

Considerations on the risk of precipitation of calcium phosphate salts following the use of Vismed® (0.18% sodium hyaluronate) formulation

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Introduction

- Recently, Bernauer^{1,2} et al. showed that exaggerated use (up to 100 times per day) of artificial tears containing high concentration (>50 mM) of phosphate buffer (i.e. Hylo-Comod®) in patients with severe epithelial defects may cause corneal calcification due to precipitation of calcium phosphate.
- Vismed® is a unique patented formulation that contains sodium hyaluronate (SH) together with essential ions (calcium, magnesium, potassium, sodium and chloride) that are found in natural tears. It also contains sodium citrate to maintain these essential ions in solution and avoid their precipitation³.
- The presence of citrate in Vismed® may also help avoid the precipitation of calcium phosphate.
- The concentration of phosphate buffer in the Vismed® formulation is low and close to that found in natural tears, thus reducing the risk of precipitation of calcium phosphate (Table 1).

Table 1: Concentrations of ions in natural tears and Vismed®

Ion	Concentration (mM)	
	Tears	Vismed®
Phosphate	8.0	8.99
Calcium	0.3 - 2.4	0.61
Magnesium	0.3 - 1.1	0.45
Potassium	15 - 35	13.9
Citrate	-	1.00
Sodium	108 - 175	65.7 (hypotonic)
Chloride	102 - 168	63.6 (hypotonic)

Study objectives

The aim of this study was to evaluate *in vitro* the precipitation of calcium phosphate salts in the Vismed® formulation in presence of increasing amount of phosphate and/or calcium.

Methods

To evaluate the risk of precipitation of calcium phosphate in Vismed®, increasing amounts of phosphate (Na₂HPO₄) were added to the original Vismed® formulation. The concentrations of phosphate added are shown in Table 2. The presence of calcium phosphate salts precipitate was assessed visually using a microscope.

Table 2: Increasing amount of phosphate added to Vismed®

Ion	Vismed (mM)	Increase phosphate (mM)						
		17.98	26.97	35.96	44.95	53.94	71.92	98.89
Phosphate	8.99	17.98	26.97	35.96	44.95	53.94	71.92	98.89
% increase	-	200	300	400	500	600	800	1100

A subsequent trial was carried out by adding increasing amounts of calcium (CaCl₂) to the original Vismed® formulation, as shown in Table 3. The concentration of phosphate was kept constant (8.99 mM). The presence of calcium phosphate precipitate was assessed visually using a microscope.

Table 3: Increasing amount of calcium added to Vismed®

Ion	Vismed (mM)	Increase calcium (mM)						
		1.22	1.83	2.44	3.05	3.66	4.27	4.88
Calcium	0.61	1.22	1.83	2.44	3.05	3.66	4.27	4.88
% increase	-	200	300	400	500	600	700	800

A final experiment was performed by both, adding twice (i.e. 1.22 mM) the initial concentration of calcium in Vismed® and increasing the amount of phosphate up to 63 mM (+700%). The presence of calcium phosphate precipitate was assessed visually using a microscope.

Results

Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of phosphate

Increasing concentration of phosphate up to 100 mM (+1100%) did not lead to any precipitation (Table 4).

Table 4: Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of phosphate

Ion	Vismed (mM)	Increase of phosphate (mM)						
		17.98	26.97	35.96	44.95	53.94	71.92	98.89
Phosphate	8.99	17.98	26.97	35.96	44.95	53.94	71.92	98.89
Calcium	0.61	Constant						
% increase	-	200	300	400	500	600	800	1100
Precipitate	-	NO	NO	NO	NO	NO	NO	NO

Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of calcium

The addition of calcium up to 2.44mM (+400%) to the Vismed® formulation did not lead to any precipitation (Table 5). A precipitate was visible at 3.05 mM (+500%) of calcium and above.

Table 5: Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of calcium

Ion	Vismed (mM)	Increase of calcium (mM)						
		1.22	1.83	2.44	3.05	3.66	4.27	4.88
Phosphate	8.99	Constant						
Calcium	0.61	1.22	1.83	2.44	3.05	3.66	4.27	4.88
% increase	-	200	300	400	500	600	700	800
Precipitate	-	NO	NO	NO	YES	YES	YES	YES

Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of phosphate and constant amount of calcium

At a concentration of 54 mM (+600%) of phosphate and above, a precipitate was visible (Table 6).

Table 6: Precipitation of calcium phosphate in Vismed® in the presence of increasing amounts of phosphate and constant amount of calcium

Ion	Vismed (mM)	Increase calcium and phosphate (mM)							
		1.22	1.22	1.22	1.22	1.22	1.22	1.22	
Calcium	0.61	1.22	1.22	1.22	1.22	1.22	1.22	1.22	
Calcium % increase	-	200	Constant						
Phosphate	8.99	8.99	17.98	26.97	35.96	44.95	53.94	62.93	
Phosphate % increase	-	-	200	300	400	500	600	700	
Precipitate	-	NO	NO	NO	NO	NO	YES	YES	

Discussion and conclusion

In conclusion, it is unlikely that the repeated use of Vismed® will cause corneal calcification *in vivo*, since:

- Increased amount of phosphate up to 100 mM (+1100%) and calcium up to 2.44 mM (+400%) did not lead to any precipitation in Vismed®.
- Vismed® contains 5 times less phosphate (8.99 mM) than commercially available products like Hylo-Comod®.
- The results of several clinical trials in a total of more than 300 patients in patients with moderate dry eye, together with post marketing surveillance show a very positive result. Vismed® has never been implicated in such side effects to date. This shows that it is safe to use over time.

These results may be attributed to the presence of sodium citrate, that maintains the ions phosphate and calcium in solution. Further experiments are ongoing to confirm this hypothesis.

References

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