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A New Spectrophotometric Method for the Determination of Chlorine-Containing Organic Compounds Using 1,5-Bis(6-methyl-4-pyrimidyl)carbazone

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A new spectrophotometric method for the determination of small amounts of chlorine ion by using 1,5-bis(6-methyl-4-pyrimidyl)carbazone (MPC) has been established.

The proposed method is based on the reduction of the absorbance of silver–MPC complex at 530 nm after the reaction of chlorine ion with the complex to form AgCl in 1 M ethanolic acetic acid solution. Silver–MPC is a violet complex ($\lambda_{max} = 530$ nm) which is soluble in water, pyridine, dimethylsulfoxide and lower alcohols, and is stable when kept in light-resistant containers. A surfactant was added to the solvent to disperse silver chloride colloid uniformly in the test solution, and this allowed direct measurement of absorbance without the separation of AgCl. The calibration curve conformed to Beer's law between 1×10^{-5} and 1×10^{-4} M chlorine ion.

This method could be applied to the determination of various chlorine-containing organic compounds listed in The Japanese Pharmacopoeia (JP X), when they were processed by the oxygen flask combustion method described in the General Test Methods of JP X.

Keywords——spectrophotometric determination; silver-complex; oxygen flask combustion; chlorine-containing compound; 1,5-bis(6-methyl-4-pyrimidyl)carbazone

Various methods have been established for the quantitation of chlorine ion by spectrophotometry, such as the mercuric thiocyanate method,[1)] mercury–diphenylcarbazone–benzene extraction method,[2)] and copper–diethyldithiocarbamate (DDTC)–benzene extraction method.[3)] In the oxygen flask combustion method adopted in The Japanese Pharmacopoeia (JP X),[4a)] a mercuric acetate titration has been applied to the analyses of chlorine-, bromine- and iodine-containing compounds. However, these methods are considered to be undesirable for the following reasons. Firstly, these methods employ harmful mercuric ions and include extraction procedures. Secondly, the titration method is generally regarded as being prone to individual errors and as having lower sensitivity than spectrophotometry.

On the other hand, the proposed method using 1,5-bis(6-methyl-4-pyrimidyl)carbazone (MPC) is a spectrophotometric method which is based on the quantitative reaction between chlorine ion and siliver–MPC complex,[5)] which results in the formation of AgCl and concomitant reduction of the absorbance of Ag–MPC complex at 530 nm. MPC is similar to diphenylcarbazone (DPC), having a carbohydrazine moiety in common, and forms complexes with various heavy metal ions. As a result of the introduction of pyrimidine groups into DPC instead of phenyl groups, MPC and its complex compounds are more stable and more soluble than DPC and its adducts in polar media. Thus, a 1 : 1 complex of silver and MPC formed in basic, neutral or weakly acidic solutions is soluble in water, pyridine, dimethylsulfoxide (DMSO) and lower alcohols. In the lower alcohols, in which the solubility of Ag–MPC is excellent and the solubility product of AgCl is small enough for the determination of Cl^-, AgCl is produced quantitatively by the addition of Cl^- to Ag–MPC solution. In the presence of appropriate surfactants, the reaction mixture can be directly measured for absorbance without removal of colloidal AgCl by filtration, or extraction of Ag–MPC.

It is the purpose of this paper to present details of this rapid and simple spectrophotometric method for the determination of chlorine-containing organic compounds.

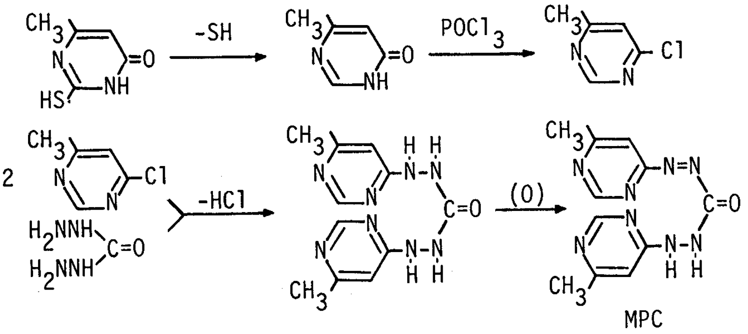

Chart 1

Experimental

Materials and Reagents

Colorimetric reagent, MPC, was prepared by the synthetic route shown in Chart 1, as reported by Ichiba *et al.*[6)]

Reagents and Solutions——One-tenth of a gram of MPC was dissolved in 100 ml of guaranteed reagent grade (GR) DMSO and the solution was stable for more than a week when kept in a dark and cool place.

Standard Cl^- solution (0.125 M) was prepared by dissolving 0.7305 g of sodium chloride (GR) in water to make 100 ml.

Silver nitrate solution (0.005 M) was prepared by 20-fold dilution of 0.1 M $AgNO_3$ standard solution with water, and was standardized by Mohr's method according to JP X.[4b)]

Ethanolic acetic acid solution (1 M) was prepared by dissolving 60 ml of glacial acetic acid (GR) and 0.1 g of polyethylene glycol mono-*p*-nonylphenyl ether (NP-10) in absolute ethyl alcohol to make 1 l.

Sodium azide solution (0.5 M) was prepared by dissolving 3.25 g of NaN_3 (GR) in water to make 100 ml.

All other materials and reagents were of analytical reagent grade and were used without further purification. All the solutions were prepared with deionized and distilled water throughout this investigation.

The pharmacopoeial materials were purchased from Sigma Chemical Company, Iwaki Seiyaku Co., Ltd. and Sumitomo Chemical Co., Ltd., or kindly supplied by Shizuoka Coffein & Co., Ltd., Yoshitomi Pharmaceutical Industries Ltd., Toho Laboratories Co., Ltd., Pfizer Taito Co., Ltd., Kodama Ltd. and Fuji Rebio Inc.

Apparatus——Absorption spectra and absrobance measurements were made with a Shimadzu model UV-240 spectrophotometer using 10 mm cells.

Method of Preparation——The sample was weighed out accurately so that it contains about 2.2 mg of chlorine. In the case of a solid sample, it was weighed on a piece of halide-free filter paper and in the case of a liquid sample, it was weighed by absorbing it on a small piece of absorbent cotton placed on the filter paper. The weighed sample was then processed by the oxygen flask combustion method using 5 ml of H_2O_2 (1→5) as an absorbing liquid. The final sample solution was prepared by transferring the H_2O_2 phase quantitatively into a 25-ml volumetric flask, adding 200 μl of 0.5 M NaN_3, and diluting it with water to make 25.0 ml.

Standard Cl^- and blank solutions were prepared by the same procedure as mentioned above except that standard NaCl solution or water was used for standard Cl^- or blank solutions, respectively, in place of the sample.

Standard Procedure——A 200 μl aliquot of 0.005 M $AgNO_3$ solution was added to 400 μl of 0.1% MPC solution in DMSO in a 10-ml light-protected volumetric flask, then 8—9 ml of 1 M ethanolic acetic acid solution was added, and mixed well. To this mixture, 200 μl of sample or standard Cl^- or blank solution was added and the final volume was adjusted to 10 ml with 1 M ethanolic acetic acid solution. After leaving the mixture to stand for 10—20 min, the absorbance was measured at the wavelength of maximum absorbance at around 530 nm.

Calculation——The amounts of chlorine-containing compounds in milligrams were calculated by using the following equation:

$$(A_o - A_s)/(A_o - A_t) \times 6.25 \times 10^{-2} \times M_r/N$$

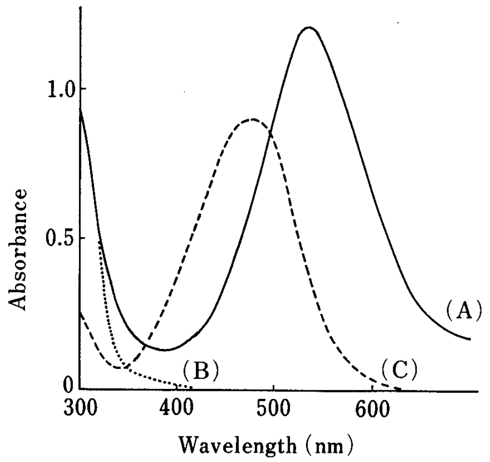

Fig. 1. Absorption Spectra of MPC and Ag–MPC

(A), 1×10^{-4} M Ag–MPC in 1 M ethanolic acetic acid solution. (B), 5×10^{-5} M MPC in 1 M ethanolic acetic acid solution. (C), 5×10^{-5} M MPC in 0.1 N sodium hydroxide solution.

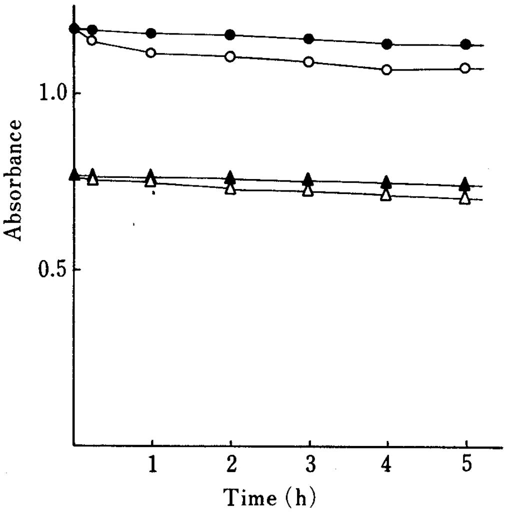

Fig. 2. Effects of Light and Standing Time on Coloration

(●), Ag–MPC in the dark; (○), Ag–MPC in the light; (▲), Ag–MPC+Cl^- in the dark; (△), Ag–MPC+Cl^- in the light.

Ag–MPC, 1×10^{-4} M; Cl^-, 5×10^{-5} M; reference, reagent blank.

Solvent, 1 M ethanolic acetic acid solution, read at 530 nm.

where,

A_s, A_t, A_0: absorbance of sample, standard Cl^- or standard Ag–MPC solutions, respectively,
M_r: molecular weight of sample,
N: number of chlorine atoms per mole of sample.

Results and Discussion

It was shown that the color reaction of MPC with Ag^+ should be carried out in acidic media since the coloration of MPC *per se* increased in neutral or basic media as compared with acidic ones.

Figure 1 shows the absorption spectra of Ag–MPC (A) and MPC (B) solutions measured in the presence of 1 M acetic acid in ethanol. The maximum molar absorptivity of the complex at 530 nm was calculated to be $1.2\times10^4 l\cdot mol^{-1}\cdot cm^{-1}$, while the absorbance of the reagent blank (B) at 530 nm was found to be negligible.

Stability of Ag–MPC

The effects of light and standing time on the coloration of Ag–MPC were examined in the presence or absence of Cl^- (Fig. 2). The color tended to fade gradually in the light due to the light-sensitive nature of Ag^+, but the coloration of the complex was stable for more than 5 h when stored in light-resistant containers at room temperature. Therefore, it is recommended that Ag–MPC should be prepared by mixing $AgNO_3$ and MPC solutions in a light-protected flask prior to use.

Effect of Organic Acids and Solvents

The effect of acidic media on the coloration was examined with various organic acids such as acetic, propionic, butyric, lactic, citric and benzoic acids. The maximum and reproducible absorbance was obtained with acetic acid in ethanol under conditions such that the absorbance of the reagent blank was sufficiently small, as mentioned above.

As shown in Fig. 3, almost constant absorbances were obtained between 0.5 and 1 M acetic acid in ethanol; it was also found that the higher the concentration of acetic acid was, the lower the reagent blank was.

The effect of various water-miscible organic solvents on the coloration was studied in the presence of 0—8×10^{-5} M Cl^- (Fig. 4). It was found that the relative absorbance was linearly related to the concentration of Cl^- when ethyl, iso-propyl, *n*-butyl or iso-butyl alcohol was used. A linear response was not obtained with *n*-propyl alcohol. Among these solvents, ethanol was a solvent of choice because of its good linear response, low viscosity and low cost.

Effect of Water Content

The effect of water content was examined because the presence of water would increase the ionization of AgCl, which would result in increased dissociation of Ag–MPC. As shown in Fig. 5, the maximum and constant absorbance could be obtained in the presence of up to 10% water. It should be noted that the water content was about 4% when the standard procedure was carried out as described above.

Effect of Surfactants and Sodium Azide

The effect of surfactants (added to disperse AgCl colloid uniformly in the solution and to stabilize the Ag–MPC complex) on the stability of the coloration was examined. Among various nonionic surfactants examined (NP-10, NP-17, Tween-20 and Triton X-100), NP-10 was found to be the most effective dispersing agent to assure good stability as well as good reproducibility (Table I). The effective range of concentrations of NP-10 was found to be between 0.005 and 0.02% in 1 M ethanolic acetic acid solution.

It was observed that there was practically no effect of light scattering by the colloidal AgCl on the absorbance, since the turbidity of AgCl colloid was less than 0.02 (A_{530}) when the standard procedure was carried out without addition of MPC.

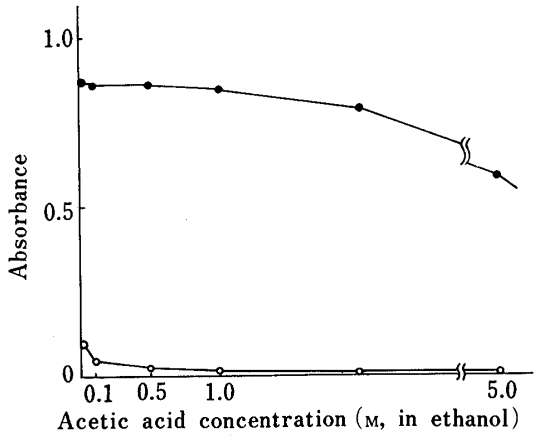

Fig. 3. Effect of Acetic Acid Content

(●): The absorbances at 530 nm of 8×10^{-5} M Ag–MPC and (○): 0.004% MPC (reagent blank) in various concentrations of acetic acid in ethanol were measured.

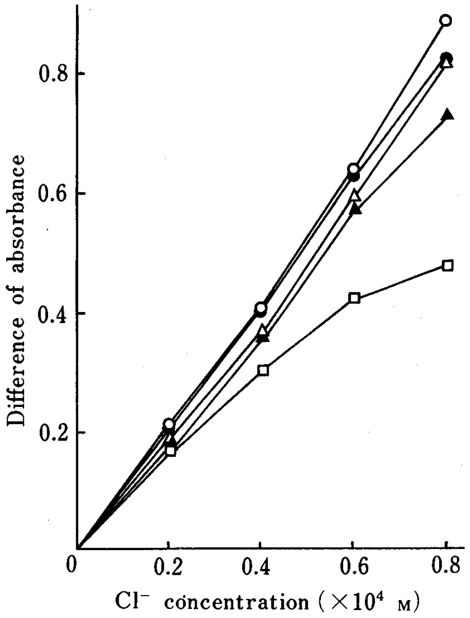

Fig. 4. Effect of Organic Solvent

Various concentrations of Cl^- (0—8×10^{-5} M) were added to 1×10^{-4} M Ag–MPC in 1 M acetic acid solution prepared in various organic solvents, and the differences of absorbance between standard Ag–MPC solution and the mixtures of Ag–MPC and Cl^- were read at 530 nm. The correlation coefficients (r) of the regression equations were: (—○—), *n*-butanol $r = 0.998$; (—△—), iso-butanol $r = 0.998$; (—□—), *n*-propanol $r = 0.985$; (—●—), iso-propanol $r = 0.998$; (—▲—), ethanol $r = 0.999$.

Table I. Effect of Surfactant[a)]

Surfactant	% (w/v)	Absorbance ± S.D. ($n=5$)	C.V. (%)
None	—	0.691 ± 0.018	2.55
NP-10	0.01	0.694 ± 0.003	0.48
NP-17	0.01	0.679 ± 0.007	1.00
Tween-20	0.20	0.669 ± 0.011	1.67
Triton X-100	0.10	0.683 ± 0.006	0.91
Triton X-100	0.02	0.687 ± 0.007	1.01

a) The absorbance of a mixture of 1×10^{-4} M Ag–MPC and 5×10^{-5} M Cl^- was measured at 530 nm in the presence of the surfactants listed in the table. S.D., standard deviation. C.V., coefficient of variation. NP-10, polyethylene glycol mono-*p*-nonylphenyl ether (ethylene oxide = 10).

Table II. Effect of Sodium Azide[a)]

Taken NaN_3 ($\times 10^{-4}$ M)	Absorbance ± S.D. ($n=5$)	C.V. (%)
0	0.624 ± 0.011	1.76
0.4	0.638 ± 0.008	1.25
0.8	0.634 ± 0.006	0.95
1.6	0.640 ± 0.003	0.47
3.2	0.647 ± 0.008	1.24

a) 0—3.2×10^{-4} M NaN_3 was added to a reagent mixture consisting of 1×10^{-4} M Ag–MPC and 5×10^{-5} M Cl^- in 1 M ethanolic acetic acid solution, and the absorbance was measured at 530 nm. S.D., standard deviation. C.V., coefficient of variation.

The effect of various concentrations of NaN_3, which is supposed to protect MPC or Ag–MPC from oxidative decomposition, is shown in Table II which indicated that the most effective range of concentrations of NaN_3 was from 0.4—1.6×10^{-4} M (final concentration).

Calibration Curve

The calibration curve for the determination of Cl^- was obtained by the standard procedure using NaCl standard solution (Fig. 6). It conformed to Beer's law up to 1×10^{-4} M Cl^-. The coefficients of variation were 0.4—1.5% ($n=5$) at each measured point and the correlation coefficient (r) of the regression equation was 0.999.

Effect of Foreign Ions

Bromine and iodine ions reacted with Ag–MPC in the same manner as chlorine ion. Although organic ions such as oxalate or citrate were able to form complexes with Ag^+, they did not interfere with the reaction since they were decomposed during the combustion process. On the other hand, sulfate ion produced by the combustion of sulfur compounds decreased the absorbance slightly due to precipitation of Ag^+ as Ag_2SO_4.

Cations such as Hg^+, Hg^{2+} and Cu^{2+} which formed complexes with MPC in acidic solution, competed with Ag^+. However, other heavy metal ions such as Zn^{2+}, Pb^{2+}, Cd^{2+} which formed complexes with MPC under neutral or basic conditions did not interfere with the determination under acidic conditions even in the presence of a 10-fold excess over Ag^+.

Light metal ions such as Al^{3+}, alkali metal ions or alkaline earth metal ions, as well as hydrogen peroxide, did not interfere with the reaction at all.

Application to Pharmaceuticals

Several chlorine-containing pharmaceuticals were determined by the MPC method. The results are summarized in Table III, and compared with those obtained by the official methods

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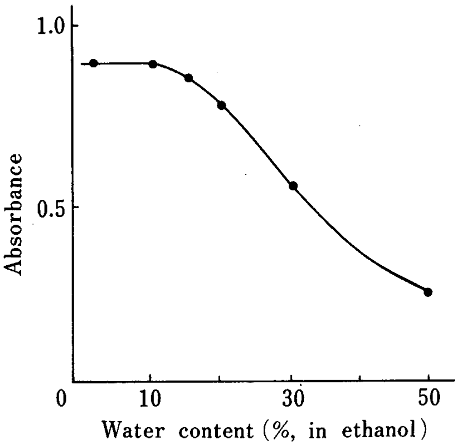

Fig. 5. Effect of Water Content

A 8×10^{-5} M solution of Ag–MPC was prepared in 1 M acetic acid in ethanol containing 4—50% water, and the absorbance was read against 0.004% MPC in 1 M ethanolic acetic acid solution at 530 nm.

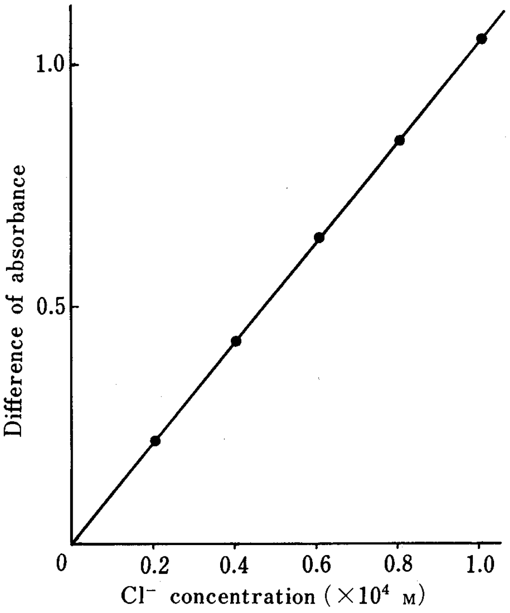

Fig. 6. Calibration Curve for Chlorine Ion

Standard Cl^- solution (0.2—1.0×10^{-4} M) was determined by the standard procedure described in the text, and the relationship between Cl^- concentration (X, M) and the difference of absorbance (Y) was calculated to be:

$Y = 10663X + 0.0006 \quad r = 0.999$,
(r : correlation coefficient).

TABLE III. Comparison of the Proposed Method with the JPX Method[a]

Sample	Proposed method			JPX method		
	Taken mg	Recovery (%) mean ± S.D.	C.V. (%)	Taken mg	Recovery (%) mean ± S.D.	Method
Clofibrate	15	98.53 ± 2.61	2.65 ($n=6$)	500	101.38 ± 0.58	[b]
Clemastine fumarate	29	100.09 ± 1.15	1.15 ($n=7$)	400	97.54 ± 2.24	[c]
Diazepam	18	101.10 ± 1.75	1.73 ($n=5$)	600	96.42 ± 0.24	[c]
Haloperidol	22	99.07 ± 1.93	1.95 ($n=6$)	600	97.99 ± 0.53	[c]
Indometacin	22	100.61 ± 0.55	0.54 ($n=4$)	700	100.62 —	[d]
Chlorpheniramine maleate	24	100.59 ± 1.73	1.72 ($n=5$)	400	99.39 ± 0.09	[c]
Medazepam	17	101.46 ± 2.12	2.09 ($n=5$)	400	97.12 ± 0.34	[c]
Hydroxyzine pamoate	48	97.75 ± 0.48	0.49 ($n=3$)	600	93.68 ± 0.59	[c]

a) Samples containing about 2.2 mg of chlorine were weighed out and the organic chloride was converted to inorganic chloride by the oxygen flask combustion method. Determination methods described in individual monographs in JPX are: *b*) 0.1 N sodium hydroxide–ethanol titration. *c*) 0.1 N perchloric acid titration. *d*) 0.1 N sodium hydroxide titration. S.D., standard deviation. C.V., coefficient of variation.

as directed in the individual monographs in JPX.

As shown in Table III, the results were satisfactory in terms of accuracy, reproducibility, simplicity and the amount of sample necessary for the determination.

Conclusion

The new spectrophotometric method for the determination of chlorine-containing organic substances established in the present investigation possesses the following

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characteristics:

i) It enabled the determination of Cl^- by the use of the oxygen flask combustion method without using harmful mercuric compounds.

ii) The presence of ethanol and a surfactant made the spectrophotometric determination simpler and reproducible by stabilizing Ag–MPC complex and dispersing colloidal AgCl uniformly in the solution so that filtration or extraction of AgCl was unnecessary.

In the proposed method, the range of determination was 0.355—3.55 ppm of Cl^- in a final volume of 10 ml, which is equal to or 2—5 times less than the ranges obtained with conventional methods such as the mercuric thiocyanate method, DPC method, and Cu–DDTC method. The coefficients of variation of the proposed method were found to be 1—2% with several pharmaceutical substances determined by the standard procedure.

It is suggested that this method could be widely applied to the determination of chlorine-containing pharmaceutical preparations or other substances found in clinical samples or in the environment. It was also found that bromine-containing substances can be determined by the proposed method. Although it is feasible to apply this method to the determination of iodine-containing substances, there are some practical problems which make direct application to such substances difficult. Investigations are in progress to cope with these problems.

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