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ARTICLES

Determination of pK_a Values of Clinically Important Perfluorochemicals in Nonaqueous Media[1]

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Abstract—Perfluorochemicals (**PFC**s) are clinically and biotechnologically important species. In this work, the potentiometric titration method in nonaqueous media is proposed for the determination of acidity constant values of six different perfluorinated compounds. The saturated and nonsaturated perfluoroacids, perfluorocarnosine, and perfluoroalkyl-β-alanine were potentiometrically titrated in acetonitrile, N,N-dimethylformamide, acetone, ethanol, methanol, and pyridine with tetrabutylammonium hydroxide. The half-neutralization potentials and acidity constants of PFCs have been calculated from the titration curves by using the computerized derivative method. Except for R_F-Carnosine, all of the potentiometric titration curves of the PFCs exhibited one stoichiometric and well-defined endpoints in all of the solvents employed. The reproducibility and sensitivity of the method were evaluated.

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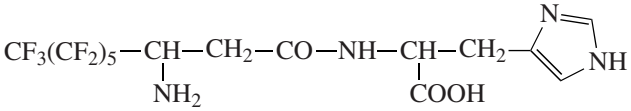
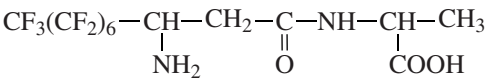
Perfluorochemicals are organic compounds in which all hydrogen atoms are replaced by fluorine. Since PFCs can dissolve large volumes of respiratory gases, their clinical and biotechnological aspect becomes increasingly important [1]. The emulsions of perfluorinated compounds were proposed as artificial blood; therefore, many works have been devoted to this area [2–4] and the efforts resulted in the commercialization of these products [5–8]. Two commercially available PFC emulsions are Flusol-DA 20% (**F-DA**) and flusol–43/Oxypherol (**FC-43**) both manufactured by Green Cross Corporation, Japan. Flusol-DA 20% was used for clinical purposes as an oxygen carrier during percutanewous transluminal coronary angioplasty (balloon angioplasty) [3, 4]. The stable emulsions of PFCs are also useful as contrast agents for biological imaging by the modalities of nuclear magnetic resonance, X-ray, and ultrasound [8]. PFCs are water insoluble, chemically and biochemically inert, and have high dissolution capacities for oxygen. Their inertness to heat and chemical reagents results from their strong C–F bonds (about 116 kcal/mole, i.e., 20 kcal/mole more than a standard C–H bond) and the dense coating of electron-rich repellent fluorine atoms, which protects the carbon backbone [4]. Knowledge of the dissociation constants (pK_as) of these compounds is of fundamental importance in order to provide information for scientists working on their chemical reactivity, salt formation, purification process, and chromatographic separations (retention times and selectivity dependence of the mobile phase pH).

When the above-mentioned characteristics of the PFCs are considered, the potentiometric titration in nonaqueous media looks like the most proper method for the pK_a determination of perfluoro compounds.

In this context, in comparison with the thermometric, amperometric, and spectrophotometric methods, the potentiometric method in nonaqueous media would yield valuable information about the acidities or basicities of the compounds. Many organic acids [9], their binary mixtures [10], enols, phenols, imides, and schiff bases [10–16] have successfully been titrated in nonaqueous media.

This paper first describes acidity constant (pK_a) determination of six clinically important PFC derivatives. The potentiometric titrations of saturated and nonsaturated perfluoroacids, perfluorocarnosine, and perfluoro-β-alanine compounds were performed with a strong quaternary ammonium base; tetrabutylammonium hydroxide (**TBAOH**) in acetonitrile, N,N-dimethylformamide (**N,N-DMF**), acetone, ethanol, methanol, and pyridine. Schematic structures of the titrated perfluoroacids and abbreviations of the employed perfluorocompounds are given below:

[1] The text was submitted by the authors in English.

Compound	Abbreviation
$CF_3(CF_2)_5–CH_2–COOH$	$C_6F_{13}C_2H_3O_2$
$CF_3(CF_2)_7–CH_2–COOH$	$C_8F_{17}C_2H_3O_2$
$CF_3(CF_2)_9–CH_2COOH$	$C_{10}F_{21}C_2H_3O_2$
$CF_3(CF_2)_4–CF=CHCOOH$	$C_6F_{12}C_2H_2O_2$
	R_F-Carnosine
	R_F-β-Ala-Ala

EXPERIMENTAL

The 0.1000 N solution of tetrabutylammonium hydroxide (for titrations in nonaqueous media) was purchased from Merck and diluted with chromatographic grade propan–2-ol (Merck). The resulting solution was standardized against freshly sublimed benzoic acid. The concentration of the solution was 0.0472 M and it was kept in a refrigerator when not in use as advised in the literature [9, 10]. Pyridine, acetonitrile, N,N-dimethylformamide, and acetone were purchased from Merck (99.9% purity) and used without further purification. Ethanol and methanol were purchased from Carlo Erba and dried with a Linde-type 4A molecular sieve. The PFCs were synthesized according to the literature [17, 18].

An Orion Model 720-A digital pH-meter with a modified combined glass electrode (Ingold, 9102 SC) was used for potentiometric titrations. A magnetic stirrer, a semimicro burette with a sensitivity of 0.01 mL, was used throughout this work. All titrations were carried out in 30-mL beakers with the temperature control at 25 ± 1 °C.

Table 1. The reported autoprotolysis constants, potential ranges of solvents used and the measured upper and lower potential limits against 0.1 M $HClO_4$ (in dioxane) and 0.1 M $(C_4H_9)_4$ NOH (in 2-propanol). The auotoprotlysis constant and potential range of water are given for comparison

Solvent	K_0	Reported Potential	Range	Measured Potential	Range
Water	10^{-14}	–300	+240	–317	+222
N,N-DMF	$\approx 10^{-18}$	–1000	+270	–900	+237
Methanol	$10^{-16.7}$	–250	+450	–273	+400
Acetonitrile	$10^{-28.6}$	–970	+590	–999	+540
Acetone	$\approx 10^{-30}$	–970	+660	–965	+598
Pyridine	–	–1000	+50	–900	+57
Ethanol	$10^{-19.8}$	–	–	–597	+60
Isopropanol	$10^{-20.6}$	–750	+400	–720	+407
Dioxane	–	–109	+750	–57	+732

The combined glass electrode was modified by emptying its internal buffer (aqueous KCl solution) and refilling it with a saturated solution of KCl in dry ethanol [9]. In order to obtain reliable and reproducible potentiometric titration curves, the potential readings must be calibrated against a buffer solution with potential readings of –17.0 mV and pH 7.0 [10]. In our case, the glass electrode was calibrated before each series of measurements against a (tetra-nbutylammonium picrate + picric acid) buffer solution at one point (pH 7.0; –17 mV) and conditioned in the solvents under investigation. The upper and lower limits of the potential readings were checked in each solvent against 0.1000 M of $HClO_4$ (in dioxane) and 0.1000 M of $(C_4H_9)_4NOH$ (in 2-propanol). The obtained results shown in Table 1 are in good agreement with the literature [19].

RESULTS AND DISCUSSION

One of the most important targets of this study is to perform the precise determination of the acidity constants of the inert and water-nonsoluble perfluoro derivatives in nonaqueous media. For this purpose, three analogs of saturated perfluoroalcanoic acids (C_nF_{2n+1}–COOH, n = 6, 8, 10), one nonsaturated perfluoroalcanoic acid (C_nF_{2n}–COOH, n = 6), one perfluorocarnosine derivative (R_F-Carnosine), and one perfluoro β-alanine (R_F-β-Ala-Ala) were titrated potentiometrically in the chosen nonaqueous solvents with a strong quaternary ammonium base, tetrabutylammonium hydroxide.

The pH scale in nonaqueous solvents is governed by the autoprotolysis (auotodissociation) constant of the solvent. The chosen solvents having quite small autoprotolysis constants ($K_s = 10^{-n}$, where n is high) are advantageous for acid titrations, because their longer millivolt scale provides a better opportunity for precise titrations of PFCs. The K_0 values of the solvents employed in this study were in the range of 10^{-18}–10^{-30}. The reported autoprotolysis constants, reported potential ranges, and the measured ones for the employed solvents are given in Table 1.

Since the acids are nonsoluble in water, it is nearly impossible to assess their acidic characteristics or acidic behavior in known water-solvent systems and a known pH scale. Additionally, both the potential range (–300 to +240 mV) and the pH scale of water (K_0: 10^{-14}; pH 0–14) are not large enough to assess their acidities. Employed solvents provided large potential ranges and exhibited potential diapasons ranging from –1000 to +660 mV (see Table 1) [9, 10, 15–21]. Among them, acetone provided the largest potential range (–970 to +660 mV) and pH scale (K_0: 10^{-30} pH 0–30). Therefore, the acidic characteristics of the perfluoro derivatives can be investigated with a better resolution in acetone. All of the recorded HNPs are in the acidic potential regions of the nonaqueous solvents employed. In acetone, the HNP of the acids were found between (–) 105 and (–) 268 mV, which corresponds to the acidic region of the long milivolt range of the solvent.

It is convenient to use the ionization constants that have been determined in aqueous solutions for various acids to predict the relative strengths. The pH scale in aqueous solutions is governed by K_w (10^{-14}) and an equilibrium in water predicts a potential change of 0.059 V (59 mV) for each unit change in the pH for the linear region of the equation $E = K - 0.059$ pH [13].

For a precise description of the endpoint, and consequently, the pK_a value, the computerized derivative method was used [20]. In the titration of a monoprotic acid with a strong base, it is often assumed that pH = pK_a when $V_b = V_{eq}/2$. At pH = pK_a, $\alpha_{A^-} = 0.5$, so that the equation (1) can be reduced to equation (2):

$$\frac{V_b}{V_a} = \frac{(c_a\alpha_{A^-}) - \Delta}{c_b + \Delta}, \quad \text{(I)}$$

$$\frac{V_b}{V_a} = \frac{(0.5c_a) - \Delta}{c_b + \Delta}. \quad \text{(II)}$$

This equation has been applied to calculate the value of V_b/V_a at pH = pK_a, where V_a and V_b refer to the initial volumes, c_a and c_b are the total analytical concentrations of the acid and base, respectively, $\Delta = [H^+] - [OH^-]$, and V_{eq} is the equivalence point where the added base exactly neutralizes the acid [20, 21].

The titration curve of an approximately 10^{-3} M solution of compound I ($C_6H_{13}C_2H_3O_2$) with 0.0470 M quaternary ammonium base TBAOH and the corresponding first derivative curve are given in Fig. 1. The titration curves of all perfluoro compounds exhibited one welldefined S-shaped stochiometric endpoint, except for that of the perfluorocarnosine derivative (see Figs. 1, 2, and 3).

The endpoints of the five perfluoroacids corresponded to one equivalent base and related to the neutralization of the COOH group. The R_F-Carnosine compound exhibits an interesting potentiometric titration curve with two inflection points (Fig. 3) related to the neutralization of the two weak acidic functions of the

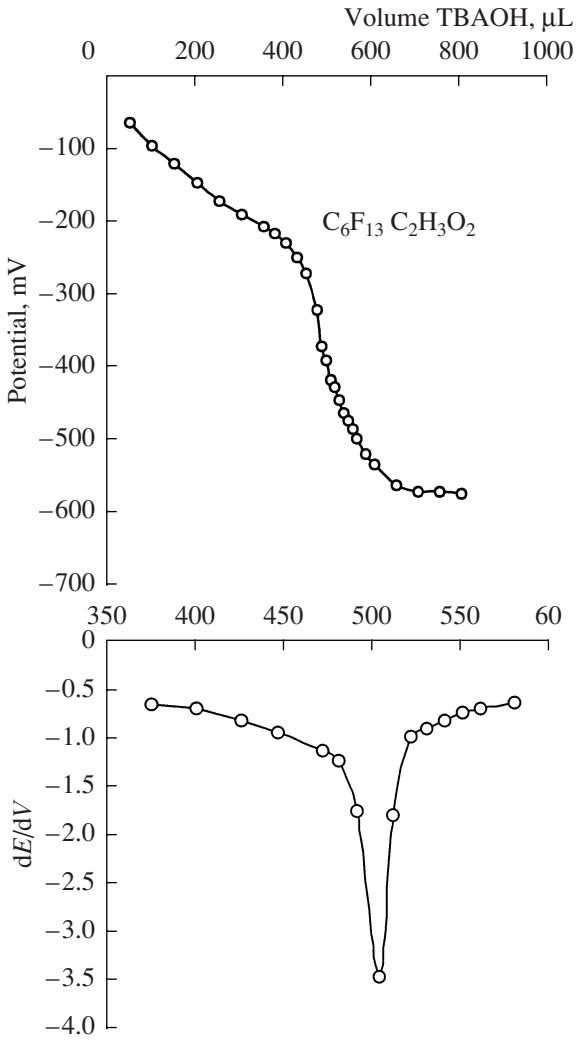


Fig. 1. The potentiometric titration plot of 10^{-3} M $C_6F_{13}C_2H_3O_2$ in acetone with 0.047 M TBAOH and the first derivative curve (theoretical equivalence point, 513 μL; experimental equivalence point, 503 μL; relative error, 1.9%).

compound in methanol. One of them corresponds to the carboxyl proton neutralized at the beginning of the titration and deprotonation of the imidazolium nitrogen followed by the deprotonation of the terminal nitrogen atom. Therefore, two different pK_a values can be reported in methanol and other solvents.

Using the titration curves and computerized derivative method, the half-neutralization potentials (**HNP**) and the related pK_a values were calculated. The half-neutralization potentials and pK_a values of the studied PFCs are listed in Table 2.

In perfluoroacids, the expected effect of the length of the perfluorocarbon skeleton on the acidity of the compounds is a slight increase in pK_a in the order of an increasing carbon number. The relative acidity order of the perfluoroacids is found to be: $C_{10}F_{21}C_2H_3O_2 > C_8F_{17}C_2H_3O_2 > C_6F_{13}C_2H_3O_2 > C_6F_{12}C_2H_3O_2 > R_F$-β-

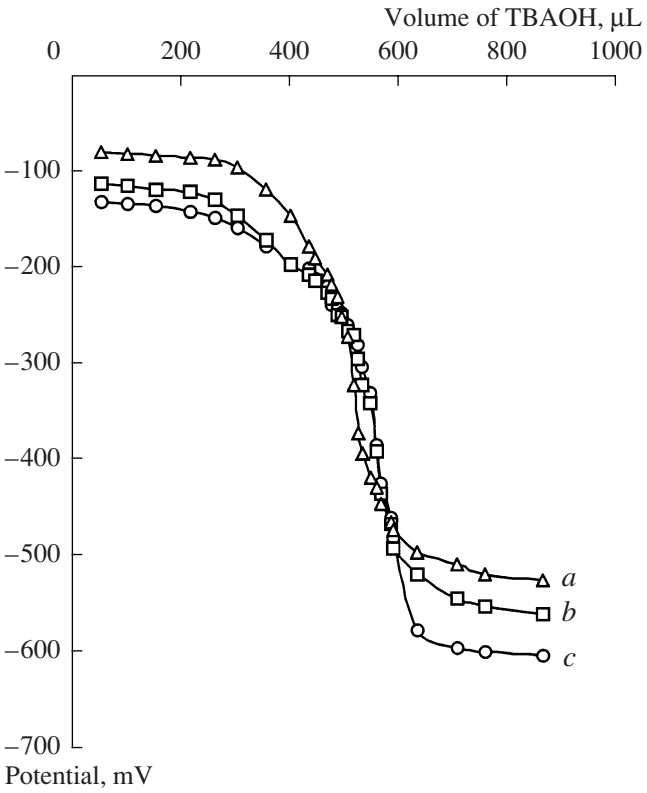


Fig. 2. The potentiometric titration plot of 10^{-3} M PFCs in acetone with 0.047 M TBAOH (*a*) $C_{10}F_{21}C_2H_3O_2$, (*b*) $C_6F_{13}C_2H_3O_2$, and (*c*) $C_8F_{17}C_2H_3O_2$.

Ala-Ala. This order is approximately the same in all of the solvents employed and is in agreement with the expected results.

Acetone provides the best resolution media for the dissociation of PFCs and excellently differentiates the pK_a values. However, the highest pK_a value is reached in methanol. When the acidities of some saturated and nonsaturated acids are compared, a remarkable decrease in the acidity of the nonsaturated analog can be observed. In acetone, the increase in the apparent pK_a value from a saturated to nonsaturated analog is the highest and is equal to 0.97 pK_a units. In pyridine and methanol, this increase is around 0.5 pK_a units. This significant difference in pK_a of the nonsaturated analog can be attributed to the existence of the double bond. The mesomeric electron release effect of the double bond overcomes its inductively electron withdrawing effect and the nonsaturated carbon skeleton attracts an acidic hydrogen stronger than the saturated analog.

The dielectric constant of the solvent, which is a measure of the electrical insulating ability of the medium, also has an effect on the dissociation and titration of the PFCs. When the dielectric constant of the chosen solvents (in the range of 12.5 to 36 Debye) are compared with an unusually high dielectric constant of water (ε = 78.5 Debye), the parameter can be considered as a disadvantage. However, this is not the sole effect on the acid dissociation. The acidic–basic characteristics of the solvent also have to be considered. In our

Table 2. The half neutralization potentials (HNP) and pK_a values of the studied PFCs

Compound	(HNP; mV) pK_a					
	acetone	acetonitrile	N,N-DMF	pyridine	Me–OH	Et–OH
$C_6F_{13}C_2H_3O_2$	(–166) 9.01	(–112) 7.29	(–143)	(–195) 8.91	(36) 5.93	(–21) 6.82
$C_8F_{17}C_2H_3O_2$	(–147) 8.89	(–100) 7.10	9.03	(–187) 8.73	(47) 5.88	(–09) 6.67
$C_{10}F_{21}C_2H_3O_2$	(–83) 8.56	(–91) 6.96	(–138)			
$C_6F_{12}C_2H_3O_2$	(–149) 9.98	(–133) 7.57	9.01	(–175) 8.70	(55) 5.82	(10) 6.02
R_F-Carnosine	Nonsoluble	Nonsoluble	(–130)			
			9.00	(–201) 9.02	(16) 6.11	(–58) 7.26
R_F-β-Ala-Ala	(–303)	(–144) 7.63	(–201)			
	10.09		9.53	Nonsoluble	(09)	Nonsoluble
			Nonsoluble		6.28	
			(–227)		(–457)	
			9.83	(–200) 9.02	11.5	(–68) 7.29
					(19) 6.19	

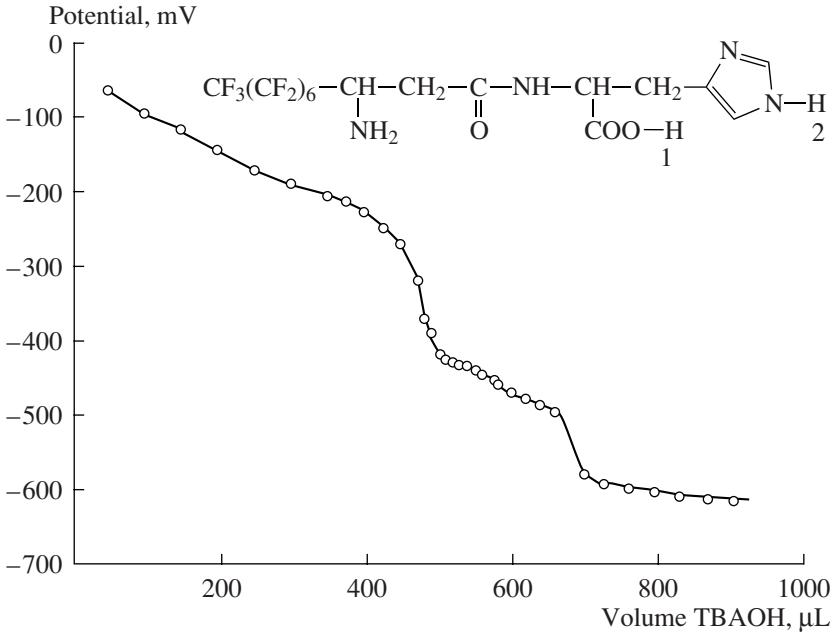


Fig. 3. Potentiometric titration plots of 10^{-3} M R_F-Carnosine in methanol.

case, the chosen solvents should not possess appreciable acidic properties and the products of titration should be soluble.

In all cases, the homocoagulation effects are not encountered when the acids were titrated either in a solvent with quite a low dielectric constant, pyridine (ε = 12.3 Debye), or with a moderate one, acetonitrile (ε = 36.0 Debye) (see Figs. 1, 2, and 3).

The reproducibility and sensitivity of the method were evaluated by successive determinations carried out on three perfluoroacids ($C_6H_{13}C_2H_3O_2$, $C_8H_{17}C_2H_3O_2$, and $C_{10}H_{23}CH_2COOH$) in acetone under the same conditions as employed for the original determinations. The results are shown in Table 3. As can be seen from the data in Table 3, the mean HNPs obtained by the reproducibility test are in good agreement with the data in Table 2. The relative standard deviations were found to be between ±2.4–4.8 %, which reveals the precision or agreement of the results for five replicate measurements.

The recovery studies of standard additions of PFCs were also carried out to determine the linearity and sensitivity of the method. In these titrations, as the amount of the standard added increases, the volume of the titrant used increases linearly. The analytical sensitivity of the method is not linearly related to the amount of the standard added to the original amounts. Thus, the recovery values (%) are found to be nearly constant. The results related to these studies are shown in Table 4.

Table 3. Titrimetric half neutralization potential (HNP) determinations of PFCs

PFC	No. tests	Original HNP, mV	Mean value	% RSD
$C_6H_{13}C_2H_3O_2$	5	–166	–170	2.4
$C_8H_{17}C_2H_3O_2$	5	–149	–155	4.0
$C_{10}H_{23}C_2H_3O_2$	5	–83	–79	4.8

Table 4. Recovery studies of standard additions for some PFCs

PFC	Added, mg	Found, mg	Recovery, %
$C_6H_{13}CH_2COOH$	2.0	2.07	103.5
	1.8	1.83	101.6
	2.3	2.35	102.1
$C_6H_{12}CHCOOH$	1.6	1.66	103.7
	1.5	1.45	96.66
	1.1	1.09	99.09
$C_{10}H_{23}CH_2COOH$	2.0	2.05	102.5
	1.6	1.63	101.8
	1.5	1.48	98.66
	1.1	1.12	101.8

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