Methodology in studying improvement of polyacrylates biodegradability

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SUMMARY : The biodegradability of polyacrylic acid derivatives may be improved through introduction of some fragility points within the main carbon chains. These weakness points can be either hydrolizable bonds or oxidizable sites. Enzymatic hydrolysis and oxydative biomimetic degradation tests have been set up in order to quickly evaluate new polymeric structures, before their possible evaluation through microbial degradation by *Candida tropicalis*.

1 - Introduction

Polycarboxylic acids such as polyacrylic acid or maleic anhydride-acrylic acid copolymers are widely used in detergent formulations where they have replaced polyphosphates whose eutrophic effect in natural aquatic media was a serious drawback.

Polyacrylates in use now do not show this effect, but their lack of rapid biodegradability leads to an accumulation in the natural medium (1,3,4). No toxicity can yet be associated with these polymers, but their long term effect is uncertain, which instigated numerous research activities aimed at improving their biodegradability.

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2 - Principles

Hydrophilic polymers such as polyvinyl alcohol are rapidly degraded by micro-organisms (2). Low molecular weight polyacrylic fragments show a better biodegradability than higher molecular weight homologues (4).
This knowledge leads to the concept of acrylic co- and ter-polymers containing vinyl monomers. Other work show that hetero-atom containing chains like polyethyleneglycol (PEG) or polyoxyethylene (POE) are more easily degraded than pure carbon chains (Paik et al., 1996).
As polyacrylate biodegradability becomes acceptable for a degree of polymerisation $\leq$ 6-8, the proposal is to introduce some weak points within the acrylic chains. These points will be then quickly broken by natural microflora enzymes, leading to the requested smaller fragments. These fragments will then be degraded and assimilated by the micro-organisms.

3 - Evaluation of biodegradability

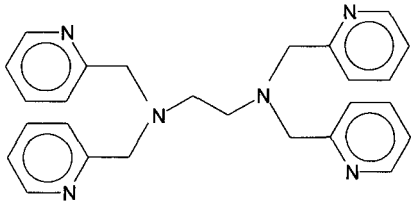
A difficulty of assessing the biodegradability of polymers is the need for long time experiments which are expensive.
This difficulty is even worse for the assessment of the degradability of newly synthetized structures : a quick answer is requested by polymer chemists to know if the new structure is better or not.
In order to solve that point, pre-screening tests have been set up which allow rapid examination of the degradability of new molecules.
The best candidates are then evaluatated by microbiological tests.

3.1 - Enzymatic hydrolysis pre-screening tests

The first way to introduce fragility points into polyacrylic acids consists in adding enzyme hydrolizable bonds within the chains. That is ester, amide, thioester, thioamide, etc. linkages. In order to test the sensitivity of the polymer to fragmentation, in vitro test conditions were designed, with known hydrolytic microbial enzymes, i.e esterases, lipases, proteases, etc. As a result of the enzyme action, the molecular weight of the degraded polyacrylates will be lowered, that can be easily observed and quantified by size exclusion chromatography (SEC).

3.2 Oxidative cleavage pre-screening test

Another way to insert weakness points into polyacrylic acids is to introduce chemical structures which can be broken by the oxidative enzymes of the microflora organisms. Such enzymes are difficult to obtain, or very expensive, or even are not active outside the micro-organisms cells.
In order to solve that point, a biomimetic oxidation system has been set up. It consists in a metal complexing agent, called TPEN : N,N,N', N', tetramethyl-pyridine-1,2-ethylene-diamine



associated with iron (FeIII).

Test conditions are as follows :

Polymer to be tested :	1 mg/ml (assay volume : 10 ml)
TPEN associated with FeIII :	0.05 mM
Free TPEN :	0.5 mM
H_2O_2 :	100 mM
pH :	7
Temperature :	50 °C
Reaction time :	4 h

3.3 *Evaluation of degradability*

Molecular weight is measured by Size Exclusion Chromatography with the following conditions :

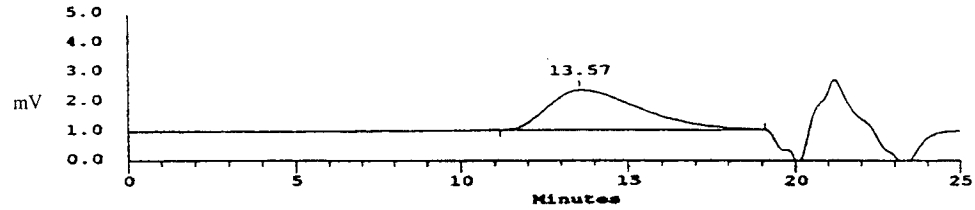
Column :	TSK 3000 Tosohaas
Eluent :	Sodium acetate 0.1 M
Flow :	0.5 ml/min.
Injection :	25 μl after 0.22 μ filtration

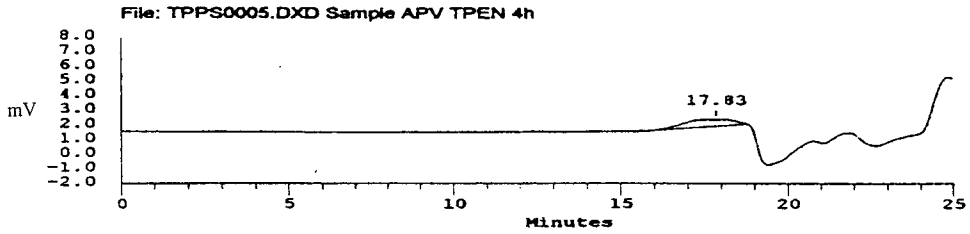
Detection : Refractive index

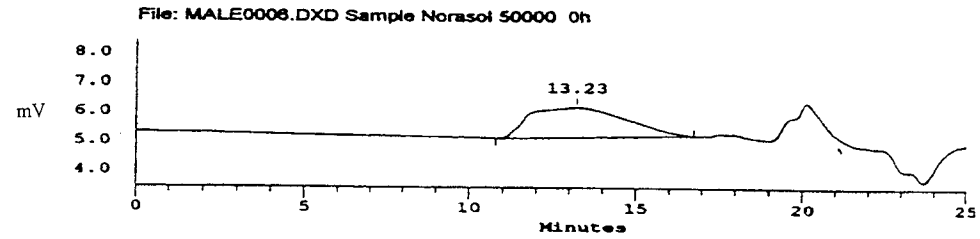
Data Acquisition : Dionex Peaknet

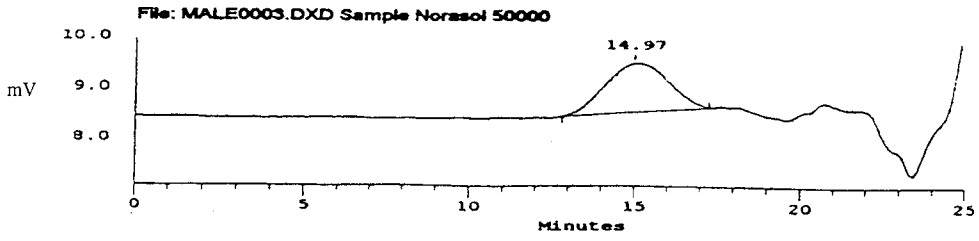
Standardization of the column is obtained through injection of polyacrylates standards (Polymer Laboratories).

Examples of chromatograms are given hereafter for polyvinyl alcohol and polyacrylic acid homopolymers :









The polymer degradation within the test conditions is quantified by the displacement of the SEC peak from the high molecular weights to the lower molecular weights. This displacement is used to calculate a degradability index I_{1000} which is independent of the initial molecular weight of the polymer :

Initial molecular weight : Mi
Final molecular weight : Mf
Number of chain cuts : nc = (Mi/Mf) - 1
Initial dp : dp = Mi/Mmono
Where Mmono is the molecular weight of the average monomer.

Degradability index : $I_{1000} = \frac{nc}{dp} x1000$ that is

$$I_{1000} = (\frac{Mi}{Mf} - 1) \times \frac{M_{mono}}{M_i} x1000$$

3.4 *Microbial degradation*

a - Experimental cultures

A *Candida tropicalis* strain is grown on a liquid culture medium with malt extract (20 g/l) and incubated at 30 °C under axial stirring during 48 h.
After 18 000 rpm 15 min. centrifugation, the cells are washed with 0.1 M pH 6 phosphate buffer, and after a new centrifugation, they are washed a second time, in order to remove any residual substrate.

b - Warburg method

Candida tropicalis respiration rate is measured through 3 ml Warburg vials containing 1.3 ml of 0.1 M pH 6 phosphate buffer, 0.5 ml of the 1.12 g/l polymer solution and 1.0 ml of the yeast suspension (roughly 3 mg dry weight).
In the center cup 0.2 ml of a 40 % KOH solution is placed to absorb the CO_2 produced.
Blank tests are run in parallel :

- One vial with only the phosphate buffer (3 ml) allows atmospheric pressure measurement.
- Endogen respiration is measured in a vial with only the yeast suspension (1 ml), the phosphate buffer (1.8 ml) and the KOH solution (0.2 ml).

- Respiration due to possible contaminants remaining in the polymer solution is measured in a vial with the polymer solution (0.5 ml), the phosphate buffer (2.3 ml) and the KOH solution (0.2 ml).

Vials are then submitted to stirring in a 30 °C water bath. Measurement of changes in pressure due to O2 absorption showing polymer assimilation by the yeast are performed every 15 min.

c – Yeast cultivation with the polymer

Two types of cultures have been performed : cultures where the polymer is the only carbon source, and cultures where the polymer is associated with some yeast extract.
The first conditions allow to show the assimilation of the polymer by the micro-organism.
The second ones are useful to improve the assimilation in order to increase the degradation yield by favouring the yeast growth.
In both cases, media are composed of :

$(NH_4)_2SO_4$	1g
Winogradsky solution*	50 ml
Phosphate buffer 0.05M pH 6	to 1 l.

* ($MgSO_4$ 2.5 g;NaCl 2.5 g;$Fe_2(SO_4)_3$ 0.05 g;$MnSO_4$ 0.05 g;distilled water to 1l.)

The polymer is tested at a final concentration of 500 ppm. When yeast extract is used, its final concentration is 200 ppm. If the growth is too weak, it can be increased to 500 ppm. Vials are incubated at 30 °C under transversal stirring and are put in new medium after 1 week. Cultures are thus maintained during 15 days in the same conditions.

4 – Inhibition of calcium sulphate precipitation

Polyacrylates have to keep their functional properties. That is, that if fragmenting sites are inserted within the polymer chains, the resulting structure must be almost as much effective against calcium as the non-modified polymer.
In order to check this functional property, the following test has been performed :

Two aqueous solutions are prepared with distilled water and the following salts :

A solution : $CaCl_2,2H_2O$ (64.9 g/l) + $MgCl_2$ (0.5 g/l)
B solution : Na_2SO_4 (62.7 g/)

A volume of 400 ml of distilled water is poured into a 500 ml beaker with 50 ml of the A solution, progressively while stirring, and then 50 ml of the B solution. Then a certain amount of the polymer to be tested is added in test vials.
A blank vial with no polymer addition is also performed. At t = 0, after homogeneization, calcium and magnesium are measured by ICP (Inductively Coupled Plasma Analysis) Emission Spectroscopy. Then vials are allowed to settle for 7 days before a new measurement of calcium and magnesium.
Results are expressed as ppm of calcium (and magnesium) remaining in solution (not precipitated as sulphate) compared to the blank.

5 - References

1) Swift G. (1994)
Water soluble polymers.
Polymer Degradation and stability, 45, 215-31.

2) Matsumura S., Shimura Y., Toshima K. (1995)
Molecular design of biodegradable functional polymers. Poly(vinyl alcohol) bloc as a biodegradable segment.
Macromol. Chem. Phys., 196, 3437-45.

3) Paik Y.H., Simon E.S., Swift G. (1996)
A review of synthetic approaches to biodegradable polymeric carboxylic acids for detergent application.
Adv. Chem. Ser., 248, 79-98.

4) Swift G. (1997)
Ecological assesment of polymers, Ch. 15, 291-306.
Hamilton J.D., Sutcliffe R. Ed. / Van Nostrand-Rheinhold, NY

5) Larson R.J., Bookland E.A., Williams R.T., Yocom K.M., Saucy D.A., Freeman M.B., Swift G. (1997)
Biodegradation of acrylic acid polymers and oligomers by mixed microbial communities in activated sludge.
J. Environmental Polymer Degradation, 5(1), 41-8.