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Determination of iodine values using 1,3-dibromo-5,5-dimethylhydantoin (DBH) without the employment of chlorinated hydrocarbons

Analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern Part 17*

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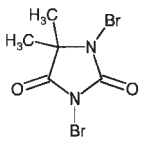
Low and medium iodine values of fixed oils and fats can be determined in glacial acetic acid within reduced waiting times of only 5 min. Highly unsaturated compounds such as those of linseed oil, cod-liver oil, sunflower oil, soybean oil, wheat germ oil and the emulsifier sorbitan trioleate result too low values in comparison to PH. EUR. 2002 [2] and USP 2000 [3]. Cocoa butter with a low iodine number is insoluble in glacial acetic acid. The iodine values of nonionogenic emulsifiers such as ceteareth-30 (Macrogol cetostearyl ether PH. EUR. 2002), oleth-10 resp. 20 (Macrogol oleyl ether PH. EUR. 2002) and polysorbate-80 PH. EUR. 2002 are obtained in aqueous solutions. Oleth-2 (Macrogol oleyl ether PH. EUR. 2002), polyoxyl-40 castor oil (Macrolglycerol ricinoleate PH. EUR. 2002), polysorbate-60 PH. EUR. 2002 and sorbitan trioleate PH. EUR. 2002 need the addition of ethyl acetate. Fixed oils even with high iodine values can be determined in an o/w emulsion with a reaction time of 5 min in most cases, when nonionogenic emulsifiers such as ceteareth-30, polyoxyl-30 glycerol monolaurate or polyoxyl-60 hydrogenated castor oil (Macrolglycerol hydroxystearate PH. EUR. 2002) are used.

1. Introduction

The iodine value (iodine number) is characteristic for the content of unsaturated fatty acids in fats, fixed oils, emulsifiers and solubilizers [4]. Bromine is added to the double bonds. After the addition of potassium iodide the excess of the halogenating agent reacts to iodine, which has to be titrated with thiosulfate. The determination of the iodine value is of important significance for pharmaceutics, food chemistry, cosmetics and others. The iodine monobromide reagent according to Hanuš [5], applied for PH. EUR. 2002 and USP 2000, can be more simply produced with DBH and potassium iodide or DBH and iodine, as recently published [6]. DBH is a stable and easy to handle crystalline compound in contrast to iodine monobromide [6, 7]. PH. EUR. 2002 and mostly USP 2000 use enhepatoxic and environmentally hazardous chloroform for the determination of the iodine value. The exchange of carbon tetrachloride or chloroform by cyclohexane [8–10] or cyclohexane/glacial acetic [11] for the Wijs method using iodine monochloride is described. However, long reaction times from 1 to 2 hours are necessary.



DBH

2. Investigations, results, and discussion

2.1. Determination in glacial acetic acid using DBH/KI

As shown in Table 1 chloroform can be replaced by glacial acetic acid analysing fats, fixed oils and emulsifiers with iodine values up to about 120. The results correspond to those of PH. EUR (see Fig. 1). The reaction time can be reduced from 30 to 5 min.

Considerably too low and intensely varying results are obtained determining linseed oil with a high iodine value. The results of cod-liver oil (IZ 169, bias = −11%), sunflower oil (IZ 134, bias = −2.0%), soybean oil (IZ 133, bias = −17.7%) and wheat germ oil (IZ 128, bias = −3.0%) are unsatisfying. Also the emulsifier sorbitan trioleate with a large variation (IZ 80 m. bias = −3.1%; RSD = 1.8) and the margarine Flora soft (IZ 64.4; RSD = 3.1) yield unsatisfactory results. Reddish colored, sticky precipitations are formed during the reaction and titration of these substances.

Linseed oil contains a high amount of multiple unsaturated fatty acids, which are obviously insoluble in glacial acetic acid after addition of one mole of bromine and form reddish colored, sticky precipitations. A quantitative bromination is no longer possible. The exchange of glacial acetic acid by propionic acid, additions of medium

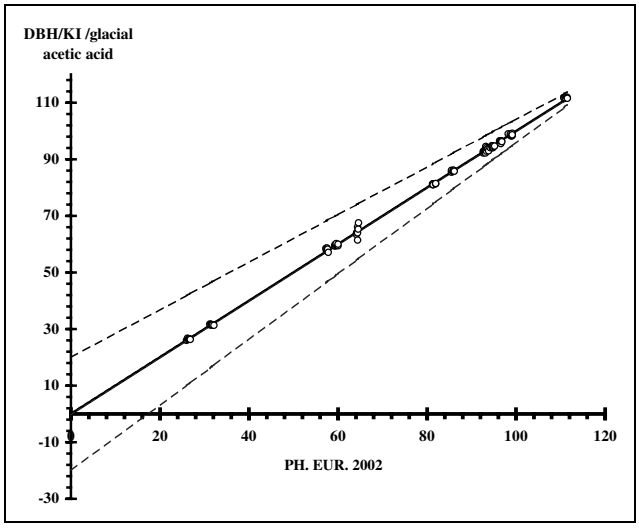


Fig. 1: Method comparison according to Passing-Bablok [12–14] of the iodine value determination using DBH/KI in glacial acetic acid by comparison to PH. EUR. 2002 (95% confidence)

Table 1: Determination of iodine values of fixed oils, fats and emulsifiers using DBH/KI in glacial acetic acid by comparison to PH. EUR. 2002

Material	Expected iodine value	Weight of sample mg	DBH/KI			PH. EUR. 2002		
			n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Almond oil	95–105[a]	132–192	7	98.7	0.39	7	98.8	0.33
Arachis oil, peanut oil	84–100[a]	109–190	7	92.7	0.43	7	92.8	0.22
Becel, margarine, 80% fat	90[b]	235–357	7	93.7	0.65	7	93.6	0.37
Butter	21–29[c]	237–344	7	26.4	1.00	7	26.4	1.12
Castor oil	83–88[a]	121–183	7	85.9	0.27	7	85.8	0.27
Cod-liver oil	145–180[a]	107–180	7	144.9	4.1	7	163.1	0.23
Flora, soft, margarine 80% fat	79[b]	256–364	7	64.6	3.1	7	64.4	0.27
Lard	46–60[d]	233–543	7	58.1	0.94	7	57.5	0.35
Lätta, margarine, 40% fat	29.2[b]	238–439	7	31.5	0.59	7	31.6	0.97
Linseed oil H. Lamotte	165–190[e]	102–158	7	177.8	1.8	7	192.4	0.18
Linseed oil Thywissen		111–168	7	165.4	2.2	7	180.8	0.07
Oleic acid	85–95[a]; 89.9[f]	194–226	7	94.5	0.33	7	94.8	0.24
Oleyl oleate	85–105[d]	216–268	6	96.3	0.40	7	96.6	0.16
Olive oil	79–88[a]	169–237	7	81.2	0.19	7	81.5	0.35
Rama, margarine, 80% fat	59.2[b]	245–361	7	59.7	0.55	7	59.6	0.50
Sesame oil	103–116[a]	102–207	7	111.7	0.15	7	111.1	0.33
Sorbitan monolaurate	$\leq$10.0[g]	743–1237	7	5.1	2.53	7	5.1	1.04
Sorbitan monooleate	62–76[a]	140–266	7	65.5	0.52	7	66.5	0.37
Sorbitan trioleate	77–85[g]	201–276	7	77.7	1.77	7	80.2	0.33
Soybean oil	120–141[a]	113–230	7	109.9	3.4	7	132.5	0.18
Sunflower oil	125–136[h]	110–158	7	131.6	0.29	7	134.3	0.27
Wheat germ oil	115–129 [17]	106–129	7	124.3	0.23	7	128.1	0.28

chain triglycerides (Miglyol® 812 N), sodium salts of sec. $-C_{13}-C_{17}$ alkane sulfonates (Marlon® PS 30 and Marlon® PS 65, Condea), sec.$-C_{14}-C_{17}$ alkane sulfonic acid (Hoechst AG), polyethylene glycol 200 or the application of DBH/I_2 do not improve the results. Cocoa butter has a low iodine value, but it is slightly soluble in glacial acetic acid, and for this reason the determination cannot be performed.

2.2. Iodine value determination of emulsifiers, solubilizers and surfactants in aqueous solutions

PH. EUR. 2002 and USP 2000 use chloroform and iodine monobromide to determine the iodine value according to Hanuš for some water soluble emulsifiers. Ceteareth-30 (Macrogol cetostearyl ether PH. EUR. 2002, Emulgin B-3), oleth-10 (Macrogol oleyl ether PH. EUR. 2002, BRIJ® 97), oleth-20 (Macrogol oleyl ether PH. EUR. 2002, BRIJ® 98) and polysorbat 80 PH. EUR. 2002 (Tween® 80) yield in aqueous solution results comparable with those of PH. EUR. 2002, as shown in Table 2. The reaction time can be reduced to 5 min. Waiting times of 5 and 30 min show no significant difference for oleth 20. The freely water soluble polyoxyl-30-50 castor oil (Macrogolglycerol ricinoleate PH. EUR. 2002, Cremophor® EL) forms a reddish colored, sticky precipitation, when KI is added after the reaction time. Therefore the addition of ethyl acetate is necessary before KI is added. Some emulsifiers such as oleth-2 are only dissolved in water after long stirring. In this case it is advantageous to dissolve the emulsifier in a small amount of ethyl acetate and then to emulsify with water. DBH/KI yields problems by formation of precipitations, which cannot be observed using DBH/I_2. Polyoxyl-30-50 castor oil (Macrogolglycerol ricinoleate PH. EUR. 2002) results in aqueous solution in the tolerance of PH. EUR. 2002 slightly higher iodine values than obtained according to the method of the pharmacopoeia.

2.3. Iodine value determinations of fixed oils in aqueous solution by application of nonionogenic emulsifiers

In contrast to the determination in glacial acetic acid iodine values corresponding to PH. EUR. 2002 and USP 2000 (see Table 1 and 2 and Fig. 2) are obtained by dissolution of fixed oils such as linseed oil, cod-liver oil, sunflower oil and cocoa butter in small amounts of ethyl acetate. Afterwards the solution is dispersed with a nonionogenic emulsifier such as ceteareth-30. The reaction times of fixed oils are also reduced generally to 5 min, if an o/w emulsion is used. Only for linseed oil with a very high iodine value, 15 min are necessary. Using waiting

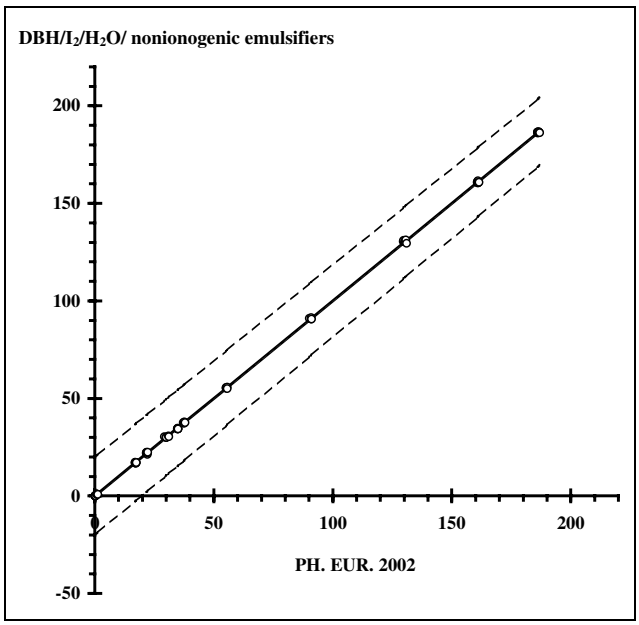


Fig. 2: Method comparison according to Passing-Bablok [12–14] of the iodine value determination using DBH/I_2/H_2O and nonionogenic emulsifiers by comparison to PH. EUR. 2002 (95% confidence)

Table 2: Determination of iodine values of fixed oils, fats and emulsifiers using DBH/I_2 in aqueous solution by comparison to PH. EUR. 2002

Material	Expected iodine value	Weight of sample (mg)	Indication	DBH/I_2				PH. EUR. 2002			Addition
				Reaction time (min)	n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)	
Arachis oil, peanut oil	84–100[a]	215–248	visual	5	4	90.9	0.43	4	90.7	0.46	Ethyl acetate, Ceteareth-30
		177–184	visual	5	7	100.7	0.16	7	101.4	0.37	Polyoxyl-60 hydrogenated castor oil
			potentiom.	5	7	100.5	0.19	7	101.2	0.23	
		172–186	visual	10	7	100.6	0.33	7	100.1	0.20	Polyoxyl-60 hydrogenated castor oil
			potentiom.	10	7	100.4	0.31	7	100.9	0.15	
		176–187	visual	15	7	100.9	0.16	7	101.3	0.13	Polyoxyl-60 hydrogenated castor oil
			potentiom.	15	7	100.6	0.15	7	101.0	0.21	
Ceteareth-30	≤2.0[g]	985–1339	visual	5	7	0.09	46.6	7	0.15	72.4	–
Cocoa butter	33–42[a]	306–347	visual	5	7	34.5	0.54	7	34.8	0.28	Ethyl acetate, Ceteareth-30
Cod-liver oil	150–180[a,g]	116–131	visual	5	7	160.9	0.25	7	161.0	0.16	Ethyl acetate, Ceteareth-30
Linseed oil	165–190[e]	105–117	visual	5	7	184.4	0.21	7	186.4	0.20	Ethy lacetate, Ceteareth-30
				15	7	186.4	0.13				
				30	7	187.3	0.18				
		99–111	visual	15	7	187.3	0.38	7	186.5	0.33	Ceteareth-30
			potentiom.	15	7	187.0	0.40	7	186.3	0.33	Ceteareth-30
		90–112	visual	15	7	187.1	0.31	7	186.9	0.16	Polyoxyl-30 glycerol monolaurate
			potentiom.	15	7	186.7	0.31	7	186.6	0.14	
		105–116	visual	5	7	183.7	0.43	7	185.9	0.27	Polyoxyl-60 hydrogenated castor oil
			potentiom.	5	7	183.0	0.51	7	185.2	0.24	
		105–119	visual	10	7	186.9	0.60	7	187.3	0.32	Polyoxyl-60 hydrogenated castor oil
			potentiom.	10	7	186.1	0.64	7	186.5	0.39	
Oleth-2	48–74[g]	391–446	visual	5	4	55.5	0.60	4	55.4	0.48	Ethyl acetate
Oleth-10	24–38[g]	425–499	visual	5	4	37.6	0.76	4	37.6	0.77	–
Oleth-20	14–24[g]	787–1278	visual	5	8	17.1	1.00	8	17.2	1.21	–
Polyoxyl 30-50 castor oil	25–35[g]	280–495	visual	5	7	34.4	0.57	7	31.2	2.3	Ethyl acetate
Polysorbate 60	≤5.0[g]	905–1435	visual	5	5	0.89	12.7	5	0.94	72.4	Ethyl acetate
Polysorbate 80	18–24[h]	712–1275	visual	5	7	22.0	1.90	7	21.9	1.01	–
Safflower oil	135–150[a]	110–119	visual	15	7	140.8	0.45	7	140.8	0.42	Ceteareth-30
			potentiom.	15	7	140.5	0.46	7	140.6	0.41	
		97–112	visual	15	7	140.3	0.24	7	140.4	0.28	Polyoxyl-30 glycerol monolaurate
			potentiom.	15	7	140.0	0.26	7	140.2	0.25	
		117–121	visual	10	7	139.7	0.18	7	140.3	0.30	Polyoxyl-60 hydrogenated castor oil
			potentiom.	10	7	139.3	0.18	7	139.8	0.23	
		115–122	visual	15	7	138.9	0.42	7	139.6	0.26	Polyoxyl-60 hydrogenated castor oil
			potentiom.	15	7	138.6	0.38	7	139.0	0.19	
Sorbitan trioleate	76–90[g]	219–282	visual	5	7	80.2	0.77	7	80.2	0.33	Ethyl acetate
Sunflower oil	120–140[h]	124–152	visual	5	7	130.6	0.43	7	130.3	0.37	Ethyl acetate, Ceteareth-30

times of 5 min yield results about 1% too low in comparison with PH. EUR. 2002. Waiting 30 min results values about 0.5% too high. Beside ceteareth-30 polyoxyl-30 glycerol monolaurate and polyoxyl-60 hydrogenated castor oil (Macrolglycerol hydroxystearate PH. EUR. 2002) also prove as suitable nonionogenic emulsifiers. For the application as emulsifier it is important that the chain of the polyether part of the molecule is large enough and the iodine value is preferably low. Thus emulsifiers with short polyethylene chains such as ceteareth-12 and ceteareth-20 are unsuitable. Fixed oils can also be determined without the addition of ethyl acetate, as shown in Table 2. Problems can arise by the slight elimination of the oily phase, if the prescription is not observed exactly. On the other hand too low values are obtained for linseed oil, when the concentration of ethyl acetate is increased. Starch solutions do not yield the characteristic blue color with iodine, if ethyl acetate or nonionogenic emulsifiers are present. Nevertheless, the change of the yellow emulsion to white at the end point can be well recognized. A potentiometric

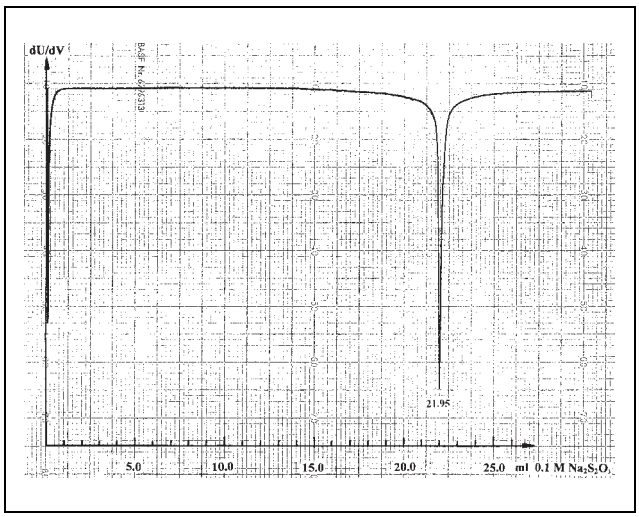
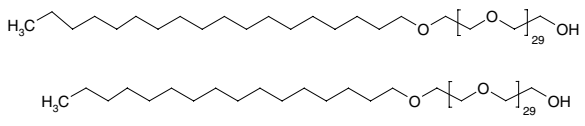
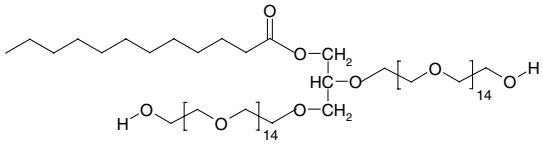


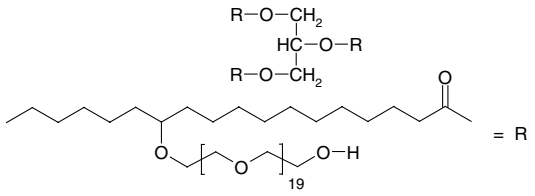
Fig. 3: Iodine value determination of linseed oil in o/w emulsion using ceteareth-30, ethyl acetate and potentiometric indication



Ceteareth-30, Macrogol cetostearyl ether PH. EUR. 2002 [68439-49-6]



Polyoxyl-30 glycerol monolaurate [51248-32-9]



Polyoxyl-60 hydrogenated castor oil,

Macrolglycerol hydroxystearate PH. EUR. 2002 [61788-85-0]

indication (see Fig. 3), which allows an automatization, is recommended.

Furthermore, it should be mentioned that oil is not eliminated in sticky form on the walls and the bottom of the flask and on the stirrer rod during the titration with thiosulfate, when emulsifiers in contrast to glacial acetic acid as solvent are used.

Ionogenic emulsifiers such as sodium dodecyl sulfate prove unsuitable. Too low concentrations of nonionogenic emulsifiers can lead to an elimination of the fixed oil, too low concentrations of KI to a prolongated change at the end point.

In conclusion, the reagent according to Hanuš can be prepared significantly simplier and less dangerous for the analyst using DBH [6]. The application of hepatotoxic and environmentally hazardous chloroform for analytical determinations is nowadays considered to be obsolete. Chlorinated solvents can be replaced by glacial acetic acid and the reaction time is reduced from 30 to 5 min. However, this method is restricted to certain fats, fixed oils, and emulsifiers with low iodine values. The application of nonionic emulsifiers also avoids chlorinated solvents and makes it possible to determine fixed oils with high iodine values. The reaction times are significantly shorter in comparison with PH. EUR. 2002 and USP 2000. Determinations using cyclohexane with long reaction times cannot be recommended for routine analyses.

3. Experimental

3.1. Instrumentation

Metrohm potentiograph E 536 with Metrohm dosimat 665, 20 and 50 ml exchange unit, platinum electrode (Pt/Ag/AgCl) Metrohm AG, No. 9100

3.2. Materials

Almond oil [8007-69-0] H. Lamotte, Bremen; arachis oil, peanut oil [8002-03-7] H. Lamotte, Bremen; avocado oil [8024-32-6], H. Lamotte, Bremen; Becel, diet-margarine, Union Deutsche Lebensmittelwerke, Hamburg; castor oil [8001-79-4], Mainland, Pharm. Fabrik, Frankfurt; ceteareth-30, Macrogol cetostearyl ether PH. EUR. 2002, Emulgin B-3 68439-49-6, Cognis, Düsseldorf; cocoa butter [8002-31-1], Caelo, Hilden; cod-liver oil [8001-69-2], H. Lamotte, Bremen; 1,3-dibromo-5,5-dimethylhydantoin = 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione [77-48-5], for synthesis Merck art. 803600 = DBH (for analytical purpose qualified); Flora soft, 80% fat, Union Deutsche Lebensmittelwerke, Hamburg; Lätta, semi-bold margarine 40% fat, Union Deutsche Lebensmittelwerke, Hamburg; lard, Mainland, Pharm. Fabrik, Frankfurt; linseed oil [8001-26-1], H. Lamotte, Bremen resp. Unimills, Hamburg; oleic acid [112-80-1], Fluka; oleth-2, Macrogol oleyl ether PH. EUR. 2002, polyoxyl-2 oleyl ether, BRIJ® 92 [9004-98-2], ICI; oleth-10, Macrogol oleyl ether PH. EUR. 2002, polyoxyl-10 oleyl ether, BRIJ® 97 [9004-98-2], ICI; oleth-20, Macrogol oleyl ether PH. EUR. 2002, polyoxyl-20 oleyl ether, BRIJ® 98 [9004-98-2], ICI; oleyl oleate, Cetiol®, Cognis, Düsseldorf; olive oil [8001-25-0], H. Lamotte, Bremen; polyoxyl-30-50 castor oil, Macrogolglycerol ricinoleate PH. EUR. 2002, Cremophor® EL, 9004-98-2, (BASF); polyoxyl-30 glycerol monolaurate [51248-32-9], Tagat L, Goldschmidt, Essen; polyoxyl-60 hydrogenated castor oil, Macrogolglycerol hydroxystearate PH. EUR. 2002, Emulgin HRE 60 [61788-85-0], Cognis, Düsseldorf; polysorbate 60 [9005-67-8], Tween® 60, ICI; polysorbate 80 [9005-65-6], Tween® 80, ICI; Rama, margarine, breakfast quality, 80% fat, Union Deutsche Lebensmittelwerke, Hamburg; safflower oil [8001-23-8], Lidl-Stiftung & Co KG, Neckarsulm; sesame oil [8008-74-0] Mainland, Pharm. Fabrik, Frankfurt; sorbitan monolaurate [1338-39-2], SPAN® 20, ICI; sorbitan monoleate PH. EUR. 2002 [1338-43-8], SPAN® 80, ICI; sorbitan trioleate [26266-58-0], SPAN® 85, ICI; soya oil [8001-22-7], H. Lamotte, Bremen; sunflower oil [8001-21-6], H. Lamotte, Bremen; triglycerides, medium-chain PH. EUR. 2002, caprylic/capric triglyceride, Miglyol® 812N, Condea, Witten; wheat germ oil [8006-95-9], Caelo, Hilden.

3.3. Solutions

0.03 M Ceteareth-30: 4.73 g (3 mmol) of ceteareth-30 are dissolved with H_2O by heating on a water bath for a short time, and after cooling to room temperature the solution is diluted with H_2O to 100 ml. *DBH/I_2:* 3.57 g (12.5 mmol) of DBH and 6.35 g (25 mmol) of iodine are dissolved in 500 ml of glacial acetic acid. When heating the iodine in glacial acetic acid on a water bath, cooling to room temperature and dissolving DBH afterwards, the preparation time can be reduced. Prepare before use. *DBH/KI:* 7.15 g (25 mmol) of DBH and 8.30 g (50 mmol) of potassium iodide are dissolved in glacial acetic acid to 500 ml; *0.02 M polyoxyl-30 glycerol monolaurate:* 6.39 g (4 mmol) of polyoxyl-30 glycerol monolaurate are dissolved with about 150 ml of H_2O by heating on a water bath for a short time. After cooling to room temperature the solution is diluted with H_2O to 200 ml. *0.05 M polyoxyl-60 hydrogenated castor oil:* 35.8 g (10 mmol) of polyoxyl-60 hydrogenated castor oil are dissolved with about 150 ml of H_2O by heating on a water bath for a short time and after cooling to room temperature the solution is diluted with H_2O to 200 ml.

3.4. Assays

Samples are put into a cut off micro test tube of about 1 cm in length and about 0.6 cm in diameter or directly into the iodine flasks. Therefore, a tared pipette with the sample to be analysed is used and weighed back after pipetting into the flask.

3.4.1. Iodine value determinations of fats, fixed oils and emulsifiers using glacial acetic acid and DBH/KI by comparison to PH. EUR. 2002

Dissolve the sample to be analysed in 20 ml of glacial acetic acid. Add 20 ml of DBH/KI and stir 5 min under light protection. Put 10 ml of 0.2 M KI into the flask and titrate with 0.1 M $Na_2S_2O_3$ with addition of 1 ml of starch solution, iodide-free, PH. EUR. 2002 [6] at the end of the titration. An indicator change from dark greyish brown to colorless can be observed.

For the dissolution of Becel in 20 ml of glacial acetic acid it is necessary to heat 2–5 min on a water bath of 40 °C, for Lätta of about 50 °C, for butter, Flora and Rama of 90 °C. Lard is dissolved in 20 ml of glacial acetic acid and 5 ml of medium chain triglycerides (Miglyol® 812N) with heating of about 40 °C. Precipitations formed from Becel, Lätta or Rama do not affect the analytical results.

3.4.2. Iodine value determinations of olive oil in glacial acetic acid with various reaction times

5 min: n = 3, mean = 81.4, RSD = 0.31; 10 min: n = 3, mean = 81.0, RSD = 0.07;
20 min: n = 3, mean = 81.2, RSD = 0.26; 30 min: n = 3, mean = 81.5 RSD = 0.35.

3.4.3. Iodine value determinations of emulsifiers, solubilizers and surfactants in aqueous solutions

Dissolve the weighed sample in 20 ml of H_2O with stirring and heat, if necessary. Add 20 ml of DBH/I_2 and stir 5 min under light protection. Pipette 10 ml of 0.5 M KI and titrate with 0.1 M $Na_2S_2O_3$ and visual (yellow to colorless) or with potentiometric indication.

3.4.4. Iodine value determinations of emulsifiers, solubilizers and surfactants in aqueous solutions with addition of ethyl acetate

Dissolve the weighed sample in 5 ml of ethyl acetate. Add 20 ml of DBH/I_2 and afterwards 15 ml of H_2O with stirring. Stir 5 min under light protection. Pipette 10 ml of 0.5 M KI and titrate with 0.1 M $Na_2S_2O_3$ and visual (yellow to colorless) or potentiometric indication. The readily water soluble polyoxyl-30-50 castor oil is dissolved at first in 20 ml of H_2O, and then 20 ml of DBH/I_2 is pipetted. After 5 min under light protection and stirring 5 ml of ethyl acetate is added before the addition of KI to avoid the formation of a precipitate.

3.4.5. Iodine value determinations of oleth-20 with various reaction times

5 min: n = 4, mean = 17.1, RSD = 0.74; 30 min: n = 4, mean = 17.3, RSD = 1.97

3.4.6. Iodine value determinations of fixed oils in o/w-emulsion using ceteareth-30 and addition of ethyl acetate

Dissolve the weighed sample in 5 ml of ethyl acetate. Add 20 ml of DBH/I_2 and afterwards 20 ml of 0.03 M ceteareth-30 with stirring. Stir 5 min under light protection. Pipette 10 ml of 0.5 M KI and titrate with 0.1 M $Na_2S_2O_3$ and visual (the yellow emulsion becomes white) or potentiometric indication. For linseed oil a reaction time of 15 min is necessary.

3.4.7. Determination of iodine values of fixed oils using nonionic emulsifiers, DBH/I_2, and without ethyl acetate

Dissolve about 0.2 g of arachis oil resp. about. 0.1 g of linseed oil resp. 0.1 g of safflower oil, accurately weighed, in 25 ml of DBH/I_2 with stirring. Add slowly 50 ml of 0.03 M ceteareth-30 or 0.02 M polyoxyl-30 glycerol monolaurate also with stirring and stir for further 5 to 15 min (see Table 2) under light protection. Pipette 10 ml of 0.5 M KI and titrate with 0.1 M $Na_2S_2O_3$ and visual (the yellow emulsion becomes white) or with potentiometric indication. Using polyoxyl-60 hydrogenated castor oil only 20 ml of DBH/I_2 and 10 ml of 0.05 M emulsifier are necessary.

3.5. Statistical methods

Evaluations and graphics have been performed with Excel 97 on an IBM-compatible PC running under Windows 95. The built-in F- and t-test routines of Excel 97 have been used. The transformation of the Passing-Bablok algorithm [12–14] to an Excel-macro according to Dr. Martin Holz, D 79395 Neuenburg has been applied.

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* Part 16 [1]

[a] USP 2000 p. 322, 461, 1499, 1544, 2412, 2439, 2486, 2487, 2488, 2512, 2521

[b] calculated to the found iodine value of the fatty phase according to Wijs and the fat content reported by the Union Deutsche Lebensmittelwerke GmbH.

[c] calculated to the fat content of 82–84% [15] of butter and the iodine value of milk fat with 26–35 [16]

[d] DAB 2000

[e] DAB 1968 p. 634

[f] calculated value.

[g] PH. EUR. 2002 p. 1498, 1501, 1505, 1783, 1944, 1946

[h] PH. EUR. 1997 p. 261, 1355

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