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Determination of acid values of fats and oils by flow injection analysis with electrochemical detection[1]

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Abstract

A new method using a flow injection system with electrochemical detection was developed to determine acid values of fats and oils. VK_3 (2-methyl-1,4-naphthoquinone) solution, i.e., ethanol containing 3 mM VK_3 and 38 mM $LiClO_4$, was used as the carrier solution. Flow signals were monitored at -0.33 V vs. Ag/AgCl. For preparation of a sample solution, an oil sample was completely dissolved in VK_3 solution, or fatty acids were extracted from the sample into this solution. Aliquots (5 μl) of the sample solution were injected into the flow injection system. Acid values were determined based on flow signals for 14 samples and the results were found to be consistent with those by potentiometric titration. Relative standard deviation was less than 2%. Samples were processed at the rate of 60 h^{-1}. The stability of fish and cod liver oils was followed by measuring acid values for 8 weeks. This method proved to be a simple and rapid means for acid value determination. © 1997 Elsevier Science B.V.

Keywords: FIA; Electrochemical detection; Acid values; Acid value determination; Stability of oil

1. Introduction

The determination of acid value, as milligrams KOH required to neutralize free acids in 1 g fat or oil sample, is essential to the quality control of fats and oils. Titration, by which a sample in ethanol and ether is neutralized with KOH or NaOH using phenolphthalein indicator, is the recommended pharmacopoeial procedure in many countries [1–5]. But this method is attended with problems such as indicator error, requirements for large sample size and the considerable time necessary for titration. For highly coloured samples, the unclear colour change in the transition range of the indicator may be a source of error. Alkaline titration is not suitable for some oils containing esters easily saponified by alkali. For acid value determination in stability tests of pharmaceutical preparations, a small sample size is desirable.

To avoid the problems described above, the authors previously developed a new voltammetric method for determining acid values based on the reduction prepeak of 2-methyl-1,4-naphthoquinone (VK_3), which appears due to the presence

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of acid in ethanol solution containing VK_3 [6]. Voltammetry involves a somewhat complicated procedure and thus is not always suitable for quality control. Flow injection analysis (FIA) using a walljet-type of electrochemical detector was previously developed by the authors. Using this method, the free fatty acid content in oils could be easily determined with a high degree of sensitivity [7].

In the present study, FIA with electrochemical detection was developed as a simple and highly accurate means of acid value determination of fats and oils and applied for this determination in stability test of pharmaceutical preparations of oils.

2. Experimental

2.1. Apparatus

The present flow injection (FI) system consists of a carrier solution reservoir, pump (Model DMX-2200-T, SNK., Tokyo, Japan), sample injection valve (Model 7125, Rheodyne, Cotati, CA, USA) with a stainless-steel sample loop and electrochemical detector. All these components were connected by polytetrafluoroethylene tubing (0.5 mm i.d.). The electrochemical cell comprised a glassy carbon working electrode, Ag/AgCl reference electrode, stainless-steel counter electrode and polychlorotrifluoroethylene cell body, cell volume 2.4 μl. The detection potential was controlled with a potentiostat (Model 312, Fuso Seisakusho, Kawasaki, Japan), and the FIA signal current was registered using a recorder (Model 807-IT, JASCO, Tokyo, Japan).

The detection potential was maintained at −0.33 V vs. Ag/AgCl. The flow rate of the carrier solution was 0.6 ml min^{-1}. Aliquots (5 μl) of sample solution and standard acid solution were injected into the FI system.

2.2. Reagents

The 2-methyl-1, 4-naphthoquinone (VK_3) was used as received. Ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$, i.e., the VK_3 solution in this study, was used as the carrier solution. Air was removed from the solution by degassing under reduced pressure.

Standard acid solution was prepared by dissolving palmitic acid (99.5% pure, Wako Pure Chemical, Osaka, Japan) in the VK_3 solution.

The following fats and oils were obtained commercially: cacao butter (Fujisawa-Astra, Osaka, Japan), camelli oil (Shiseido Seiyaku, Tokyo, Japan), corn oil (Hayashi Chemicals, Tokyo, Japan), glyceryl monostearate (Wako Pure Chemical, Osaka, Japan), mentha, olive and sesame oils (Miyazawa Yakuhin, Tokyo Japan), peanut oil (Kozakai Seiyaku, Tokyo, Japan), soy bean oil (Kanto Chemical, Tokyo, Japan), DHA Pawakyu (Shin Nihon Kanpo, Osaka, Japan), DHA 45% (Jard, Tokyo, Japan), Fish oil EPA with DHA (Otsuka, Tokyo, Japan), and Natural DHA (Taisho Seiyaku, Tokyo, Japan).

2.3. Procedure

The sample size was determined from Table 1 and the sample weighed into a 20-ml round-bottom centrifuge tube. Then 5 ml VK_3 solution was added. Sample solution was prepared by completely dissolving sample in the VK_3 solution, or by extracting free fatty acids from the sample oil into this solution.

Following this, 5 μl of sample solution was injected into the FI system and the signal height recorded and 5 μl 0.1 mM standard acid solution was injected into the FI system and the signal height recorded.

Table 1
Sample size required for fats and oils

Acid value	Sample weight (± 10) (g)
0–0.1	0.4
0.1–1	0.1
1–5	0.01
5–20	0.002
>20	0.001

2.4. Calculation

The acid value, as mg KOH g^{-1} of sample, was calculated as follows.

$$\text{Acid value} = 56.11\, A/(I_S\, W)$$

where A is the signal height for the sample solution (μA); I_S the signal height per unit concentration of standard palmitic acid (μA M^{-1}); and W, grams of oil samples in 1-ml sample solution (g ml^{-1}).

2.5. Stability test

To demonstrate the utility of the method, the stability of pharmaceutical preparations, as acid values of sample oils, was followed for 8 weeks. The samples were placed in a box at a relative humidity of 75% and 40°C [8].

3. Results and discussion

3.1. Optimization of the flow injection system

Any higher fatty acids present in the VK_3 solution caused a reduction wave of protonated VK_3 at a negative potential less than the reduction potential of VK_3 on a voltammogram of the solution. The height of the reduction wave was proportional to acid concentration. The same reduction wave potentials were noted for the higher fatty acids whose dissociation constants were essentially the same. Free fatty acid content in fats and oils, or acid values of fats and oils, could thus be determined based on wave height [6]. Based on the electrochemical reduction of protonated VK_3, FIA was carried out for determination of free fatty acid [7]. Using the walljet-type electrochemical cell (2.4-μl cell volume) as the detector in the FI system, the effect of injection volume was examined by measuring signals for palmitic acid solution. Signal height increased with injection volume, but at larger injection volumes the reproducibility of signal height became progressively worse in replicate measurements. Thus, injection volume in the FI system was set at 5 μl. Signal height was maximum from −0.33 to −0.35 V vs. Ag/AgCl on the hydrodynamic voltammogram obtained for 0.1 mM palmitic acid solution and thus a potential of −0.33 V was chosen as the detection potential for monitoring acid amount. On increasing the flow rate of carrier solution from 0.2 to 1 ml min^{-1}, signal height reached a plateau at 0.6 ml min^{-1} and then decreased slightly (Fig. 1). Signal height could be determined most precisely at this flow rate. Fig. 2 shows flow signals at 0.6 ml min^{-1} for various amounts of palmitic acid. A peak appeared for each amount and then decreased to the base line within 1 min following sample injection. Sixty samples could be analyzed in 1 h. The equation for the calibration curve for signal height (y) vs. acid amount (x) is,

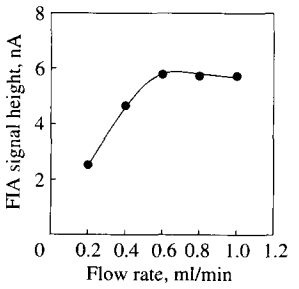

Fig. 1. Effect of flow rate of carrier solution on FIA signal height for 0.1 mM palmitic acid. Injection volume, 5 μl; detection potential, −0.33 V vs. Ag/AgCl; carrier solution, ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$.

$$y(nA) = 7.75x(\text{nmol}) + 0.0302.$$

The correlation coefficient(r) was 0.998 from 0.025 to 1.50 nmol.

3.2. Comparison of the present FIA method with a conventional method for acid value determination

Acid values of camellia oil, glyceryl monostearate, corn oil and saury oil were determined by both the present FIA method and a conventional titration using phenolphthalein as indicator. Though the results for camellia oil were the same, differences by as much as 10% were noted for other oils. To estimate indicator error by colour change in phenolphthalein, four sample oils were

titrated with standard 0.02 M KOH using the potentiometric end-point measured with a pH meter (Fig. 3). End-point breaks were compared with indicator colour changes. As shown in Fig. 3B, C and D, for glyceryl monosterate, corn oil and saury oil, end point breaks could not be detected by phenolphthalein colour change to show the cause of error in the conventional method. The volume of 0.02 M KOH corresponding to acid value by FIA for each sample is shown in Fig. 3 by arrow c, and coincided with that of the end point break of the potentiometric titration curve (arrow b). Acid values determined by FIA are plotted against those be potentiometric titration in Fig. 4. The correlation coefficient was 0.999, suggesting that is a FIA suitable and accurate method for determining the acid values of fats and oils.

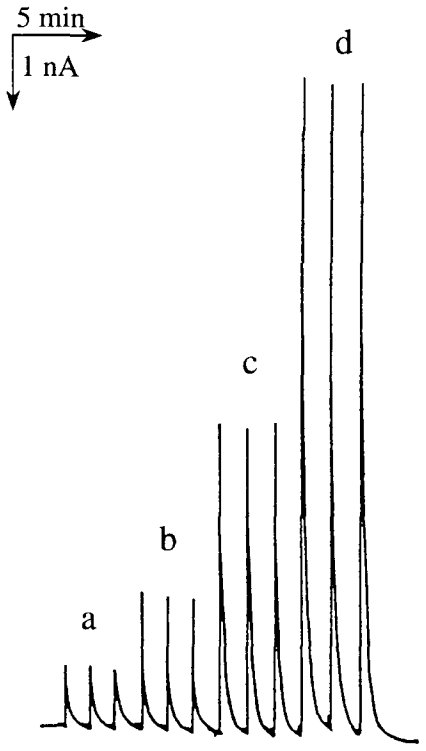

Fig. 2. Signals obtained for: (a) 0.10; (b) 0.20; (c) 0.50 and (d) 1.00 nmol palmitic acid. Injection volume, 5 μl; flow rate, 0.6 ml min^{-1}; detection potential, −0.33 V vs. Ag/AgCl; carrier solution, ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$.

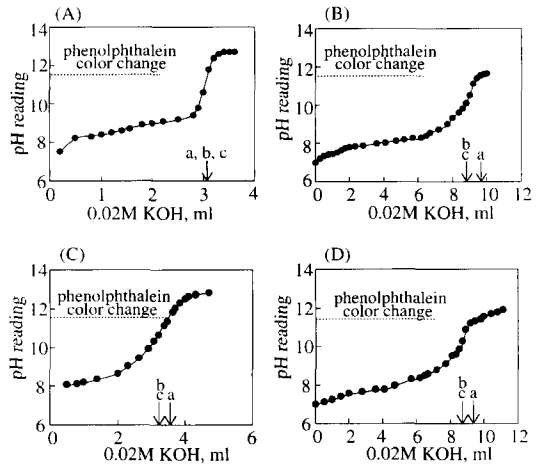

Fig. 3. Titration curves for free fatty acids in: (A) camellia oil; (B) glyceryl monostearate; (C) corn oil; (D) saury oil, all against 0.02 M KOH. Arrow a indicates the end-point by phenolphthalein colour change and arrow b, the potentiometric end-point break. Arrow c shows the volume of 0.02 M KOH corresponding to the acid value determined by FIA.

3.3. Acid values of fats and oils

Acid values of fats and oils for 14 samples determined by FIA are shown in Table 2. The R.S.D. was less than 2.0% ($n = 5$). Sample size was not more than 0.4 g, this amount being only 2–4% that required for a conventional titration. Consequently, the time taken for sample solution

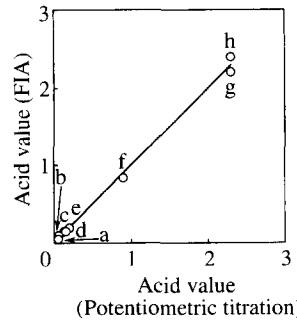

Fig. 4. Correlation of acid values of oil samples determined by the present FIA method with potentiometric titration. Acid values FIA (y) are plotted against those by titration (x), using the regression equation, $y = 0.993\ x + 0.00547$, The correlation coefficient was 0.999. Oil samples are: (a) peanut oil, (b) sesame oil, (c) olive oil, (d) corn oil, (e) mentha oil, (f) glyceryl monostearte, (g) camellia oil and (h) cacao butter.

Table 2
Acid value of fats and oils obtained by FIA

Sample	Acid value	R.S.D., (%) ($n = 5$)
Cacao butter	2.4	2.0
Camellia oil	2.1	1.9
Corn oil	0.15	0.5
Glyceryl monostearate	0.84	1.0
Mentha oil	0.20	1.3
Olive oil	0.15	1.2
Peanut oil	0.057	2.0
Sesame oil	0.095	1.9
Soya bean oil	0.11	1.4
Cod liver oil	0.45	1.8
Fish oil A	2.5	1.6
Fish oil B	0.79	1.7
Fish oil C	0.24	1.2
Fish oil D	0.18	1.1

Fish oil A: DHA Pawakyu (Shin Nihon Kanpou, Osaka).
Fish oil B: DHA 45% (Jard, Tokyo).
Fish oil C: Fish oil EPa with DHA (Otsuka, Tokyo).
Fish oil D: Natural DHA (Taisho Seiyaku, Tokyo).

preparation was reduced. FIA could be conducted much more easily than voltammetry, with data precision being ca. 0.5% better than that by voltammetry.

3.4. Change of acid values of pharmaceutical preparations of oils with time

FIA is clearly shown by the present results to be a simple and rapid means for determining acid values of fats and oils with high sensitivity and accuracy. To confirm the applicability of FIA to stability tests of pharmaceutical preparations, acid values of fish and cod liver oils of pharmaceutical preparations were determined using FIA and followed as a function of time at a relative humidity of 75% and 40°C (Fig. 5). In both cases, the values increased gradually with time, suggesting that degradation occurred. FIA may thus be concluded as being a suitable means for the quality control of pharmaceutical preparations of fats and oils.

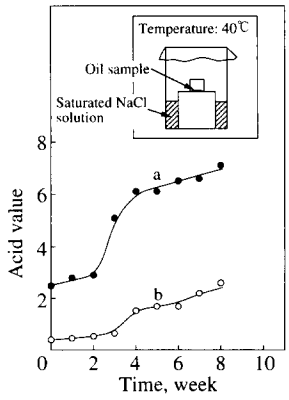

Fig. 5. Effects of time elapsed on acid values of (a) fish oil and (b) cod liver oil. The sample oils were maintained at a humidity of 75% and 40°C.

4. Conclusions

A novel FIA method was developed to determine the acid values of fats and oils with good accuracy, high sensitivity, simplicity of operation and in less time than conventional titration. Accurate quality and stability assessment of fats and oils over time is possible by this method.

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