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Influence of storage time and temperature on the stability of indomethacin Pluronic® F-127 gels

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The stability of 20 topical gel formulations containing drug, 1% w/w indomethacin (IND), and 20% w/w Pluronic® (PF-127) as a gel-forming agent, hexylene glycol (HG) and polyethylene glycol 300 (PEG) in different amounts (16, 20 and 24% w/w) as solvents and 1% w/w polyvinyl pyrrolidone (PVP, K-25) and Tween® as excipients was determined by appearance and consistency of the gels, microscopy, pH and rheological measurements after 1 and 4 weeks storage, at 6 °C, 20 ± 2 °C and 45 °C. Viscosity values were determined from rheograms by a Haake Rotovisco sensor at shear rates of 1000 to 10000 1/s. The relationship between effectors (temperature and storage time) and response (viscosity) was determined using multiple regression analysis. All formulations were stable at room temperature (20 ± 2 °C). The consistency of the gels containing HG and PEG decreased during storage at 6 °C. Storing the gels at 6 °C resulted in the precipitation of IND, but when PVP was incorporated into the IND-PF-127 gels, the stability of the gels was improved. All IND gels sustained their pseudoplastic flow behaviour. The viscosity decreased as storage time increased. A statistically significant model was obtained, showing that the effect of storage temperatures on the viscosity was much less than the effect of storage time.

1. Introduction

Gels are semi-solid aqueous preparations that are widely used as dermatological vehicles. Besides oleogels and hydrogels, transparent gels are used extensively in cosmetics and pharmaceuticals [1].

Heterogeneous systems suffer from phase changes when stored incorrectly. Ointments and gels may bleed as their matrices contract and squeeze out the constituents. High temperatures can produce or accelerate such adjustments. The apparent pH of a topical product may also change during storage [2]. Drug products must remain stable over a sufficient period of time to cover supply and use of the finished products following their manufacture. Consequently, much effort is expended on research to assure the stability not only of the drug, but of the formulation itself [3]. Indomethacin (IND) is stable at neutral pH, but rapidly decomposes in an alkaline environment because of the OH^- reactive amide bond. Furthermore, IND is a water-insoluble, weak acid, but is soluble in alkaline solutions (e.g, Pluronics). This compound is unstable in alkaline media and is liable to base-catalyzed hydrolysis. Therefore, it is necessary to characterize the effects of Pluronic F-127 on the stability of IND in a finished product. Studies have been published on the stability of IND in the aqueous form of some Pluronics [4–7] as well as of IND in aqueous solutions in the presence of alkali [1, 4, 5], alkali and Pluronics [4, 6], and alkali and surfactants [7–9]. Pluronics, stabilize IND in alkaline solutions. The stabilization of IND in the presence of Pluronics was attributed to micellar solubilization [4, 6]. The nonionic surfactant (Tween® 80) and co-solvent polyethylene glycol 400 were found more effective than glycerin and propyleneglycol in stabilizing IND against alkaline degradation [10]. Polysorbates decrease the decomposition rate of IND due to a decrease of the medium polarity [9], while the presence of PEG (400) as a cosolvent causes a decrease of the dielectric constant [10]. The presence of Pluronic (PF-127) and cosolvents (glycols), besides increasing the solubility, significantly enhance the stability of IND.

Several authors have noted that consistency is a significant factor in a patient's assessment of a product and hence its acceptability. Such a need for product acceptability is by no means restricted to cosmetic preparations; pharmaceuticals also have to be formulated so that the patients see the product as being of high quality [11]. Lervolino et al. [12] showed that crystallization is one of the most destabilizing physical processes in drug formulations. It can be influenced by a number of external parameters such as solvent, supersaturation, impurities, temperature, pH and hydrodynamics [12]. Destabilization is promoted by temperature changes during storage, especially if the solubility of the drug is strongly dependent on temperature [13].

This study is a continuation of an earlier study [14] where topical gel formulations of 1% w/w IND were prepared (Table 1) using 20% w/w PF-127 as a gel-forming agent and HG and PEG 300 in different amounts (16, 20 and 24% w/w) as solvents and 1% w/w of PVP and Tween as excipients [14]. Storage tests were made on 20 gel formu-

Table 1: 1% Indomethacin gel formulae containing Pluronic F-127

No.	IND	F-127	HG	PEG 300	PVP 25	Tween 80	Ster. Water
1	–	20	16	–	–	–	64
2	1	20	16	–	–	–	63
3	1	20	16	–	1	–	62
4	1	20	16	–	–	1	62
5	1	20	20	–	–	–	59
6	1	20	20	–	1	–	58
7	1	20	20	–	–	1	58
8	1	20	24	–	–	–	55
9	1	20	24	–	1	–	54
10	1	20	24	–	–	1	54
11	1	20	–	16	–	–	63
12	1	20	–	16	1	–	62
13	1	20	–	16	–	1	62
14	1	20	–	20	–	–	59
15	1	20	–	20	1	–	58
16	1	20	–	20	–	1	58
17	1	20	–	24	–	–	55
18	1	20	–	24	1	–	54
19	1	20	–	24	–	1	54
20	–	20	–	16	–	–	64

lations over a period of 1 and 4 weeks at 6 °C, 20 ± 2 °C and 45 °C.

The aim of this study was to examine the influence of storage time and storage conditions on the stability of the gels. The stability was determined by appearance and consistency of the gels, microscopy, pH and rheological measurements.

2. Investigations, results and discussion

2.1. Macroscopic analysis

The results of the consistency of the gels are summarized in Tables 2 and 3. The effect of storage on the appearance and consistency was quite clear. No bacterial growth were detected by visual inspection in all gel samples after storage. All of the gels made with HG stored in a refrigator were of low viscosity at all storage times, while all other gels made with PEG were semi-solid after storage at 6 °C for 1 and 4 weeks. It was noted that PEG gels were highly structured semi-solid gels which showed changes in consistency after storage; their final consistency was thicker than that of HG gels. This can be attributed to the molecular weight of PEG. Placebo PEG gel (no. 20) was a more viscous gel when stored at 45 °C. Attwood (1985) suggested that an increase in temperature would dehydrate the oxyethylene chains of PF-127, and would thus be expected to decrease the extent and rate of water uptake [11].

Some HG gels stored at 6 °C were turbid due to the precipitation of IND in the gel. All IND gels remained yellow and transparent at 20 ± 2 °C and 45 °C after 1 week of storage. The appearance of a pale yellow to dark yellow color in samples stored at 45 °C for 4 weeks is most likely due to ion bonding or hydrolysis of some IND, which is known to darken slightly at high temperatures.

2.2. Crystallinity

No IND crystals were observed microscopically during 4 weeks of storage at 20 ± 2 °C or 45 °C. IND crystals were observed in the formulations stored at 6 °C without additive (PVP) for 1 week and increased with time after 4 weeks of storage under the same conditions. Solubility is not the only factor affecting the drug crystallization process. Storage of the gels at low temperatures may affect IND crystallization through other factors. In our previous work [15] we showed that the solvents HG and PEG 300 gave the best solubility for IND. Using PVP as an excipient improved the solubility of IND [15]. PVP has been used as a drug crystallization inhibitor in pharmaceutical formulations for many years. For instance, Ziller and Rupprecht [16] suggested that the inhibitory effect of PVP on drug crystallization in aqueous suspension may be primarily attributed to the protective PVP

Table 2: Effect of storage on physico-chemical characteristics of HG gels

No.	Temp	Consistency			Crystallization			pH		
		0 time	1 week	4 weeks	0 time	1 week	4 weeks	0 time	1 week	4 weeks
1	6 °C		1	1		–	–		7.13	7.25
	22 °C	1	1	1	–	–	–	7.37	7.13	7.28
	45 °C		2	2		–	–		7.2	7.35
2	6 °C		1	1		(++)	(+++)		4.62	4.76
	22 °C	2	2	2	–	(+)	(+)	4.68	4.65	4.6
	45 °C		2	2		–	–		4.4	4.4
3	6 °C		1	1		–	–		4.58	4.54
	22 °C	2	2	2	–	–	–	4.58	4.59	4.58
	45 °C		2	2		–	–		4.35	4.3
4	6 °C		1	1		(+)	(++)		4.71	4.7
	22 °C	2	2	2	–	–	–	4.73	4.72	4.74
	45 °C		2	2		–	–		4.48	4.43
5	6 °C		1	1		(+)	(++)		4.64	4.68
	22 °C	2	2	2	–	–	–	4.67	4.62	4.62
	45 °C		2	2		–	–		4.38	4.35
6	6 °C		1	1		–	–		4.5	4.54
	22 °C	2	2	2	–	–	–	4.52	4.58	4.54
	45 °C		2	2		–	–		4.48	4.31
7	6 °C		1	1		–	(+)		4.73	4.8
	22 °C	2	2	2	–	–	–	4.75	4.75	4.8
	45 °C		2	2		–	–		4.58	4.47
8	6 °C		1	1		–	–		4.68	4.67
	22 °C	1	1	1	–	–	–	4.62	4.6	4.64
	45 °C		1	1		–	–		4.4	4.45
9	6 °C		1	1		–	–		4.53	4.5
	22 °C	1	1	1	–	–	–	4.53	4.56	4.55
	45 °C		1	1		–	–		4.45	4.41
10	6 °C		1	1		–	–		4.75	4.78
	22 °C	1	1	1	–	–	–	4.72	4.75	4.7
	45 °C		1	1		–	–		4.42	4.44

Table 3: Effect of storage on physico-chemical characteristics of PEG gels

No.	Temp	Consistency			Crystallization			pH		
		0 time	1 week	4 weeks	0 time	1 week	4 weeks	0 time	1 week	4 weeks
11	6 °C		2	2		(+)	(++)		5.07	5.02
	22 °C	3	3	3	–	–	–	5.02	5.08	5.03
	45 °C		2	2		–	–		4.94	4.96
12	6 °C		2	2		–	–		4.99	5.03
	22 °C	3	3	3	–	–	–	5.02	4.99	4.98
	45 °C		2	2		–	–		5	4.91
13	6 °C		2	2		–	(+)		5.08	5.1
	22 °C	3	3	3	–	–	–	5	4.98	5.05
	45 °C		2	2		–	–		4.94	4.81
14	6 °C		2	2		–	(+)		5.15	5.2
	22 °C	3	3	3	–	–	–	5.15	5.1	5.01
	45 °C		2	2		–	–		4.9	4.8
15	6 °C		2	2		–	–		5.22	5.25
	22 °C	3	3	3	–	–	–	5.2	5.2	5.18
	45 °C		2	2		–	–		5.01	5
16	6 °C		2	2		–	(+)		5.15	5.18
	22 °C	3	3	3	–	–	–	5.14	5.1	5.04
	45 °C		2	2		–	–		4.98	4.88
17	6 °C		2	2		–	–		5.24	5.2
	22 °C	3	3	3	–	–	–	5.25	5.2	5.22
	45 °C		2	2		–	–		5.02	5
18	6 °C		2	2		–	–		5.21	5.18
	22 °C	3	3	3	–	–	–	5.2	5.2	5.18
	45 °C		2	2		–	–		5.1	5.09
19	6 °C		2	2		–	(+)		5.32	5.28
	22 °C	3	3	3	–	–	–	5.3	5.23	5.25
	45 °C		2	2		–	–		5.1	5.05
20	6 °C		2	2		–	–		6.65	6.8
	22 °C	3	2	2	–	–	–	6.79	6.75	6.7
	45 °C		3	3		–	–		6.65	6.6

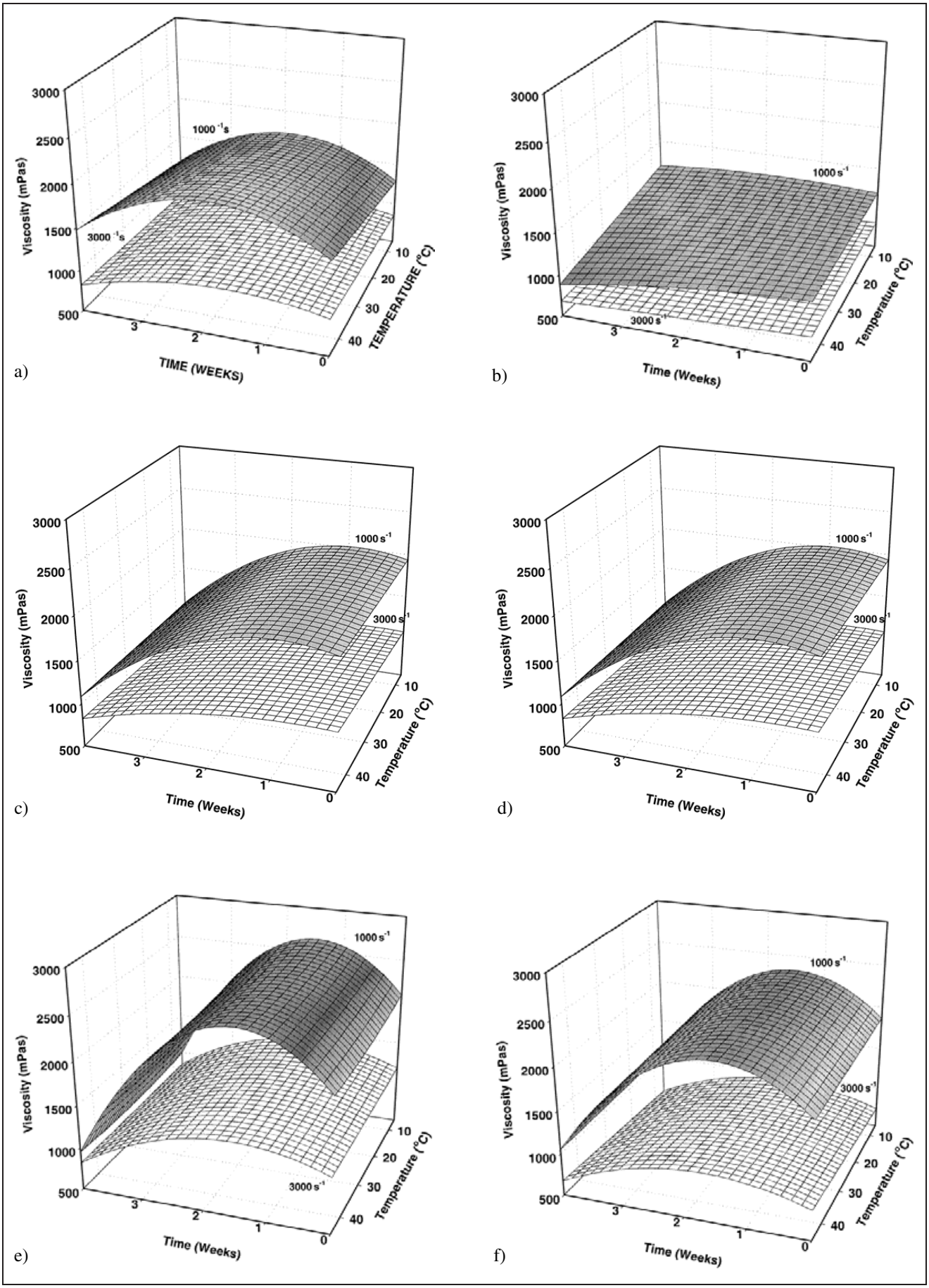

Fig.: Surface plots of the effect of temperature and storage time on the viscosity of a: Pluronic F-127- IND16% HG – PVP gel; b: Pluronic F-127- IND 20% HG gel; c: Pluronic F-127- IND16% PEG – PVP gel; d: Pluronic F-127- IND16% PEG – Tween gel; e: Pluronic F-127- IND 20% PEG – PVP gel; f. Pluronic F-127- IND 24% PEG gel

layers adsorbed on the crystal surfaces. In the case of a transdermal matrix system, PVP may interact and adsorb onto the Norethindrone acetate (NETA) nuclei or initial crystals, and thus prevent drug crystal growth [17]. Richards [2] has attributed this precipitation to the solubility of the unionized species, which is less than that of the ionized form. If the pH of a solution of a weakly acidic drug like IND is reduced then the proportion of unionized acid molecules in the solution increases [2]. The pH of the gels stored at 6 °C was unchanged during storage at this temperature.

2.3. pH

The results on the effects of storage temperature and time on the pH of the formulations are summarized in Tables 2 and 3. No changes occurred within the mild acidic pH range which is suitable for human skin. Initially, the pH values of each formulation were relatively stable for 1 and 4 weeks, indicating that no hydrolysis of IND occurred during the storage of the gels at 6 °C and 20 ± 2 °C. At 45 °C, the pH values of each formulation were slightly decreased by ≈ 0.1 to 0.3 units during the storage. These results indicated that the storage temperature plays an important role in the stability of the IND gels, which is in agreement with previous studies [4, 6].

2.4. Viscosity

The viscosity values obtained for the different variables involved in the study (shear rate and shear stress) were graphically represented at different shear rates [14, 18]. All the rheograms showed a non-Newtonian behaviour of the pseudoplastic flow with yield values. However, the behaviour of the flow curves for all the gels did not change. The unusual rheological behaviour (pseudoplastic flow with yield value) found in the poloxamer gels studied may be due to the association of glycols (PEG and HG) with IND in the polymeric structure of the gel. Miller and Drabik [19] could not define the flow of different poloxamer gels. These data displayed neither a clearly pseudoplastic or a simple plastic flow [19].

All systems showed minor changes in viscosities after storage but at different rates and to differing extents. The net change of the viscosity of the gels was due to the thermoreversibility of PF-127. 20–30% PF-127 gels are fluid at 4–5 °C and highly viscous at room temperature and body temperature, but the presence of additives affects the gel strength [20].

The viscosity of gels stored at 6 °C was clearly lower than that of gels stored at warmer temperatures. Viscosity was lower after 4 weeks than it was initially or after 1 week storage at every storage temperature investigated. Rassing and Attwood (1983) and Vadnere et al. (1984) showed that below 10 °C the polymer chains exist as extended coils surrounded by a hydration layer. At the higher temperature the hydrogen bonding, especially between poly(oxypropylene) (PPO) units and water (i.e. more hydrophobic at higher temperatures), becomes unstable and leads to desolvation. Thus the structure of the solution shows a more random pattern and contributes to the increase in solution entropy. This is reflected as an increase in viscosity [21, 22]. Spancake et al. (1991) showed at 60 °C a decrease in viscosity of the gel and break-down of the gel at higher temperatures [13, 21–23].

2.5. Response surfaces

Stability was determined for several formulations: 3 (16% HG,1% PVP), 5 (20% HG), 12 (16% PEG-1% PVP),13 (16% PEG-1% Tween), 15 (20% PEG-1% PVP) and 17 (24% PEG). Figures a–f show the response surfaces of storage temperature and time on the viscosity of the six batches of PF-127-IND gels at low shear rates (1000 and 3000 1/s). A statistically significant model was generated from these data. It can been seen that changes in temperature and storage time have little effect on viscosity (Fig. b), whereas changes in storage time to 1 week increases the viscosity of all gels at all temperatures, with the greatest effect for gels 12, 13, 15 and 17. Any further increase in storage time (4 weeks) causes a reduction in viscosity (Figs. a, c–f). Gel viscosity decreased progressively on increasing storage time. The exception was the first 1 week after preparation, when the gels appeared to complete their structurization. However, the stability of the preparation was maintained throughout the experiment. The response surfaces in Figs. a and c–f show a dependence of storage temperature on viscosity, while in Fig. b the contribution was negligible. The reduction in viscosity after 4 weeks of storage was likely caused by minor dehydration of the gels due to the polyoxyethylene chains of PF-127, which are known to have high hydration properties. Also because the difference in viscosity between initial and 4 weeks storage is significant, it seems impossible to predict long-term stability.

It was concluded that 1% w/w IND-20% w/w PF-127 gels are stable at 20 ± 2 °C and should not be subjected to a low temperature (6 °C) because storing gels in a low temperature results in the precipitation of IND. Crystallization of IND in PF-127 gel is dependent on the additives, storage temperature and storage time. Crystal formation can be controlled by adjusting these factors. PVP was the most effective crystallization inhibitor. Under these experimental conditions the gels containing PEG were more stable than those containing HG. The gel pH varied between 4.30 and 5.30; greatest stability was noted for gels stored at room temperature. In this study, viscosity increased during the first week of storage. Viscosities of the gels decreased between 1 week of storage and 4 weeks of storage. The effect of storage temperature on the viscosity seems to be much smaller than the effect of storage time. Only in gels containing 20% HG (5) the storage temperature had a noticeable effect on the viscosity. The viscosity decreased with increasing storage time.

3. Experimental

3.1. Materials

Indomethacin IND (particle size <5 μ) was supplied by Orion Corporation, Espoo, Finland. Pluronic PF-127 Basf Chemicals, Germany and hexylene glycol HG, polyethylene glycol 300 PEG and Tween® 80 by Fluka Chemical, Switzerland, and polyvinyl pyrrolidone (PVP) Plasdone®, K-25 by GAF Chemicals, USA.

3.2. Preparation of pluronic gels

The composition of the different IND gels is shown in Table 1. The gels were prepared by the cold method of Schmolka [20]. The weighted amount of PF-127 was slowly added to cold water (4–6 °C) under constant agitation. Thereafter the dispersion was stored overnight in a refrigerator. With time, a clear viscous solution was formed.

IND was previously dissolved in the solvents (HG, PEG). The solution of IND was added to the viscous solution of Pluronic by mixing gently to facilitate the formation of IND gel. The gels were packed in Amber glass containers and stored at room temperature (20 ± 2 °C) for 24 h before storing at three different temperatures (6 °C, 20 ± 2 °C and 45 °C).

3.3. Stability study

The stability of the gels was determined during a 4-week period in three different conditions. Measurements were made 24 h after gel preparation (0-sample) as well as 1 and 4 weeks after gel preparation. The storage conditions studied were cold (6 °C), room temperature (20 ± 2 °C) and accelerated conditions (45 °C).

3.3.1. Macroscopic analysis

The appearance of the model formulations was inspected for changes in colour and clarity of the gel. The consistency was assigned as liquid (1), semi-solid (2) or more viscous semi-solid (3).

3.3.2. Microscopy

The gels were analysed for the presence of crystals by optical microscopy (Olympus VANOX-T, Tokyo, Japan), using a magnification of 500 X.

3.3.3. pH

The pH was measured for each gel using a portable pH meter (PHM 80, Copenhagen, Denmark) which was calibrated at room temperature (20 ± 2 °C) before each use with buffered solutions of pH 4 and 7.

3.3.4. Rheological characteristics

The rheograms were measured by the cone and plate method (Haake Rotovisco sensor PKI/0.5 degree PK100/RV100, Haake GmbH, Karlsruhe, Germany). Determinations were made at 20.0 ± 0.1 °C. A sample of approximately 1 g was carefully placed on the plate. The measuring time was 2 min during which the shear rate was increased from zero to 10000 1/s. The viscosities were calculated from the rheograms at shear rates of 1000, 2000, 3000, 6000 and 9000 1/s. All determinations were made in triplicate.

3.3.5. Response surfaces methodology

The effects of storing time (Time) and storing temperature (Temperature) to the viscosity (Visc) were modelled using a normal second order polynomial equation:

$$\text{Visc} = a_1 \cdot \text{Time} + a_2 \cdot \text{Temperature} + a_3 \cdot \text{Time}^2 + a_4 \cdot \text{Temperature}^2 + a_5 \cdot \text{Time} \cdot \text{Temperature} + a_6,$$

where a_1 to a_6 are coefficients. The model was simplified with a multilinear backward, stepwise regression technique. The validity of every term was tested by t-test. Only statistically significant terms ($p < 0.05$) were chosen for the final model. Data were modelled using M-mode for Windows (Version 3.0, Umetri AB, Sweden) and the figures were plotted with Sigma Plot PC program (Version 5.0, SPSS Inc., USA).

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