



A novel validated method for estimation of Carbachol in ophthalmic preparations by spectrophotometer

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ABSTRACT

A simple and selective novel spectrophotometric method has been proposed for determination of Carbachol in pure and pharmaceutical ophthalmic preparations. The method is developed on formation of ion-pair complex between Bromophenol blue (BPB) and Carbachol (Carb), which is blue in color. The variables which effect the complex formation were thoroughly studied and optimized. The Beer's and Lambert's plots were obeyed in a concentration range of 10-100 ppm with regression co-efficient 0.9999. The LOD and LOQ are obtained respectively 5ppm and 10ppm. The proposed method was successfully applied to the analysis of the cited drugs in ophthalmic preparations. The Linearity, precision, specificity, robustness and ruggedness of proposed method were evaluated as per ICH and USP guidelines.

Key words; - BPB (Bromophenol blue), Quaternary Ammonium, Ion-Pairing, Method validation

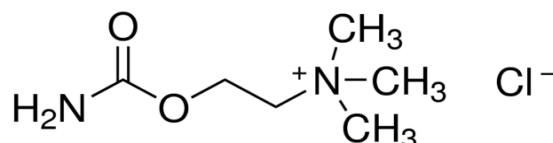
1. INTRODUCTION

Carbachol is a positively charged quaternary ammonium compound, it is also called choline carbamate or carbamylcholine its chemical name is 2-(carbamoyloxy)-N, N, N-trimethylethanaminium. Quaternary ammonium compounds are an important class of



zwitter surfactants, because their amines of quaternary ammonium compounds have with four rest groups, which tender it permanently positively charged and they only occur as complexes with anion. Similarly Carbachol behaves as zwitter ion its positive charge may be permanent or only exist in certain pH range ^(1,8).

It is generally used in medicine especially for treatment of glaucoma and its surgery. In human body it binds and activates choline, nicotinic and muscarinic receptors^(2,3). Due to this relationship it is classified in cholinergic agonist. In recent research it was found that It's absorbance in gastro intestinal tract is nearly zero and it does not cross the blood and brain barrier, its absorbance is increased by mixed using with Benzalkonium chloride⁽²⁾. As a drug it is used as intraocular injection or as eye drop. Its chemical structure of molecule is shown in Fig; 01. It has side effects also the most serious side effects are headache, flushing, cloudy sight, sweating, stomach pain, irritation of the eye, frequent urge to urinate, salivation and diarrhea has been reported.



Fig; 01; - Carbachol

2. MATERIALS AND METHOD

Materials

Sodium phosphate monobasic, Sodium phosphate dibasic, Sodium Acetate, and Sodium citrate and Carbachol were purchased from sigma Aldrich, while BPB was purchased from BDH. The all chemical were analytical grade, no any further purification was carried out.

Instrumentation:

The UV-visible spectrophotometer 1601 (SHIMADZU) have matched 1cm quartz cells, attached with EPSON LX-300 printer and electronic balance LIBROR AEX-120G (SHIMADZU) were used throughout working.

Reagent Preparation:

Acetate Buffer pH 5.0 - 5.3:-

Weighed accurately 7.75gm sodium acetate and transferred it into 500ml volumetric flask, made-up volume with distilled water. pH was adjusted 5.0 to 5.3 with acetic acid.

Stock Solution of 0.1% Bromophenol Blue Dye:-

Accurately weighed 0.1gm of Bromophenol blue and transferred into 250ml volumetric flask, added 75ml of 0.1N sodium hydroxide, made-up the volume with deionized water.

Bromophenol Blue Solution of pH 3.4 - 3.9:-

The 20ml of 0.1% Bromophenol solution was taken from stock solution, and accurately transferred into 500ml volumetric flask, diluted the solution with 75ml of 96% ethanol and acetate buffer of pH 5.0 to 5.3. The final pH of solution was adjusted between 3.4 – 3.9 with Trichloroacetic acid and NaOH.

Preparation of Carbachol Reference Standard:-

A 25mg Carbachol RS was weighed accurately and carefully transferred into 250ml volumetric flask. The end concentration of stock solution was 100ppm. The volume was made-up by acetate buffer of pH 5.3.

Sample preparation:-

The 100ppm per ml sample solution was prepared from intraocular ophthalmic solutions which were purchased from local pharmacy.



Pharmaceutical Preparations;-

The purposed method was applied on different pharmaceutical preparations of Carbachol, which were available in local pharmacy. Some of them are listed as follows

MIOCHOL	ETHICAL LABORATORIES (PVT) LTD.
MIOSTAT	NOVARTIS PHARMA (PAK) LTD
OCUSTAT	FARMIGEA PAK (PVT) LTD.
Ophth Carb	Ophth Pharma (PVT) Ltd

General Procedure:-

A serial dilutions 50,,100,150 and 200 ppm were prepared in 10 ml volumetric flasks from standard stock solution,0.1% BPB solution having pH 3.4-3.9 was added in each dilution a blue coloration was developed by formation of ion-pairing between BPB and Carbachol, and samples were diluted with distilled water up to the mark.

3. RESULTS AND DISCUSSIONS**Absorption Spectra (λ max);-**

The ion-pairing complex between BPB⁻²and Carbachol⁺¹ showed a blue colored product which was recorded at 700—400 nm wave length against corresponding blank solution. The resultant complex showed absorbance at 590 nm. The obtained spectra has been shown in Fig-02.

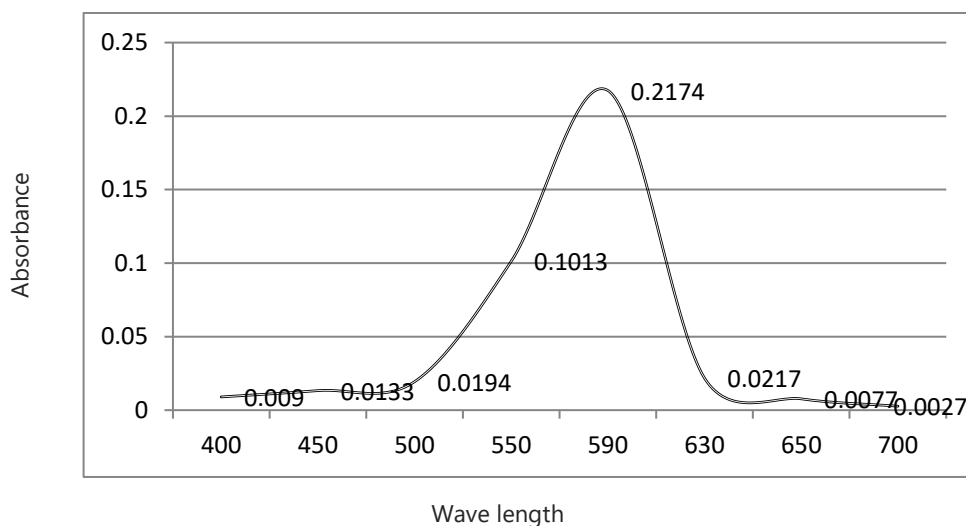


Fig-02;- Spectra of Ion-pair complex

Table-01;-Optimization parameter

S.NO	Parameters	Observations
1	Linear Equation	$Y=0.0631x +0.0181$
2	Slop	0.000645
3	Absorptivity	2000L/PPM
4	Intercept	0.081
5	LOD	5 PPM
6	LOQ	10 PPM
7	Regression Co-efficient(R^2)	0.9999
8	Linearity	10-100 ppm
9	Correlation Co-efficient	0.9999
10	λ max	590 nm

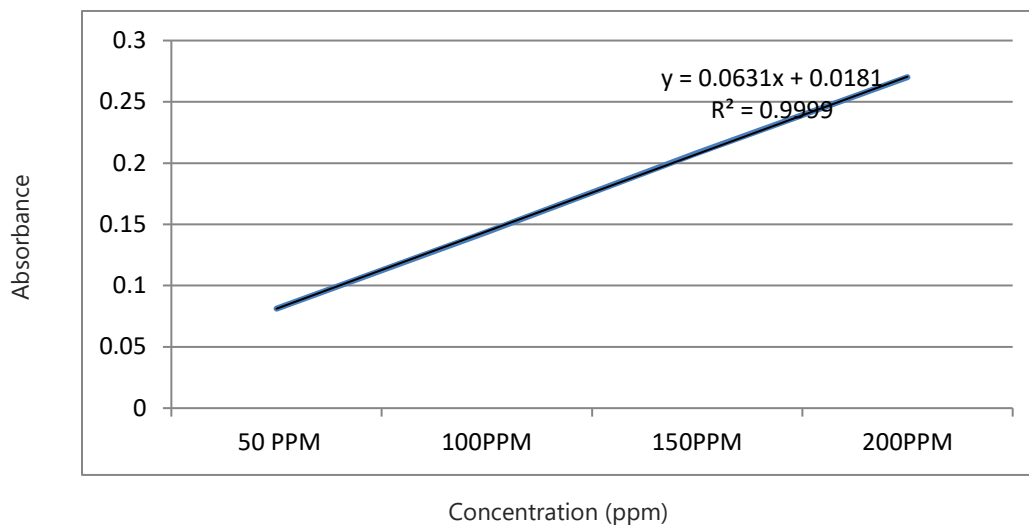


Fig-03;- Calibration curve of Carbachol

Optimization variables;-

Effect of pH on ion- pairing between BPB and Carbachol;-

The Carbachol solution was prepared in acetate phosphate and citrate buffers having pH 5.0, 7.4 and 4.0 respectively. The concentration of carb was 76.5 ppm in each solution. The ion-pair complex was prepared as per general procedure in 10 ml volumetric flasks and volume was made up with distilled water. The absorbances of solutions were measured and plotted against buffer pH the plot is shown in Fig-04.

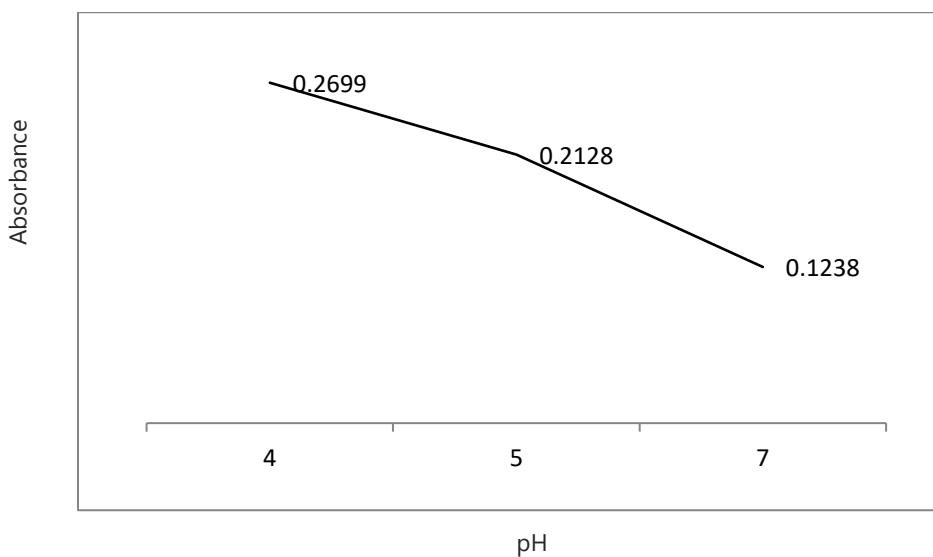


Fig-04;-Effect of pH on Ion Pairing Complex of $\{BPB^{-2}\}\{Carb^{+1}\}$

Medium Effect on BPB Reagent Solution;-

BPB 0.1% was prepared in acetate, phosphate and citrate medium, the final pH was adjusted between 3.4-3.9. The ion pair complex was prepared as per general procedure in 10ml volumetric flask; the volume was made up with distilled water. The recorded results of absorbance were plotted against medium. The plot is shown in Fig 05;-

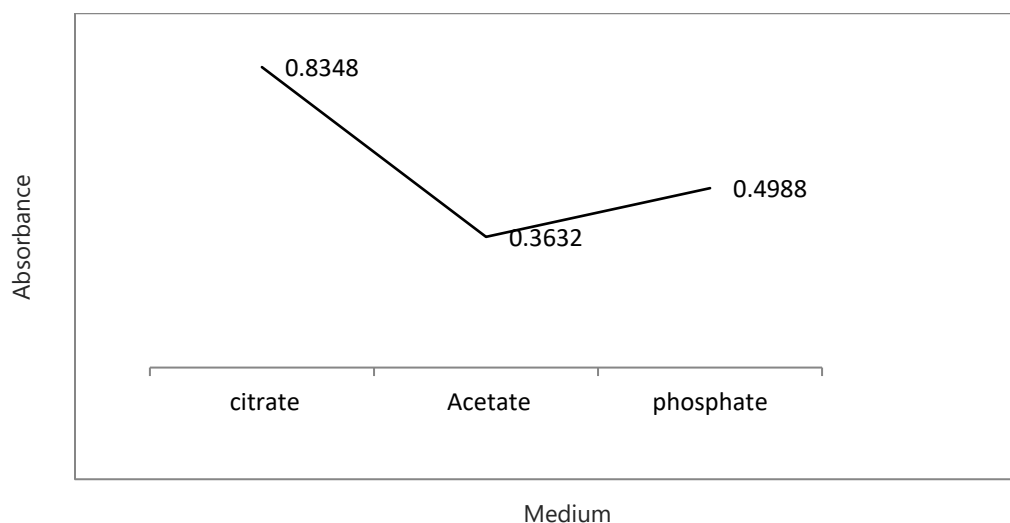


Fig-05;-Medium Effect and Absorbance relation

Variation and interaction of medium and buffer change for ion-pairing complex formation;-

0.1% BPB was prepared in citrate, acetate and phosphate medium and pH was adjusted as per procedure given in BPB reagent preparation proposed method. The solutions of Carbachol of 76.5 ppm concentration were prepared while the Carbachol solutions in Citrate, acetate and phosphate buffer with pH 4.0, 5.0 and 7.0 respectively. The absorbances of prepared complexes were recorded and is shown in Table-02;-

Table-02;- Effect of BPB medium and Carb in buffers

Medium for Dye at pH(3.4-3.6)	Observed Absorbances	Buffer medium for carb samples	Concentration of Carb in each solution(ppm)	
Phosphate medium	0.4930 0.4927 0.4922	Phosphate Buffer pH 7.0	76.5	
	0.5237 0.5218 0.5222	Citrate Buffer pH 4.0	76.5	
	0.5931 0.5936 0.5933	Acetate Buffer pH 5.0	76.5	
	0.2998 0.2996 0.2998	Phosphate Buffer pH 7.0	76.5	
	0.4126 0.4124 0.4124	Citrate Buffer pH 4.0	76.5	
	0.3517 0.3516 0.3514	Acetate Buffer pH 5.0	76.5	
Acetate Medium	0.8121 0.8247 0.8243	Phosphate Buffer pH 7.0	76.5	
	0.8247 0.8247 0.8243	Acetate Buffer pH 5.0	76.5	
	Citrate Medium	0.8121 0.8247 0.8243	Phosphate Buffer pH 7.0	76.5
		0.8247 0.8247 0.8243	Acetate Buffer pH 5.0	76.5

	1.0962	Citrate Buffer pH 4.0	76.5
	1.0962		
	1.0958		

Effect of Dye Concentrations and ion –pair complex;-

Three concentration levels, 0.1%, 0.2% and 0.3% of BPB solutions were prepared in acetate medium as per general procedure of BPB reagent method. The three samples of having 200ppm concentration levels of Carbachol were tested with each solution of BPB. The concentration of ion-pair complex formation was measured by means of absorbances. The recorded results indicated that 0.2% and 0.3% PBP solutions did not show any significant changes with respect to 0.1%BPB solution. The plot of absorbances of ion-pair complex and BPB concentration were shown in Fig-06.

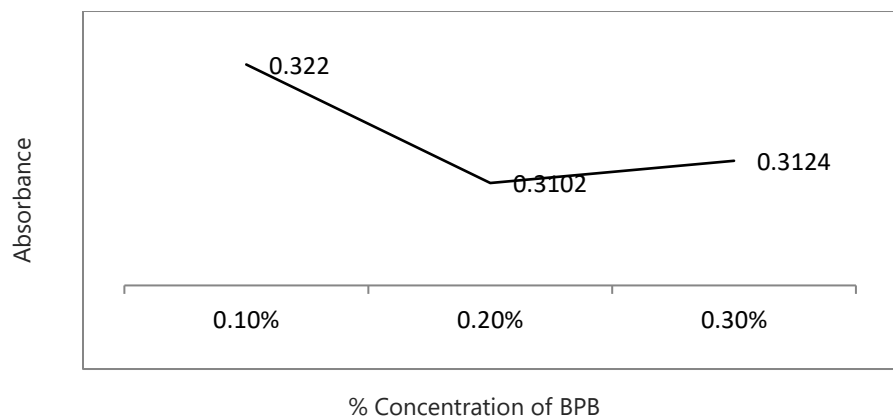


Fig-06;- Effect of Dye Concentrations and ion –pair complex

Stability of Ion-Pair complex;-

Different time intervals were tested after formation of ion-pair complex by taking absorbance of reaction mixture at 10 min, 30min, 60min and 120 min. The obtained results are shown in Fig-07.

Here Eq-01 is for estimation LOD and Eq-02 is for LOQ.

Where α is the standard deviation of intercept, and S is the slop of calibration curve. The calculated detection limits and quantification limits of studied drugs were 5ppm and 10ppm respectively, indicating good sensitivity of proposed method.

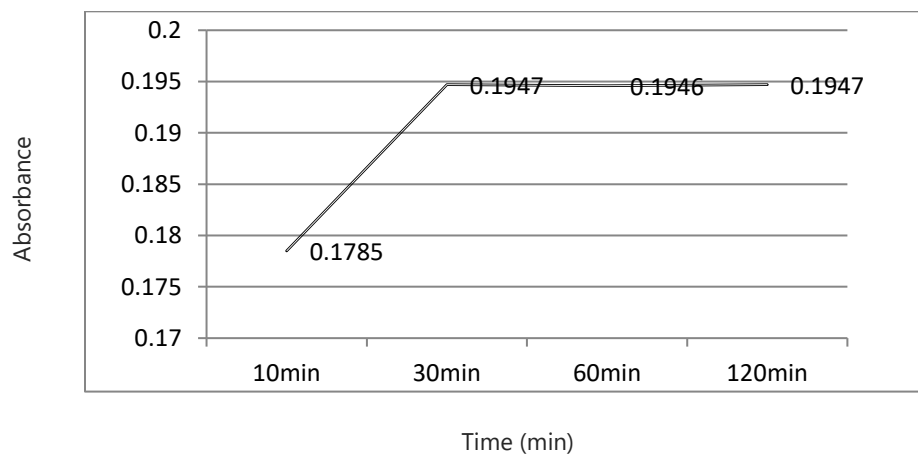


Fig-07;- Stability of ion –pair complex

Validation of proposed Method;-

The proposed method was validated according to guidelines of ICH and USP-40, with respect to linearity, precision, robustness and ruggedness.

Linearity:-

The regression Co-efficient $R^2(0.9999)$ indicated a high linearity of proposed method. The limit of Quantification (LOQ) and limit of detection (LOD) were determined by using the following equation (USP-40).

$$\text{LOD} = 3.3\alpha/S \quad \longrightarrow \quad \text{Eq-01}$$

$$\text{LOQ} = 10\alpha/S \quad \longrightarrow \quad \text{Eq-02}$$

Accuracy:-

The accuracy of proposed method was evaluated at five different concentration levels. Three replicates of each concentration level were recorded. Meanwhile the investigated drug was tested and compared with reported method (Doulakas J et al 1975). The good percent recoveries were obtained show close agreement with the true values.

Table-3; - Evaluation of Accuracy

Concentration Levels (ppm)	% Recovery of Reported Method (Doulakas J. et al 1975)	% Recovery of Proposed Method
50	100.1	99.94
75	99.89	100.06
100	100.02	100.14
150	99.99	99.58
200	100.07	99.96

Precision:-

The precision of proposed method was checked with three concentration levels. The three (n=3) replicate measurements were recorded of each concentration level, having less than 2.0% RSD with respect to intraday and intraday precision. The obtained results were summarized in table 4.

Table-4; Evaluation of Precision (interday and intraday precision)

Parameters	20ug/ml	60ug/ml	100ug/ml
Interday			
1	20.41	59.97	99.67
2	20.23	60.2	99.98
3	19.99	60.05	99.46
Mean	20.21	60.07	99.703
±SD	21.7	12.22	26.16
±RSD	1.04	0.21	0.26
Intraday			
1	20.2	60.1	100.42
2	20.01	60.01	100.17
3	20.13	60.27	99.99
Mean	20.11	60.11	100.19
±SD	0.58	0.58	21.59
±RSD	0.29	0.01	0.22

Robustness:-

The assessment of robustness was evaluated by the influence of small changes in the analytical method. Then the performance of method was checked by estimation of % recoveries.

In these experiments, the pH of Carbachol solution was slightly changed, while the other all variables kept unchanged. The obtained % recoveries were tabulated in table-5. The small variations in variables of proposed method did not show any significant change in results. It gave an indication for reliability of the proposed method, during routine testing.

Table-5;- Evaluation of Robustness

<i>PH Change in Carbachol</i>	<i>%Recovery</i>	<i>±RSD</i>
5.0	100.13	0.25%
5.3	99.76	
5.4	99.65	
<i>pH Chang in BPB Solution</i>		
3.4	100.21	0.18%
3.6	100.17	
3.7	99.96	
3.8	99.83	

Ruggedness;-

The ruggedness of analytical method was assessed by changing the temperature of reagent at 4c, 15c, 25c and 40c. The results were evaluated as shown in Table - 6;-The %RSD which was less than 2.0%, fairly indicated, the high precession of proposed method.

Table-6;- Ruggedness

Temperature change	% Recoveries	% RSD
4 C	99.86	0.49%
15C	98.99	
25C	100.12	
40C	99.48	

% Recovery;-

The proposed and reported methods (Doulakas J.et al 1975) were applied for investigated drug of different commercial brands of ophthalmic preparations which were available in local market. Good percent recoveries were obtained having 1.2 %RSD are shown in Table-7.

Table-7;- % Recovery, comparison with proposed and reported method for determination of Carbachol in pharmaceutical Dosage forms.

Pharmaceutical Dosage forms	% Recovery of proposed method	% Recovery of Reported method
<i>Ophth Carb</i>	99.80 ±0.17	99.81±0.21
MIOCHOL	99.46 ±0.17	99.48 ±0.21
<i>Miostat</i>	99.42 ±0.17	99.67 ±0.21
<i>Ocustat</i>	99.58 ±0.17	99.98 ±0.21

*Average of Five Determinations

5. CONCLUSION

A simple and easy to handle, precise, accurate and specific spectrophotometric method was developed for routine testing of studied drugs in ophthalmic preparations.

Funding:

This study has not received any external funding.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Masayuki Kohashi, Koichi Kawahara & Yoshiko Yamauchi, "Carbachol-induced Suppression of Contraction Rhythm in Spontaneously Beating Cultured Cardiac Myocytes from Neonatal Rats" *Biological Rhythm Research*, 09 Aug 2010, Pages 367-381
2. Sen Hu, Jin-Wei Che, Yi-Jun Tian, and Zhi-Yong Sheng. "Carbachol promotes gastrointestinal function during oral resuscitation of burn shock" *World J Gastroenterol*, 2011 Apr 7; 17(13): 1746–1752
3. R. Atcheson, K. Bjornstrom, R. A. Hirst, D. J. Rowbotham And D. G. Lambert, 'Effect of halothane on K⁺ and Carbachol stimulated [³H]noradrenaline release and increased [Ca²⁺]_i in SH-SY5Y human neuroblastomacells" *British Journal of Anesthesia*, 1997; 79: 78–83
4. Almamoun Abdelkader "Influence of different concentrations of Carbachol drops on the outcome of presbyopia treatment – A randomized study", *International Journal of Ophthalmic Research*, 2019, Vol 5, No 1, 317-320
5. Stahel, P.F., Moore, E.E. "Peer review for biomedical publications: we can improve the system" *BMC Med*, 2014, 12, 179
6. Doulakas J. Spectrophotometric method for estimation of carbachol in eye drops in presence of its degradation products. *Pharm ActaHelv*. 1975;50(12):447-50.
7. Mannan A, Khan AJ, Khan M, Abass G, Roohullah S, Khan Z. A Validated RP-HPLC Method for Determination of Rose Bengal in Bulk and Surgical Strips. *RADS J. Pharm. Pharm. Sci.* 2018; 6(3): 185-189
8. Carol J. Ormond, Sandra Orgeig, Christopher B. Daniels, William K. Milsom. "Thermal acclimation of surfactant secretion and its regulation by adrenergic and cholinergic agonists in type II cells isolated from warm-active and torpid golden mantled ground squirrels, *Spermophilus lateralis*" *The Journal of Experimental Biology*, 206, 3031-3041

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