Analytica Chimica Acta, 116 (1980) 69—76

POLAROGRAPHIC ANALYSIS FOR CORTICOSTEROIDS
Part 4. Determination of Corticosteroids in Multicomponent and Complex Pharmaceutical Preparations

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(Received 25th September 1979)

SUMMARY

Differential pulse polarographic methods for determination of corticosteroids in multicomponent and complex pharmaceutical preparations are described. The influence of other reducible common components of such preparations and excipients on the height of the reduction peaks of corticosteroids and the accuracy of the results was investigated, as well as the interference of excipients by adsorption processes at the electrode or at solid particles of the preparations. In the procedures developed, variations in composition of the supporting electrolyte according to the lipophilicity of the preparation, and the use of standard additions, produce results quickly and reliably. Standard deviations vary from 0.8 to 2.8%.

Earlier parts of this series dealt with polarographic analysis for corticosteroids in single-component pharmaceutical preparations [1, 2]. The main advantages of the described methods are the sensitivity and the speed of the determination. These characteristics are achieved by the application of differential pulse polarography (d.p.p.) and standard addition methods, combined with a simple clean-up procedure. Corticosteroids are frequently combined with antibiotics, analgesics and local anesthetics. Some of these compounds are electro-active, as are some excipients of lipophilic preparations. Furthermore, some of the excipients of lipophilic and hydrophilic preparations may interfere because of their adsorptive properties.

The aim of this work was to investigate the application of the clean-up procedure to the polarographic analysis of multicomponent preparations and preparations with complex mixtures of excipients.

EXPERIMENTAL

Apparatus

Three polarographs were used: a PAR model 174 polarograph and a Bruker E310 modular electrochemical system, both of which were equipped with a drop timer and a Houston model 2200-3-3 *x-y* recorder, and a Metrohm Polarecord E506 equipped with a polarographic stand E505. A

water-jacketed 10-ml polarographic cell (Metrohm EA 880-T-5) was employed with a dropping mercury electrode, a Metrohm EA 436 Ag/AgCl reference electrode and platinum wire auxiliary electrode. The cell was maintained at 20 ± 0.2°C. The "salt bridge" of the reference electrode was filled with the supporting electrolyte. Standard additions were made by a piston microburette (Metrohm E457-0.6) and a 100-μl or 50-μl micropipette (Oxford P7000).

Chemicals

The steroid preparations used are listed together with their sources in Table 1. The solvents and materials for the supporting electrolytes were: methanol (zur Analyse), dimethylformamide (DMF; Uvasol), tetramethylammonium hydroxide (Me_4NOH; 10% zur Polarographie), acetic acid (99% reinst), phosphoric acid (99% reinst) (all from Merck); toluene and boric acid (reagent grade; Baker). Toluene was distilled twice before use. All other reagents were used without further purification.

Procedure

Because of the variety of dosage forms of corticosteroids, three solvents were used with different lipophilic properties: a mixture of Britton—Robinson buffer in methanol (50% v/v) at pH 10, 0.03 M Me_4NOH in methanol and 0.02 M Me_4NOH in a mixture of DMF (87% v/v) and water. A d.p.p. curve of 10 ml of the supporting electrolyte (solvent) was recorded after deaeration with oxygen-free nitrogen [3] for 15 min. Then, a suitable amount of the sample was added, to obtain a concentration between 10^{-3} and 10^{-5} M steroid in the polarographic cell. After further deaeration for 1 min, the polarogram was recorded. A small volume of the standard solution containing about the same amount of steroid was then added and the polarogram was recorded again. Deaeration for 1 min was sufficient. Dilution by addition of the standard should not exceed 1%. Standard solutions of the steroids were prepared in methanol or DMF depending on the supporting electrolyte used.

The normal scan parameters were: drop time 2 s, scan rate 2 mV s^{-1}, modulation amplitude 100 mV.

RESULTS

Ointments

A suitable amount of ointment was dissolved completely in 5.00 ml of toluene. An aliquot was added to the supporting electrolyte, consisting of 0.02 M tetramethylammonium hydroxide in a mixture of DMF (87% v/v) and water. The fatty constituents did not dissolve in the electrolyte solution, whereas the active compound was extracted into the aqueous DMF. This layer was analyzed by d.p.p.; the results are listed in Table 1. Gentamycin, neomycin and bacitracin in preparation Nos. 1, 2, 4 and 5 are not reducible

TABLE 1

Compositions and analytical results for some multicomponent preparations[a]

No.	Name	Source	Active constituents	Content (mg g^{-1})	Found (mg g^{-1})	R.s.d. (%)
Ointments						
1	Celestoderm-V with garamycin	Schering	Bethamethasone[b] (as 17-valerate)	1	0.98	2.7
			Gentamycin	1		
2	Celestoderm-V with neomycin	Schering	Bethamethasone[b] (as 17-valerate)	1	0.96	2.8
			Neomycin	5		
3	Dexatopic	Organon	Dexamethasone[c]	0.4	0.412	2.7
			Nandrolone decanoate	0.4		
			Chlorhexidine-HCl	10		
4	Corneo	Centrapharm	Prednisolone[c]	2	2.00	2.1
			Neomycin (as sulphate)	5		
			Bacitracin	200		
Cream						
5	Hydrocortisone with neomycin	—[d]	Hydrocortisone acetate[c]	10.0	10.3	1.7
			Neomycin	5		
Alcoholic solutions						
6	Composed triamcinolone acetonide solution	—[d]	Triamcinolone acetonide[c]	1.01[e]	1.01[e]	0.8
			Salicylic acid	20[e]		
			Benzalkonium chloride	0.1[e]		
7	Kenalog Tincture	Squibb	Triamcinolone acetonide[c]	2[e]	1.91[e]	1.3
			Salicylic acid	20[e]		
			Benzalkonium chloride	0.5[e]		
Tablets						
8	Delta-butazolidine	Geigy	Prednisone[c]	1.25[f]	1.26[f]	2.0
			Phenylbutazone	50[f]		
9	Rheulysin	Organon	Prednisone[c]	0.5[f]	0.3[f]	
			Acetylsalicylic acid	300[f]		
Suppositories						
10	Proctosedyl	Roussel	Hydrocortisone[c]	5[g]	5.24[g]	1.3
			Cinchocaine-HCl	5[g]	4.96[g]	1.4
			Framycetin sulphate	10[g]		
11	Tri-Anal	Will Pharma	Triamcinolone acetonide[c]	0.5[g]	0.50[g]	1.5
			Lidocaine-HCl	50[g]		
12	Triamcinoione acetonide	—[d]	Triamcinolone acetonide[c]	0.5[g]	0.50[g]	1.9
			Lidocaine-HCl[c]	50[g]		
Enema						
13	Predniment enema	Pharmachemie	Prednisone[c]	25[e]	28.5[e]	1.7
14	Prednisone enema	—[d]	Prednisone[c]	100[e]	102[e]	1.6

[a]Relative standard deviations of 5 separate determinations with individual sampling. [b]Obtained in pure form from Glaxo. [c]Obtained in pure form from Nogepha. [d]Made in the laboratory. [e]Content in mg ml^{-1}. [f]Content in mg/tablet. [g]Content in mg/suppository.

in the potential range studied and do not interfere. Nandrolone decanoate in preparation No. 3 is a Δ^4-3-ketosteroid. The half-wave potentials of the enones lie about 200 mV more negative than those of dienones. Synthetic mixtures of equimolar amounts of a dienone and an enone showed a small increase in current immediately before and after the d.p.p. peak of the

dienone. This affected the measurement of the peak of the dienone and resulted in a systematic error of +3% of the amount of the dienone. No better results were obtained with normal pulse polarography, in spite of the additivity of the individual waves. The amount of the enone cannot be determined by d.p.p. because of the overlap of the reduction peak of the enone with the more negative peak of the dienone (Fig. 1). Chlorhexidine in preparation No. 3 is also reducible, but the reduction occurs at potentials 250 mV more negative than those for dexamethasone (Fig. 1). Furthermore, the interference of chlorhexidine is diminished by its limited solubility in toluene. Thus none of the other components interferes in the determination of dexamethasone in preparation No. 3.

Some excipients of ointments cause an increase in the base-line, resulting in high results for the corticosteroids. The effects of several common excipients are listed in Table 2. These results make it possible to predict which preparations other than those listed in Table 1 can be successfully analyzed by pulse polarography. For example, the Locacorten ointment (Ciba) contains white beeswax and the results can be expected to have limited

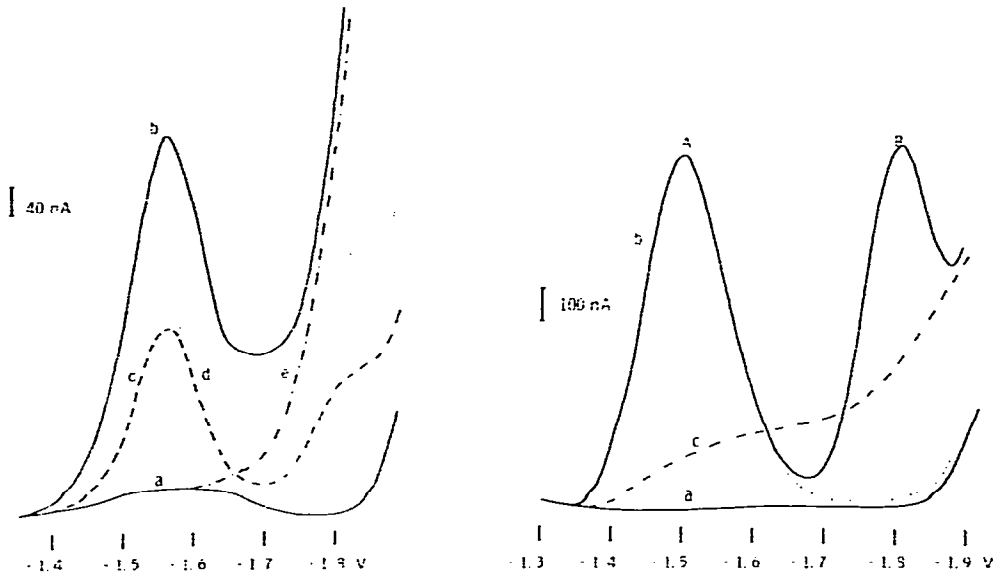

Fig. 1. Differential pulse polarogram of Dexatopic ointment with 0.02 M Me_4NOH in a mixture of DMF (87% v/v) and water. (a) Blank solution; (b) blank solution + sample addition; (c) blank solution + dexamethasone; (d) blank solution + dexamethasone + equimolar amount of an enone; (e) blank solution + chlorhexidine-HCl.

Fig. 2. Differential pulse polarogram of Proctosedyl suppositories with 0.02 M Me_4NOH in a mixture of DMF (87% v/v) and water. (a) Blank solution; (b) blank solution + sample addition, in which peak A is the reduction peak of cinchocaine-HCl and peak B of hydrocortisone and base-line increase by cinchocaine-HCl (dotted line); (c) blank solution + framycetin.

TABLE 2

Effects of lipophilic excipients on the determination of corticosteroids (0.1% corticosteroid)

Compound	Normal content (%) (when present)	Positive systematic error (%)		
		<1	1—5	>5
Arachis oil	50	X		
Cetiol V	20	X		
Cetomacrogol	15	X		
Lanette wax	15	X		
Liquid paraffin	10		X	
Methylhydroxybenzoate	0.2	X		
Monolein	6			X
Polyethylene glycol 400	60		X	
Polyethylene glycol 4000	40		X	
Polysorbate 80	8			X
Propylene glycol	10	X		
Propylhydroxybenzoate	0.2	X		
Sorbic acid	0.2		X	
Sorbitol	4	X		
Span 80	8			X
Spermaceti	10			X
Stearic acid	15			X
Triethanolamine	2	X		
White beeswax	5			X
White soft paraffin	90	X		
Witepsol	100	X		
Wool fat	10		X	
Yellow soft paraffin	90	X		

accuracy, whereas Tumeson ointment (Hoechst) contains wool fat and liquid paraffin, which should have a smaller effect on the accuracy of the results. An extended (mostly chromatographic) clean-up procedure is needed for preparations where large positive errors are expected [4—6].

Creams, alcoholic solutions and tablets

The analyzed cream (preparation No. 5, Table 1) is based on an oil-in-water emulsion in which the steroid ester and neomycin are dispersed; the composition is given in Table 3. The cream (250 mg) was dispersed in 5.00 ml of methanol, and the hydrocortisone dissolved in the methanolic solution; a 1.00-ml aliquot of this solution was transferred to 0.03 M tetramethylammonium hydroxide in methanol and analyzed as described before [1]. A small increase in the base-line was observed, resulting in a positive systematic error of about +3%.

With regard to the alcoholic solutions, neither salicylic acid nor benzalkonium chloride are reducible in the potential range studied and thus do not interfere in the determination of triamcinoline acetonide. An aliquot of the sample (containing 70% ethanol) of preparation Nos. 6 and 7 (Table 1) was

TABLE 3

Complete composition of some complex preparations

Name	Constituent	Content	
Hydrocortisone cream	Hydrocortisone	10	mg g^{-1}
	Neomycin	5	mg g^{-1}
	Cetomacrogol	75	g
	Cetiol V	100	g
	Sorbitol (aq. 70% v/v)	20	g
	Sorbic acid	1	g
	Water ad	500	g
Predniment enema	Prednisone	25	mg
	Sorbitol	5.5	g
	Polysorbate 80		
	Methyloxybenzoate		
	Propyloxybenzoate		
	Sodium carragenate		
	Water ad	100	ml
Prednisone enema	Prednisone	100	mg
	Na_3PO_4	20.5	mg
	Na_2HPO_4	30.0	mg
	NaCl	850	mg
	Methylhydroxybenzoate	150	mg
	Polividon	2.5	g
	Water ad	100	ml
Rheulysin tablets	Prednisone	0.5	mg/tablet
	Acetylsalicylic acid	300	mg/tablet
	$Al(OH)_3$	30	mg/tablet
	Magnesium trisilicate	50	mg/tablet
Triamcinolone acetonide suppositories	Triamcinolone acetonide	0.5	mg/supp.
	Lidocaine HCl	50	mg/supp.
	Witepsol ad	2	g

added to 0.03 M tetramethylammonium hydroxide in methanol and determined as described before [1]. The results agree with the claimed content.

The coated tablets of Delta-butazolidin (preparation No. 8, Table 1) had to be crushed mechanically before dispersion and dissolution in methanol. An aliquot was analyzed in 0.03 M tetramethylammonium hydroxide in methanol as described earlier [2]. Phenylbutazone is not reducible and therefore does not interfere [7]. Rheulysin tablets (preparation No. 9, Table 1) gave low results for prednisone. Neither the excipients nor the salicylic acid are reducible in the voltage range studied and therefore cannot affect the results; adsorption on aluminium hydroxide (Table 3) seems to be a plausible explanation for the low results found. In this particular case, a modification of the extraction procedure is needed.

Suppositories

Because of limited solubility of the hydrocortisone present in the suppository, a relatively high volume (100 ml) of toluene was needed for dissolution of the Proctosedyl suppositories (preparation No. 10, Table 1). Framycetin is itself not electro-active, but gives rise to excessively high results for hydrocortisone by d.p.p., probably because of adsorption processes. When the suppository is treated with 100 ml of toluene containing 1 ml of water, the framycetin dissolves in the aqueous layer and does not interfere in the analysis of the toluene phase. Such analyses were completed by using standard addition in 0.02 M tetramethylammonium hydroxide in DMF (87% v/v) and water. Cinchocaine-HCl is reduced at the DME at potentials 200 mV more positive than those for the hydrocortisone reduction and causes slightly high (about 3%) results for hydrocortisone by d.p.p. (Fig. 2). As can be expected, normal pulse polarography gave very little improvement. Tri-Anal suppositories (preparation No. 11) were analyzed by the procedure developed for ointments. The presence of lidocaine-HCl did not interfere (Table 3). High results were caused by relatively large amounts of Witepsol when the amount of steroid was low (0.25%). When laboratory-made suppositories of the same composition were analyzed, the systematic error could be diminished by adding some Witepsol to the electrolyte solution (Fig. 3). The results obtained by this procedure are listed in Table 1.

Enemas

When an aliquot of the sample was transferred in a methanolic solution containing 0.03 M tetramethylammonium hydroxide, high results were found for prednisone in Predniment enema (preparation No. 13), because of the polysorbate 80 present. Analysis of the prednisone enema (preparation

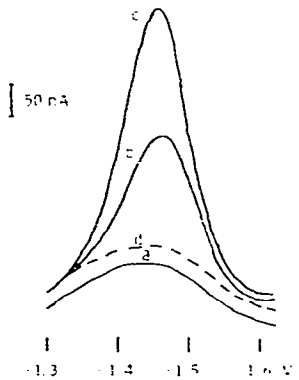

Fig. 3. Differential pulse polarogram of Tri-Anal suppositories with 0.02 M Me_4NOH in a mixture of DMF (87% v/v) and water. (a) Blank solution; (b) blank solution + sample addition; (c) blank solution + sample addition + standard addition; (d) blank solution + Witepsol.

No. 14) in 0.03 M tetramethylammonium hydroxide in methanol yielded a 102% recovery (Tables 1 and 3).

DISCUSSION

Most corticosteroids studied can be determined by differential or normal pulse polarography in the presence of other non-reducible active compounds (Table 1). Sometimes polarographic analyses are possible even in the presence of reducible species, when the waves are well separated, but in some cases interferences cannot be prevented by using the simple treatment proposed in this paper, because of similar reduction potentials and/or similar lipophilic properties. Compounds like iodochlorohydroxyquinoline present in Celestoform-V ointment (Schering), chloramphenicol in Bacicoline eye drops (Bournonville) or dextrochloropheniramine maleate in Polaronil tablets (Schering) interfere in steroid determinations. An analysis of these preparations involves a special sample pre-treatment. Some of the constituents of lipophilic preparations lead to high results, because of adsorption processes or reduction. The systematic error introduced is negligibly small in most cases. As the effects of likely excipients on this analytical procedure are known (Table 2), it is possible to predict situations involving limited accuracy. Tablet excipients, such as lactose, starch, gelatin, talc, magnesium stearate and trisilicate, sodium carboxymethylcellulose, calcium phosphate, acacia, hydroxypropylmethylcelluiose, aerosil and aluminium hydroxide usually do not cause an increase in the base-line. At high contents, some of them (like aerosil and aluminium hydroxide) can interfere because of adsorption processes, either at the electrode or by a decrease in the bulk concentration. In the majority of cases, however, the method is reliable, sufficiently accurate and fast.

The authors thank Prof. Dr. P. Zuman (Potsdam, N.Y.) for his critical reading of the manuscript and his enthusiastic cooperation.

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