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Osmolality of antisense oligonucleotide parenteral formulations: Implications on counterion dissociation and recommended osmometry techniques

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ABSTRACT

The intrinsic osmolality of aqueous solutions of sodium salt antisense oligonucleotides (ASOs) has been studied to inform formulation practices, understand the molecular basis underlying the difference between theoretical and empirical results, and determine suitable measurement methods. It was found that regardless of nucleotide sequence, ASO concentration of ~140 mg/mL has isotonic osmolality of ~290 mOsm/kg water (SI unit: mmol osmotically-active particles/kg water), such that lower concentration formulations require excipients for tonicity adjustment. The range of osmolality values at a given active ingredient concentration can be ascribed to drug substance lot-to-lot purity differences impacting total oligonucleotide content (i.e., including oligonucleotide-related impurities). Empirical osmolality measurements were found to be ~70% of theoretical values, which corresponds to an osmotic coefficient value of ~0.7, thus inferring incomplete counterion dissociation. When comparing theoretical (ideal) osmolality of multiple sequences with various nucleotide compositions and chemistries at the same w/v concentration, the "average osmolar mass" (molar mass of the oligonucleotide, including the sodium counterions, divided by the ideal Van't Hoff factor, i^{id}) appears to be the strongest factor governing theoretical osmolality values. Other factors examined were the sequence length, backbone chemistry, 2′ sugar chemistry, and nucleotide composition. A head-to-head comparison between two osmolality techniques showed that vapor pressure osmometry is generally more suitable than freezing point osmometry for oligonucleotide solutions greater than ~150 mg/mL due to viscosity effects, but the two techniques are comparable otherwise.

1. Introduction

Osmolality is defined as the moles of osmotically-active particles per mass of solvent, with one osmole being the osmotic pressure of a 1.0 molal solution of an ideal non-electrolyte (Sweeney and Beuchat, 1993). Osmolality is commonly expressed in osmoles or milliosmoles per kilogram of solvent, i.e., Osm/kg solvent or mOsm/kg solvent, respectively, as defined by the United States Pharmacopeia and National Formulary, Chapter 785 (i.e., USP ⟨785⟩ 2014). In this article, the solvent is water. The International System of Units (SI) defines the SI unit of osmolality as mmol osmotically-active particles/kg water. Osmolality is not to be confused with osmolarity, which is defined as osmoles per *volume of solution*, analogous to the difference between molality and molarity terms. The two terms may be used interchangeably for dilute solutions, but in concentrated solutions they are appreciably different, and confusing the terms may lead to significant errors (Avis et al., 1993). Osmolality can be precisely determined by the use of a perfectly semi-permeable membrane that only permits solvent movement between two chambers; however, due to the difficulty of manufacturing such a membrane, osmolality is typically measured by exploiting its linear relationship to two colligative properties of a solution: freezing point and vapor pressure (Martin, 1993; Sweeney and Beuchat, 1993; Avis et al., 1993; USP ⟨785⟩ 2014).

Osmolality is an important factor to consider during parenteral drug product formulation development due to its impact on the "tone" of cells upon dose administration (Avis et al., 1986; Desai and Lee, 2007). Cell lysis is induced by hypotonic solutions, while cell crenation is induced by hypertonic solutions (Avis et al., 1986). Serum osmolality is typically approximately 290 mOsm/kg water (Papadakis and McPhee, 2015). The actual physiological range has

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Nomenclature

A	Adenine
$\%Ac$	Percent sodium acetate in drug substance
A.I.	Advanced Instruments
ASO	Antisense oligonucleotide
Average osmolar mass	Average mass per osmole which for an ASO is the molar mass of the ASO including the sodium counterions divided by i^{id}
$\%B$	Percent assay value of finished product
C^{Act}	Target active ingredient concentration of sodium salt form ASO (g/L or mg/mL). For ASO finished product this value is equivalent to the label claim concentration
C_{DS}	Drug substance concentration including native water and impurities (g/L or mg/mL)
cEt	Constrained-ethyl
C^{Tot}	Total oligonucleotide concentration (g/L or mg/mL)
$FL+(P=O)_1$	Full-length, fully thioated ASO plus full-length sequences containing a single desulfurized nucleotide
FPO	Freezing point osmometer/osmometry
G	Guanine
GMP	Good manufacturing practice
i^{id}	Theoretical (ideal) Van't Hoff factor assuming complete counterion dissociation
i^{obs}	Empirical (observed) Van't Hoff factor
IM	Intramuscular
Label Claim	Target nominal concentration of drug product (i.e. finished product) solutions. For practical purposes this concentration is equivalent to C^{Act} (see above)
M	Molar mass (g/mol) (SI unit: kg/mol)
mC	5-Methylcytosine
-mer	Refers to the ASO nucleotide length; e.g. sequences with 20 nucleotides are known as 20-mers
MOE	Methoxyethyl
$\overline{M}_{osm}$	Average osmolar mass (defined above)
m^{Tot}	Total oligonucleotide molality (mol/kg water) with respect to the polyion
M^{Tot}	Total oligonucleotide molarity (mol/L) with respect to the polyion
Na^{+}_{free}	Dissociated sodium counterions
Na^{+}_{Tot}	Total number of sodium counterions per oligonucleotide strand
Osmolar Mass	See definition for average osmolar mass
Osmoles	Moles of osmotically-active particles
$\%P$	Percent purity of drug substance
PO	Phosphodiester
PS	Phosphorothioate
$\%S$	Percent residual solvents in drug substance
SI	International system of units
T	Thymine
VPO	Vapor pressure osmometer/osmometry
w	Water content in a solution (kg water/L)
$\%W$	Percent native water in oligonucleotide drug substance
w/v	Weight per volume
α	Degree of counterion dissociation
ξ^{id}	Theoretical (ideal) osmolality assuming complete counterion dissociation (mOsm/kg water) (SI unit: mmol/kg water)
ξ^{obs}	Empirical (observed) osmolality (mOsm/kg water) (SI unit: mmol/kg water)
ρ	Solution density (g/mL) (SI unit: kg/m^3)
Φ_m	Molal osmotic coefficient

been reported by multiple authors: 250–350 mOsm/kg water (Waymouth 1970), 282–290 mOsm/kg water (Glasser et al., 1973), and 285–310 mOsm/kg water (USP ⟨785⟩ 2014). The osmotic pressure exerted by plasma corresponds to that of 0.9% w/v NaCl (Avis et al., 1993).

The range of acceptable parenteral formulation tonicity with respect to injection pain depends on parameters such as dose volume, injection rate, and route of administration, as has been addressed in several published reports. In one such report, an intramuscular (IM) dose of 0.5 mL vaccine solution formulated at 300–1100 mOsm/kg water elicited indistinguishable pain responses with respect to osmolality (Nony et al., 2001). In a separate report, an IM dose of 0.2 mL saline solution formulated with 0.6–3.0% w/v NaCl (osmolality ~200–1000 mOsm/kg water) was found to elicit "very few pain responses" (Jarvik and Wolff, 1962). In a review article discussing the tolerability of hypertonic injections (Wang, 2015), intradermal doses of 300–600 mM NaCl (osmolality ~560–1120 mOsm/kg water) was found to induce pain proportional to tonicity, and IM administration of 5% w/v NaCl (osmolality ~1600 mOsm/kg water) induced significantly more pain than isotonic saline; regarding the effect of injection volume, a 0.4 mL IM injection was found to be significantly more painful than a 0.1 mL IM injection.

In some cases, parenteral drug product formulations reach hypertonic levels due to the active ingredient and excipient compositions necessary to produce therapeutic benefit and ensure solution stability, such as for high-concentration biologics, and amino acid perfusions (Avis et al., 1993; Wang, 2015). Being hypertonic is considered acceptable in such cases where therapeutic benefits outweigh the injection discomfort, and problems of tonicity can be mitigated by administering the doses slowly (Avis et al., 1993). The United States Food and Drug Administration requires that drug product labels list injection pain under Adverse Reactions, and if pain is a significant safety concern, it should be listed under the Warnings and Precautions (Wang, 2015).

Presently, antisense oligonucleotide (ASO) therapeutics developed by Ionis Pharmaceuticals, Inc. (formerly Isis Pharmaceuticals, Inc.) are manufactured as the sodium salt and formulated as aqueous solutions for parenteral administration, most commonly delivered via subcutaneous or intrathecal injection. Dissolved ASO molecules impart tonicity due to sodium counterions that