PetVisionPro®: An - *In Vivo* - study

(The Ophthalmic Option to Reduce Canine and Feline Cataracts)

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Abstract

Cataract is defined as any opacification of the eye lens, most cataracts in canines and felines are inherited, although they may be caused by congenital defects, nutritional deficiencies, toxic substances, uveal adhesions, senility and diabetes mellitus.
The basic abnormality in cataract formation is the degeneration of the normal protein structure of the lens fibers. As such cataract formation affects predominantly the lens cortex.
The glycation of eye lens proteins (aggregation of alpha, beta and gamma crystallins) occurs in vivo and may contribute to cataractogenesis, Anti-glycation compounds such as **PetVisionPro®** are preventive, but interestingly **PetVisionPro®** reverses eye lens opacity in our in vivo study.

We investigated this effect using canine eyes with incipient cataracts, (glycated eye lens proteins), and after the application of **PetVisionPro®** for 36 days the size of the glycation-aggregates decreased.

Our data analysis supports this hypothesis; **PetVisionPro®** disaggregates glycated proteins (alpha, beta and gamma crystalline) reducing any opacification on the eye lens and assist in maintaining its clarity.

Key words: Cataracts, Proteins, Glycation, **PetVisionPro®**

Cataracts

Any opacity of the eye lens or its capsule.

Pathogenesis

Related to alterations in lens metabolism, any irregularity in the 3-dimensional spacing of the lens fibers resulting from changes in lens hydration, protein conformation, cell metabolism, electrolyte imbalances, or cell membrane stability can disrupt lens transparency and result in light scattering (cataract).

When they occur

1). Congenital - Present at birth. Tend to be nuclear and may have clear cortex around them. They may or may not progress or be inherited.

2). Juvenile - Developing before adulthood (1-2 yrs of age in dogs).

3). Adult - Occur in adult animals.

4). Senile - Aged animals. (Beyond nuclear sclerosis).

Where they occur

Important in that many inherited cataracts occur in the posterior cortex area.

1). Structure affected - capsule, cortex or nucleus.

2). Location in the lens - anterior or posterior, equatorial or polar (axial), zone (capsular, subcapsular, cortical, nuclear).

Stage of development

1). Incipient - Small opacity and vision is maintained.

2). Incomplete (immature) - Vision is impaired and the fundus is seen indistinctly. A tapetal reflex, however, is still seen.

3). Intumescent - A swollen, opaque lens which may cause secondary glaucoma due to mechanical compression of the drainage angle.

4). Complete (mature) - Entire lens is opaque with no tapetal reflex or fundus visible.

5). Resorbing (hypermature) - The cortex may liquefy and permit visualization of the fundus around the opacity. These can be identified by noting wrinkles in the anterior lens capsule or "sparkles" in the lens which consist of very fine particles that reflect light much as snow reflects sunlight. Often these types of cataracts are associated with lens-induced uveitis. May also see when the cortex "dries out" resulting in a smaller lens that may pull away from the zonules and luxate. Occasionally limited vision is restored in some patients but this is very unpredictable.

6). Morgagnian cataract - A hypermature lens with liquefied cortex and the solid nucleus sinks to the bottom.

Lens consistency

Cataracts in young animals tend to be soft whereas those in older animals tend to be hard.

Etiology

The most useful method but the most difficult to determine.

Inherited

May/may not be congenital and is probably the most common cause of cataracts in dogs. Can appear at any age and may involve just the lens or be part of multiple ocular abnormalities. In dogs the location, age of onset, progression pattern, and inheritance differ by breed. Diagnosis and prognosis is based on breed, clinical appearance, age of onset, evaluation of related animals, and test breading.

Nutritional

Especially protein deficiencies.

a). Amino acid deficiencies in puppies fed kittens fed milk replacers.

b). Protein deficient diets and starvation states.

c). Various amino acid and vitamin deficiencies (A, E and riboflavin) as well as electrolyte imbalances.

Toxic

Either congenital or acquired. Clinically significant toxins include:

a). Disophenol (dewormer) - transient or permanent cataracts, especially in pups.

b). Hygromycin B (anthelmintic) - Pigs.

c). In humans and in experimental animals, corticosteroids, epinephrine, chlorpromazine, and parasympathomimetic miotics (especially phospholine iodine).

Inflammatory/infectious

Usually due to altered aqueous humor or lens metabolism.

a). Uveitis-induced cataracts - Probably the most common cause of cataracts in cats and adult horses.

b). BVD in cattle - acquired in utero.

Senile degeneration

May be seen in elderly animals secondary to the cumulative effects of chronic exposure to ultraviolet light and perhaps oxidizing stress.

Radiation and electric shock

Usually seen in patients undergoing radiation therapy for neoplasia in which the eye was in the field - or in animals that have experienced an electric shock from chewing on electric cords.

Metabolic

a). Diabetes mellitus - Common. Diabetic cataracts are often intumescent with a rapid onset. Blood glucose should be performed on all dogs with rapid onset bilateral cataracts, especially if they have "water" or "Y" suture clefts.
Elevated glucose in the aqueous overloads the enzyme hexokinase in glycolysis and forces glucose into the aldose reductase pathway thereby producing impermeable sorbitol and drawing water into the lens making "water cleft" cataracts.
Eventually coagulation of lens protein occurs. Good control of blood glucose can prevent diabetic cataracts.

b). Cataracts have also been reported in cats and dogs with hypocalcemia (hypoparathyroidism, and nutritional secondary hyperparathyroidism).

Traumatic

Lens capsule rupture and lens fiber disruption draws aqueous into the lens, resulting in opacification. Lens protein release also may lead to lens-induced uveitis.

Therapy of Cataracts

Palliative

Atropine - Pupil dilation may permit vision around an axial cataract (careful using in breeds prone to angle closure glaucoma). Use once every 2-3 days.

Anti-inflammatories

Topical/oral corticosteroids and aspirin - May decrease lens-induced uveitis associated with lens resumption. Occasionally, in very young dogs (<1 yr of age) limited vision may be restored as the cataract resorbs. Lens-induced uveitis, however, must be controlled to avoid secondary glaucoma, retinal detachments etc. which may again cause blindness.

Materials and Equipment

Materials

76 canine eyes with opacity in the eye lens, Phenylephrine (2.5%) and 190 droppers of **PetVisionPro®** with 8 milliliters each dropper (This Ophthalmic solutions are sterile, free from foreign particles and especially prepared for instillation into the canine eye).

Equipment

Opacity Lensmeter – IntraOptics Model 701, Manufacturer Schlieren / Switzerland,

Slit Lamp: Topcon - Model SL D8Z -, Manufacturer Topcon Medical Systems, Inc. USA.

Methodology

Procedure of preparation of PetVisionPro®

All preparation must be done in a clean-air environment, such as a laminar flow hood, by qualified aseptic compounding pharmacists.

The source of all the ingredients must be the highest grade (USP, Reagent grade or NF).

- Accurately weigh/measure each of the Ingredients:

Lubricant

Carboxymethylcellulose of Sodium, 0.5% w/v

Antioxidants

3 salts of essential aminoacids, 8.0% w/v

Preservative

Benzalkonium Chloride, 0.01% w/v

Sterile water for injection

Ophthalmic grade, pH range 6.8-7.2

- Dissolve the ingredients in about 3/4 of the quantity of Sterile Water for Injection and mix well.

- Add sufficient Sterile Water for Injection to volume and mix well.

- Determine the pH, clarity, bioburden and other quality control factors from a sample of the solution.

- Filter through a sterile 0.2 micron filter into a sterile ophthalmic container.

- Second sterilization using gamma radiation.

- Package and label.

Direction for use of PetVisionPro®

Apply 1-2 drops in the effected eye or eyes, minimum 3 times daily, for a minimum of 36 days to see measurable improvements.

Procedure of application of PetVisionPro® into the canine eye.

Lean canine's head back so that it's looking upward and apply a single drop; repeat this procedure for each drop of each eye as directed above.

Application of eye drops into canine eye

Slit Lamp Examination

- The slit lamp is a microscope with a light attached that allows the veterinary ophthalmologist to examine canine eye under high magnification. This instrument is primarily used to view the anterior structures of the eye such as the cornea, iris, and lens.

- To open the pupil the ophthalmologist applies Phenylephrine (2.5%) to produce Mydriasis (dilatation of the pupil) in the canine eye.

- Canine cataract was determined by visual inspection using a Slit Lamp Topcon - Model SL D8Z-, Manufacturer Topcon Medical Systems, Inc. USA.

Slit Lamp Examination

NOTE: PetVisionPro® is not to be used on pets taking a Corticosteroid, or derivative, as the benefits from PetVisionPro will be cancelled.

Allow 2- 3 weeks for the steroid to pass from the pet's system before applying the drops. There will be no detrimental effect on the pet in the event PetVisionPro eye drops are introduced into a pet who is on a steroid.

Measurement of Opacification of the Eye Lens

The opacity lensmeter 701 is a new instrument for quantifying the lens opacity.

The basic concept is the measurement of stray light transmitted by the lens.

The handling of the instrument is very simple. It is mounted on a slit lamp-like table.

After adjusting the instrument to the canine eye, the measurement is initiated by depressing a console button.

The present study was undertaken to determine whether the scattered light of lenses measured with the Opacity Lensmeter 701 used for objective, quantitative assessment of lens opacities.

Visual acuity and scattered light were determined in 76 canine eyes with the Opacity Lensmeter 701 set at 700 nm.

In the group with nuclear cataract there was a significant correlation between visual acuity and the amount of scattered light measured by the Lensmeter 701.

In the groups with posterior cataract and cortical cataract there was no significant correlation between visual acuity and scattered light measurements.

The influence of pupillary diameter on the scattered light measurement was examined in 76 canine eyes.

In all the canine eyes significant correlations were found between pupillary diameter and scattered light measurements.

Therefore, when measuring stray light in canine cataract cases, the pupillary diameter should be noted.

Results

Measurement of Opacification of the Eye Lens

76 canine eyes with opacity in the eye lens were measure for light scattering using an Opacity Lensmeter Model 701, Manufacturer Schlieren / Switzerland.´

And the results are:

- **41** Subcapsular Posterior Cataracts
- **22** Senile Cataract (Cupuliform Cataracts)
- **9** Cortical Cataracts
- **4** mixed Cataracts

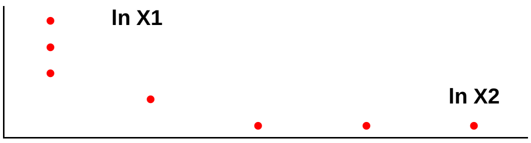
A correlation between initial opacity and final opacity was found in a first order reaction or exponential model:

$$X2 = X1 \{e^{(-)k(Y2 - Y1)}\}$$

Where:

X1	Initial Opacity
X2	Final Opacity
(-) k	Rate Constant
(Y2 - Y1)	Days of treatment

Exponential graph of the mathematic model



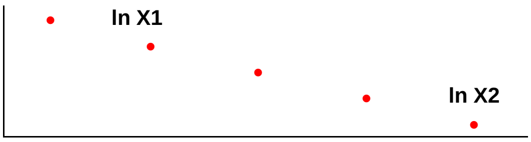
Linear representation of this mathematic model

$$\ln X2 = \ln X1 - k (Y2 - Y1)$$

Where:

ln X1	Initial Opacity
ln X2	Final Opacity
(-) k	Proportionality Constant
(Y2 - Y1)	Days of treatment

Linear graph of the mathematic model



The rate constant should be realized using the information obtained during the taking of physical readings, the value of the rate constant will be obtained calculating the pending straight line in accordance with the following equation:

$$k = m = (Y2 - Y1) / (X2 - X1)$$

Units of rate constant

Units of rate constant = units of rate /(units of concentration) 2 = (M/s) / M2 = M-1 • s-1

Calculating the half-life (λ) of the reaction

The half-life of the reaction (λ), is defined how the time necessary for the initial concentration to reach half of the initial concentration according to the following equation:

$$\lambda = (\ln 2) / k \text{ or}$$

$$\lambda = (\ln 2) / (Y2 - Y1) / (X2 - X1)$$

APPLICATION - *In Vivo* - OF THE MATHEMATICAL MODEL TO REAL CASE

Mathematic model

X2 = X1 {*e* (-) k (Y2 – Y1)}

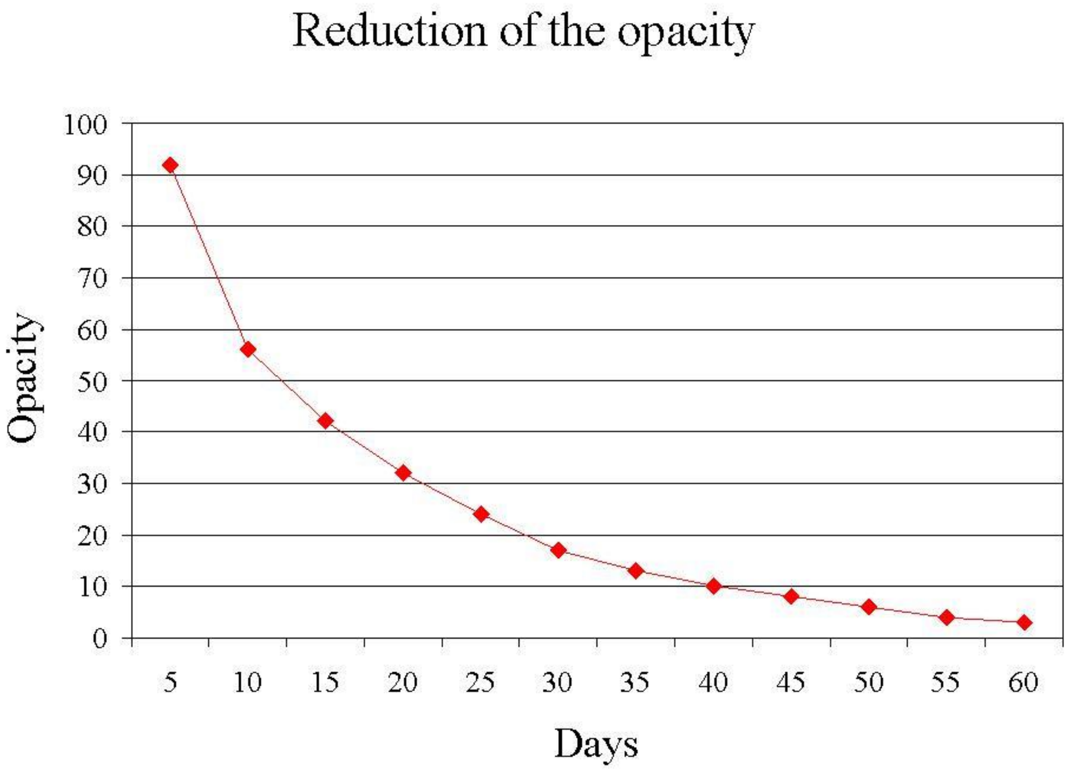
Data Table

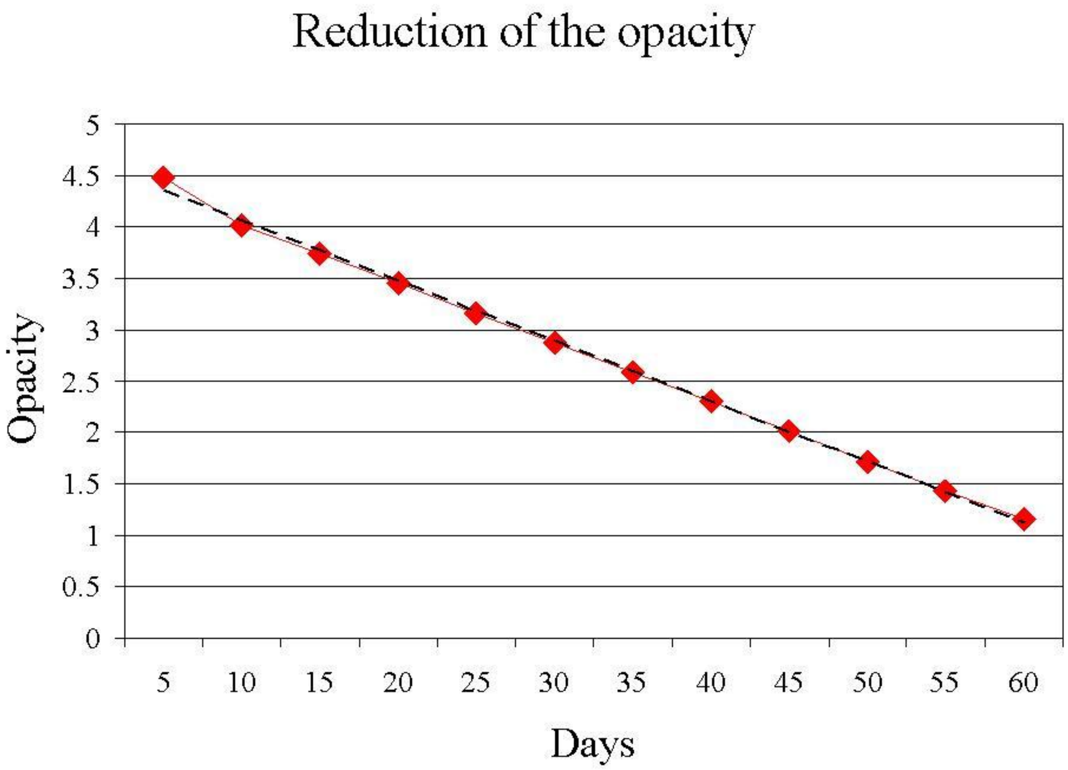
(Y2-Y1)	- k (Y2-Y1)	*e* – k (Y2 –Y1)	X1 *e* (-) k (Y2 – Y1)	ln X2
2	- 0.1151	0.8912	89.12	4.48
4	- 0.2303	0.7942	79.42	4.37
6	- 0.3454	0.7079	70.70	4.25
8	- 0.4606	0.6309	63.09	4.14
10	- 0.5757	0.5623	56.23	4.02
15	- 0.8636	0.4216	42.16	3.74
20	- 1.1515	0.3161	31.61	3.45
25	- 1.4393	0.2370	23.70	3.16
30	- 1.7272	0.1777	17.77	2.87
35	- 2.0151	0.1333	13.33	2.59
40	- 2.3030	0.0999	9.99	2.30
45	- 2.5908	0.0749	7.49	2.01
50	- 2.8787	0.0562	5.62	1.72
55	- 3.1666	0.0421	4.21	1.43
60	- 3.4545	0.0316	3.16	1.15

Where:

X1 Initial Opacity
X2 Final Opacity
(-) k Rate Constant
(Y2 - Y1) Days of treatment

Exponential and linear graph of the Data Table





Calculating the rate constant from:

$\ln X2 = \ln X1 - k (Y2 - Y1)$

Then

$k = (\ln X2 - \ln X1) / (Y2 - Y1)$

In this particular case the value of k is

K = (0.057575)

Calculus of the reduction on canine eye lens opacity:

From:

$X2 = X1 \{ e^{(-) k (Y2 - Y1)} \}$

Opacity final = opacity initial { e-k (Final time – Initial time)}

WHERE:

Opacity final	Unknown (continued to improve; last reading estimted at 15% opacity)
Opacity initial	98% opacity (Measured using an Opacity Lensmeter Model 701)
Value of (- k)	- 0.057575 M-1 • s-1
Initial time	0 days of application of **PetVisionPro®**
Final time	2 days after inicited the application of **PetVisionPro®**

For 2 days of application:

% Opacity Final = 98 % {e – (0.057575) (2-0)}

% Opacity Final = 98 % (e – 0.11515)

% Opacity Final = 98 % (0.8912)

- **% Opacity Final = 87.33 %**

For 15 days of application:

% Opacity Final = 98 % {e – (0.057575) (15-0)}

- **% Opacity = 41.31 %**

For 25 days of application:

% Opacity Final = 98 % {e – (0.057575) (25-0)}

- **% Opacity = 23.23 %**

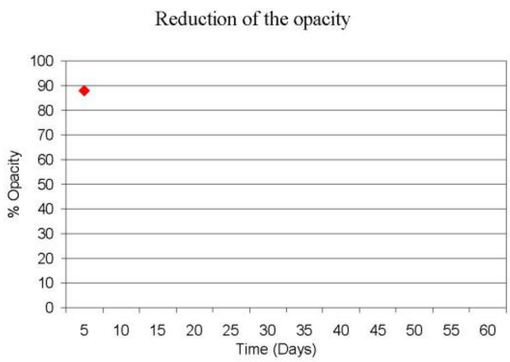
For 36 days of application:

% Opacity Final = 98 % {e – (0.057575) (36-0)}

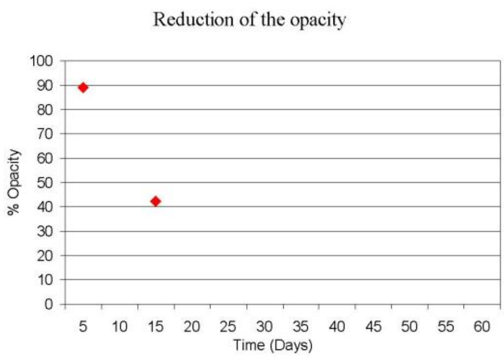
- **% Opacity = 12.33 %**

Photo Gallery of the reduction of the opacity

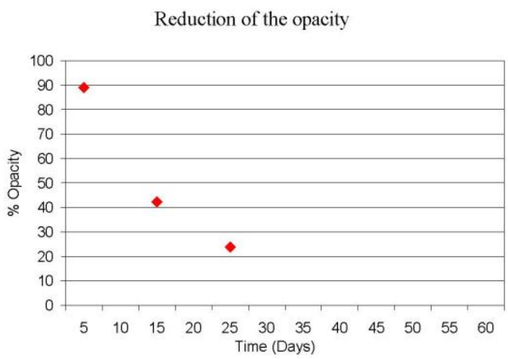
2 days after PetVisionPro® application (% Opacity = 87.33 %)



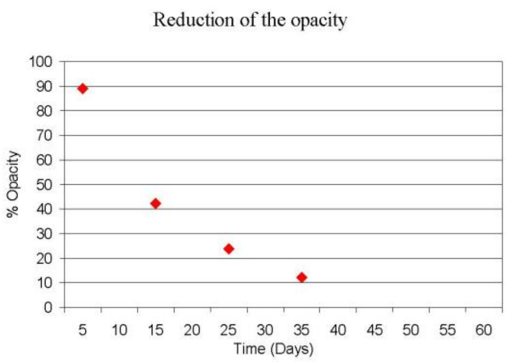
15 days after PetVisionPro® application (% Opacity = 41.31 %)



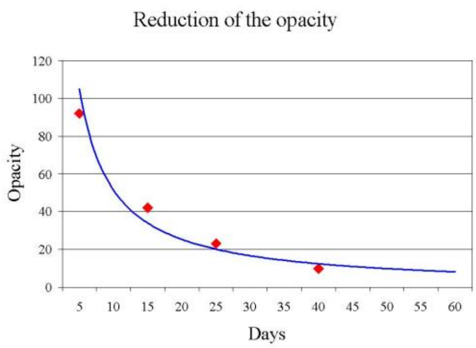
25 days after PetVisionPro® application (% Opacity = 23.23 %)



36 days after PetVisionPro® application (% Opacity = 12.33 %)



40 days after PetVisionPro® application.



Calculating the half-life (λ) of the reaction

The half-life of the reaction (λ), is the time required for a reactant to drop to one half of its initial value, [A] t1/2 = ½ [A] .,

The half – life is a convenient way to describe how fast occurs, especially if is a first order reaction, a fast reaction will have a short half – life.

We can determine the half – life of a first – order reaction by:

$$\lambda = (\ln 2) / k$$

Where:

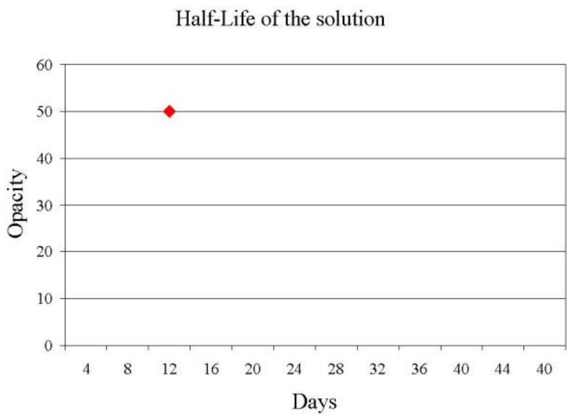
ln2 = 0.693
k = 0.057575

$$\lambda = 0.693147 / 0.057575$$

or

$$\lambda = 12.03 \text{ days}$$

Graph of the mode half-life (λ) of the reaction



DOSE INFORMATION

Effective dose (ED)

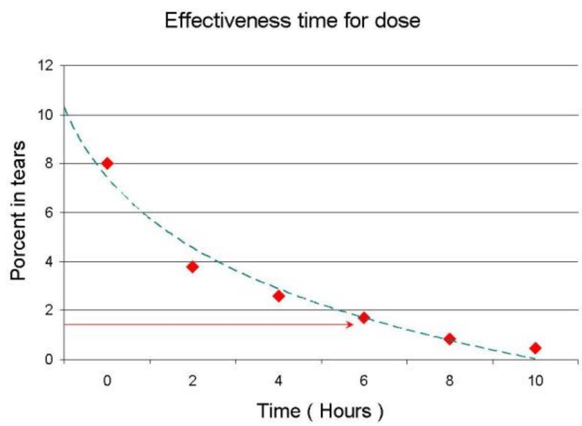
Drop volume of 0.05 milliliters

Median effective dose (ED 50)

A container with 8 milliliters of solution

Effectiveness time of dose

6 hours



Indirect determination of PetVisionPro® in tears

A high-performance liquid chromatographic (HPLC) method is described for the indirect determination of PetVisionPro® in tears.

The technique involves direct injection of the sample on a 5-microns Spherisorb-CN column. The mobile phase is acetonitrile-triethylamine (0.1%, v/v) in water (pH 2.5; 40:60, v/v).

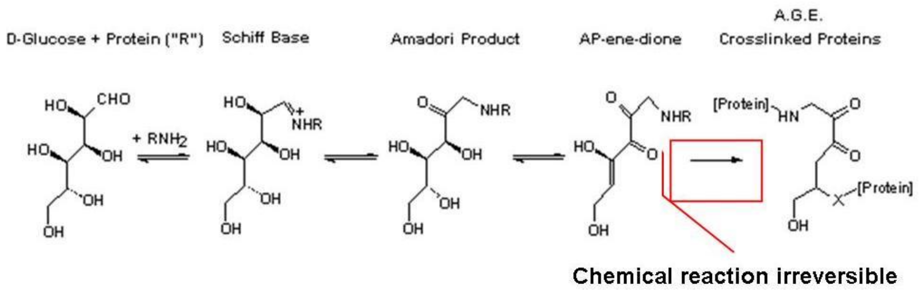
The detection is carried out at 215 nm. This method is rapid, specific, reproducible and simple.

Discussion

In the early 1900's Maillard and later Amadori described the formation of complexes between sugars and the amino acids of proteins that were believed to cause the toughening and discoloration of food during the cooking process and after prolonged storage.

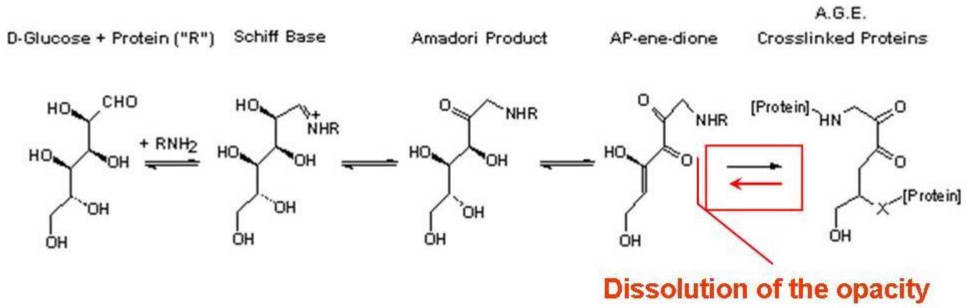
Subsequent studies led to the hypothesis that formation of these amino-sugar structures was an important step along a new biochemical pathway in which permanent glucose structures, called "Advanced Glycosylation Endproducts" (A.G.E.s), were formed on the surface of proteins.

The next biochemical reaction involved the conversion of glucose and an amino acid to an A.G.E. s



The current in vivo study presents evidence to support the hypothesis that PetVisionPro® is an effective disaggregating agent of crosslinked proteins into the canine eye lens.

In canine study PetVisionPro® reverses the lens opacity associated with cataracts. The authors of that study suggested that PetVisionPro® anti-glycation properties are responsible for reversal of lens opacity, according the following biochemical reaction:



This in vivo study may not fully represent the complexities of cataract formation, which involves complex mixtures of diverse proteins.

Nonetheless, there is evidence to support the significant contribution of protein glycation to aggregation in cataractogenesis.

Our findings that **PetVisionPro®** disaggregated glycated eye lens proteins suggest a new ophthalmic option to reduce canine and feline cataracts.

References

Upon Request.