]. Also, ethanol shows adverse synergistic effects on the central nervous system if combined with other drugs. For example, ethanol and dextromethorphan can cause an infant's death [10].

The United States Pediatric Formulation Initiative (US PFI) suggested applying new technologies for paediatric drug formulations. Nanotechnology is considered an innovative approach for reducing drug toxicity and improving drug efficacy. US PFI also argued for future applications of polymeric nanoparticles as a controlled drug delivery system over a long period [11]. This review aims to assess the nanoparticles (NPs) used or suggested as drug delivery systems in the paediatric field to tackle paediatric diseases' needs. The excipients used and the technological and biological properties of the nanosystems are reported.

2. Nanoparticles as Paediatric Formulations

NPs are particles with a size range from one to several hundred nanometers. The active agents are incorporated in the core or on the surface of the particles [12]. They can be used as delivery vehicles for small drugs, diagnostic imaging agents, genes, proteins and peptides. NPs can positively alter the pharmacokinetics of drugs and, if compared to conventional pharmaceutical dosage forms, lead to a different dose-response [13]. So, there are challenges in paediatric nanomedicine.

Regarding pharmacokinetics, the drugs formulated in nanosystems displayed a prolonged circulation time, increased half-life, reduced clearance, and increased mean residence time [14]. These improvements were obtained, for example, by loading doxorubicin, daunorubicin, vincristine and amphotericin B, in liposomes [12,13,14]. NPs show a fundamental role in modifying physicochemical properties, improving water solubility, stability and permeability of the drug [15]. Moreover, NPs provide advantages in paediatrics because they could reduce the dose of antineoplastic drugs and their side effects [16].

NPs can be classified according to their chemical and physical characteristics into polymeric NPs, lipid-based NPs, metal NPs, ceramic NPs, semiconductor NPs and carbon-based NPs [17]. Polymeric and lipid NPs (**Figure 2**) are the leading candidates in paediatrics because of their composition. Compared to

other NPs, the materials used to make them impart to NPs biocompatibility and biodegradability, non-immunogenicity and non-toxicity, and a high drug entrapment efficiency. Polymeric and lipid NPs are the most clinically approved NPs in therapy [18].

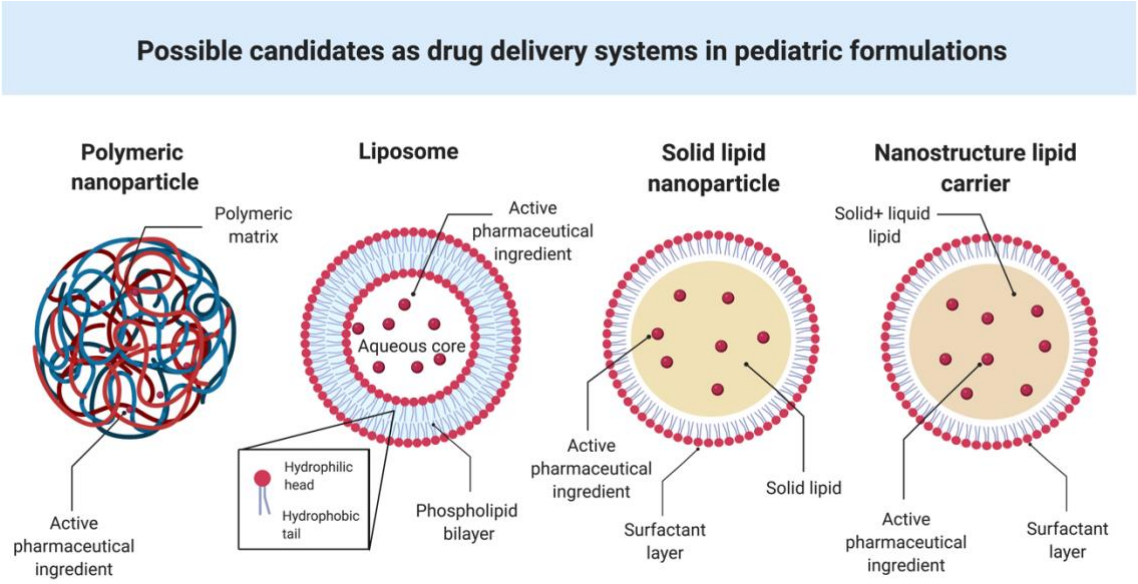

Figure 2. Schematic representation of possible candidates as drug delivery systems in paediatric formulations. It was created with *Biorender.*

Other kinds of NPs show promising results, such as gold and silver NPs or quantum dots. Metallic NPs are promising for cancer diagnosis and for inhibiting tumour growth. For instance, Liu and co-workers [19] developed gold NPs coated with polyethylene glycol, chitosan and polyethyleneimine loaded with siRNA to reduce paediatric brain tumour resistance radiation therapy. Also, titanium nanohybrids were used to fabricate palatable paediatric formulation azithromycin, masking its bitter taste [20]. Despite good prospects as drug delivery systems, the use of metal NPs is compromised by their biosafety. Several studies have revealed that they can alter the cell cycle, reducing the cell survival rate [21,22]. The development of NPs for paediatric use must consider paediatric physiology [13]. NPs smaller than 5.5 nm are filtered by the kidney [23], whereas if they are between 200 and 500 nm they are filtered by the spleen, like adults; all NPs show an increased accumulation in children's lungs regardless of the size, due to the airway diminished caliber and increased ventilation rate. Because the plasma

protein level is lower in neonates and young infants, opsonization may be decreased. Pharmacokinetic parameters (circulation time, area under the curve and distribution) of NPs (100–200 nm) could be altered because children exhibit an increased cardiac output, a reduced glomerular filtration rate and an increased organ surface area to weight ratio [13]. Both the innate and adaptive immune systems are immature in newborns and mature and acquire memory as they grow. Monocytes and macrophages is immature, as well as neutrophil functions, and the efficiency of the adaptive immune system to respond to T-cell-dependent antigens early are impaired in neonates compared with older children and adults [24]. Therefore, the mononuclear phagocyte system (MPS) clearance of negatively charged particles larger than 20 nm may be decreased as well as the shape-dependent uptake; also, the serum protein binding of positively charged particles could be altered because the serum protein distribution is age-dependent [13]. The immaturity of cytochrome enzymes modifies the toxicity and degradation of NPs in paediatric patients, depending on their composition [13]. As regards the composition, poly(ethylene glycol) (PEG) is tolerated in children, and the lipid composition allows an increased half-life of the NPs, avoiding macrophage uptake; adults and children older than one year react in a similar manner [13]. The fenestration sizes of healthy and tumour tissues do not differ in adults and children, so the size of NPs could be considered for exploiting the enhanced permeability and retention (EPR) effect [25].

3. Polymeric Nanoparticles

Polymeric NPs are a kind of solid NP whose matrix consists of natural, semi-synthetic or synthetic polymers. They can be biodegradable or non-biodegradable. Because non-biodegradable polymeric NPs can induce chronic toxicity and high immunological response after chronic use [26,27], biodegradable polymers are of great interest in paediatric nanomedicine. According to their origin, biodegradable polymers can be classified as natural and synthetic (**Figure 3**).

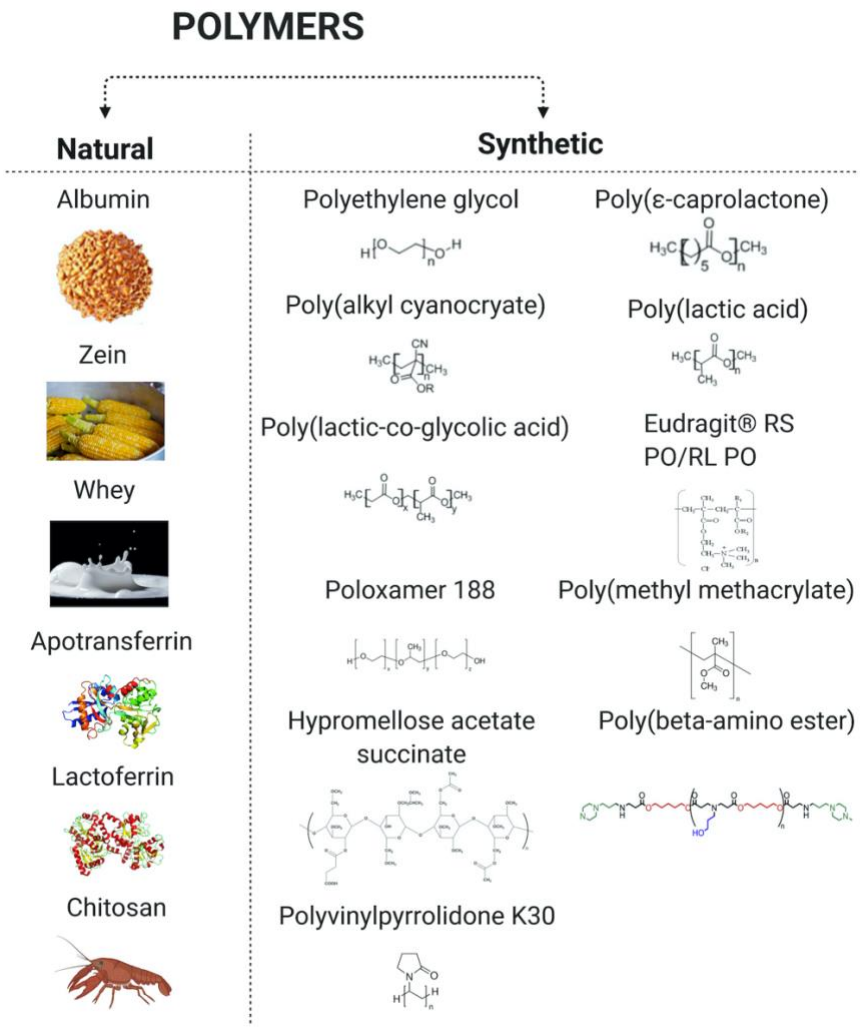

Figure 3. Natural and synthetic polymers used to make paediatric nanoparticles. It was created with *Biorender.*

Synthetic polymers, like aliphatic polyesters, such as poly(lactic-co-glycolic acid) (PLGA), poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), have been approved by the US Food and Drug Administration (FDA) as they are degraded as carbon dioxide and water by the human body [26,28]. Natural polymers can be proteins and polysaccharides. They are obtained from animal and vegetable sources. Examples of proteins are collagen, albumin, zein, and gluten, whereas polysaccharides can be chitosan, hyaluronate, cellulose, alginate, starch, etc. NPs based on these polymers display great properties such as biodegradability and low toxicity due to their metabolism [29]. Polymeric NPs possess a high drug loading capacity [27] and can improve both the pharmacodynamics and

pharmacokinetics of formulated drugs, enhance safety, and obtain more available and suitable formulations in children [13].

3.1. Natural Polymeric Nanoparticles

Albumin is a water-soluble and biodegradable animal protein. It can be bovine, human serum albumin, and ovalbumin (from egg white). This protein improves the release of drug bioavailability and decreases drug resistance in the body [29]. It is non-toxic and non-immunogenic [30]. Albumin-bound technology (named nab-technology) was developed and used for the delivery of drugs in the treatment of various types of tumours [31].

Paclitaxel bound-albumin NPs (Abraxane®) have demonstrated efficacy in adults with solid tumours [32]. This formulation is the first approved product based on protein nanotechnology chemotherapeutics. Furthermore, Abraxane® is the first natural polymeric nanoparticle used in paediatric patients. Abraxane® is a lyophilized powder administered as a suspension in sodium chloride solution [33]. The diameter of paclitaxel bound-albumin NPs was 130 nm, which prevents any risk of capillary obstruction [33]. There is a clinical trial (ClinicalTrials.gov Identifier: NCT01962103) in phase I/II in paediatric patients with recurrent or refractory solid tumours, in phase I of which the maximum tolerated dose was determined. Compared to the conventional paclitaxel formulation containing Cremophor EL and ethanol as solvents, Abraxane® has an improved safety profile [34]. Wagner and co-authors studied Abraxane® for the treatment of paediatric bone sarcoma. These albumin NPs produced *in vivo* a temporary regression of an osteosarcoma model and increased survival [35].

Currently, a phase I clinical trial is being developed by Children's Oncology Group based on albumin-bound rapamycin NPs administered by the intravenous route in association with irinotecan hydrochloride temozolomide, for the treatment of children with recurrent or refractory solid tumours (ClinicalTrials.gov Identifier: NCT02975882).

Catanzaro and collaborators developed redox-responsive bovine serum albumin NPs for cisplatin release in medulloblastoma treatment. This brain tumour is a primary central nervous system cancer in the paediatric population. The desolvation method was applied to prepare them to use N, N'-bis(acryloyl)cystamine (BAC) as a crosslinker. BAC led to the release of cisplatin

inside tumour cells where high glutathione levels were present. Spherical NPs showed a size range of 83 nm and a polydispersity index (PDI) of 0.3. Albumin nanocarriers were *in vitro* tested on Daoy medulloblastoma and healthy cells. Studies revealed a rapid and efficient internalization of the NPs and higher cytotoxicity on Daoy medulloblastoma cells than healthy controls. These redox-responsive bovine serum albumin NPs could improve the safety and efficacy of antineoplastic drugs in treating paediatric brain tumours [36].

NPs based on bovine serum albumin, hydroxyapatite, and paclitaxel were prepared for in situ treatment of osteosarcoma by Liu and collaborators. NPs were prepared by wet coprecipitation method, purified and freeze-dried; the particles obtained had 55 nm diameter, good drug loading (32.17%), sustained release properties of drug and calcium and low cytotoxicity. Moreover, NPs showed significant anticancer and inhibition of tumour metastasis activities and osteogenesis effects [37].

Islam and co-workers studied safe and effective zein-whey NPs (ZWP) for the oral delivery of lopinavir and fenretinide in children. Zein is a biopolymer derived from corn cell endosperm. It is composed of glutamic acid and hydrophobic amino acids, leucine, proline and alanine [38]. Zein has been certified by the FDA as Generally Recognized As Safe (GRAS) polymer [29]. This corn protein is used as a preservative coating for some food and pharmaceuticals [39]. In nanotechnology, zein NPs show great potential in providing a controlled release of the drug. Due to their hydrophobicity, zein NPs can protect the encapsulated contents from stomach acid [38]. Whey protein is a by-product of the manufacture of cheese or casein. This protein is interesting as a delivery material because of its GRAS status. It is composed of various globular proteins. Its main properties are the quality of binding lipophilic bioactive compounds and the ability to form hydrogel [40]. NPs were obtained by the phase separation method based on the different solubility of whey protein and zein in an aqueous solution and hydroalcoholic solvent. Zein formed the core and whey protein of the shell. The ZWP size ranged from 200 to 250 nm, and the encapsulation efficiency was over 70%. ZWP showed potent *ex vivo* and *in vivo* bioadhesive properties. *In vivo*, gastrointestinal distribution and pharmacokinetic studies were performed. As a result, ZWP had good sensory properties and sustained drug release; they

improved the oral bioavailability of lopinavir and fenretinide and raised their half-life without showing any immunogenicity in mice [41].

Ahmed et al. proposed apotransferrin or lactoferrin NPs containing carboplatin to treat retinoblastoma in children representing a common paediatric malignant intraocular cancer. Apotransferrin and lactoferrin are iron transporting proteins characterized by high biocompatibility. Nanoformulations were prepared by the sol-oil method. Apotransferrin-carboplatin and lactoferrin-carboplatin nanoparticle sizes were 140 nm and 263 nm, and the PDI were 0.155 and 0.340, respectively. The encapsulation efficiency was 50% for Apo- transferrin and 52% for lactoferrin due to the electrostatic interaction of carboplatin with proteins. Both protein NPs were able to enter into the cells, probably by receptor mediated endocytosis, and showed *in vitro* sustained intracellular drug retention and, thus, antiproliferative activity in retinoblastoma cells compared to the free drug [42]. These natural biodegradable proteins provide a formulation with a low toxicity profile for children.

Another natural polymer used for preparing paediatric NPs is chitosan, a chitin derivative characterized by biocompatibility and biodegradability [27]. Chen and collaborators proposed prednisolone-loaded chitosan NPs formulated in oral dispersible tablets for asthma treatment. Ionotropic external gelation method was used to prepare these NPs with sizes between 130 and 450 nm and a PDI of 0.310. The surfactant used was Tween® 80, and the crosslinker was tripolyphosphate. The chitosan-tripolyphosphate weight ratios affected the encapsulation efficiency and prednisolone solubility most when a 1:1 ratio was employed. NPs were spherical and with a smooth surface [43]. The oral dispersible tablets obtained by direct compression of NPs could represent an efficient delivery system for paediatric populations with swallowing problems.

3.2. Synthetic Polymeric Nanoparticles

As regards synthetic polymers, PEG is a biodegradable polymer often used in nanomedicine. The FDA approved it because of its biocompatibility and biodegradability [26]. Its hydrophilic nature does not accumulate in the tissues and can increase the drug solubility and stability in aqueous media [27].

Krishnan and co-authors developed NPs based on an amphiphilic block of the biodegradable copolymer consisting of PEG and poly(ε-caprolactone) (PCL). The

Nano- precipitation method was used to prepare dexamethasone-loaded block copolymer NPs to treat leukaemia in the paediatric population. This blood cancer is the most common in children and teens. The average particle size was 110 nm. *In vitro* cell and *in vivo* studies in mice from 4 to 8 weeks of age demonstrated no toxicity of unloaded nanoformulation. It was also possible to study dose reduction compared to the free drug, and it was observed a better quality of life and survival of mice [16].

Polymeric NPs of poly(isobutylcyanoacrylate) (PIBCA) based on the grafting of the anti-CD99 antibodies on the chitosan corona were developed by Ramon et al. The association of the antibodies on NPs' surface can be achieved by designing targeted NPs with biotinylated ligands and using the biotin-streptavidin coupling method. Redox radical emulsion polymerization was used to synthesize PIBCA/chitosan NPs. The dimensions were less than 100 nm, which enables a better distribution on the body. These polymeric NPs release siRNA, which acts on cell membrane glycoprotein CD99, and is overexpressed in these tumour cells. *In vivo* studies in mice revealed that the inhibition of Ewing sarcoma's gene expression was 78%. The different polymers allowed siRNA, a polyanionic hydrophilic molecule, to bond to the cationic and hydrophilic shell of the nanoparticle chitosan corona resulting in a longer blood circulation time [44].

Yu and collaborators prepared salinomycin-loaded PEGylated PLGA nanoparticles (SAL-NP). SAL-NP conjugated with CD133 aptamers (Ap-SAL-NP) have the potential to deliver the drug and attack CD133+ osteosarcoma cancer stem cells. Osteosarcoma is another childhood bone cancer. The FDA has approved PLGA as a safe material, and it is often employed in drug delivery systems that improve circulation time and permeability [27]. NPs were prepared by emulsion solvent evaporation. Particle size was around 150 nm, and drug encapsulation efficiency was nearly 50%. *In vitro* and *in vivo* studies in mice 4–6 weeks of age showed that Ap-SAL-NP could selectively kill CD133+ osteosarcoma cancer stem cells [45].

Majumdar et al. developed a formulation of luteolin loaded in PLA-PEG NPs. The FDA already approves the polymers used. PLA is characterized by hydrophobicity, biodegradability, biocompatibility and a safe profile [27]. PLA is the core of the nanoparticle. Meanwhile, PEG is the hydrophilic shell and luteolin is located inside the inner core of the nanoparticle. Luteolin is a flavonoid that

inhibits the activity of lung cancer and head and neck cancer cells; it has been investigated for paediatric use in chemoprevention (ClinicalTrials.gov Identifier: NCT03288298). This natural compound has low solubility in water, so it was incorporated in synthesized NPs. PLA-PEG NPs were prepared by
the same method mentioned above [46]; luteolin and the diblock copolymer PLA-PEG methanolic solution were added dropwise to 1% polyvinyl alcohol solution, then, nanoparticle suspension was filtrated and purified. The diameter of the particles was about 115 nm. Anticancer activity was demonstrated in *in vitro* studies. *In vivo* studies in mice aged from 4 to 6 weeks showed that PLA-PEG NPs inhibited lung cancer cells' growth and squamous cell carcinoma of head and neck cells compared with free luteolin [46].

Etoposide (VP-16) is a chemotherapy drug for glioma treatment; it is hydrophobic and has a poor brain parenchyma distribution. NPs of PLGA or PLGA/Poloxamer 188 loaded with VP-16 were developed by Callewaert and co-workers [47]. The particles were obtained by a modified spontaneous emulsification-solvent diffusion method; the size of NPs obtained was between 110 and 130 nm and a PDI below 0.2. The results on two glioma cell lines showed an enhanced antitumor efficacy: in fact, PLGA and PLGA/Poloxamer 188 particles induce an improvement in the drug's cytotoxicity activity.

Seremeta et al. studied micro-and NPs that consist of Eudragit® RS PO and Eudragit® RL PO loaded with benznidazole, a drug with low solubility, were studied to treat Chagas' disease in neonates and young children. Eudragit® RS PO polymers with low permeability and RL PO polymers with high permeability are copolymers of acrylic and methacrylic acid esters. Spray-drying was used to prepare microparticles, while NPs were prepared by nanoprecipitation and freeze-drying. Pluronic® F68 was used as a steric stabilizer in NPs and was suitable for keeping the optimal particle size. The nanoparticle size was between 200 and 300 nm and a PDI below 0.2, whereas microparticles were below 2 μm. The *in vitro* dissolution rate of benznidazole was increased, and the nanocarriers' release was due to the diffusion from the polymer matrix [48].

Deng and co-workers recently developed lopinavir and ritonavir NPs based on Eudragit® E PO as new orodispersible drug delivery systems. NPs were obtained by nano- precipitation method using methanol for dissolving drugs and polymer and a Kolliphor® P 188 aqueous solution. After the removal of the organic

solvent, nanosystems were purified and freeze-dried. The powders were rapidly dispersible in water (in about 7 s) with a particle size of 158.2 ± 1.1 nm and 125.2 ± 3.7 nm for lopinavir and ritonavir NPs, respectively. NPs were able to mask the unpleasant taste and improve the dissolution and oral bioavailability of drugs compared to commercial tablets (Kaletra®) and raw drugs [49].

Zaritski and collaborators studied galactomannan-based NPs for obtaining selective intratumoral accumulation in paediatric sarcomas. Galactomannan was hydrolysed and grafted with poly(methyl methacrylate) using a 2:1 weight ratio. Galactomannan is a biocompatible and biodegradable polysaccharide, while poly(methyl methacrylate) is a biocompatible and non-biodegradable polymer. NPs of 141 nm in size and a PDI of 0.11 were selected for *in vitro* and *in vivo* studies due to their suitable size. The encapsulation capacity was demonstrated with imatinib. NPs were internalized in most of the cells that overexpress actively target glucose transporter-1 (GLUT-1). This intratumoral accumulation was also confirmed in paediatric patient-derived models of solid tumours [50].

Monterrubio et al. developed polymeric NPs conjugated to anti-GD2 antibodies and loaded with topoisomerase-I inhibitor SN-38 to treat paediatric neuroblastoma. PLGA and carboxymethyl-poly(ethylene glycol)-b-poly(lactic acid) (PLA-PEG-COOH) were chosen as polymers. The surfactant used was polyvinyl alcohol. NPs were obtained by a single oil-in-water emulsion method. The mean size of particles was about 300 nm and a PDI below 0.3, and zeta potential of about −22 mV. High loading efficiency was obtained and an *in vitro* controlled drug release (48–72 h). The *in vivo* tumour penetration of the drug was increased in mice when released from targeted NPs compared with non-targeted ones or free SN-38, prolonging mice's survival [51].

Clofazimine NPs based on hypromellose acetate succinate (HPMCAS), lecithin and zein were studied by Zhang and co-authors. This poorly soluble drug is used in treating Cryptosporidiosis, a disease that can be lethal in children. HPMCAS enhances the drug solubility and, in conjunction with lecithin and zein, provides stability to the NPs. These NPs were prepared by the flash nanoprecipitation process and dried by freeze-drying or spray-drying to produce redispersible powders. HPMCAS NPs showed the greatest particle size stability. Particles with sizes in the range of 70–100 nm, PDI of 0.19–0.26 and high encapsulation efficiency of over 92% were obtained. This formulation improved the dissolution

rate of clofazimine and the supersaturation levels compared to the marketed formulation and, thus, was proposed as a paediatric fast-releasing oral formulation [52].

An oral nanosuspension for mefenamic acid delivery was investigated by Perween and collaborators. To improve its poor water solubility, mefenamic acid was incorporated in NPs composed of hydroxypropyl methylcellulose and Tween 80 or sodium dodecyl sulfate as surfactants. Formulations were prepared by antisolvent precipitation method. NPs, with a size diameter of 510 nm and a PDI of 0.329, were stable for more than three weeks; the drug content was 84.41%. *In vitro* release study demonstrated that the drug release increased when high surfactant concentration was used; a reduction in the particle size was also obtained [53]. Further studies are necessary to explore their complete potential for paediatric delivery.

Uckun et al. characterized multi-functional NPs as a novel nanoformulation anti-acute lymphoblastic leukaemia. CD22-RTM (RNA trans-splicing molecules) was complexed with PVBLG-8, a cationic polypeptide, in a weight ratio of 1:10. This polypeptide displayed safety, biocompatibility, pharmacodynamic and biodistribution profiles. It was suitable for nucleic acid delivery *in vivo* due to its ability to enhance membrane permeability. The particle size was 105 nm and a PDI of 0.192. This nanosystem revealed anti-leukaemia activity *in vitro* [54].

A cancer gene therapy based on poly(beta-amino ester) (PBAE) NPs was studied by Choi and collaborators to treat brain tumours. PBAE was self-assembled with plasmid DNA encoding the suicide gene herpes simplex virus I thymidine kinase at a 1:1 volume ratio through transfection. The size range was 100–200 nm. These polymers are an interesting biomaterial for non-viral gene delivery due to their fast degrading under cytotoxicity. *In vivo* studies revealed an increased survival compared to previous studies, so it is a promising paediatric nanomedicine [55].

Table 1. Polymeric nanoparticles studied as drug carriers in paediatrics.

Drug	Composition	Paediatric use/ indication	Reference
Paclitaxel	Albumin	Recurrent or refractory solid tumours	[31-34]
		Bone Sarcoma	[35]
Cisplatin	Bovine serum albumin and BAC	Medulloblastoma	[36]
Paclitaxel	Bovine serum albumin and hydroxyapatite	Osteosarcoma	[37]
Lopinavir / Fenretinide	Zein and whey protein	Human immunodeficiency virus (HIV)	[41]
Carboplatin	Apo-transferrin and lactoferrin	Retinoblastoma	[42]
Prednisolone	Chitosan, Tween® 80 and tripolyphosphate	Asthma	[43]
Dexamethasone	Poly(ethylene glycol) and poly (ε-caprolactone)	Leukaemia	[16]
siRNA	Poly(isobutylcyanoacrylate) and chitosan	Ewing sarcoma's	[44]
Salinomycin	PEG-Poly(lactic-co-glycolic acid) and CD133 aptamers	Osteosarcoma	[45]
Luteolin	Poly(lactic acid), PEG and polyvinyl alcohol	Carcinoma of head and neck	[46]
Etoposide (VP-16)	Poly(lactide-co- glycolide) and poloxamer 188	Glioma	[47]
Benznidazole	Eudragit®RS PO, Eudragit®RL PO and Pluronic®F68	Chagas	[48]
Lopinavir / Ritonavir	Eudragit® E PO and Kolliphor® P 188	Human immunodeficiency virus (HIV)	[49]
Imatinib	Hydrolyzed galactomannan and poly(methyl methacrylate)	Sarcoma	[50]
SN-38 (irinotecan analog)	Poly(lactic-co-glycolic acid), carboxymethyl-poly(ethylene glycol)-b-poly(lactic acid) and polyvinyl alcohol	Neuroblastoma	[51]
Clofazimine	Hypromellose acetate succinate, lecithin and zein	Cryptosporidiosis	[52]
Mefenamic acid	Hydroxypropyl methylcellulose and Tween 80® or sodium dodecyl sulfate	Antipyretic	[53]
CD22-RTM	PVBLG-8 cationic polypeptide	Acute lymphoblastic leukaemia	[54]
Herpes simplex virus I thymidine kinasa (HSVtk)	Poly(beta-amino ester)	Brain tumours	[55]
Praziquantel	Polyvinilpirrolydone K30 and polaxamer 188	Schistosomiasis	[56]

González et al. developed a dry nanosuspension of praziquantel, using Poloxamer 188 and Polyvinylpyrrolidone K30 as stabilizers, to treat schistosomiasis. Preschool-age children are involved in the transmission of this parasitic infection. A marketed formulation is a tablet form, and it is not suitable for paediatric patients. Praziquantel is characterized by its low aqueous solubility and a solidly bitter and metal taste. High-pressure homogenization was used to prepare NPs that were dried by spray-drying and then redispersed in a sweet vehicle. This system improves the aqueous solubility and dissolution performance of praziquantel [56].

The polymeric NPs studied for the treatment of paediatric disease are displayed in **Table 1**.

4. Lipid Nanoparticles

Lipid-based NPs form colloidal dispersions. These drug delivery systems can be emulsions, vesicular systems, and lipid particulate systems [57]. Liposomes are vesicles having one or more phospholipid bilayers. Phospholipids consist of a hydrophilic head and a hydrophobic tail: the apolar chains face each other, whereas the polar heads are oriented towards the aqueous medium and in the central compartment forming the liposome. The hydrophilic drugs occupy the aqueous compartment; meanwhile, the lipophilic ones are attached to the liposomal lipid membrane bilayer by the hydrophobic interaction [57]. Liposomes range in size between 20 nm and 10 μm [58]. Solid lipid nanoparticles (SLNs) were identified as a viable alternative to various colloidal systems. SLNs have a spherical shape with a dimension ranging from 50 nm to 1000 nm, and they are composed of a solid lipid core stabilized in the aqueous medium by surfactants [57]. They present high biocompatibility because lipids are well tolerated by the body and have reduced cellular and systemic toxicity. SLNs improve drug stability due to the drug's protection from chemical and enzymatic degradation and enhance its bioavailability [59]. Due to the high lipid biocompatibility, they can be administered through all existing routes of administration. Since they consist of hardcore solid lipids at room temperature, SLNs can guarantee stability and controlled drug release and improve the drug delivery to its target.

Table 2. Lipid nanoparticles studied as drug carriers in paediatrics.

Drug	Composition	Paediatric use/ indication	Reference
Vincristine sulfate	Liposome based on Sphingomyelin:Cholesterol (60:40)	Lymphoblastic leukaemia	[61-63]
Cytarabin	Liposome based on Cholesterol:Triolein:Dioleoil phosphatidylcholine: Dipalmitoyl phosphatidyl glycerol (11:1:7:1)	Leptomeningeal dissemination	[25,61,64]
Daunorubicin	Liposome based on Distearoyl phosphatidylcholine: Cholesterol (2:1)	Acute myeloid leukaemia	[61,65]
Doxorubicin	Pegylated liposomal based on Hydrogenated soybean phosphatidylcholine:Cholesterol:PEG 200-DSPE (56:39:5)	Hodgkin lymphoma	[66-68]
Doxorubicin	Non-pegylated liposomal composed by Phosphatidylcholine:Cholesterol (55:45)	Non-Hodgkin lymphoma	[66,68]
Mifamurtide	Liposome based on Dioleoyl-sn-glycero-phosphoserine:Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (3:7)	Osteosarcoma	[61,69]
Daunorubicin/Cytarabine	Liposome composed by 1,2-Distearoyl-sn-glycero-3-phosphocholine:1,2-distearoyl-sn-glycero-3-phospho- (1-rac-glycerol):Cholesterol (7:2:1)	Acute myeloid leukaemia	[70]
Amphotericin B	Liposome based on Hydrogenated soy phosphatidylcholine: Cholesterol: Distearoyl phosphatidylglycerol (2:1:0.8)	Systemic fungal infections	[61,71,72]
Edelfosine/methotrexate	Lipid Nanoparticle based on Precirol® ATO 5 and Tween® 80	Osteosarcoma	[73]
Doxorubicin	Precirol® ATO 5, triethanolamine, oleic acid, Tween® 80 and EDTA	Osteosarcoma	[74]
Hydrochlorothiazide	Solid lipid Nanoparticle based on HP-beta-cyclodextrin, Precirol® ATO5 and Pluronic® F78	Hypertension	[75]
Hydrochlorothiazide	Nanostructure Lipid Carrier based on Precirol® ATO5, Tween® 80, Tween® 20 and castor oil	Hypertension	[76]
Hydrochlorothiazide	Solid lipid nanoparticle and nanostructure lipid carrier based on Precirol® ATO5 , Transcutol® HP, Gelucire® 44/14 and PluronicF68 or Tween® 80	Hypertension	[77]
Lopinavir/Ritonavir	Nanocapsule containing Oleic acid and-α- tocopheryl polyethylene glycol 1000 succinate and Aeropearl® 300	Human immunodeficiency virus (HIV)	[78]
Gemcitabine/Edelfosine	Nanoassembly based on squalenic acid and ether lipid	Osteosarcoma and neuroblastoma	[79]

Nanostructured lipid carriers (NLCs) differ from SLNs because their core is made up of both solid and liquid lipids. NLCs show higher drug loading capacity than SLNs because their nucleus is less organized [57,60].

Lipid drug delivery nanosystems are good candidates to encapsulate poorly water soluble, lipophilic drugs and poorly permeable drugs [60]; particular attention should be paid to the choice and concentration of lipids because, as mentioned above, they can reduce the clearance and increase the plasma half-life of the formulation [13,60].

Lipid-based NPs studied for the treatment of paediatric diseases are summarized in **Table 2**.

4.1. Liposomes

Several liposomes are on the market and have been evaluated in children (**Table 3**). They were developed for use in the adult population, but they have been used in clinical trials in the paediatric population. The administration route is intravenous, most of which are used in anticancer therapy. Vincristine sulphate liposome injection (marketed as Marqibo®) is a formulation used in treating lymphoblastic leukaemia. Marqibo® liposome is composed of sphingomyelin and cholesterol in approximately 60:40 molar ratio with a particle size around 100 nm [61]. It was approved in adults by the FDA in 2012, and a phase I clinical trial was reported utilizing Marqibo® (ClinicalTrials.gov Identifier: NTC02879643) in paediatric patients aged <21 years with relapsed or chemotherapy-refractory solid tumours or leukaemia [62]. The study did not show dose-limiting neurotoxicity with the adult FDA-approved dose. Non clinical studies demonstrated that these liposomes could increase the residential time, accumulation in the tumour tissue and controlled release of vincristine in the tumour compared with conventional vincristine [63].

Intrathecal liposomal cytarabine (marketed as DepoCyt® in 1999) has been approved in children with leptomeningeal dissemination [25]. Currently, a clinical trial in phase 2 (ClinicalTrials.gov Identifier NCT02393157) by New York Medical College is in progress. Depocyt® is administered intrathecally in children, adolescents, and young adults with relapsed CD20 positive B-cell Non-Hodgkin Lymphoma. This multivesicular liposome platform composed of cholesterol, triolein, dioleoil phosphatidylcholine and dipalmitoyl phosphatidyl glycerol allowed

a more extended drug-controlled release with higher exposure than standard cytarabine, avoiding frequent multiple injections of this drug characterized by its short half-life into the cerebrospinal fluid [61]. Pharmacokinetics and toxicity were evaluated in children and adolescents with malignant brain tumours because the variation in intracranial volume with age is a difficulty in dosage in the paediatric population. The doses studied exhibited sufficient drug exposure for at least one week and appeared well tolerated [64].

In 1996, daunorubicin (DaunoXome®) was approved by the FDA to treat advanced human immunodeficiency virus (HIV)-related Kaposi's sarcoma in adults. A phase III clinical trial of liposomal daunorubicin added to granulocyte colony-stimulating factor showed a good result for paediatric relapsed acute myeloid leukaemia [65]. Studies suggest that liposomal formulation is effective and less cardiotoxic in paediatric patients. Daunorubicin was encapsulated into small unilamellar liposomes with a range of 45 nm, composed of highly purified distearoyl phosphatidylcholine and cholesterol, in a concentration of 2:1 mole ratio, respectively [61]. The liposome structure enhances the stability and increases the half-life of DaunoXome® compared with free Daunorubicin [61].

Doxil® (approved 1995), the first nanomedicine available on the market, is pegylated liposomal doxorubicin (90 nm) consisting of hydrogenated soybean phosphatidylcholine, cholesterol and N-(carbonyl-methoxypolyethyleneglycol2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium (56:39:5 molar ratio), whereas Myocet® (marketed in 2000) is non-pegylated liposomal doxorubicin (100–200 nm) consisting of phosphatidylcholine and cholesterol (55:45 mole per cent) [66]. Both liposomes have been studied in clinical trials in paediatric patients. Doxil® was combined with other drugs in a clinical trial (ClinicalTrials.gov Identifier: NCT00006029) to treat Hodgkin lymphoma. The results have shown a well-tolerated regimen for relapsed Hodgkin lymphoma with similar results to more toxic regimens [67]. However, Myocet® with other drugs have been evaluated in patients cardiopathic affected by non-Hodgkin lymphoma (ClinicalTrials.gov Identifier: NCT01009970). The results are not yet publicly available. These two formulations have different pharmacokinetics and toxicity profiles, but doxorubicin free liposomal reduces this drug's cardiotoxicity characteristics [68]. Doxil® pharmacokinetics was evaluated in paediatric patients. The studies

demonstrated differences compared to adults; a low half-life may be associated with decreased protein binding of the drug due to reduced plasma protein concentrations in the paediatric population [13].

Mepact® is a liposomal formulation containing mifamurtide, a synthetic analogue of a cell wall component of Mycobacterium species that stimulates the immune system to destroy the tumour cells. Mepact® is composed of multilamellar liposomal formulation under 100 nm, based on synthetic lipids such as 1,2-dioleoyl-sn-glycero-phosphoserine and palmitoyl-2-oleoyl-sn-glycero-3-phosphocoline (3:7 molar ratio) [61]. Mepact® is indicated for treating high-grade resectable non-metastatic osteosarcoma after macroscopically complete surgical resection in children, adolescents, and young adults; the market authorization was in 2009 as a second-line treatment for children [69].

In 2017, Vyxeos® or CPX35 was approved for recurrent leukaemia in adults [70]. This lipid formulation is the first liposome loaded with Daunorubicin and Cytarabine. This medicine contains bilamellar liposome composed of 1,2-Distearoyl-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-glycero-3-phospho-(1-rac-glycerol), and cholesterol corresponds to the molar ratio 7:2:1 and with a diameter of 100 nm [70]. A phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT02642965) reports that Vyxeos® can reduce the heart's side effects and studies the best dose liposome-encapsulated in a paediatric population from 1 to 21 years. Currently, a phase I pilot study (ClinicalTrials.gov Identifier: NCT03826992) is testing the safety and tolerability of venetoclax combined with Vyxeos®. The liposomal formulation exerts a synergistic effect on leukaemia cells compared with free drugs that show a different pharmacokinetics profile. An enhancement in overall survival has been observed in adult patients treated with the liposomal formulation compared to those tested with the free drug [70]. In the future, these data could be extrapolated to the paediatric population.

Liposomal Amphotericin B (marketed as AmBisome®) is used in treating systemic fungal infections, approved in 1997 in adults [61]. AmBisome® are small unilamellar liposomes of 100 nm made up of hydrogenated soy phosphatidylcholine, cholesterol and distearoyl phosphatidylglycerol (2:1:0.8 molar ratio) [61,71]. The lipidic complex interacts with Amphotericin B providing stability to the formulation, reducing toxicity and achieving a prolonged blood residence time [71]. Immunocompromised children and adolescents were

admitted to a study to assess the safety, tolerability and pharmacokinetics of AmBisome®. The results show that a similar liposomal formulation dose can be used in adult and paediatric patients, but the latter can develop azotemia [72].

Table 3. Nanoparticle formulations in the market.

	Drug	Approval	Composition	Paediatric use/ indication	Reference
Abraxane®	Paclitaxel	2005	Natural polymer: albumin	Solid tumours	[31-34]
Marqibo®	Vincristine sulfate	2012	Sphingomyelin:Cholesterol (60:40)	Lymphoblastic leukaemia	[61-63]
DepoCyte®	Cytarabin	1999	Cholesterol:Triolein:Dioleoil phosphatidylcholine: Dipalmitoyl phosphatidyl glycerol (11:1:7:1)	Leptomeningeal dissemination	[24,61,64]
DaunoXome®	Daunorubicin	1996	Distearoyl phosphatidylcholine: Cholesterol(2:1)	Acute myeloid leukaemia	[61,65]
Doxil®	Doxorubicin	1995	Hydrogenated soybean phosphatidylcholine:Cholesterol:PEG 200-DSPE (56:39:5)	Hodgkin lymphoma	[66-68]
Myocet®	Doxorubicin	2000	Phosphatidylcholine: Cholesterol (55:45)	Non-Hodgkin lymphoma	[66,68]
Mepact®	Mifamurtide	2009	Dioleoyl-sn-glycero-phosphoserine:Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine(3:7)	Osteosarcoma	[61,69]
Vyxeos® o CPX35	Daunorubicin/Cytarabine	2017	1,2-Distearoyl-sn-glycero-3-phosphocholine:1,2-distearoyl-sn-glycero-3-phospho- (1-rac-glycerol):Cholesterol (7:2:1)	Acute myeloid leukaemia	[70]
AmBisome®	Amphotericin B	1997	Hydrogenated soy phosphatidylcholine: Cholesterol: Distearoyl phosphatidylglycerol (2:1:0.8)	Systemic fungal infections	[61,71,72]

4.2. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers

González-Fernández et al. proposed different oral lipid NPs to deliver anti-osteosarcoma drugs [73]. Firstly, they prepared lipid NPs encapsulating edelfosine or methotrexate and evaluated the *in vitro* activity in various primary and commercial osteosarcoma cell lines. Edelfosine-based lipid NPs were obtained using Precirol® ATO 5 and Tween® 80 by hot homogenization method

followed by high shear homogenization and ultrasonication. The size and the zeta potential of the obtained particles are about 137 nm and −20 mV, respectively. Osteosarcoma cell studies demonstrated that the encapsulation of edelfosine in lipid NPs increased the antitumor efficacy due to the higher uptake in primary and metastatic cells [73]. The same group prepared doxorubicin lipid NPs and studied the antitumor efficacy alone and combined with edelfosine lipid NPs. Doxorubicin lipid NPs were prepared by three different methods (O/W emulsion, W/O/W emulsion, both followed by solvent evaporation and hot melting homogenization), but only the hot melting homogenization allowed them to obtain small homogeneous particles (95 nm size and 0.2 PDI) and high drug loading [74]. The lipid phase was constituted of Precirol® ATO 5, triethanolamine, oleic acid and doxorubicin; the aqueous phase contained Tween® 80 and EDTA. This nanoformulation was able to counteract neoplastic cells' drug resistance by increasing cellular absorption and reducing doxorubicin's efflux. Moreover, a synergistic effect of doxorubicin and edelfosine lipid NPs was demonstrated [74]. A paediatric oral liquid formulation of hydrochlorothiazide for paediatric hypertension was investigated by Cirri et al. It is a drug characterized by low solubility and poor permeability. Drug–cyclodextrin complex was loaded in solid lipid NPs. SLNs were obtained by a hot high-shear homogenization method followed by ultrasonication. The leader formulation was prepared with Precirol® ATO5 as lipid and Pluronic®F78 as surfactant. SLNs revealed a size of 380 nm, and hydroxylpropyl-beta-cyclodextrin improved NPs stability. This nanoformulation was tested on Sprague–Dawley rats showing a controlled drug delivery and improved oral bioavailability compared with drug suspension [75]. Afterwards, they studied nanostructured lipid carriers (NLC) instead of SLNs. The microemulsion technique was chosen to produce hydrochlorothiazide lipid NPs with a 300–400 nm range size. The NLC that demonstrated better results consisted of Precirol® ATO5 as solid lipid, Tween® 80 and Tween® 20 as a surfactant and co-surfactant, and castor oil as liquid lipid. Formulation orally administered in Sprague–Dawley rats showed an enhanced therapeutic effect. Moreover, this NLC showed better sustained drug release and increased diuretic effect than SLNs [76]. Starting from these results, the same authors recently published a new formulative study regarding SLN and NLC as carriers of hydrochlorothiazide based on Precirol® ATO5 and Transcutol® HP as solid and

liquid lipids, respectively, and Gelucire® 44/14, PluronicF68 or Tween® 80 as surfactants. As demonstrated previously, NLC showed a better loading capacity and drug release rate than SLN; in particular, Gelucire® 44/14 improved particle size, entrapment efficiency, drug release properties and storage stability of new SLN and NLC and also concerning previously developed formulations [75,76]. Due to their non-toxicity and the ability to pass the Caco-2 cell monolayer, these formulations appear suitable for developing oral liquid paediatric formulations [77].

4.3. Other Types of Lipid Nanoparticles

In situ self-assembly nanoparticles (ISNPs) were also proposed. Lopinavir and ritonavir loaded in self-assembly nanocapsules were studied by Pham and collaborators. They developed solid palatable granules based on oleic acid and α-tocopheryl polyethylene glycol 1000 succinate (1:2 ratio) plus colloidal silicon dioxide (Aeropearl® 300); when in contact with water or biological fluids, oleic acid and α-tocopheryl polyethylene glycol 1000 succinate (1:2 ratio) self-assemble to form ISNPs encapsulating both drugs. The size of ISNPs obtained was 158 nm. When orally administered in rats, improved lopinavir bioavailability and concentration in the brain and lymphoid tissues, sanctuary sites of HIV, were observed [78].

Rodríguez-Nogales and co-workers studied a suitable candidate for childhood cancer therapy. They prepared and characterized nanoassemblies using squalenoyl-gemcitabine and alkyl-lysophospholipid edelfosine by nanoprecipitation method. The nanoassemblies with a mean size of 50 nm showed high uptake in human osteosarcoma cells and an excellent antitumor profile. *In vivo* studies were carried out in 6–8 weeks old mice and revealed an enhancement in gemcitabine and edelfosine pharmacokinetic profiles [79].

5. Considerations and Challenges in the Application of Polymeric and Lipid Nanoparticles in Paediatrics

The literature analysis showed that polymeric and lipid NPs have been widely studied to treat paediatric diseases. In particular, albumin NPs and liposomes are also used in clinical practice or are the object of clinical trials conducted with the paediatric population.

Several final considerations can be drawn. Firstly, all prepared formulations use excipients approved as food ingredients generally recognized as safe (GRAS) or as inactive ingredient for drug products. Knowledge of the excipient safety profile is one of the regulatory agencies' mandatory requirements for avoiding any potentially toxic or unsuitable excipients for children. Moreover, the preparation methods reported were also techniques that limit the use of organic solvents, mainly chlorinated ones. Concerning the size of NPs, most of the particles proposed/used have a mean diameter of 150 nm, mainly those intravenously administered, to avoid capillary obstruction and increase the loaded drug's pharmacokinetics. Secondly, all nanoformulations prepared are almost exclusively focused on cancer treatment, which is a challenge both for the adult and paediatric populations. Only a few NPs are intended to treat paediatric chronic diseases (e.g., hypertension, asthma). Therefore, nanomedicine applied in paediatrics remains a significant challenge in the near future.

NPs could indeed solve many of paediatric medicines' needs, such as developing age-appropriate formulations for each paediatric group, due to their ability: (i) to modify the physicochemical and pharmacokinetic properties of loaded drugs; (ii) to mask the unpleasant taste of drugs, thus improving the palatability; (iii) to be easily swallowable even for newborns; (iv) to achieve a prolonged drug release and, thus, the reduction of the number of doses and the enhancement of compliance both of patient and parents; (v) in drug targeting; (vi) to facilitate easy dose adjustments as children grow; (vii) and enable the combination of more drugs in one formulation (Drug Combination Nanoparticle Platform). Many of these advantages have been highlighted in a recent report, about innovative delivery systems for paediatric medicine, by the World Health Organization and Unitaid, which reports the NPs as a technology that may be applied to more than one dosage form type and/or route of administration, and which may have relevance for paediatric product development [80]. Moreover, foundations, such as The Center for Paediatric Nanotechnology of Emory University School of Medicine and Children's Healthcare of Atlanta, promote the development of paediatric nanomedicine focus on paediatric heart disease, thrombosis and infectious diseases [81].

The promising results of *in vitro* and *ex vivo* studies obtained with NPs, reported here, are certainly an encouraging basis for further research and their application

in clinical practice. However, the unavailable data on pharmacokinetics, dose, safety and efficacy of many drugs in paediatric patients limits the development of NP formulations. The need for clinical trials on the paediatric population was widely recognized and is stimulated by EU legislation. Currently, 1,534 trials with a EudraCT protocol are reported in the EU Clinical Trials Register database; an increase in the next years is desirable (https://www.clinicaltrialsregister.eu/; accessed on 26 April 2021).

For the effective use of NPs in paediatrics, an increase *in vivo* studies on nanoparticles' efficacy and safety are necessary. In addition to the trials listed above, recent clinical trials are scarce. Looking at the trials reported in the EU Clinical Trials Register database, only 31 trials concern nanoparticles and only 2 trials involve paediatric patients: one is related to the safety, tolerability, potential efficacy, and dose finding of INP20, an oral NPs for treatment of immunotherapy in peanut-allergic patients (EudraCT Number: 2018-003665-34); and the other the immunogenicity and safety of a respiratory Syncytial Virus (RSV) nanoparticle vaccine with aluminium in pregnant women and their infants. In the US ClinicalTrials.gov, three new clinical trials are also reported concerning the clinical assessment of oxiconazole SLNs in gel against tinea fungal infection (ClinicalTrials.gov Identifier: NCT03823040), the *in vivo* susceptibility of root canal bacteria to chitosan NPs (ClinicalTrials.gov Identifier: NCT03588351), and the side effects and best dose of albumin-bound rapamycin NPs when given together with temozolomide and irinotecan hydrochloride in treating paediatric patients with solid tumours (ClinicalTrials.gov Identifier: NCT02975882). The breakthrough of nanotechnology in paediatrics is also associated with nanomedicine development in adults, and adult physiologically based pharmacokinetic (PBPK) models continue to prevail over paediatric models.

Finally, the development of new drug delivery for the paediatric population is strongly related to the progress of *in vitro* models simulating characteristics of the targeted paediatric population, with the purpose of facilitating *in vitro–in vivo* correlations (IVIVC).

6. Conclusions

Polymeric and lipid NPs have been widely studied to treat paediatric diseases, but only a few are used in therapy and only to treat paediatric cancers. Great attention is paid to the excipients used, to the safe and easy-to-scale-up preparation methods and, of course, to the size in order to obtain safe carriers.

This state of art demonstrates that NPs are able to modify the physicochemical and pharmacokinetic properties, mask the unpleasant taste, obtain a prolonged or accelerated release and increase the efficacy of loaded drugs. Therefore, NPs could be favourable when developing new paediatric medicines not just to treat cancer but also for chronic disease, avoiding off-label use and extemporaneous formulations. Furthermore, NPs can be used as platforms for developing age-appropriate formulations for each paediatric group as required by the EMA and FDA.

The development of *in vitro* models to facilitate IVIVC, as well as the increase of *in vivo* studies on pharmacokinetics, safety and efficacy of both drugs and nanoparticles in paediatric patients, are necessary for guiding the research towards new paediatric drug delivery nanosystems.

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2.3. Surfactant-Free Chitosan/Cellulose Acetate Phthalate Nanoparticles: An Attempt to Solve the Needs of Captopril Administration in Paediatrics

Adapted from:

Surfactant-Free Chitosan/Cellulose Acetate Phthalate Nanoparticles: An Attempt to Solve the Needs of Captopril Administration in Paediatrics

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1. Introduction

One of the roles of the Paediatric Committee (PDCO) of the European Medicines Agency is establishing and regularly updating an inventory of paediatric medicine needs in various therapeutic areas. For the cardiovascular therapeutic area, the development of an age-appropriate formulation of captopril (CAT) is required for the treatment of hypertension and heart failure as well as for the treatment and prevention of diabetic nephropathy [1]. The childhood hypertension is becoming a worldwide public health problem due to the significant increase in the paediatric population. The prevalence of arterial hypertension in children and adolescents, in apparent good health, is estimated to be about 3.5% and increases to 25% if we consider overweight and obese children [2].

CAT is an angiotensin-converting enzyme (ACE) inhibitor with a short half-life; thus, it is administered frequently during the day [3]. It is a water-soluble drug, stable as solid, but unstable in solution where CAT undergoes an oxidative degradation of the sulfhydryl group [4]. At the moment, CAT is administered using an extemporaneous liquid formulation due to its limited water stability and the absence of a suitable approved formulation for the paediatric population [5]. These extemporaneous formulations usually contain antioxidants to reduce captopril degradation. The use of extemporaneous preparation may result in a degree of content variability, which in turn may lead to a risk of toxicity due to overdosing or sub-therapeutic effects due to underdosing [6,7]. Variable bioavailability and lack of documented information about the physical, chemical and microbial stability of the preparation are also potential issues. Furthermore, the off-label prescriptions of adult dosage forms are widespread to treat

congenital and acquired cardiovascular disease in paediatrics [8], with a high risk of adverse events, including hospitalisation and death [9,10].

Liquid dosage forms are the favourite dosage form of the age groups 0–6 and 7–12 years [11]. The acceptability of the dosage form is crucial for achieving good patient compliance. In particular, the relevant attributes of medicine are the tastiness of the medicine, the swallowability and the practicality. Swallowing a whole solid dosage form may be problematic for children, depending on their age [12,13]. Therefore, there is a need to develop a more appropriate paediatric formulation of CAT.

In the development of formulations, knowledge of the excipient safety profile is one of the regulatory agencies' mandatory requirements for avoiding potentially toxic or unsuitable excipients for children [14]. All excipients used in pharmaceutical formulations, and particularly those intended for use in paediatric products, should be demonstrated to be necessary for the formulation and should be used at the minimum required level. The age of the child needs to be considered when choosing the excipients to use and their use levels. In the context of this paper, whilst polysorbate 80 is widely used as a surfactant in paediatric formulations, its use can be associated with toxicity, especially in neonates [15]. Thus, we investigated whether a suitable nanoparticulate formulation could be produced without the need for surfactants.

Polymeric nanoparticles (NPs) have been used or suggested to tackle paediatric formulation needs, such as developing age-appropriate formulations for each paediatric age group, mainly due to their ability to modify the physicochemical and pharmacokinetic properties of drugs loaded into them, to be easily swallowable and to facilitate easy dose adjustments as children grow [16]. Polymeric nanoparticles are therefore proposed as innovative carriers for CAT. Our recently published paper proposed the preparation nanoparticles by only exploiting the interaction between cellulose acetate phthalate (CAP) and chitosan (CH) without using surfactants to obtain a nanoparticle dispersion in an aqueous medium; this can be utilised as such or for preparing other solid or liquid dosage forms for CAT delivery in paediatrics, allowing an easy dose adjustment as children grow. CAP is a coating material characterised by pH-dependent solubility that is resistant to acidic gastric fluids, but dissolves in a mildly acidic or neutral environment [17]. In addition, CAP presents negative charge groups in its

structure [18]. CH is a natural, non-toxic polysaccharide able to form an electrostatic complex with synthetic or natural polymers. CAP and CH are biodegradable and FDA-approved biocompatible polymers [19,20]. Previous authors investigated the ability of CAP and CH to form an interpolymer complex for developing packaging film [21], coating film [22], microspheres [23] and composite blends [24]. We hypothesised that CAP and CH interact due to negative charges of CAP and cationic amino groups of CH, leading to a polymeric matrix that entraps and protects CAT from chemical degradation in water. After administration, the interpolymer complex gradually dissociates in contact with biological fluids due to its pH-dependent solubility and releases CAT. Consequently, the *in vivo* biodistribution/accumulation of nanoparticles and surfactants is avoided, as well as their potential toxicity in children. Thus, a formulation study was conducted to identify the correct CAP: CH polymer ratio and select the best formulation.

2. Results and Discussion

The preliminary results of our research have shown that it is possible to prepare NPs based on CAP and CH loaded with a hydrophilic drug (captopril) without the need for surfactants using the nanoprecipitation method–dropping technique. One interesting finding was the ability of this easy and reproducible method to encapsulate captopril, as nanoprecipitation is mainly employed for the encapsulation of hydrophobic drugs [25,26]. The absence of surfactants could contribute to a safer formulation for paediatric patients [15].

A summary of the physicochemical characteristics is shown in **Table 1**. Based on the results obtained, NP-C dispersion was selected as the lead formulation and further characterised. The particle size of CAP:CH nanoparticles was influenced by the CH concentration. Indeed, NP-Cb showed a higher mean size than NP-Bb and NP-Ab ($p < 0.0001$); the same was true for NP-C compared to the other loaded NPs ($p < 0.0001$). These results agree with previous findings that show that the CH concentration is the most important factor affecting the NP size [27]. Whereas no significant size differences were found between the drug-free NP-Ab and NP-Bb and their drug-loaded counterparts (NP-A and NP-B ($p > 0.05$)), the loading of CAT significantly decreased the mean diameter of NP-C compared with NP-Cb ($p < 0.0001$). As reported by other authors [28,29], this phenomenon

could be due to an increase in the internal cross-linking during the preparation process between CH chains in the presence of the negatively charged CAT [30], inducing a contraction of NPs. All PDI values were around 0.2, indicating a homogeneous size distribution [28].

NP-Cb and NP-C exhibited a zeta potential of +55 ± 1.08 mV and +53.30 ± 0.95 mV, respectively, due to the presence of chitosan on the surface. The high zeta potential ensures the good stability of NPs in dispersion [31].

As reported in Table 1, NP-A had a low encapsulation efficiency. The presence of CH allowed an increase in the encapsulation of CAT, with the highest encapsulation efficiency being obtained when the 1:3 *w*/*w* CAP:CH ratio was used ($p < 0.0001$). This could be attributed to the electrostatic interactions between the CAT and CH [27].

Table 1. Composition and physicochemical properties of the NPs.

Formulation	Drug	Polymer	Size (nm)	PDI	Total drug in dispersion (%)	Encapsulation Efficiency (%)
NP-Ab	-	CAP	259.2±30.0$^{\Delta}$	0.10±0.07$^{\bullet}$	-	-
NP-Bb	-	CAP:CH 1:1 w/w	252.5±16.4$^{\Delta}$	0.21±0.09$^{\bullet}$	-	-
NP-Cb	-	CAP:CH 1:3 w/w	478.6±21.1$^{*\Delta}$	0.16±0.06	-	-
NP-A	CAT	CAP	259.8±27.7$^{¥}$	0.06±0.03§	103.9±3.2	11.7±8.8$^{\#}$
NP-B	CAT	CAP:CH 1:1 w/w	248.3±31.9$^{¥}$	0.23±0.04§	100.3±8.0	32.6±2.3$^{\#\$}$
NP-C	CAT	CAP:CH 1:3 w/w	427.1±32.7$^{*¥}$	0.17±0.09§	99.16±0.5	61.0±6.5$^{\#\$}$

*p value < 0.0001: NP-Cb vs NP-C. ¥p value < 0.0001: NP-C vs NP-A and NP-B. Δ p value < 0.0001: NP-Cb vs NP-Ab and NP-Bb. §p value < 0.05: NP-A vs NP-B and NP-C. •p value < 0.05: NP-Ab vs NP-Bb. #p value < 0.001: NP-A vs NP-B and NP-C; $p value < 0.0001: NP-B vs NP-C.

Morphological analyses were conducted on NP-C and NP-Cb dispersion by Scanning Electron Microscope (SEM). NPs were spherical in shape with a smooth surface (**Figure 1**). The drug did not affect the shape of these NPs and no drug crystals were observed. NPs appear smaller than the mean diameters measured by dynamic light scattering due to the dry condition during SEM studies [32].

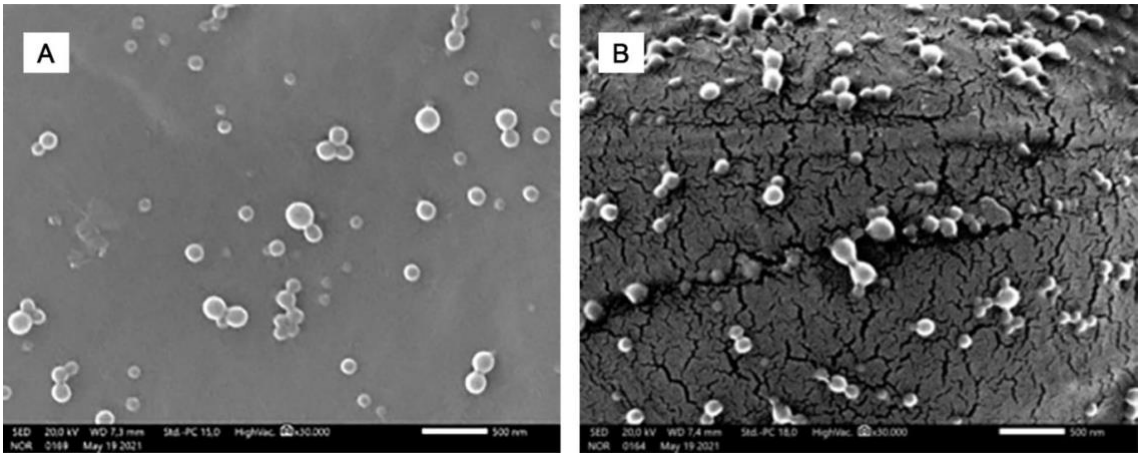

Figure 1. SEM images of NP-Cb (A) and NP-C (B) were acquired at 20 kV. Magnification: 30,000 times. Scale bar: 500 nm.

Subsequently, physical stability was investigated by monitoring the NP size and PDI over time (**Figure S1**). Good stability of NPs in dispersion was confirmed even without the presence of a surfactant due to the high zeta potential. The size was stable over 28 days regardless of the storage temperature ($p > 0.05$). Additionally, the PDI did show no significant variations from day 0 to day 28, whether stored at 25 °C or 4 °C (from 0.17 ± 0.09 to 0.15 ± 0.10) ($p > 0.05$).

Simultaneously, the chemical stability of CAT was evaluated in NP-C dispersion as compared to CAT in the aqueous solution (**Figure 2**). The total amount of CAT in the NP-C dispersion remained stable up to 28 days of storage both when stored at 25 °C and 4 °C ($p > 0.05$). Meanwhile, in the aqueous solution, the percentage of CAT on day 28 significantly decreased compared with CAT on day 0 ($p < 0.01$) and CAT on day 1 at 25 °C ($p < 0.05$). The main reason for CAT instability in the water solution is the oxidative degradation of the sulfhydryl group [33]. Perhaps surprisingly, degradation of CAT was avoided in the NP-C dispersion for both the encapsulated and free drug present. In other words, NP-C avoided CAT

degradation of both CAT loaded in the NPs and CAT dissolved in the medium. A weak ionic interaction between the drug, negatively charged, and the positive surface charge of nanoparticles could be involved. Free-CAT in the NP-C dispersion could also interact electrostatically with free CH chains.

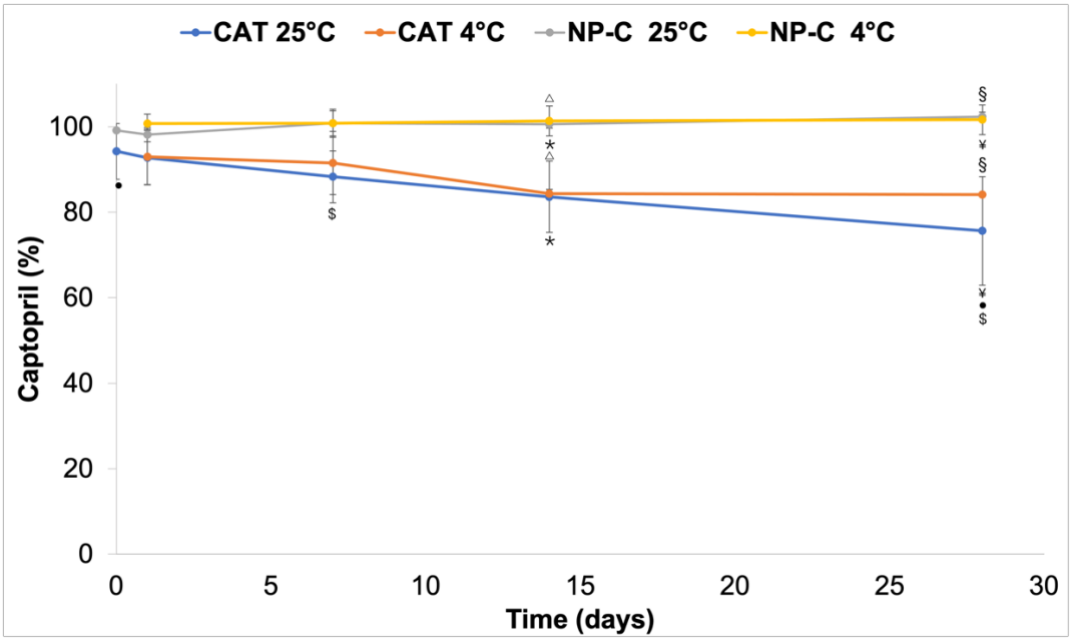

Figure 2. Chemical stability of CAT in aqueous solution (pH 3) and NP-C over 28 days at 4° and 25°C. *p value < 0.01: NP-C day 14 at 25°C vs. CAT day 14 at 25°C. ¥p value < 0.0001: NP-C day 28 at 25°C vs. CAT day 28 at 25°C. Δp value < 0.05: NP-C day 14 at 4°C vs. CAT day 14 at 4°C. §p value < 0.01: NP-C day 28 at 4°C vs. CAT day 28 at 4°C. •p value < 0.01: CAT day 0 vs CAT day 28 at 25°C. $p value < 0.05: CAT day 1 vs CAT day 28 at 25°C.

In order to evaluate the solid state, Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed. FT-IR spectra of CAT, NP-C, NP-Cb and the corresponding physical mixture are reported in **Figure 3**. The spectra of CAP and CH are shown in **Figure 4**. NP-Cb showed a wide band between 3000–3500 cm^{-1} due to the NH and OH stretching of CH and CAP, a shift of C=O stretching (from 1740 to 1732 cm−1), –C–O–C– stretching (from 1259 to 1248 cm^{-1}) and –C–O– stretching (from 1039 to 1031 cm^{-1}) peaks of CAP, as well as an intensity increase of amide bands of CH (1634 and 1521 cm^{-1}) (**Figure 4B**).

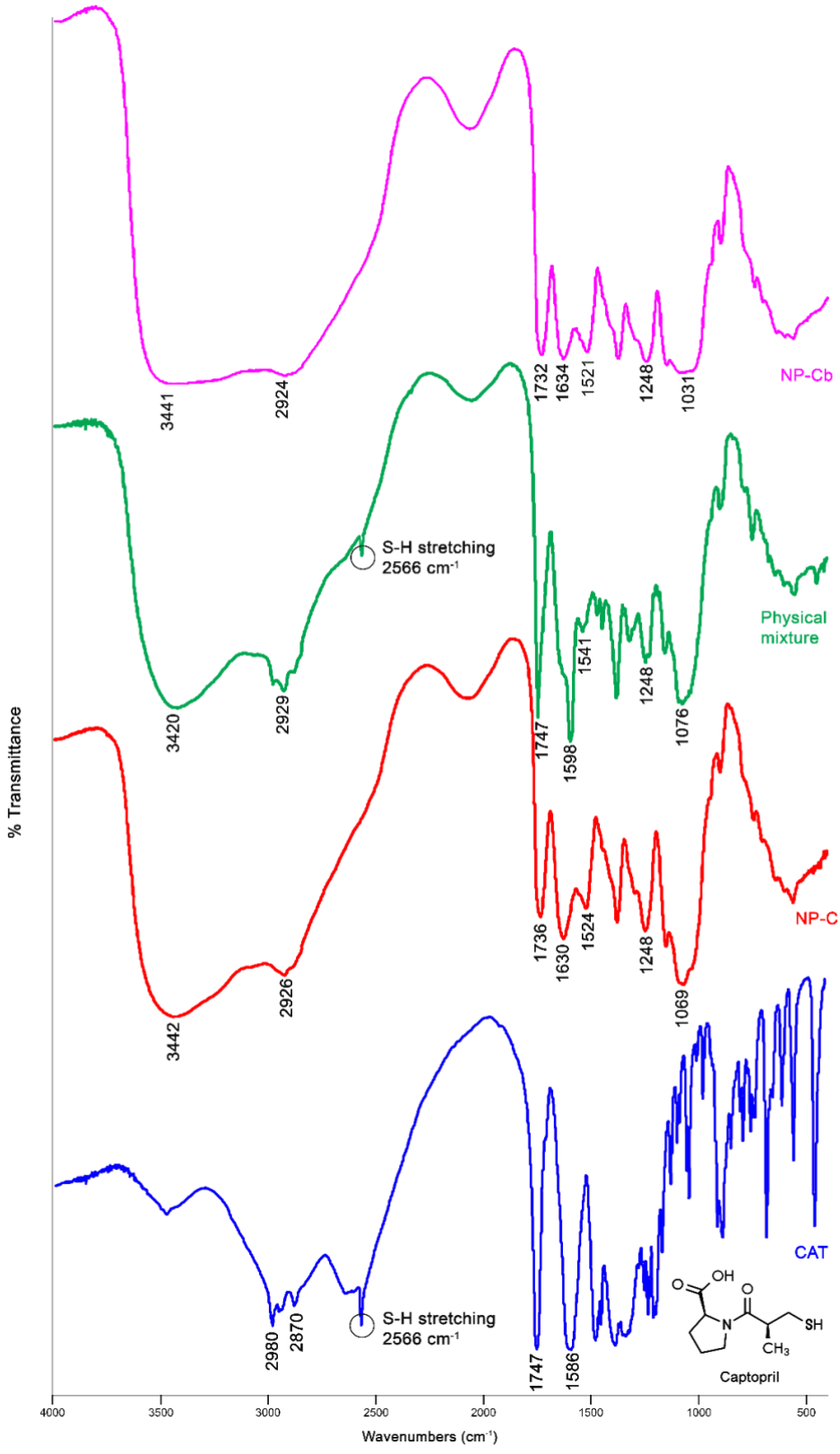

Figure 3. FTIR spectra of CAT, NP-C, NP-Cb and the physical mixture.

These changes to CH and CAP spectra are indicative of intermolecular interaction between carbonyl groups of CAP and OH groups in CH and between COO– group of CAP and the amine group of CH in the polymer matrix [21,34] The NP-C spectrum exhibits similar bands to NP-Cb, and it does not show the characteristic peak of CAT at 2566 cm^{-1} which is, however, visible in the physical mixture. This absence could be ascribed to the loading of CAT in the polymer matrix [35] or molecular interactions with the NP components that are not developed in the physical mixture and could be responsible for the shoulder at 2600–2450 cm^{-1} (**Figure 3**). In the physical mixture, the peak at 1747 cm^{-1} related to the carboxylic group of CAT is also evident.

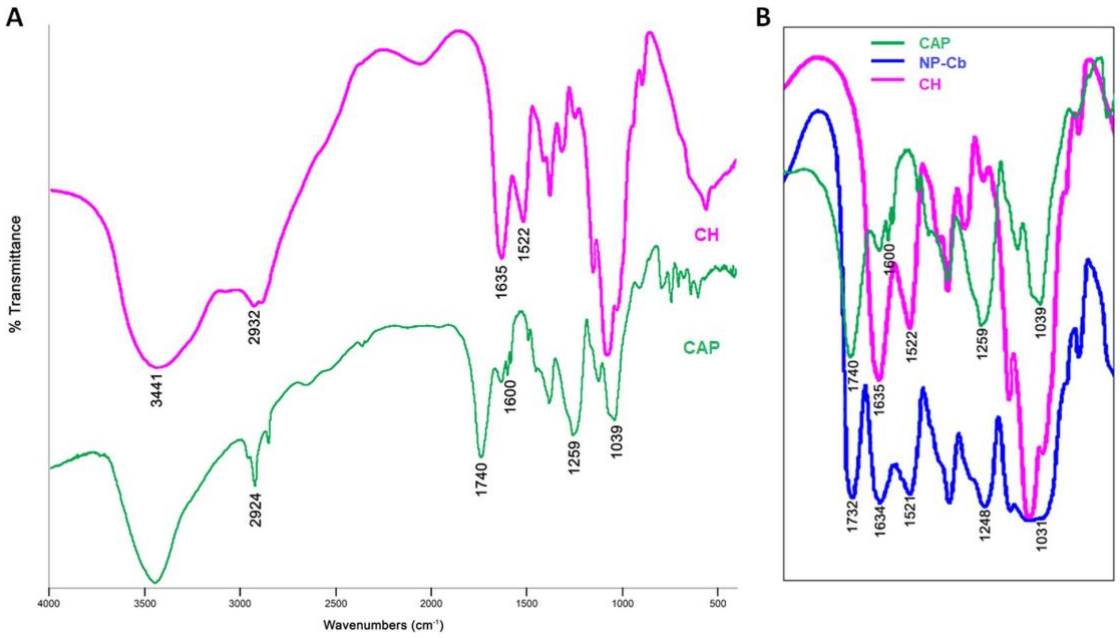

Figure 4. a) FTIR spectra of CAP and CH; b) zoom of the overlap of CAP, CH and NP-Cb spectra in the 1800-1000 cm^{-1} region.

In addition, X-ray Diffraction (XRD) analysis support our hypothesis. NP-Cb and NP-C are amorphous and show similar patterns (**Figure 5**), with humps located at 9.7, 12.0, 19.0 and 22.5°. The absence of captopril peaks in NP-C demonstrates that the drug contained in the NPs does not have a crystalline structure; this means that the preparation process of the nanoparticles has determined captopril amorphization. NP-Cb and NP-C are amorphous and show similar patterns (**Figure 5**), with humps located at 9.7, 12.0, 19.0 and 22.5°. The

absence of captopril peaks in NP-C demonstrates that the drug contained in the NPs does not have a crystalline structure; this means that the preparation process of the nanoparticles has determined captopril amorphization. No change in the therapeutic properties of CAT is expected after the administration of NP-C. It has been reported in the literature that amorphous CAT complexed by cyclodextrin administered *in vivo* reduced mean arterial pressure similar to that upon crystalline CAT [36].

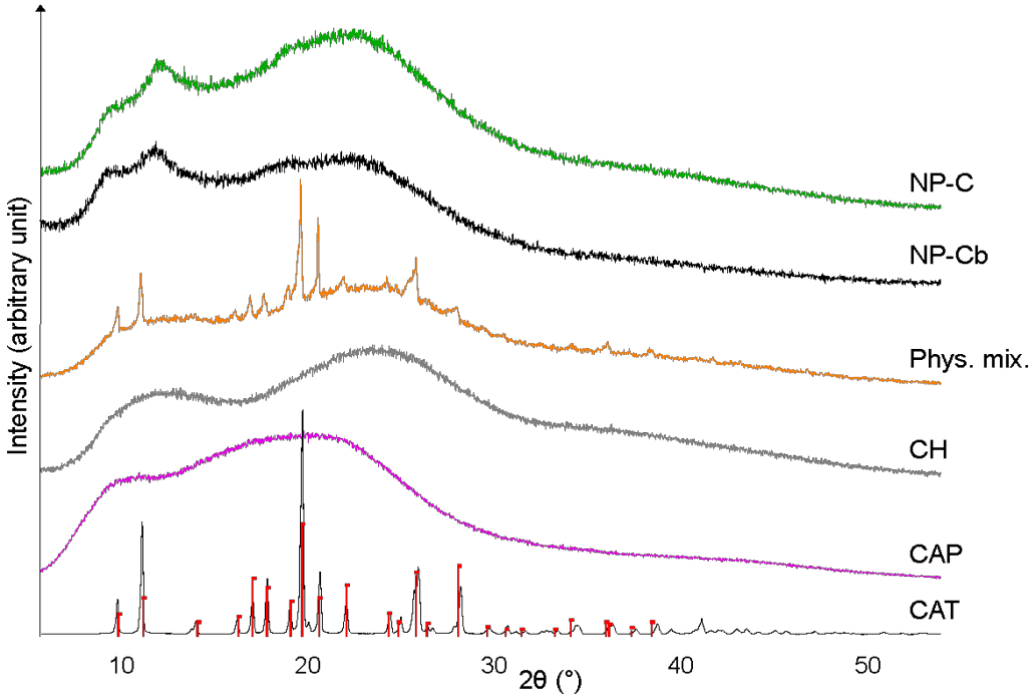

Figure 5. XRD patterns of CAT, CAP, CH, Physical mixture, NP-Cb and NP-C; red bars: Powder Diffraction File 35-1916 (captopril).

Thermal analysis (TG-DTG-DTA) revealed that the DTA curve of CAT shows a sharp endothermic peak at 109 °C, determined by the melting of captopril (**Figure 6a**). The physical mixture displays curves similar to CH (**Figure 6c,d**). The presence of crystalline captopril is revealed by the weak but sharp melting endotherm at 107 °C along the DTA. TG and DTG curves of NP-Cb and NP-C are superimposable (**Figure 6e,f**). Their profiles are similar to those of the physical mixture, although the main DTG peak appears sharper and exhibits a slightly lower temperature (212 °C vs. 221 °C). The DTA profiles of NP-Cb and NP-C are also similar (**Figure 6e,f**), except for the weak endothermic peak at 195

°C (due to CAP, see above), which is only noticeable in NP-Cb. NP-C contains the same amount of captopril as the physical mixture, but the drug in the nanoparticles is amorphous, this explains the absence of the captopril melting peak along the DTA of NP-C.

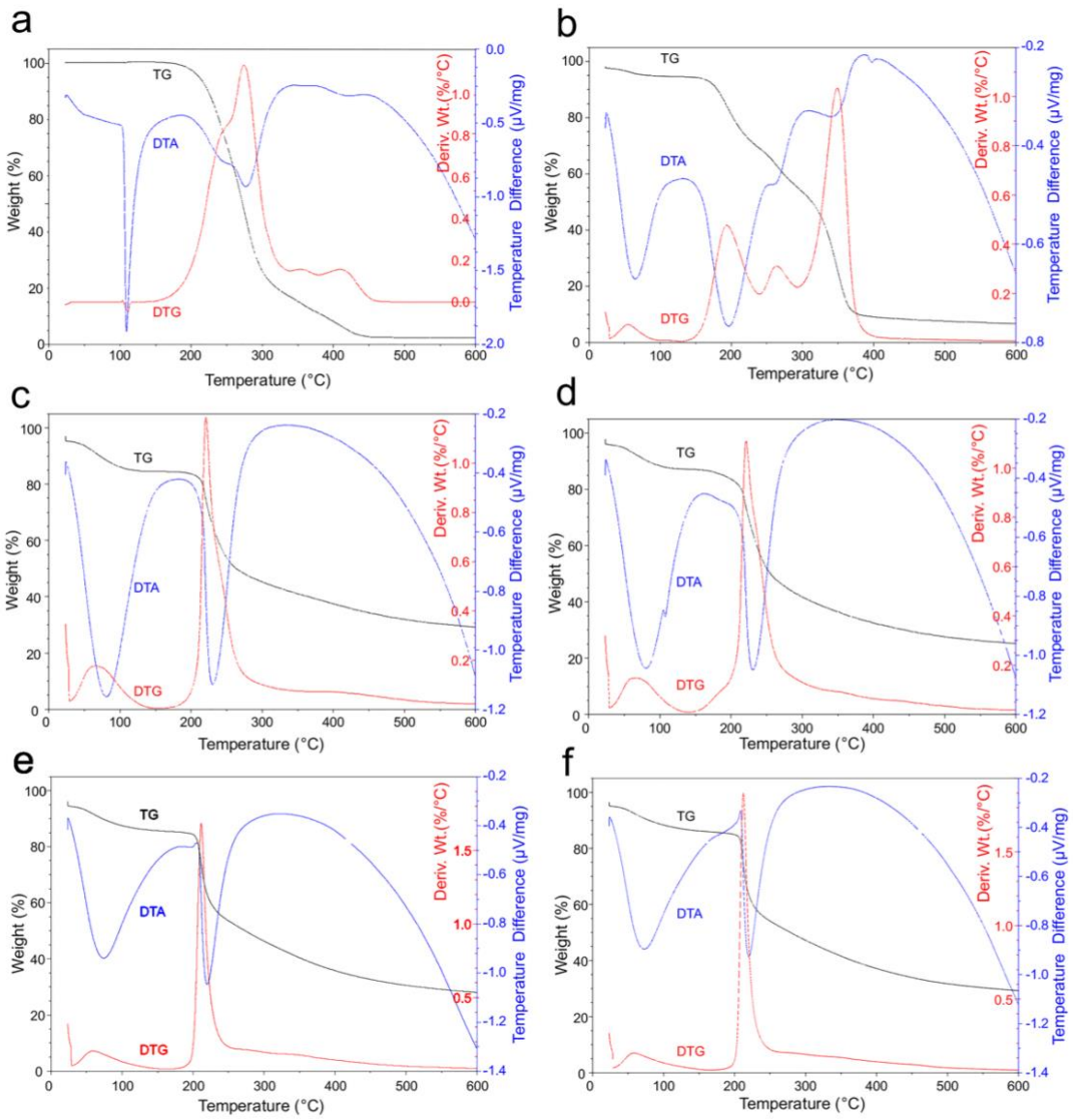

Figure 6. TG, DTG, and DTA curves of: a) CAT; b) CAP; c) CH; d) Physical mixture; e) NP-Cb; f) NP-C.

In vitro drug release study of NP-C dispersion (**Figure 7**) was performed using pH 7.4 phosphate buffer as medium to simulate the biological fluid regardless of the administration route. CAT is released from NP-C with linear kinetic (R^2 = 0.983) from 0 to 2 h, reaching 70% of the amount tested after 2 h and 84.81 ± 6.07% after 24 h. CAT from the CAT solution, in comparison, reached 67.47 ± 3.24% of the amount expected at the end of the test; statistical analysis of 24 h release data indicated a significant difference ($p < 0.05$). Furthermore, these results demonstrated that NP-C in contact with pH 7.4 gradually dissociates due to the changes in the charges of both polymers and CAT resulting in CAT release [30]. This behaviour was also confirmed by the size analysis of NP-C at different pHs: as reported in **Figure 2S** (APPENDIX 4.2: Section 2.3), the mean diameter decreased as the pH of the medium increased from 3 to 7, confirming the pH dependent of the interpolymer complex. In fact, NP preparation takes place at pH 3, where the amino groups of CH are protonated, so the CH chains from the surface of the nanoparticles are in an extended conformation. At pH = 7.4, the carboxylic groups of CAP are dissociated, but the CH is in neutral form, so the interpolymeric complex dissociates. This dissociation leads to the disappearance of the NPs, and any possible toxicity of the nanodelivery system for children is prevented.

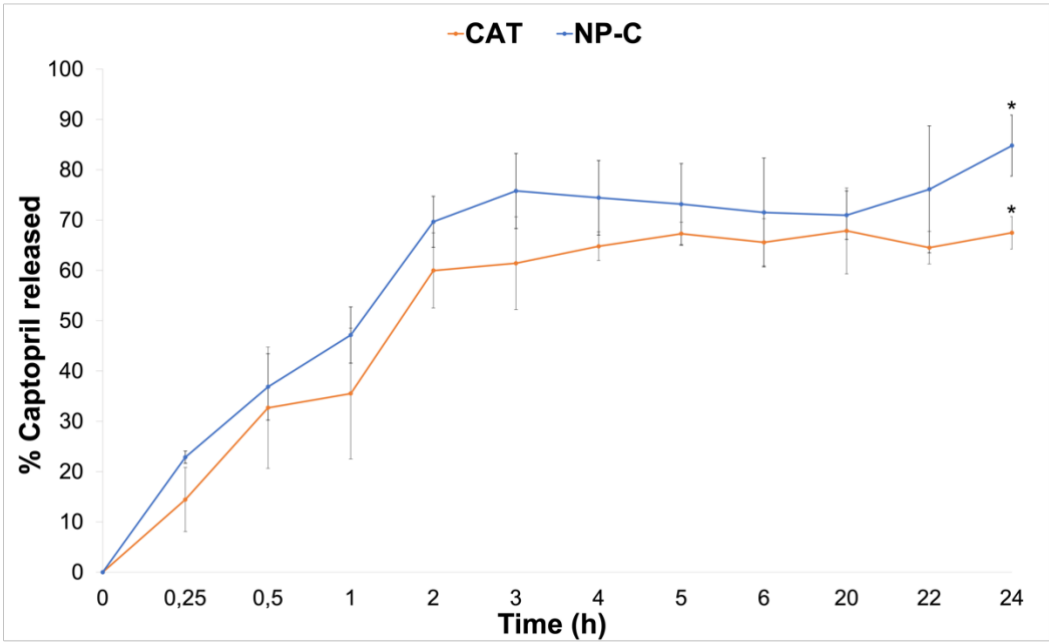

Figure 7. *In vitro* drug release profiles from NP-C and CAT solution in comparison. *p value < 0.05: CAT vs. NP-C after 24 h.

To complete this study, *in vitro* cytotoxicity of the NP-C dispersion was evaluated on neonatal human fibroblasts as this cell line is considered an important target for NP toxicity studies [37]. As shown in **Figure 8**, HFF-1 cells treated for 24 h with NP-Cb (50–1380 µg/mL) or NP-C (100–2759 µg/mL) equivalent to 13, 25, 51, 101, 202, 405 µM CAT concentrations did not show significant cytotoxic effects compared to the control groups of DMSO alone ($p > 0.05$). In fact, the viability >70% is considered as "no toxicity". After 48 h, NP-Cb at the highest concentration (1380 µg/mL) and NP-C (2759 µg/mL) equivalent to 405 µM CAT slightly reduced cell viability by around 64% and 59%, respectively, indicating dose-dependent cytotoxicity ($p < 0.05$). No significant differences in cytotoxicity were found between NP-Cb and NP-C after 24 and 48 h ($p > 0.05$). Free-CAT (equivalent to 100 µM and 1000 µM) did not display changes on HFF-1 cells viability after 24 h, whereas after 48 h, cell viability was reduced to 61% for a CAT concentration of 100 µM and 65% for 1000 µM (**Figure S3**: APPENDIX 4.2: Section 2.3). Even so, there was no statistically significant difference after 48 h ($p > 0.05$).

As reported in the literature, CH is a polymer that contributes to increasing cell viability [38]. This finding was also reported by other authors who showed that fibroblast viability decreased minimally in a stepwise manner as the NP concentration increase [39,40]. Comparison of the findings with those of other studies confirms that this natural polysaccharide in the appropriate conditions exhibits low toxicity in the µg/mL range. At therapeutic doses, chitosan's *in vivo* toxicity was shown to be largely benign. Although some toxicity was found at high doses, the researchers determined that chitosan was safe for *in vivo* use [41]

Assuming drug therapeutic concentrations from clinical studies may approach the 1 mg/L range (5 µM), these concentrations match NP concentrations in the low range of **Figure 8**, hence NP-C would not be very toxic *in vivo*.

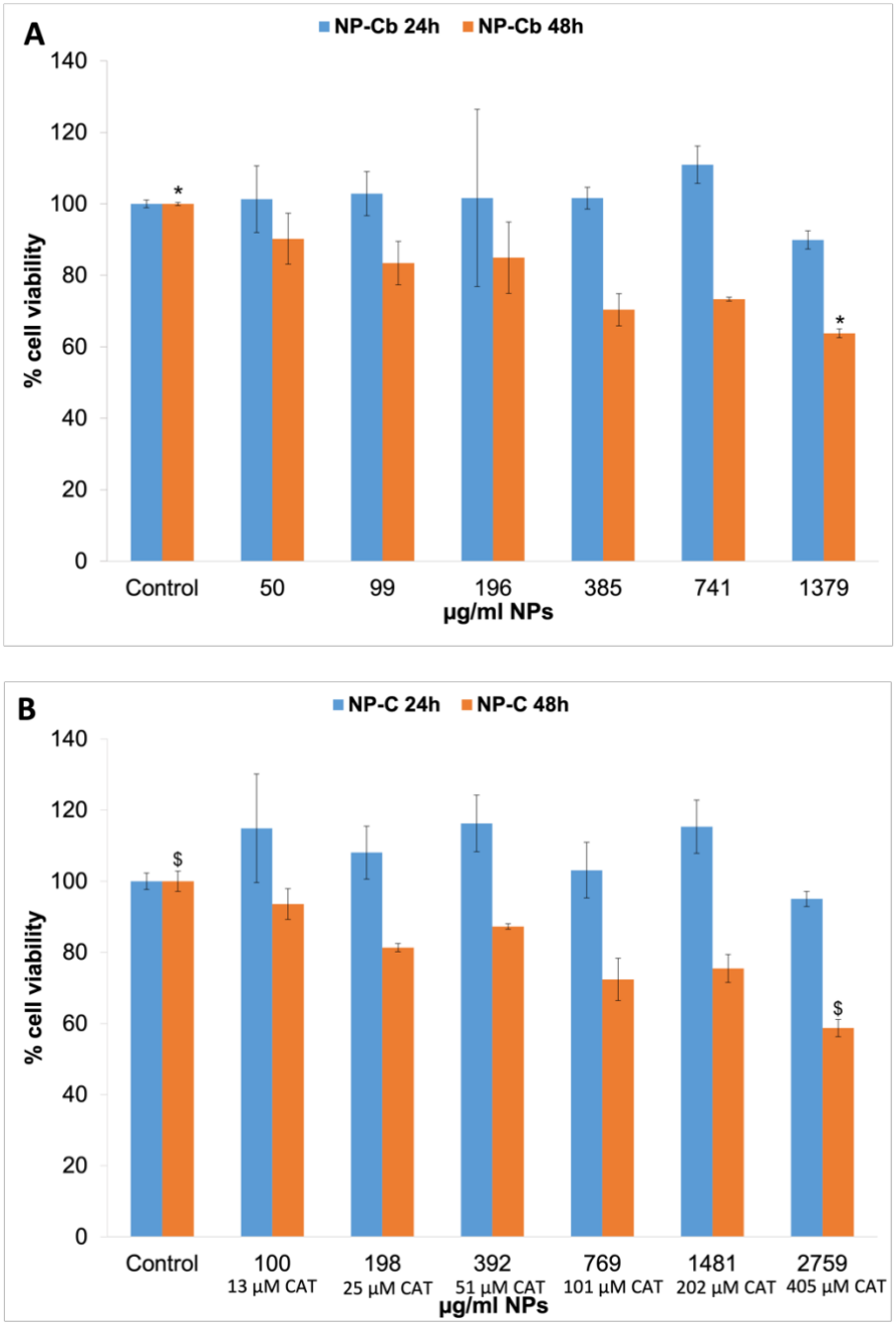

Figure 8. *In vitro* cytotoxicity. (A) Effect of NP-Cb on HFF-1 cells after 24 h (blue bars) and 48 h (orange bars) of incubation: *p value < 0.05: the effect of 1.379 µg/ml NP-Cb vs. control. (B) Effect of NP-C on HFF-1 cells after 24 (blue bars) and 48 h (orange bars) of incubation: $p value < 0.05: the effect of 2.759 µg/ml NP-C vs. control.

3. Materials and Methods

3.1. Materials

Captopril (CAT, 98% HPLC) and cellulose acetate phthalate (CAP, Mw: 2534.12 g/mol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan chloride (CH, Protasan UP CL 113, Mw: 160,000 g/mol, deacetylation degree, 86%) was obtained from Novamatrix (Drammen, Norway). Acetone was bought from Carlo Erba Reagents (Val de Reuil, France). Ultrapure bi-distilled water was obtained by a MilliQ R4 system, Millipore (Milan, Italy). Acetonitrile and ethanol of chromatographic grade were purchased from Merck (Darmstadt, Germany). Hydrochloric acid 37% was acquired from Honeywell Fluka™ (Seelze, Germany). Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5,diphenyltetrazoliumbromide (MTT) were acquired from Merck (Madrid, Spain). HFF-1 cells were obtained from the ATCC (Barcelona, Spain).

3.2. Preparation of Polymeric Nanoparticles

Drug-free and loaded nanoparticles were prepared, without using surfactants, by the nanoprecipitation method/dropping technique (**Figure 9**) [42]. The polymer used was either CAP alone (NP-Ab and NP-A, respectively) or CAP with CH at a 1:1 *w*/*w* ratio (NP-Bb and NP-B) or 1:3 *w*/*w* (NP-Cb and NP-C) ratio (**Table 1**). NPs consisting of CH and CAT alone cannot be obtained with this technique. CAP (50 mg) was dissolved in 5 mL of acetone under magnetic stirring to provide the organic phase. The aqueous phase was 20mL Milli Q water pH 3, acidified with hydrochloric acid 0.01% (calibrated pH meter, Eutech Instruments, pH 510, Singapore). Where CH was used, it was solubilised in the aqueous phase. Ten milligrams of CAT were dissolved in the acetone along with the CAP to prepare loaded NPs (NP-A, NP-B, NP-C). Then, the aqueous phase was added drop by drop to the organic phase under mild magnetic stirring. Acetone was evaporated for 2 h in a water bath at 60 °C under magnetic agitation. After that, the rotary evaporation method (Rotavapor RE111, Bünchi Labortechnik AG, Flawil, Switzerland) at 60 °C under vacuum for 5 min was used to remove any remaining acetone. Finally, the dispersion was sonicated in an ultrasonic bath for 40 s (60/120 W and 35 kHz, Bandelin sonorex RK52H, Heinrichstrabe, Berlin, Germany).

This preparation method was defined based on the pre-formulation studies reported in the supplementary data (APPENDIX 4.2: Section 2.3).

NPs prepared were directly characterised both in dispersion and after isolation by ultrafiltration for solid state characterisation as described in Section 3.9.

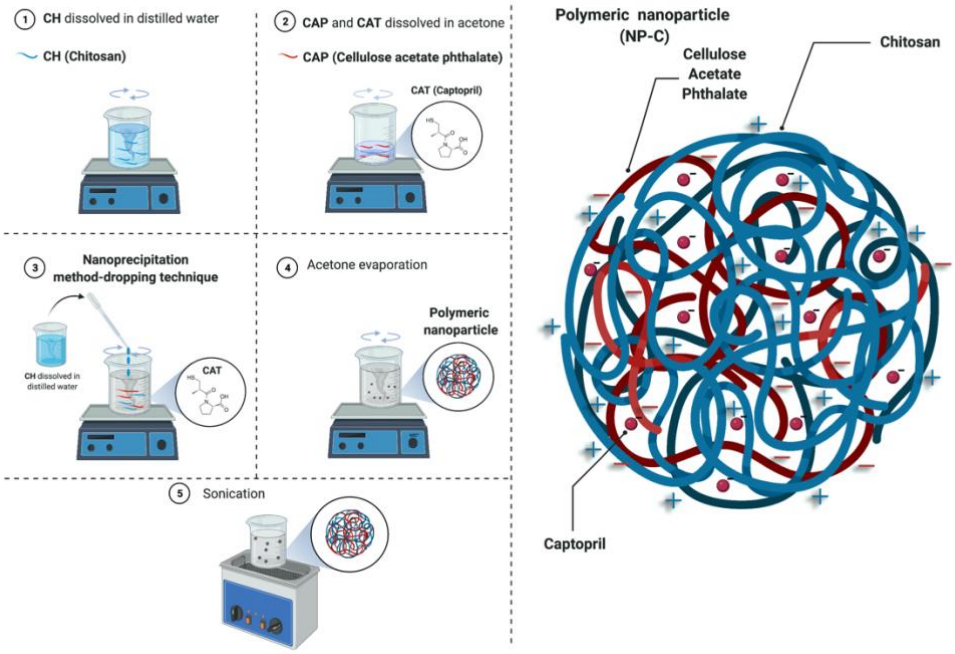

Figure 9. Nanoprecipitation method-dropping technique. Created with *BioRender*.

3.3. Particle Size, Polydispersity Index and Zeta Potential Measurements

Mean particle size and PDI were measured by the dynamic light scattering method using a Coulter Submicron Particle Sizer N5 (Beckman-Coulter Inc. Miami, Florida, USA). The measurements were performed after dilution of NP dispersion in MilliQ water adjusted to pH 3 (with 0.01% hydrochloric acid) previously filtered (regenerated cellulose syringe filter, pore size: 0.20 µm, filter size: 15 mm, Albet LabScience, Dassel, Germany). Triplicate NP formulations were prepared, and each sample was analysed three times (n = 9).

Zeta potential of NP dispersions was evaluated in Malvern Zetasizer Nano ZS ZEN 3600 (Malvern Instrument, Worcestershire, UK).

The particle size of NP-C dispersion was also measured using dispersion media at different pHs. The measurements were performed immediately after dilution of

NP-C dispersions in Milli Q water acidified to pH 5 with 0.01% hydrochloric acid and in water pH 7 previously filtered as reported above.

3.4. Quantitative Analysis of Captopril

For quantitative determination of CAT, a rapid and sensitive high-performance liquid chromatographic (HPLC)-modified method was used [43]. A Varian HPLC–DAD system (Palo Alto, CA, USA) consisting of two ProStar 210 pumps, a ProStar 410 autosampler and a DAD Varian 330 detector was employed. Varian workstation version 6.2 software was used for data acquisition and processing. The chromatographic separation was performed on a Water Spherisorb Nucleosil C_8 column (250 mm × 4.6 mm ID and 5 µm of particle size, Supelco, Milan, Italy). The binary mobile phase consisted of acetonitrile and water pH 2.0 (45:55 *v*/*v*) (per H_3PO_4) was filtered through 0.22 µm cellulose regenerated membrane filters (Sartorius, Goettingen, Germany) prior to use and was pumped at a flow rate of 1.5 mL/min at room temperature. The injection volume was 10 µL and the analysis time was 3 min per sample. Peak areas in the chromatography were recorded and measured at 205 nm. Concentrations of CAT were correlated through a calibration curve ($y = 70{,}963{,}714x - 41{,}338$; $R^2 = 0.9999$) obtained from standard solutions of drug (range 0.005–0.2 mg/mL) in phosphate-buffered solution 7.4.

3.5. Total Drug in Dispersion and Encapsulation Efficiency

The total drug in dispersion was determined. A dispersion volume (0.5 mL) was diluted in 2 mL of acetonitrile in a 5 mL volumetric flask and sonicated in an ultrasonic bath for 10 min. After that, 3 mL of 0.1% (*v*/*v*) phosphoric acid aqueous solution was added to ensure an acidic pH and avoid the formation of disulphide bridges; the dispersion was sonicated for 10 min to extract the drug. The sample was centrifugated at 14,000 rpm, and the supernatant was analysed by HPLC as described in the supplementary data (APPENDIX 4.2: Section 2.3). Then, the total drug in dispersion was calculated as a percentage with respect to the amount of CAT used for preparing NPs.

Furthermore, the assessment of encapsulation efficiency was performed by quantification of the free drug separated from NPs by ultrafiltration with Amicon Ultra-15 centrifugal filters at 4400 rpm (Eppendorf Centrifuge 5702 R, Hamburg, Germany) for 10 min at 4 °C. The non-encapsulated drug in the filtrate was

measured by HPLC and the intensity check using the Particle Sizer N5 was also evaluated. To validate the centrifugal ultrafiltration method, a CAT solution was centrifuged, and the filtrate was collected for HPLC assay. As a result, it was confirmed that absorption/adsorption of the drug to the membrane filter or any NPs in the filtrate did not occur. Encapsulation efficiency (EE) was calculated according to Equation (1):

$$EE(\%) = \frac{total\ \ CAT\ in\ dispersion - free\ CAT}{total\ \ CAT\ in\ dispersion} \times 100 \qquad (1)$$

The results obtained are the average of three NP formulations analysed 2 times (n = 6).

3.6. Morphological Analysis

The morphology of NP-Cb and NP-C dispersion was observed with JSM-IT500 InTouchScope™ Scanning Electron Microscope (SEM) (Jeol Ltd., Akishima, Tokyo). SEM studies were carried out on sputter gold-coated NP pellets (Polaron E-5000) obtained after dilution, centrifugation and gradual drying on top of aluminium stubs.

3.7. Physical Stability

The physical stability of NP-Cb and NP-C dispersion was studied by analysing the mean diameter and PDI at 1, 7, 14 and 28 days after preparation under different storage conditions (4 °C and 25 °C). The test was carried out on three NP formulation samples. Each sample was analysed twice (n = 6).

3.8. Chemical Drug Stability

The chemical stability of CAT in NP-C dispersion and aqueous solution at pH 3 was evaluated for 1, 7, 14 and 28 days under different storage conditions (4 °C and 25 °C). The total CAT was assessed by HPLC as reported in Section 3.4. Values are the mean of three independent analyses (n = 6).

3.9. Solid State Characterisation

NP-Cb and NP-C were isolated from dispersion by ultrafiltration with Amicon Ultra-15 centrifugal filters at 4400 rpm (Eppendorf Centrifuge 5702 R, Hamburg, Germany) for 10 min at 4 °C and dried at room temperature before the analysis of the chemical and crystalline structures as well as thermal behaviour.

3.9.1. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Sample discs (NP-Cb, NP-C, physical mixture and CAT) were prepared by intimately mixing about 1 mg of each one with 100 mg of potassium bromide using a pestle and mortar and compressing the mixture in a 15T Manual Hydraulic Press. Fourier transform infrared spectroscopy (FTIR) was performed on a Nicolet Avatar 320 FTIR spectrometer (Nicolet Instrument Corporation, Madison, WI, USA) equipped with EzOminic version 6.0 software (Ezonics, Fremont, CA, USA).

3.9.2. X-ray Diffraction (XRD) Analysis

X-Ray diffraction (XRD) was performed using a diffractometer Bruker D2 Phaser (Billerica, MA, USA) equipped with a Cu tube and a LynxEye PSD detector (angular opening 5°). Working conditions were as follows: 30 kV, 10 mA, scanning in the 2θ range of 5.8–60°, step size of 0.02°, counting time of 1 s per step and spinner at 15 rpm. Bruker low background Si-crystal specimen holders were employed. Bruker EVA 14.2 software (Billerica, MA, USA) and the PDF-2 (ICDD) database were used to evaluate the X-ray patterns.

3.9.3. Thermal Analysis (TG-DTG-DTA)

A TA Instrument Q600 (CeSAR—Centro Servizi di Ateneo per la Ricerca, Sassari University) was used to perform thermogravimetric (TG), derivative thermogravimetric (DTG) and differential thermal analysis (DTA). The samples, about 10 mg in weight, were brought from room temperature (approx. 17 °C, RH approx. 50%) to 24 °C with a gradient of 1 °C/min, held for 10 min at this temperature, and then heated to 600 °C with a rate of 10 °C/min. Analyses were carried out under a nitrogen flow of 100 mL/min and using Al_2O_3 crucibles (an empty crucible was used as a reference). The results were evaluated through the TA-Universal software.

3.10. In Vitro Drug Release Study

In vitro drug release test of NP-C dispersion was performed in pH 7.4 phosphate buffer (100 mL) using the dialysis bag method at 37 °C, under stirring at 100 rpm, assuring sink condition [44]. Cellulose acetate dialysis bags (12,500 cut-off, Sigma-Aldrich St. Louis, MO, USA) were filled with 2 mL of NP-C dispersion or CAT solution (0.5 mg/mL) as a comparison. At predetermined time intervals (0.25–24 h), samples of acceptor fluid were withdrawn and replaced by equal

volumes of fresh buffer. The quantification of CAT released was assayed by HPLC. The release studies were performed in triplicate.

3.11. In Vitro Cytotoxicity

Neonatal human dermal foreskin fibroblast-1 (HFF-1) cells were procured from ATCC (American Type Culture Collection) and grown in Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) to a final concentration of 15%. The cells were maintained at 37 °C in a humidified 5% CO_2 incubator using standard cell culture procedures. The cytotoxicity effect of NPs on the HFF-1 cell line was determined by MTT assay. Cells were seeded at 15,000 viable cells/well density in 24-well plates with the medium containing 15% FBS and incubated for 48 h at 37 °C and 5% CO_2. Later, cells were incubated ($n \geq 4$) for 48 h with different concentrations of free-CAT aqueous solution (equivalent to 100 µM and 1000 µM) and variable volumes (2.5–80 µL) of NP-Cb and NP-C dispersion equivalent to 13, 25, 51, 101, 202, 405 µM CAT concentrations and 50–1380 µg/mL and 100–2759 µg/mL for NP-Cb and NP-C dispersion, respectively. After the treatment and incubation period, 10 µL of MTT was added to each well and incubated for 4 h. Purple colour formazan crystals formed were then dissolved in DMSO. Solution absorbance was determined at 570 nm, with a 690 nm reference wavelength, employing an ELISA reader. Cells treated with 5 µL DMSO were used as a control. Cell viability was calculated using the following Equation (2):

$$\%\, Viability = \frac{Abs\ sample \times 100}{Abs\ control} \qquad (2)$$

4. Statistical Analysis

Statistical analyses were exported to the GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA, USA). Unpaired t-test was performed for the statistical difference between two treatment groups and one-way ANOVA analysis of variance test followed by a Tukey's multiple comparison test in case of multiple comparisons. Statistical significance was set at $p < 0.05$.

5. Conclusions

The present study demonstrated that nanoparticles based on CAP and CH can be prepared by exploiting only the interaction between the two polymers without using surfactants. The loading of the hydrophilic drug CAT was affected by the polymer weight ratio, and a high amount of CH was necessary for obtaining a good encapsulation efficiency. NP-C had a spherical shape and good physical stability in dispersion thanks to the high zeta potential. Furthermore, NP-C protected CAT, both encapsulated and free, from chemical degradation in water. When in contact with fluids with physiological pH, the polymer matrix gradually dissociated and released CAT. The nanoparticles prepared did not show *in vitro* cytotoxic effects except at the highest dose tested after 48 h. Therefore, NP-C can be proposed as a platform for preparing different dosage forms for CAT delivery in paediatrics. Nanoparticle dispersion could be used for preparing (i) oral formulation by adding, e.g., sweeteners and flavourings to improve palatability, (ii) enteral formulation and (iii) intravenous or parenteral injection. For applicability, further studies are necessary.

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2.4. A thermosensitive nasal hydrogel: A new approach for dimethyl fumarate delivery in paediatric multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a chronic, relapsing autoimmune disease of the central nervous system and one of the most common non-traumatic disabling diseases affecting mainly young adults [1]. According to a systematic review (1965 - 2018) of the epidemiology of paediatric-onset MS revealed that the overall incidence of this condition ranged from 0.05 to 2.85 per 100,000 children and adolescents [2]. The female-male ratio increased from 1.2:1 in children <12 years old to 2.8:1 in children ≥12 years old [2]. The large range in incidence and prevalence rates may be due to a variety of factors, including ethnic and regional diversity and case ascertainment. Several studies show that children constitute a minimum of 5% of the overall number of MS patients [3–5]. The paediatric MS incidence in Sardinia is estimated to be significantly higher than those reported elsewhere and the prevalence in this region is among the highest values worldwide [6]. A genetically predisposed person and a specific environmental trigger are risk factors for its pathogenesis [7]. Environmental factors can be vitamin D deficiency or Epstein–Barr virus [8,9]. The main pathogenic processes causing the clinical symptoms include inflammation, demyelination, and axonal degeneration [10,11]. Adult and paediatric MS share the same basic disease mechanisms [12,13]. Moreover, paediatric patients often develop relapsing-remitting MS with a significant relapse rate [14]. Currently, there are no curative treatments for MS. Fingolimod is the only MS therapy approved by both the FDA and EMA for use in paediatric patients 10–17 years of age [15]. EMA authorised dimethyl fumarate (DMF) in children and adolescents from 13 years of age [16]. There is the evidence that the employment of disease-modifying therapies (DMTs) such as rituximab, fingolimod, or dimethyl fumarate (DMF) work better than the more traditional injectable platform, but with the probability of a higher rate of serious adverse events [17,18]. DMF hard gelatin capsules (Tecfidera®) is the methyl ester of fumaric acid administered orally, whose active metabolite is monomethylfumarate [19]. Preclinical studies indicate that the combination of neuroprotective and immunomodulatory mechanisms is implicated in the mode of action of DMF

through the activation of the Nrf2 (nuclear factor (erythroid-derived 2)-like 2 (Nrf2)) anti-oxidant pathway in the central nervous system [20,21]. Clinical trials revealed that DMF significantly decreased relapse rates and the development of new brain lesions on magnetic resonance imaging and reduced the rate of disability progression [22,23]. Furthermore, the FOCUS study (registered at ClinicalTrials.gov NCT02410200; NCT02555215) revealed that paediatric and adolescent patients with MS may benefit from DMF treatment [24,25]. A pharmacokinetic and safety profiles consistent with those in adults were shown by this clinical trial. However, gastrointestinal and flushing events were often reported, which has led to decreased compliance and discontinuation of therapy [16]. Additionally, in January 2020, DMF was designated as an orphan medicine for the treatment of adrenoleukodystrophy by the European Commission to Consorcio Centro de Investigación Biomédica en Red, M.P, Spain [26] The most severe variant of this disease is infantile cerebral adrenoleukodystrophy, which affects mostly male children. This rare genetic condition is a progressive neurodegenerative disorder that leads to death in children between 5 and 12 years of age [27].

The present purpose of this study is to implement an alternative route of administration to the oral route to increase the efficacy of therapy and patient compliance. Nasal administration is an excellent alternative for drug delivery for the treatment of neurological diseases [28], as well as a patient-friendly strategy for treating chronic illnesses because of its quick beginning of effect, painless therapy, non-invasiveness, and simplicity of administration (self-medication) [29]. Stimuli responsive in situ nasal gel increases drug retention and lessen mucociliary clearance; and also increases drug bioavailability in the brain through olfactory and trigeminal nerve passage [29]. The gel-based drug delivery system is liquid when administered but possesses the capacity to undergo into a sol - gel transition upon contact with the body. Hence, the sol - gel transition occurs as a result of an external stimulus, such as a change in temperature [30]. For this reason, a thermosensitive chitosan/glycerophosphate hydrogel for nose-to-brain delivery containing DMF was developed to increase the bioavailability of the drug into the brain and reduce side effects. DMF was complexed with hydroxypropyl-β-cyclodextrin (HP-β-CD) to increase the solubility of the drug in water.

Chitosan (CH) is an amino polysaccharide with excellent mucosal adhesive properties due to the interaction between its positive charge and the negative charge of mucin. This cationic polymer can act as a penetration enhancer opening tight junctions between mucosa cells and promoting the absorption of drugs. Hence, CH is a good candidate for nasal drug delivery based on its high biocompatibility, mucoadhesive, and penetration enhancement properties [30,31]. The critical problem with the application of CH is its low pKa (6.3); thus, CH cannot be kept in solution up to physiological pH because this polymer has no charge above pH 6 and precipitates. For the study, glycerophosphate (GP) was used as an agent for the formation of a thermosensitive gel [29]. GP allows to obtain of a liquid solution at room temperature with a pH range of 6.8 to 7 and exhibits mild alkalinity (pKa 6.34), permitting proper buffering without causing quick precipitation or gelation at low temperatures (between 4 and 15 ºC), promoting the protective hydration of CH chains [30]. HP-β-CD can increase drug solubility, behave as a penetration enhancer and improve drug delivery through biological membranes and has also shown potential therapeutic use in neurodegenerative diseases [32]. Different types of interactions occur during gelation: CH in an acid environment is subject to electrostatic repulsion due to the close presence of the positive charges of the polymer's amine groups; water molecules interpose themselves between the chains, forming a regular arrangement; the addition of β-GP results in the formation of hydrogen bonds as a consequence of the neutralisation of repulsion due to the presence of the negatively charged phosphate groups; as well as CH hydrophobic interactions, which, together with electrostatic interactions, are mainly responsible for gel formation. The increase in temperature causes the rearrangement of the chitosan chains, and this results in more polymer-polymer interactions than solvent-polymer interactions, which leads to gel formation [29,30,33]. The characterization of the DMF-HP-β-CD complex is in progress at the University of Pavia (data not reported). The hydrogel was formulated in different conditions to achieve the optimal formulation. The performance attributes investigated included drug content, chemical stability, thermogelling properties, pH, osmolality, viscosity, water uptake determination, mucoadhesion studies and permeability behaviour.

2. Results and Discussion

2.1. Preparation of thermosensitive nasal hydrogel

The phase solubility study was carried out using the method developed by Higuchi and Connors [34], as described in Section 3.2. The inclusion complex of DMF:HP-β-CD improved the water solubility of DMF. DMF solubility increased in the molar ratio DMF: HP-β-CD of 1:0.5, 1:1 and 1:2, from 1.68 g/L to 1.89±0.01, 2.17±0.10, and 2.94±0.06 g/L, respectively. The 1:1 molar ratio was selected for G1 and G2 preparation in order not to interfere with the gel formation.

CH/GP solution 2/10 ratio (% w/v) was selected to prepare thermosensitive nasal hydrogels (Formulations G1 and G2) according to pre-formulation studies reported in the supplementary data (APPENDIX 4.2: Section 2.4). As reported in **Table 1**, G1 showed a higher drug content compared with G2 because of the preparation method employed ($p < 0.05$). Indeed, during the preparation of the G2 solution, the DMF:HP-β-CD complex was solubilised in 0.1 N HCl for 24 h until complete solubilisation. On the other hand, DMF:HP-β-CD complex was solubilised in an aqueous medium for 48 h for G1 preparation and the solution was acidified before the addition of CH, minimising the residence time of DMF at acidic pH. DMF is easily susceptible to hydrolysis under both acidic and alkaline conditions with decreasing stability in the following pH order 7>5>3>1>9 [35]. For this reason, the DMF:HP-β-CD complex solubilised in the acidic environment of G2 for (pH 1) may undergo a degradation compared to DMF:HP-β-CD complex solubilised in the aqueous medium of G1 (pH 4.3). In both formulations, after the addition of CH and β-GP, the pH of the liquid solution obtained was between 6.8 and 7.2, avoiding precipitation or gelation at low temperatures (between 4 and 15 ºC) [30,31].

Table 1. Composition of the thermosensitive nasal hydrogel. Drug content results are expressed as the mean ± SD (*n* = 6). * *p* value < 0.05: drug content (%) G1 vs G2.

Formulation	DMF:HP-β-CD ratio	CH/GP ratio (w/v) (%)	Medium	Drug content (%)
G1	1:1	2/10	MilliQ water	92.52 ± 5.21*
G2	1:1	2/10	HCl 0.1 N	36.52 ± 13.84*

2.2. Chemical stability of DMF

Figure 1 shows the chemical stability of DMF in G1 and G2, as well as DMF:HP-β-CD complex and free DMF in aqueous solution (DMFw and DMF:HP-β-CDw) and acid medium (0.1 N HCl) (DMFa and DMF:HP-β-CDa).

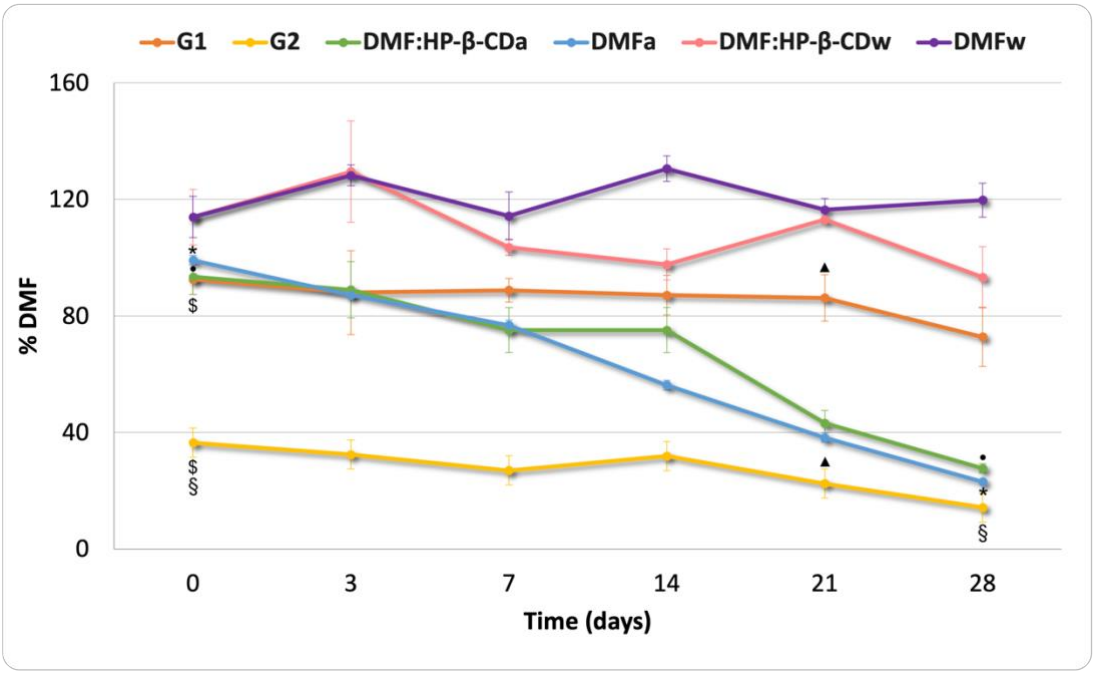

Figure 1. Chemical stability of DMF in G1, G2, DMF:HP-β-CD complex and free DMF in aqueous solution (DMFw and DMF:HP-β-CDw) and acid medium (DMFa and DMF:HP-β-CDa) over a period of 28 days. Values are expressed as the mean ± SD (*n* = 6). $ *p* value < 0.05: G1 day 0 vs G2 day 0. ▲ *p* value < 0.05: G1 day 21 vs G2 day 21. § *p* value < 0.05: G2 day 0 vs. G2 day 28. * *p* value < 0.05: DMFa day 0 vs DMFa day 28. · *p* value < 0.05 DMF:HP-β-CDa day 0 vs DMF:HP-β-CDa day 28.

The percentage of DMF in G1 was stable for 21 days ($p > 0.05$). However, the low concentration of DMF from G2 decreased over time ($p < 0.05$). As well as DMF:HP-β-CD complex and free DMF in acid medium decreased significantly over time ($p < 0.05$). The degradation did indeed lead to the presence of FA in HPLC chromatograms. The absence of the FA peak in the G1 chromatograms indicated that the aqueous environment of this formulation, acidified during the addition of chitosan could protect DMF from hydrolysis. This data was essential to prevent DMF degradation in the formulation. Furthermore, to confirm the method of leader formulation, DMF and DMF:HP-β-CD complex in an aqueous solution were analysed. Both solutions, DMFa and DMF:HP-β-CDa were stable over a period of time, as >90% of DMF was found at 28 days ($p > 0.05$).

2.3. Gelation time of formulation G1 and G1b

The gelation time for G1 and G1b was evaluated for 28 days (**Table 2**), as described in Section 3.6. Formation of G1 hydrogel took around 2 min but decreased at 1 min over time, remaining temperature-sensitive after 4 weeks of storage at 4°C. Hydrogen bonding between CH chains, electrostatic attraction between CH ammonium groups and GP phosphate groups and CH-CH hydrophobic interactions may be involved in the gelation process [28]. In addition, the blank solution of the leader formulation (G1b) revealed a gelation time of 1 min over time. Therefore, the gelation time of G1 was considered appropriate to overcome the nasal mucociliary clearance that results in around 15-20 min [36].

Table 2. Gelation time of G1 and G1b performed with inverted tube test during the time

	Gelation time (min:s)					
Days	0	3	7	14	21	28
G1b	1:00	1:00	1:00	1:00	1:00	1:00
G1	2:00	1:30	1:00	1:00	1:00	1:00

[Video]: Simulation of DMF thermosensitive gel (G1) nasal delivery.
https://drive.google.com/drive/folders/19Oq5MgU-b8pp4Jk8Pa9kZPzBTyUfhLqs?usp=share_link

2.4. pH and Osmolality measurements

The pH of thermosensitive nasal hydrogels was 6.9 ± 0.05 for G1b and 6.8 ± 0.06 for G1. As a result, incorporating GP provides a thermosensitive hydrogel as well as raises its pH to around 6.5 - 7 and enhances its biocompatibility [37].

Simulated nasal mucus revealed an osmolality of 0.447 ± 0.002 Osm/kg. The osmolality changes in the simulated nasal medium using the extraction method were measured. The osmolality of the simulated nasal medium for G1b and G1 was 0.613 ± 0.05 and 0.585 ± 0.016 Osm/kg, respectively. The results showed that hydrogels did not significantly modify the simulated nasal medium ($p > 0.05$). This thermosensitive nasal hydrogel may avoid nasal irritation.

2.5. Viscosity measurements

As reported in **Figure 2**, the viscosity of solution G1 was 65.84 ± 8.82 cP and varied slightly. Thus, the hydrogel remained stable for 28 days at 4 °C ($p > 0.05$). In addition, viscosity analysis revealed that the presence of HP-β-CD in solution G1b conferred a higher viscosity compared to the blank solution without HP-β-CD ($p < 0.05$). Thus, the formation of the inclusion complex with DMF may influence the viscosity profile. G1 exhibited optimum viscosity to facilitate nasal administration, aiding in increasing residence time by reducing mucociliary clearance [38,39].

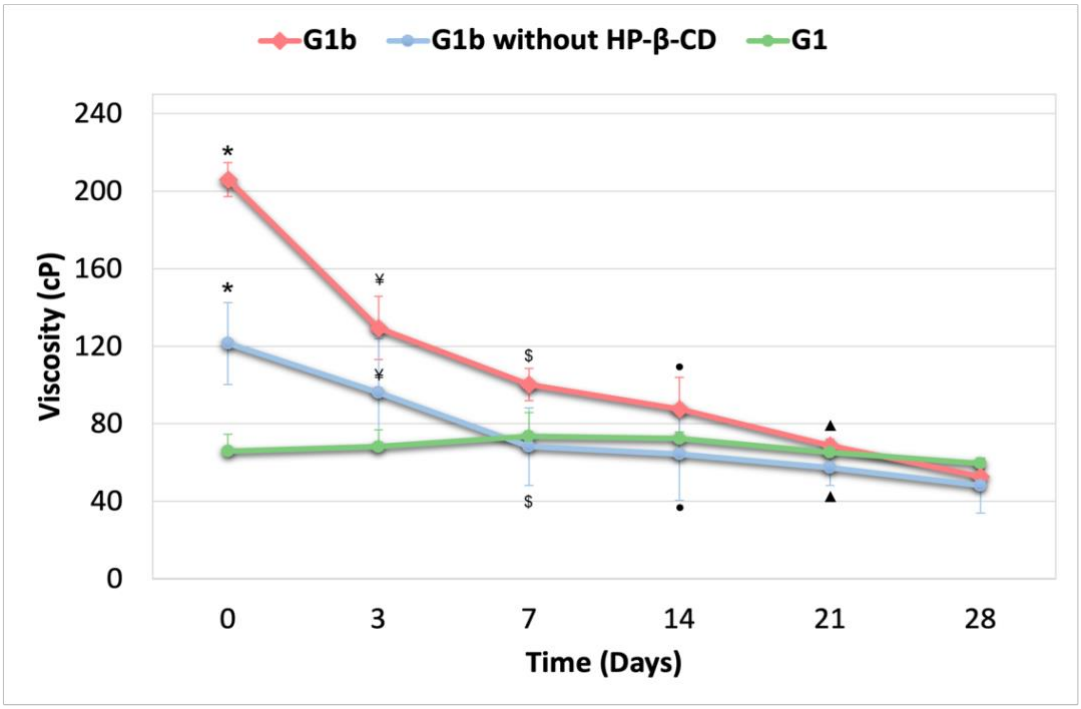

Figure 2. Viscosity stability of solution G1b, G1b without HP-β-CD and G1.

Values are expressed as the mean ± SD (n = 6). * p value < 0.05: G1b vs G1b without HP-β-CD at day 0. ¥ p value < 0.05: G1b vs G1b without HP-β-CD at day 3. $ p value < 0.05: G1b vs G1b without HP-β-CD at day 7. · p value < 0.05: G1b vs G1b without HP-β-CD at day 14. ▲ p value < 0.05: G1b vs G1b without HP-β-CD at day 21.

2.6. Water uptake determination

The water uptake for formulation G1 is presented in **Figure 3**. After 180 min, G1 water absorption was very low. According to the literature, this fact can be attributed to a compact structure with a protective polyol layer that forms around the CH chains due to interchain hydrogen bonding. The presence of GP may have caused a reduction in the solubility of the polymer due to hydrophobic interactions and intrachain electrostatic repulsion [40,41]. Another element that may affect water absorption is the pH of the hydrogel. The amine groups were deprotonated with an increase in pH, which reduced the repulsion between the polymer chains in hydrogels. This causes hydrogels to have a rigid structure and reduces their absorption ability [42,43]. G1 can avoid dehydration of the nasal mucosa due to the low water absorption by this thermosensitive hydrogel.

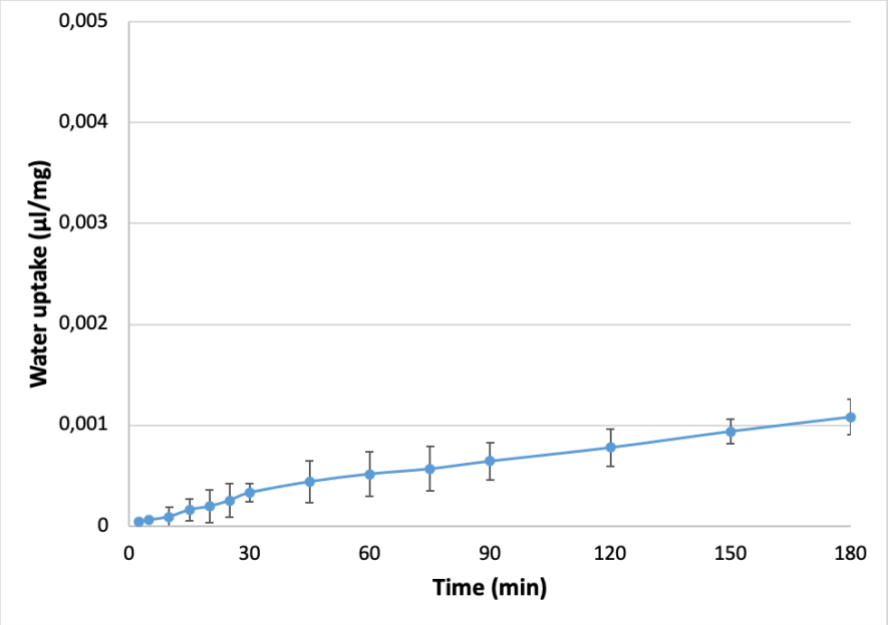

Figure 3. The water uptake capacity (µl/mg) of the hydrogel. Results are reported as the mean value ± SD (n = 3).

2.7. Mucoadhesion studies

The detachment force needed for the separation of the two surfaces (G1/filter paper) was 263.25 ± 2.51 mN/cm^2. This thermosensitive hydrogel (G1) composed of CH and DMF:HP-β-CD complex showed good *in vitro* adhesion properties, as reported in the literature [44]. Likewise, chitosan can increase mucoadhesive properties and enhance drug penetration across the nasal mucosa due to its cationic nature and the interaction with negatively charged mucin [45–47].

2.8. In vitro permeation studies

In vitro permeation studies of G1 and a DMF:HP-β-CD aqueous solution as a comparison was performed using synthetic membranes simulating the nasal mucosa (**Figure 4**). The test was performed on G1 formulation and an aqueous solution of DMF:HP-β-CD complex using a phosphate buffer pH 6.5 as the acceptor fluid. As illustrated in Figure 4 (dotted lines), after 15 min, the percentage of DMF permeated from formulation G1 and DMF:HP-β-CD solution was 16.77 ± 2.31% and 55.41 ± 6.08%, respectively. G1 reached 37.28 ± 9.11 % after 120 min; meanwhile, DMF permeated from the DMF:HP-β-CD decreased to 29.03 ± 9.58 % after 120 min. The degradation of DMF had an impact on the data gathered; hence continuous lines in **Figure 4** provide the corrected permeated profile to consider the DMF degradation. After 20 min, around 30% of DMF from G1 and approximately 90% from DMF:HP-β-CD solution was permeated. Thus, DMF from G1 revealed a significantly more sustained permeated profile compared to DMF:HP-β-CD solution (p<0.05) and was able to permeate through the synthetic membrane within 2 hours. Permeation-enhancing capabilities of CH and HP-β-CD may potentially contribute to nose-to-brain DMF delivery [32,48]. Therefore, this profile is optimal for a nasal formulation [49].

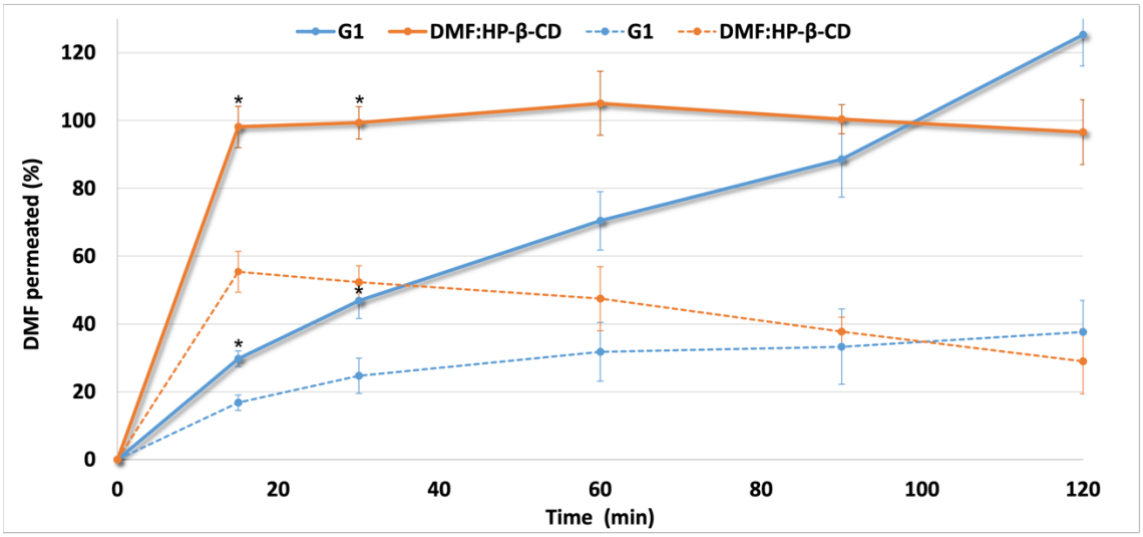

Figure 4. *In vitro* permeation profile of DMF from G1 and DMF:HP-β-CD. Data corrected (continuous lines) and data non corrected (dotted lines). Results are the mean of three experiments. Data are reported as the mean ± SD. * *p* value < 0.05 G1 vs DMF:HP-β-CD after 15 and 30 min.

3. Materials and Methods

3.1. Material

Dimethyl fumarate (DMF, 97%), fumaric acid (FA, ≥99% HPLC) and monomethyl fumarate (MMF, 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD, Cavasol® W7 HP Pharma, Mw: 1,400 g/mol) was bought from Wacker-Chemie GmbH (Munich, Germany). Chitosan (CH, ChitoClear® TM 1358, Mw: 103 kDa, deacetylation degree, 94%) was obtained from PRIMEX EHF (Siglufjordur, Iceland). Hydrated disodic salt (GP) was acquired from Sigma Aldrich (St. Louis, USA). Mucin from porcine type II was purchased from Sigma-Aldrich® (Darmstadt, Germany). Hydrochloric acid (HCl) 37% was bought from Honeywell Fluka™ (Seelze, Germany). Ultrapure bi-distilled water was obtained by a MilliQ R4 system, Millipore (Milan, Italy). Acetonitrile, ethanol and methanol of chromatographic grade were acquired from Merck (Darmstadt, Germany).

3.2. Phase solubility analysis

Phase solubility studies were performed according to the method reported by Higuchi and Connors [34]. An excess amount of DMF (0.050 g) was added into

the vial containing MilliQ water and various concentrations of HP-β-CD (molar ratio DMF:HP-β-CD of 1:0.5, 1:1 and 1:2). These flasks were shaken in a rotary shaker for 72 h at 25ºC; then, solutions were centrifuged in a centrifuge at 4400 rpm for 10 minutes at 20°C, as well as in a microcentrifuge at 14000 rpm for a further 10 minutes. The amount of DMF in the solution was determined by an HPLC method described below to determine the solubility.

3.3. Preparation of thermosensitive nasal hydrogel

CH/GP 2/10 ratio (% w/v) blank solutions (G1b and G2b) and containing DMF solutions (G1 and G2) was prepared following the method reported by Salis et al. (2015) with some modifications (**Figure 5**) [30]. This volumetric mixing ratio of the CH and β-GP solutions were optimised previously in terms of thermogelling properties, rheological behaviour, and biodegradability. The preparation method was defined based on the pre-formulation studies reported in the supplementary data (APPENDIX 4.2: Section 2.4.). To prepare formulation G1, DMF (0.021 g) and HP-β-CD were complex using a molar ratio 1:1 (DMF:HP-β-CD) by manual grinding and then solubilised in MilliQ water in a closed system for 48 h under constant agitation in a rotary shaker at room temperature until the complex was completely solubilised; meanwhile, G2 was prepared to solubilise DMF:HP-β-CD complex in HCl 0.1 N for 24 h under constant agitation (**Table 1**). Afterwards, CH (2% w/v) was added into the aqueous solution (10 ml) containing DMF:HP-β-CD of G1, previously acidified with 100 µl HCl, as well as CH was added directly to the acidic medium containing DMF:HP-β-CD (1:1) of G2. GP (10% w/v) was solubilised in 1 ml of Milli Q water at room temperature. Solutions were put into an ice bath until the temperature of 4°C was reached. Thereafter, the GP solution was dropwise into the CH solution under vigorous magnetic stirring for 15 min at 4°C, and the thermosensitive nasal hydrogels were stored at 4°C.

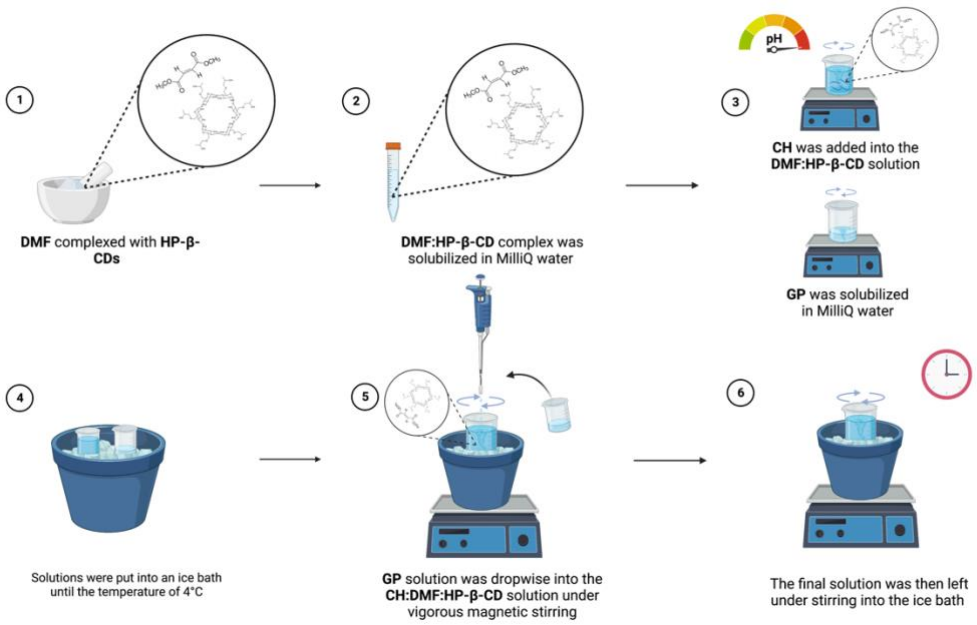

Figure 5. Preparation of thermosensitive nasal hydrogel. Created with *BioRender*.

3.4. HPLC analysis of DMF

DMF was analysed by a rapid and sensitive high performance liquid chromatographic (HPLC) modified method [50]. A Varian HPLC–DAD system (Palo Alto, CA, USA) consisting of two ProStar 210 pumps, a ProStar 410 autosampler and a DAD Varian 330 detector was employed. Varian workstation version 6.2 software was used for data acquisition and processing. The chromatographic separation was performed on Nucleosil C_{18} column (150 mm × 4.6 mm ID and 5 µm of particle size, Alltech, IL, USA). The binary mobile phase consisted of acetonitrile and water (50:50 v/v) was filtered through 0.22 µm cellulose regenerated membrane filters (Sartorius, Goettingen, Germany) prior to use. Flow rate was fixed at 1.5 ml/min at room temperature and the detection wavelength was set at 210 nm. The injection volume was 10 µl and the analysis time was 4 min per sample. The assay was linear in the concentration range of 2.5-50 mg/L (y=214078x-14146; R^2=0.9998).

3.5. Drug content

Drug content of G1 and G2 was determined by HPLC analysis. A solution volume (100 µl) was diluted in 10 mL of acetonitrile in a volumetric flask. The resulting dispersion was sonicated in an ultrasonic bath for 30 min and centrifuged in a

microcentrifuge at 14,000 rpm for 10 minutes for the first extraction. After that, the supernatant was analysed by HPLC. Moreover, a second extraction was performed, and the precipitate present in the Eppendorf was withdrawn with acetonitrile, vortexed and centrifuged at 14,000 rpm for 10 minutes. The supernatant was analysed to quantify any DMF residue present, and drug content was calculated according to equation 1:

$$DC(\%) = \frac{real\ amount\ of\ DMF}{theorical\ amount\ of\ DMF} \times 100 \qquad (1)$$

3.6. Chemical stability of DMF

The percentage of DMF in the G1 and G2 formulations stored at 4 °C was evaluated during the time (1, 3, 7, 14, 21 and 28 days), as well as free DMF and DMF:HP-β-CD complex in aqueous solution and acid medium (0.1 N HCl) as a comparison. The amount of DMF was calculated in solutions according to the calibration curve described previously.

3.7. Gelation time

Gelation time of G1b and G1 was studied using the inverted tube test method [30]. Chromatographic glass tubes of 2 ml were thermo-stabilized in a temperature-controlled bath at 35°C. Sample solutions (500 µl) were placed into the tubes and incubated in the bath. At predetermined time intervals of 30 s, the tubes were taken out of the water bath and horizontally inverted in order to verify the solution flowability. If the solution did not flow within 15 s, the time is recorded as gelation time (**Figure 6**). Analysis were performed in order to assess the stability of the solution and any changes in the gelation time over a period of 28 days.

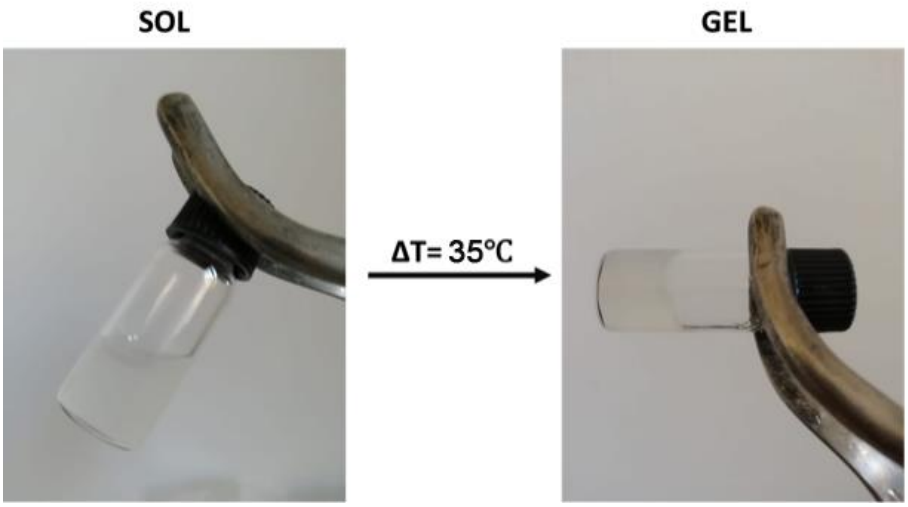

Figure 6. Inverted tube test.

3.8. pH and Osmolality measurements

The pH of thermosensitive nasal hydrogels was examined to verify an appropriate pH for nasal delivery. Measurements were carried out at 4 °C with a calibrated pH meter (Hanna Instruments, HI 8417, Singapore).

Furthermore, the extraction method was used to measure the osmolality changes in the nasal medium [51]. Nasal mucus was prepared by dissolving 8% (w/w) of porcine mucin-type II in a simulated nasal electrolyte solution with 7.5 mg/mL sodium chloride, 1.3 mg/mL of potassium chloride, and 0.3 mg/mL of calcium chloride dihydrate. NaOH solution was used to adjust its pH to around 6.4 [52]. G1b and G1 (1 ml) were soaked in simulated nasal mucus (9 ml) and placed for 24h at 37 °C. Samples (50 µl) were analysed with The OSMOMAT 030 Cryoscopic Osmometer (Genotec, Germany). Each analysis was carried out in triplicate (n = 3).

3.9. Viscosity measurements

The solution viscosity of G1 during the time (0, 1, 3, 7, 14, and 28 days) was determined in order to assess the solution stability. A rotational viscosimeter Fungilab (Barcelona, Spain) was used with spindles L1-2 at 100 rpm to ensure proper calibration. Likewise, the solution viscosity of G1b with and without HP-β-CD was determined. The test was conducted by keeping the solutions at a temperature of 4 °C in an ice bath to avoid viscosity changes due to the temperature difference between the refrigerator and the working environment.

3.10. Water uptake determination

The capability to absorb water was carried out using a modified Enslin apparatus [53]. A fritted glass support was connected with a graduated capillary through a three-way channel. The top of the support was horizontally aligned with the capillary and water diffused freely into the system. An applied sample could take up water by capillary forces, and the amount absorbed was measured by the graduated capillary. The water uptake was measured at predetermined times (2.5, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min). The test was performed at 34 °C using ultra-pure water and a 0.1 mL (1/ 1000 mL, 20°C) glass capillary. A weighted amount of G1 was spread uniformly on a pre-saturated cellulose filter (d = 1 cm), lying on top of the fritted glass support. Evaporation was avoided by covering the glass holder with parafilm.

3.11. Mucoadhesion studies

The mucoadhesion of the thermosensitive hydrogel (G1) was evaluated using a modified precision balance [54]. The detachment forces were measured between samples of G1 and a filter paper conditioned with 2% mucin solution (from the porcine stomach, type II, Sigma) or with phosphate buffer, pH 6.5 (blank) previously kept at 34 °C 60 s after the contact is established. Results were expressed as detachment force (in mN/cm^2) required to detach the two systems (G1/ filter paper). The measurements of detachment force were performed in triplicate, and the results were reported as mean ± SD.

3.12. In vitro permeation studies

In vitro permeation studies were performed on vertical diffusion cells with receptor and donor compartments connected to a continuous flow system, thermostated at 37 °C (EP3909667A1, University of Sassari) and with a permeation area of 1.5 cm^2 [55]. The test was performed for G1 formulation and for an aqueous solution containing the DMF:HP-β-CD complex used as a comparison. Cellulose acetate membranes with 0.45 µm pore size (Sartorius, Goettingen, Germany) conditioned with pH 6.5 phosphate buffer were used and placed between the cell compartments. The receptor compartment was filled with 50 ml of pH 6.5 phosphate buffer, simulating the nasal mucosa pH; meanwhile, G1 and DMF:HP-β-CD aqueous solution (500 µL) was placed on the donor compartment. Aliquots (600 µL) of the receptor medium were withdrawn at 15 min, 30 min, 60 min, 90

min and 120 min. After the collection of the sample, an equal amount of fresh acceptor fluid was added to ensure sink conditions. The amount of permeated DMF was determined using the HPLC method reported in Section 3.3.

Considering the permeation profile of DMF:HP-β-CD, the equation of the linear portion from 15 to 120 min was calculated. Thus, release profiles were corrected to remove the degradation aspect of DMF in the acceptor medium. According to Rassu et al. (2016), the technique used for the correction can be described by the equation below:

$$Ftn = Ft \times \frac{100}{-0.2514 \times t + 60.237} \qquad (2)$$

where Ftn is the corrected percentage release, Ft is the cumulative percentage release, and t is the time at which Ft level was taken [56]. The results were expressed as a percentage of DMF permeated versus time.

4. Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA, USA). Comparisons were made using the nonparametric Kruskal–Wallis test followed by a Dunn's test for individual differences and Mann-Whitney test. A value $p < 0.05$ was considered statistically significant.

5. Conclusions

In this study, HP-β-CD contributes to increasing the solubility of DMF in water. The preparation method is crucial to determine the performance of the hydrogel system. G1 formulation is optimal for DMF incorporation, as well as provides stability to DMF for 21 days. Furthermore, the gelation time is appropriate for nasal administration and remains constant over time when stored at 4 °C. The viscosity may favour the residence time of the hydrogel within the nasal cavity, counteracting mucociliary clearance and promoting DMF absorption. In addition, the presence of penetration enhancers such as CH and HP-β-CD could favour the opening of tight junctions between cells, promoting paracellular absorption of the drug through the mucosa. The *in vitro* permeation studies demonstrated a sustained permeated profile of G1 when compared with the control. These characteristics allow nasal administration as a thermally sensitive pH-dependent

solution, which will then undergo rapid sol to gel conversion upon contact with the body. G1 is proposed as a potential new approach to exploit the nose-to-brain absorption mechanism of DMF in the treatment of multiple sclerosis.

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CHAPTER 3. *CONSIDERATIONS FOR ORAL SUSPENSION ADMINISTRATION VIA ENTERAL FEEDING TUBE TO PAEDIATRIC POPULATION: A SYSTEMATIC REVIEW AND HOLISTIC PROOF OF CONCEPT STUDY*

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3.1. Introduction

Enteral feeding for the preterm infant or critically ill neonate is a necessary means of providing nutrition and medications to this fragile patient group who are otherwise unable to breast or bottle feed. In paediatric hospital settings, enteral feeds, fluids and oral medicinal products are regularly administered to paediatric patients via enteral feeding tubes (EFTs) due to the rising prevalence of children living with severe illness or disability that results in reduced swallowing capacity and limited gastrointestinal access [1,2]. Administration of medication through EFTs offers several advantages such as economy, convenience, compliance, and, most importantly, lower probability of infections. However, it also is associated with several challenges and issues, such as drug administration errors associated with the use of IV syringes for enteral drug administration; dose variability due to potential drug adsorption onto the tube material or method of administration [3–5], the obstruction of feeding tubes with inappropriate drug formulations; the risk of cross-contamination from sharing of tablet crushing devices; and the risks of occupational exposure to drug powders through inappropriate handling. In addition, a variety of feeding tubes with different diameters, openings, lengths, and polymer compositions are employed in a hospital setting, thus adding to the complexity of drug administration. The practitioner may select a tube of a specific bore size from a variety of materials and lengths when giving oral medicinal products to paediatric patients via nasogastric (NG) tubes in order to adapt to various situations without compromising clinical outcomes. Generally, between 4-12 Fr bore size is recommended for paediatric age [6]. However, the narrow bore sizes of neonatal/paediatric tubes present the risk of occlusion [7]. Successful drug delivery through EFTs requires a thorough assessment of factors and practical aspects of administration such as the dosage form to be used, formulation characteristics, administration technique, whether any interaction with the feed may occur, the type of tube and placement site, the site of drug absorption etc. Additionally, an assessment demonstrating delivery of the entire drug dose with no (or minimal) loss is necessary and should include the volume of solution to be used to rinse the feeding tube to ensure complete administration of the medication. Likewise, if the product is likely to be administered via an enteral (e.g., NG, nasojejunal (NJ)) tube, issues such as viscosity of formulation (to

permit flow of the product through neonatal tubes), size of particles, and adsorption to commonly used enteral tubes should be investigated. There is a risk that the active pharmaceutical ingredient (API) may interact with the material of the tube leading to the adsorption of the API. If the dose delivered to the patient is low, there may be a significant loss of therapeutic activity. Pharmacotherapy problems related to drug bioavailability, effectiveness and safety are often associated with product device incompatibilities, drug-nutrient interactions or the use of incorrect feeding tubes and administration techniques [8]. Despite these issues, the majority of information available on drug administration via EFTs cannot be supported except by anecdotal experiences, practical or theoretical considerations, or very limited anecdotal evidence in the literature [9]. There is literature available about the pharmaceutical and technical implications of medication administered via EFTs, but there is limited evidence of how the pharmaceutical aspects affect the performance of the oral medicinal product when administered to paediatric patients via different EFTs. Establishing guidelines is needed to help the industry to study the pharmaceutical aspects during the pharmaceutical development of medicines to minimize potential complications and limitations associated with this method of drug delivery. In the EMA questions and answers (Q&As) document, a risk-based approach is recommended and suggests considering tube types/materials and all components of the medicinal product [10].

This Q&As states that companies should include information in the summary of product characteristics (SmPC) if administration through an enteral tube is very likely.

3.2. Project rationale

Pharmaceutical companies developing medicinal products for the paediatric patient population are required to devise an appropriate formulation strategy and a thorough evaluation of factors that may influence the delivery of drugs via EFTs [11]. The Q&A document developed by EMA quality working party in Dec 2018 provides the feasibility studies to be addressed when the feasibility of administering a particular medicinal product through an EFT is to be investigated [10]. Furthermore, the guideline indicates it is not necessary to evaluate all tube types, flush and dose volumes etc, but a bracketing approach or worst-case

evaluation can be acceptable. In addition, the FDA has provided some guidance for in vitro studies to evaluate the bioequivalence of generic products via feeding tubes [12]. Hospitals develop their own policies and procedures to inform their practitioners of their responsibility in the safe and effective administration of medicines via EFTs [13,14]. However, these procedures focus mainly on the clinical practice of administrating the medication through EFT [15]. The administration of drugs via EFTs is an area that has implications for each member of the multidisciplinary team, including scientists, clinicians, nurses, and pharmacists. Without a holistic view, issues such as particle size, viscosity, dosing and rinse volume(s), chemical compatibility of the oral medicinal product with the tube material and the risk of physical blockage of the tube may be overlooked. This can lead to medication error and tube obstruction, reduced drug effectiveness, and an increased risk of toxicity. Hence it is necessary to understand the effect of different formulation characteristics and tube characteristics on the performance of medication and administration of medication via EFTs. The scope of this project is limited to NG tubes only.

3.3. Aims and Objectives

3.3.1. Overall aim

The overall aim of the project is to develop evidence-based recommendations/guidance for pharmaceutical companies to evaluate the feasibility of administration of oral paediatric drug products via NG tubes to paediatric patient group. The study is conducted in two parts.

Part 1 aim (Section 3.4)

To provide a systematic review on different factors affecting the performance of the oral medicinal product when administered to paediatric patients via different EFTs. This will help to determine what information is in the public domain on the administration of drug products via different EFTs and highlight the gaps.

Part 2 aim (Section 3.5)

On the basis of the systematic review, conduct a feasibility study (*in vitro* study) to evaluate the factors and their effect on the performance of drug products when administered via NG tube.

3.3.2. Overall objective

The overall objective is to provide a systematic review of existing information and practices regarding the assessment of EFTs during drug product development and recommend possible prioritisation of practices or steps for the assessment of EFTs during the drug product development process. Besides, technically assess commonly available feeding tubes and practices taking into account healthcare professional input.

Part 1 objective (Section 3.4)

In part 1, a systematic review was carried out to identify factors (associated with the formulation and EFTs), their effect on the performance of drug products and studies needed to demonstrate the feasibility of administering medication via EFTs to paediatric population. The determination of factors and their evaluations could support development of evidence-based recommendations or guidance for pharmaceutical companies to evaluate the administration of new oral paediatric drug products via EFTs.

Part 2 objective (Section 3.5)

Part 2 was conducted to assess and streamline the key factors that impacted the administration of medication via NG tubes. The key quality/critical attributes of oral suspension (i.e. dose strength), NG tubes (i.e. bore size and tube length), and administration methods (i.e. flush volume and dose dilution volume) affecting the product performance (dose recovery) when administered via paediatric NG tubes were evaluated. This study is part of a more global study with several dosage forms, forms, different EFTs and administration factors to determine the key quality/critical attributes that may affect the administration through EFTs and the possibility of providing a design space (*allowable operational flexibility*).

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3.4. Considerations for oral dosage form administration via enteral feeding tube in paediatric patients – A systematic review

1. Introduction

Medication administration via the oral route is the most preferred, and common practice in hospital settings due to its convenience, cost-effectiveness, safety, and compliance. However, for paediatric patients living with severe illness or disability that results in reduced swallowing capacity and limited gastrointestinal access, oral medicinal products are regularly administered via enteral feeding tubes (EFTs) [1,2]. There is evidence of increasing prevalence of patients on enteral tube feeding across the globe [3]. Enteral nutrition was delivered to intubated and ventilated patients with COVID 19 through different EFTs [4]. Up to 40-70% of children with chronic illness are estimated to have feeding issues and gastric feeding via a nasogastric (NG) tube is usually the initial approach for such patients. NG tubes are commonly used in the Neonatal Intensive Care Unit (NICU) due to immaturity of coordination of sucking, swallowing and breathing or to respiratory, cardiovascular, gastrointestinal, or neurologic disease [1].

Feeding tubes are classified according to the site of insertion, or the route by which they can be inserted [5,6]. For example, nasoenteric or oroenteric tubes start in the nose or mouth, respectively, and those which end into the stomach are referred to as nasogastric (NG) tubes, whilst those ending in the small intestine are termed nasoduodenal/jejunal (NJ) tubes. They are available in different diameters (4- 20 French (Fr) units), lengths (40 – 160cm), and materials (e.g. polyvinyl chloride (PVC), polyurethane (PU), silicone). Although it is feasible to administer medications through EFTs, the process can be complex due to the different types, sizes and materials of tubes, as well as the properties of different dosage forms [7]. Medication administration to patients via EFT is not risk free and may render the patient three times more likely to be involved in a drug administration error [8]. Incorrect medication preparation processes used for administering through feeding tubes often disregard the pharmaceutical properties of the active pharmaceutical ingredient (API)/drug product and its biopharmaceutical characteristics. This can lead to physical and chemical incompatibilities, resulting in precipitation, flocculation, adsorption, colour

changes, chelation, and drug-nutrient interactions, which may cause changes in drug effectiveness or compromise nutritional therapy [9].

The American Society for Parenteral and Enteral Nutrition (ASPEN) developed evidence-based guidelines for safe medication administration [10,11], and the British Association For Parenteral And Enteral Nutrition (BAPEN) produced guidance on the safe administration of medication via EFTs for healthcare professionals and patients [12,13]. Moreover, the British Pharmaceutical Nutrition Group (BPNG) with the support of BAPEN took the initiative to prepare *The Handbook of Drug Administration via Enteral Feeding Tube* [14]. Hospitals develop their own policies and procedures to inform their practitioners of their responsibility in the safe and effective administration of medicines via EFTs [15,16]. However, most of these procedures focus mainly on the clinical practice (eg. placement of tubes) of administrating the medication through EFT and neither of the guidelines referred to above are specific for neonates, children and adolescents [11]. Since administering drugs via an EFT usually falls outside of the terms of the drug's product license, this has implications for the professionals responsible for prescribing, supplying and administering the drug as they become liable for any adverse event that the patient may experience. The technical difficulties resulting from administration of substances directly to the stomach or small intestine are often overlooked, especially in the paediatric population.

There are differing requirements regarding the properties of different dosage forms for successful administration via EFT. There is a risk that the API may interact with the material of the tube leading to adsorption of the API [17]. Insoluble APIs and excipients in oral dosage forms can be associated with tube blockage [18]. If the dose delivered to the patient is low, there may be a significant loss of therapeutic activity [19]. Clinical practices such as crushing modified release dosage forms or enteric-coated products may cause serious adverse effects in patients [20]. Pharmacotherapy problems related to drug bioavailability, effectiveness and safety are often associated with product device incompatibilities or the use of incorrect feeding tubes and administration techniques [21,22]. Authors from many different fields have documented issues related to drug bioavailability, effectiveness, and safety when drugs are administered through EFTs [11,23–25].

Therefore, pharmaceutical companies developing drug products for the paediatric patient population are required to devise an appropriate formulation strategy and a thorough evaluation of factors that may influence the delivery of their product via EFTs [26]. The European Medicines Agency (EMA) developed and published questions and answers (Q&As) on the administration of oral immediate release medicinal products through EFTs in Dec 2018 [27]. The Q&A document provides a list of factors to be addressed when the feasibility of administering a particular medicinal product through an EFT is to be investigated. Furthermore, the Q&A indicates that it is not necessary to evaluate all tube types, flush and dose volumes, but a bracketing approach or worst-case evaluation can be acceptable. The FDA has a guidance for *in vitro* studies to evaluate bioequivalence of generic products via feeding tubes [28]. Despite these available guidelines, EFTs are associated with several challenges/issues, which can be even more complicated when the medication is administered to neonates, infants or children.

The quality of data published relating to medication administration via EFTs is largely anecdotal and individual case report based [29]. In most cases, no published data exist to confirm the safety of these suggestions, and most drugs are not licensed for administration to the paediatric population via EFTs [30]. The pharmaceutical companies can only provide very limited information on product licenses, while medication administration to the paediatric population often involves off-label use [31]. Emerging research has documented variations in product performance associated with the administration of medication via EFT, by either one or a combination of formulation and tube related factors. However, the extent to which various factors influence product performance in paediatric populations has not been systematically assessed. Only a few articles discuss formulation characteristics, and interplay of different factors that affect the administration and overall paediatric treatment [32–34]. Without a holistic view of all the different factors involved in the administration of medication via EFTs, issues such as particle size, viscosity, dosing, rinse volume(s), patient's characteristics, administration methods or chemical compatibility of the oral medicinal product with the tube material and the risk of physical blockage of the tube may be overlooked. This can adversely impact the outcome of patients receiving their medications via this route resulting in medication errors, a risk of

inadequate dosing, reduced medication effectiveness, increased toxicity, and the occlusion of feeding tubes [20,35,36]. Many studies conclude that a lack of information contributed to the problems with administering drugs to patients on feeding tubes and suggested that practice could be improved by establishing guidelines [32,34,37]. Establishing guidelines will help minimize potential complications and limitations associated with this method of drug delivery.

This systematic review was carried out to identify factors (associated with the formulation and tubes), their effect on the performance of drug products and studies needed to demonstrate the feasibility of administering medication via EFTs to paediatric population. The determination of factors and their evaluations could support development of evidence-based recommendations or guidance for pharmaceutical companies to evaluate the administration of new oral paediatric drug products via EFTs.

2. Method

This systematic review has been registered in the International prospective register of systematic reviews PROSPERO, on March 1, 2022, under the number CRD42022299418 (Available at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022299418) [38]. It was performed according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) defined by Moher et al. [39].

2.1. Data source and search strategy

The literature search strategy was developed using keywords, synonyms and Medical Subject Headings that focused on the PIO criteria (Population of interest, Intervention, and Outcomes). For this review, these components were paediatric patients on enteral tube feeding involving different types of tubes and various types of oral medicines (P), the effect of dosage form characteristics of the medication administered via EFTs (I) and specified outcomes regarding the evaluation of dosage form characteristics such as dose accuracy, dose recovery, flowability, and tube blockage (O). The search terms from each category were combined using Boolean operators (And/Or) in order to locate all relevant literature using the following databases: PubMed, Embase, Web of Science and Scopus. Additionally, the European Medicines Agency (EMA) website was searched for the European public assessment reports (EPARs) that discuss the

product administration via EFTS, and clinical trial registration databases (ClinicalTrials.gov, EudraCT and International Clinical Trials Registry Platform) were searched for trials at any stage of completion that are relevant to this review. Reference lists from review articles were identified during this search to identify additional potentially eligible studies. Patents granted and pending applications that were published in the USPTO (United States Patent and Trademark Office) and EPO (European patent office) databases were searched using the Google Patents search engine (Google Inc., Mountain View, California, USA). The USPTO database is publicly available at www.uspto.gov (United States Patent and Trademark Office, Alexandria, Virginia, USA). The Keywords and phrases used to query the databases are available in https://drive.google.com/drive/folders/19Oq5MgU-b8pp4Jk8Pa9kZPzBTyUfhLqs?usp=share_link .The search was conducted from December 2021 until December 2022 and covered studies published in all languages and all years up until December 2022. The searches were conducted by one researcher and cross checked by another researcher.

2.2. Study selection

Due to the nature of the area being reviewed (enteral tube feeding and formulation characteristics), a range of study designs including randomized controlled trials, cross-sectional studies, prospective cohort studies, uncontrolled clinical trial, retrospective reviews, in vitro studies, observational and comparative studies, and case reports were included in this review. Moreover, letters, patents, and EPARs that evaluated either the feasibility, accuracy and/or stability of administering oral dosage forms via EFTs to paediatric patients, as well as studies that quantitatively investigated the impact of formulation methods on transit and dose recovery of oral dosage forms through EFT used in paediatric patients, were considered for inclusion. However, any literature that discussed the instructions for EFTs insertion, verifying tube placement, surgery for insertion and removal of EFTs were excluded. Additionally, studies were excluded if their main research focus was the formulation of enteral nutrition and studies assessing oral medicine product administration via EFTs in animals. Only English-language publications or articles in other languages with full English translation were included in this review. The preferred reporting items for

systematic reviews and meta-analyses (PRISMA) flow chart (**Figure 1**) explains the literature search strategy and the selected studies [39]. Articles from each database were downloaded to the EndNote library, duplicates were removed, and the remaining articles screened by the primary reviewer for relevance to the review based on the information contained in the title, abstract and subject descriptor/MeSH heading. All articles deemed relevant were retrieved in full text and appraised for inclusion. In addition, if the title and abstract were inconclusive, the full text of the paper was retrieved and appraised for inclusion.

2.3. Methodological Quality Assessment

After the second stage of the search process was complete and likely studies for inclusion had been identified, each article was closely scrutinised by two independent reviewers for adherence to the inclusion criteria. The reviewers were not blinded to the authorship of the studies. There was no disagreement between the reviewers. An adapted version of the Academy Evidence Analysis Library risk of bias tool called the Quality Criteria Checklist (QCC), Evidence Coding Scheme, and GRADE (Grading of Recommendations, Assessment, Development and Evaluations) was then used by the two reviewers to assign the level of evidence for each study and to identify those for inclusion in the review [40]. Methods for assessing clinical (human) studies are well developed. However, reporting standards and checklists to support the adequate reporting of scientific efforts *in vitro* research still have no generally accepted criteria [41,42]. There have been a number of initiatives aimed at improving and standardising the quality and reporting of *in vitro* research, such as cell culture studies, but there are no universal or consistent references to any particular set of guidance for assessing the quality of other *in vitro* research areas such as pharmaceutical formulation development. Considering the majority of criteria are generic and could be applied to this area, we were considered appropriate and adopted for assessment of the quality of *in vitro* studies included in this study. The three criteria identified for the assessment of the quality of studies are defined below:

1. EFT parameters listed or discussed e.g. type of tube, bore size, composition/material, length, internal and external diameter, site of tube blockage or outlet type/design.

2. Formulation characteristics discussed e.g., dosage form, dosage strength, particle size, viscosity, flowability, osmolarity, stability, acid resistance, sedimentation volume, reconstitution, disintegration or holding time.
3. Evaluation parameters discussed e.g., flush volume, dispersion medium, preparation method, co-administration with enteral feeding solutions/nutrition, tube blockage or dose recovery.

Accordingly following grading scale was devised:

- Grade I (good/strong evidence if the article discussed all three criteria listed above and is for/applicable to paediatric patients.
- Grade II (fair evidence if the article discussed at least two criteria listed below and is for/applicable to paediatric patients)
- Grade III (limited/weak if the article discussed any of the one criterion listed below and is for/applicable to paediatric patients).

Through the use of the QCC, the quality rating of each study was determined. The relevance and validity questions on the QCC were answered with 'yes' if the quality criteria were adequately addressed in the study or 'no' if they were not, and not applicable (N/A) when the questions were not relevant to the specific study (https://drive.google.com/drive/folders/19Oq5MgU-b8pp4Jk8Pa9kZPzBTyUfhLqs?usp=share_link). Likewise, the checklist included the final quality rating (minus -, plus + or neutral ∅). If most (five or more) of the answers to the above relevance and validity questions are "No," the report should be designated with a minus (-). The report should be designated with a plus symbol (+) if most of the answers to the above relevance and validity questions are "Yes" (must include criteria 1, 2, 3, 4 and 5). If the answer to any relevance questions (1-5) is "No," but other criteria indicate strengths, the study should be designated with a neutral (∅) symbol.

All data from the quality assessment and **Table 1,** which summarises the included studies, are presented in https://drive.google.com/drive/folders/19Oq5MgU-b8pp4Jk8Pa9kZPzBTyUfhLqs?usp=share_link .

2.4. Data collection and synthesis

Two reviewers independently carried out data extraction from the included studies. A meta-analytic synthesis was not appropriate in this review due to the heterogenicity of studies and outcomes, and hence a narrative synthesis of the included studies has been provided. A narrative synthesis was useful to provide a greater understanding of the current needs, issues, gaps, and opportunities in

the administration of medication via EFTs to paediatric patients. The discussion incorporated issues such as the effects of medicine characteristics (such as particle size, or viscosity) on the administration of medication to the paediatric population via EFTs, as well as the interplay of the formulation, enteral tube, and administration characteristics on performance of the paediatric drug product. Furthermore, without a holistic view, these issues could be overlooked in daily practices. The analysis and the synthesis of the data highlight the limitations of the data and gaps in the evidence. The information from this systematic review will help in the development of guidance to the pharmaceutical industry for demonstration of the feasibility of administration of drug products to children through EFTs.

3. Results

The search resulted in a total of 993 articles, of which 780 papers were identified after removing duplicates and screening for inclusion criteria. All 780 records were assessed by the primary reviewer for potential inclusion from their titles and abstracts only. A total of 213 records were found to be not relevant to the review. Of the remaining records, some were clearly identified as possible inclusions, whereas for others the possible inclusion was questionable. After reviewing the full text of the questionable records, 66 records were included. Of these 66 papers, the studies that were considered relevant for further assessment included 39 in vitro studies, 9 European public assessment reports, 4 patents, 4 randomized clinical trials, 3 comparative studies, 3 case reports, 3 observational studies and one letter. Relevant interventions and considerations included studies that considered the use of medications in different dosage forms, methods used to administer medications via EFTs, flushing of EFTs when administering medication, tube-related issues, (such as size and tube material), and patency and occlusion issues. The outcome predominantly measured was dose accuracy, recovery and tube occlusion; however, other outcomes such as flow rate, rates of occlusion and medication administration were also considered. Most of these studies were from the United States (US) and were conducted in academic settings in collaboration with hospitals and, sometimes, pharmaceutical companies. Several factors were found to affect the performance of the medication and administration of medication through EFTs. These included

patient characteristics such as age and existing conditions, a variety of tubes of different lengths, sizes (outer diameter), and materials, different volumes of flushing media, characteristics of the API, dosage form, extemporaneous manipulations of solid oral dose formulations. In addition, clinical practices such as verifying tube placement before administration, methods used to give medication, and methods used to flush tubes varied and impacted product and patient performance. Specific practices to prevent possible complications related to the administration of enteral medications, such as whether to dilute the medication and what syringe size to use, characteristics of the exit site, and enteral feeding regime were also found to influence the correct administration of medication via EFT. Furthermore, the extent of this effect varied between different dosage forms and their characteristics. All these factors influencing the performance of the medication and correct administration of medication via EFT were summarised into three categories 1) Formulation/Medication Characteristics, 2) Enteral Feeding Tube Characteristics, and 3) Administration Characteristics. When these occurred in two or more studies, positive and negative influences on medication administration via EFTs are summarised as evidence synthesis in **Figure 2 and Table 1** (APPENDIX 4.1: Section 3.4).

Using the QCC, a quality assessment of 65 studies was carried out. 64% of the studies were assigned a positive (+) rating indicating that the studies were focused on the paediatric population and clearly address issues of inclusion/exclusion, bias, generalizability, and data collection and analysis. 36 % of the studies were assigned a neutral (∅) rating, indicating that the studies were assessed based on tube size assumed to be commonly used in the paediatric and clearly addressed issues of inclusion/exclusion, bias, generalizability, and data collection and analysis (https://drive.google.com/drive/folders/19Og5MgU-b8pp4Jk8Pa9kZPzBTyUfhLqs?usp=share_link). GRADE was assigned to indicate the overall strength or weakness of the evidence. 91% of the studies rated Grade I, indicating good/strong evidence. These results were important and consistent, with minor exceptions at most and free of serious doubts about generalizability, bias, and flaws in research design. Meanwhile, 6% of studies rated Grade II, indicating fair evidence. The results were consistent, with minor exceptions at most, but they were from weaker designs for the questions addressed. On the other hand, 3% of studies rated Grade III, indicating

limited/weak evidence because the evidence consisted of results from a limited number of studies of weak design for answering the questions addressed. In conclusion, most studies are generally of high quality and low bias for containing quantitative analysis of through tube transit of pharmaceutical formulations using feasible and reproducible methods.

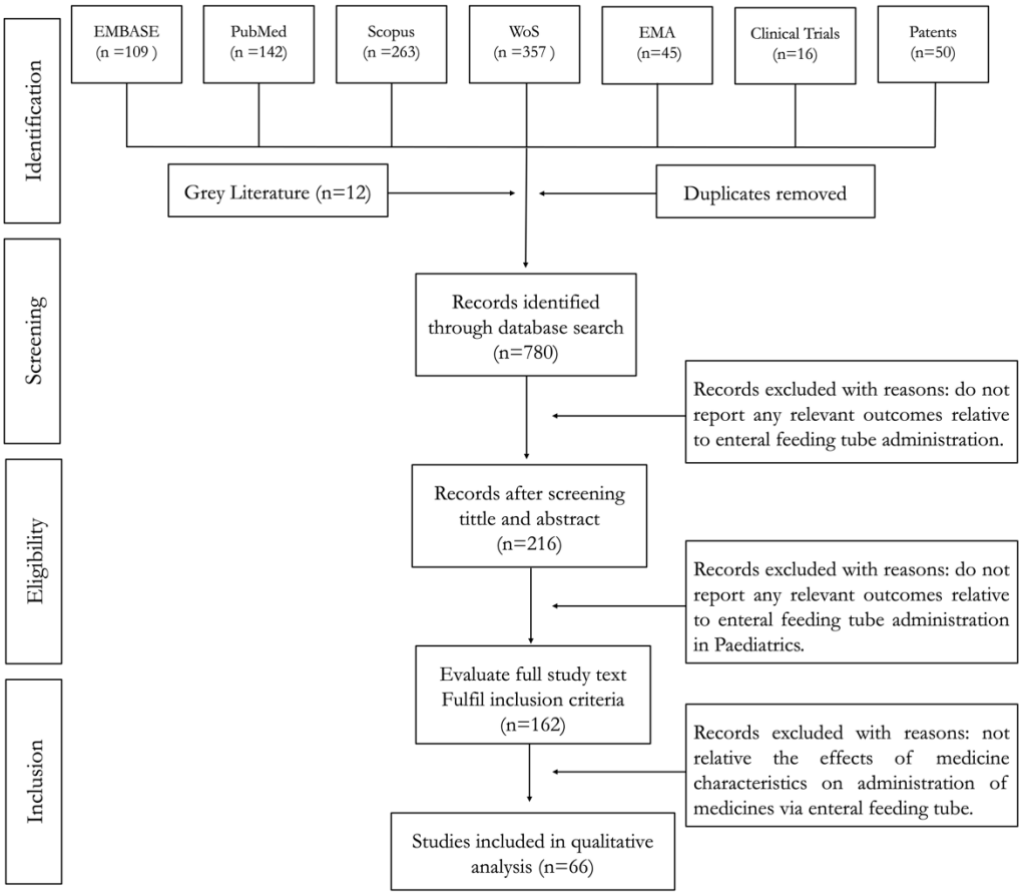

Figure 1. PRISMA flow chart to explain the literature search strategy and study selection

A total of 43 factors were identified and evaluated for data regarding enteral tube administration to paediatric population. The data on the effect of the factors on the primary outcome (tube cogging and dose recovery) were available for 28 of the 43 factors, though it is not known if those factors are suitable for paediatric population NG or gastric tube administration as for most of *the in vitro* studies, the factors were assessed on the basis of tube size assumed to commonly used in paediatric population. Most of the studies involved NG tube administration. Of

note, the factors analysed had data for enteral tube administration to paediatric population. Whereas 15 factors (35 %) listed in **Table 2** only had narrative/review data (e.g., *Handbook of Drug Administration via Enteral Feeding*) to support administration but no systematic study data on evaluating those particular factors on tube administration.

The level of evidence for each factor varied, with some factors having data on effect of extemporaneous preparations that are extrapolated to enteral tube administration, while others have effect on dose recovery and /or tube clogging to support administration via this route. For example, **Table 3** denotes the level of evidence available for each factor.

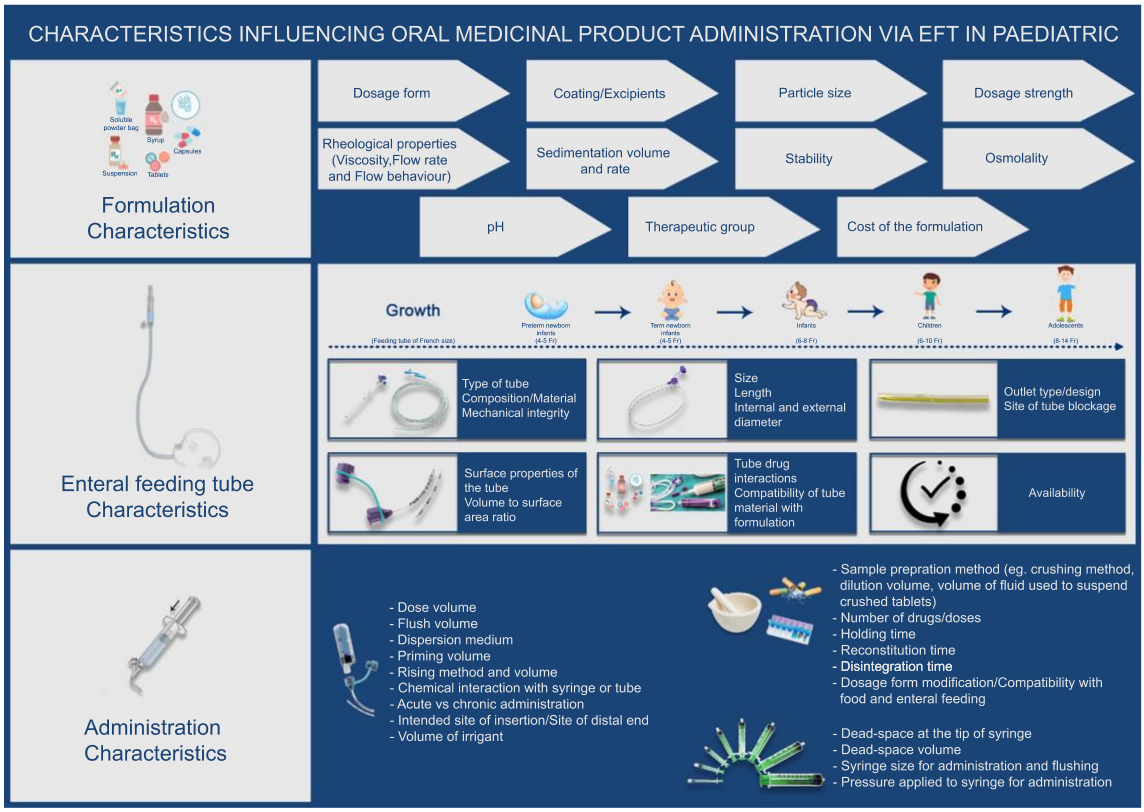

Figure 2. Characteristics/factors influencing the performance of the medication and correct administration of medication via EFT.

Table 2. Characteristics/factors that are supportive with narrative/review data for administration but no systematic study data on evaluating these particular factors for tube administration to paediatric population.

Formulation characteristics	Tube characteristics	Administration characteristics
Cost of the formulation	Mechanical Integrity	Dead-space at the tip of syringe
	Surface properties of the tube	Dead-space volume
	Volume to surface area ratio	Syringe size for administration and flushing
	Availability	Pressure applied to syringe for administration
		Priming volume
		Rising method and volume
		Volume of irrigant
		Intended site of insertion/ Site of distal end
		Acute vs chronic administration
		Chemical interactions with syringe or tube

3.1. Formulation/Medication characteristics

A total of 41 studies linked variation in medication performance or administration of medication via EFTs to the formulation characteristics. A full list of these studies' citations and summaries can be found in **Table** 1 (APPENDIX 4.1: Section 3.4). A total of 11 factors were identified and categorized under formulation characteristics (**Figure 1**). Factors, the number of studies and the type of studies that support each factor are shown in **Table 3**. The majority of the formulation factors (90%) had data available to demonstrate the effect on tube administration to paediatric population.

Table 3. Formulation characteristics. *In vitro* study (IVT), Randomized Controlled Trial (RCT), Comparative study (C), Observational study (O), Case Report (CR), Patent (P) and EPAR (ER).

Factors	Number of supporting studies	Type of study	No. of studies in which no effect was found on the primary outcome (tube clogging or dose recovery)
Therapeutic group	62	IVT, C, RCT,O,CR,P,ER	20
Dosage forms	61	IVT, C, RCT,O,CR,P,ER	20
Dosage strength	8	IVT	5
Particle size	12	IVT	0
Rheological properties	7	IVT, P	1
Sedimentation volume and rate	4	IVT	1
Chemical and physical stability	24	IVT, P, ER	0

3.1.1. Therapeutic group

Several medicinal products were identified in the studies included in the systematic review and according to The Anatomical Therapeutic Chemical (ATC) Classification System were classified (https://www.whocc.no/atc_ddd_index/). 21 studies were identified in the alimentary tract and metabolism group [32,43–58], 16 studies in the nervous system group [19,34,58–71], 8 studies in blood and blood forming organs [17,57,58,72–76] , 8 studies in cardiovascular system group [33,58,68,75,77–79], 5 studies in antiinfectives for systemic use group [76,80–83], 5 studies in antineoplastic and immunomodulating agents [37,58,84–86], 3 studies in systemic hormonal preparations, excl. sex hormones and insulins group [87–89], 2 studies in antiparasitic products, insecticides and repellents group [18,58], as well as 1 study in various group [90]. Of the 22 studies in the alimentary tract and metabolism group, about 11 were focused on Proton pump inhibitors (PPIs). As a result, the transit of PPIs via EFTs was the therapeutic group more investigated in paediatric patients.

3.1.2. Dosage forms

There is a general awareness that all dosage forms are not suitable for administration via EFTs, but there is still insufficient evidence on dosage forms that are safe and effective if administered via EFTs to paediatric patients. 14 studies noted that no suitable paediatric dosage form is available for certain therapeutic areas such as oncology drugs for administration to young children or to allow adaptation of precisely prescribed doses [18,25,32–34,37,45,46,65,68,71,75,77,84]. 61 studies determined the suitability of dosage form and indicate that preferred dosage forms for administration via EFTs were appropriate liquids (particularly elixirs and suspensions), followed by solid drugs with liquid administration form and solid drugs that could be crushed and/or suspended (**Table 3**). 13 studies used liquid forms for enteral administration and discussed the factors and challenges associated with administrating liquid dosage forms via EFTs to paediatric patients. Liquid dosage forms include solutions (7 studies), suspensions (5 studies), syrup (3 studies) and elixir (1 study). 56 studies included solid dosage forms for enteral administration for paediatric population. This includes tablets (33 studies), capsules (20 studies), granules (7 studies), and powder forms (4). In relation to the tablets administered, immediate-release tablets were the most common (n= 24; 69%), followed by enteric coated tablets (n= 4; 11%), orally disintegrating tablets (ODTs) (n= 4; 11%), soluble tablets (n= 2, 6%), and modified-release tablets (n= 1; 3%). The use of each dosage form comes with its own challenges, and several factors associated with the formulation have been shown to affect the product performance and administration of the oral medicinal product via EFT (**Figure 3**). Though liquids are most recommended (13 studies) and known to be more easily dosed through enteral tubes, 4 studies have highlighted issues with liquid formulations and indicated that some liquid medications may be unsuitable for administration by enteral tube to paediatric patients. Elixirs or suspensions are generally favoured over syrups since syrups are more likely to cause tube occlusion when exposed to enteral nutrition. There is evidence that certain liquid medications, such as clarithromycin, and ciprofloxacin suspension, readily occlude feeding tubes, especially paediatric enteral tubes. In addition, the oil-based suspension does not mix with aqueous solutions and cannot be easily flushed with water [91,92]. Not all syrups are considered suitable for

administration via EFTs. Daniel et al. revealed that hydrocortisone suspension administered via NG tube showed a variable dose recovery. It could be associated with the suspension viscosity and hydrocortisone low solubility [45]. Clark-Schmidt et al. discussed that four of the six methods in which the undiluted suspension was administered via NG tube showed a significant decrease in carbamazepine compared with the diluted suspension [60]. Klang et.al studied 62 commercially available solutions and suspensions (i.e., 58 liquid medications and 4 vehicles) and found that depending on the dose, the osmotic load of a liquid medication may cause cramping and diarrhoea. It also reported that many drugs are weak bases and require acidic vehicles for optimal stability [58]. The acidic liquids are especially reactive with enteral formulas that contain intact proteins. The result of this interaction can result in an occlusion of the feeding tube as the proteins form a gel-like clogs. The study also demonstrated that obstruction of a feeding tube due to the interactions of liquid medication and nutrition products is not solely dependent on the pH of the medication. Other causes have been speculated upon for obstruction formation factors, such as soap formation, viscosity of ingredients, and reactions with other excipients. Osmolality and the formulation tested (elixir, solution, or suspension) did not have any relationship to the occurrence of precipitate formation. Dilution of the syrup with water cannot prevent the physical incompatibilities between enteral feed and syrups. However, flushing the tube before and after the administration may help separate the medication from the enteral feed. The osmotic effect of high concentrated glycerol has to be considered in paediatric use with EFTs because of gastrointestinal adverse effects (diarrhoea). In studies of patients who have contracted diarrhoea while receiving enteral nutrition, many of these cases were found to be caused by the excipients found in the liquid formulations rather than intolerance to the nutrition. Edes et al. evaluated 32 episodes of diarrhoea in hospitalized patients receiving enteral nutrition support to determine the causes of diarrhoea in tube-fed patients and found that medicinal elixirs containing sorbitol were responsible for 13 cases of osmotic diarrhoea [93]. It is necessary to evaluate each liquid medication individually, as even generically equivalent products may have different excipients and formulation properties present. Daniell et al. has discussed the adverse effects caused by liquid preparations that are extremely hyperosmolar or contain large amounts of sorbitol [94]. This is particularly

troublesome when a large volume of drug is dispensed per dose. The study recommends that hypertonic medications, especially when undiluted, should not be administered directly into the small intestine, such as with a jejunostomy tube as this may cause bloating, nausea, cramping, and diarrhoea. Diluting the hyperosmolar liquid medication with 10–30 mL of sterile water before delivery through the feeding tube may help prevent these intestinal intolerances. However, more water may be needed when diluting medications that are either extremely hypertonic or being delivered directly into the small intestine and this can be unsuitably high for the target age especially if fluid restricted. A balance between the needs of a fluid-restricted patient and the minimal volume required to dilute medications for EFT administration must be realized. The viscosity and osmolality of the liquid dosage form, the internal diameter and length of the tube, and the location of the distal tip will all determine the final diluted volume of the liquid drug formulation. The lowest necessary volume needed to clear the tube is recommended for neonates, pediatric patients, and fluid-restricted patients. A study by Meakin et al. found that temazepam elixir significantly increase both gastric volume and pH compared to temazepam capsules although the age of the capsule group was higher with a mean age of 9.1 years versus 6.6 years in the elixir group [95]. This increase in gastric volume and pH was thought due to the composition of the elixir vehicle (ethanol 9%, sorbitol 45% and glycerol 50%). Glycerol is an irritant which stimulates mucus secretion, and both glycerol and sorbitol have osmotic properties which can cause the influx of water into the stomach. Both mechanisms dilute and increase the volume of the stomach contents. Due to the mechanism of action, these effects will also occur in adults or older children, and it is simply the ratio of dose volume to stomach content volume that renders them more significant in young children. Jones-Saete et al. suggested the replacement of valproate syrup due to gastric irritation with sprinkle valproate in paediatric patients [64].

The lack of licensed liquid formulations for paediatric means extemporaneous preparation of solid oral medications into other forms for administration, such as crushing or opening of capsules or off-label use of medications are required [96]. There are a number of potential limitations associated with the preparation and administration of these extemporaneous formulations, such as the time and complexity associated with both compounding and administering them, their

propensity to accumulate and occlude in the EFT, the potential for binding to enteral nutrition or other medications, a bioavailability that is lower than that of the oral capsule, a risk for product contamination particularly if preparation occurs at the bedside, and a lack of data regarding the stability of these extemporaneously prepared suspensions [22,97].

Pellets or granules and ODTs are a viable alternative to crushing dose forms because their reduced diameter allows them to flow through the tube diameter and avoid modification during the preparation method before the administration [32,33,48,49,98]. Out of 4 patents included, 1 patent discussed the suitability of the powder product to be administered via EFT [76]. It reported that the powder product should be dispersed uniformly in a short period of time and should not plug in the syringe or tube. Powder products which are coated with a pH-dependent polymer such as methacrylic acid copolymer cohere in a non-electrolyte liquid such as purified water or glucose solution, resulting in clogging in the syringe or tube. Therefore, such powders are not suitable for administration using EFT. Similarly, powder products which are formed by the use of sugar serving as an excipient, such as lactose, also cause clogging in the syringe or enteral tube and thus are not suitable for administration using tubes. Another patent studied the suitability of administration of multiparticulate sprinkle dosage forms through a NG tube or gastrostomy tube of at least 12 Fr and has high acid resistance stability after passage through NG tube [71]. These multiparticulate dosage forms had higher acid resistance stability as compared to the marketed duloxetine delayed release capsules after recovery through a combination of syringe and the feeding tube. The enteric coat of the discrete units remained stable after passage through the EFT.

Out of 61 studies, 3 studies compared the effect of different solid dosage forms on product performance when administered via EFTs. One study studied the differences in collection rates among different dosage forms for topiramate and antiepileptic drugs to determine the more suitable dosage forms for administration via feeding tube to newborn infants [67]. Three dosage forms: powder, fine granules or dry syrup (powdery form) drugs, powdery form drugs that have been pulverized (pulverized powdery forms), and pulverized tablets were investigated. Considering stability and pharmacokinetics, the more suitable dosage form for topiramate was pulverized tablets, whereas the more suitable

dosage form for other antiepileptic drugs was powdery form. The study indicated that the loss of drug during administration via feeding tube has a large influence on treatment efficacy. It also highlighted the importance of evaluating collection rates in addition to the passage through the tube, when investigating the more suitable dosage forms and drugs for administration via feeding tube [67]. Ponrouch et al. study different PPIs dosage forms (tablets, ODTs, and capsules). Only lansoprazole ODT were reliably and reproducibly delivered through small-bore (8 Fr) NG tubes, even when using small volumes of water to dissolve the tablet in children. Esomeprazole microgranules from tablets stuck to the inner walls of the tube, and many of the omeprazole and lansoprazole granules in the capsules may have been unable to penetrate the tube [32]. The study carried out by Sivén et al. studied the effect of spironolactone tablets (compounded to sachets and capsules). They concluded that the choice of excipients and the drug solubility should be considered as they can affect the conformity of the dosage form. In addition, from the therapeutic point of view, a smaller amount of excipients would be preferable as the safety of many excipients in very young patients is unknown. Likewise, a more frequent blockage was observed in the formulation where slightly soluble drugs and insoluble excipients were combined [75]. While the other study and 3 EPARs reported the investigation of the potential administration of the granules through nasogastric tubes [45,89,90,99]. The results indicated that the granules stick to the tubes (6 Fr and 8 Fr) and are not expelled following flushing. Removing the granules by creating a suspension in a universal tube shaken and left for 15 minutes, then aspirating using a sampling needle to avoid granules and administered down a NG tube resulted in a dose recovery of 74%-98%, which was comparable to and less variable than the other hydrocortisone formulations; however, this is not a licensed method of administration for hydrocortisone granules [45]. It was concluded that the smaller tubes do not allow proper administration of hydrocortisone. EPAR of Alkindi® concluded that granules were not to be suitable for tube feeding considering the high risk of tube blocking due to the granule size and impossibility of crushing without affecting the release profile [99]. Another product discussed in EPAR was Mimpara® which is presented as granules in capsules. This EPAR reported that this new formulation (granules filled in gelatine capsules that are intended to be sprinkled out on food or liquid or administered through gastric or gastro-nasal

tubes) could be favourable in paediatric population and also in adult patients with difficulties in swallowing [89]. However, the handling of the capsules (to open the capsules to administer just the granules) may not be optimal way of administration. Another EPAR reported that the prescribed dose of Velphoro® oral powder suspended in water may be administered via an EFT >6 Fr [90]. One nonblinded, two-phase cross-over trial study compared the pharmacokinetics of omeprazole administered through the gastrostomy tube as a suspension in paediatric patients versus the pharmacokinetics of omeprazole administered as a multi-unit-pellet system (MUPS®). The study concluded that omeprazole MUPS® had no advantage over the more easily administered suspension formulation [44].In an *in vitro* study, multiparticulate formulation of Qudexy® XR (topiramate), beads from opened capsules were able to pass through ≥14-Fr gastrostomy and jejunostomy tubes with tube clogging minimized by portioning the dose and using diluents for smaller tubes [59]. Another *in vitro* study showed that topiramate beads could be administered via a feeding tube, but clogging was likely to occur if an entire capsule content was added in one go or if a right-angled bend feeding tube was used [100]. In a study of six children, Carbatrol™ capsules (an extended release formulation of carbamazepine) were opened and administered via a feeding tube [65]. Two children had to withdraw due to tube occlusion. It was noted that these two children had longstanding problems with constipation and increasing intra-abdominal pressure that may have slowed the rate of flow through the tube. In one clinical study, four of 8 children who received valproate sprinkles (VPA-S) through feeding gastrostomies developed problems with recurrent external leakage around the gastrostomy site. It was hypothesized that the administration of VPA-S through the tubes results in wetting of the particles at the distal end of the feeding tube, promoting clumping of the material [64]. This clumping of particles may have caused acute occlusion of the tube and thus discourage gastrotomy feedings of VPA-S to children. These studies indicated that before an oral liquid formulation is prepared extemporaneously, it is essential to have an adequate understanding of the pharmacokinetic characteristics of the drug, the chemical compatibility of an active drug with excipients, as well as stability, ease of administration, and safety concerns. Manipulations are even more critical if they affect modified release formulations. Chua et al. demonstrated that the crushing of Circadin® (a prolonged-release

tablet formulation of melatonin) led to an immediate release of melatonin rather than the desired prolonged release [101]. Since 2019, the PUMA product Slenyto® (prolonged-release tablets of melatonin) has been available in the German market. However, although this medicinal product is licensed especially for paediatric patients with developmental disabilities, data for administration via EFT is lacking. Therefore, administration via EFT is considered off-label according to the manufacturer [102]. Another study reported that out of 21 paediatric products studied, only 4 products [Desitren® (levetiracetam), Nexium® (esomeprazole), Vistogar® (uridine triacetate), Prevacid® (lansoprazole)] mentioned that an NG tube may be used [30]. All four give instructions on its use but are not necessarily clear on details such as flush volume, composition/frequency of flushing, tube lumen diameter, compatibility data of the prepared formulation with the tube material or blockage issues. This illustrates that health professionals are often left alone in their decision-making when drugs need to be administered via EFT.

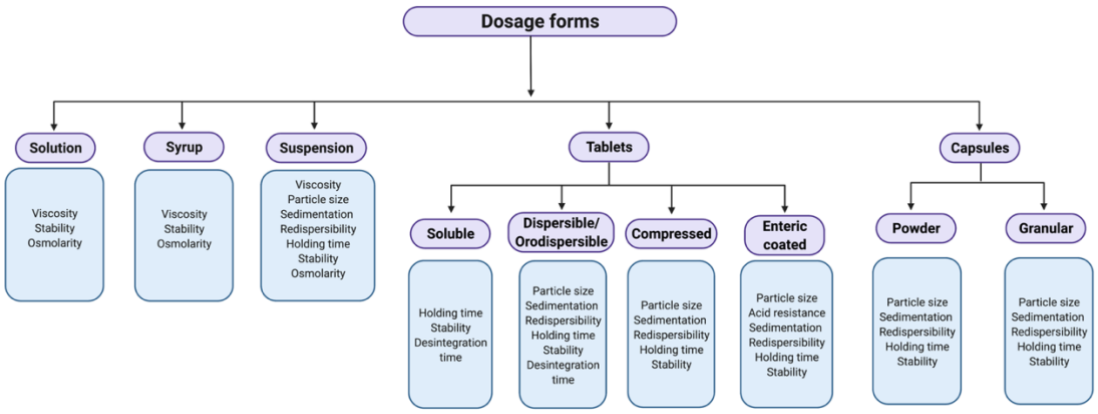

Figure 3. Characterisation of different dosage forms to support administration via EFTs.

3.1.3. Particle size

The manipulation process may change the performance of product by influencing the particle size and the size distribution. The combination of the range of particle sizes resulting from tablet crushing or dispersal, with the stickiness of different ingredients found in tablets and capsules, increases the likelihood of tube

blockage [103]. Twelve studies were identified as assessing the particle size of oral medicinal products (**Table 3**). Four studies evaluated tablets [19,34,49,68], and three studies analysed pellets [56,62,98]. As well, two studies and one EPAR evaluated the mean particle size of granules [48,62,104], one study assessed capsule powder [46], and another study analysed nano-emulsions [77]. These studies indicate that particle size is a critical factor to consider when administering medicines via fine bore tubes, which are commonly used in paediatric population. Most of blockages can be reversed, the process can be unpleasant for the patient and can ultimately result in unnecessary use of resources if the tube has to be re-sited. Hoover et al. (2021) observed more irreversible clogs due to the larger particle size and the increased amount of insoluble excipients. Also, the dosage with the higher dose contained larger microgranules (170 and 503 μm) than the dosage form with the lowest dose (130 and 425 μm) after enteral tube delivery, which increases the risk of tube obstruction [49]. Reindel et al. reported that lower particle size is important to avoid rapid sedimentation of particles and obtain a physically stable suspension. This study displayed that crushed tablets using standard mortar/pestle produced powders with significantly lower mean particle size, approximately 70 μm compared to pillcrusher (~ 400 μm) [19]. Particle size of different tablets using three crush protocols was studied by Salmon et al. Granulometric measurements suggested a strong dependency on the overall composition of the tablets [68]. Swedrowska et al. demonstrated the difference in particle size of powders produced by different crushers is caused by device geometry. The size of particles was affected by the comminution method and the number of manoeuvres performed. Rubbed and conical crushing tablets using five crushing manoeuvres (King's 5-5-5 protocol) reduced particle size below 350 μm, meanwhile spheroidal produced a particle size below 500 μm. Mortar and pestle reduced the particle size to 170 μm and two spoons above 700 μm. However, these last ones are not recommended as explained in the preparation method paragraph. The evaluation confirmed that five crushing manoeuvres (King's 5-5-5 protocol) are sufficient to produce powders with particles < 500 μm which were not prone to aggregation or tube-blocking when suspended in 5 mL water and delivered through a 6 Fr nasogastric tube with a pre and post administration flush using 5 mL water [34]. In contrast, Karkossa et al. observed that the inner diameter of the feeding tube is not accurate indicator for pellet

administration via EFTs (pellets with diameters ranging from 1363 to 1653 μm were unable to pass through 9 and 10 Fr tube diameters of 1900, 2100, or 2300 m, respectively). Also, pellets made from the 1000 μm starter cores were administered successfully through 12 Fr or larger tubes, meanwhile, the 500 μm formulations presented more problems, sometimes even in 15 Fr tubes [98]. However, White et al. affirmed that the efficient transit of esomeprazole pellets was better in the larger diameter tubes than in 8 Fr NG tubes but not clinically significant. The study showed that the higher dose recovery of the pellets may have been due to the smaller size (0.53 mm) and weight of the esomeprazole pellet (6.63 mg) compared with omeprazole [56]. Fleming et al. reported that the difficulty of pellets passage through the gastrostomy tube could be due to pellet size (>1 mm) compared to microspheres of approximately 300 μm that did not block the tubes [62], as well as the EPAR of Sibnayal® concluded that the finished product was not to be suitable for tube feeding considering the high risk of tube blocking due to the granule size and impossibility of crushing without affecting the release profile [104]. On the other hand, the median particle size granules ranging from 552 to 594 μm studied by Hoover et al. (2017) transited through a PU 8 Fr NG tube, and the risk of blockage was low. Some lots of 20 mg and 40 mg demonstrated smaller and larger particles after delivery through NG tube with a 15-min incubation time in DI water compared to pellets without incubation. The smaller particles may be the consequence of debris from the damaged enteric coating, and the larger ones could result from particle and coating agglomeration [48]. De Villiers et al. obtained with a novel compounding mixer a particle size between 15.6 and 24.4 mcm in different vehicles, meanwhile with the manual mortar and pestle method, particle size was between 47.6 and 57.6 mcm. It was found that the compounding mixer produced smaller and more uniform particles. The suspensions prepared from compounding mixer also did not cause blockage of 8 Fr NG tube, whereas those prepared using mortar and pestle left residuals and eventually blocked the tube, suggesting smaller particle size may be more suitable for nasogastric dosing [46]. Nanometric emulsions of 165 nm studied via EFTs by Hallouard et al. did not show adsorption in NG tubes, 95% of the drug was recovered by flushing with 15 ml of water [77].

3.1.4. Dosage strength

There were 8 studies that assessed the effect of dosage strengths on the drug recovery after delivery through EFTs (**Table 3**). Moore et.al assessed both the 10 and 2.5 mg dose strengths of crushed rivaroxaban tablets to determine the NG tube adsorption characteristics of rivaroxaban and found that rivaroxaban did not adsorb to the NG tube. Daniel et al. investigated hydrocortisone recovery after passage through 6, 8, 10 and 12Fr NG tubes in vitro for three formulations in two different doses (0.5mg and 2mg) [45]. The hydrocortisone granules and the liquid suspension showed similar results throughout the range of NG tube sizes, whereas the crushed hydrocortisone tablets gave greater variability for both doses. The delivery of hydrocortisone with hydrocortisone granules was significantly better than crushed hydrocortisone for the 0.5 mg dose ($p < 0.01$) and the 2 mg dose ($p < 0.01$), and the liquid suspension was better than the crushed hydrocortisone for the 2 mg dose ($p < 0.01$). One reason attributed to the variability in the recovery of the administered hydrocortisone dose was the problem with the practice of preparing small doses from adult dose tablets. Klang et al. looked at the changes by adding 5, 10 or 15 mg of warfarin to the same amount of chopped feeding tubes in simulated gastric fluid. The fraction dissolved of warfarin revealed a linear progression, and the recovery of warfarin was lower when the dose and chopped feeding tubes were increased in the study [17]. Hoover et al. (2021) observed more clogs with the 30 mg dose of lansoprazole ODTs than with the 15 mg dose during NG tube delivery and noted the build-up of lansoprazole particles during repeat dosing conditions could increase the risk of tube obstruction [49]. The greater clogging risk with the 30mg dose was attributed to the presence of a greater number of granules which can enter or exit the enteral tube at the same time. Ferrari et.al and Wojcinski et al. examined the recovery of suspensions of different dosage strengths administered through different EFTs. Ferrari et.al studied two strengths (100mg and 200mg) of cenobamate for five different EFTs. Approximately 100% of cenobamate was recovered after ex vivo administration of the suspension through EFTs [61]. There was no significant drug recovery loss with any of the EFT sizes or dosage strengths used in the study. Wojcinski et al. studied two strengths of lurasidone suspensions, 1 and 8 mg/mL prepared from tablets and found that both strengths of lurasidone suspensions passed through all 3 tube types without any clogging

issues, and the trend of flowability was consistent with the tube diameter [66]. Bladh et al. observed that the complete transit of esomeprazole pellets from the packet formulation via different small NG and gastric tubes was found to be sufficient for the 2.5 and 40 mg dosage strengths after suspension in a small volume of water (5 and 15 mL, respectively), followed by flushing with an equivalent volume was sufficient [43]. Santangelo et al. confirmed a method for consistently making suspensions from azithromycin tablets and capsules, ranging from 250 to 2000 mg, which may be administered via NG tube (8 and 12 Fr) [83]. Hoover et al. reported that 20 and 40 mg strengths were successfully delivered through an 8 Fr PU NG tube [48]. Johnson et al. verified that the different strength doses of omeprazole-sodium bicarbonate suspension (2.5, 5 and 10 mg) were easily administered through 5,6 and 8 Fr paediatric feeding tubes [50].

3.1.5. Rheological properties (Viscosity, Flow Rate and Flow Behaviour)

Seven studies analysed and discussed the rheological properties of different formulations, dispersion medium and effect on medication administration via EFT (**Table 3**). Viscous medications are more difficult to flush and increase the risk of tube obstruction [10]. The increased viscosity raises the concern of tube occlusion if the bore size is inadequate**.** Reindel et al. revealed that liquids with viscosities of 1000 cps or below demonstrated acceptable flow through the enteric tubes, and dose strength influenced suspension viscosity [19]. De Villier et al. evaluated the use characteristics of compounded Rifampicin suspension and found that due to the viscous nature of the liquid diluent (syrup), it was more difficult to remove the remaining Rifampicin suspension from the end of the tube and eventually blocked the 8Fr PU NG tube [46]. One patent reported a solid composition comprising a thickener that formed a viscous medium after mixing with an aqueous vehicle, increased the "floating" property and allowed the pellets to pass through thinner tubes [105]. Shlieout et al. observed that for the administration of pancrelipase pellets by gastrostomy tube, a high-viscosity vehicle is necessary to ensure that they are correctly suspended and pass through the gastrostomy tube without sedimentation, which might lead to tube blockage [53]. However, another patent stated that the use of low viscosity oily ingredients allowed for the administration of a higher dose with a lower amount

of baby food compared to what was reported by Shileout et al [106]. Wojcinski et al. analysed by visual observation flowability when lurasidone suspension was administered through different NG tubes. Flowability for PU 12 Fr and PVC 10 Fr NG tubes was excellent; meanwhile, for silicone 8 Fr NG tubes, flowability was satisfactory [66]. And Elia et al. measured the flow of glycerol solutions using different NG tubes and observed that was evident that irrespective of the solution's viscosity, modest changes in tube diameter can result in significant variations in flow. However, there were little to no differences in flow when the holes on the sides of NG tubes (6 and 9 Fr) and with only one side hole were occluded and cut, respectively [107].

3.1.6. Sedimentation volume and rate

Four studies evaluated sedimentation as a factor to consider for administration of medications via EFTs to paediatric population (**Table 3**). Two different lansoprazole ODTs studied by Hoover et al. (2021) showed that a high dose strength and the presence of different excipients in the dosage form increased the sedimentation volume. Furthermore, the angle at which the syringe and enteral tube were held during drug administration could be critical [49]. When the syringe was held in a vertical position, sedimentation of the granules to the bottom of the syringe tip occurred and concomitantly increased the risk of clogging the EFT. Karkosssa et al. demonstrated that administering the entire dose as a bolus would facilitate clogging of the syringe tip due to sedimentation during the administration process [98]. Additionally, rifampin suspensions prepared in the more viscous vehicles showed slower sedimentation rates than water, with a higher degree of flocculation indicating that they were better at resisting sedimentation and had better injectability during NG tube administration [46]. Sedimentation and redispersibility are more related to ensuring dose to dose content uniformity. While a large degree of sedimentation may be tolerable, redispersion should occur readily and rapidly upon shaking. Hallouard et al. developed spironolactone-loaded-nanoemulsion and observed that, notably, oil droplets or nanoscale particles could improve the dissolution rate and also cause stabilisation against sedimentation.

3.1.7. Chemical and physical stability

There were 26 studies that evaluated the suspension stability before the administration via EFTs (**Table 3**). Several studies confirmed acceptable suspensions for administration via EFTs: Enalapril orodispersible minitablets [33], potassium canrenoate film-coated tablets [78], azithromycin capsules and tablets [83], lurasidone tablets [66], lenalidomide tablets and capsules [85], valganciclovir tablets [82], apixaban tablets [72], eslicarbazepine acetate tablets[19], bisoprolol, ramipril and gabapentin tablets [68], tedizolid phosphate tablets [81], rivaroxaban tablets [74], pancrelipase tablets [106], temozolomide suspension [84], duloxetine capsules [71], esomeprazole pellets [43], morphine pellets [63], spironolactone nanoemulsions [77], sachets of omeprazole-sodium bicarbonate for oral suspension [50], glycopyrronium bromide oral solution [108], larotrectibib oral solution [86], sucroferric oxyhydroxide powder [90], cinacalcet hydrochloride granules [89], vigabatrin tablets [70], sodium phenylbutyrate granules [109], and fenfluramine hydrochloride oral solution [69]. The quality and safety of medicinal products should be assured by chemical and physical stability studies of the drugs in the dispersion medium [28]. Solid pharmacological formulations are often used in paediatric patients with EFTs [25]. One of the main challenges when preparing extemporaneous formulations is stability issues. Disadvantages of solutions include the increased potential for drug instability due to hydrolysis or oxidation [11].

3.2. Enteral feeding tube characteristics

As reported in **Figure 2**, a total of 13 factors were identified and categorized under enteral feeding tube characteristics. A full list of these studies' citations and summaries can be found in **Table 1** (APPENDIX 4.1: Section 3.4). 69% of factors had data available to demonstrate the effect on tube administration to paediatric population. The rest of the factors only had narrative/review data (e.g., Handbook of Drug Administration via Enteral Feeding) to support administration but no systematic study data on evaluating those particular factors on tube administration. In some cases, data relevant to these factors were either insufficiently reported or not investigated in the included studies.

The most commonly used/studied NG tube sizes in paediatric patients were 8 Fr (n=26), 12 Fr (n=18), 10 Fr (n=14), 6 Fr size (n=14), 5 Fr (n=6), and 4 Fr (n=3) (**Table 4**).

Gastrostomy tube sizes were 16 Fr (n=8), 14 Fr size (n=6), 18 Fr (n=6), 20 Fr (n=3 studies), 12 Fr (3 studies), 15 Fr (n=2), and 8 Fr (n=1) (**Table 4**).

Table 4. Enteral Feeding Tube Characteristics.

Enteral Feeding Tube Characteristics	Number of supporting studies	References
NG Tube Sizes		
4 Fr	3	[51,71,86]
5 Fr	6	[33,50,60,67,71,86]
6 Fr	14	[32,32,34,43,45,50,54,61,71,75,82,86,99,105,107]
8 Fr	26	[32,35,43,45–50,52,56,57,66,71,79,80,82–84,86,87,90,99,105,107,110]
10 Fr	14	[19,33,37,45,62,66,71–73,81,98,105,107,110]
12 Fr	18	[17,34,37,45,49,60,61,63,66,71,74,78,82,83,90,98,107,110]
Gastrostomy Tube Sizes		
8 Fr	1	[85]
12 Fr	3	[48,71,85]
14 Fr	6	[53,59,65,71,88,106]
15 Fr	2	[44,71]
16 Fr	8	[53,59,61–63,65,71,106]
18 Fr	6	[53,59,65,71,81,106]
20 Fr	3	[56,71,85,106]
Material		
PU	33	[19,32–34,37,43,46,48–50,54,56–58,61,63,66,70,72,73,75,77–81,83–85,87,89,98,105,110]
PVC	14	[19,33,43,49,60,61,63,66,74,80,83,85,89,105]
Silicone	14	[33,49,53,56,61,65,66,70,74,89,98,105,106,110]

Tube materials investigated were PU (n=33), PVC (n=14) and silicone (n=14) (**Table 4**). The length of tubes used in the studies was between 40-125 cm. Many studies did not disclose information on length (n=36), material (n=25) or size of

the tube (n=11). While most of the studies assessed the size of PU tubes, information on different materials or lengths was lacking. Thus, only a few studies assessed the effect of tube material or tube length on the administration of commonly used medication for children via EFTs. For many commonly used drugs in paediatric population, the appropriate dosage form for administration via EFTs and information on appropriate size/material of the tube is lacking.

Hoover at al. (2021) studied different EFTs: PU and PVC 8 Fr NG tube, silicone 12 Fr gastrostomy tube and plastic and silicone 12 Fr jejunostomy tube. Results reported that gastrostomy tubes had the greatest risk of clogging compared to NG and jejunostomy tubes [49]. However, Jones Saete et al. observed that children with "button" feeding tubes experienced some difficulties when receiving VPA-S and recommended not VPA-S administration to children with that device. Reducing external leakage related to VPA-S administration would be likely possible by changing tubes more frequently and/or using larger tubes [64]. As well as Strobbe et al. suggested the change from NG tube to a gastrostomy tube in a 3-year-old girl due to the long duration of mitotane treatment and thus reduce discomfort [37]. Karkossa et al. revealed that gastrostomy tubes (9, 10, 14, 15, 18, and 20 Fr) had fewer administration issues than the NG tube. It was noted in NG and gastrostomy tubes that the tube diameter significantly influenced the success of the pellet delivery [98]. The geometry and design of the enteral tube, specifically the size, location of the eyes and open/closed distal tip, and the type of tube used as directed by the intended *site of insertion and site of the distal end* should be considered [49,83,98]. One study observed that tubes with lateral outlets had more probable blocks than a linear outlet [98]. Hallouard et al. affirmed that the distal end part of tubes could eventually be responsible for a siphon effect during emulsions administration [77]. Furthermore, Johnson et al. observed that lansoprazole bead suspension partially clogged the 6 Fr feeding tube and completely clogged the 5 Fr feeding tube. Microgranules were stuck in the feeding tube hub-barrel junction [50]. Several authors confirmed that the occlusion was caused by the small diameter of the tubes [45,48,54,55,65]. On the other hand, Elia et al. determined that there little to no differences in flow when the holes on the sides of NG tubes (6 and 9 Fr) and NG tube with only one side hole were occluded and cut, respectively [107]. In paediatrics, lengthy NG tubes are used to prevent cluttering up the space around the patient's head with

tubes and tube system equipment. However, Ponrouch et al. found that tube length had no statistically significant impact in drug delivery via EFTs [32]. Elia et al. observed that the length (70-120 cm) of feeding tubes and the discharge rate of fluid (solution with a viscosity of 4.25 cps) through them showed a linear relationship, and for tubes with smaller diameters, the impact was more noticeable [107]. Additionally, the flow of glycerol solutions via different NG tubes (6,7, 8,10 and 12 Fr) revealed that irrespective of the solution viscosity, modest changes in tube internal diameter could result in significant variations in flow. Therefore, the internal diameter of the NG tube had a far greater impact on the discharge rate than its length [107]. Likewise, tube diameter should also be considered due to obstructions and cracks caused by the pressure applied to the syringe during the injection [37,54]. A case report discussed the breakage of a nasoenteral tube in a 5-year-old boy. Different factors may have caused the breakage, for instance, an acute increase in intraluminal pressure induced by vigorous flushing or the kinking of the EFT in the child's stomach [111]. The capacity of feeding tubes to bend without kinking, or what is known as bending stiffness, is a crucial factor to consider in a clinical setting. Selecting feeding tubes made of different materials that are less prone to cause tube blockage is an important factor. Silicone is flexible, extra material is needed to give support and prevent stretching or collapsing. As a result of the thicker walls, the inner diameter of the tube is smaller than that of PVC or PU tubes of the same Fr size [6]. Metheny et al. confirmed that polyurethane was consistently superior to silicone as a tube material in a comparative study [110]. Karkossa et al. reported that pellets administered via PU tubes had fewer problems during administration than silicone tubes. Moreover, Thabet et al. observed that silicone NG tubes achieved a slightly lower dose recovery than PVC and PU NG tubes [33]. Interactions between EFT and drug was reported by Klang et al. The study discussed that warfarin binded directly and irreversibly to PU EFTs. Binding to plastic surfaces can depend on pH, temperature, the concentration of the warfarin in solution, and rate of administration. Therefore, the overall quantity of warfarin that will reach patients would be decreased by feeding tube delivery [17]. Hence, the characteristics mentioned above may substantially impact drug delivery through EFTs.

3.3. Administration characteristics

A total of 19 factors were identified and categorized under administration characteristics (**Figure 2)**. A full list of these studies' citations and summaries can be found in **Table 1** (APPENDIX 4.1: Section 3.4). Data were obtained from 47% of factors to show the impact of tube administration to paediatric patients. The other factors lacked systematic study data for evaluating them specifically on tube administration and were only supported by narrative or review data (e.g., Handbook of Drug Administration by Enteral Feeding). In other instances, data pertinent to these factors were either insufficiently reported or not investigated in the included studies.

3.3.1. Preparation method

Seventeen studies were identified that investigated and discussed the preparation and dispersion methods as a factor to consider for administration of medications via EFTs to paediatric population. Salmon et al. discussed that the recovery of a maximum of powder after the preparation method was better using a confined crushing and suspending protocol (pill crusher or combined crushing and suspending device) compared with open protocols (mortar and pestle) [68]. Cross contamination, aerosolization of the drug powder and drug loss during drug transfer are some disadvantages of mortar and pestle method. Swedrowska et al. developed a protocol to pulverise tablets with 5 crushes using a pill crusher. This preparation method showed good results in delivering melatonin to paediatric patients through NG tubes [34]. De Villiers et al. proposed a compounding machine that employs wet milling based on the positive results that suspensions revealed prior to their administration via EFT. Preparing these suspensions using other devices was more resistant to sedimentation and was easier to redisperse than mortar and pestle [46]. During the verification of the dose Santangelo et al. observed that the lowest dose strength in crushed tablets revealed loss of azithromycin attributed to the preparation process compared to intact tablets and capsules [83]. Likewise, Masaoka et al. observed that cracking the surface films of valganciclovir tablets was more effective than making powder from tablets and could reduce the loss of the drug [82]. Logrippo et al. associated drug loss with solid oral dosage form manipulation before administration via EFTs due to the chemical and physical characteristics, such as freely water-solubility

of APIs and excipients [78]. Also, Sivén et al. affirmed that the solubility of the drug and excipients should be considered, as this affected the simulated drug dose in administration [75]. Manessis et al. discussed that some levothyroxine doses were lost during the preparation method (crushing, mixing, and diluting of the drug), and hydrophobic drugs showed compromised solubility in water, and it was very difficult to mix [88]. Daniel et al. observed a loss of hydrocortisone mass due to its slight solubility in water and adhesion to the compounding equipment [45]. Musazzi et al. proposed the adoption of proper procedures for purifying (filtration and centrifugation) the preparation from the insoluble excipients and highly water-soluble drugs. This study observed that compounded oral suspensions containing chloroquine and hydroxychloroquine could be filtered and/or centrifuged without altering the drug assay of the preparation [18]. However, despite a slight loss of drug and variability, Caraballo et al. confirmed that mortar/pestle method, pill crusher and crushing syringe are alternative methods to prepare apixaban suspensions of acceptable dosage accuracy [72]. Strobbe et al. developed a new reproducible and effective method using mortar/pestle to administer a narrow therapeutic range and lipophilic drug (mitotane tablets) that is usually complicated for healthcare teams, especially when dealing with children [37]. Wojcinski et al. revealed a viable administration via NG tube preparing suspensions from lurasidone tablets using the standard mortar-pestle method. Rinsing the mortar and pestle, the study achieved an acceptable drug concentration delivery via NG tube [66]. The in vitro study by Thabet et al. showed the differences between two preparation methods, ODTs dispersed using only a syringe or diluting in a container [33] . Using only the oral syringe allowed dose titration and uniform doses. Morita et al. investigated an effective, simple suspension method based on disintegrating lenalidomide tablets and capsules in hot water without crushing or opening them [85]. Niimura et al. also revealed that the simple suspension method without crushing amlodipine did not show the loss of drug during the preparation. On the contrary, the drug adhered to the mortar and pestle and solidified when hot water was added compared to Campo's simple suspension procedure [79]. Naya et al. observed that the preparation method combining clopidogrel + MgO tablets as a suspension produced changes in the drug’s physical properties and increased the risk of blockage compared with the method that contained only clopidogrel.

Physical properties of clopidogrel to water-insoluble and viscous clopidogrel via neutralization under alkaline conditions due to MgO tablet's presence [57]. Avoiding the mixture of different medicinal products is recommended in the Enteral Nutrition Practice Recommendations due to physical and chemical incompatibilities [10,36].

3.3.2. Dose volume and number of doses

The dosage of drugs for newborn infants is precisely determined based on the infant's body weight. Because the dosage is much lower in infants than in adults, the loss of the drug during the feeding tube must be carefully considered. Four studies evaluated the dose volume as a factor to consider for administration of medications via EFTs to paediatric population. Clark-Schmidt et al. studied the administration via NG tube 10 mL of the carbamazepine suspension undiluted and diluted with an equal volume of different diluents (water, 5% dextrose solution and 0.9% NaCl solution). Results revealed that hydrocortisone adhered to PVC NG tubes and a significant loss of carbamazepine administering undiluted suspension was detected compared with diluted suspension that underwent viscosity changes upon dilution [60]. Ponrouch et al. evaluated the influence of the volume of water (2 or 5 ml) used to suspend the different PPIs dosage forms. Lansoprazole ODTs were delivered via paediatric NG tube using small volumes of water to dissolve the tablet and flush the tube after the PPI. However, the amounts of water employed in this study to dissolve the esomeprazole tablets were insufficient to produce a suitably fluid solution, leading to a "paste" that was challenging to administer and plugged into the tube [32]. Thus, compounding and administering are potential limitations associated with PPI. Thabet et al. found uniform doses for 0.025 mg and 0.125 mg of enalapril minitablet, with the administration of 1 or 5 mL of the dispersion, avoiding manipulation of the dosage form and errors during the administration [33]. Considering that minimal volumes are required for paediatric patients, Swedrowska et al. evaluated a procedure in which Circadin® tablets were suspended in 5 mL water and administered via a paediatric NG tube [34]. Two studies discussed the effect of the number of doses of PPIs when administered via paediatric EFTs [49,54]. Stewart et al. found that after administering the 11th dosage of esomeprazole pellets on the sixth day of continuous feeding, the size 6 Fr tube became blocked, but the 8 and 10 Fr tubes

remained clear [54]. Hoover et al. (2021) noted a low recovery of lansoprazole, indicating that particles remain in a 12 Fr jejunostomy tube after the administration and the build-up of lansoprazole particles during repeat dosing conditions could increase the risk of tube obstruction [49].

3.3.3. Holding, reconstitution and disintegration time

Three studies analysed the holding time. Karkossa et al. showed that pellets with polymethacrylate-based coatings had a higher tendency for agglomeration and sticking to the syringe after a holding time of 30 minutes compared to formulations coated with PVA and EC that are insoluble in water [98]. Thus, pellets should be administered after the dispersion to avoid blocking devices. On the contrary, the allowed holding time of 30 minutes during the suspension in water was maintained for esomeprazole pellets studied by Bladh et al. [43]. Also, sucroferric oxyhydroxide granules (Velphoro®) should be taken within 30 minutes after being suspended, and if necessary, the suspension should be resuspended because the compound does not dissolve completely.

There were two studies that assessed the reconstitution time. Daniel et al. evaluated a method for the preparation of hydrocortisone granules studying the best reconstitution time (60 min) resulting in the maximum dose delivery [45]. Meanwhile, Bladh et al. observed that reconstitution time in water and apple or orange juice was 2 and 9-10 minutes, respectively [43]. Likewise, four studies observed the disintegration time. Thabet et al. observed that in ODTs disintegration time is dependent on the medium. In an aqueous solution, the time was less, meanwhile in different juices and milk, was higher, which could result in an increased risk of choking in children. The study recommended the dispersion in tap water [33]. Hoover et al. found that lansoprazole had a short disintegration time in the water [49]. In addition, Patent nº ES2338009T3 revealed that omeprazole pellets as tablets were disintegrated in syrup or yoghurt in 2 minutes [105] and the disintegration time of granules studied by Patent nº US20050152975A1 was 5 minutes [76].

3.3.4. Flush volume

A critical procedure parameter having a significant impact on medication transit through EFTs is flush volume, which is the volume of fluids used for rinsing a feeding tube immediately before and after drug administration [112]. Flushing is

a standard clinical practice for prevention blockage and flushing or diluting with water is necessary during the administration of viscous formulations**.** As *The Handbook of Drug Administration via Enteral Feeding* recommends water is the most appropriate flushing agent. Metheny et al. found that water and cola as a flushing agent were superior to cranberry juice, but cola can increase tube occlusion by forcing feed to coagulate or protein to denaturize, both of which can lead to tube blockage [110]. Although there isn't much research on the best type of water to use, for patients with tubes beyond the stomach, it is best to use sterile water, and for gastric tubes, tap water or sterile water, depending on local policy [112]. ASPEN recommend for infants to flush the enteral access device with sterile water before and after administration of enteral formula and medication [11]. Around 15 ml of suspending volume and 15–30mL water flush are recommended in adults [14,34]. Special attention should be taken in paediatric patients and patients with renal or cardiac disease. To safely deliver medicines through EFTs, the volume of liquids used for rinsing tubes and the volume of suspension need to be reduced to prevent gastric overload in paediatrics due to the smaller stomach size [112]. Several authors reported that the sum of liquid volumes administered in paediatric patients should not exceed 15 ml [32,34,113]. The total drug administration volumes in neonates patients should be minimal with lower flush volumes (1–3 mL) to limit excessive free water administration [36,113].

19 studies focused on the paediatric population investigated the flush volume administered through paediatric EFTs. Johnson et al. administered the flush volume according to this population, 10 mL NaCl 0.9% flush with 8 and 6 Fr NG feeding tubes and 5 mL NaCl 0.9% flush with 5 Fr feeding tube using 5 ml of powder and microgranules as suspension. Flush volume was unable to administer in 5 Fr tube due to the blockage caused by lansoprazole microgranules suspension but could clear the 6 Fr tube. However, omeprazole sodium bicarbonate suspension made from packets administered through all 3 sizes of neonatal/paediatric feeding tubes was easily administered [50]. Bladh et al. used relatively small volumes (5 and 10 ml for 2.5 and 40 mg, respectively) for full delivery of esomeprazole packet through a variety of small calibre and standard enteral tubes, including tubes of diameters as small as 6 Fr [43]. Stewart et al. simulated drug administration for a patient with volume restriction and

observed the risk of blockage with esomeprazole sachet as suspension delivered through 6 French NG feeding tubes due to the small-diameter feeding tubes and low flush volumes [54]. Ponrouch et al. studied low flush volumes to prevent gastric overload, as these should not exceed 15 ml. The authors evaluated the influence of 3 flush-through volumes (2 ml, 5 ml and 10 ml) in 8 Fr NG tube. The highest flush volume with omeprazole granules showed the best results, although the low quantities recovered. On the other hand, esomeprazole did not show a better result increasing the flush volume and the microgranules stuck to the inner walls of the tube. On the contrary, lansoprazole granules did not reveal that the largest flush volume yielded an adequate dose recovery. However, lansoprazole ODTs were able to optimize the full dose delivery using the highest flush volume [32]. Sivén et al. evaluated different solid dosage forms through 6 Fr NGT flushing with 2 ml of water before and after drug administration [75]. The study revealed drug loss during the preparation method or due to the solubility of the drug and excipients. Thabet et al. studied the flush volume necessary to recover enalapril ODTs in 10 Fr NG tubes. Studies revealed that at least flush with 2 ml of tap water was necessary to dose recovery [33]. Daniel et al. affirmed that flushing the administrating syringe is important to maximize the dose recovery. According to the recommendations given by healthcare professionals specialising in neonatal and paediatric care, *in vitro* testing with hydrocortisone was carried out with 2 mL for the 6Fr and 8Fr tubes, 5 mL for the 10Fr tubes and 10 mL for the 12Fr tubes [45]. Shimizu et al. studied several antiepileptic drugs administered through 5 Fr J-feed feeding tubes maintaining low flush volumes, around 10 ml [67]. Topiramate pulverized tablets and powdery forms of other antiepileptic drugs showed a dose recovery of > 90%. Following the same recommendations, crushed melatonin tablets as suspension were administered via 6 Fr NG tube by Swedrowska et al., where the pre- and post-flush volumes, as well as the volume to suspend the drug, were limited to 5ml [34]. After administering paediatric temozolomide suspension through 8 Fr NG tube, Annereau et al. performed one or two cycles of washing with 5 or 10 mL of water, achieving a dosage recovery of 100% [84]. 5 EPARs that developed medicinal products for paediatrics patients recommend flush EFTs for dose delivery, even though experiments are unavailable since confidentiality policies [69,70,86,89,90]. One case report described a protocol that included flushing the tube after administration of

mitotane crushed tablets as a suspension in milk via NG tube in a 3 year old girl [37]. Two clinical studies using pre-flush and post-flush volumes for administration were identified. Pre-flush volume of around 10 ml and post-flush volumes of around 10-20 ml were used to administer carbamazepine capsules as a granular suspension in patients ranging from 6 to 20 years old and Multiple Unit Pellet System (MUPS®) tablets and capsules as a suspension in patients between 8 ad 27 years through gastrostomy tubes [44,65]. In addition, an observational study by Jones-Saete et al. studied gastrostomy tube administration of valproate suspended in formula in children aged 3 to 17 years, during which flushes were performed before and after administration [64]. According to Wollmer et al. research assessing the ingested fluid volume co-administered with oral dose forms is needed across all paediatric age groups [114]. Flushing the EFTs is a recommended clinical practice to avoid tube blockage and ensure maximum drug delivery because insufficient flushing could significantly impact the effective dose. Nevertheless, the flush volumes used are recommended by the guidelines' policies for adults, but these conditions would not be realistically applicable in all paediatric age groups.

3.3.5. Dispersion medium

Most of the studies selected water as a dispersion medium for the preparation and administration of medicinal products via EFTs used in paediatric patients. 15 studies used purified water [18,35,48,49,54,59,61,63,66,75,76,78,80,82,85], 9 studies used tap water [33,48,49,52,56,67,70,98,105], 5 studies used sterile water [34,45,60,81,88], 2 studies used commercial mineral water [68,83], two studies used dextrose in water [60,73], one study used sparkling water [59], and the rest of the studies did not specify the type of water. In contrast, a few studies were identified that used different dispersion mediums such as milk [37,62], formula feeds [51,53,59,62,64,65], apple juice [65], sugar free-NF [46], Ora-Sweet [46]. Hoover et al. (2017) observed no statistical difference between suspending the granules in deionized water or tap water because most granules were recovered after EFT administration [48]. Riss et al. found that carbamazepine suspended in water or apple juice rather than formula was considerably less likely to occlude the tubes [65]. Clark et al. observed that

topiramate beads diluted in a feeding formula minimized the blockage in 14 Fr Gastrostomy tube compared to deionized water, but the dose recovery using deionized water was nearly 100% in 16 Fr, 18 Fr gastrostomy and 18 Fr jejunostomy tubes [59]. Some studies demonstrated success delivery via EFT with the aid of different liquid vehicles such as milk or formula feed [51,62]. Strobbe et al. demonstrated a simple, effective, and reproducible method using a fatty substance (milk) as a dispersion vehicle to allow the administration via EFTs of lipophilic drugs in a paediatric patient [37]. In addition, 5 studies reported using Ora-Plus as a satisfactory alternative to water for administration via EFTs [19,46,52,66,72]. Therefore, water was the preferred dispersion medium for preparing and administering medicinal products via EFTs.

As noted in this systematic review, some drugs can be co-administered with certain juices, beverages, milk, formula feed or syrup vehicles. In literature is reported that several drugs, such as phenytoin, carbamazepine or warfarin can interact with feeding formulas [22]. Thus, chemical stability and pharmacokinetics studies should support the compatibility of the medicinal oral product with different dispersion mediums.

4. Discussion

This review has identified and summarised several important factors that may affect the performance of medication when administered via EFTs to paediatric population. Among the extensive list of putative risks or factors reviewed, few had sufficient evidence to draw firm conclusions about their association with tube clogging or altered dose recovery. This does not mean that the remaining factors do not play a role in medication administration via EFTs to paediatric population, but only that evidence was insufficient to draw such a conclusion. It was also recognised that despite the regularity with which the medications are administered to paediatric population via EFTs, there is a surprising lack of empirically derived evidence to guide the assessment of the feasibility of medication administration via EFTs to paediatric products especially neonates. Much of current practice has been borrowed or modified from practice in adults. This is unacceptable, given the importance of these tubes for the safety and feeding of these vulnerable infants. Most studies found that the suitability of

dosage form and tube size are the dominant factors in tube administration for paediatric population. It is the characteristics of the dosage form that play a critical role. Different dosage forms may differ in their potential to be administered via EFTs. However, significant factors associated with one dosage form may not be significant in another. For example, for suspension, the viscosity particle size, and sedimentation were found to be critical factors that had an effect on the performance of tube administration. The paucity of data related to the effect of manipulation of commercial dosage forms for administration via EFT to paediatric population is further confounded by the multitude of drug specific properties that could affect the tube administration to paediatric population.

Drugs subject to hydrolysis or pH effects, such as temozolamide, lansoprazole are important to consider when utilising an aqueous vehicle for suspension. Water is the preferred dispersion medium for preparing and administering medicinal products administered via EFTs. When lansoprazole was mixed with water or apple juice and administered via an NG tube, it was associated with a 42% incidence of clotting [115]. The lansoprazole-packet suspension had varying changes in pH, the suspension was more acidic with apple juice, orange juice, and water and more alkaline with cranberry juice. The greatest increase in acidity was with the packet in water [116]. The packet suspension immediately thickened following reconstitution with these vehicles only a very small amount was injectable from the syringe into the NG tube, causing blockage of the NG tube. The relative impact of vehicle or diluent pH is difficult to assess but may be dependent on drug specific chemical properties affecting ionization, such as pKa. The excipients can also differ, which might lead to differences in solubility. As the data of the literature implies, particle size is a very important parameter influencing the final recoverable quantity after passage through EFTs, as well as the viscosity that could become critical in the preparation and administration of medication via EFTs. Osmolarity must be adjusted by dilution since high osmolarity may increase the incidence of gastrointestinal side effects. The quality and safety of medicinal products should be assured by sedimentation, holding time, reconstitution time, acid resistance and chemical and physical stability. Insoluble components of a drug product have the potential for sedimentation that may increase the risk of clogging. Therefore, to avoid tube blockage and a significant loss of dose delivery and therapeutic activity due to the interference of

the above factors, it is recommended to administer oral medicinal product after dispersion. Also, a lack of data regarding stability could compromise the administration via EFTs. The preparation and dispersion methods are associated with potential risks such as alterations to the physicochemical stability of drugs, the loss of drugs due to adhesion or adsorption to the inside tube surface or crushing apparatus, variations in the compounding ratio, tube occlusions, drug and nutrition incompatibilities.

The age of a child can have an impact on tube administration; newborns and infants are the most challenging groups due to the need for dose adjustments based on body weight [26,33,45]. The drug concentration of oral liquids should enable flexible administration of different doses without exceeding a dose volume. Dose titrations of enalapril maleate doses in the range of 0.025–4 mg were manageable with orodispersible mini tablets of two different strengths (0.25 and 1 mg, respectively), facilitating flexible dosing for different patient groups [33]. It was shown that content uniformity and dose accuracy of the orodispersible films were at least equally good as for dose powders currently used for paediatrics. Both orodispersible mini tablets and films dispersed in water were also suitable for administration through a NG tube without causing blockage of the tube. Daniel et al. reported low recovery of hydrocortisone or a higher amount than the target dose after passing through EFTs, highlighting issues with the existing process of preparing small doses from adult dose tablets [45]. As well as Hoover et al. observed more clogs with the 30 mg dose of lansoprazole orally disintegrating tablets than with the 15 mg dose during enteral tube delivery [49] . Flush volume or dilution volume are crucial factors when individual fluid restrictions place limitations on the amount of liquid administered to a child. To safely deliver medicines through EFTs, the volume of liquids used for rinsing tubes and the volume of suspension need to be reduced to prevent gastric overload in paediatric due to the smaller stomach size [112]. Several authors reported the sum of liquid volumes administered in paediatric patients should not go over 15 ml [32,34,113]. Nurses should use the laboratory's water volume recommendations. Although it is focused on adult patients, it could be too large in several paediatric applications [32]. It is important to consider these requirements in drug development.

Tube blocking is a particular concern for paediatric NG tubes used for neonate feeding as they have an exceptionally narrow bore e.g., 6 Fr have an external diameter of 2000 µm and an internal diameter of ~1300 µm [32]. Infants will often have small diameter feeding tubes which are not compatible with the size of commercially available multiparticulates. Administration of the viscous suspension through the size 6 Fr tubes required significant pressure on the syringe. In addition, the size of the syringe used to deliver medication can affect the drug delivery due to the differences in the force applied to the syringe. Smaller syringes could produce high pressure and could damage or even rupture the tube [14]. In addition to the size the types of tube, the number of medications given through a tube, the length of time a tube was *in situ,* and the rate of administration of an enteral feed are all significant factors in the development of tube occlusions. ASPEN does recommend tubes with internal diameter of 10 Fr for administering crushed or dissolved solid dosage medications; for smaller diameters, only liquid formulations, with few residuals, should be used. However, only few specialised liquid solutions for NG delivery are available. Off-license use of solid dosage forms that have been crushed and can be administered as a suspension is often required, resulting in dosing accuracy challenges or tube blockages. One study revealed that nearly half of the manipulations observed affected patients with a feeding tube. More than 60% of the manipulations affecting these patients were not covered by the respective SmPC [117]. These numbers demonstrate the importance of drug-specific information on administration via tube provided by the manufacturer, which is particularly relevant for special patient groups, such as children. Hence, it recommends that information on feasible manipulations be made available in the SmPC. This information should address the divisibility of tablets, especially whether a tablet may be divided into equal doses, mortarability, and the possibility of dispersing a tablet in liquid. Furthermore, manufacturers are encouraged to address the option of administering a medicinal product via an EFT and provide guidance, e.g., solvent or size of the tube. Innovative age-appropriate dosage forms such as minitablets, multiparticulates, and granules developed especially for paediatric population are not always suitable for feeding tube administration. For example, Alkindi® granules were found to stick to the tubes (6 Fr and 8 Fr) and are not expelled following flushing [99]. Sibnayal® concluded that the finished product was concluded not to be suitable for tube

feeding considering the high risk of tube blocking due to the granule size and impossibility of crushing without affecting the release profile [104]. Current sachets cannot be applied in case of the presence of a feeding tube, while this is often applied in infants. Another relevant example is esomeprazole enteric granules, which are formulated to swell after dispersion in water so that they are homogenous prior to administration, as outlined in the SmPCs. In order to avoid the granules swelling and clogging the tube, in a clinical setting, enteric tablets crushed and dispersed or administered the dispersed granules before the swelling was complete are used, resulting in inaccurate dosing due to sedimentation or depending on tube distal end position [118]. It is also not always true to assume that a liquid is better than a tablet; undesired side-effects of a liquid formulation's excipients must be considered. Critical attributes of dosage forms and how the interplay of tube, administration and formulation factors affect the performance of the oral medicinal product when administered to paediatric patients via different enteral tubes are needed, as research in this area is limited. The geometry and design of the enteral tube have a substantial impact on drug delivery through EFTs. 8 and 12 Fr are the most studied paediatric size tubes, as well as PU is the leading material in the literature. Particularly, tubes with small diameters designed for paediatric feeding are susceptible to obstructions. For many commonly used drugs in paediatric population, the appropriate dosage form for administration via EFT and information on the appropriate size/material of the tube is lacking. Feeding tube blockage can be caused by a variety of factors, including tube design, insufficient flushes, and off-label usage of medicinal products. Indeed, formulation characteristics for different dosage forms, administration characteristics, and enteral feeding tube characteristics must be considered to administer medical products via EFTs.

5. Conclusions

The results of this review suggest that more studies around the understanding of critical attributes of dosage forms and how the interchange of tube, administration and formulation factors affect the performance of the oral medicinal product when administered to paediatric patients via different EFTs are needed, as research in this area is limited. To address this gap, research is needed to determine the design space for different factors for medication administered by EFTs.

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3.5. Considerations for oral suspension administration via enteral feeding tube to paediatric population: A holistic proof of concept study

1. Materials

Ibuprofen in the form of Nurofen for Children (NfC) suspensions (100 mg/ 5 ml) as the marketed formulation was selected as the model drug for this study as the current guidance does not provide a specific indication for EFT administration. Ibuprofen standard was obtained from Cambridge Bioscience (Cambridge, UK). Sodium hydroxide was purchased from Fisher Scientific (Loughborough, UK). Polyurethane (PU) NG tubes (GBUK Enteral, UK) with a bore size of 6 and 8 Fr and lengths of 50, 75 and 120 cm were utilised for the NG tube dose recovery studies, as well as a 20 ml Enteral Syringe (GBUK Enteral, UK).

2. Methods

2.1. Characterisation of marketed Ibuprofen suspension for children: viscosity, sedimentation volume, particle size and osmolality

The viscosity of the undiluted and diluted paediatric suspension was determined on a rotational rheometer (Bohlin Gemini HR nano, Malvern, UK), applying a shear rate from 0 to 100 1/s at 25°C. The slope (n) was determined in order to evaluate the changes in the viscosity increasing the shear rate. The gap between the fixed bottom and the measuring plate (conical, 2°/55mm) was set to 150 µm. The data were collected with the aid of Bohlin software. All determinations were carried out in triplicates, and the results obtained were expressed as the mean values.

The sedimentation volume of 2.5 ml of suspension diluted with different volumes (0.5, 1, 1.5, 2, and 2.5 ml) was measured into a graduated cylinder. To simulate the time after the dose preparation and before the suspension administration via NG tube, the sedimentation volume was recorded using the volume markings on a cylinder by plotting sediment volume as a function of time after 15 min [1,2]. The sedimentation volume (F) was calculated using the relationship:

$$F = \frac{V_u}{V_o}$$

V_u is the ultimate volume of sediment, and V_o is the original volume of the suspension before settling occurs.

Malvern Mastersizer 3000 laser diffraction system with the Hydro MV unit

(Malvern Panalytical, UK) was used to assess the particle size distributions of NfC suspension. Deionised water was used as the dispersant with a stirring speed of 1000 rpm. Drops of the sample to be analysed were introduced into the dispersion unit and unit to produce at least an obscuration of 10%, and the bar colour turned green. Each sample was measured as 6 (ISO guidelines) consecutive 30-s signal collections, and the average values were reported. Dv10, Dv50 and Dv90 represent the statistical parameters from the cumulative particle size distribution below which 10%, 50% or 90% of the sample lies.

The osmolality of NfC suspension was determined in Osm/kg using an osmometer (OSMOMAT 030 Cryoscopic Osmometer, Genotec, Germany). System calibration was performed with standard calibration solutions with an osmolality of 100, 300, 500, 850 and 2000 mOsmol/kg. Measures were carried out in triplicate with a 1:5 dilution to avoid the carried to avoid exceeding the osmometer capacity.

2.2. Quantification of Ibuprofen

For the standard curve of Ibuprofen, a stock solution was prepared by taking 50 mg of ibuprofen in 100 ml of sodium hydroxide 0.1 N in a volumetric flask [3]. The drug was dissolved in the solvent under magnetic stirring. Different standard solutions were prepared of the stock solution having concentrations of 0.4 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.10 mg/ml and 0.05 mg/ml, respectively. Spectrophotometrically (Cary UV/V–s - 320), ibuprofen was determined by measuring at 264 nm using sodium hydroxide 0.1 N as background solvent and calculated through the calibration curve prepared ($y= 1.7986x+0.0118$; $R^2=0.999$) obtained from standard solutions of drug.

2.3. NfC suspension administration via NG tube

Polyurethane (PU) NG tubes with a bore size of 6 and 8 Fr and lengths of 50, 75 and 120 cm were utilised. The suspension was administered through the NG tube positioned vertically on a retort stand, with the nasal end placed securely on a clamp and the gastric end in a volumetric flask. The NG tube was pre-flush with 1 ml distilled water using the volumes stated in the flushing protocol [4]. The dose was previously diluted/suspended in an equal volume of water (2.5 or 7.5 ml) and entered the tube from the adaptor at the nasal end via syringe, and exited through small openings near the gastric tip, followed by NG tube post-flushing and the

administrating syringe with distilled water. The total flush volume (pre-flushing, suspending, post-flushing) used was 15 ml. Upon administration, the NG tubes were left to drain all administered materials into the collecting tube at the gastric end of each NG for 3 minutes [5], and dose recovery was determined through an UV-Visible spectrophotometer.

2.4. Dose recovery determination

Each NG tube output was collected in a 50 mL volumetric flask, and all flasks were made up to volume with sodium hydroxide 0.1 N. Subsequently, the dispersions were sonicated for 10 minutes, and the fully clear solutions obtained were diluted 1:10 in sodium hydroxide 0.1 N. Ibuprofen concentration was determined through an UV-Visible spectrophotometer (Cary UV/V–s - 320) at 264 nm [3] and calculated through the calibration curve prepared ($y= 1.7986x+0.0118$; $R^2=0.999$) obtained from standard solutions of drug (range 0.05–0.4 mg/ml) in sodium hydroxide 0.1 N.

2.5. Experimental factorial design – Effect of dose strength, NG tube size and length: dose recovery from nasogastric tubes

The application of an experimental factorial design as an instrument to investigate the influence of NG tube and formulation factors product on the dose recovery was evaluated using a 2^3 factorial design, composed of 3 variables: Dose strength (A), Tube size (B) and Tube length (C), which were set at 2-levels (high and low) each (**Table 1**).

Table 1. The factors selected for the experimental factorial design.

Factors	Low level (-1)	High level (+1)
Dose strength (A)	2.5 ml	7.5 ml
NG tube size (B)	6 Fr	8 Fr
NG tube length (C)	50 or 75 cm	120 cm

Two replications were run at each setting leading to a 2^3 full factorial design which comprised 16 experimental runs (or treatments) (**Table 2**). The data were analysed using Minitab® Statistical software. The most important parameters were identified by plotting the effects against their cumulative probability

distribution on a normal probability plot. Statistical significance of each effect was also evaluated by analysis of variance (ANOVA).

Table 2. *Design of Experiments (DoE)*: design table.

Design Table (randomized)				
Run	**Blk**	**A**	**B**	**C**
1	1	-	+	+
2	1	+	-	-
3	1	-	-	-
4	1	+	+	+
5	1	-	+	-
6	1	+	-	+
7	1	+	+	-
8	1	-	-	-
9	1	+	-	+
10	1	-	-	+
11	1	+	+	-
12	1	-	-	+
13	1	+	+	+
14	1	-	+	-
15	1	-	+	+
16	1	+	-	-

2.6. Effect of flush volume on dose recovery

To investigate the effect of different flush volumes on dose recovery, 2.5 ml of the NfC suspension was administered via NG tube. Flush volumes tested in this study were 0, 2, 4, 6, 8, and 10 ml with a PU NG tube size of 8Fr 50 cm [5,6]. The ibuprofen concentration was determined using UV–Visible spectrophotometer. The experiment was performed in triplicate, and the statistical analyses run on the results were performed using Minitab software on one-way ANOVAs.

2.7. Effect of dose dilution volume on viscosity and dose recovery

An assessment of the rheological profile of NfC suspension was carried out. The effect of different dose dilution volumes (0, 0.5, 1, 1.5, 2, 2.5 ml) on viscosity was evaluated. Likewise, the effect of the mentioned different dose dilution volumes on dose recovery was determined. The ibuprofen dose selected was 2.5 ml, and a PU NG tube size of 8 Fr 50 cm. Ibuprofen administration was tested without dose dilution and with small volumes of water (0.5, 1, 1.5, 2 ml) up to a volume of water equal to the dose (2.5 ml). NG tubes were pre-flushed with 1 ml and post-flushed with 8 ml of distilled water according to the previous method.

Ibuprofen was quantitatively analysed by UV–vis . The experiment was performed in triplicate, and the statistical analyses run on the results were performed using Minitab software on one-way ANOVAs.

3. Results

3.1. Suspension characteristics: viscosity, sedimentation volume, particle size and osmolality

Table 3 shows the characterisation of the marketed NfC suspension, the dose of which was 100 mg/5 ml. The content assay was found to be 107.08 ± 4.22% and the viscosity was found as 201.17 ± 0.231 cP (0.201 Pa·s), which correlates with other similar suspensions [7]. The average particle size of 90% of the product was below 105.91±2.66 μm with a distribution between 1 and 100 μm, a common size to elaborate pharmaceutical suspension. Meanwhile, 50% and 10 % of the product were below 52.12 ± 2.53 μm and 20.74 ± 1.88 μm, respectively. The density obtained was 1.058 ± 0.014 g/cm^3. No sedimentation was observed for 2.5 ml of NfC suspension and 2.5 ml of suspension diluted with different volumes (0.5, 1, 1.5, 2, and 2.5 ml) after 15 min. Due to the high osmolality (> 2000 mOsm/kg), as reported in the literature, the NfC suspension osmolality was measured by diluting the sample [8]. Considering the dilution, the osmolality of this paediatric suspension was 476 ± 0.002 mOsm/kg.

Table 3. Characterisation of marketed Ibuprofen paediatric suspension.

	NfC suspension
Ibuprofen content (%)	107.08 ± 4.22
Viscosity(cP)	201.17 ± 0.231
Particle Size (μm)	105.91 ± 2.66
Density (g/cm^3)	1.058 ± 0.014
Sedimentation volume (ml)	0
Osmolality (mOsm/kg)	476 ± 0.002

3.2. Experimental factorial design - Effect of dose strength, NG tube size and length: dose recovery from nasogastric tubes

Full factorial design (**Figure 1**) showed that there was no statistically significant effect of the dose strength, tube size and tube length on dose recovery of the marketed NfC suspension ($p > 0.05$). The dose of NfC suspension diluted did not result in tube obstruction, and dose recovery for each setting was 90% - 106% after passage through the NG tubes (**Table 4**). According to the Q&A document developed by EMA, dose recoveries above 90% are usually considered acceptable [9].

Table 4. Design of Experiments (DoE): 2^3 Full factorial design. The corresponding value for response (Dose recovery: % Ibuprofen): results are mean ± SD, n = 2.

StdOrder	RunOrder	Dose (ml)	Size (Fr)	Length (cm)	Dose recovery (%)
4	1	7.5	8	50	103.43 ± 2.55
16	2	7.5	8	120	106.19 ± 1.34
8	3	7.5	8	120	98.59 ± 3.83
5	4	2.5	6	75	102.23 ± 4.64
9	5	2.5	6	50	91.06 ± 4.81
15	6	2.5	8	120	94.83 ± 0.93
12	7	7.5	8	50	96.09 ± 9.56
2	8	7.5	6	50	98.36 ± 3.52
6	9	7.5	6	75	91.18 ± 3.26
11	10	2.5	8	50	93.70 ± 1.78
1	11	2.5	6	50	94.29 ± 4.49
7	12	2.5	8	120	90.54 ± 3.85
13	13	2.5	6	75	96.05 ± 0.67
3	14	2.5	8	50	97.92 ± 4.78
14	15	7.5	6	75	95.05 ± 1.02
10	16	7.5	6	50	93.49 ± 0.74

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	186.189	26.598	1.85	0.204
Linear	3	55.129	18.376	1.28	0.346
Dose	1	29.079	29.079	2.02	0.193
Size	1	23.401	23.401	1.63	0.238
Length	1	2.649	2.649	0.18	0.679

$p > 0.05$

Figure 1. Full factorial design: Analysis of variance**.**

As reported in **Figure 2**, the dose recovery was better with the large dose (7.5 ml), with the wide bore (French size 8), and with the long length (120 or 75 cm). The interaction plot (**Figure 3**) showed that the large dose (7.5 ml) revealed a better dose recovery in 8 Fr NG tube and the low dose (2.5ml) in 6 Fr NG tube; both lengths displayed better dose recovery with the large dose, as well as with 8 Fr NG tube. However, there was no statistically significant difference in dose recovery ($p > 0.05$).

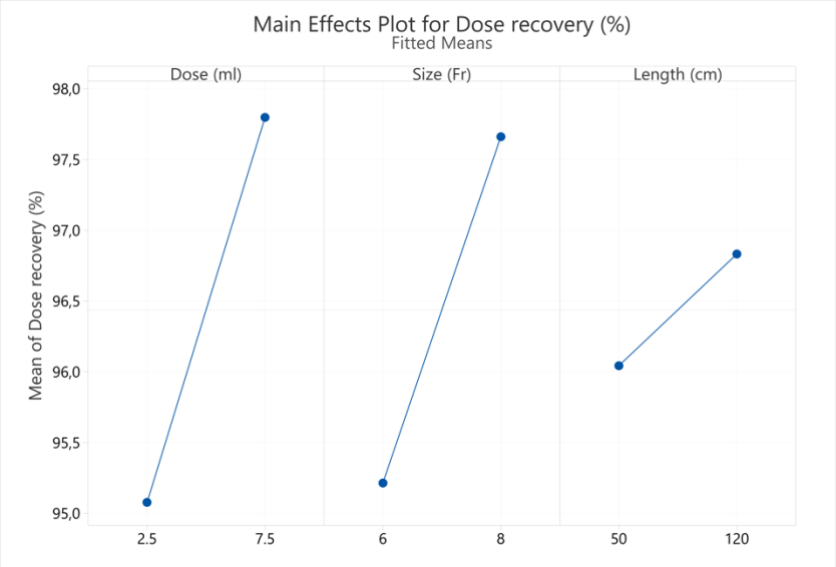

Figure 2. Main effects plot for dose recovery.

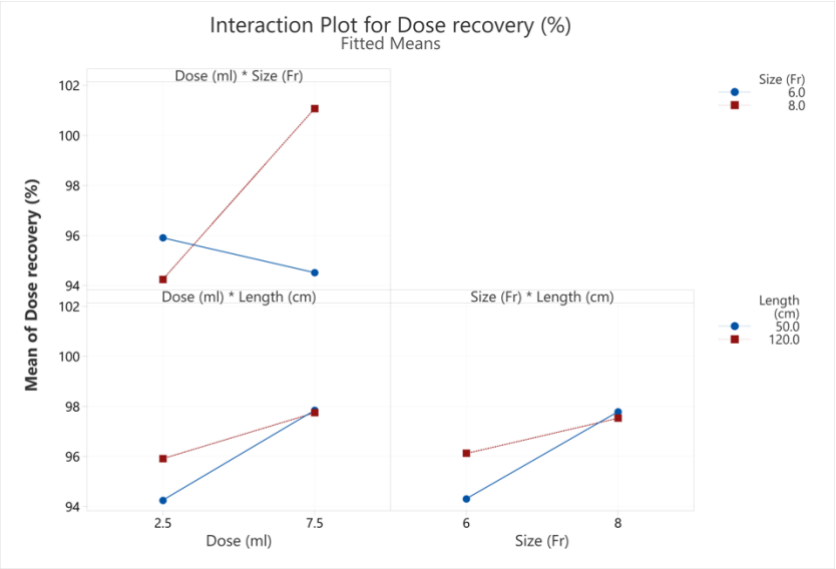

Figure 3. Interaction plot for dose recovery.

3.3. Effect of flush volume on dose recovery

The flush volume study (**Figure 4**) revealed that the percentage of ibuprofen recovered without flush volume was around 83 %; meanwhile, using different flush volumes, the ibuprofen recovered was approximately between 93 – 97 %.

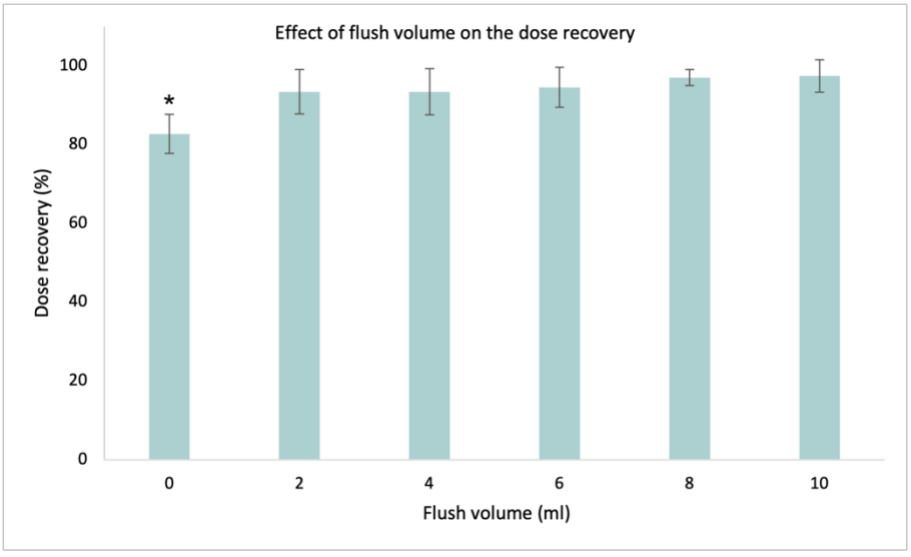

Figure 4. Percentage ibuprofen recovered corresponding to different flush. volumes. **p* value < 0.05: 0 ml vs 2, 4, 6, 8 and 10 ml.

Hence, there is a significant effect on dose recovery without and with flush volumes (0 ml vs 2, 4, 6, 8 and 10 ml p <0.05). However, there was no significant

effect of flush volume on dose recovery between 2 and 10ml (p >0.05). Pre-flush volume of 1 ml and post-flush volume of 2 ml and above were adequate for dose recovery of more than 93% through an 8 Fr NG tube, according to Q&A document developed by EMA [9].

3.4. The effect of dose dilution volume on viscosity and dose recovery

Figure 5 shows the effect of dose dilution volume on viscosity. For each dose dilution volume (0.5 – 2.5 ml) analysed by applying a shear rate from 0 to 100 1/s, the viscosity resulting from mixing the dose with different volumes of water decreased by increasing the volume of water used to dilute the dose. In addition, as the shear rate increased, the viscosity dropped. This well-known phenomenon is seen in most non-Newtonian liquids and is referred to as "shear-thinning" [10]. Likewise, determining the slope (n) of viscosity curves involves understanding suspension resistance to flow. As reported in **figure 5**, the slope increased with the rate of dose dilution volume. The slope corresponding to the undiluted suspension (blue line) and diluted suspension 1:1 v/v NfC:water ratio (green line) was -0.2113±0.0302 and -0.0180±0.0003, respectively. Low flow resistance is determined by the high slope [11]; thus, 2.5 ml of water to dilute the dose represented by the green line in **figure 5** exhibits better flowability.

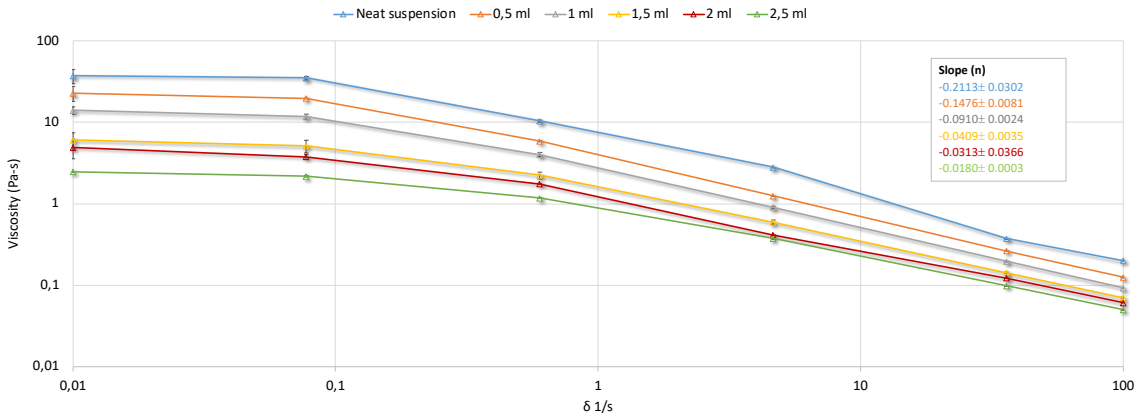

Figure 5. Rheological profile of NfC suspension: Effect of different dose dilution volumes on viscosity and slope (n).

On the other hand, the effect of different dose dilution volumes was studied on dose recovery because diluting the dose with water could become a challenge in paediatrics due to fluid volume restrictions (**Figure 6**). The percentage of

ibuprofen delivered after administration via NG tube without dose dilution was around 95%; this percentage remained constant (95%) using 1 and 1.5 ml of water to dilute the dose, meanwhile increased to 98% with 2 ml and around 99% of ibuprofen using 2.5 ml of water to dilute the dose as recommended [12]. Diluting 2.5 ml of ibuprofen dose with 2 and 2.5 ml of water is clearly clinically significant as almost the whole dose was recovered. In addition, dose dilution implied a decrease in osmolality, as reported in Section 3.1. Therefore, the dose diluted in a low volume of water improved the drug recovery after passing through the NG tube [13]. However, from a statistical point of view, the analysis revealed no significant effect of dose dilution volume on the dose recovery (p >0.05) (**Figure 6**). The flush volume and suspension characteristics probably make it possible to deliver a high percentage of ibuprofen without dose dilution and prevent tube blockage.

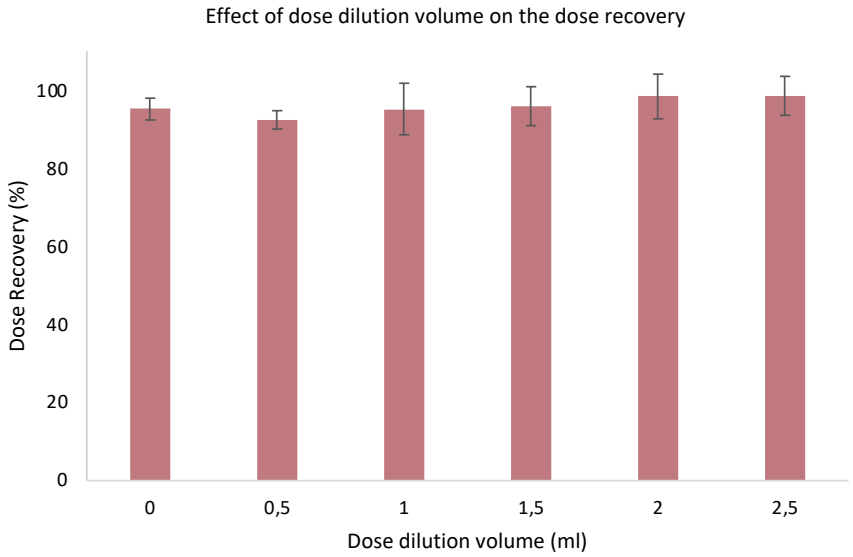

Figure 6. Recovered ibuprofen percentage corresponding to different dose dilution volumes.

4. General Discussion

Manipulation of dosage forms is common in paediatrics, and extemporaneous preparations are often used in these patients due to the lack of dosage forms or dose strength suitable for children. However, manipulation frequently occurred for treatment administered through EFTs for both paediatric and adult patients

with severe illness or disability that limits their ability to swallow and gastrointestinal access [14,15]. The assessment of EFTs during drug product development is required to demonstrate the feasibility of medication administration through these medical devices to avoid their widespread improper use. EMA stated key considerations in a Q&A, as well as FDA, provided recommendations for the design of an *in vitro* study.

As identified in the systematic review (Section 3.4), a holistic view of the effect of factors such as formulation characteristics for different dosage forms, enteral feeding tube characteristics and administration characteristics is needed and should be considered in medication administration via EFTs. Formulations for the paediatric population should conduct feasibility studies to examine safe and effective administration through EFTs. Identifying the drug product compatibility with EFTs and understanding the critical attributes of dosage forms and how the interchange of tube, administration and formulation factors affect the performance of the oral medicinal product when administered to paediatric patients is crucial to ensure successful delivery through EFTs. Different factors must be considered depending on the dosage form to be administered. For instance, for tablets or capsules, you should consider the sample preparation method (e.g. crushing method, volume for suspended), particle size obtained when tablets are crushed, reconstitution and disintegration time or excipients. Moreover, insoluble excipients could be associated with tube blockage [16].

A feasibility study (*in vitro* study) was conducted in part 2 of this study to assess and streamline the key factors that affect product performance (dose recovery) when administered via paediatric NG tubes.

NfC suspension was characterised before administration via NG tube. Physical properties of an oral pharmaceutical suspension depend on multiple factors, including the rheological profile, sedimentation or particle size, as indicated in **Table 4**. For the administration of an oral suspension via NG tube, viscosity is an important formulation characteristic that must be taken into account [12]. In the present study, the viscosity of NfC suspension correlates with other similar suspensions [7]. However, viscous medications are more difficult to flush, increasing the risk of tube obstruction [17]. One study reported that the liquids with viscosities of 1000 cps or below showed adequate flow through the EFTs, and administration factors like dose strength may affect suspension viscosity [18].

Sedimentation is another crucial formulation characteristic to support enteral tube administration. This study showed the absence of this settling behaviour prior to administration via NG tube. Additionally, particle size is also a critical factor that must be considered in paediatric formulations for whom fine tubes are often needed. Particle size showed a distribution between 1 and 100 μm, a common size to elaborate pharmaceutical suspension. Tube obstruction is associated with large particle sizes and insoluble excipients [19]; thus, small particle sizes may be more suitable for nasogastric administration [1].On the other hand, small particle size is important to formulate a physically stable suspension and avoid rapid sedimentation of particles, as well as the viscosity needs to be controlled within a range that offers slow sedimentation but also facilitates redispersion [10,18]. Osmolality is a formulation characteristic that should be considered. Due to the high osmolality (> 2000 mOsm/kg), as reported in the literature, the NfC suspension osmolality was measured by diluting the sample [8]. Hyperosmolar oral medicinal products are associated with gastrointestinal adverse events. However, manufacturers often do not provide medication osmolality, and limited information can be found in the literature. For gastric administration, an osmolality of less than 700 mOsm should be used [8]. Considering the dilution, the osmolality of this paediatric suspension was 476 ± 0.002 mOsm/kg. Hence, diluting the dose with an equal volume of water prior to administration is recommended to reduce the osmolality of the suspension [12,20,21]. Additionally, flushing properly with water before and after dosing could help reduce the suspension's osmolality and minimise the probability of enteral tube blockage [12].

The present study applied the utilisation of an experimental factorial design as an instrument to investigate the effect of dose strength, NG tube size and NG tube length on the product performance (dose recovery) when administered via paediatric NG tube. Full factorial design (**Figure 1**) revealed that there was no statistically significant effect of the dose strength, tube size and tube length on dose recovery of the marketed NfC suspension ($p > 0.05$). Dose strength factor revealed that the dose recovery with 2.5 and 7.5 ml was around 95% and 98%, respectively (**Figure 2**). Different dose strengths of omeprazole suspension showed similar drug recovery after EFT administration, which is consistent with the present study's findings [22]. In contrast, dose recovery was variable for the

doses of hydrocortisone suspension investigated. 6 Fr and 8 Fr NG tubes showed a better dose recovery with the low dose; meanwhile, 10 and 12 Fr displayed a better dose recovery with the high dose [5]. Furthermore, dose strength varies in children of different ages. For instance, the lower dose required in neonates or newborns could affect dose recovery when passed through different lengths or sizes of EFTs [6]. Dose recovery with both bore sizes was higher than 90% and not statistically significant, as reported in **Table 5** and **Figure 1**. In the literature, similar results for hydrocortisone liquid suspensions were reported. The recovery of the administered dose was unaffected by tube size (6,10,8 and 12 Fr) [5]. However, the enteral tube size is another factor affecting product performance when administered via EFTs. Narrow bore NG tubes are more prone to blockage [23]. Consequently, the dose delivered to the patient will be low, and there will be a significant loss of therapeutic activity. Likewise, the type of dosage form may cause blockage in the tube [24]. Johnson et al. observed that suspensions prepared from lansoprazole tablets partially clogged the 6 Fr tube and completely clogged the 5 Fr tube, meanwhile omeprazole sodium bicarbonate powder for suspension were easily administered through 5, 6, and 8 Fr feeding tubes [22].

The NG tube length did not have a significant effect on dose recovery. Similar results were discussed in the literature by Ponrouch et al. [6]. Lansoprazole study also looked at the transit of different crushed suspensions through different length tubes (50 cm and 125 cm), and the results showed no correlation between dose recovery and tube length, but the recovery was slightly higher with the long length [6]. This finding may be advantageous because it prevents feeding or other activities close to the patient's head when using a long NG tube.

In addition, administration factors such as flush volume and dose dilution volume were studied individually. Lower volumes were included because it is crucial to maintain proper volumes to impede gastric overload [25]. According to hospital care practice, feeding tubes were flushed before and after drug administration to keep them from getting blockage and ensure maximum drug delivery because insufficient flushing could significantly impact the effective dose [13]. Pre-flush volume of 1 ml and post-flush volume of 2 ml were adequate for dose recovery of more than 93% in a small-bore (8 Fr) NG tube. In paediatric care, the flushing and suspension volumes are recommended to be kept low to avoid gastric overload because children have smaller gastric volumes. The total flush volume

(pre-flushing, suspending, post-flushing) administered through NG tubes should not exceed 15 ml in paediatric patients [4,6,26]. Flush volumes of 1-3 mL are often required for neonatal patients and could need to be limited to the smallest volume needed [9]. However, paediatric patients with fluid volume restriction require air flushing or minimal volume flushing for the successful delivery of medicines through the EFTs. Hence, the same volume cannot be administered to neonates compared to children due to the heterogeneity of this population.

The *Handbook of Drug Administration via Enteral Feeding Tubes* recommends diluting the dose with an equal volume of water immediately prior to administration to reduce viscosity and osmolality. However, since paediatric patients may have fluid volume restriction, it might be difficult to dilute with an equal volume of water. Hence, the effect of diluting the dose with small volumes was investigated [4]. Results demonstrated that diluting 2.5 ml of dose with 2 and 2.5 ml of water (1:1 v/v NfC:water ratio) is clearly clinically significant as almost the whole dose was recovered after administration via 8 Fr NG tube. On the other hand, dose recovery was around 95% using 1 and 1.5 ml of water to dilute the dose and without dose dilution. According to the Q&A document developed by EMA, dose recoveries above 90% are usually considered acceptable [9]. The rheological profile (**Figure 5**) revealed that the lower slope of the diluted suspension (1:1 v/v NfC:water ratio) represented as a green line and low viscosity suggested better flowability in comparison to the undiluted suspension (blue line). Moreover, dose dilution implies a decrease in osmolality that would prevent gastrointestinal adverse events, as reported in Section 3.1. Therefore, NfC suspension can be reliably administered through small-bore (8 Fr) NG tubes, even when using small volumes of water to dilute the dose and to flush the NG tube after the administration.

5. Limitations and Recommendations

Experimental protocol reflects the standard clinical practice and complies with FDA guidance on in vitro testing of oral drug products administered via EFTs. The experiment considered: NG tube characteristics such as tube bore size (6 and 8 Fr) and length (50, 75 and 120 cm); a high and a low dose strength; and pre-flushing and post-flushing during the administration adjusted to the available paediatric information in the literature. Due to volume restriction in paediatrics,

flush volume is a crucial consideration since it is a limitation in these patients. Air flushes are an alternative option for fluid-restricted patients, but sometimes water is required to decrease the osmolality and avoid gastrointestinal effects [8,13].

Future studies should also include *in vitro* techniques that replicate conditions and procedures relevant to clinical settings more accurately. Additional research should assess commercially available medicinal suspensions containing another active pharmaceutical ingredient (API) and excipients to provide more background, as well as the study of required physical characteristics of suspensions made from tablets/capsule content for use in EFT administration. More studies combining different tube materials, such as PVC or silicone, would be interesting to observe possible changes in the material or tube-drug interaction [27] Although water is the main medium for administration, testing the interaction with food and tube feeding formula would be another interesting factor [8,24]. In order to identify the main factors with worst-case scenarios and the possibility of providing a design space (*allowable operational flexibility*) for different factors for administration by EFTs, this holistic proof of concept establishes the bases for a global study with several dosage forms, different EFTs and administration factors

6. Conclusions

NfC suspension showed suitable characteristics like viscosity, particle size and absence of sedimentation for administration via NG tube. Full factorial design revealed no statistically significant effect of the dose, size and length on dose recovery of the NfC suspension. After passing via the NG tube (8 and 6 Fr) and utilising a total flush volume (pre-flushing, suspending, post-flushing) of 15 ml, the dose recovery for each setting ranged from 90% to 106%. In paediatrics, maintaining proper flush volumes is crucial to impede gastric overload. NG tube (8 Fr) should be washed with at least 2 mL tap water to ensure a dose recovery of 93% or more. Dose dilution volume (1:1 v/v NfC:water ratio) improved flowability and its use was clearly clinically significant as almost the whole dose was recovered. Additionally, as the dose was diluted, osmolality was reduced, which could prevent gastrointestinal adverse effects in children. Proper flush and dilution volumes must be taken into account for optimal NfC suspension administration via NG tubes since they may impact the formulation performance when given to paediatric patients.

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APPENDIX 4.2. SUPPLEMENTARY DATA

2.3. Surfactant-Free Chitosan/Cellulose Acetate Phthalate Nanoparticles: An Attempt to Solve the Needs of Captopril Administration in Paediatrics

1. Methods

1.1. Pre-formulation studies

The amount of CAP to use for the preparation of NPs was studied. Firstly, 20 mg (CAP 20) or 50 mg (CAP 50A) of CAP were dissolved in 5 ml of acetone under magnetic stirring and then 15 ml of Milli Q water was added drop by drop [11]. Acetone was evaporated for 2 h at 60 °C under magnetic stirring. After that, the rotary evaporation (Rotavapor RE111, Bünchi Labortechnik AG, Flawil, Switzerland) at 60°C for 5 min was used to remove any remaining acetone. Finally, the dispersion was sonicated in an ultrasonic bath for 40 s and analysed by dynamic light scattering method (Coulter Submicron Particle Sizer N5, Beckman-Coulter Inc. Miami, Florida, USA). Also, CAP 50B was prepared under the same conditions as CAP 50A, but 20 ml of Milli Q water was added drop by drop in the organic phase.

Furthermore, the optimal solvent of CAT was evaluated. CAT was dissolved in water (CAP 50B1) or in acetone (CAP 50B2); then, the preparation method was the same.

The evaporation method to eliminate the organic solvent was also assessed: evaporation was performed at room temperature for 4 h or for 2 h in a water bath at 60 °C both under magnetic stirring (200 rpm).

2. *Results*

2.1. Pre-formulation studies

CAP 20 and CAP 50A showed a mean particle size of 279.1±3.3 nm and 457.4±6.0nm and a PDI of 0.01±0.09 and 0.14±0.09, respectively. However, CAP 20A dispersion displayed a slight opalescence and a low NP concentration: therefore, the high CAP amount was selected.

CAP 50B, prepared with 20 ml of Milli Q water exhibited a lower mean size, 248.4±1.2 nm and PDI of 0.070±0.003 than CAP 50A.

Results of CAT dissolved in water or acetone (CAP 50B1 and CAP 50B2) are shown in **Table 1S**. Formulation studies showed that CAP 50B1 have no loading

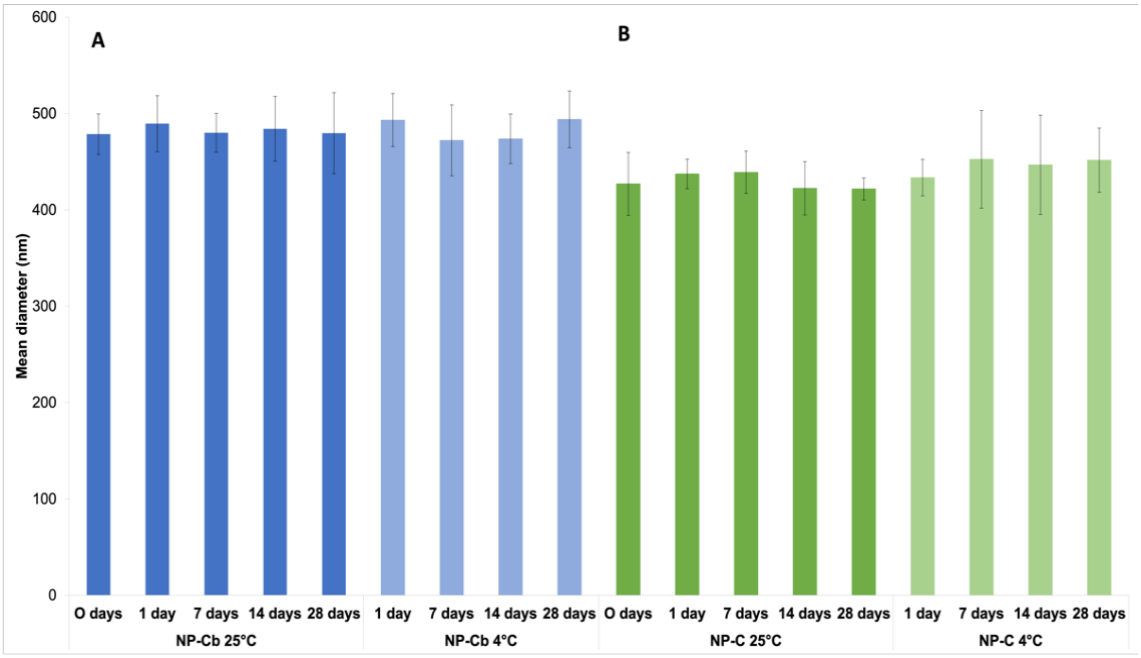
capacity compared to CAP 50B2.

CH1 evaporated at room temperature for 4 h showed a mean size of 449.1±47.5 nm, a PDI of 0.12±0.01 and encapsulation efficiency of 51.6±3.0 %.

Furthermore, the best evaporation method resulted the evaporation for 2 h in a water bath at 60 °C allowed to obtain reproducible results.

Table S1. Influence of the solvent of CAT in CAP NPs.

Formulation	Drug	Polymer	Size (nm)	PDI	Encapsulation Efficiency (%)
CAP 50B1	CAT in water	CAP	263.1±2.9	0.076±0.017	0
CAP 50B2	CAT in acetone	CAP	245.0±3.1	0.100±0.003	10.2±1.5



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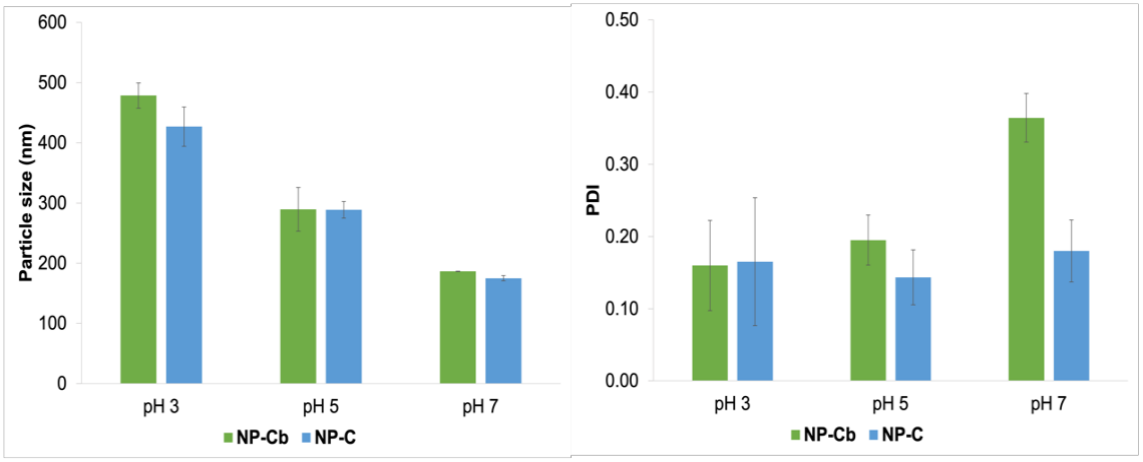

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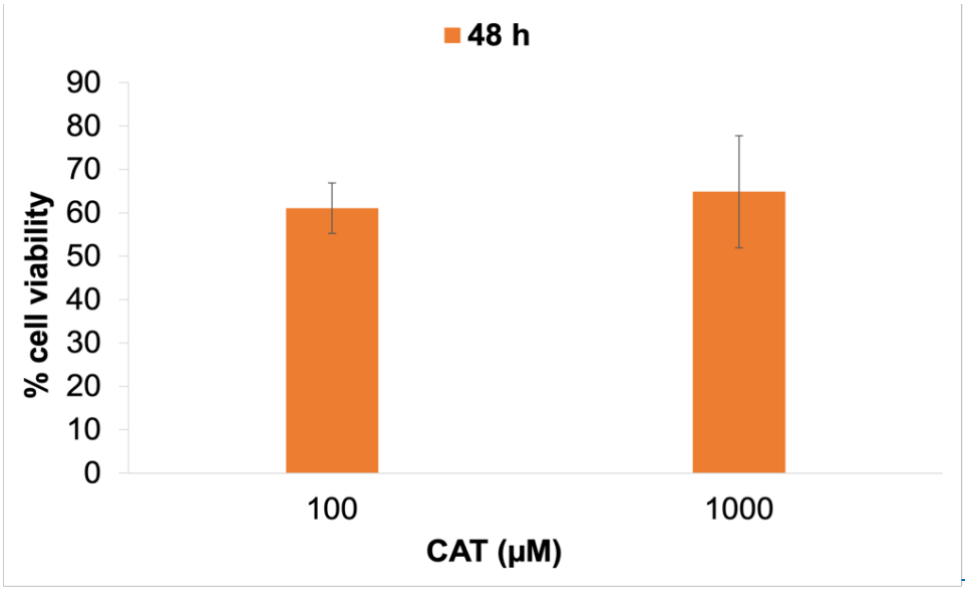

Figure S3. *In vitro* cytotoxicity. Effect of free-CAT (100 μM and 1000 μM) on HFF-1 cells after and 48 h of incubation.

2.4. A thermosensitive nasal hydrogel: A new approach for dimethyl fumarate delivery in paediatric multiple sclerosis

1. Method

1.1. Pre-formulation studies

1.1.1. Selection of chitosan

Two thermosensitive nasal hydrogels were prepared using different varieties of chitosan (CH), Chitoclear TM1358 (63 cP, Mw: 103 kDa, deacetylation degree, 94%) and Chitoclear TM1283 (224 cP, Mw: 282 kDa, deacetylation degree, 96%), at various molecular weights and viscosities to evaluate which type of CH was most suited for the development of a hydrogel for nasal administration. β-GP was used as the crosslinking agent, following the method proposed by Salis et al. (2015). CH/GP 2/12 ratio (% w/v) was the ratio selected to test CH.

0.2 g of CH was solubilised in 9 mL of 0.1 N HCl. Separately, 1.2 g of β-GP was dissolved in 1 mL of Milli Q water. Solutions were put into an ice bath until the temperature of 4°C was reached. Subsequently, the solution containing β-GP was added, under constant stirring, drop by drop, to the CH solution at 4 °C. The final solution of CH and β-GP was under constant stirring for 15 minutes at 4 °C, and samples were placed and incubated in the bath at 37 °C to achieve gelation.

1.1.2. Preparation of CH/GP solutions

Different blank CH/GP solutions (A1b, A2b, A3b, and G2b) were prepared using the method reported by Salis et al. (2015) in order to make a gel with an appropriate viscosity for nasal administration. CH was solubilised in 0.1 N HCl and GP in 1 mL of MilliQ water. Solutions were cooled in an ice bath at 4 °C and GP solution was dropwise into the CH solution under vigorous magnetic stirring for 15 min at 4 °C. The different concentrations of CH and β-GP used to prepare the solutions are shown in Table 1. In addition, the same solutions were prepared by adding DMF:HP-β-CD complex using a molar ratio 1:1 (A1, A2, and G2). The complex was solubilised in 0.1 N HCl for 24 hours under constant agitation in a rotary shaker, then CH was added, and the solutions were prepared as previously described. All solutions were subjected to an inverted tube test to assess their gelation time and identify the most suitable composition for preparing thermosensitive nasal hydrogel.

2. Results

2.2. Pre-formulation studies

2.2.1. Selection of chitosan

The viscosity of two hydrogels consisting of CH and β-GP using different types of CH with different molecular weights (Chitoclear TM1358 and TM1283). CH TM1358 and TM1283 possess molecular weights of 103 and 282 kDa and viscosity of 63 and 224 cP, respectively.

The hydrogel obtained using Chitoclear TM1283 showed a high viscosity compared to that formed with Chitoclear 1358. For this reason, further experiments were conducted using Chitoclear TM1358, as it was felt that it could provide gelling systems with a more suitable viscosity for nasal administration.

2.2.2. Preparation of CH/GP solutions

Gelation time of blank CH/GP solutions (A1b, A2b, A3b and G2b) and CH/GP solutions with DMF:HP-β-CD (1:1) complex (A1, A2, and G2) are shown in Table 1. Solutions revealed that an increase in β-GP content decreased the gelation time, while as the % CH decreased, the gelation time increased. Long gelation times were excluded. A very short gelation period is required because mucoadhesion is facilitated, and mucociliary clearance is inhibited. CH/GP solution 2/10 ratio (% w/v) containing the DMF:HP-β-CD complex (solution G2) was selected as the gelation time is suitable for nasal administration. Therefore, this composition was used to prepare thermosensitive nasal hydrogels (Formulations G1 and G2).

Table 1. Composition of CH/GP solutions (% w/v) and gelation time performed with invested tube test.

Solution	CH (% w/v)	GP (% w/v)	DMF (g)	HP-β-CD (g)	Gelation time (min:s)
A1b	2.0	12.0	-	-	6:00
A2b	1.6	15.0	-	-	3:00
A3b	2.0	7.0	-	-	14:00
G2b	2.0	10.0	-	-	8:00
A1	2.0	12.0	0.021	0.203	20:00
A2	1.6	15.0	0.021	0.203	-
G2	2.0	10.0	0.021	0.203	3:00

APPENDIX 4.3. ABBREVIATIONS

Absorption, distribution, metabolism, and elimination (ADME)
Active pharmaceutical ingredient (API)
Angiotensin-converting enzyme (ACE)
Blank solution to gel (G1b)
Blank solution to gel (G2b)
British Association For Parenteral And Enteral Nutrition (BAPEN)
British Pharmaceutical Nutrition Group (BPNG)
Captopril (CAT)
Cellulose acetate phthalate (CAP)
Chitosan (CH)
Design of Experiments (DoE)
Dimethyl fumarate (DMF)
Drug delivery systems (DDSs)
Eagle's medium (DMEM)
Encapsulation efficiency (EE)
Enhanced permeability and retention (EPR)
Enteral feeding tubes (EFTs)
European Commission (EC)
European Paediatric Formulation Initiative (EuPFI)
European public assessment reports (EPARs)
European Union (EU)
Evidence Coding Scheme, and GRADE (Grading of Recommendations, Assessment, Development and Evaluations)
Drug-free cellulose acetate phthalate nanoparticles (NP-Ab)
Drug-free cellulose acetate phthalate:chitosan 1:1 w/w ratio (NP-Bb)
Drug-free cellulose acetate phthalate:chitosan 1:3 w/w ratio (NP-Cb)
Fetal bovine serum (FBS)
Foreskin fibroblast-1 (HFF-1)
Fourier Transform Infrared Spectroscopy (FTIR)
French (Fr)
Generally Recognized As Safe (GRAS)
Glycerophosphate (GP)
High-performance liquid chromatographic (HPLC)
Hydroxypropyl-β-cyclodextrin (HP-β-CD)
Hypromellose acetate succinate (HPMCAS),
In situ self-assembly nanoparticles (ISNPs)
Quality Criteria Checklist (QCC)
Questions and answers (Q&As)
Loaded cellulose acetate phthalate nanoparticles (NP-A)
Loaded cellulose acetate phthalate:chitosan 1:1 w/w ratio (NP-B)
Loaded cellulose acetate phthalate:chitosan 1:3 w/w ratio (NP-C)
Mononuclear phagocyte system (MPS)
Multiple sclerosis (MS)
Multi-unit-pellet system (MUPS®)
N, N'-bis(acryloyl)cystamine (BAC)
Nanoparticles (NPs)
Nanostructured lipid carriers (NLCs)
Nasogastric (NG) tube
Nasojejunal(NJ) tube
Neonatal Intensive Care Unit (NICU)

Nurofen for Children (NfC) suspension
Orally disintegrating tablets (ODTs)
Paediatric Committee (PDCO)
Paediatric investigation plans (PIPs)
PDI (polydispersity index)
Pharmaceutical ingredients (APIs)
PIO criteria (Population of interest, Intervention, and Outcomes)
PLGA and carboxymethyl-poly(ethylene glycol)-b-poly(lactic acid) (PLA-PEG-COOH)
Poly(beta-amino ester) (PBAE)
Poly(ethylene glycol) (PEG)
Poly(ethylene glycol) (PEG)
Poly(glycolic acid) (PGA)
Poly(lactic acid) (PLA)
Poly(lactic-co-glycolic acid) (PLGA)
Poly(ε-caprolactone) (PCL).
Polydispersity index (PDI)
Polymeric NPs of poly(isobutylcyanoacrylate) (PIBCA)
Polyurethane (PU)
Polyvinyl chloride (PVC)
Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA)
Proton pump inhibitors (PPIs)
Safety and Toxicity of Excipients for Paediatrics database ("STEP")
Scanning Electron Microscope (SEM)
Slope (n)
Solid lipid nanoparticles (SLNs)
Solution (sol)
Solution to gel containing DMF (G1)
Solution to gel containing DMF (G2)
Summary of product characteristics (SmPC)
The American Society for Parenteral and Enteral Nutrition (ASPEN)
The enhanced permeability and retention (EPR)
The mononuclear phagocyte system (MPS)
United States Pediatric Formulation Initiative (US PFI)
US Food and Drug Administration (FDA)
Valproate sprinkles (VPA-S)
Zein-whey NPs (ZWP)

APPENDIX 4.4. PUBLICATIONS FROM THIS AUTHOR

Nieto González N, Obinu A, Rassu G, Giunchedi P, Gavini E. Polymeric and Lipid Nanoparticles: Which Applications in Pediatrics? *Pharmaceutics*. 2021; 13(5):67 https://doi.org/10.3390/pharmaceutics13050670

Nieto González N, Cerri G, Molpeceres J, Cossu M, Rassu G, Giunchedi P, Gavini E. Surfactant-Free Chitosan/Cellulose Acetate Phthalate Nanoparticles: An Attempt to Solve the Needs of Captopril Administration in Paediatrics. *Pharmaceuticals*. 2022; 15(6):662. https://doi.org/10.3390/ph15060662

Salunke, S.; Nieto González, N.; Walsh, J.; Hermans, E.; Rassu, G. Effect of Formulation Characteristics of Oral Paediatric Products on Administration via Enteral Feeding Tubes to Paediatric Patient (e-PET): A Systematic Review. PROSPERO CRD42022299418. 2022.

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