



Review

If Euhydic and Isotonic Do Not Work, What Are Acceptable pH and Osmolality for Parenteral Drug Dosage Forms?



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ABSTRACT

Parenteral products should aim toward being isotonic and euhydic (physiological pH). Yet, due to other considerations, this goal is often not reasonable or doable. There are no clear allowable ranges related to pH and osmolality, and thus, the objective of this review was to provide a better understanding of acceptable formulation pH, buffer strength, and osmolality taking into account the administration route (i.e., intramuscular, intravenous, subcutaneous) and administration technique (i.e., bolus, push, infusion). This evaluation was based on 3 different approaches: conventional, experimental, and parametric. The conventional way of defining formulation limits was based on standard pH and osmolality ranges. Experimental determination of titratable acidity or *in vitro* hemolysis testing provided additional drug product information. Finally, the parametric approach was based on the calculation of theoretical values such as (1) the maximal volume of injection which cannot shift the blood's pH or its molarity out of the physiological range and (b) a dilution ratio at the injection site and by verifying that threshold values are not exceeded. The combination of all 3 approaches can support the definition of acceptable pH, buffer strength, and osmolality of formulations and thus may reduce the risk of failure during preclinical and clinical development.

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Introduction

During drug product development, nonclinical safety studies are performed to support clinical trials and marketing authorization for pharmaceuticals.^{1,2} As an added precaution, the first clinical trials ("entry into human") usually start with a relatively low systemic exposure in a small number of healthy volunteers. The formulation that is being brought forward into nonclinical and clinical testing needs to take into account physical and chemical stability, manufacturability, and local and systemic tolerability, in addition to regulatory and pharmacopeial requirements. Although physico-chemical parameters like pH, osmolality, buffer concentration, their process-related acceptance criteria, and impact on stability are part of a state of the art drug product formulation development, the definition of what is accepted in terms of tolerability in a clinical setting is more controversial and not straightforward.

Solutions for injection of infusion may require a pH or osmolality, which are clearly outside the physiological (euhydic and isotonic)

range, often for solubility or stability reasons. In those cases, only a sound understanding of physiological, anatomic, physical, and chemical mechanisms of the parenteral administration at the injection site and during infusion will provide enough insight into the suitability of a formulation for the nonclinical and clinical studies. Especially, small molecule parenteral dosage forms are often characterized by a pH and osmolality significantly deviating from ideal target values. Although biologics in most cases can be developed toward isotonicity, the pH values may also deviate from euhydic pH because of stability reasons. For example, antibodies are often formulated around pH 5.5–6.5, and G-CSF is around pH 3–4. Biologic formulations often contain a buffer, and buffering capacity thus also needs to be considered in connection with target pH. In cases where lyophilisates are reconstituted in less volume, for example, to achieve higher concentration antibody formulations for administration,³ the tonicity may also be hypertonic.

Parenteral products should aim toward being isotonic and euhydic (physiological pH). If this is not achievable, as a general rule, excessive values of pH and osmolality should be avoided as much as possible to minimize or prevent local damage on vascular endothelium and circulating blood cells. However, because many parameters play a crucial role in terms of local tolerance (e.g., administration site and route of administration, vein selected, related venous blood flow, injection volume, infusion time, infusion

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duration, residence time in subcutaneous [s.c.] or intramuscular [i.m.] tissue, diffusion into surrounding tissues),⁴ no well-defined and generally recognized pH, buffer strength, and osmolality limits are available.

To evaluate the appropriateness of a formulation with regard to pH, buffer, and osmolality related to sufficient systemic and local tolerance, 3 different approaches can be considered: conventional, experimental, and parametric.

The *conventional approach* is knowledge based and consists in choosing pH and osmolality within the usual ranges of literature. The difficulty of this approach is that those ranges can vary considerably depending on the reference chosen as shown in later sections. Furthermore, they often do not sufficiently take other crucial parameters into consideration such as buffer capacity of the formulation, administration site, injection volume, injection duration, or frequency of administration.

A second approach is based on *experimental* data from either (1) titratable acidity measurements or (2) *in vitro* hemolysis tests. The titratable acidity measurements are used to determine the buffer strength of a solution at nonphysiological pH values, whereas the *in vitro* hemolysis test characterizes the risk of cell membrane damage after contact with a parenteral injection solution. The advantage of the experimental approach is that it is product specific and, at least for hemolysis testing, includes a biological component such as a direct contact with the (red blood) cell membrane. Hemolysis testing can be considered as a simple *in vitro* model for local tolerance. However, it provides no information about systemic toxicity, for example, interaction with the whole blood volume.

Finally, the *parametric approach* is based on the calculation of theoretical threshold values such as the maximal volume of injection which will still keep the blood's pH or its molarity within the physiological range or an estimate of the dilution ratio at the injection site. As long as those threshold values are not exceeded, the formulation and its administration condition have at least no theoretical concern and may be considered within an experimental design for further assessment. The parametric approach provides some additional information but does not replace the conventional or the experimental approach. In fact, all approaches are complementary. Of course, such an evaluation is specific for a given product, formulation, and administration scheme, and only nonclinical and clinical studies will finally confirm the appropriateness of the drug product related to systemic and local tolerance and the assessment of safety (vs. efficacy).

Assessment via the Conventional Approach

pH Limits of Small- and Large-Volume Parenterals

For large-volume intravenous (i.v.) infusion administration, pH and osmolality recommendations are summarized in Table 1.⁵

For small-volume i.v. injection solutions (<100-mL nominal volume), broader pH ranges can be envisaged depending on the source of information: pH 4–9,⁶ 3–10.5,⁷ or 3–11.^{4,8} On the other hand, when the risk of infiltration in subcutaneous tissue cannot be

excluded, more restrictive pH ranges such as 5.5–8.5⁹ are suggested, to avoid any risk of tissue damage.

As summarized in Table 2, drug products within a broad range of pH values (2.55–11.15) are on the market, most likely to overcome solubility or stability constraints.

There are no major warnings about pain or irritation in the packaging inserts of the drug products listed in Table 2, although an injection solution with an extreme pH value is more likely to induce vascular irritation, inflammatory reactions, or pain. However, the physiological local reaction cascade depends on too many factors (injection volume, infusion rate, local blood rate, duration of infusion, needle diameter, injection depth, buffering capacity, viscosity, active ingredient, cosolvents, and so forth) to permit a direct correlation between the pH (as isolated parameter) and pain or local irritation, unless all other factors would be kept constant in a homologous test series. Therefore, in case of a low buffering capacity, a low injection volume, a slow injection rate (favoring an rapid dilution by blood close to the injection site), and a nonirritating active drug substance even an extreme pH (high or low) can be locally well tolerated and produce neither pain nor irritation. In contrast, for an injection solution such as promethazine hydrochloride, which has a relatively harmless pH (4.0–5.5), adverse reactions including burning, pain, thrombophlebitis, tissue necrosis, and gangrene are mentioned in the packaging insert.

Those examples show that a too restrictive use of pH acceptance criteria could unnecessarily jeopardize the feasibility of a parenteral drug product.

For small-volume injections, not only the dilution factor but also the time factor plays an important role with regard to local i.v. tolerance. In contrast to the almost instantaneous solubilization of lipidic cell membrane components in presence of a high concentration of cosolvents, acid or basic hydrolysis needs time. This is the reason why small-volume bolus injections can often be administered i.v. in a very broad pH range. This has been confirmed by animal studies^{5,10} that concluded that a solution with a pH of 3–11 did not induce phlebotic changes when drugs were administered over a few minutes. However, the same studies showed that the local tolerance was highly dependent on the pH in case of a 6-hour infusion through peripheral vessels. Indeed, a solution with a pH of 4.5 resulted in a 100% incidence of severe phlebotic changes, a pH of 5.9 caused mild-to-moderate phlebotic changes in 50% of the animal subjects, a pH of 6.3 still caused mild damage in 20% of those subjects, and a pH of 6.5 caused no significant damage.^{8,11,12}

To keep the risk of local irritation low, pH values should nevertheless be inside the target pH range of $3.5 \leq \text{pH} \leq 9.0$ (according to DailyMed database) unless for very compelling reason. Especially, alkaline solutions with significant buffering capacity should be preferably be avoided. In case of borderline pH, a slower infusion rate (e.g., 5-minute push instead of 1-minute bolus injection) will contribute to overcome or reduce the risk of local irritation and vein damage. The reason of a better local tolerance in that case is related to the infusion duration but to the increased drug product dilution by the blood flow at the injection site.

Osmolality Limits of Blood and Infusion Solutions

With regard to osmolality, hypertonic injection solutions with an osmolality >600 mOsm/kg¹³ have been reported to possibly cause crenation (shriveling up) of red blood cells and significant pain. Hypotonic solutions with an osmolality about <150 mOsm/kg in contrast may cause hemolysis and pain at the site of injection. The limit of 240 mOsm/kg given in the European Pharmacopoeia for monoclonal antibodies¹⁴ has already a considerable safety margin and seems a bit arbitrary acceptance criterion but is not the physiologically lowest limit conceivable for these products. Indeed, a hypotonic sodium chloride 0.45% infusion solution (154 mOsm/L)

Table 1

Recommendations of Infusion Nursing Society for Minimization or Prevention of Vascular Damage From Extremes in Infusate pH or Osmolarity⁵

Vessel	Blood Flow (mL/min)	Osmolality (mOsm/L)	Solution pH
Superior vena cava	2000	>900	<5 or >9
Subclavian vein and proximal axillary vein	800	500–900	<5 or >9
Cephalic and basilica veins in the upper arms	40–95	<500	5–9

Table 2
Marketed Drug Products With Extreme Formulation pH

Active Pharmaceutical Ingredient	Trade Name	Company	Administration Route	pH ^a
Doxycycline hyclate	Doxycycline	Mylan Institutional LLC	i.v.	2.55 ± 0.75 (1.8–3.3)
Dipyridamole	Dipyridamole injection USP	Baxter Healthcare Corporation	i.v.	2.7 ± 0.5 (2.2–3.2)
Midazolam HCl	Midazolam	West-ward Pharmaceutical Corp.	i.v., i.m.	3.1 ± 0.6 (2.5–3.7)
Morphine Sulfate	Morphine Sulfate	Hospira, Inc.	i.v.	3.25 ± 0.75 (2.5–4.0)
Minocycline HCl	Minocin	Rempex Pharmaceuticals, Inc	i.v.	3.25 ± 0.75 (2.5–4)
Mycophenolate mofetil HCl	CellCept	Roche	i.v.	3.25 ± 0.85 (2.4–4.1)
Nicardipine HCl	Nicardipine Hydrochloride	Emcure Pharmaceuticals Ltd.	i.v.	~3.5
Dolasetron mesylate	Anzemet	Sanofi Aventis	i.v.	3.5 ± 0.3 (3.2–3.8)
Odansetron HCl	Odansetron	Remedyrepack.	i.v.	3.65 ± 0.35 (3.3–4.0)
Dopamine HCl	Dopamine Hydrochloride And Dextrose	Hospira, Inc.	i.v.	3.5 ± 1.0 (2.5–4.5)
Vancomycin hydrochloride	Vancomycin	Pfizer Laboratories	i.v.	3.5 ± 1.0 (2.5–4.5)
Insulin glargine	Lantus	Sanofi-Aventis	s.c.	~4
Isoproterenol hydrochloride	Isuprel	Hospira	i.v., i.m., s.c.	4.0 ± 0.5 (3.5–4.5)
Sumatriptan succinate	Sumatriptan succinate	Teva Parenteral Medicines, Inc	s.c.	4.75 ± 0.55 (4.2–5.3)
Pentobarbital sodium	Pentobarbital sodium	West-ward Pharmaceutical Corp.	i.v., i.m.	9.7 ± 0.5 (9.2–10.2)
Pantoprazole sodium	Pantoprazole Sodium	Akorn, Inc.	i.v.	9.75 ± 0.75 (9.0–10.5)
Esomeprazole sodium	Esomeprazole Sodium injection	Sun Pharmaceutical Industries Limited	i.v.	10.0 ± 1.0 (9–11)
Phenytoin sodium	Phenytoin Sodium	West-Ward Pharmaceutical Corp.	i.v.	11.15 ± 1.15 (10.0–12.3)

^a pH values taken from the online drug product database “DailyMed,” the official provider of FDA label information (package inserts), <http://dailymed.nlm.nih.gov/dailymed/about.cfm>.

could for instance be administered i.v. in case of dehydration. Dextrose solution is another hypotonic solution for infusion. Because it is a permeant solute (in nondiabetic patients), it will rapidly enter cells. This explains why a 5% dextrose is hypotonic despite being isosmolar. As a consequence of local hypotonicity and increased blood concentration, red blood cells can aggregate and form so-called erythrocyte rouleaux.¹⁵

To complete those recommendations regarding osmolality, a close look at already marketed formulations and drug products is useful. As summarized in Table 3, osmolality values (623–2018 mOsm/kg) mentioned in the package inserts of some drug products are significantly outside of the “expected ranges.”

Osmolality is an estimation of the osmolar concentration of plasma and is proportional to the number of particles per kilogram of solvent; it is expressed as mOsmol/kg. (The SI unit is mmol/kg, but mOsmol/kg is still widely used.) According to the USP (<785>¹⁶), the osmolality of blood ranges between 285 and 310 mOsmol/kg. The average physiological osmolality is therefore about 297.5 mOsmol/kg.

Infusion solutions with an osmolality >800¹⁷ or >850 mOsmol/L¹⁸ should be infused by the central venous route. This is a common practice in parenteral nutrition. In general, infusion solutions with an osmolality <600 mOsm/kg and a pH close to the physiological value have been suggested to have a low-to-moderate risk of phlebitis.^{5,19}

Osmolality limits have nevertheless to be interpreted with caution because not only the selection of the vein and the volume of infusion but also the infusion duration will play a role. Thus, the tolerance osmolality of peripheral venous endothelial cells has been reported to be 820 mOsm/kg for 8 hours, 690 mOsm/kg for 12 hours, and 550 mOsm/kg for 24 hours, with poor blood flow.²⁰

Injection Volume of Small-Volume Injections (Bolus)

As long as a parenteral injection has not to be administered over a prolonged time as infusion, the quotient of the target dose (*D*) divided by the maximal injection volume (*V_{inj}*) corresponds to the minimal solubility (*S_{min}*) needed to get the drug substance in solution.

The usual injection volume ranges for dosage forms applied as bolus injection to adults, depending on the route of administration,

are summarized in Table 4, completed with some pediatric examples.

$$S_{\min} = \frac{D}{V_{\text{inj}}}; [\text{mg/mL}] = \frac{[\text{mg}]}{[\text{mL}]}$$

The volume administered by subcutaneous bolus injection is typically not exceeding 2 mL in products currently on the market. However, the administration of up to 3 mL and more has been reported²³ and has been evaluated to not be increasing injection pain.²⁴ Any upper limit of s.c. bolus injection volume is likely limited by tissue backpressure²⁵ and injection site induration and especially leakage.

This upper bolus injection volume limit can even be significantly extended in case of coadministration or prior administration of a recombinant hyaluronidase. This enzyme can temporarily hydrolyze the connective tissue in the subcutaneous space and thus decreases tissue backpressure and ensure spreading of the injected volume. This increases the practical s.c. injection volume to tens, and possibly hundreds, of mL.^{21,22}

The maximal injection volume for the different administration routes is however not an absolute physiological limit and has to be adjusted drug product specifically depending on the local tolerance and pain measured in nonclinical and clinical studies.

Given the maximal injection volumes (*V_{inj}*) mentioned previously, the minimal solubility of a drug (*S_{min}*) needed for s.c. administration is the quotient of the target dose (*D*) divided by *V_{inj}*.

Local Tolerance

The probability of local tissue damage on injection of a drug product, although critical to the clinical success of parenteral drug products, is relatively thorny to assess being dependent on manifold parameters such as injection volume, infusion rate, choice of the injection site, injection duration, injection technique, and frequency of administration, as shown in the literature.^{26–34} Also, the active ingredient itself can have an impact on local tolerance and injection pain, which is assessed in preclinical and clinical studies.

The administration route plays an essential role in local tolerability of injections especially due to the dilution effect by blood flow which is missing in s.c. and i.m. administration. Tissue damage, inflammation, and pain at the injection site are mainly related to

Table 3
Marketed Drug Products With Significant Hyperosmolality

API	Trade Name	Company	Administration Route	Osmolality	Reference
Amino Acids	Aminosyn-HBC 7%	Hospira, Inc.	i.v.	623 mOsmol/L	^a
Dextrose 5% monohydrate and sodium chloride 0.9%	Dextrose 5% and sodium chloride 0.9%	Baxter Healthcare Corporation	i.v.	585 mOsmol/L	^c
N(2)-L-Alanyl-L-Glutamin	Dipeptiven	Fresenius Kabi	i.v.	921 mOsmol/L	^b
Aminoven 10%	Aminoven 10%	Fresenius Kabi	i.v.	990 mOsmol/L	^b
Mannitol 20%	20% OSMITROL	Baxter Healthcare Corporation	i.v.	1098 mOsmol/L	^a
Amino acids	Aminoven 15%	Fresenius Kabi	i.v.	1505 mOsmol/L	^b
Amino acids	Aminomix N 1	Fresenius Kabi	i.v.	1826–2018 mosm/kg	^b

^a Drug Product Database “DailyMed” (USA), <http://dailymed.nlm.nih.gov/dailymed/about.cfm>.^b Drug Product Database “Arzneimittelkompendium der Schweiz” (CH), <http://compendium.ch/home/de?nocookie=true>.^c Drug Product Database “Electronic Medicines Compendium” (UK), <http://www.medicines.org.uk/EMC/AdvancedSearchPage.aspx>.

physical (osmotic pressure) and chemical stress (pH, surfactant concentration, solvent content, increased sodium and potassium concentration) and their interaction with vascular endothelial cells, circulating blood cells, adipocytes, skeletal muscular cells, and nerve cells, depending on the route of administration.

In a vein, this can result in a vein inflammation (phlebitis), initiate a coagulation cascade, form a repairing thrombus, and evolve in a local inflammation (thrombophlebitis), as well as a systemic inflammatory reaction with significantly increased levels of white blood cells and plasma C-reactive protein.³⁵

From the biochemical point of view, an acidic or basic injection solution will be harmful for the cell membranes mainly by their cytotoxic and cytolytic properties, by protonation or deprotonation and destabilization of the extracellular portion of membrane proteins, and by hydrolysis of both phospholipids and proteins. The phospholipid ester bonds connected to free fatty acids and situated directly beneath the phosphate group are sufficiently accessible to the injection solution to be hydrolyzed. The damage or dissolution of cell membranes can result in the release of cellular components into plasma,³⁶ pain, vascular irritation, necrosis, and phlebitis.^{37,38} In case of injection of alkaline solutions, an additional chemical injury is possible: cell membrane fatty acids are saponified causing cell disruption and cellular death.³⁹

Arachidonic acid can for instance be released from the cell membranes and be transformed into powerful cellular mediators such as thromboxanes, prostaglandins and leukotrienes, hydroxy fatty acids, and lipoxins. These compounds beside a broad range of activities will initiate a pain signal in nerve cells.⁴⁰ Some main pain-inducing mechanisms are summarized here⁴¹:

- Damaged tissue releases globulin and protein kinases, which are believed to be among the most active pain-producing substances. Minute s.c. injections of globulin induce severe pain.
- Tissue damage stimulates the mast cells to release histamine to the surrounding area. Histamine excites the nociceptors. Minute s.c. injections of histamine elicit pain.
- Most tissue damage results in an increase in extracellular potassium ions. There is a good correlation between pain intensity and local potassium ion concentration.

Table 4
Typical Injection Volumes of Bolus Injections

Administration Route	Subcutaneous	Intravenous	Intramuscular	Intradermal
Injection Volume	≤3 mL (>>5 mL with Halozyme, e.g., 11.7 mL for Mabthera s.c.) ^{21,22}	≤5 mL	≤5 mL (thigh) ≤2 mL (deltoid)	≤0.5 mL

- After drug-induced intramuscular tissue damage (rhabdomyolysis), the injured muscle cells leak myoglobin.⁴²
- It has been reported that intramuscular pain is increased by temporal (repeated injections) and spatial summation (injections given at different sites).⁴³

All i.v., i.m., and the s.c. administration routes have of course also specific characteristics, among which the most important will be recalled in the following sections.

Intravenous Administration

The i.v. administration has the advantage that, in case of emergency, there is no delay before the drug reaches the circulation and a bioavailability of 100% is defined for i.v. administration.⁴⁴ An i.v. injection of a drug product with some anaphylactoid potential will in contrast bear a higher risk of severe response than when using other administration routes. This risk can be decreased by reduction of the infusion rate or of the dose of excipients with anaphylactoid potential. The local blood flow dilution effect is still a major advantage of the i.v. administration and explains why too irritating drugs by the i.m. or s.c. route can be given intravenously.²⁶

A direct i.v. injection (bolus) is in general given over shorter periods (e.g., 2–3 minutes), observing the patient and the injection site for signs of adverse reaction. The volume of injection is usually ≤5 mL. In case of an i.v. infusion, the volume used to dilute ranges in general from 50 to 500 mL. In clinical practice, most drugs are given in 100 mL and are set to infuse over some more extended period, for example, 20–30 minutes (Table 5).

In the example mentioned previously, the infusion rate limitation is based on a volume/time base independently of the formulation and the excipients. The importance of the volumetric

Table 5
Intravenous Injection Techniques

Injection Technique	General Infusion Rates and Points to Consider
i.v. Bolus injection	Infusion rate = 1 mL/10 s or 6 mL/min, that is, 5 mL/50 s, duration: ≤1 min; max. volume about 5 mL; No cannula needed
i.v. Push medication	Low infusion rate (0.6–1 mL/min), over 5–8 minutes; max. volume about 5 mL; to avoid pain or injury at the injection site due to the needle, the use of a catheter and a short tubing are necessary ^a
i.v. Infusion	Prolonged infusion time, 15–60 min (or more); infusion rate: 30 mL/10 min or, e.g., 180 mL/1 h corresponding to 1 mL/20 s (3 mL/min); the infusion rate is controlled either by a bag with a drop chamber or by an infusion pump or syringe pump ^a

^a The solution being in contact with an infusion set (tubing, connectors, filter, and cannula), the absence of leachables or absorption during application has to be verified by means of a standardized compatibility test.

infusion rate will be discussed more in detail in the parametric chapter under “local dilution ratio.”

There are however special cases where an excipient-specific limitation should be considered as well. This is the case with polymeric surfactants such as Cremophor EL^{45–47} or polysorbates^{48,49} which are prone to induce pseudoallergic reactions. Such hypersensitivity reactions do not involve IgE immunoglobulins, arise as a consequence of activation of the complement system, are (excipient-) dose-dependent, and do not need any presensitization. Therefore, to ensure a safe administration of parenteral formulations containing surfactants, not only the excipient dose and its concentration in view of a good local i.v. tolerability but also the excipient-infusion rate (mg/min) should be taken into consideration. A good practice consists in verifying that the intended excipient infusion rate is already covered by a marketed drug product for the same administration route.

For other excipients such as cosolvents or sucrose, a closer look at the excipient infusion rate is also advisable. In 1999, the Food and Drug Administration (FDA) issued a warning to physicians about the safety of intravenous immunoglobulins (IVIG) products. In this document, the FDA also provided advice regarding proper patient management surrounding the administration of IVIG. For sucrose-containing IVIGs, FDA recommended a maximum infusion rate of 3 mg sucrose/kg/min.⁵⁰

Intramuscular Administration

Absorption by the i.m. route is relatively fast. Nevertheless, a delayed onset of action is usually associated with that administration route. Furthermore, bioavailability differences between i.m. and i.v. administration may be observed. After drug-induced intramuscular tissue damage (rhabdomyolysis) beside the manifold inflammatory mediators,⁵¹ the injured muscle cells leak in addition myoglobin.⁴²

To ensure an i.m. administration of a drug, a sufficient needle length is essential. Suboptimal hospital practice of i.m. injections can indeed result in a majority of unintentional s.c. administration.⁵²

The i.m. site is more often associated with pain on injection compared to i.v. or s.c. administration. This most likely results from the prevalence of nerves in muscle tissue compared to subcutaneous tissue and the rapid dilution of the drug by blood when administered i.v., which may limit the concentration at the injection site.²⁶ Intramuscular injections require a longer needle than s.c. injections to reach the desired muscle. In addition, the needle diameter is in general larger (22–25 G) as shown in vaccination practice. Although it is generally assumed that larger needles mean larger pain sensation in patients, the contrast may also be true. Contrary to the belief that smaller needles cause less pain, study findings revealed however that vaccination with smaller bore needle (25 G) caused more pain compared to larger needles (23 G).⁵³ This could be explained by a higher jet pressure created through thinner needles in comparison to larger ones. To reduce this pressure-induced pain and muscle trauma, an i.m. injection should be administered slowly (e.g. 1 mL in 10 or 20 seconds).⁵⁴ However, in matter of i.m. injection technique and vaccination, there is no general consensus. A systematic review of randomized controlled trials has for instance shown that rapid injection might be less painful.⁵⁵

Subcutaneous Administration

In s.c. administration, absorption is slower than by the i.m. route but, nevertheless, can be prompt with some drugs. The slower rate of absorption by the s.c. route is usually the reason for choosing the route. Drugs such as insulin and sodium heparin for instance are given by this route because it is desirable to distribute their pharmacological effect over several hours, to avoid frequent injections. Irritant medications should not be administered s.c. as they may

cause tissue necrosis or a sterile abscess. Not only increased injection volume but also the buffering strength at a nonphysiological pH or the buffer choice⁵⁶ can be related to pain perception at the injection site.²⁶

A sound understanding and standard ranges of pH, buffer strength, osmolality, and injection volume are essential to develop parenteral formulations for the different administration routes with a high likelihood of good local and systemic tolerance. Complementary quantitative parameters can further facilitate the selection of the right composition will be discussed in the parametric approach.

Assessment via the Experimental Approach

The term experimental approach is used to describe additional assays allowing better prediction of either the likelihood of a systemic pH shift outside the physiological pH range or the damaging potential toward cell membranes rather than the common experimental physicochemical measurements such as pH and osmolality.

Titrateable Acidity Limits for Infusion Solutions

The difference between the pH of a parenteral injection solution and the physiological pH and also the buffer concentration of the injection solution can be quantified by means of the measurement of the titrateable acidity which is defined as the number of mmoles of NaOH required to titrate an acidic solution back to a pH of 7.4. For injection solutions having a pH >7.4, a titrateable basicity can be defined in a similar way using an HCl solution as titrant. For slightly acidic large-volume infusion solutions which are infused over a prolonged duration, the titrateable acidity is a good marker of a potential risk of local inflammation reactions and phlebitis. A higher titrateable acidity will also increase the risk of irritation of endothelial cells over a longer distance from the catheter tip. Therefore, it is common practice to mention titration acidity on package inserts of large-volume infusion solutions (Table 6).

The lower the titration acidity, the better. However, the titrateable acidity is not the only factor influencing the time to return to pH homeostasis because downregulation of carbon dioxide excretion in the lung and increased reabsorption of bicarbonate and excretion of acids in the kidney will support the regulation of the acid–base balance of blood.

There are 2 important disadvantages with this experimental parameter: the titrateable acidity is rarely mentioned in parenteral drug product information sheets and there are no generally recognized threshold values. The parametric approach is therefore easier to handle especially during early formulation development.

Table 6
Titrateable Acidity of Hyperosmolar Drug Products

Trade Name	Company	Titration Acidity (pH 7.4; mmol NaOH/L)	pH	Reference
Aminomix	Fresenius Kabi	18.0–33.0	5.5–6.0	^a
Aminoplasmal 15%	B. Braun Medical	31	5.7–6.3	^b
Aminoven 15%	Fresenius Kabi	44	5.5–6.3	^a
Aminoven infant 10%	Fresenius Kabi	27–40	5.5–6.0	^a
Dipeptiven	Fresenius Kabi	90–105	5.4–6.0	^b
Glucosaline 1:1	B. Braun Medical	<1	3.5–6.5	^a
Ringer-Acetate	Fresenius Kabi	1.0–10.0	5.0–7.0	^a
Voluven 6% balanced	Fresenius Kabi	<2.5	5.7–6.5	^a

^a Drug Product Database “Arzneimittelkompendium der Schweiz” (CH), <http://compendium.ch/home/de?nocookie=true>.

^b Drug Product Data sheet, Google search.

In Vitro Hemolysis Test

At the injection site, the damage of vascular endothelial cells or even the rupture of any circulating cell (e.g., erythrocytes, lymphocytes, thrombocytes) will initiate an inflammatory reaction and result in the release of cellular mediators³⁶ producing pain, vascular irritation, and phlebitis or necrosis.

Erythrocytes are particularly suitable to be used as surrogate marker in an *in vitro* hemolysis test for membrane compatibilities, which provides an indication of the risk of vascular membrane irritation (e.g., thrombophlebitis). It should not be confused with another *in vitro* assay used for hemocompatibility testing which is commonly used during the development of new synthetic materials.⁵⁷ Its purpose is quite different: studying the compatibility of foreign surfaces with blood and its impact on the coagulation system and complement activation.

The hemolysis test is performed with whole blood of rodent (rat) and nonrodent species (minipig, cyno) or human blood and is largely described in literature (G. Schmitt, unpublished data, 2013).^{38,57–62} The analytical end points are hemolysis, plasma precipitation, and turbidity. The data have however to be interpreted with caution because they can be overpredictive because of the static nature of the *in vitro* test (minutes of incubation vs. seconds in a vein under dynamic *in vivo* conditions). The *in vitro* test can be helpful if there is a specific concern for a particular formulation regarding local tolerance. This is often the case for small-molecule injectable formulations when significant amounts of potentially irritating excipient concentrations are needed (e.g., surfactants or cosolvents).⁶³

Assessment via the Parametric Approach

The systemic toxicity of a parenteral drug product is related to the pharmacological and toxicologic effects of a drug substance but will also depend on the composition of the vehicle (e.g., buffer concentration, pH, cosolvent, surfactants, etc.).

In a 1-compartmental pharmacokinetic model, the body is depicted as a kinetically homogenous unit.⁶⁴ This assumes that the drug achieves instantaneous distribution throughout the body. Based on that assumption and knowing the volume and the buffering capacity of blood of an adult, it is possible to calculate the effect of an acidic or basic i.v. injection of defined volume and buffer concentration on the pH homeostasis.

Physiological pH Regulation and pH Limits

The human body is designed to maintain a very delicate pH balance in its fluids, tissues, and systems. The normal range of the pH of blood is between 7.35 and 7.45; that range is necessary for the normal functioning of critical metabolic processes. A pH outside this range is physiologically stabilized by 3 primary mechanisms: the action of buffer systems, respiratory control, and renal control. Buffer systems use bicarbonate, phosphate, and proteins including hemoglobin. The carbonic acid–bicarbonate system of the body is the main buffer system and is responsible for about 80% of extracellular buffering.⁶⁵ Its pH regulation mechanism can be described by the Henderson–Hasselbalch equation⁶⁶ (Eqs. 1 and 2).

$$\text{pH} = 6.1 + \log \left(\frac{[\text{HCO}_3^-]}{\alpha \times \text{pCO}_2} \right) \quad (1)$$

where, $[\text{HCO}_3^-]$ = in mmoles/L; normal value = 24 mM; pCO_2 = in kPa (normal value = 5.3 kPa) or mm Hg (normal value = 40 mm Hg); α = solubility coefficient of Henry law = 0.03 when pCO_2 is in kPa or 0.225 when pCO_2 is in mm Hg.

Chemical equilibrium: $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$

Equation 1 contains the 2 species which allow the regulation of the pH of blood.

1. The partial pressure of arterial carbon dioxide pCO_2 is reduced by hyperventilation ($\Rightarrow$ increased CO_2 elimination $\Rightarrow$ respiratory alkalosis) or by hypoventilation ($\Rightarrow$ reduced CO_2 elimination $\Rightarrow$ respiratory acidosis).
2. In the kidney, the elimination of bicarbonate $[\text{HCO}_3^-]$ can be either increased ($\Rightarrow$ metabolic acidosis) or reduced ($\Rightarrow$ metabolic alkalosis).

Although the physiological pH of human arterial blood is between 7.35 and 7.45, acidosis is said to occur when arterial pH falls <7.35 and alkalosis when arterial pH is >7.45 .

To calculate the pH of blood, the standard Henderson–Hasselbalch equation can be used:

$$\text{pH} = \text{pKa} + \log \left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \right) \quad (2)$$

where,

- pKa is the negative logarithm of the acid dissociation constant of carbonic acid and is equal to 6.1 (at 37°C)
- $[\text{HCO}_3^-]$ is concentration of bicarbonate for example in mmoles/L arterial blood, normal value = 24 mM
- $[\text{H}_2\text{CO}_3]$ is the concentration of carbonic acid for example in mmoles/L in arterial blood, normal value = 1.2 mM

$$\text{Control : pH} = 6.1 + \log \left(\frac{24}{1.2} \right) = 7.4$$

From Equation 2, the normal ratio of base bicarbonate to carbonic acid can be extracted as follows:

$$\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = 10^{(\text{pH} - \text{pKa})} \quad (3)$$

This means that to obtain a pH of 7.4, a ratio of $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ of about 20 (Excel: $= 10^{(7.4 - 6.1)} = 19.95$) is necessary. With that ratio, 95.2% (Excel: $= 100 - \text{ROUND} [100/(1 + \text{ROUND} \{10^{(7.4 - 6.1)}; 2\}); 1] = 95.2\%$) of the buffer system is bicarbonate and only 4.8% (Excel: $= \text{ROUND} [100/(1 + \text{ROUND} \{10^{(7.4 - 6.1)}; 2\}); 1] = 4.8\%$) carbonic acid.

From the chemical point of view, the selection of a buffer with a pKa of 6.1 to maintain a constant pH at 7.4 is suboptimal because it will ensure a 20 times lower resistance against a basic pH shift than against an acidic one.

The blood buffering system has, however, still a major physiological advantage because it is “open at both ends” (meaning that both HCO_3^- and pCO_2 can be adjusted). Indeed, this greatly increases its buffering effectiveness and enables a faster return to a physiological pH.

Acidobasic Acceptance Criteria Related to Systemic Toxicity

Acceptance limits for the parenteral administration of an injection solution which will not jeopardize the buffering capacity of the entire blood volume can be defined by means of maximal (allowable) injection volume v_{max} representing the theoretical amount of acid or base (in mL injection solution of a given concentration of acid in mmoles/liter) which can be administered i.v. The absolute amount of buffering molecules (i.e., mainly

bicarbonate and acid carbonic) of the body will be directly proportional to the total blood volume, which is on the average 5 L for an adult who weighs 70 kg.⁶⁷ For children, the blood volume can be estimated based on the body surface and the growth charts of the Centers for Disease Control and Prevention, National Center for Health Statistics, USA. Thus, $v_{\max}$ can also be calculated for pediatric populations and provide a first estimation of the risk of any significant impact on the physiological pH of blood.

The estimate is based on following premises:

1. The buffering capacity is calculated *after* homogeneous distribution of the injection solution in the total blood volume. The question of the local tolerance is neglected at this stage but will be discussed later.
2. Only the main buffering system (bicarbonate/carbonic acid) is considered.
3. The compensatory mechanisms (lung and kidney) will not be taken into account and blood will be considered as a closed buffer system.
4. The dissociation of a weak acid or a weak base will be handled as a strong acid, meaning that each molecule has a direct impact on the pH equilibrium.

The premises 2–4 are all worst-case assumptions, so that, the conclusion which will be deduced will be on the safe side.

Based on those premises, Equation 2 becomes:

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-] - a}{[\text{H}_2\text{CO}_3]}\right) \quad (4)$$

or

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-] - \frac{c}{1000} \times v}{[\text{H}_2\text{CO}_3]}\right) \quad (5)$$

where,

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- HCO_3^- = amount of bicarbonate in mmoles in V L of blood = 24 mM $\times$ V = 120 mmoles
- H_2CO_3 = amount of carbonic acid in mmoles in V L of blood = 1.2 mM $\times$ V = 6 mmoles
- V = blood volume in L (5 L for adult)
- v = volume of i.v. injection or infusion in mL
- c = concentration in mmoles of acid in injection solution
- a = amount of mmoles of acid in v mL of injection solution

and finally,

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-] \times V - \frac{c}{1000} \times v}{[\text{H}_2\text{CO}_3] \times V}\right) \quad (6)$$

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- HCO_3^- = concentration (mM) of bicarbonate of blood = 24 mM
- H_2CO_3 = concentration (mM) of carbonic acid of blood = 1.2 mM
- V = blood volume in L (5 L for adult)
- v = volume of i.v. injection or infusion in mL
- c = concentration (mM) of acidic injection solution

After rearrangement of Equation 6, $v_{\max}$ of an acidic injection of concentration c which would reduce the pH from 7.4 to 7.35 can be calculated:

$$V_{\max} = \left(\frac{1000}{c}\right) \times ([\text{HCO}_3^-]) \times V - (10^{(\text{pH}-\text{pKa})}) \times [\text{H}_2\text{CO}_3] \times V \quad (7)$$

where,

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- pH = 7.35
- HCO_3^- = concentration (mM) of bicarbonate of blood = 24 mM
- H_2CO_3 = concentration (mM) of carbonic acid of blood = 1.2 mM
- V = blood volume in L (5 L for adult)
- $v_{\max}$ = maximal allowable injection volume = volume of acidic solution in mL which would reduce the pH from 7.4 to 7.35
- c = concentration (mM) of acidic in injection solution

Example 1:

- c = 50 mM, V = 5 L
- $\Rightarrow v_{\max} = 266 \text{ mL} (= [1000/50 \times (24 \times 5 - 10^{\{7.35 - 6.1\}} \times 1.2 \times 5)] = 266)$ of an acid 50 mM solution for an adult

Maximal Injection Volume of Bases

Using the same approach for acid injections, the pH shift after injection of a basic solution can be defined as the following:

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3] - b}\right) \quad (8)$$

or

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3] - \frac{c}{1000} \times v}\right) \quad (9)$$

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- HCO_3^- = amount of bicarbonate in mmoles in V L of blood = 24 mmoles $\times$ V = 120 mmoles
- H_2CO_3 = amount of carbonic acid in mmoles in V L of blood = 1.2 mmoles $\times$ V = 6 mmoles
- V = blood volume in L (5 L for adult)
- v = volume of i.v. injection or infusion in mL
- c = concentration in mmoles of base in injection volume
- b = amount of mmoles of base in v mL injection solution

and finally,

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{HCO}_3^-] \times V}{[\text{H}_2\text{CO}_3] \times V - \frac{c}{1000} \times v}\right) \quad (10)$$

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- HCO_3^- = concentration (mM) of bicarbonate of blood = 24 mM
- H_2CO_3 = concentration (mM) of carbonic acid of blood = 1.2 mM
- V = blood volume in L (5 L for adult)

- v = volume of i.v. injection or infusion in mL
- c = concentration (mM) of basic injection solution

After rearrangement of Equation 10, $v_{\max}$ of a basic injection of concentration “ c ” which would increase the pH from 7.4 to 7.45 can be calculated:

$$V_{\max} = \left(\frac{1000}{c \times 10^{(\text{pH}-\text{pKa})}} \right) \times ([\text{H}_2\text{CO}_3]) \times V \times (10^{(\text{pH}-\text{pKa})} - [\text{HCO}_3^-] \times V) \quad (11)$$

where,

- pKa = negative logarithm of the acid dissociation constant of carbonic acid = 6.1
- pH = 7.45
- HCO_3^- = concentration (mM) of bicarbonate in blood = 24 mM
- H_2CO_3 = concentration (mM) of carbonic acid in blood = 1.2 mM
- V = blood volume in L (5 L for adult)
- $v_{\max}$ = maximal allowable injection volume = volume of basic solution in mL which would increase the pH from 7.4 to 7.45
- c = concentration (mM) of base in injection solution

Example 2:

- $c = 50$ mM, $V = 5$ L

$\Rightarrow v_{\max} = 13$ mL ($= 1000/[50 \times 10^{(7.45 - 6.1)}] \times [1.2 \times 5 \times 10^{(7.45 - 6.1)} - 24 \times 5] = 13$) of a basic 50 mM solution.

The reason for the much lower $v_{\max}$ for basic solutions than for acidic solutions is discussed in the following sections.

Asymmetry of Acidobasic Risk

Examples 1 and 2 show that the maximal injection volume of a 50 mM acidic solution is 266 mL, and $v_{\max}$ of a basic solution of same concentration (50 mM) is only about 13 mL. This asymmetry, respectively, the ratio of both volumes ($266/13 = 20$), is not surprising. Indeed, it corresponds exactly to the ratio of $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ in blood.

Because the absolute amount of acidic or basic buffer molecules injected is the cause of a potential acidosis or alkalosis, it is good to recall that in case of administration of large infusion volumes, 2 situations have to be clearly distinguished:

A large volume of infusion solution with a nonphysiological pH does not represent a systemic risk if it was prepared by diluting a bolus i.v. injection (1–5 mL) in a saline infusion (e.g., 100 or 250 mL).

In contrast, if a 50 mM basic solution was used undilutedly as infusion, even a relatively small volume (e.g., 25 mL) would be already above the maximal injection volume (13 mL).

The infusion of undiluted large volumes of acidic solutions is less critical. Nevertheless, a risk of acidobasic imbalance is likely, when using rather concentrated solutions (e.g., 200 mM). In that case, using Equation 7, a maximal injection volume of 67 mL would be obtained (Excel: $= [1000/50 \times (24 \times 5 - 10^{\{7.35 - 6.1\}} \times 1.2 \times 5)] = 67$).

It can be concluded that, thanks to the high buffering capacity of human blood, there is in practice no acidobasic restriction:

- Neither for small-volume injection solutions (e.g., <5 mL), as far as only the total blood volume buffering capacity is considered.
- Nor for infusion solutions prepared by dilution of a small volume bolus injection in a larger volume of NaCl 0.9% or dextrose 5%.

In contrast, for large-infusion volumes or in case of pediatric use, the risk of an acidosis or alkalosis has to be excluded by means of $v_{\max}$ calculations. The $v_{\max}$ approach does however not represent an “all-clear” signal. Indeed, it concerns only the systemic suitability but does not take into consideration the local tolerance at the injection site, which will be discussed later.

Local Dilution Ratio

The better tolerability of i.v. administration in comparison to i.m. or s.c. injection is explained by the dilution effect of blood directly after injection. The physical laws of fluid dynamics defined by Ohm's and Poiseuille's equations underline the impact of the vein diameter on the local blood flow, although both equations are only approximations based on assumptions which are not totally fulfilled (e.g., Newtonian fluid, laminar flow, nonelastic cylindrical pipe of constant cross section).

In Ohm's formula, $P = Q \times R$, P represents the change in pressure along the vessel, Q the blood flow, and R the resistance to the flow. Therefore, an increase in both flow and resistance will result in a pressure increase. Resistance can be expressed according to Poiseuille's law:

$$R = \frac{8\eta L}{\pi r^4}$$

where η represents the viscosity coefficient, L the length of the vessel, and r its radius.

As blood viscosity and the length of the vessel usually do not change significantly, a change in resistance is usually caused by a change in the vessel diameter. The power of 4 relationship means that a small reduction in the vessel diameter provokes a dramatic increase in resistance.

A small segment of an arm vein measuring, for example, 15 cm with a diameter of 3 mm would contain only about 1 mL ($= \text{Pl}() \times (0.3/2)^2 \times 15 = 1.1$) blood. At the injection site, within a few centimeters of the vein, the buffering capacity of blood is therefore far too little to ensure a good local tolerance. The only factor which will compensate nonphysiological pH values is the local and immediate dilution by peripheral blood flow. For that reason, the injection/infusion rate has a crucial influence on the local i.v. tolerance and is worth to be parametrized.

To minimize local irritation at the internal vein wall, the injection/infusion rate is generally not higher than

- 1 mL/10 s (6 mL/min) for small injection volumes (≤ 5 mL).
- 1 mL/20 s (corresponding to 3 mL/min, 30 mL/10 min, or 180 mL/h) for large-volume infusion solutions (≥ 25 mL). For maintenance and replacement fluids however, higher infusion rates, close to upper rate limit, for small injection volumes can be applied (i.e., 3 mL/m² body surface area/min, corresponding to approximately 5.5 mL/min).⁶⁸
- For s.c. injections, a faster injection rate is possible (e.g., 1 mL in 1–10 seconds).

Three veins are commonly recommended for venipuncture⁶⁹: the median cubital, the cephalic, and the basilic vein.

A scoring system distinguishing between gentle, borderline, and aggressive i.v. administration conditions can be used based on a more anatomic model and the following data⁷⁰:

- blood velocity in cm/min in cephalic vein from controls: 7–15 cm/s
- cephalic vein diameter: 0.15–0.35 cm

The blood flow in mL/min can be calculated as follows:

$$F_{bl} = v_{bl} \times \pi r^2 = v_{bl} \times \pi \times \left(\frac{d}{2}\right)^2 \quad (12)$$

where, F_{bl} = blood flow (mL/min); v_{bl} = blood velocity (cm/min); d = vein diameter (cm); r = radius of vein diameter (cm)

$$\text{unit control: } \frac{mL}{min} = \frac{cm}{min} \times cm^2$$

The ratio of blood flow divided by infusion rate, called below dilution ratio, is defined by

$$\text{Dilution ratio} = \frac{F_{bl}}{F_{inf}} = \frac{v_{bl} \times \pi \times \left(\frac{d}{2}\right)^2}{\frac{v_{in}}{t}} \quad (13)$$

where, v_{in} = infusion volume (mL); t = infusion time (min); F_{bl} = blood flow (mL/min); F_{inf} = infusion solution flow = infusion rate (mL/min).

Because of physiological and anatomic variability, neither vein diameter nor blood flow can be defined by a value but have to be defined as a range. The dilution ratio will therefore be between following 2 values

$$\text{Worst case dilution ratio} = \frac{F_{bl}}{F_{inf}} = \frac{v_{blmin} \times \pi \times \left(\frac{d_{min}}{2}\right)^2}{\frac{v_{in}}{t}} \quad (14)$$

$$\text{Best case dilution ratio} = \frac{F_{bl}}{F_{inf}} = \frac{v_{blmax} \times \pi \times \left(\frac{d_{max}}{2}\right)^2}{\frac{v_{in}}{t}} \quad (15)$$

Those 2 dilution ratios can be used to estimate whether an infusion rate is sufficient to compensate a potential local irritation. When both dilution ratios (worst and best case) are >3 and the intravenous infusion rate is not faster than the standard conditions mentioned previously, the infusion rate does not represent any particular concern. If one of both dilution ratios is >2 , the administration conditions are borderline and should be avoided. A dilution ratio <2 can be considered as aggressive.

Physiological Osmolar Regulation

In the steady state, our total body water and salt content remain constant. A change in water and salt intake is paralleled by an equivalent change in renal water and salt excretion through the process of glomerular filtration of plasma.

As for blood buffering system, the electrolyte–water balance is under physiological control (osmoregulation). Increased osmolality in the blood will stimulate secretion of antidiuretic hormone (ADH) resulting in increased water reabsorption, more concentrated urine, and less concentrated blood plasma. A low serum osmolality in contrast would suppress the release of ADH, resulting in decreased water reabsorption and more concentrated plasma. An increase of only 2%–3% in plasma osmolality will produce a strong desire to drink. A change of 10%–15% in blood volume and arterial pressure is required to produce the same response.

Osmolality Acceptance Criteria of Hypotonic Solutions

Knowing the human blood volume and assuming a standard osmolality of 297.5 mOsm/kg, a maximal injection volume v_{max} can be calculated in connection with hypotonic and hypertonic solutions in a similar way as proposed for acids and bases, based on a 1-compartmental pharmacokinetic model and neglecting compensatory mechanisms (secretion of antidiuretic hormone).

For hypotonic solutions, v_{max} represents the theoretical amount solution (in mL) which can be administered i.v. to an adult, decreasing the blood osmolality to its lower limit of 285 mOsm/kg.

$$V_{max} = 1000 \times V \times \frac{[O_{norm}] - [O_{min}]}{[O_{min}] - [O_{inj}]} \quad (16)$$

where, V = blood volume in L (5 L for adult); O_{inj} = osmolality in mOsm/L of hypotonic injection solution (<285 mOsm/kg); O_{min} = lower isotonic osmolality limit of blood = 285 mOsm/kg; O_{norm} = normal osmolality of blood in mOsm/L prior injection = 297.5 mOsm/kg = $(310 + 285)/2$.

Example 3: O_{inj} = 0 mM (water for injection), V = 5 L (adult)
 v_{max} = maximal injection volume of hypotonic i.v. injection in mL = 219 mL (Excel: = $1000 \times 5 \times (297.5 - 285)/(285 - 0)$ = 219) of water for injection.

Example 4: O_{inj} = 150 mM, V = 5 L (adult)
 v_i = 463 mL (Excel: = $1000 \times 5 \times (297.5 - 285)/(285 - 150)$ = 463) of 150 mM solution.

Osmolality Acceptance Criteria of Hypertonic Solutions

The v_{max} of a hypertonic infusion solution is calculated in a similar way.

$$V_{max} = 1000 \times V \times \frac{[O_{norm}] - [O_{max}]}{[O_{max}] - [O_{inj}]} \quad (17)$$

V = blood volume in L (5 L for adult); O_{inj} = osmolality in mOsm/L of hypertonic injection solution (>310 mOsm/kg); O_{max} = upper isotonic osmolality limit of blood = 310 mOsm/kg; O_{norm} = normal osmolality of blood in mOsm/L prior injection = 297.5 mOsm/kg = $(310 + 285)/2$.

Example 5: O_{inj} = 600 mM, V = 5 L (adult)
 v_{max} = maximal volume of hypertonic i.v. injection or infusion in mL = 216 mL (Excel: = $1000 \times 5 \times (310 - 297.5)/(600 - 310)$ = 216) of 600 mM solution.

Examples 4 and 5 show that from a systemic point of view, the hypo-osmotic or hyperosmotic risk is not relevant at least for small-volume bolus injections.

Nevertheless, this is not an all-clear signal for nonisotonic solutions. Indeed, the local situation at the injection site is in fact the critical quality parameter to address.

Conclusion

Parenteral products should aim toward being isotonic and euhydric (physiological pH). Yet, because of other considerations, this goal is often not reasonable or doable. There are no clear allowable ranges related to pH and osmolality. The objective of this review was to provide a better understanding of acceptable pH, buffer strength, and osmolality of parenteral formulations. This evaluation is based on 3 different approaches: conventional, parametric, and experimental:

- The conventional way of defining formulation limits is based on standard pH and osmolality ranges.
- The parametric approach is based on the calculation of theoretical values such as the maximal volume of injection which does not shift the blood's pH or its molarity out of the physiological range and a dilution ratio at the injection site.
- Experimental determination of titratable acidity or *in vitro* hemolysis testing provides additional drug product information. The combination of all 3 approaches can support the definition of acceptable pH, buffer strength, and osmolality of parenteral formulations and thus may reduce the risk of failure during preclinical and clinical development.

pH and osmolality considerations for parenteral formulations will help to guide development scientists in selecting formulations that are not only adequate to achieve acceptable product stability but will also be well-tolerated in preclinical and clinical studies.

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