**International Journal of Chemical and Biomolecular Science**
Vol. 1, No. 2, 2015, pp. 43-48
http://www.aiscience.org/journal/ijcbs

# Bioinorganic and Pharmacological Study of Fe(II)-Coumarin Complex

**Rakesh Choure*, Neelam Vaidya**

Department of Chemistry, Hari Singh Gour University, Sagar, India

## Abstract

The formation of complexes of Coumarin and Fe(II) was studied in aqueous media at pH 8.2±0.1 by polarography and spectroscopy. The polarogram indicated formation of complexes between coumarin and Fe(II). Coumarin produces a well-defined direct current polarogram and differential pulse polarogram in 1M Ammonium tartrate (supporting electrolyte) at pH 8.2±0.1. The stoichiometry of the Fe(II)-Coumarin complex is 1:1. Anticoagulant studies on the drug and its metal complex have been performed in albino mice. Revealing the complex to be more potent in anticoagulation activity compared to the parent drug.

## Keywords

Coumarin, DCP, DPP, Prothrombin Time, Iron Complex

Received: June 18, 2015 / Accepted: June 29, 2015 / Published online: July 15, 2015



## 1. Introduction

Coumarin is the lactone of o-hydroxycinnamic acid, it occurs as colourless, prismatic crystals and has a characteristic fragrance bitter taste, and aromatic, burning[1-4]. It is soluble in alcohol.

Coumarin has a widespread occurrence in natural products, generally being liberated from the corresponding glycoside (melilotoside) on drying coumarin containing herb material. Dicoumarol is a microbiological biotransformation product in spoiled melilotus clover and other hay products and its presences in fodder at >10 ppm is cause for concern, as it is responsible for fatalities by hemorrhaging in cattle. This is because dicoumarol interfores with vitamin k reductase in the liver and the liver is unable to reactivate vitamin K, which leads to a decrease in vitamin K dependent clotting proteins. Coumarin can be synthesized readily[5]. Coumarin is rather widely distributed in nature and in addition to it's occurrence in tonka beans[6-10],. it has been isolated from sweet vernal grass(11-13) (Anthaxanthum adaratum Linne, Fam. Gramineac).

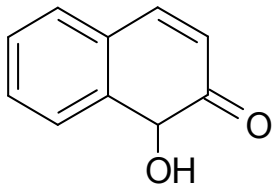



**Fig. 1.** Coumarin.

Cynosurus cristatus (Crested Dog's- Tail),Anthoxylum odoratum L. and L. Narcissus spp, Clary sage Salvia sclarea L.[19,20] sweet clover (Melilatus albus Medicuss and M. officinalis Lamanck, Fam. Leguminosac), Coumarin finds its use as an anticoagulant it's easy availability from plants and in synthetic form it has now became a popular drug among the physician[14], looking at the easy availability of the drug, I have attempted to modify, the coumarin molecule to improve it's anticoagulation potency. The results of which

* Corresponding author
E-mail address: choure_chem@rediffmail.com (R. Choure)

have been discussed in the present paper.

# 2. Experimental Section

## 2.1. Instrumentation

*(1) Polarography:* All polarograms were recorded on Microprocessor (µp)polarographic analyzer model CL-362. An Elico digital pH meter model 335 was used for pH measurement. The polarographic cell consisted of a three-electrode assembly with a saturated calomel electrode (reference electrode) as working electrode.

*(2) Spectroscopy:* The IR spectrum of solid complex was recorded using KBr pellets on a model 8400s IR-spectrophotometer Shimadzu, Japan.

## 2.2. Chemicals

Coumarin chemical used for the present work were of CDH grade. And ammonium tartrate from Himedia grade was used. Stock solutions of the reagents were prepared in requisite amount of distilled water.

## 2.3. Preparation of Complex

Qualitative and quantitative studies on coumarin were carried out using direct current polarography (DCP) and differential pulse polarography (DPP). The pH of the test solution was adjusted to 8.2±0.1 to avoid a matrix effect for electrochemical behavior of coumarin.

Coumarin (0.230g) was dissolved in 100mL ethanol and a set of solution containing varying concentration of coumarin were prepared in 0.01M overall concentration of ammonium tartrate at pH 8.2±0.1.

For study of stoichiometry and formation of the complex, Lingane's polarographic method was used, a simple method over the entire range of ligand concentration.

Experimental solutions were prepared by keeping overall iron (metal ion) and ammonium tartrate concentration fixed at 0.2M and 1M respectively, while varying the ligand concentration from 0 to 15mM. The pH was adjusted to 8.2±0.1, and the solution was deaerated with purified $H_2$ gas. Polarogram was recorded keeping the initial potential set to -1100mV.

## 2.4. Synthesis of Solid Complex

A white solid was synthesized by refluxing 1:1 aqueous solution of ferrous ammonium sulfate and coumarin in water and ethanol (55:45 v/v) for about 4h. the complexation was marked by precipitation after reducing the volume to one fourth of the original volume. The product was filtered, washed, dried over $P_4O_{10}$ and stored.

## 2.5. In-Vivo Study on Fe(II)-Coumarin Complex

*Pharmacological study [Prothrombin Time]*

Pharmacological screening of coumarin and its complex was done in-vivo through the study of anticoagulation activity to check efficacy and safety of prepared complex of coumarin. Since coumarin is an indirect acting anticoagulant which inhibits the clotting of blood in-vivo, thus the mode of screening of new complex of coumarin was screened through same pharmacological test i.e. average plasma prothrombin time determination. The in-vivo experiments were done using albino mice animals.

Coumarin anticoagulant acts by inhibiting the synthesis of vitamin K dependent clotting factors, which include Factors II, VII, IX and X. Vitamin K is an essential cofactor for the post ribosomal synthesis of vitamin K dependent clotting factors. The vitamin promotes the biosynthesis of -carboxyglutamic acid residues in the proteins which are essential for biological activity. The anticoagulation effect generally occurs within 24 hours after drug administration. However, peak anticoagulation effect may be delayed for 72 to 96 hours. Anticoagulants have no direct effect on an established thrombus, nor do they reverse ischemic tissue damage. The plasma prothrombin times is a test to assess hemostatic function of blood coagulation mechanism and screening of patients suffering bleeding disorders or have gone anticoagulation therapy. The plasma prothrombin time (P.T.) is tests for prothrombin activity in addition to factors VII and X are sensitive to the presence of coumarin in blood. Thus author has screened anticoagulation activity of coumarin and its complex through average plasma P.T. by Quick's method [15] in different plasma samples. The time required for coagulation of citrated plasma after addition of thromboplastin calcium mixture, is known as prothrombin time. The clotting of citrated plasma involves a few coagulation factors like II, VII, IX and X in presence of thromboplastin and calcium.

*Quick's Method:* One stage plasma prothrombin time was calculated by Quick's method. For this, 2ml of fresh citrated plasma obtained as mentioned in the experimental part. 0.1ml of this citrated plasma was mixed with 0.1ml of thromboplastin in a glass tube or in a dish placed in a water bath at 37°C. After a delay of 2min 0.1ml of prewarmed 0.025M $CaCl_2$ solution was added to this mixture carefully and mixed well. A stopwatch was started and the tube was held with its lower end submerged in water bath at 37°C. The tube was continuously but gently inclined from the vertical to just short of the horizontal so that its contents could be observed for the first sign of clotting and time was noted. The test was repeated three times with the same citrated plasma

sample and average P.T. was calculated.

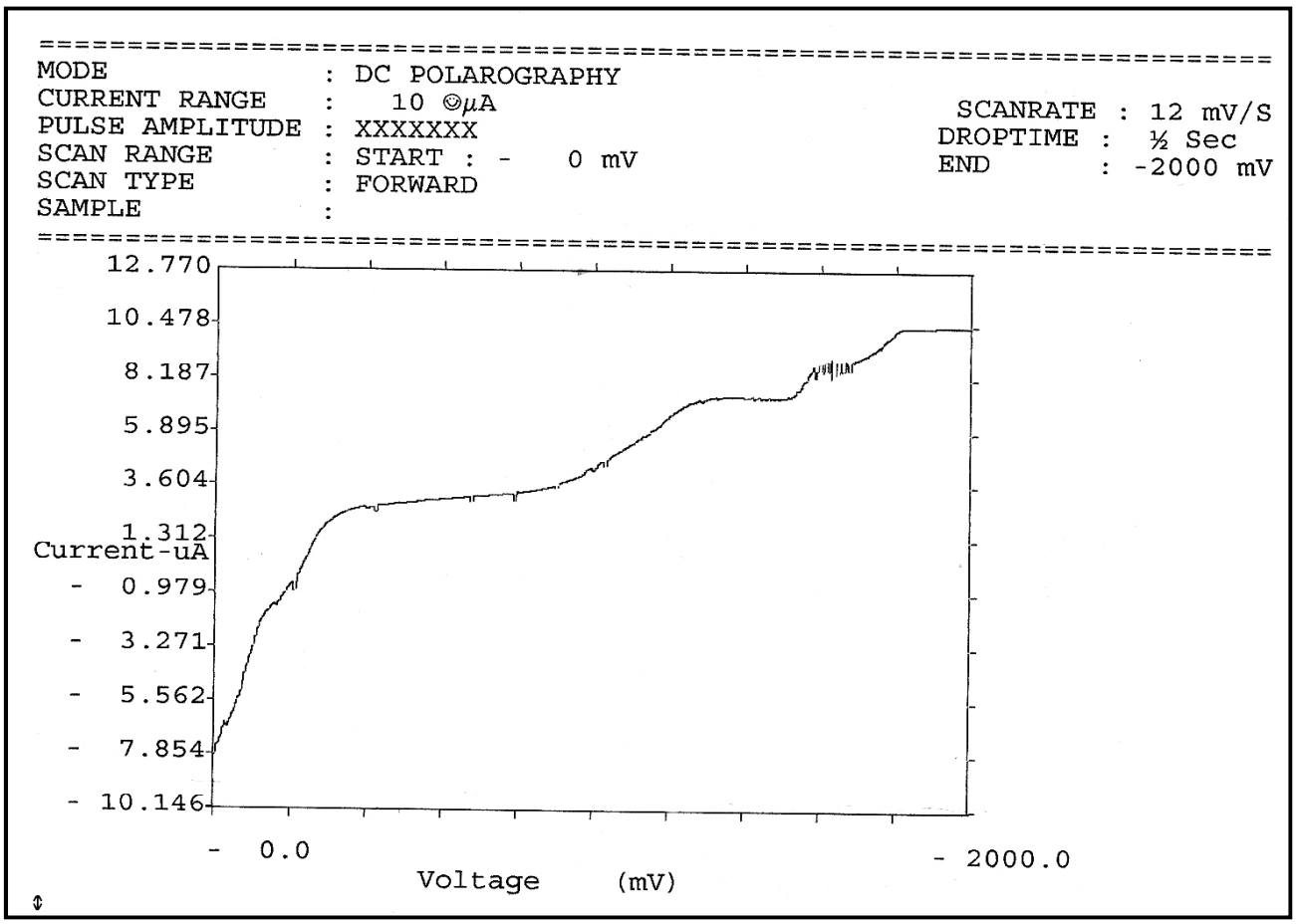
*In-vivo (Albino Mice Plasma):* Coumarin dose shows a relationship between anticoagulation effect and antithrombotic efficacy. The anticoagulation activity of coumarin and complex was screened in albino mice animals for in vivo effect. For this, a standard procedure was adopted for dose of drugs under study and for in-vitro studies the dose was designed as 1mg/100kg. Each drug was administrated orally, on a set of four healthy animals according to their weight (albino mice). 2 ml of venous blood samples of the mice were collected through orbital puncture after 24 hours of dose. Without delaying blood samples were mixed with sodium citrate in a requisite amount and citrated plasma was obtained from each blood sample. This citrated plasma obtained from albino mice for each newly designed coumarin molecule was used to determine average P.T. in sec. by Quick's method.

## 3. Results and Discussion

The direct current polarogram (DCP) and differential pulse polarogram (DPP) of the authentic sample solution of coumarin in ammonium tartrate (1M) at pH 8.2±0.1, produced a well-defined polarographic wave/peak with $E_{1/2}$ / $E_p$= -1.60V/-1.60V vs SCE. Coumarin is polarographically active in both acidic and basic environments

### 3.1. Polarographic Study of M: L Complexation Equilibrium

Both Fe(II) and its complex with coumarin produce a reversible two-electron reduction wave in 1M ammonium tartrate at pH 8.2±0.1. Complex formation between Fe(II) and coumarin (Supplementary Material) was revealed by the shift in half-wave potential and peak potential to a more negative value and decrease in the height of the diffusion current with gradual increase of the coumarin concentration. Plots of $\Delta E_{1/2}$ (shift in the half wave potential). $\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s$ against log Cx (Logarithm of the concentration of the ligand) resulted in a linear plot (Figure 3), showing formation of a single complex in solution. Lingane treatment of the observed polarographic data revealed 1:1 Fe(II)-coumarin complex with formation constant log $\beta_1$= 5.85.



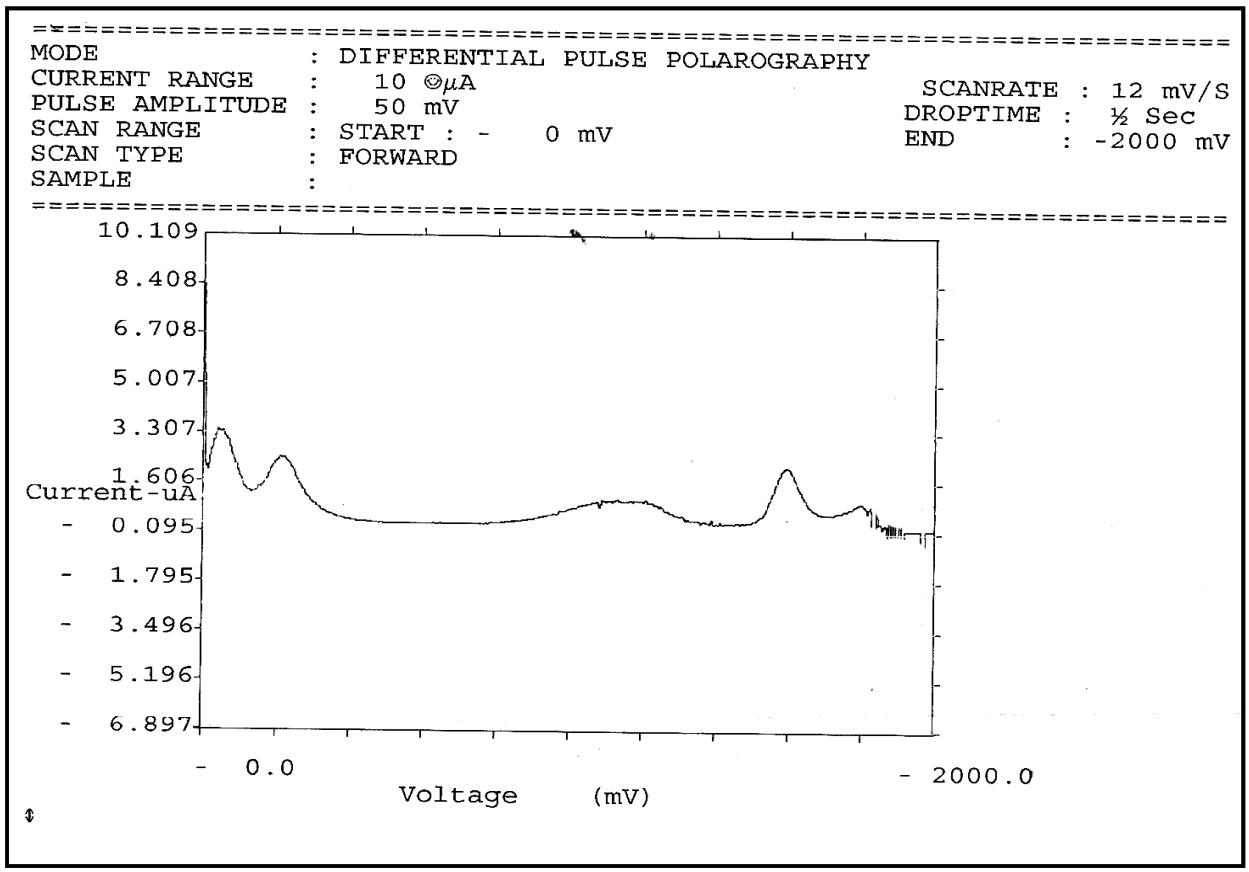



**Fig. 2.** DCP and DPP of 1mM coumarin in 0.2M ammonium tartrate, pH 8.2±0.1.

**Table 1.** *In Vivo* Anticoagulation Action of Fe(II)- Coumarin Complex in Albino mice.

| S.No. | Body Weight of Animal | Dose administered | Plasma Prothrombin Time in sec. | | | Average P.T. Sec. |
|---|---|---|---|---|---|---|
| | Grams | mL | a | b | c | (a+b+c)/3 |
| 1. | 40.44 | 0.40 | 21.02 | 21.16 | 21.16 | 21.16 |
| 2. | 43.52 | 0.43 | 21.22 | 21.22 | 23.78 | 21.22 |
| 3. | 42.12 | 0.42 | 21.35 | 21.32 | 21.35 | 21.35 |
| 4. | 40.11 | 0.40 | 21.20 | 21.23 | 21.23 | 21.23 |
| 5. | Mean average P.T. | | | | | 21.24 |

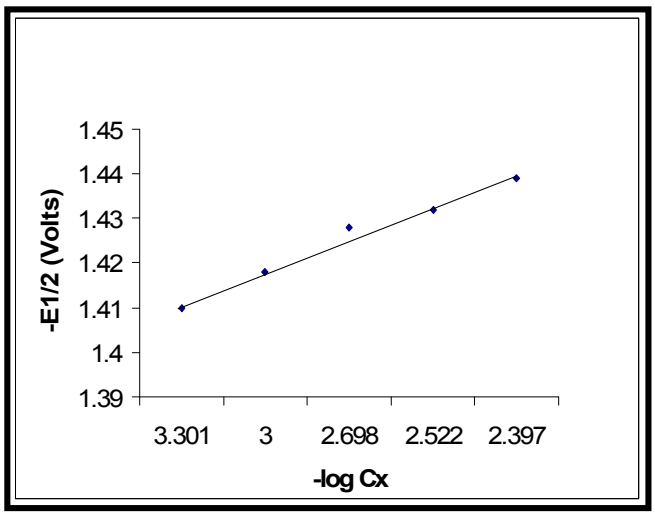



**Fig. 3.** Plot of $-E_{1/2}$ VS, log Cx for Fe(II)-coumarin complex.

## 3.2. IR Spectral Analysis of Fe(II)-Coumarin Complex

On comparing the IR spectra of coumarin and its Fe(II) complex, it was observe that the band at 1674 $cm^{-1}$ due to C=O group in the spectrum of pure drug and this band disappear in the spectrum of its Fe(II) complex. The sharp –OH signal at 3115 $cm^{-1}$ is observed in coumarin. This band is not shifted in the spectrum of Fe(II)-coumarin complex, which confirms involvement of C=O in the complexation of Fe(II). Thus on the basis of polarographic and IR studies a tentative structure to 1:1, Fe(II)-coumarin complex may be as under:

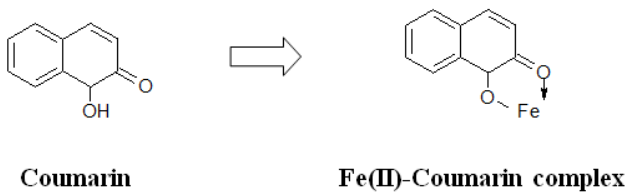



**Fig 4.** Coumarin and Fe(II)-Coumarin complex.

### 3.3. Pharmacological Experiments (Average Prothrombin Time)

*In vivo (Albino Mice) Anticoagulation Action:* The in vivo anticoagulation action of coumarin and its complex was screened on albino mice. The results of anticoagulation activity obtained for normal and each test sample species of drugs are shown in respective table. The average prothrombin time as observed using complex of coumarin is depicted in the bar diagram. It is quite clear from the bar diagram that, though the mean average prothrombin time using coumarin as anticoagulant increases to 20.74sec as compared to that observed with pure blood plasma of the animal which is 11.94sec (without drug dose), but coumarin modified molecule shows increased anticoagulation activity to 21.24sec.

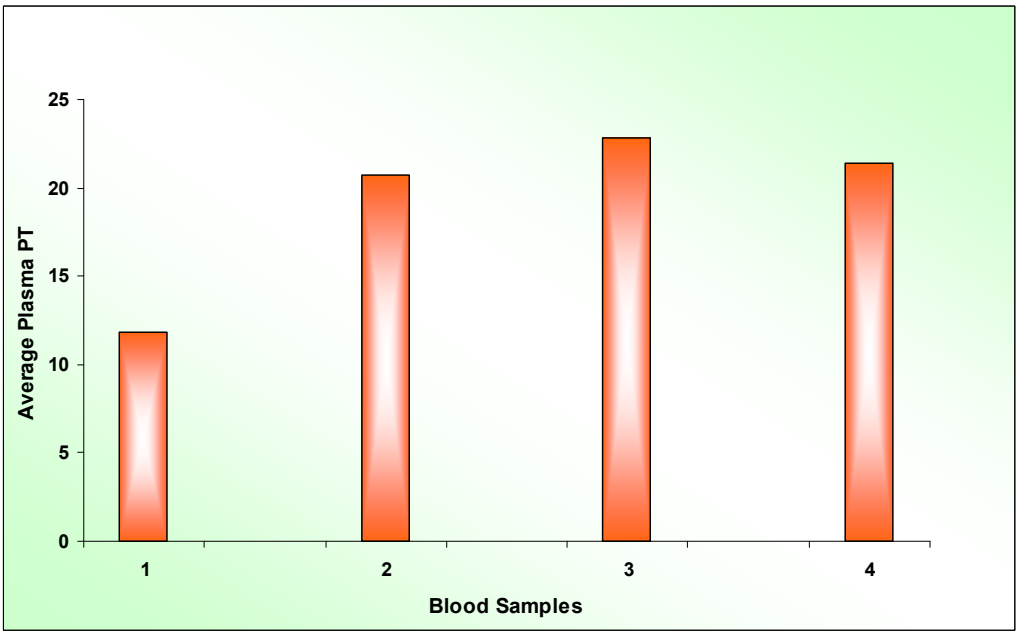



**Fig. 5.** Bar Diagram of Plasma Prothrombin Time (Sec.).

Blood Samples
1= Normal blood plasma, 2= Blood plasma sample of Coumarin, 3= Blood plasma sample of Fe- Coumarin, 4= Blood plasma sample of Zn- Coumarin

## 4. Conclusion

The data show stoichiometric ratio of 1:1 for the Fe(II) coumarin complex. The polarographic method is used for qualitative and quantitative analysis of coumarin and is recommended for quality control in the drug industry. The increased potency of the complex may allow use as a potent anticoagulant drug.

## References

[1] F. Floc' h, F. Mauger, J R. Desmurs, coumarin in plants and fruits, 27: 32-37 2002.

[2] Gwynne., "P. Drug", Discovery Development (5),50-55 2002.

[3] C. W. Tornoe, C. Christensen, M. J. Meldal, Org. Chem. 67,3057-3064 2002.

[4] H. R. Dharmaratne, W. M. Wanigasakera, E. Mata – Greenwood, J. M., Pezzoto, Planta Med. 64, 460-461 1998.

[5] H. X. Wang, Planta Med., 67, 669-672 2001.

[6] A. J. Vlietinck, T. De Bruyne, S. Apers, L. A. Pieters, Planta Med. 64, 97-109 1998.

[7] R. A. Katz, A. M. Skalka, Annu. Rev. Biochem. 63, 133-173 1994.

[8] B. Lake, Coumarin metabolism, toxicity, and carcinogenicity: relevance for human risk assessment, Food and Chemical Toxicology,37,423- 453 1999.

[9] G. Feuer, The metabolism & biological actions of coumarins, Progress in Medicinal Chem, 10, 85-158 1974.

[10] H. R. Dharmaratne, G. T. Tan, G. P. Marasinghe, J. M. Pezzuto, Planta Med., 68, 86-87 2002.

[11] Z. Q. Xu, T. R. Jenta, M.T. Flavin, Current Opinion for Drug Discovery & Development, 3,155-166 2000.

[12] K. H. Lee, S. L. Morris-Natschke, Pure Appl. Chem. 71, 53-57 1999.

[13] S. L. Born, P. E. Rodriguez, C. L. Eddy, & L. D. Lehman-McKeeman, Drug Metabolism and Disposition. 25, 1318-1323 1997.

[14] M. Vocanson, C. Goujon, G. Chabeau, M. Castelain, M., Floc'h, F. Valeyrie, Maliverney, C. Gard, A., & J. F. Nicolas, The skin allergenic properties of chemicals may depend on contaminants, Int Arch Allergy Immunol 140, 231-238 2006.

[15] A. J. Quick's, Am. J. Clin. Pathol. 45,105 1966.