TURNER SCIENTIFIC
PRECLINICAL HEARING RESEARCH

1351 Lincoln Avenue
Jacksonville, IL 62650

(217) 602-0306
Email: info@turnerscientific.com

Background Solutions for Injections into the Inner Ear.

Alec Salt
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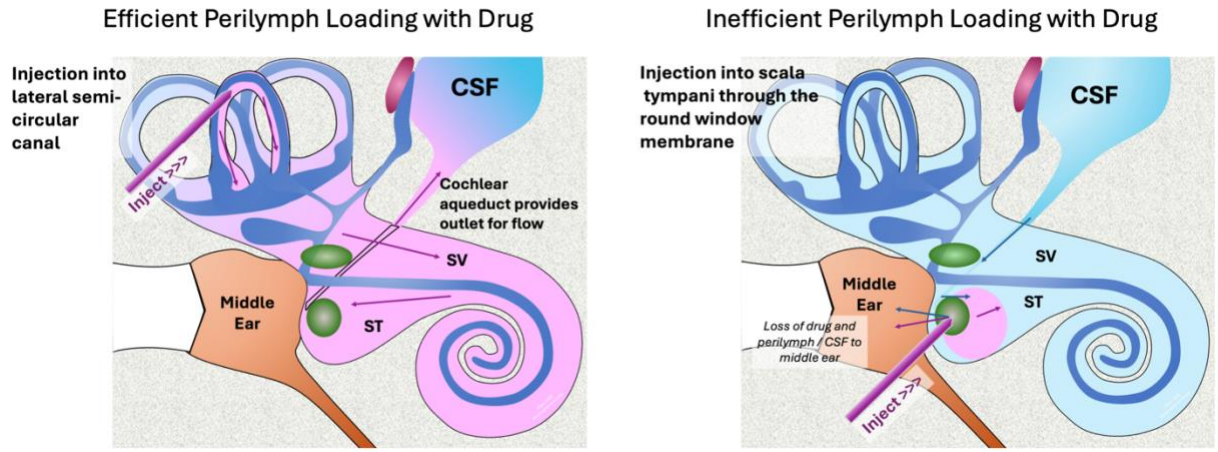
There is an increasing interest in the injection of drugs or other therapeutics directly into perilymph of the ear. Direct applications allow precise control of the drug concentration in perilymph compared to intratympanic (middle ear) delivery. With intratympanic applications the perilymph concentration is more variable as it depends on the ability of the drug to pass through the membranes of the round window and stapes. Distribution of drug along the cochlea may also be improved with direct applications.

When drug solution is administered intravenously the background medium is not of great significance physiologically as the drug in its background medium is only briefly in contact with biological tissues (specifically the tissues of the vein at the injection site and cells in the blood). The formulation is rapidly stirred, washed away and diluted by blood flow as it becomes evenly distributed throughout the vasculature.

Many other fluid systems of the body (urine in the collecting tubule, cerebrospinal fluid, aqueous humor of the eye) have “volume turnover” processes, in which new fluid is secreted or generated by specific tissues and taken up or lost to other structures (such as to the lymphatic system) resulting in turnover of the fluid. Cerebrospinal fluid is continually secreted in volume by the choroid plexus and is primarily cleared from the cranium by lymphatic vessels. Aqueous humor, the fluid in the anterior chamber of the eye, is generated by the ciliary body, located behind the iris, and drains through the trabecular meshwork, located in the angle between the iris and the cornea. The vitreous humor, the jelly-like contents in the globe of the eye behind the lens, is not secreted and resorbed in volume. Although the volume is “stagnant” the composition is actively maintained by local homeostatic mechanisms.

The perilymph of the ear is not gelatinous but is also “stagnant”, i.e. not stirred or mixed, with **almost no volume turnover**. Perilymph is influenced by limited interactions with CSF at the base of scala tympani through the cochlear aqueduct, providing some local dilution and CSF-perilymph exchange there. As with vitreous humor of the eye, there are local homeostatic mechanisms for different perilymph components, but each has individual different kinetic properties.

As there is no volume turnover of perilymph, how long the “full-strength” formulation injected stays in contact with the living tissues of the ear depends on how efficiently the solution was delivered. There are some very efficient methods of delivery and there are other inefficient methods. Delivery methods are compared in detail in a separate document, but below are given examples of the most efficient delivery method (injection from a pipette sealed into the semi-circular canal) compared with an inefficient delivery method (injection through the round window membrane).



Injection into a semi-circular canal *(left side)* drives the solution in both directions through the perilymphatic spaces of the canal, entering the vestibule, passing apically along scala vestibuli (SV) to the helicotrema at the cochlear apex, then basally down scala tympani (ST) to exit into the cranium through the cochlear aqueduct at the base of ST. This fills almost the entire perilymph space with concentrated, undiluted drug solution. Depending on the elimination characteristics of the drug there may be a gradient with highest concentration near the injection site and declining towards the exit at the base of ST. Nevertheless, many of the tissues of the ear are exposed to almost undiluted background medium of the injection which may influence the outcome.

With canal injections there may also a major difference between species. The mouse ear contains about 1 uL of perilymph, which can be replaced by injection at 1 µL/min for 4 mins (2 µL total). The guinea pig ear, in comparison, contains 15 µL of perilymph and requires a 30 min injection at 1 µL for replacement (30 µL total). The canal tissues of the mouse are therefore exposed to undiluted medium for 4 mins, while the guinea pig canal tissues are exposed for 30 min. Medium that causes no problems when injected into the mouse may cause problems when injected into the guinea pig or other larger species.

In contrast, injection through the round window membrane *(right side)* allows perilymph to leak out around the pipette (which is not sealed in place). The leaking perilymph is being replaced by CSF entering ST adjacent to the round window, a flow which dilutes both the drug level and background medium in ST. Drug distributes along ST by diffusion contributing to the concentration decline in the basal part of ST. When the pipette is removed, it leaves a hole in the round window membrane, allowing further

washout with time. With this method the peak drug level at the base of ST may only be a small proportion of the injected concentration and will decline quickly with time. The background medium is similarly diluted, so the tissues are only exposed to diluted medium for a brief period. This method be suitable for large components in the formulation (antibodies, adenoviruses, etc) which are retained well in perilymph, but is typically not suitable for the delivery of small molecules. With this type of injection the composition of the background medium is of lesser importance.

The pH buffers commonly used **for IV drug formulations** include:

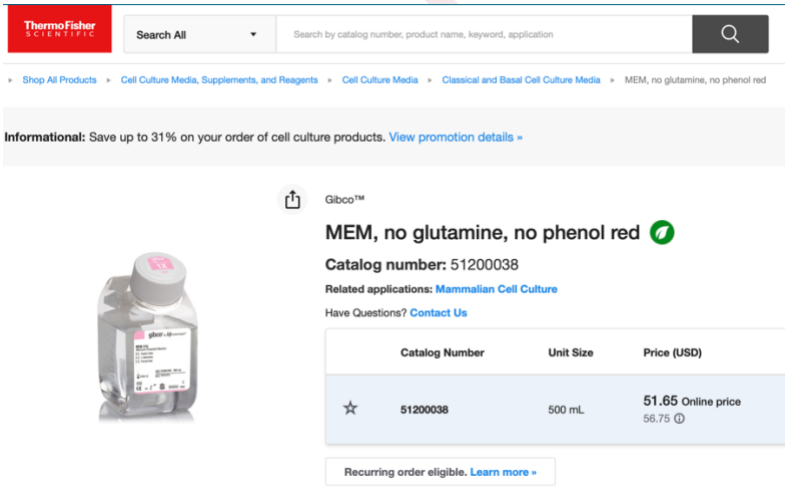
Phosphate Buffer (NaH2PO4/Na2HPO4): Effective in the physiological pH range, approximately from pH 6.0 to 8.0.
Citrate Buffer (Citric Acid/Sodium Citrate): More used with acidic drugs in the pH range from pH 3.0 to 6.0.
Acetate Buffer (Acetic Acid/Sodium Acetate): Suitable for acidic drugs, across the pH range of 3.5 to 5.6.
His Buffer (Imidazole/Histidine): Prevents aggregation of proteins; Used for formulations containing proteins tagged with histidine residues.

For cell culture, a variety of other buffers are widely used.

1. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). pH range: 6.8 to 8.2
2. Tris (tris(hydroxymethyl)aminomethane). pH range: 7.0 to 9.0
3. MOPS (3-(N-morpholino)propanesulfonic acid) pH range: 6.5 to 7.9

Each provides good buffering capacity in the physiological pH range, relatively low toxicity to cells, and are compatible with many cell culture media. However, they may interfere with certain enzymatic reactions and in some cases affect cell growth.

For perilymphatic injections, a bicarbonate buffer is preferred, such as provided by MEM-alpha. This can be purchased sterile and without phenol red indicator dye.



The detailed composition of MEM-alpha is as follows: Importantly, it contains physiological levels of most electrolytes, including Na, K, Ca, Mg Cl, HCO3.

Components	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
L-Arginine hydrochloride	211.0	126.0	0.5971564
L-Cystine 2HCl	313.0	31.0	0.09904154
L-Histidine hydrochloride-H2O	210.0	42.0	0.2
L-Isoleucine	131.0	52.0	0.39694658
L-Leucine	131.0	52.0	0.39694658
L-Lysine hydrochloride	183.0	73.0	0.3989071
L-Methionine	149.0	15.0	0.10067114
L-Phenylalanine	165.0	32.0	0.19393939
L-Threonine	119.0	48.0	0.40336135
L-Tryptophan	204.0	10.0	0.04901961
L-Tyrosine disodium salt dihydrate	261.0	52.0	0.19923371
L-Valine	117.0	46.0	0.3931624
Vitamins			
Choline chloride	140.0	1.0	0.007142857
D-Calcium pantothenate	477.0	1.0	0.002096436
Folic Acid	441.0	1.0	0.0022675737
Niacinamide	122.0	1.0	0.008196721
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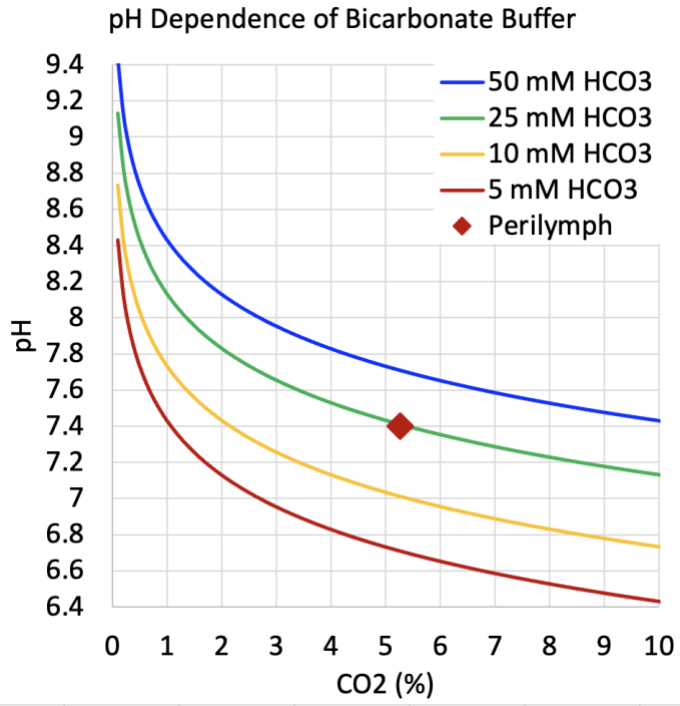
Pyridoxal hydrochloride	204.0	1.0	0.004901961
Riboflavin	376.0	0.1	2.6595744E-4
Thiamine hydrochloride	337.0	1.0	0.002967359
i-Inositol	180.0	2.0	0.011111111
Inorganic Salts			
Calcium Chloride (CaCl2) (anhyd.)	111.0	200.0	1.8018018
Magnesium Sulfate (MgSO4) (anhyd.)	120.0	97.67	0.8139166
Potassium Chloride (KCl)	75.0	400.0	5.3333335
Sodium Bicarbonate (NaHCO3)	84.0	2200.0	26.190475
Sodium Chloride (NaCl)	58.0	6800.0	117.24138
Sodium Phosphate monobasic (NaH2PO4-H2O)	138.0	140.0	1.0144928
Other Components			
D-Glucose (Dextrose)	180.0	1000.0	5.5555553

MEM-alpha contains 26.2 mM sodium bicarbonate, so the pH dependence on CO_2 level is close to that shown for the green curve (25 mM) in the following chart.



Out of the bottle, MEM-alpha has a pH above 8 but when injected into the ear this instantly becomes around 7.4 due to the near 5% prevailing CO_2 content of perilymph inside the ear. If, for drug solubility or other issues (protein conformation, etc), injection of the alkaline formulation is undesirable then it can be pre-equilibrated and stored under a CO_2 environment. For example, if an acid pH is desired for the formulation, then handling and storage under a 100% CO_2 environment (pH around 6.5) may be appropriate. The injected formulation will immediately change to physiologic pH when injected into perilymph.

Injecting zero-bicarbonate solutions into the ear (such as PBS, HEPES- TRIS-buffered) should generally be avoided. The ear has massive amounts of carbonic anhydrase in the spiral ligament (necessary for endolymph bicarbonate regulation). When bicarbonate is low this catalyzes

$$CO_2 + H_2O \;<>\; H^+ + HCO_3^-$$

generating bicarbonate and also acid (H^+). The acid generation is so active that it swamps any pH buffering of the solution and perilymph become very acid. (This is based on perilymph pH measurements we made with microelectrodes *in vivo* in the 1990's but never published).

Our experience is that the ear tolerates MEM-alpha injections very well. If another buffer system is to be used for formulation reasons, we will need to perform control injections

(vehicle only) to determine how well the background solution is tolerated. This primarily applies to semi-circular canal injections, but may also be relevant to other forms of direct injection into perilymph.

We have experts fully trained in all aspects of perilymph drug delivery and sampling. If you need help, call (217) 602-0306 for assistance.

TURNER SCIENTIFIC
PRECLINICAL HEARING RESEARCH

Project Design, Setup and Management: Amanda Henton, CSO
ahenton@turnerscientific.com

Sampling / Analysis Questions: Alec Salt
asalt@turnerscientific.com

Technical Questions: Jared Hartsock
jhartsock@turnerscientific.com