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Preformulation stability screening of ivermectin with non-ionic emulsion excipients

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As an important and complementary step during a preformulation study differential scanning calorimetry (DSC) and high-pressure liquid chromatography (HPLC) were used to determine the compatibility between ivermectin and commonly used excipients for preparing non-ionic emulsions. Ivermectin was found to exhibit interactions with 21 excipients, while it was compatible with 25 excipients. HPLC showed a significant decrease in drug amount, ±20%, when substances interacted with invermectin.

1. Introduction

With potency far exceeding those of other anthelmintics and the drug's wide range of activity against nematodes and anthropods, ivermectin has been registered as a veterinary medicine in numerous countries since 1985 [1]. Ivermectin is in particular used for the treatment of infections caused by the nematode *Onchocerca volvulus* that is the major cause of blindness and associated severe dermatological pathology prevalent in inhabitants of some tropical areas [2]. The drug is usually administered orally for the systemic treatment of onchocerciacis with little or no emphasis on external or topical application of the drug for alleviating onchodermatitis.

From a pharmaceutical viewpoint the design and formulation of a suitable topical vehicle for invermectin can be important but the stability of such a formulation will depend, among other factors on the compatibility of the active component with other ingredients in the formulation [3]. Unless incompatibility is glaringly evident it is necessary to carry out a stability study that usually requires weeks or months. Using Differential Scanning Calorimetry (DSC) it is possible to obtain significant data in a short period of time. Although this technique will not soon replace a classical stability program it can be regarded as a qualitative, rapid screening process which can provide an early alert to compatibility problems, especially when combined with a quantitative assay [4–6].

The thermal properties of a physical prepared mixture are the sum of the individual components, and this thermogram can be compared with those of the drug and/or the excipient alone. An interaction on DSC will show as changes in melting point, peak slope and area and/or the appearance of a phase transition [7]. Interactions observed by DSC can be confirmed by chemical assays using TLC or HPLC [3]. A loss in potency in 1 : 1 physical mixtures can indicate incompatibility.

The purpose of this study was to investigate the combined use of DSC and HLPC to determine the compatibility of ivermectin with commonly used non-ionic excipients, prior to formulating a cream. Drug-excipient mixtures

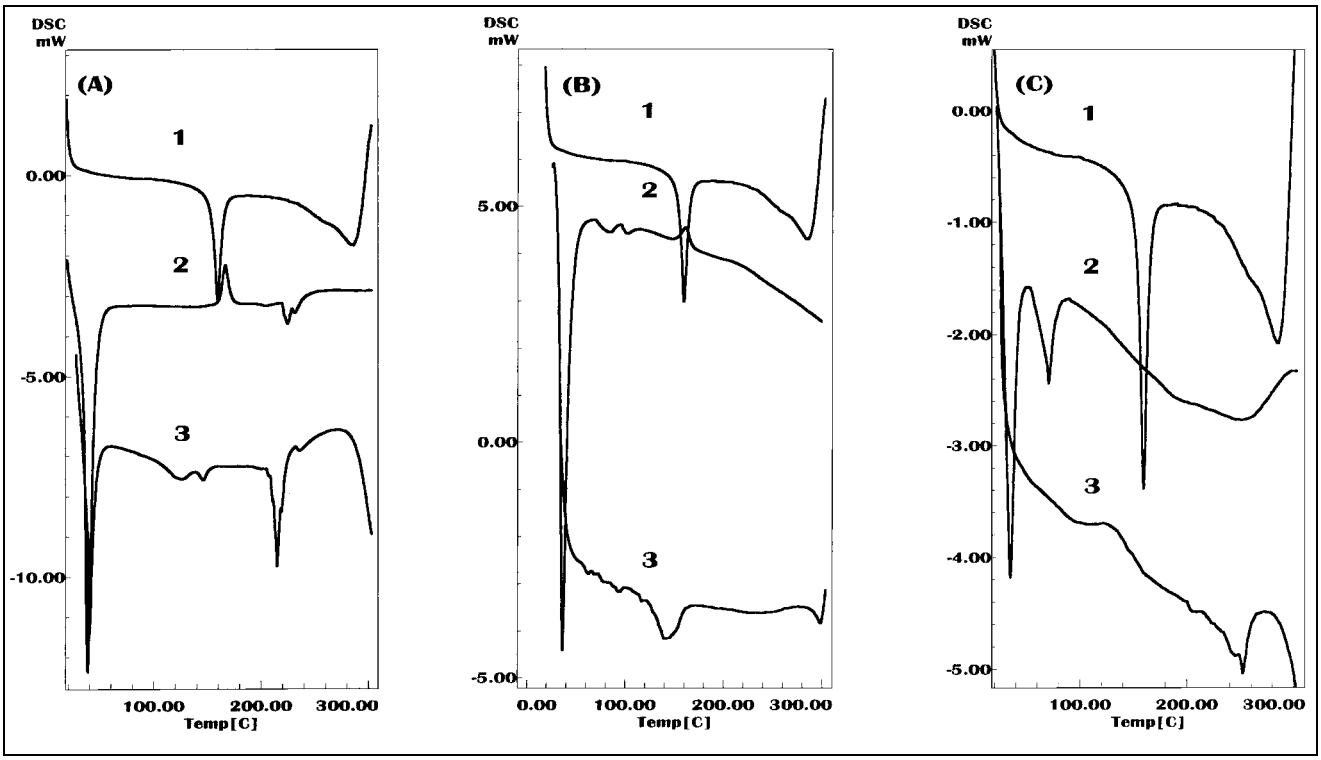

Fig. 1. DSC thermograms of ivermectin and 1 : 1 physical mixtures with polyoxyethylene 10 (A), PEG-8 distearate (B) and PEG-8 stearate (C). 1 ivermectin; 2 excipient; 3 1 : 1 physical mixture

were prepared in 1 : 1 physical mixtures. Both DSC and HPLC analyses were used to evaluate mixtures for potential interactions. The results were compared statistically to determine the effects of analytical technique, sample composition and preparation on the outcome of the compatibility assessment.

2. Investigations, results and discussion

DSC analysis results of 1 : 1 physical mixtures of ivermectin and a specific excipient were compared to that of the drug. Changes in DSC thermograms, indicating possible incompatibilities listed in Table 1, where shifts in melting points, appearance or disappearance of peaks or additional peaks and elongation or broadening of endotherms and exotherms as a result of thermal behaviour.

Only 7 of the 46 excipients evaluated showed potential interactions with ivermectin. The excipients indicating possible interactions were polyoxyethylene 10 (Fig. 1), PEG-8 distearate (Fig. 1), PEG-8 stearate (Fig. 1), propylene glycol (Fig. 2), cholesterol (Fig. 2) and the sodium salts of methyl-4-hydroxybenzoate and propyl-4-hydroxybenzoate (Fig. 3). Each figure shows thermograms of (1) ivermectin, (2) the excipient, and (3) an 1 : 1 physical mixture of ivermectin and excipient.

These changes may indicate interactions but do not necessarily indicate incompatibilities because they could also be the result of differences in the sample geometry of mixtures. Changes such as these were not observed when other mixtures were studied, indicating that ivermectin was compatible with these excipients.

In Table 2, mean assay results of the ivermectin-excipient mixtures are listed. The assay for pure ivermectin in tetrahydrofuran was $100.30 \pm 0.23\%$. Assay results for the mixtures were statistically compared to the assay results of the ivermectin solution. Significant differences were ta-

Table 1: Changes in DSC thermograms indicating possible incompatibilities

Excipients/Materials		Changes in DSC Thermograms*				Interaction**
Chemical Names	Trade names	1	2	3	4	
Laureth-4	Volpo L4	−	−	+	−	−
Oleth-10	Volpo N10	−	−	+	−	−
Steareth-10	Volpo S10	−	−	+	−	−
Ceteareth-25	Cremophor A25	−	−	+	−	−
Ceteareth-6	Cremophor A6	−	−	+	−	−
Sorbitan laurate	Crill 1	−	−	+	−	−
Sorbitan palmitate	Crill 2	−	−	+	−	−
Sorbitan stearate	Crill 3	−	−	+	−	−
Sorbitan oleate	Crill 4	−	−	+	−	−
Sorbitan isostearate	Crill 6	−	−	+	−	−
Polysorbate 80 N/F	Crillet 4	−	−	+	−	−
PEG-8-distearate	Cithrol 4DS	+	+	−	+	+
PEG-8-stearate	Cremophor S9	−	−	+	−	+
PEG-660-OH-stearate	Solutol HS15	−	−	+	−	−
PEG-4000	Lutrol E 4000	−	−	+	−	−
Polyoxyethylene 10	POE 10	−	+	+	−	+
GMS S/E		−	−	+	−	−
Propylene glycol		+	+	+	+	+
Propylene glycol dicaprylate	Crodamol PC	+	−	+	+	−
Cetyl alcohol		−	−	+	−	−
Cetearyl octanoate	Luvitol EHO	+	−	−	+	−
Stearyl alcohol		−	−	+	−	−
Oleyl alcohol	Novol	−	−	+	−	−
Nonoxynol-10	Cremophor NP 10	−	−	+	−	−
Nonoxynol-14	Cremophor NP 14	−	−	+	−	−
Isopropyl myrsitate		+	−	−	+	−
Isopropyl palmitate		+	+	−	+	−
Polyoxyl 40-caster oil	Etocas 40	−	−	+	−	−
Polyoxyl 40 hydrogenated caster oil	Cremophor RH 40	−	−	+	−	−
Polyglyceryl-3 dioleate	Cremophor GO 32	−	−	+	−	−
Glycerol		−	−	−	+	−
Lanolin USP	Lanolin	−	−	+	−	−
Emulsifying wax NF	Polawax GP 200	−	−	+	−	−
$C_{18}-C_{36}$ Triglycerides	Syncrowax	−	−	−	+	−
Gamma cyclodextrin		+	−	−	+	−
Lecithin soya		+	−	−	+	−
Cholesterol		+	+	+	+	+
Stearic acid		−	−	+	−	−
Carbomer	Carbopol 940	+	−	−	+	−
Hydroxyethylcellulose	Natrosol	+	−	−	+	−
Hydroxypropylmethyl cellulose		+	−	−	+	−
D-Panthenol		−	−	+	−	−
Na-Methylparaben		+	+	+	+	+
Na-Propylparaben		+	+	−	+	+
Triethanolamine		−	−	+	−	−
Sodium hydroxide		−	+	−	+	−

* 1. Shifting in Melting Point, 2. Appearance of New Peaks, 3. Disappearance of Peaks, 4. Peak Broadening
** +: possibe interaction; −: no interaction

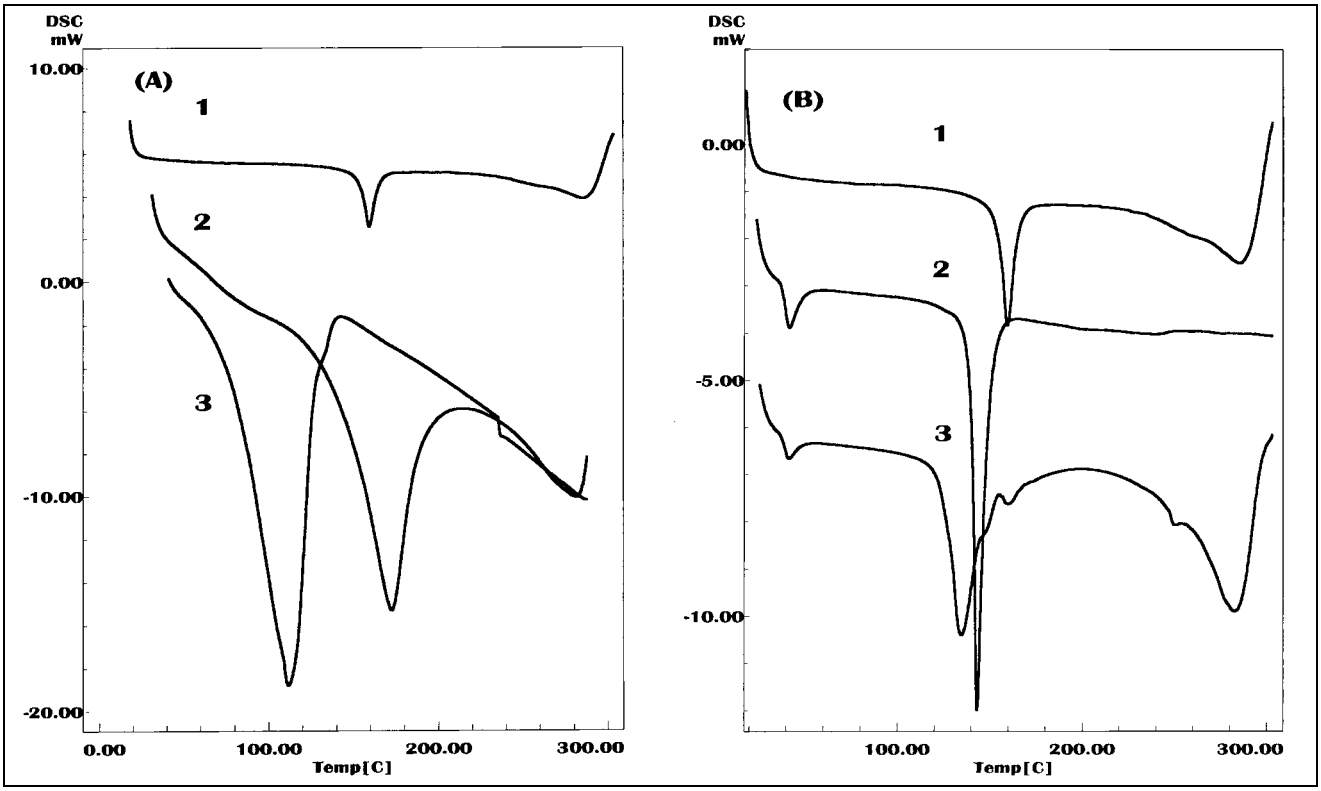

Fig. 2: DSC thermograms of ivermectin and 1 : 1 physical mixtures with propylene glycol (A) and cholesterol (B). 1: ivermectin; 2: excipient; 3: 1 : 1 physical mixture

ken as p values < 0.05, Fig. 4. The assay results for mixtures containing ceteareth-25, ceteareth-6, PEG-8-distearate, PEG-8-stearate, PEG-660-OH-stearate, PEG-4000, polyoxyethylene-10, glycerol monostearate S/E, propylene glycol dicaprylate, cetearyl octanoate, poly-glycerol-3-dioleate, a mixture of C18–C36 triglycerides, gamma cyclodextrin, lecithin soya, cholesterol, stearic acid and sodium salts of methyl and propyl-4-hydroxybenzoate were significantly lower than that for an ivermectin solution.

Normally the pharmacopoeial specification for assay results is between 90–110% or 95–105% of the labelled amount. The assay results for the incompatible mixtures

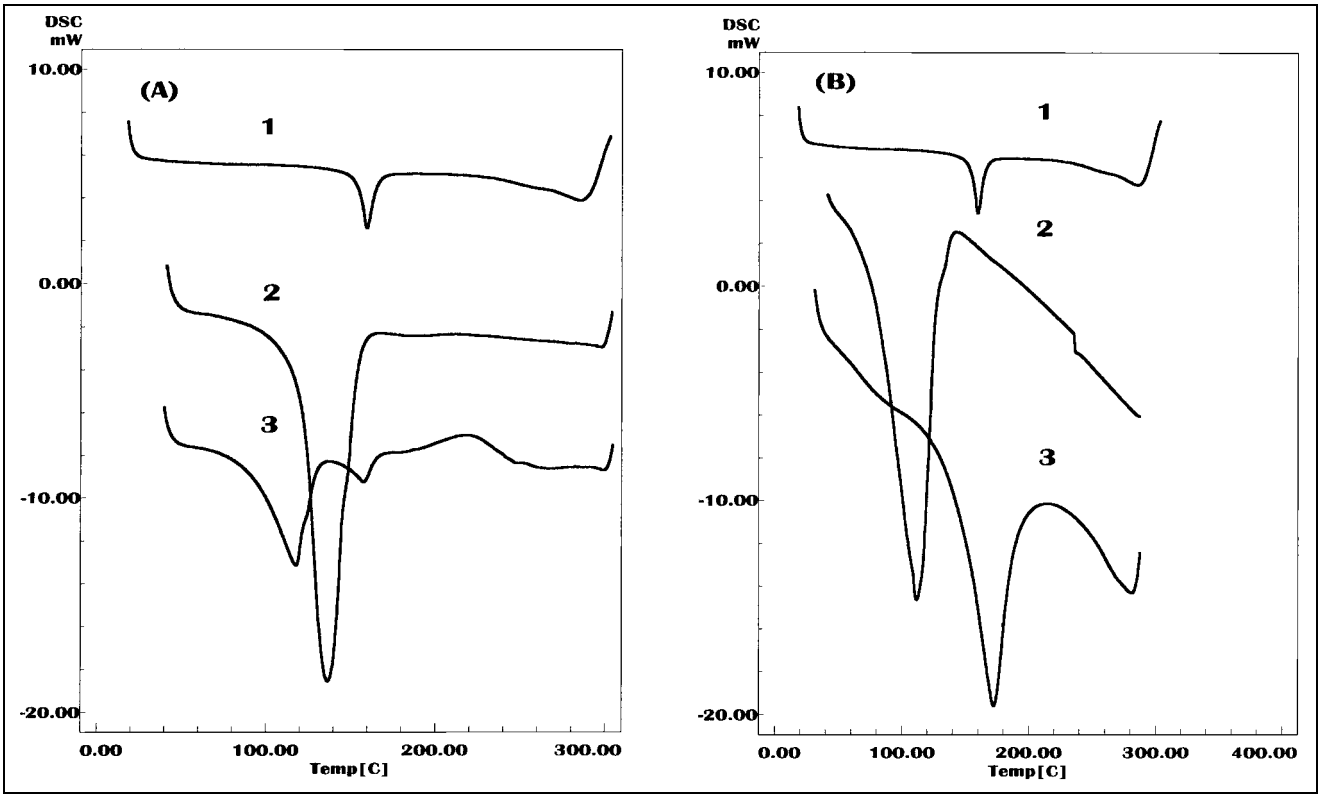

Fig. 3: DSC thermograms of ivermectin and 1 : 1 physical mixtures with two preservatives sodium methyl-4-hydroxybenzoate (A) and sodium propyl-4-hydroxybenzoate (B). 1: ivermectin; 2: excipient; 3: 1 : 1 physical mixture

Table 2: HPLC results for ivermectin in ivermectin-excipient mixtures

Excipients/Materials		HPLC Assay Results		Interaction*
Chemical Names	Trade Names	(%)	(p)	
Laureth-4	Volpo L4	99.91 ± 0.47	0.9760	−
Oleth-10	Volpo N10	99.05 ± 0.13	0.9496	−
Steareth-10	Volpo S10	103.04 ± 0.04	0.0619	−
Ceteareth-25	Cremophor A25	78.43 ± 0.01	0.0001	+
Ceteareth-6	Cremophor A6	84.96 ± 6.68	0.0001	+
Sorbitan laurate	Crill 1	102.85 ± 0.05	0.9501	−
Sorbitan palmitate	Crill 2	103.15 ± 0.73	0.9609	−
Sorbitan stearate	Crill 3	101.01 ± 0.12	0.9943	−
Sorbitan oleate	Crill 4	102.52 ± 0.04	0.9567	−
Sorbitan isostearate	Crill 6	100.74 ± 0.09	0.9749	−
Polysorbate 80 N/F	Crillet 4	106.01 ± 0.51	0.4750	−
PEG-8-distearate	Cithrol 4DS	80.80 ± 0.37	0.0001	+
PEG-8-stearate	Cremophor S9	78.42 ± 0.23	0.0002	+
PEG.660-OH-stearate	Solutol HS15	80.24 ± 0.35	0.0002	+
PEG-4000	Lutrol E 4000	78.30 ± 0.01	0.0001	+
Polyoxyethylene 10	POE 10	78.63 ± 0.30	0.0001	+
GMS S/E		79.87 ± 0.03	0.0001	+
Propylene glycol		101.83 ± 0.71	0.9809	−
Propylene glycol dicaprylate	Crodamol PC	77.63 ± 0.31	0.0002	+
Cetyl alcohol		101.35 ± 0.38	0.9907	−
Cetearyl octanoate	Luvitol EHO	78.30 ± 0.18	0.0001	+
Stearyl alcohol		106.29 ± 0.55	0.4129	−
Oleyl alcohol	Novol	105.88 ± 1.39	0.4969	−
Nonoxynol-10	Cremophor NP 10	102.88 ± 0.82	0.9563	−
Nonoxynol-14	Cremophor NP 14	102.48 ± 0.21	0.9465	−
Isopropyl myrsitate		102.88 ± 0.06	0.9662	−
Isopropyl palmitate		99.96 ± 0.71	0.9925	−
Polyoxyl 40-caster oil	Etocas 40	102.88 ± 0.93	0.9578	−
Polyoxyl 40 hydrogenated caster oil	Cremophor RH 40	103.27 ± 0.15	0.9501	−
Polyglyceryl-3 dioleate	Cremophor GO 32	78.76 ± 0.14	0.0001	+
Glycerol		103.35 ± 0.45	0.9935	−
Lanolin USP	Lanolin	99.53 ± 0.13	0.9641	−
Emulsifying wax NF	Polawax GP 200	105.63 ± 0.14	0.5489	−
$C_{18}-C_{36}$ Triglycerides	Syncrowax	115.72 ± 0.04	0.0002	+
Gamma cyclodextrin		78.80 ± 0.16	0.0001	+
Lecithin soya		79.75 ± 0.04	0.0002	+
Cholesterol		78.57 ± 0.11	0.0002	+
Stearic acid		78.03 ± 0.18	0.0002	+
Carbomer	Carbopol 940	103.48 ± 0.13	0.9604	−
Hydroxyethylcellulose	Natrosol	100.75 ± 0.81	0.9883	−
Hydroxypropylmethyl cellulose		103.52 ± 0.54	0.9627	−
D-Panthenol		100.82 ± 0.92	0.9935	−
Methyl-4-hydroxybenzoate sodium		77.18 ± 0.20	0.0002	+
Propyl-4-hydroxybenzoate sodium		77.11 ± 0.70	0.0002	+
Triethanolamine		103.77 ± 0.03	0.9463	−
Sodium hydroxide		103.37 ± 0.37	0.9634	−

* + indicate loss in potency, − No loss in potency
Significant differences in ivermectin content, compared to pure ivermectin, are indicated by $p < 0.05$

fell outside this specification (Fig. 4). The concentration of ivermectin in compatible mixtures was between 95–105%. Except for the interaction between propylene glycol and ivermectin, HPLC confirmed the ivermectin/excipient incompatibilities predicted by DSC evaluation. However, HPLC predicted more interactions than DSC did. Especially for mixtures containing liquid excipients, evaluation with DSC was impossible and HPLC proved to be more useful. The overall correlation between the two methods was good and HPLC complimented DSC in predicting incompatibilities in mixtures that could not be evaluated by DSC alone.

Stability tests of developed dosage forms are expensive. These results demonstrate the usefulness of DSC and HPLC as quick and rapid methods of ascertaining that some non-ionic emulsion excipients are likely to be unsuitable for an ivermectin topical formulation.

DSC predicted incompatibilities between ivermectin and a

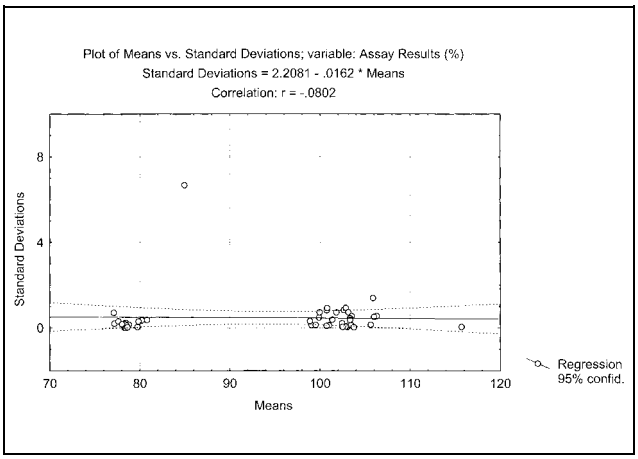

Fig. 4: HPLC assay results for 1 : 1 physical mixtures of ivermectin and non-ionic emulsion excipients

Table 3: Excipients found to be compatible with ivermectin

Excipients		Description	Manufacturer/Supplier
Chemical names	Trade names		
Laureth-4	Volpo L4	Clear liquid	Croda chemicals
Oleth-10	Volpo N10	Pale straw paste	Croda chemicals
Sorbitan laureate	Crill 1	Clear viscous liquid	Croda chemicals
Sorbitan palmitate	Crill 2	Tan waxy solid	Croda chemicals
Sorbitan stearate	Crill 3	Tan waxy solid	Croda chemicals
Sorbitan oleate	Crill 4	Amber viscous liquid	Croda chemicals
Sorbitan isostearate	Crill 6	Amber viscous liquid	Croda chemicals
Polysorbate 80 N/F	Crillet 4	Clear amber liquid	Croda chemicals
Cetyl alcohol		White flake Granules	Saarchem, South Africa
Stearyl alcohol		Granules	Croda Chemicals
Oleyl alcohol	Novol	Clear liquid	Croda Chemicals
Nonoxynol-10	Cremophor NP 10	Clear liquid	BASF
Nonoxynol-14	Cremophor NP 14	Clear liquid	BASF
Isopropyl myristate		Clear liquid	Henkel
Isopropyl palmitate		Clear liquid	Henkel
Polyoxyl 40-caster oil	Etocas 40	Tan viscous liquid	Croda Chemicals
Polyoxyl 40-hydrogenated castor oil	Cremophor RH 40	White viscous liquid to soft paste	BASF
Glycerol		Clear liquid	Saarchem, South Africa
Lanolin USP	Lanolin anhydrous	Amber grease base	BDH
Emulsifying Wax NF	Polawax GP 200	White waxy pellet	Croda Chemicals
Carbomer	Carbopol 940	White fine powder	Edenchem, South Africa
Hydroxyethylcellulose	Natrosol	White coarse powder	Aqualon, United States of America
Hydroxypropylmethylcellulose		White fine powder	Dow Chemicals
D-Panthenol		Clear viscous sticky liquid	BASF, South Africa
Triethanolamine		Clear liquid	Saarchem, South Africa

number of excipients. These substances include non-ionic surfactants, co-solvents, emulsifiers and preservatives. HPLC assay results confirmed DSC results and showed that ivermectin assay results fell by approximately 20% when the drug was mixed with these excipients (Fig. 4). Therefore, if ivermectin will be combined with these excipients in an emulsion formulation, the resulting product will not comply with any pharmacopoeial specification for drug assay.

Although it cannot be conclusive that these results indicate incompatibilities or predict stability problems during storage of formulations at room temperature there are sufficient alternatives being compatible with ivermectin (Table 3).

3. Experimental

3.1. Materials

Ivermectin [consists of two components, 22,23-dihydroavermectin $B_{1a}(H_2B_{1a} \geq 80\%)$ and 22,23-dihydroavermectin $B_{1b}(H_2B_{1b}, \leq 20\%)$] was obtained from Sanvet (South Africa). All organic solvents were of HPLC grade from BDH (Poole, United Kingdom). All other reagents were of analytical-grade purity. Excipients were obtained from companies distributing it in South Africa where possible. Details are listed in Table 1.

3.2. Preparation of samples

The excipients and materials analysed in the current study had various physical states ranging from liquids to solids, semi-solids and coarse or finely ground powders. The mixed samples consisted of ivermectin in a 1 : 1 w/w ratio with each of the excipients listed in Table 1.

3.3. DSC Analysis of samples

Shimadzu Corporation DSC-50 Differential Scanning Calorimeter and Shimadzu Corporation Thermal Analysis Software TA-50. The instrument was calibrated using indium as a reference standard (melting point 156.4 °C). Samples (2–8 mg) were weighed to the nearest 0.001 mg and crimped in aluminium seal pans. The heating rate of $10\,^{\circ}C \cdot min^{-1}$ under nitrogen purge with a flow rate of $45\ ml \cdot min^{-1}$. The DSC scans were performed from ambient temperature to 300 °C.

3.4. HPLC analysis of samples

Thermal Separation Products Inc. High Pressure Liquid chromatograph (HPLC); Scientific Software, Inc., EZChrom™ Chromatography Data System. This chromatograph consisted of a SpectraSERIES™ Model P 200 gradient pump; SpectraSERIES™ Model AS 300 autosampler and a SpectraSERIES™ Model 150 ultraviolet detector. The chromatograph was equipped with a Rheodyne sample valve and a 100 µl sample loop.

Normal-phase HPLC (NP-HPLC): A Macherey-Nagel analytical column (4.6 mm × 250 mm ODS, 5 µm particle size) was used. The mobile phase was a mixture of acetonitrile and water 95 : 5 v/v, at a flow rate of $1.2\ ml \cdot min^{-1}$. All chromatograms were monitored by UV detection at 240 nm. To prepare standard solutions, an accurately weighed quantity of ivermectin was dissolved in tetrahydrofuran. Dilutions were made from this solution and injected into the chromatograph. Samples taken from mixtures of ivermectin and excipients were similarly prepared in a way that the drug concentration in the samples, injected into the chromatograph, fell within the range of standard concentrations.

3.5. Calculations and statistical analysis

Mean HPLC results were compared according to the Student-Newman-Keuls multiple range tests (Statistica CSS 3.1 Statsoft, USA). A 95% confidence level ($p \leq 0.05$) was considered satisfactory for indicating significant differences.

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