Influence of locust bean gum on the rheological properties of kappa-carrageenan systems in the vicinity of the gel point

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Viscosity and oscillatory shear measurements have been performed on kappa-carrageenan alone and on kappa-carrageenan/galactomannan (guar gum or locust bean gum) blends in the vicinity of the sol–gel transition. From these measurements, a phase diagram showing the boundary limits for kappa-carrageenan/locust bean gum and kappa-carrageenan/guar gum was established. The viscoelastic behaviour at the transition showed that this process was sharp for the kappa-carrageenan/locust bean gum blend, while for the kappa-carrageenan/guar gum blend, it was more progressive. The kappa-carrageenan content at which the transition took place was estimated and was found to be constant, whatever the total polymer concentration of the mixture studied. This phase transition results in a system whose rheological properties are far removed from those of the original biopolymers. However, it is shown that kappa-carrageenan is primarily responsible for gel network formation.

INTRODUCTION

The synergistic effects of various hydrocolloids are frequently encountered in the food industry. Expensive polymers may be replaced by cheaper alternative formulations, and mixtures may be used to generate new functionalities or to manipulate texture and rheology of food products. The most exploited of these synergistic systems is the one containing kappa-carrageenan and locust bean gum.

Carrageenan, a water soluble galactan, is extracted from red seaweeds. Three main fractions, referred to as kappa, iota and lambda, respectively, can be distinguished depending upon sulphate content. The kappa-carrageenan fraction (κ-car) is characterized by an alternating disaccharide unit of (1→3)-linked β-D-galactose-4-sulphate and (1→4)-linked 3,6-anhydro-α-D-galactose. This polysaccharide shows a coil–helix conformational transition promoted by a temperature decrease in the presence of cations such as potassium, which affects polymer solubility and hence induces aggregation phenomena (Rochas & Rinaudo, 1984). The ordered conformation of κ-car molecules has been described as a single helix (Smidsrod, 1980), a double helix (Morris et al., 1980), or a helical dimer (Rochas & Rinaudo, 1984; Rochas & Landry, 1987). However, the double helix structure appears now to be widely accepted (Millane et al., 1988; Cairns et al., 1991; Lee et al., 1992). The role of cations in the conformation of κ-car at the sol–gel transition has been extensively investigated (Rochas & Rinaudo, 1982; Norton et al., 1983; Plashchina et al., 1986; Day et al., 1988; Nilsson &
Piculell, 1989). Of particular interest is the work reported by Rochas and Rinaudo (1980, 1984) who evidenced that the inverse of the transition temperature upon gelling and melting, in absolute units, was linearly related to the logarithm of the total potassium concentration. The resulting phase diagram can be separated in two domains, the first one corresponding to the coil-type macromolecular system, the second one to an ordered conformation. Each domain corresponds to a specific state, namely a sol-state, on one hand, and a gel-state or an aggregated-state depending upon the total ionic concentration, on the other hand (Rochas & Rinaudo, 1984; Fernandes et al., 1991a).

Galactomannans consist of linear chains of (1→4)-linked β-D-mannopyranosyl residues, to which are attached (1→6)-linked α-D-galactopyranosyl groups as single-unit side chains. The two major galactomannans exploited commercially are locust bean gum (LBG) and guar gum (GG). These polysaccharides differ primarily in terms of their galactose content. GG contains around 33% galactose, whereas LBG has a galactose content of about 20%. Another galactomannan that may be of interest in the future is tara gum (TG) whose galactose content is around 25%. These polysaccharides do not exhibit gelling properties by themselves. They display interesting thickening properties that are widely exploited in the industry. The rheology of aqueous galactomannan solutions has been extensively studied (Sabater de Sabates, 1979; Dourbi et al., 1981; Morris et al., 1981). It has been found that these properties are typical of macromolecular solutions and are primarily governed by the degree of polymerization of the mannan backbone. A consequence of the higher mannose to galactose (M/G) ratio of LBG as compared to GG is its ability to form gels by itself when submitted to freeze–thaw cycles (Dea et al., 1977). However, the main difference between galactomannans lies on the synergistic properties of LBG, and to a lesser extent of TG, with other polysaccharides such as κ-carrageenan or xanthan gum.

Locust bean gum/κ-car blends can gel at total polymer concentrations lower than the concentration at which carrageenan alone will gel. This synergism does not occur with GG because of structural differences. However, it has been shown that LBG can be replaced in the mixture by enzymatically modified guar gum (McCleary & Neukom, 1982), or by tara gum (Cairns et al., 1986; Fernandes et al., 1991b; Tako, 1991). The mechanism of gelation of these mixed systems is still a matter of debate and several models have been proposed (Dea et Morrison, 1975; Tako & Nakamura, 1986; Cairns et al., 1987, 1991). Of particular interest is the description of viscoelastic properties of the mixed system upon varying the polymer composition in the vicinity of the sol–gel transition as has been performed for xanthan gum/LBG mixtures (Cuvelier & Launay, 1988). Sol–gel phase diagrams can thus be described that are useful to improve our knowledge on the mechanism of gelation between both polysaccharides.

The aim of the present investigation was to characterize the rheological properties of κ-car alone and κ-car/LBG mixtures in the vicinity of the sol–gel transition. For this purpose, the systems were studied using viscometric and dynamic measurements. For comparison, a similar approach was applied to κ-car/GG mixtures.

### MATERIALS AND METHODS

#### Materials

The carrageenan sample in the K⁺ form was extracted from Eucheuma cottonii and was a gift of SBI (France). Its intrinsic viscosity in NaCl (0.1 M) was 7.10 dL/g. This sample was used without further purification. Despite the fact that this sample is said to be in the K⁺ form, a small amount of the acidic sulphated function (~20%) is in the Na⁺ form. One Portuguese carob flour was obtained according to Goncalves et al. (1988) and one commercial grade guar gum flour was supplied by SBI (France). Only the carob gum sample had to be purified as described by Fernandes et al. (1991b). The main physicochemical characteristics of the galactomannans are given in Table 1.

#### Methods

**Preparation of the solutions and mixtures**

Galactomannans and kappa-carrageenan were first dispersed in water under strong stirring. The agitation was maintained at room temperature for 1 h and then at 90°C for 30 min. After cooling, the polysaccharide content was determined using the phenol–sulphuric method (Dubois et al., 1956).

For κ-car alone the concentration ranged from 0.3% to 1%. Mixed systems were prepared at 90°C by blending the hot solutions of the galactomannan and the kappa-carrageenan in the desired proportion so as to yield overall concentrations ranging from 0.3 to 1.2%, for LBG, and from 0.6 to 1.2% for GG. The mixture was kept for 15 min at 90°C, under stirring, and then poured into glass moulds as described in Fernandes et al. (1991b). The glass moulds were then covered with paraffin in order to avoid dehydration problems. These mixtures were aged for approximately 14 h, at 15°C. Before performing rheological measurements, the mixture was transferred to the measuring system of the rheometer, covered with light oil in order to eliminate dehydration problems, and allowed to set for approximately 2 h, at 15°C. In the particular cases of κ-car at 0.8 and 1%, the hot solutions were poured directly on the plate of the rheometer and aged for 14 h, thereafter...
Table 1. Composition and macromolecular characteristics of the galactomannan samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>H₂O</th>
<th>Ash</th>
<th>Protein</th>
<th>M/G</th>
<th>[η]</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBG</td>
<td>12.40</td>
<td>0.35</td>
<td>0.49</td>
<td>4.04</td>
<td>15.3</td>
<td>1.8 × 10⁶</td>
</tr>
<tr>
<td>GG</td>
<td>8.32</td>
<td>0.10</td>
<td>0.22</td>
<td>1.68</td>
<td>12.0</td>
<td>1.7 × 10⁶</td>
</tr>
</tbody>
</table>

*Kjeldahl determination (5.87 N₂ according to Anderson (1986)).
*M/G: mannose to galactose ratio by GLC according to Blakeney et al. (1983).
*[η]: Intrinsic viscosity in dl/g.
*Viscosity-average molecular weight from [η] as described in Fernandes et al. (1991b).
*Values are on a dry weight basis.

The rheological measurements were performed, also at 15°C.

Rheological measurements

Mixtures of galactomannan/kappa-carrageenan. Measurements were performed on a controlled stress rheometer Carri-Med CS-50 (CM) and a Rheometrics Fluids Spectrometer (RFS II), both fitted with cone and plate geometry (4° cone angle and 5.0 cm diameter for CM and 2° cone angle and 5.0 cm diameter for RFS II) at the fixed temperature of 15°C. From the oscillatory shear measurements, the two rheological functions, G' (storage modulus) and G'' (loss modulus), were obtained over the frequency range of 0.01–100 rad/s for the Rheometrics and 0.06–60 rad/s for the Carri-Med. The strain amplitude was fixed at 0.05.

RESULTS

Behaviour of kappa-carrageenan

Viscosity measurements could be performed only for concentrations lower than 0.51%. Beyond this value, gelation occurred. The flow curves evidenced a shear-thinning behaviour with a limiting zero shear rate viscosity η₀. Such behaviour is typical of a macromolecular solution. Variations in η₉₀₀ (where η₉₀₀ is the zero shear rate specific viscosity) as a function of concentration (c), in logarithmic scales, are illustrated in Fig. 1. Two linear regions were evidenced with gradients of 3.82 for c < 0.43% and 5.23 for 0.43% < c < 0.48%, respectively. The first zone corresponds to the classical η₉₀₀ vs concentration dependence of usual macromolecular solutions below c* (critical concentration), whereas the higher gradient (c > 0.43%) may indicate the early beginnings of gelation. This is confirmed by viscoelastic measurements. Figure 2 shows G'(ω) and G''(ω) curves plotted in logarithmic scales for three concentrations, namely 0.48, 0.51 and 0.8%. The viscoelastic properties of the 0.48% κ-carr system were typical of a macromolecular solution, confirming viscosity measurements. At 0.8%, the behaviour was typical of a weak gel with a slight G' dependence upon ω and G' > G''. At 0.51%, the G'(ω) and G''(ω) curves were almost superimposed to those of 0.48% at the highest frequencies (ω > 5 rad/s), whereas they differed strongly towards low values of ω, with G'' reaching a steady value, an elastic plateau, and G' > G''. Such a phenomenon indicates that a slight increase in the concentration from 0.48 to 0.51% has yielded a structured system. This means that the system is just at the boundary of the sol–gel transition. This clearly illustrates that dynamic measurements provide a good tool to investigate accurately the rheological results of such phenomena.

Properties of kappa-carrageenan/LBG mixtures

Figure 3 shows the flow curves of two mixtures taken as examples. These curves are compared to that of LBG.

![Image](image-url)
Fig. 3. Flow curves of 21/79 and 22/78 kappa-carrageenan/locust bean gum mixtures. Temperature: 15°C; total polysaccharide concentration: 1-0% (w/w); continuous line: 21/79 mixture; (○), 22/78 mixture, compared with (△), locust bean gum 1% (w/w).

Fig. 4. Complex moduli, $G'(\omega)$ and $G''(\omega)$ of a 21/79 kappa-carrageenan/locust bean gum mixture. Same conditions as in Fig. 3; continuous line: $G'$; dashed line: $G''$; compared with locust bean gum: (●), $G'$; (○), $G''$.

Fig. 5. Complex moduli, $G'(\omega)$ and $G''(\omega)$ of kappa-carrageenan/locust bean gum mixtures. Same conditions as in Fig. 3; 23/77 ratio: (—), $G'$ and (— —), $G''$; 24/76 ratio: (○), $G'$ and (●), $G''$.

Fig. 6. Complex moduli $G'(\omega)$ and $G''(\omega)$ as a function of kappa-carrageenan/locust bean gum ratio. Temperature: 15°C; total polysaccharide concentration: 1% (w/w); frequency: 0.2 rad/s; (○), $G'$ and (●), $G''$. The dashed zone defines the sol–gel transition.

The curve of the κ-carr/LBG mixture at a 21:79 ratio did not differ very much from that of LBG alone. However, a slight deviation appeared towards the lower shear rate range. Increasing the ratio to 22:78 drastically modified the flow curve with a dramatic increase in the apparent viscosity at low shear rates. This is another indication of the beginning of the development of structure in the system.

Figures 4 and 5 display the viscoelastic properties of the same mixtures. As is seen in Fig. 4, the 21:79 mixture did not differ very much from that of LBG. However, a slight increase of $G'$ was exhibited towards the low frequency range. In contrast, the 23:77 and 24:76 mixtures shown in Fig. 5 provide clearer evidence of the beginnings of the gelation process. The change appeared progressive and, before reaching a well-defined gel state with $G' > G''$ and $G'(\omega)$ almost constant, an intermediate step was found. For the 24:76 blend, $G'$ was higher than $G''$ throughout the frequency range and still depended upon frequency, but to a lesser extent. This reflects the properties of a weak gel. The spectrum exhibited by the 23:77 mixture was 'spindle-shaped', with two cross-over points at ~0.06 and 6 rad/s, respectively. This spectrum has been found for all the concentrations investigated.

Variations in $G'$ and $G''$ at 0.2 rad/s as a function of the κ-carr/LBG ratio are illustrated in Fig. 6 for 1% total polysaccharide concentration. $G'$ and $G''$ slightly decreased when κ-carr/LBG ratios increased from 0:100 to 17:83 but increased sharply beyond 21:79. The dashed zone defines the sol–gel transition as estimated from the departure from the viscoelastic behaviour typical of macromolecular solutions. Its lower limit is for the 21:79 ratio and its upper limit at 23:77 coincides with the $G''$–$G'$ cross-over.

Properties of kappa-carrageenan/guar gum mixtures

It was interesting to check whether guar gum could yield the same behaviour when mixed with κ-carr. Figure 7 shows the flow curves of two mixtures which are compared with GG alone at 1%. The 28:72 κ-carr/GG ratio resulted in a classical shear-thinning behaviour with a limiting zero shear rate plateau. The value of $\eta_0$
was lower than for GG alone, which reflects a dilution effect of guar gum. The actual galactomannan concentration being 0.72%. For the 33/67 mixture, the flow curve was almost superimposed on the previous one at high shear rate. However, a slight increase of the apparent viscosity was evidenced at low shear rate similar to that previously described for k-car/LBG mixtures (see Fig. 3).

Figure 8 shows the mechanical spectra at the 46/54 and 48/52 k-car/GG ratios. The result obtained for the 46:54 ratio did not differ very much from that of GG alone at 1% (not shown) unless the curves had been shifted to lower values probably owing to a dilution effect. Such behaviour is typical of a macromolecular solution and the gradients at low frequencies, defined as the terminal region, are of the order of 2 for $G'(w)$ and 1 for $G''(w)$, as expected for such systems. It was only beyond this ratio that significant changes in viscoelastic properties were experienced. For the 48:52 ratio, no change in $G'(w)$ and 1 for $G''(w)$ was noticed at high frequency. However, at low frequencies, a large increase in the storage and loss moduli was found. The increase was much larger for $G'$ than for $G''$. Furthermore, $G'$ and $G''$ at low frequencies varied more slowly with frequency than for GG alone or the 46/54 mixture, the limiting gradients of $G'(w)$ and $G''(w)$ being both of the order of 0.66. It is clear, however, that the overall shape of the spectrum was not strongly modified and that changes induced in the properties of GG by the presence of k-car are not comparable to those observed with LBG. Figure 9 shows the $G'$ and $G''$ variations as a function of the k-car/GG ratio at 0.2 rad/s. $G'$ and $G''$ first decreased up to a ratio of 46:54 and then both increased sharply (but $G' < G''$). The first part of the curves is to be ascribed to dilution effects, k-car not taking part in the properties of the system since it is too diluted. Beyond the 46:54 ratio, on the other hand, the significant increase of the values of viscoelastic parameters, particularly $G'$, provides clear evidence of the effects of k-car in the properties of the mixture. It is also clear that gelation never took place in admixtures of GG and kappa-carrageenan.

DISCUSSION

Gelation of kappa-carrageenan

The present results have to be interpreted in the light of the phase diagram as described by Rochas and Rinaudo (1980, 1984). This diagram defines the relationship between the total free potassium ions concentration and the melting temperature. It was established on the basis of conductivity and optical measurements for the detection of the helix-coil conformational transition. From this plot, the free potassium ions concentration ($C_f$) required for gelation to take place at 15°C is $4.5 \times 10^{-3}$ eq/litre. Since no KCl is added, the only K+ ions in the medium come from the counterions of the
carrageenan. We can estimate the corresponding carrageenan concentration \(C_p\) from the expression \(C_p = C_t / \gamma\) with \(\gamma\) the activity coefficient, equal to 0.55 (Rochas & Rinaudo, 1980). This will give a carrageenan concentration of 0.35%. However, we must take into account the fact that only 80% of the sulphated groups are neutralized by K\(^+\) ions. Hence, the amount of carrageenan required for the transition at 15°C might be of the order of 0.44%. In the present work, we observed a macromolecular solution at 0.48% and the gel state was evidenced at 0.51%. The sol–gel transition is thus likely to take place between these two values. There is thus a slight difference which may be explained by the fact that the prerequisite for the transition to be detected by rheological means is that helices are aggregated.

The break point in Fig. 1 at ~0.4% may indicate the early beginnings of the gelation process with a certain amount of helices. But this amount would be still too low for gel formation to be induced. An intermediate state may thus exist between ~0.4% and 0.5%. However, this should be confirmed by additional experiments. An important result of the present observations is that viscoelastic measurements in oscillatory shear provide an excellent means for determining the sol–gel transition of \(\kappa\)-car in rheological terms as the point where the behaviour deviates from that of a macromolecular solution. Increasing the concentration very slightly, from 0.48 to 0.51%, made the system pass through the transition if we assume the critical behaviour at the gel point to be characterized by a power-law dependence of \(G'\) and \(G''\) upon frequency with a gradient of the order of 0.5–0.7 (Axelos & Kolb, 1990; Cuvelier et al., 1990; Lin et al., 1991). In the present case, the transition was so fast that we could not detect the parallelism between \(G'\) and \(G''\) described by the latter authors. However, the appearance of an elastic plateau for \(G'\) at low frequency values indicates that the system passed beyond the gel point and therefore was in the gel state. The evolution shown by the spectra of \(\kappa\)-car at 0.48% and 0.51% means that the parallelism of \(G'\) and \(G''\) should probably occur between these two concentrations. Similar data have been recently reported by Coviello and Burchard (1992) who demonstrated that gelation of an exopolysaccharide from Rhizobium leguminosarum can be detected by rheological means owing to the fact that the transition from solution to gel behaviour is very sharp.

**Gelation of kappa-carrageenan/galactomannan mixtures**

The two galactomannans by themselves indeed yield macromolecular solutions. Replacing part of the galactomannan by \(\kappa\)-car does not result in spectacular changes initially. It is only beyond a certain \(\kappa\)-car content that strong modifications are experienced, as can be seen from viscosity and dynamic measurements. The phase diagrams relating the total polymer concentration to the \(\kappa\)-car/galactomannan ratio have been plotted using two criteria for assessing the transition (Figs 10 and 11). The viscosity criterion was taken as the deviation from the limiting zero shear viscosity \(\eta_0\) in the flow curve (see Figs 3 and 7). The viscoelastic one was defined as the deviation of the \(G'\) vs composition curves (see Figs 6 and 9). For LBG (Fig. 10), the two criteria resulted in the same transition line. This is to be related to the sharpness of the transition. In contrast, in the case of GG, two distinct transition lines were obtained according to the criterion employed (Fig. 11). A second difference between the two systems is that, for LBG, the transition coincided with the appearance of a gel-like structure. No such effect was evidenced in case of GG.

We previously described the thermal behaviour of \(\kappa\)-car/galactomannan mixtures at a high ratio of \(\kappa\)-car (\(\kappa\)-car/galactomannan ranging from 4/1 to ~0.6/1) (Fernandes et al., 1992). The basic mechanism taking

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**Fig. 10. Phase diagram relating the total polymer concentration to the kappa-carrageenan/locust bean gum ratio. Temperature: 15°C. The isolated point (○) is for kappa-carrageenan alone. Criterion of transition: see text.**

**Fig. 11. Phase diagram relating the total polymer concentration to the kappa-carrageenan/guar gum ratio. Temperature: 15°C. The isolated point (○) is for kappa-carrageenan alone. Criteria of transition: viscosity (curve 1) and viscoelasticity (curve 2).**
place in these blends would be a volume exclusion between unlike polysaccharides. Those mixed systems could be described as the result of two interpenetrating phases, one being composed of κ-car in the gel state, whereas the other contained the galactomannan. Under certain conditions, the two phases were gelled and the resulting system was an interpenetrating network, the primary network being the κ-car gel with the galactomannan forming a secondary network. The present results can be interpreted on a similar basis despite the low content of κ-car which results in this polysaccharide being at its gelation threshold.

Comparable effects to those reported here have been found with blends of synthetic polymers upon phase separation (Mani et al., 1992). The $G'(ω)$ and $G''(ω)$ curves were similarly shifted in the terminal region, at low frequency, while no effect was noticed at high frequency. This dramatic change at low frequency was mostly ascribed to the enormous differences between the moduli of the two phases in the medium. The present data with GG may equally be interpreted on the basis of a phase separation process. The first part of the diagram of Fig. 11 below line 1 corresponds to the one-phase region. Beyond this lies the two-phase region. The viscosity boundary limit (line 1) corresponds to a slight change in the flow behaviour at low shear rates. This may arise from a liquid–liquid phase separation. A similar mechanism has been reported for protein–polysaccharide systems, the properties of which could be regarded as similar to those of a suspension (Castellain et al., 1986). The polysaccharide filled most of the system, whereas the proteins were concentrated in droplets dispersed in the polysaccharide continuous phase. In the present case, we suggest that the GG-rich phase is the continuous medium in which κ-car-enriched droplets are dispersed. Upon passing through line 2, the carrageenan-enriched phase remains dispersed but is brought to a gel inside droplets which become much more rigid than the GG-rich continuous phase. The resulting carrageenan-rich beads will therefore contribute more efficiently to the rheology of the system particularly at low frequencies, or long times, as illustrated in Fig. 8. In the case of LBG, the same phenomenon will occur as a first step. However, gelation does take place as soon as the transition line is crossed. LBG probably plays a complementary role owing to its structural specificity. This galactomannan is known to be able to gel by itself. Since the system gels during the phase transition, this suggests that the continuous phase of the system, the LBG-rich one, is a macro-molecular network. It is also likely that LBG induces the coil–helix transition of κ-car molecules and stimulates the subsequent aggregation of helices, a prerequisite for gelation of κ-car as discussed above. Another possibility would be that both gelled phases interpenetrate each other.

We could also estimate the κ-car content at which the transition occurred for both types of mixture (Table 2). It is worthy to note that these values are almost constant whatever the total polymer concentration considered. They were 0.23% for mixtures with LBG irrespective of the criterion used, against ~0.33% and ~0.45% for GG on the basis of viscosity and viscoelasticity criteria, respectively. These amounts are below the limit with κ-car alone, as discussed above (~0.5%). In the presence of LBG, the κ-car content required to form a gel is half that of κ-car alone. This is consistent with the fact that LBG stimulates gelation of κ-car. The phenomena with GG are less spectacular and gelation does not occur with low amounts of κ-car. This means that, although volume exclusion does take place, the continuous phase will not gel. Such a liquid–liquid or solid–liquid biphasic system will have properties that are comparable to those of the galactomannan, although κ-car plays a role in enhancing the overall rheology of the system.

<table>
<thead>
<tr>
<th>Blend</th>
<th>Total polymer concentration (%)</th>
<th>Conc. of κ-car at transition (%)</th>
<th>Criterion of transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>κ-car/LBG</td>
<td>0.40–1.20</td>
<td>0.22–0.34</td>
<td>$a$, $b$</td>
</tr>
<tr>
<td>κ-car/GG</td>
<td>0.60–1.20</td>
<td>0.32–0.34</td>
<td>$a$</td>
</tr>
<tr>
<td>κ-car</td>
<td>0.80–1.20</td>
<td>0.42–0.47</td>
<td>$b$</td>
</tr>
</tbody>
</table>

$a$: viscometry; $b$: viscoelasticity.

CONCLUSION

It is clearly shown that the viscoelastic behavior of κ-car/galactomannan blends at the transition zone is influenced both by LBG and GG. Furthermore, the constant κ-car content found at the transition zone suggests that the transition corresponds primarily to the active participation of κ-car in the rheology of the system. Such an effect indeed does not depend upon the type of galactomannan. The only difference between both systems lies in the κ-car content at which the transition appears. The additional effect of LBG is a structuration of the medium by way of a mechanism which is similar to that observed for synergistic interactions between κ-car and LBG.

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