Notes

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Accelerated Degradation of Non-steroid Anti-inflammatory Drugs at Carbon Black/Water Interface[1,2]

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In the course of investigating the adsorption of non-steroid anti-inflammatory drugs at carbon black (CB)/water interface, it was found that the degradation of some of these drugs was accelerated. It was also found that this acceleration was observed in the existense of graphite in the place of CB, but not observed in a simple aqueous solution nor in the presence of such hydrophilic powder as silica gel.

The results obtained from solutions of different pH and temperature showed that the *Rf* values of thin-layer chromatography of the degradation products of the respective drugs were coincident to both cases of the presence of CB at 30° and the absence of CB at 60°, while the rate of degradation was observed greater in the presence of CB than in the absence of CB.

As various kinds of interaction of drugs at hydrophobic interfaces in body are considered to give influence on the onset of pharmacological action, physico-chemical investigations have been carried out as to adsorption phenomena of drugs at the carbon black(CB)/water interface as a model of hydrophobic interfaces.[4]

Extending a similar investigation to non-steroid anti-inflammatory drugs, the authors found that the degradation of some of these drugs in aqueous solution was accelerated in the presence of carbon black, while the similar acceleration was not observed in simple aqueous solution or in the presence of such hydrophilic powder as silica gel. In the present study, therefore, the effects of the pH of solution and the reaction temperature on this degradation were investigated, examining the reaction products in comparison with the *Rf* values of thin-layer chromatography (TLC) reported by several workers.[5—9]

Experimental

Materials——Highly purified samples of phenylbutazone, indomethacin and azapropazone used were supplied by Adobans Kasei Co. Ltd., Sumitomo Chemical Ind. and Nippon Chemiphar Co. Ltd., respec-

1) This paper forms Part XXVIII of "Physico-chemical Approach to Biopharmaceutical Phenomena." Preceding paper, Part XXVII: N. Nambu, S. Sakurai, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **22**, 1405 (1974).
2) A part of this work was presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972.
3) Location: *Ebara-2-4-41, Sinagawa-ku, Tokyo, 142, Japan.*
4) *a*) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968); *b*) H. Nogami, T. Nagai, and H, Uchida, *ibid.*, **17**, 176 (1969); *c*) H. Nogami, T. Nagai, and S. Wada, *ibid.*, **18**, 348 (1970); *d*) H. Nogami, T. Nagai, and N. Nambu, *ibid.*, **18**, 1643 (1970); *e*) H. Umeyama, T. Nagai, S. Wada, and H. Uchida, *Yakuzaigaku*, **31**, 194 (1971).
5) H.D. Beckstead, K.K. Kaistha, and S.J. Smith, *J. Pharm. Sci.*, **57**, 1952 (1968).
6) D.V.C. Awang, A. Vincent, and F. Matsui, *J. Pharm. Sci.*, **62**, 1673 (1973).
7) R.E. Harman, M.A.P. Meisinger, G.E. Davis, and F.A. Kuehl, Jr., *J. Pharmacol. Exptl. Therap.*, **143**, 215 (1964).
8) D.W. Yesair and C.B. Coutinho, *Biochem. Pharmacol.*, **19**, 1569 (1970).
9) S. Goto, W.Y. Tseng, M. Kai, S. Aizawa, and S. Iguchi, *Yakuzaigaku*, **29**, 118 (1969).

tively, as were recognized to conform to the standards. Carbon Black (CB), graphite and silica gel were used after the same treatment as described in a previous paper.[4a] The rest of the materials were of the reagent grade.

Procedure for Measurement of Degradation of Drugs——Five mg of CB, 25 mg of graphite or 25 mg of silica gel was added to 25 ml of 1×10^{-4}M solution of each drug in 1/30M phosphate buffer at different pH values, shaken at different temperatures for a certain period, and filtered very rapidly with a glass filter. The ultraviolet (UV) absorption of the filtrate was measured with a Shimazu UV-200 spectrophotometer.

Thin-Layer Chromatography (TLC) of the Reaction Products——The filtrate mentioned above was concentrated by a rotary evaporater below 55°.[10] The concentrated solution was subjected to TLC according to the methods reported by Beckstead, *et al.*[5] and Harman, *et al.*[7] for phenylbutazone and indomethacin, respectively. In the case of azapropazone, modifying the method by Beckstead, *et al.*,[5] HCl/ethanol/water (1:8:2, v/v) as the extracting solvent was added to the concentrated solution mentioned above, and the extract after concentrating was developed by TLC in $CHCl_3$/methanol (9:1, v/v).

Result and Discussion

Fig. 1—3 show the changes in UV absorbance and spectrum of phenylbutazone, indomethacin and azapropazone, respectively, in aqueous solution in the presence of CB at 30° with the lapse of time.

Similar changes were not observed in the absence of CB at the same temperature, though they were done after a long time at an elevated temperature, as shown in Fig. 4—6. Generally, the adsorption equilibrium of drug is rapidly reached at CB/water interface and the drug is kept satisfactorily stable during the experimental procedure, as has been reported in previous papers.[4]

It was, therefore, clear that the degradation of drug was accelerated on account of CB in the present system. Furthermore, small changes in UV absorption similar in shape to Fig. 1—3 were observed in the presence of graphite while none in the presence of silica gel, suggesting that the degradation of drug might be accelerated at hydrophobic interfaces. The results as the respective drugs will be described in the following.

i) Phenylbutazone

The absorbance at λ_{max} 264 mμ decreased and that at a new λ_{max} 234 mμ developed with the lapse of time in the absence of CB in both acidic and alkaline solutions at 60°, as shown in Fig. 4. The rate of degradation was greater in acidic solution than in alkaline one.

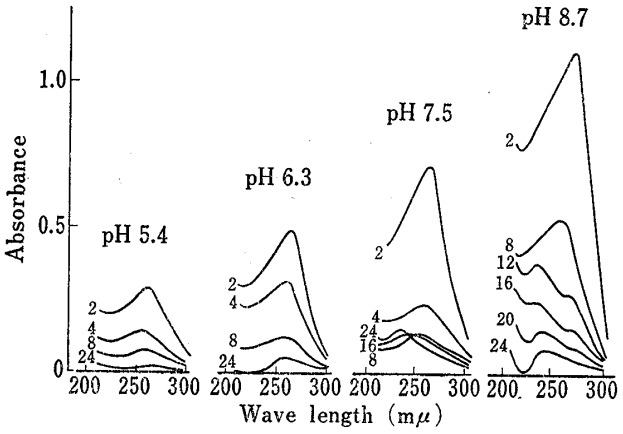

Fig. 1. Changes in UV Absorption of Phenylbutazone in 1/30M Phosphate Buffer Solution at Different pH Values in the Presence of CB at 30°

The respective numbers close to the curves mean the lapse of time in hr.

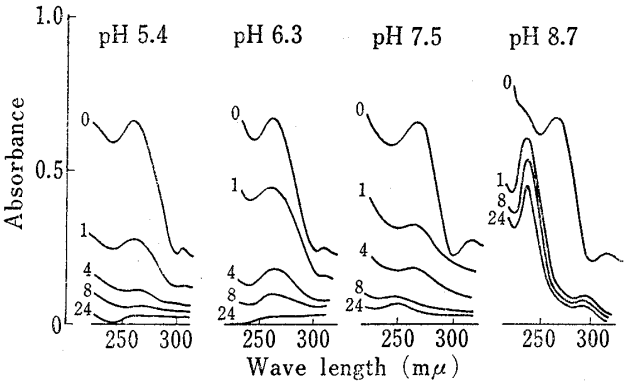

Fig. 2. Changes in UV Absorption of Indomethacin in 1/30M Phosphate Buffer Solution at Different pH Values in the Presence of CB at 30°

The respective numbers close to the curves mean the lapse of time in hr. The respective absorbances at 0 hr are shown on 1/2.5 scale.

10) It was ascertained preliminarily that the UV absorption of the solution did not change during this procedure.

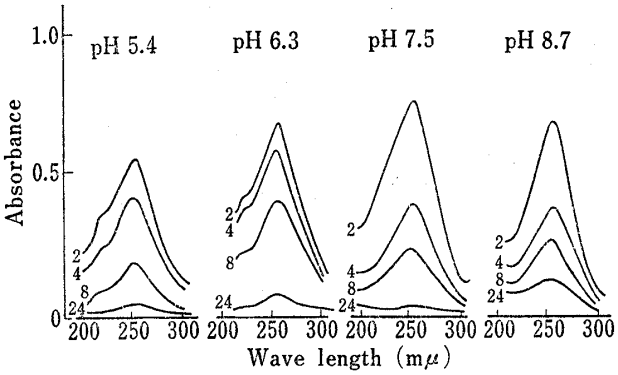

Fig. 3. Changes in UV Absorption of Azapropazone in 1/30M Phosphate Buffer Solution at Different pH Values in the Presence of CB at 30°

The respective numbers close to the curves mean the lapse of time in hr.

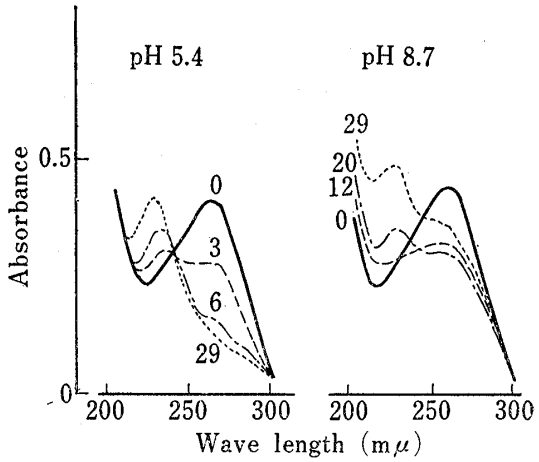

Fig. 4. Changes in UV Absorption of Phenylbutazone in 1/30M Phosphate Buffer Solution at Different pH Values in the Absence of CB at 60°

The respective numbers close to the curves mean the lapse of time in days.

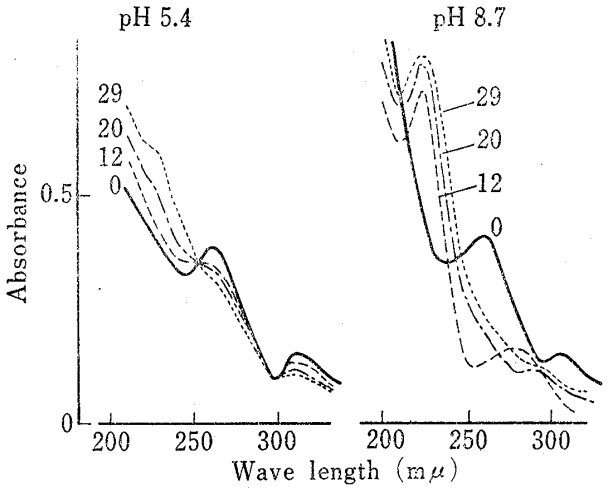

Fig. 5. Changes in UV Absorption of Indomethacin in 1/30M Phosphate Buffer Solution at Different pH Values in the Absence of CB at 60°

The respective numbers close to the curves mean the lapse of time in days.

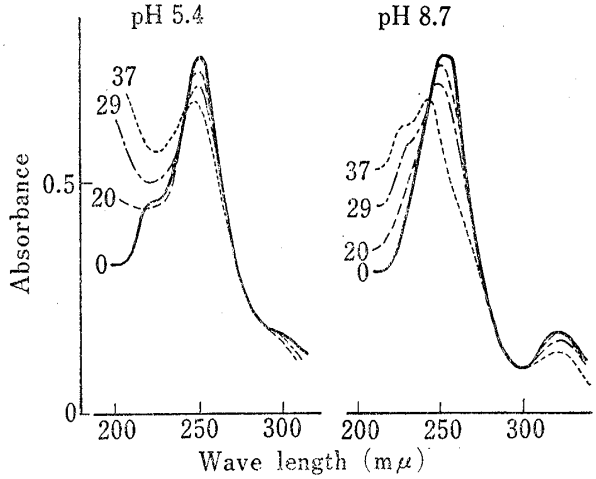

Fig. 6. Changes in UV Absorption of Azapropazone in 1/30M Phosphate Buffer Solution at Different pH Values in the Absence of CB at 60°

The respective numbers close to the curves mean the lapse of time in days.

The change in absorption spectrum in alkaline solution in the presence of CB at 30° was similar to that in the absence of CB at 60°, as shown in Fig. 1 and 4, though a similar change was not detected in acidic solution because the drug was more adsorbed by CB than from alkaline solution and thus a low concentration of drug remained in solution.

The degradation products of phenylbutazone in the presence of CB at 30° and in the absence of CB at 60° were detected by TLC and the results are shown in Table I. The *Rf* values of five products a, b, c, d and e were common to both cases of the presence and the absence of CB and those of a, b, c and d were considered to correspond to II, III, IV and V, respectively, reported by Beckstead, *et al.*[5]

ii) Indomethacin

Indomethacin was very unstable in higher alkaline solution, as had been reported by Goto, *et al.*[9] As shown in Fig. 2 and 5, the absorbance at λ_{max} 267 mμ decreased immediately

Table I. Degradation Products of Phenylbutazone detected by TLC

Present experimental data			Existing data			
Symbol	*Rf* values at pH 6.3[a]	*Rf* values at pH 8.7[a]	Symbol	Compound	λ_{max}[b] mμ	*Rf* values[b]
			I	2-butyl-3-keto-3-α,β-diphenyl hydrazino-2-hydroxypropionic acid	232	0.13
a	0.20		II	2-butyl-3-keto-3-α,β-diphenyl hydrazinopropionic acid	232	0.18
b	0.25	0.25	III	4-butyl-1,2-diphenyl-4-hydroxy-3,4-pyrazolidinedione	232	0.25
c	0.38		IV	α,β-diphenylhexanohydrazine	236	0.39
d	0.50	0.49	V	4-butyl-1,2-diphenyl-3,5-pyrazolidinedione (phenylbutazone)	264	0.49
e	0.60	0.59				

a) Degradation products at the respective pH values both in the presence of CB at 30° and in the absence of CB at 60°.
b) See reference 5).

Table II. Degradation Products of Indomethacin detected by TLC

Present experimental data			Existing data			
Symbol	*Rf* values at pH 6.3[a]	*Rf* values at pH 8.7[a]	Symbol	Compound	λ_{max}[b] mμ	*Rf* values[c]
a	0.13		I	1-(*p*-chlorobenzoyl)-5-hydroxy-2-methylindole-3-acetic acid	265	0.15
b		0.22				
c	0.28					
d	0.36	0.38	II	5-methoxy-2-methylindole-3-acetic acid	280	0.37
e	0.57	0.57	III	1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid	265	0.57
f	0.64		IV	5-methoxy-2,3-dimethylindole		0.63
g	0.69					
h		0.76				
i		0.82	V	1-(*p*-chlorobenzoyl)-5-methoxy-2,3-dimethylindole		0.80
j		0.89				

a) Degradation products at the respective pH values both in the presence of CB at 30° and in the absence of CB at 60°.
b) See reference 8).
c) See reference 7, 9).

and that at a new λ_{max} 267 mμ developed in the absence of CB at 60° in alkaline solution. In acidic solution, the absorbance at λ_{max} 267 mμ decreased gradually and that at the shoulder 230 mμ developed with the lapse of time in the absence of CB at 60°.

The change in absorption spectrum in alkaline solution in the presence of CB at 30° was similar to that in the absence of CB at 60°, as shown in Fig. 2 and 5, though a similar change was not detected in acidic solution because of the same reason as described in the case of phenylbutazone.

The degradation products of indomethacin in the presence of CB at 30° and in the absence of CB at 60° were detected by TLC and the results are shown in Table II. The *Rf* values of ten products a, b, c, d, e, f, g, h, i and j were common to both cases of the presence and the absence of CB and those of a, d, e, f and i correspond to I, II, III, IV and V, respectively, reported by Harman, *et al.*[7] and Goto, *et al.*[9]

iii) Azapropazone

As shown in Fig. 6, the absorbance at λ_{max} 255 mμ decreased and that at a new λ_{max} 235 mμ developed with the lapse of time in the absence of CB at 60°.

This phenomenon was accelerated in the presence of CB even at 30°, as shown in Fig. 3. The *Rf* values of three degradation products detected by TLC were common to both cases of the presence and the absence of CB, as shown in Table III.

TABLE III. Degradation Products of Azapropazone detected by TLC

Symbol	*Rf* values			
	in the presence of CB at 30°		in the absence of CB at 60°	
	at pH 6.3[a)]	at pH 8.7[a)]	at pH 6.3[a)]	at pH 8.7[a)]
a	0.03	0.04	0.03	0.04
b			0.08	
c[b)]	0.12	0.15		
d			0.22	0.26
e			0.48	0.42
f				0.53
g	0.58	0.59		
h	0.63	0.64	0.63	0.64
i	0.69	0.71	0.69	0.71

a) degradation products at the respective pH values
b) azapropazone

Conclusively, the changes in absorption spectra in solution and the *Rf* values of the degradation products of the respective drugs were common to both cases of the presence of CB at 30° and the absence of CB at 60° except that the rate of degradation was greater in the presence of CB than in the absence of CB.

Therefore, it was considered that the hydrolysis reaction of drugs was accelerated at the CB/water interface.

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