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Application Note

Determination of the osmolarity of salt solutions for the production of eye drops

Category	Pharmaceutical analysis
Matrix	Eye drops
Method	Osmometry
Keywords	hypotonic, isotonic, hypertonic, salt solution, osmotic pressure, eye drops, osmolarity, semi-micro osmometer, freezing point osmometer, tear fluid, dry eye
Analytes	Sodium chloride solution
ID	VPH0061N_O_E

Summary

This application note presents a simple and fast method to determine the osmolarity of salt solutions for the production of eye drops. By measuring the osmolarity of solutions, these can be divided into hypotonic, isotonic, and hypertonic; each of which can be used as eye drops for the various applications. This determination can be carried out with the KNAUER semi-micro osmometer (freezing point osmometer) K-7400.

Introduction

Dry eye is one of the most common complaints in ophthalmologic practice. External influences such as air pollution, central heating, air conditioning, air travel or the computer can dry out the eyes. This often leads to a deficit or to a concentration of tear fluid. [1]

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Tear fluid has an osmotic pressure due to its colloid electrolyte content. The osmotic pressure can be determined with the Pepper's cell, however this method is complex and laborious. The osmotic pressure is - like the boiling point increase, the lowering of vapor pressure and the freezing point depression - a colligative property. Therefore, it is possible, by measuring the freezing point, which is proportional to the osmotic pressure, to determine the osmotic pressure. This determination is made with a freezing point osmometer and can be specified as osmolarity in mOsmol* per liter (mOsmol/l). [2]

*Osmol= amount of osmotic active particles

The tear film in ophthalmic samples of healthy subjects typically ranges between 290–300 mOsmol/l. The osmolarity increases with a decrease in the liquid content, and an extreme liquid content decrease in tear fluids may lead to permanent eye damage. In various publications the following classification and limits can be found:

Values below 308 mOsmol/l are considered normal; 308–328 mOsmol/l indicate the range for mild to moderate dry eye; and values above 328 mOsmol/l are expected for a serious illness. [3,4]

It is well known that a 0.9% sodium chloride (NaCl) solution is isotonic to tears. A hypertonic or hypotonic solution can cause pain in the eye. The eye has a large tonicity tolerance range, which goes from 0.7 to 1.4% NaCl solution (=225–430 mOsmol/l). Within these limits, no pain is to be expected. Therefore it is very important to check the osmolarity of the prepared solutions with a freezing point osmometer. [5]

If eye drops are applied with different tonocity, irritation occurs. The tolerable range is larger for hypertonic solutions, as these are diluted by the tear fluid. Hypotonic eye drops must be made isotonic. For this, an appropriate solution assures that the same number of osmotic active particles is present in the eye drops as in the tear fluids. Most commonly, NaCl is used as a isotonization additive. [3]

Method parameters

Instrument	KNAUER semi-micro osmometer K-7400
Sample tubes	glass vials
Sample volume	150 µl
Monitoring view (min)	3.0
Measurement view (min)	3.0
Freezing level (°C)	-5.5
Cooling limit (°C)	-12.0
Units	mOsmol

Preparation of standards

For a two-point calibration, deionized water (corresponding to the 0 mOsmol point) and a 300 mOsmol/l calibration standard (corresponding to the 300 mOsmol point) are used. In this context, the expected measurement values will be higher than 300 mOsmol/l.

Sample preparation

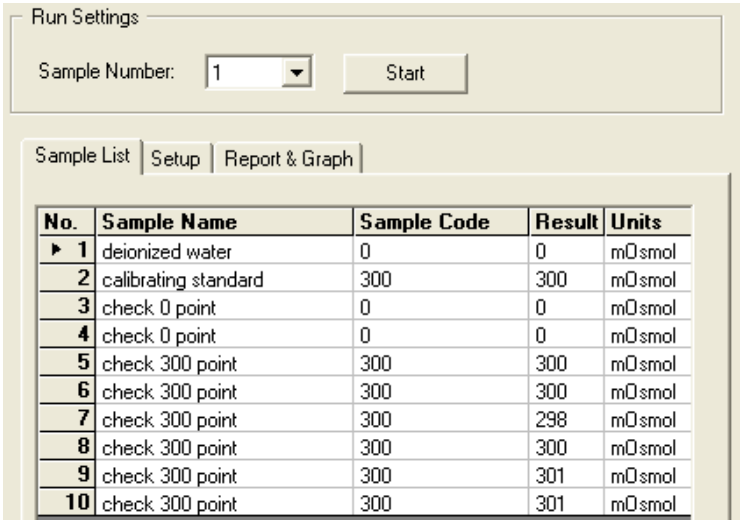
The first sample is a 0.9% NaCl solution. To prepare this sample, 0.9 g of NaCl were dissolved in 100 ml of deionized water and filtered through a 0.45 micron filter. The second sample is an eye drop solution purchased at the pharmacy. Both samples can be measured directly after the calibration of the osmometer.

Procedure

1. Place 0.150 ml sample or calibration solution into a clean and dry measurement vial.
2. Put the vial all the way into the adapter. The meniscus of the liquid must be horizontal.
3. Place the measuring head on the instrument in such a way that the vial extends into the cooling cavity. Thus, the instrument is ready for calibrating or measuring.
4. Press the start key on the instrument or the start button in the software.
5. After finishing the run, carefully remove the measurement vial from the thermistor.

Calibration

Table 1: Result table generated by EuroOsmo 7400 software



No.	Sample Name	Sample Code	Result	Units
▸ 1	deionized water	0	0	mOsmol
2	calibrating standard	300	300	mOsmol
3	check 0 point	0	0	mOsmol
4	check 0 point	0	0	mOsmol
5	check 300 point	300	300	mOsmol
6	check 300 point	300	300	mOsmol
7	check 300 point	300	298	mOsmol
8	check 300 point	300	300	mOsmol
9	check 300 point	300	301	mOsmol
10	check 300 point	300	301	mOsmol

The instrument calibration shows a measurement error of <1% for the acquisition of data (see Table 2). This corresponds to the device policy for the KNAUER osmometer.

After successful instrument calibration, the determination of the freezing point of the samples can be performed analogously. Optionally, the acquisition of data can be done with the EuroOsmo 7400 software (see Table 1).

Results

Table. 2: Data of the sample measurements

	Osmolarity of a 0.9% sodium chloride solution (mOsmol/l)	Osmolarity of eye drops from the pharmacy (mOsmol/l)
Measurement 1	290	308
Measurement 2	289	310
Measurement 3	290	310
Measurement 4	291	309
Measurement 5	289	308
Measurement 6	290	308
Mean value	289.83	308.83
Measurement error	0.33%	0.33%

The six independent measurements of osmolarity values of the 0.9% NaCl solution resulted in a mean value of 290 mOsmol/l (Table 2). Since the tear fluid normally has an osmolarity of 290–300 mOsmol/l, the measured sodium chloride solution can be referred to as isotonic.

The multiple determination of eye drops from the pharmacy gave an average reading of 309 mOsmol/l. Thus, the eye drops can be described as slightly hypertonic. During use, there is a dilution of the solution by the tear fluid, the osmolarity decreases resulting in the eye to be in the isotonic range.

The accuracy and reproducibility are well within the permitted levels of 1 %, as they are listed in the technical specifications of the KNAUER semi-micro osmometer. This demonstrates a very precise operation of the device.

Conclusion

The method of determination of osmolarity in salt solutions described here is widely used as a standard test method for the preparation of eye drops. According to the measured osmolarity value, hypotonic solutions will be made isotonic by adding NaCl and hypertonic solutions will be made isotonic by adding water. With the help of KNAUER semi-micro (freezing point) osmometer K-7400, the determination of the osmolarity of eye drops is simple, fast and reliable.

References

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Recommended instrumental equipment

For the current application, a semi-micro osmometer K-7400 is required. The instrument can be operated as standalone device, in combination with an external printer or by software (EuroOsmo 7400) and PC. Furthermore, the measuring head available in two different types: glass or plastic vessels. Please contact KNAUER, to provide you with an individual system configuration according to your requirements.

Description	Order No.
Semi-micro osmometer K-7400 with measuring head for glass vessels	A3707**
EuroOsmo7400 software	A3705
Calibration standard 300 mOsmol/l	Y1240

** Alternatively, the following units can be selected:
A3707-1 Semi-micro osmometer with printer
A3709 Semi-micro osmometer (measuring head for plastic vessels)
A3709-1 Semi-micro osmometer with printer (measuring head for plastic vessels)

Contact

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