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Studies on gynaecological hydrophilic lactic acid preparations

Part 3: Effects of chitosan on the properties of methylcellulose gels

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Methylcellulose gels comprising lactic acid, similarly to its water solutions, have pH values between 2.0 and 2.6, i.e. out of the physiological range. Addition of Chitosan enables preparation of gels with pH values from 2.2 to 4.9 similar to the natural vagina pH range, 3.8–4.4. The absolute viscosity of the gels, ranging from 27 up to 700 mPa · s, depends on chemical composition and on excess of lactic acid used. Measurement of adhesion enabled the selection of gels which remain located at the point of application under simulated biopharmaceutical conditions. The behavior of those preparations in the true environment of the vagina needs to be verified *in vivo*.

1. Introduction

An earlies study [1] presented results of investigation of complexes of Eudragit® E-100 and lactic acid. Attention was now paid to chitosan as a suitable carriers for lactic acid, in gynaecological therapy. Chitosan and lactic acid form transparent gels, thoroughly examined by Knapczyk [2–4], Tokura [5], Gallo and Hassan [6], and Takeuchi [7], looking for possible applications in dermatology as antimycotic preparations, in tablets and as microspheres.

2. Investigations and results

Stepwise addition of 5–20% lactic acid solutions to suspensions of chitosan did not change reaction of the complex, and the pH remained within the range of 5.7–6.1.

Table 1: Effect the amounts of chitosan, lactic acid and hydrophilizing agents on pH of methylcellulose gels

1 MC (g)	2 CH (g)	3 LA (g)	4 G (g)	pH	4′ PG (g)	pH	4 PEG-200 (g)	pH
4.00	0.83	0.50		3.93				
4.00	0.83	1.00		3.48				
4.00	0.83	2.00		2.75				
4.00	0.83	4.00		2.65				
4.00	0.83	0.50	5.00	4.21	5.00	4.56	5.00	4.27
4.00	0.83	1.00	5.00	3.30	5.00	3.45	5.00	3.41
4.00	0.83	2.00	5.00	2.73	5.00	2.70	5.00	2.83
4.00	0.83	4.00	5.00	2.25	5.00	2.29	5.00	2.35
4.00	0.83	0.50	10.00	4.80	10.00	4.83	10.00	4.10
4.00	0.83	1.00	10.00	3.48	10.00	3.52	10.00	3.38
4.00	0.83	2.00	10.00	3.05	10.00	2.74	10.00	2.92
4.00	0.83	4.00	10.00	2.52	10.00	2.38	10.00	2.47
4.00	0.83	0.50	15.00	4.89	15.00	3.98	15.00	3.75
4.00	0.83	1.00	15.00	3.47	15.00	3.11	15.00	2.98
4.00	0.83	2.00	15.00	2.83	15.00	2.86	15.00	2.65
4.00	0.83	4.00	15.00	2.34	15.00	2.34	15.00	2.24
4.00	0.83	0.50	20.00	4.47	20.00	4.12	20.00	3.84
4.00	0.83	1.00	20.00	3.15	20.00	3.59	20.00	2.96
4.00	0.83	2.00	20.00	2.51	20.00	2.83	20.00	2.69
4.00	0.83	4.00	20.00	2.16	20.00	2.41	20.00	2.28
4.00	0.83	0.50	25.00	4.51	25.00	4.95	25.00	3.62
4.00	0.83	1.00	25.00	3.50	25.00	3.22	25.00	3.31
4.00	0.83	2.00	25.00	2.78	25.00	2.86	25.00	3.02
4.00	0.83	4.00	25.00	2.24	25.00	2.31	25.00	2.57

MC: methylcellulose; CH: chitosan; LA: lactic acid; G: glycerol; PG: 1,2-propylene glycol

After addition of an equivalent volume of lactic acid, the neutralization point remained within the pH range of 3.7–4.0, in dependence upon concentration. The chitosan/lactic acid complex forms a clear transparent solution. To reach the point of stoichiometric neutralization, the solution required 0.61 g of lactic acid (calculated for 100%). This ratio of lactic acid/chitosan was assumed as the molar ratio 1 : 1. The pH value of the gel prepared in stoichiometric proportion (1 : 1) was 3.9 (see Table 1). A rise in the molar ratio of lactic acid to chitosan from 2 : 1 to 8 : 1 resulted in a reduction of pH from 3.5 to 2.7.

Addition of hydrophilizing agents increased pH values of the gels by 0.02–1.02, in dependence upon chemical composition.

The absolute viscosity of the 1 : 1 – gels was 354 mPa · s, for the maximum shearing rate of 4860 (Table 2). The 2 : 1 – gels reveal an absolute viscosity of 397 mPa · s under the same conditions. The 4 : 1 – gels have an absolute viscosity of 236 mPa · s, whereas the value for 8 : 1 – gels is 219 mPa · s.

Application of hydrophilizing agents insignificantly changed the data. The absolute viscosity of the gels comprising 5–25% of glycerol remains within the range 31–195 mPa · s, at maximum shearing speed. Addition of 1,2-propylene glycol results in viscosity values from 27 to 700 mPa · s, whereas the gels comprising PEG-200 revealed an absolute viscosity within the range of 329–641 mPa · s.

Methylcellulose gels comprising lactic acid and chitosan in stoichiometric proportions from 1 : 1 to 8 : 1, after 5 min changed their positions 0.3–1.5 cm. Similar mobility reveal the gels comprising 5% of glycerol, gels with 5, 10 and 25% of 1,2-propylene glycol and gels comprising 10% of PEG-200 (data not shown). The remaining gels are not so mobile and remain in their points of application.

3. Discussion

Selection of the lactic acid/chitosan ratios enables the preparation of gels with physiological pH values. An increased lactic acid/chitosan ratio leads to gels with lower pH values, comprising some acid reserve, which is able to neutralize the excess of base compounds, occurring in advanced inflammatory states of the vagina. The gels differ in their viscosity. However, rheological data alone do not enable prediction of their behavior *in vivo*. This may be overcome by means of adhesion measurements which provide information on the mobility of the gels under condi-

Table 2: Effect the amounts of chitosan, lactic acid and hydrophilizing agents on the viscosity methylcellulose gels

1 MC (g)	2 CH (g)	3 LA (g)	4 G (g)	η (mPa · s)	4′ PG (g)	η (mPa · s)	4″ PEG-200 (g)	η (mPa · s)
4.00	0.83	0.50		354.41				
4.00	0.83	1.00		396.60				
4.00	0.83	2.00		236.27				
4.00	0.83	4.00		219.40				
4.00	0.83	0.50	5.00	286.90	5.00	151.89	5.00	506.30
4.00	0.83	1.00	5.00	295.34	5.00	253.15	5.00	556.93
4.00	0.83	2.00	5.00	99.69	5.00	60.34	5.00	514.73
4.00	0.83	4.00	5.00	139.92	5.00	160.33	5.00	514.73
4.00	0.83	0.50	10.00	253.15	10.00	118.93	10.00	548.49
4.00	0.83	1.00	10.00	70.83	10.00	168.77	10.00	379.72
4.00	0.83	2.00	10.00	269.96	10.00	210.96	10.00	607.56
4.00	0.83	4.00	10.00	99.69	10.00	27.11	10.00	464.10
4.00	0.83	0.50	15.00	31.48	15.00	675.06	15.00	497.86
4.00	0.83	1.00	15.00	52.47	15.00	405.04	15.00	489.42
4.00	0.83	2.00	15.00	48.97	15.00	531.61	15.00	540.05
4.00	0.83	4.00	15.00	101.44	15.00	540.05	15.00	480.98
4.00	0.83	0.50	20.00	103.19	20.00	556.93	20.00	455.67
4.00	0.83	1.00	20.00	171.40	20.00	531.61	20.00	464.10
4.00	0.83	2.00	20.00	127.67	20.00	556.93	20.00	329.09
4.00	0.83	4.00	20.00	39.35	20.00	540.05	20.00	556.93
4.00	0.83	0.50	25.00	34.98	25.00	700.38	25.00	641.31
4.00	0.83	1.00	25.00	52.47	25.00	379.72	25.00	641.31
4.00	0.83	2.00	25.00	73.46	25.00	219.40	25.00	573.80
4.00	0.83	4.00	25.00	95.32	25.00	303.78	25.00	523.17

tions close to the physiological situation. Tests have shown that a number of gels is characterized by good adhesion and low mobility. It may be assumed that these gels will be well maintained in the place of application. This assumption needs to be verified *in vivo*.

4. Experimental

4.1. Materials

Aqua purificata, acc. To FP V. Lactic acid, PZF Cefarm, Wrocław. Methylcellulose, Aldrich Chemical Company Ltd. Gillingham-Dorest SP 84 SL-England. 1,2-Propylene glycol, Polskie Odczynniki Chemiczne, Gliwice. Polyoxyethylene glycol 200, LOBA-Chemie, Wien-Fishamend. Glycerol pro analysis, Polskie Odczynniki Chemiczne, Gliwice. Chitosan, deacetylated in 93.5%, MIR, Gdynia.

4.2. Methods

4.2.1. Measurements of pH and absolute viscosity

pH values and viscosity were determined as reported earlies [8].

4.2.2. Determination of the stoichiometric neutralization point for the chitosan − lactic acid system

Chitosan (0.2 g) pulverized in a mortar was poured into 9.8 g water in an Erlenmeyer flask. The suspension was titrated with a 5% solution of lactic acid, added in 0.1 ml portions, or with equivalent volumes of 10, 15 and 20% solutions. After addition of each portion of acid, the mixture was heated up to 40 °C to enable the reaction of lactic acid with chitosan, then cooled down to room temperature and made up with water, to replace the water evaporated. Finally, pH was measured.

4.2.3. Gel preparation technique

Chitosan was dissolved at 40 °C in an aqueous solution of lactic acid, prepared with half of the prescribed amount of water. After cooling, the hydrophilizing agent was added and the remaining amount of water was poured in. To the mixture, methylcellulose was added. The gels obtained comprised 4% of methylcellulose, 0.83% chitosan, from 0.5 to 4% lactic acid and 5–25% of particular hydrophilizing agents.
For specification of the gels see Table 1.

4.2.4. Measurement of adhesion

The equipment consisted of a round-bottomed flask, used for measuring of the gel flow rate under simulated *in vivo* conditions [8].

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