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Efficiency of stabilization of low level of coloration of Castellani's paint without fuchsine with disodium edetate

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Addition of 0.03% of disodium edetate dihydrate (DED) was determined by observation of colour differences ΔE^* in the CIELAB system to give more effective stabilisation of a low level of coloration of Castellani's paint without fuchsine than did 0.02% DED. Increase of the DED addition to 0.04% did not lead to further retardation of the increase in coloration of the preparation.

Castellani's paint without fuchsine is a cosmetically more acceptable and less irritating variant of this classical medicinal preparation containing fuchsine (Shah 2003). Although it is generally referred to as colorless, even when freshly prepared it can and in practice often does, have a slightly drab, tawny or reddish colour. This coloration originates from coloration of the resorcinol and phenol solutions, which is permitted by pharmacopoeias. The slight initial coloration of Castellani's paint without fuchsine gradually increases over time in spite of storing the preparation in brown coloured glass containers until it has an intense tawny or ferruginous colour. This is probably a result of the oxidation of resorcinol and phenol. Variations in intensity and sometimes also type of this coloration cause doubt as to the quality of the preparation, which is why it is desirable to stabilize it's composition. Addition of not less than 0.01% disodium edetate dihydrate (DED) has been documented to give effective stabilization of a low level of coloration of Castellani's paint of the composition specified by the Czech Pharmacopoeia 2002 and the Slovak Pharmaceutical codex 1997 (Šubert and Cieslarová 2006). However, it is unknown whether an increase of DED content to 0.02%, 0.03% and 0.04% further raises the effectiveness of stabilisation. The aim of this study was to resolve this question by observation of changes in color of the preparation with different contents of DED. Spectrophotometric tristimulus colorimetry was performed by measurement of the transmittance of samples in the visible spectral region followed by description of their colour using values of the coordinates L^*, a^*, b^* in uniform CIELAB space (Wyszecki and Stiles 2000; Krishna Prasad et al. 1996) and by calculation of colour differences ΔE^*. Tristimulus colorimetry can be more sensitive than HPLC assays in stability studies (Shephard et al. 1999). Results of observation of colour changes of Castellani's paint without fuchsine over 31 weeks are summarized in the Table.

Table: Colour differences (ΔE^* CIELAB)[a] of Castellani's paint without fuchsine 2–31 weeks after addition of 0.02–0.04% of DED

DED (%)	Weeks										
	2	4	6	8	11	l4	19	22	25	28	31
0.02	0.58	0.54	1.0[b]	2.0[b]	2.7[b]	4.1[b]	6.2	7.4	11.6	11.6	12.2
0.03	0.41[b]	0.59	0.97	1.7	2.6	3.9	5.7	6.9	9.2	11.4	11.6
0.04	0.44	0.53	0.95	1.3	2.5	3.9	5.9	7.2	9.3	11.4	12.1

[a] $\bar{x}$ from $n = 5$ except [b] where $n = 4$

The differences in ΔE^* values are not large, because the aim of stabilization is to slow the increase of coloration of the preparation, and thus the increase of ΔE^* during time. This was successful with all concentrations of DED tested. The highest determined value of ΔE^* was 12.2 CIE units, while a non-stabilized preparation reached a difference as great as 109 CIE units within 8.5 months after its manufacturing, which is a period comparable to that of the duration of effectivness of DED (Šubert and Cieslarová 2006). Testing differences in ΔE^* values for DED contents of 0.02%, 0.03% and 0.04% with the one-sided t-test led to results statistically significant at the $\alpha = 0.05$ level, where for $n = 11$ is tabulated $t = 1.81$ and calculated t was 2.19 and 1.99 respectively. Testing the differences between 0.03% and 0.04% DED gave $t = 0.71$. Similar results were obtained by the non-parametric sign test, in which the tabulated value for $\alpha = 0.05$ and $n = 11$ is 9 and the observed values were 10, 11 and 7. It is possible to deduce from these results, that 0.03% of DED is more effective in stabilizing a low level coloration of Castellani's paint without fuchsine in the composition according to the Czech Pharmacopoeia 2002 and the Slovak Pharmaceutical codex 1997, but that an increase of DED content from 0.03% to 0.04% produces no further improvement.

Experimental

Stabilization studies were performed on a sample of Castellani's paint without fuchsine of composition according to the Czech Pharmacopoeia 2002 and the Slovak Pharmaceutical codex 1997 (i.e. a solution of 3.6% of phenol, 8.0% of resorcinol, 0.8% of boric acid, 4.0% of acetone and 6.9% of ethanol in purified water) from an industrial manufacturer (VAKOS XT, Prague, container of 1000 g, batch No. 120305); DED p.a. (Penta) was added to this prepation 6 weeks after its manufacturing date. Portions of the preparation stabilized with DED were filled into 200 ml brown glass bottles closed with plastic screw caps. The temperature during their storage ranged between 18 and 26 °C. Transmittances of samples with different DED contents were measured in the range 380–770 nm with a Helios Beta spectrophotometer (ThermoSpectronic) in 1 cm Plastibrand® plastic cells cat. No. 7591 50 (Brand) in Colour mode, 600 nm/min, against purified water. Values of the coordinates L^*, a^*, b^* of CIELAB uniform colour space were calculated with Chroma v. 2.0 Colour Measurement software, Part No: 9423 UV8 50910E (ChromSpec). From these values, differences of colour in CIE units, related in each case to the first measured values, were calculated according to the formula $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Wyszecki and Stiles 2000; Krishna Prasad et al. 1996). Coordinates L^*, a^*, b^* were calculated for normalized C light corresponding to average daylight and for 10 °C standard observer. Results of 5 single measurements were statistically processed by testing for outliers from the range, calculation of arithmetic mean, estimation of relative standard deviation of measurement ΔE^* from range, paired t-test and paired non-parametric sign test based on the binomial distribution (Sachs 1974).

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Effect of β-(1,3)-glucan on rheological properties and stability of topical formulations

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The paper deals with an effect of insoluble fungal β-(1,3)-glucan on rheological properties of topical preparations. Two types of hydrogels (based on carbomer and polyacrylamide) and two types of hydrocreams (based on polysorbate 80/Span 80™ and Brij 721™/Brij 72™) were prepared and investigated. The rheological properties of all these preparations were compared with the properties of placebos and they were measured after preparation and after 5 months of storage under different conditions : at 20 °C and 35 °C and after a triplicate freeze-thaw cycling process (−20° C/+20° C). In general it can be stated that with the exception of polyacrylamide hydrogel the β-(1,3)-glucan presence increased the apparent viscosity of assessed preparations by approximately 10–20%. In the case of hydrocreams it was observed that the triplicate freeze-thaw cycling process increased the apparent viscosity of β-(1,3)-glucan preparations by about 20–30%.

β-(1,3)-Glucans are natural polysaccharides, often used in various preparations mainly due to their immunostimulating properties. During the last years they were increasingly used as active substances in different topical preparations (Davis 1992, Klein 1999).

In water, β-(1,3)-glucans alone (similarly as other natural and semisynthetic polysaccharides) create viscous solutions, dispersions or gels (Böhm and Kulicke 1999; Burkus and Temelli 2005; Colleoni-Sirghie et al. 2003; Doublier and Wood 1995; Lee et al. 2005; Skendi et al. 2003). Our aim was to ascertain how the rheological properties and stability of topical preparations are influenced by the presence of fungal β-(1,3)-glucan.

We used insoluble fungal β-(1,3)-glucan from ligniperdous mushroom *Pleurotus ostreatus* with particle sizes below 100 μm (Natures Ltd., Slovakia). It creates viscous suspensions in water at about a 1–1.5% dry substance content. Four different types of topical preparations were prepared: (1) carbomer hydrogel (Carbopol 940™ 1%), (2) polyacrylamide hydrogel (Sepigel 305™ 2%), (3) body milk (polysorbate 80 2.75%, Span 80™ 2.25%) and (4) hydrocream (Brij 721™ 2.5%, Brij 72™ 2.5%). The body milk and hydrocream contained liquid paraffin 20% and 15%, respectively, as an oil phase. All the preparations were preserved by a mixture of methyl-, ethyl-, propyl-,