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PROCYANIDINS GELS : INFLUENCE OF IONS ON THE GELIFICATION OF CARRAGHENAN SOLUTIONS

Marie-Andrée Bos, Brigitte Vennat and Aimée Pourrat*
Laboratoire de Pharmacie Galénique et Pharmacotechnie
Faculté de Pharmacie, 28, Place Henri-Dunant
63001 Clermont-Ferrand Cedex

ABSTRACT

The formulation of procyanidin gels based on carraghenans is described. Gelling vehicles were selected to obtain stable, limpid gels of satisfactory viscosity and pH compatible with anti-ulcer therapy. Effort was focused on the study of the influence of cations (Na^+, K^+, Ca^{++}) and associated anions (chloride, citrate, gluconate), on the gelification : the best results were obtained with trisodium-citrate.

INTRODUCTION

In previous works, we studied the valorisation of procyanidins obtained by fermentation of an extract of Fragaria vesca (1-7). These procyanidins proved to possess marked anti-ulcer properties in Rat (8). The first results from procyanidin gel dosage forms, particularly suited to administer antacids, have recently been published (9,10). The work reported here concerns the formulation of semi-fluid gels based on carraghenans, derivatives wich possess a marked antipepsin activity. Effort is focused on the

* Author to whom correspondence should be addressed

study of the influence of cations (Na^+, K^+, Ca^{++}) and associated anions (chloride, citrate, gluconate), on the gelification of the colloïdal solutions.

MATERIAL AND METHODS

Raw Materials

The following carraghenans were tested : Aubygum X_2®, Aubygel X52®, Satiagel HV®, Satiagum standard® (Sanofi Bio-Industries).

The procyanidins incorpored into the gels were prepared by us (3).

Methods

Preparation of Gels

The gelling agent was dispersed in water with an IKA RW20 helical stirrer at 35°C ; after dispersion the solution was warmed at 50°C for 5 min. The stirring rate was set at 500 r.p.m.

Determination of Spreadability

One gram of gel 24 hours old was pressed between two horizontal plates 20 cm square, of which the upper one weighed 125 g and its diameter was measured against time (9).

Evaluation of Opalescence

The opalescence of the gels was studied using the method described in the French Pharmacopoeia (11), and spectrophotometric measurement of the transmission at 610 nm of reference solutions and tested gels.

Rheological Study

A rheological study of the procyanidin gels was carried out at 21°C using a Brookfiel RVTD V_2® instrument fitted with an SC_4-28/13R small adapter.

RESULTS AND DISCUSSION

132 gels were prepared, each containing 0.15 % Nipagine® together with different types and concentrations of carraghenans with and without added cations.

TABLE 1

Influence of Carraghenan Concentration on Stiffness and Limpidity

Formulation n°	Gelling Agent	Concentration % (w/w)	pH	Spreading Diameter After 1 min (mm)	T % at 610 nm
1	Aubygum X_2 ®	2	9.2	89	89
2	Aubygum X_2 ®	2.3	9.2	64	89
3	Aubygum X_2 ®	2.4	9.4	61.5	89
4	Aubygum X_2 ®	2.5	9.4	56	88
5	Aubygum X_2 ®	2.6	9.4	52	88
6	Aubygel X 52®	1.25	10.5	77	81
7	Aubygel X 52®	1.4	9.7	60.5	81
8	Aubygel X 52®	1.5	9.7	56.5	79
9	Aubygel X 52®	1.75	9.8	51	76
10	Satiagum standard®	2	10.2	72.5	52
11	Satiagum standard®	2.5	10.3	63.5	32
12	Satiagum standard®	3	10.5	57	24
13	Satiagum standard®	3.5	10.3	50	20
14	Satiagel HV®	2	10.2	72.5	48
15	Satiagel HV®	2.5	10.3	67.5	39
16	Satiagel HV®	3	10.2	61	29
17	Satiagel HV®	3.5	10.3	57.5	22
18	Satiagel HV®	4	10.3	46.5	14

The technical literature for the four colloids tested (12) indicates that their gelling power depends on the carraghenate concentration and on whether or not certain dissolved ions are presents ; K^+, Ca^{++} and NH_4^+ markedly increase gelling power while Na^+ makes it difficult to obtain a stiff gel. Accordingly, we studied the influence of three cations , Na^+, K^+ and Ca^{++} on the rheological behaviour of the gels.

Influence of Carraghenan Concentration

The 18 formulations tested were evaluated according to two main characteristics ; spreading diameter and percent transmission at 610 nm.

TABLE 2

Influence of Na^+ on Stiffness and Limpidity

Formulation n°	Gelling Agent % (w/w)	NaCl % (w/w)	Tri-Sodium Citrate % (w/w)	pH	Spreading Diameter After 1 min (mm)	T % at 610 nm
19	Aubygum X_1 ® 1.5	0.75		9.1	65.5	80.5
20	Aubygum X_2 ® 1.5		1.11	9.6	70.5	92
21	Aubygum X_1 ® 1.7	0.85		9.1	60	80
22	Aubygum X_2 ® 1.7		1.25	9.7	62	90
23	Aubygum X_1 ® 1.8	0.9		9.1	55	80
24	Aubygum X_2 ® 1.8		1.33	9.6	59.5	90
25	Aubygum X_1 ® 2	1		9.1	49	79
26	Aubygum X_2 ® 2		1.47	9.9	5	92
27	Aubygum X_1 ® 2.5	1.25		9.7	44	77
28	Aubygum X_2 ® 2.5		1.82	9.7	49.5	89
29	Aubygel X 52 ® 1	0.5		8.6	78	91
30	Aubygel X 52 ® 1		0.735	9.6	62	97
31	Aubygel X 52 ® 1.3	0.65		8.6	68.5	66
32	Aubygel X 52 ® 1.3		0.95	9.7	52	93
33	Aubygel X 52 ® 1.4	0.7		8.6	52.5	57
34	Aubygel X 52 ® 1.4		1.03	9.6	51.5	84
35	Aubygel X 52 ® 1.6	0.8		8.7	47.5	49
36	Aubygel X 52 ® 1.6		1.17	9.7	50.5	78
37	Satiagum standard® 1.5	0.75		9.7	82	54
38	Satiagum standard® 1.5		1.11	9.6	76	73
39	Satiagum standard® 2	1		10.2	68.5	38
40	Satiagum standard® 2		1.47	9.5	64.5	64
41	Satiagum standard® 2.5	1.25		9.7	58.5	23.5
42	Satiagum standard® 2.5		1.82	9.8	54.5	53
43	Satiagum standard® 3	1.5		9.6	46	20
44	Satiagum standard® 3		2.2	9.6	49	49
45	Satiagel HV ® 2	1		9.9	70.5	31
46	Satiagel HV ® 2		1.47	9.7	66	45
47	Satiagel HV ® 2.5	1.25		9.8	57.5	15
48	Satiagel HV ® 2.5		1.82	9.8	57	32
49	Satiagel HV ® 3	1.5		9.8	55.5	11
50	Satiagel HV ® 3		2.2	9.8	48	28

Spreading diameter at 1 min. is a measure of stiffness ; under The limpidity of the gel can be assessed using the method described in the French Pharmacopoeia (11), together with spectrophotometric measurement of transmission at 610 nm (T %).

As snown in Table I, all the gels were markedly alkaline with pH values between 9.2 and 10.5 compatible with anti-ulcer therapy.

TABLE 3

Influence of K^+ on Stiffness and Limpidity

Formulation n°	Gelling Agent % (w/w)	KCl % (w/w)	Tri-Potassium Citrate % (w/w)	pH	Spreading Diameter After 1 min (mm)	T % at 610 nm
51	Aubygum X_2 ® 1.3	0.65		9.1	77.5	80
52	Aubygum X_2 ® 1.3		0.89	9.4	65	87
53	Aubygum X_2 ® 1.5	0.75		9.3	59	70.5
54	Aubygum X_2 ® 1.5		1.03	9.8	58	81
55	Aubygum X_2 ® 1.7	0.85		9.4	63	35
56	Aubygum X_2 ® 1.7		1.16	9.9	53	75
57	Aubygum X_2 ® 1.8	0.9		9.4	61	37.5
58	Aubygum X_2 ® 1.8		1.23	9.8	52	72
59	Aubygum X_2 ® 1.9	0.95		9.3	54	35
60	Aubygum X_2 ® 1.9		1.30	9.9	49	52
61	Aubygel X 52®0.6	0.3		9.2	69.5	94
62	Aubygel X 52®0.6		0.41	9.4	70	95
63	Aubygel X 52®0.8	0.4		9.4	65.5	76
64	Aubygel X 52®0.8		0.55	9.6	63.5	96
65	Aubygel X 52®1	0.5		9.3	61	54
66	Aubygel X 52®1		0.68	9.6	60.5	84
67	Aubygel X 52®1.2	0.6		9.2	55.5	56
68	Aubygel X 52®1.2		0.82	9.5	55	79
69	Aubygel X 52®1.4	0.7		9.4	57.5	46.5
70	Aubygel X 52®1.4		0.96	9.5	54	65
71	Aubygel X 52®1.6	0.8		9.2	48	43
72	Aubygel X 52®1.6		1.1	3.4	50	59
73	Satiagum standard ®1	0.5		9.4	82	63
74	Satiagum standard ®1		0.68	9.3	81	73
75	Satiagum standard ®1.5	0.75		9.3	70	35
76	Satiagum standard ®1.5		1.03	9.6	59	65
77	Satiagum standard ®2	1		9.3	59	18
78	Satiagum standard ®2		1.37	9.8	47.5	49
79	Satiagum standard ®2.5	1.25		9.3	53	9.5
80	Satiagum standard ®2.5		1.71	9.9	44.5	38
81	Satiagel HV ® 1	0.5		9.3	67	46
82	Satiagel HV ® 1		0.68	9.6	70	63.5
83	Satiagel HV ® 1.5	0.75		9.2	68	18
84	Satiagel HV ® 1.5		1.03	9.8	56	44
85	Satiagel HV ® 2	1		9.2	65	8.5
86	Satiagel HV ® 2		1.37	9.8	48	29
87	Satiagel HV ® 2.5	1.25		9.0	58	4
88	Satiagel HV ® 2.5		1.71	9.9	47	15
89	Satiagel HV ® 3	1.5		9.2	53.5	2.5
90	Satiagel HV ® 3		2.05	9.9	51	6

- The gelling power of calcium gluconate was especially marked and much greater than that of $CaCl_2$.

TABLE 4

Influence of Ca^{++} on Stiffness and Limpidity

Formulation n°	Gelling Agent % (w/w)	$CaCl_2$ % (w/w)	Calcium gluconate % (w/w)	pH	Spreading diameter after 1 min (mm)	T % at 610 nm
91	Aubygum X_2® 1.3	0.65		9.4	78.5	54
92	Aubygum X_2® 1.3		2.53	9.3	80	90
93	Aubygum X_2® 1.5	0.75		9.0	61	43.5
94	Aubygum X_2® 1.5		2.89	9.1	74	82
95	Aubygum X_2® 1.7	0.85		9.1	67	10.5
96	Aubygum X_2® 1.7		3.25	9.2	61	43
97	Aubygum X_2® 1.8	0.9		9.4	60	4.5
98	Aubygum X_2® 1.8		3.47	9.2	58.5	37
99	Aubygum X_2® 1.9	0.95		9.2	58	3.5
100	Aubygum X_2® 1.9		3.69	9.2	54	35
101	Aubygum X_2® 2	1		9.5	60.5	2
102	Aubygum X_2® 2		3.87	9.1	50	31
103	Aubygum X_2® 2.2	1.1		9.2	53	1.5
104	Aubygum X_2® 2.2		4.27	9.1	49	18
105	Aubygum X_2® 2.5	1.25		9.1	48	1.5
106	Aubygum X_2® 2.5		4.8	9.1	43	18
107	Aubygel X_{52}® 0.8	0.4		9.1	> 100	14
108	Aubygel X_{52}® 0.8		1.55	9.2	91	10
109	Aubygel X_{52}® 1	0.5		9.1	> 100	12
110	Aubygel X_{52}® 1		1.94	9.3	68	10
111	Aubygel X_{52}® 1.2	0.6		9.0	> 100	7
112	Aubygel X_{52}® 1.2		2.32	9.3	61.5	8.5
113	Aubygel X_{52}® 1.3	0.65		9.2	> 100	8
114	Aubygel X_{52}® 1.3		2.53	9.3	54.5	9.5
115	Aubygel X_{52}® 1.4	0.7		8.9	> 100	4.5
116	Aubygel X_{52}® 1.4		2.7	9.2	56	6.5
117	Aubygel X_{52}® 1.5	0.75		9.0	> 100	4
118	Aubygel X_{52}® 1.5		2.89	9.1	52	9
119	Satiagum standard® 1.5	0.75		8.9	76.5	46
120	Satiagum standard® 1.5		2.89	9.1	68	37
121	Satiagum standard® 2	1		9.1	67	17
122	Satiagum standard® 2		3.87	9.0	57	26
123	Satiagum standard® 2.5	1.25		9.1	55.5	10.5
124	Satiagum standard® 2.5		4.8	9.0	47	18
125	Satiagum standard® 3	1.5		9.1	48	8
126	Satiagum standard® 3		5.8	9.0	43	11
127	Satiagel HV® 2	1		9.1	73	14
128	Satiagel HV® 2		3.87	9.2	57	9.5
129	Satiagel HV® 2.5	1.25		9.1	55.5	10.5
130	Satiagel HV® 2.5		4.8	9.1	46.5	8
131	Satiagel HV® 3	1.5		9.0	45	9.5
132	Satiagel HV® 3		5.8	9.0	37.5	8

Using the two selection criteria, spreading diameter and percent transmission at 610 nm, two formulations scored well : (i) formulation n°3 containing 2.4 % Aubygum X_2® and (ii) formulation n°7 containing 1.4 % Aubygel X52®. Because of their marked opalescence, none of the gels containing Satiagum Standard® or Satiagel Standard® were selected.

Influence of Ions on Gelling

For Na^+ we added half a part of NaCl per part of gelling agent as recommended in the technical literature for the carraghenans tested. The quantity of sodium citrate was calculated so as to afford the same Na^+ concentration as the NaCl. The same procedure was followed for the formulations containing K^+ and Ca^{++}.

The following conclusions can be drawn from the data presented in Tables 2-4.

As before, all the gels were markedly alkaline (pH between 8.6 and 10.2), practically unaffected by the nature of the added salt and compatible with anti-ulcer therapy.

- Adding Na^+ markedly modified gel stiffness regardless of the gelling agent tested.

In most cases, sodium citrate afforded stiffer gels than NaCl. It also gave more limpid gels characterised by a percent transmission at 610 nm which was often greater than that of the reference gels containing carraghenans alone.

- The influence of K^+ varied according to the salt used. Gels with KCl were stiffer than the reference gels.

Potassium citrate was much more efficient than KCl and in certain cases more even than sodium citrate. It also afforded more limpid gels than KCl, though these were still more opalescent than either the reference gels or those with added sodium citrate.

Though Ca^{++} favoured the gelling of carraghenan solutions overall, it adversely affected their limpidity ; most of the gels obtained were very opalescent.

From the results of the influence of Na^+, K^+ and Ca^{++} the four formulations with the most favourable characteristics were : (i) formulation n° 22 containing Aubygum X_2® and trisodium citrate, (ii) formulation n° 30 containing Aubygel X52® and trisodium

citrate, (iii) formulation n° 54 containing Aubygum X_2® and tripotassium citrate and (iv) formulation n° 66 containing Aubygel X52® and tripotassium citrate.

Formulations n° 22 and n° 30 advantageously combine the antipepsin activity of the carraghenates with the antacid properties of trisodium citrate.

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