AAPS PharmSciTech, Vol. 11, No. 3, September 2010 (© 2010)
DOI: 10.1208/s12249-010-9490-0

Research Article

Evaluation of Polymer Mucoadhesiveness by the Use of Acoustic Spectroscopy

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Received 8 October 2009; accepted 23 July 2010; published online 5 August 2010

Abstract. An innovative and simple methodology has been developed and used for the evaluation of mucoadhesive properties of several polymers by means of sound speed measurements using high-resolution acoustic spectroscopy. In systems made of polymers in water, variations in hydration shell of polymeric chains determine changes of dispersions compressibility, and this phenomenon can be monitored by sound speed measurements. Four different polymers have been selected, namely PEG 6000, Carbopol 974, HPMC K4M, and Pectin 200/USP, all characterised by very different mucoadhesive properties. Samples made of each polymer alone (0.3–1.0% *w*/*w*) or in mixture with mucin (mucin fixed at 1.0% *w*/*w*) in water were investigated while using high-resolution ultrasonic spectrometer at two different frequencies (5.2 and 8.2 MHz). Polymer–mucin interaction was evaluated comparing experimental sound speed values of polymer–mucin samples with their theoretical values derived from the addition of sound speeds obtained while analysing each component alone. Results demonstrated the ability of the acoustic method to discriminate between mucoadhesive and no mucoadhesive polymer–mucin dispersions and allowed also the comparison between their mucoadhesive strengths. The study has therefore demonstrated the potential of using high-resolution ultrasonic spectroscopy to evaluate the polymers' mucoadhesiveness, with the great advantage of testing small amount of samples even if opaque.

KEY WORDS: high-resolution ultrasonic spectroscopy; mucoadhesion; polymer–mucin interaction; sound speed.

INTRODUCTION

The ability of polymers to adhere to soft biological tissues has been used in the last three decades in order to optimise drug delivery in specific body sites and improve systemic drug adsorption. In fact, increased bioavailability and site-specific release was observed for dosage forms able to remain in contact with a certain tissue for a prolonged period of time (1).

Prediction of polymer mucoadhesiveness represents a fundamental task during evaluation of novel mucoadhesive dosage forms and new chemical entities. For this purpose, in the last 20–25 years, a very large number of works have been carried out for the development of *in vitro* mucoadhesive tests (2–8). Despite this great effort, the data available are often inconsistent and contradict each other. Sigurdsson (8) relates this difficulty to the existence of an elusive concept of mucoadhesion and argued that at least two different kinds of mucoadhesion can be listed, namely "wet on wet" and "dry on wet". The former refers to adhesion of a polymeric hydrogel to another one in the presence of excess of liquid, while the latter refers to the stickiness of a dry hydrophilic polymer to a wet or humid biological surface. In the "wet on wet" adhesion, fully hydrated polymer chains interact with mucus glycoproteins even in the presence of an excess of water. Applying the two definitions of bioadhesion proposed by Sigurdsson, all the methods found in literature might be classified as "wet on wet" and "dry on wet". Therefore, data comparisons should be carefully carried out while using different methodologies.

The oldest and most common method suitable for "wet on wet" mucoadhesion determinations is rheology which allows the evaluation of changes into rheological properties of mucoadhesive polymers when they are mixed with mucus (4,9–12). Despite its popularity, several authors criticised such method as being strongly dependent on the test conditions and thus not always reliable (8,13). Other methods have been recently developed such as zeta potential (5), light scattering analysis (5,14), turbidity measurements (14,15), TEM analysis (14,16), Biacore (5,6,9), and resonant biosensor methods (8). The last three methods are probably the most accurate ones; however, they require highly specialised and expensive equipment.

The aim of this work is to develop an alternative method of analysis based on the variation of sound speed when polymeric hydrogels are mixed with mucin. As previously reported (17), when no interaction occurs between polymers, the relative sound speed of the whole system is equal to the sum of the relative sound speeds of each component since the hydration layer of the molecules remains unchanged. The term relative indicates that sound speed refers only to a pure

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component within a solution. Whereas, when an interaction occurs, the hydration layer of the polymers is reduced, leading to a decrease of the resultant sound speed compared to its theoretical value. This behaviour is due to the different compressibility of the free and bound water; in particular, bound water is much less or not at all compressible (18–22). Therefore, dehydration processes are always characterised by an increase of free water and consequently of compressibility. As described by Laplace in Eq. (1), compressibility is inversely related to sound speed:

$$U = \frac{1}{\sqrt{\beta_s \cdot \rho}} \tag{1}$$

where β_s is the adiabatic compressibility and ρ is the density of the system.

High-resolution ultrasonic spectroscopy is a technique suitable for such measurement offering also several advantages such as working with small amount of samples, even if opaque. This last feature is particularly important while working with mucin dispersions which might be difficult to analyse using other techniques, i.e. light scattering. Moreover, ultrasonic spectroscopy is widely spreading fast in the research laboratories due to its large applicability in different fields (23–27).

In this paper, several polymers, characterised by different degrees of mucoadhesiveness, are analysed either alone or mixed with mucin by using high-resolution ultrasound spectroscopy, and interactions occurring between polymer–mucin are evaluated through the comparison of experimental and theoretical sound speed values of their water dispersions.

MATERIALS AND METHODS

Materials

Carbopol® 974 PNF (Lubrizol Corporation, USA), PEG 6000 (Lipoxol 6000 med powder, Sasol, Germany), Hypromellose (HPMC, Methocel K4M Premium EP, Colorcon, USA), pectin (Genu® Pectin USP/200, CP Kelco, USA), and porcine gastric mucin (type II, Sigma–Aldrich, USA) were used as received. Deionised water was obtained from an ion-exchange system GAMMA 3 s.n.c. (Castelverde, CR, Italy).

Samples Preparation

Stock solutions made of polymer alone or mixed with mucin (2% *w*/*w*) were prepared by dispersing the powders in deionised water (DW) under magnetic stirring at room temperature, except HPMC which was prepared following the "hot/cold" technique (28). All the samples were stored at 5°C for at least 24 h before testing; whereas for Carbopol samples, 4-day storage was adopted.

Ultrasonic Measurements

Ultrasonic velocity was measured using a high-resolution ultrasonic spectrometer (HR-US) 102 (Ultrasonic Scientific, Ireland) fitted with two 1 ml ultrasonic cells. The reference cell was filled with water, while the other cell, with polymer, mucin, and polymer–mucin samples. All the dispersions were analysed at the selected frequencies of 5.2 and 8.2 MHz (chosen after a preliminary frequency scan) and temperature control of 37±0.1°C was achieved using a HAAKE C25P water bath. The limiting resolution was 0.2 mm/s for ultrasound velocity. In all the following, text sound speed is reported as relative parameter obtained by subtracting the contribution of the pure solvent to the total sound speed ($\Delta U = U_{\text{sample}} - U_{\text{solvent}}$).

Stability Study

Sound speed values are particularly susceptible to polymer solvation so their incomplete hydration generates a certain variation of the measured parameter, and an erroneous interpretation of the data is obtained. Thus, an initial evaluation of stability of pure polymers and pure mucin dispersions has to be carried out by measuring the sound speed values. For this purpose, 1% *w*/*w* dispersions were prepared through dilution of stock solutions (all stored for 24 h at 5°C) and analysed within the first week after preparation. All the samples were equilibrated in an incubator at 37°C for 1 h before loading the HR-US 102 spectrometer cells.

Three different samples for each composition were analysed.

Mucoahdesion Study

Dilute dispersions made of mucin, polymer, and mucin–polymer mixtures were prepared from stock solutions according to the concentrations reported in Table I. Every dilute dispersion was prepared and analysed during the same day. Moreover, each stock solution was used for a maximum of 3 days after the initial storage at 5°C, according to earlier stability study results.

Three different samples for each composition were analysed.

Mucoadhesion degree (MD) was calculated using the rule of additivity. According to this rule, in a system containing one or more polymers dispersed in water, if no interaction occurs between the polymeric chains, the relative sound speed of the whole system is equal to the sum of the relative sound speed of each polymer dispersion; while, if an interaction occurs, the total relative sound speed measured is lower than the theoretical one. Following this assumption, the MD (%) is calculated using Eq. (2):

$$\text{MD}(\%) = \frac{(\Delta U_t - \Delta U_b)}{\Delta U_t} \times 100 \tag{2}$$

Where ΔU_t is the theoretical relative sound speed equal to the sum of the experimental relative sound speed of mucin and polymer alone, and ΔU_b is the experimental sound speed of mucin–polymer blends. A value of 0% MD indicates no interactions between polymeric chains.

MD represents the mucoadhesive attitude of a polymer, which is its ability to interact with mucin. This parameter does not express the interaction qualitatively, which is in terms of strength, but it is a quantitative index related to total number of bounds between mucin and polymers.

Table I. Analysed Samples

System	Concentration (% *w/w*)		
Mucin		1	
Polymer	1	0.6	0.3
Mucin + polymer	1+1	1+0.6	1+0.3

The MD calculation can also be used to monitor the modification of the aggregation state of a single polymer when its concentration is changed. In fact, if no aggregation state variation occurs, the total relative sound speed measured follows a linear trend meaning that the theoretical sound speed (relative to two different concentrations) is equal to the experimental one (for a concentration equal to the sum of the two previously selected concentrations). In this situation, the MD can be defined as 'Interaction degree'.

RESULTS AND DISCUSSION

Stability Study

Stability study was performed in order to plan the mucoadhesive tests effectively and avoid any risk of misinterpretation of the data due to partial hydration of the polymers. In general, increase of sound speeds in polymer dispersions during storage time indicates a progressive increase of compressibility due to an occurring hydration process, whereas a constant value suggests no change of polymer hydration layer. Results of sound speeds of PEG, pectin, HPMC, and mucin in DW, over time, showed that the sound speeds (expressed in m/s) were almost constant during the first 3 days (see Fig. 1). Whereas, for Carbopol dispersions, a different trend was seen with an increase of the sound speed during the first 3 days and a certain plateau from the fourth day. The trend observed for Carbopol dispersions showed that its hydration was completed only after the third day; while, for all the other dispersions, their hydration was completed within the first day. Overall, for all the systems in study, no significant difference was found while measuring sound speeds at two different frequencies (5.2 MHz and 8.2 MHz; data not shown at 8.2 MHz).

Mucoadhesion Study

The four polymers in study (pectin, Carbopol, HPMC, and PEG) were selected for their peculiar mucoadhesive properties. PEG is surely a non-mucoadhesive polymer (7), has poor swelling ability, and is rapidly dissolved in water; Carbopol is a well-known mucoadhesive polymer (independently from the technique used for the evaluation of this feature (5–9)); it swells and is not rapidly soluble in water. In this work, these two polymers have been selected as model materials in order to verify the reliability of the method in use. Moreover, other polymers were studied, namely pectin (with a high esterification degree) and HPMC. In literature, for both of them, there is no great agreement of their data about mucoadhesiveness. Different authors described HPMC as having poor (5,8) or medium (6,9), or strong mucoadhesive properties (29); whereas pectin is described as a more (30,31) or less (32) mucoadhesive polymer. For pectin, the situation is even more complicated due to the presence of the different types available and reported as having different mucoadhesive properties in relation to their esterification degree. Also, in the latter case, there is no agreement on the effects of this parameter on the mucoadhesiveness (30,31). Moreover, it has not been reported that there's any variation of the aggregation state for the polymers selected as a function of the concentration. This behaviour allows the comparison of different polymer concentrations.

The results of the mucoadhesiveness tests are reported in Fig. 2 for the two frequencies in use (5.2 and 8.2 MHz).

For PEG–mucin dispersions, values of MD indicated a poor interaction between polymer and mucin, with results

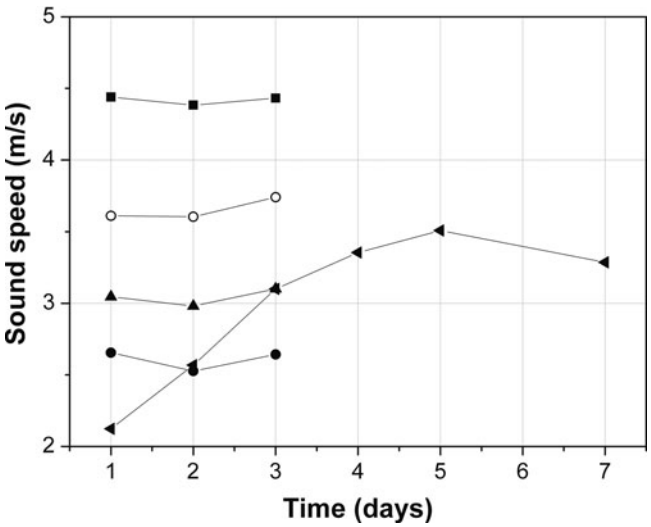

Fig. 1. Variation of polymer dispersions sound speeds (m/s) as a function of time (days) at frequency of 5.2 MHz. PEG (*filled square*), pectin (*filled circle*), Carbopol (*black-left pointing finger*), HPMC (*black-up pointing triangle*), and mucin alone (*white circle*), respectively

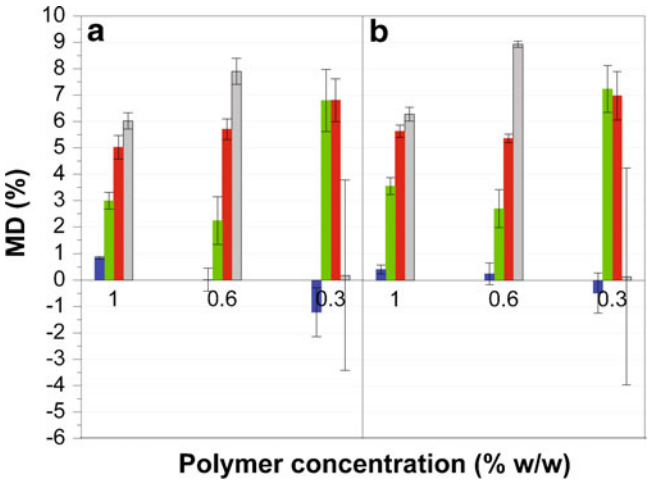

Fig. 2. Mucoadhesion degree (MD %) of polymer–mucin dispersions as function of their concentrations (% *w/w*) calculated at the frequencies of **a** 5.2 MHz and **b** 8.2 MHz. In each system, mucin was at 1% *w/w*. Colours *blue*, *green*, *red* and *gray* refer to PEG, pectin, HPMC, and Carbopol, respectively. *Error bars* refer to standard deviation

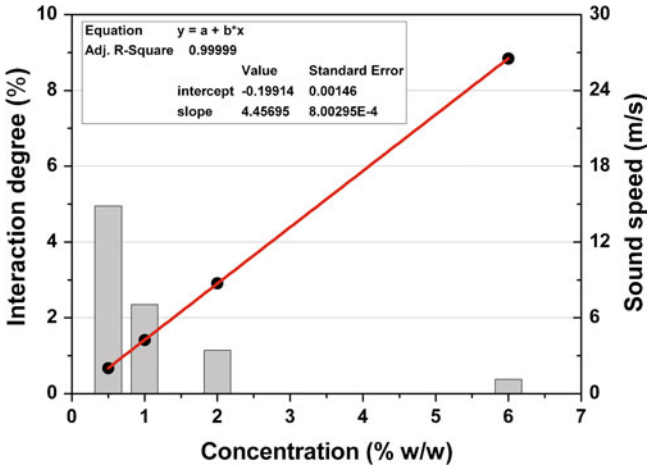

Fig. 3. Sound speed (m/s) of PEG dispersions at different concentrations (experimental values (*filled circle*) and fitting line (*filled rectangle*)) and interaction degree (*full block*) assuming an error of 0.1 m/s on the absolute PEG sound speed measurements

always lower than 1.0% *w/w*. For the lowest concentration in study (0.3% *w/w*), negative values of MD were observed which would theoretically indicate an increase of the polymers' hydration. However, this result was considered to be not very reliable, also taking into account that such value was observed only for the lowest concentration tested. Therefore, it was concluded that a MD value around 0.0%±1.0% indicated no interaction between polymer chains.

All Carbopol–mucin systems analysed showed the highest MD compared to the other systems. Using the same test conditions, the only exception was the system at 0.3% *w/w* which showed MD values around zero, but with a quite high standard deviation (SD±4.0%). Thus, this system was excluded from further data analysis.

This result suggested a possible correlation between error measurement and dispersion concentration. In fact, as the concentration varied, the absolute sound speed error remained almost constant, while the relative error changed with the values of the measured sound speed. In order to better understand this concept, PEG dispersions were analysed in more detail (see Fig. 3). In this figure, the sound speed values of PEG samples were reported in a concentration range of 0.5–6.0% *w/w*, *versus* interaction degree. It is important to clarify that when a single polymer is considered, its MD should be defined as 'interaction degree' since it refers to interactions occurring between molecules of the same polymer rather than with mucin (as explained in the method section). The perfect linearity of the results (see Fig. 3) suggested that no interaction occurred even at increasing concentrations (up to 6.0% *w/w*), which corresponded to an interaction degree equal to zero. In order to verify the effect of a possible experimental error as a function of polymers concentration, it was decided to add a value of 0.1 m/s to the sound speed values measured for each PEG concentration tested. The value of 0.1 m/s represented the worst case scenario, since from repeated measurements performed on each polymer dispersion, an error between 0.02–0.08 m/s was always observed. This data manipulation (see Fig. 3) showed an increase of the error in PEG "interaction degree" from 0.5% to 5.0% as the polymer concentration decreased from 6.0% to 0.5% *w/w*. Therefore, the reliability of the MD obtained from sound speed measurements increased with the polymer dispersion concentration.

The results obtained for PEG–mucin and Carbopol–mucin dispersions confirmed the validity of sound speed measurements for mucoadhesiveness analysis. However, for very low polymer–mucin concentrations, the measurement was considered less reliable due to the influence of the experimental error.

The MD results obtained for pectin– and HPMC–mucin dispersions showed lower values than Carbopol–mucin systems, but they were definitively higher than PEG–mucin dispersions. Among them, HPMC showed higher mucoadhesiveness compared to pectin dispersions. For pectin– and HPMC–mucin dispersions, no relevant differences were observed at the concentration 1.0% and 0.6% *w/w*, while at 0.3% *w/w*, the results were very different. This trend was similar with Carbopol–mucin system; however, in this case, the extremely high standard deviation for the 0.3% concentration makes comparison among the different polymers quite difficult. Hence, a comparison among different polymers should be carried out only at concentrations equal or higher than 1.0% *w/w*.

Overall, the mucoadhesiveness rank for the systems and the concentrations under investigation was: Carbopol > HPMC > pectin >> PEG, independently from the frequency in use. This trend was in agreement with the general scenario found in literature, in particular, with data from methodologies suitable for studies of "wet on wet" interactions, a part from the criticised rheological measurements for which strong disagreement exists. It is of utmost importance to highlight that the data obtained in this study are valid for the specific kind of polymers analysed, and that modifications on the polymers' structure could strongly influence their mucoadhesiveness, as previously reported for pectin (30–32).

CONCLUSIONS

Sound speed measurements performed in this study demonstrate the use of high-resolution ultrasonic spectrometer for the evaluation of mucoadhesiveness of several polymer–mucin dispersions. The proposed method seems less reliable when samples at very low concentrations (below 1.0% *w/w*) were tested. It was demonstrated that the use of acoustic spectroscopy was very effective in the discrimination between mucoadhesive and non-mucoadhesive systems, allowing comparisons between their mucoadhesive attitude and discrimination between mucoadhesive and non-mucoadhesive polymers, independently from the frequency in use.

Overall, sound speed measurements can be considered as a novel method for polymer mucoadhesive evaluations, with the advantage that also opaque samples, for which traditional techniques might be not suitable, can be analysed. In addition, ultrasonic measurements do not require any specific sample treatment or complicated instrument set-up. Finally, this method allows us to work with small sample volumes, particularly useful when only small amounts of material are available.

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