Characterization of Povidone Products by Means of ^{13}C-NMR, MALDI-TOF, and Electrospray Mass Spectrometry

Klaus Raith,[1,3] Andrea V. Kühn,[1] Fred Rosche,[2] Raik Wolf,[2] and Reinhard H. H. Neubert[1]

Received October 9, 2001; accepted January 4, 2002

KEY WORDS: povidone; polyvinylpyrrolidone; mass apectrometry; ^{13}C-NMR; MALDI; electrospray.

INTRODUCTION

Polyvinylpyrrolidone is one of the most widely used classes of pharmaceutical excipients, including the linear soluble povidone, the crosslinked insoluble crospovidone as well as copolymers known as copovidone (1).

Nowadays, povidone products are widely used as excipients mainly for oral dosage forms such as tablets (2). Depending on their chain lengths, they serve as binders, disintegrant, diluent, or coating agent. Furthermore, they can be used as suspending, stabilizing, or viscosity-increasing agents, and also as a drug carrier. Special pyrogen-free povidones are available for parenteral formulations. Povidone is also used as a complexing carrier for iodine in PVP-Iodine, an important topical disinfectant.

This extraordinarily broad range of pharmaceutical applications makes efficient characterization of the substances at the molecular level essential. However, apart from a number of general specifications from pharmacopeias, the only available measure for the properties of povidones is the *K* value (1,2). This parameter is calculated from relative viscosity data with Fikentscher's equation (3). Three methods have been described to determine the average molecular weight of povidone: light scattering, osmometry, and viscosity (4,5). Molecular weight distribution can be monitored with gel permeation chromatography (2). A recent paper (6) describes the qualitative characterization of povidone types with near-infrared spectroscopy.

Mass spectrometric methods have been successfully applied to characterize a variety of polymers (7), including biopolymers (8). New solvent-free sample preparation schemes permit the MALDI ionization of insoluble analytes as well (9,10). Recently, an endgroup determination assay for synthetic polymers of the poly(oxyalkylene) type using electrospray-fourier transform ion cyclotron resonance MS has been presented (11).

The present study investigated different commercially available povidone products with respect to structural aspects such as chain length and end groups. The central question raising was, whether products with the same *K* value are structurally identical. Instrumental analytical methods such as ^{13}C-NMR, electrospray, and MALDI-TOF mass spectrometry were applied to characterize the structure. Moreover, the iodine binding has been quantified.

MATERIALS AND METHODS

Substances

Samples of three different types of soluble polyvinylpyrrolidone have been included in this study: Kollidon 12 PF, Kollidon 17 PF (BASF, Ludwigshafen, Germany), and Plasdone C-15 (ISP, Wayne, NJ). The *K* value of Kollidon 12 PF is 12, of Kollidon 17 PF, and for Plasdone C-15, it is 17. Methanol was obtained from J.T. Baker (Deventer, The Netherlands). Further chemicals used are described under the concerning method.

Electrospray Mass Spectrometry

Ion Trap experiments were performed with a Finnigan LCQ mass spectrometer (ThermoFinnigan, San Jose, CA). Time-of-flight experiments were carried out with a Q-Tof 2 instrument (Micromass, Manchester, UK).

Sample solutions have been introduced using a syringe pump at 8 μL/min. Optimal concentrations for qualitative determinations were 10 μg/mL for Kollidon® products and 100 μg/mL in case of Plasdone®, both in methanol or in methanol/water 80:20.

Most experiments were performed in positive-ion mode using an electrospray voltage of 4.0 kV (LCQ) and 3.5 kV (Q-Tof), respectively. Sample solutions containing 2 mM ammonium formate (Sigma, Taufkirchen, Germany) were used to obtain negative-ion electrospray mass spectra.

MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption/ ionization mass spectrometry was performed with a Hewlett-Packard G 2025A mass spectrometer (Agilent Technologies, Waldbronn, Germany) with a linear time-of-flight analyzer. The instrument was equipped with a 337 nm nitrogen laser, a high-potential acceleration source (5 kV) and a 1-m flight tube. Detector operation was in the positive-ion mode and signals were recorded and filtered using a LeCroy 9350 M (LeCroy Europe, Heidelberg, Germany) digital storage oscilloscope linked to a personal computer. The spectrometer was calibrated externally using the synthetic polypeptide $(Gly\text{-}Pro\text{-}Ala)_n$ (Bachem, Heidelberg, Germany; n = 3–50).

Samples (5 μL) were mixed with equal volumes of the matrix solution. For matrix solution we used DHAP/DAHC, prepared by solving 30 mg 2′,6′-dihydroxyacetophenone (DHAP, Aldrich, Taufkirchen, Germany) and 44 mg of diammonium hydrogen citrate (DAHC, Fluka, Taufkirchen, Germany) in 1 mL of acetonitrile/0.1% trichloroacetic acid in water (1/1, v/v). A small volume (approximately 1 μL) of the matrix-analyte mixture was transferred to a probe tip and immediately evaporated in a vacuum chamber (Hewlett-Packard G2024A sample prep accessory) to ensure rapid and

[1] Martin Luther University Halle-Wittenberg, Institute of Pharmaceutics and Biopharmaceutics, Wolfgang-Langenbeck-Str. 4, D-06120 Halle (S.), Germany.
[2] Probiodrug GmbH, Weinbergweg 22, D-06120 Halle (S.), Germany.
[3] To whom correspondence should be addressed. (e-mail: Raith@pharmazie.uni-halle.de)

0724-8741/02/0400-0556/0 © 2002 Plenum Publishing Corporation

homogeneous sample crystallization. All spectra were obtained by accumulating data generated by single shots with laser power between 2.5 and 4.5 μJ.

^{13}C-NMR Spectroscopy

Spectra were obtained with a Gemini 2000 (Varian, Darmstadt, Germany) at 100.58 MHz. The samples were prepared in methanol-d_4 (CD_3OD).

UV-VIS Spectroscopy

Determinations were performed on a UV-VIS-spectrophotometer UV-1202 (Shimadzu, Kyoto, Japan). The calibration was performed as follows: a 50-mL sample solution was mixed with 25 mL of 0.2 M citric acid solution. Ten milliliters of 0.006 N iodine solution was added, and after 10 min the absorbance of the solution measured at 470 nm against a blank.

RESULTS

The present study focused on commercially available povidone products with a relatively small molecular weight; this is because of their better suitability for NMR as well as mass spectrometric investigation. However, it can be expected, that the results obtained can also be extrapolated to higher homologues.

NMR samples need to be highly concentrated to be able

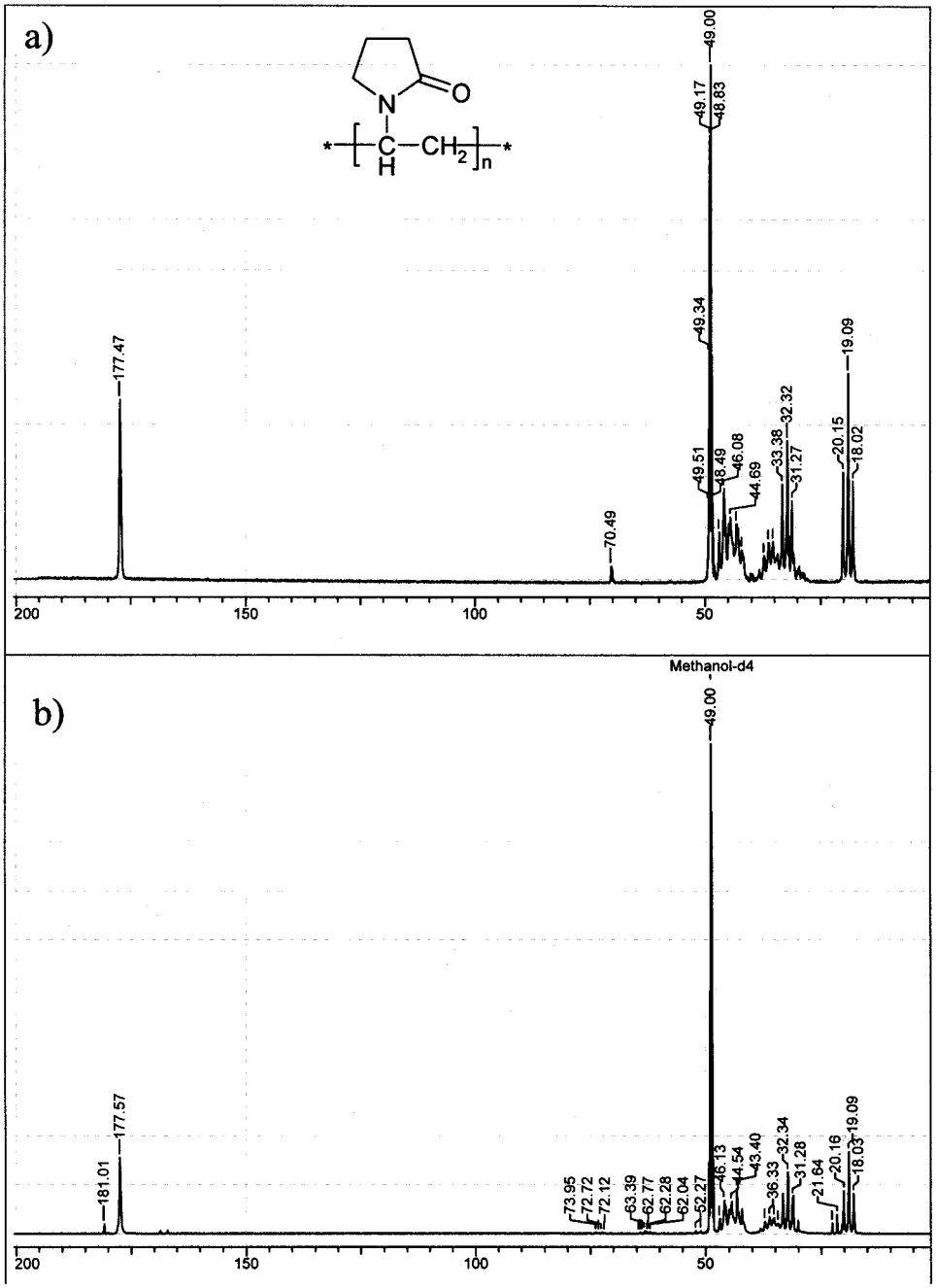

Fig. 1. ^{13}C-NMR spectra of (a) Kollidon PF 12 and (b) Plasdone C-15. Inset: general formula of polyvinylpyrrolidone.

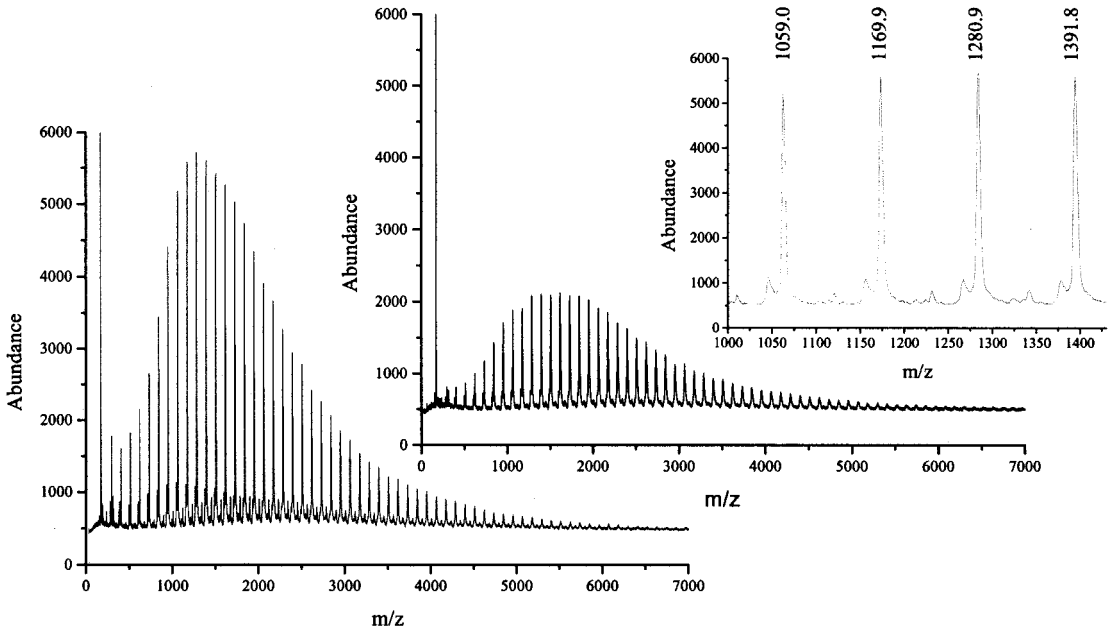

Fig. 2. MALDI-TOF mass spectra of Kollidon PF 12 (left) and Kollidon PF 17 (center). Right: enlarged section.

to detect the end groups in the presence of excessive amounts of the repeating units. ^{13}C-NMR analyses were performed to search for carbon atoms bound to oxygen, which should cause a peak shifted deep field. In case of Kollidon 12PF (Fig. 1a) and Kollidon 17PF, a singlet peak indicated a ternary C next to the oxygen. In case of Plasdone C15 (Fig. 1b), a triplet indicated a primary C next to the oxygen. These findings were confirmed in attached proton test spectra.

MALDI-TOF mass spectrometry was proven to be very suitable in obtaining the molecular weight distribution of the analyzed povidones. All ions bear a single charge. A typical pattern with peaks in a distance of 111 u is observed (Figs. 2 and 3). It is evident that in Kollidon samples one large peak is accompanied by a much smaller peak following at a distance of 58 u (Fig. 2). In contrast, the plasdone sample shows sets of five peaks in the typical 111 u distance, with 14 u in between (Fig. 3). This indicates additional methylene groups.

Electrospray mass spectra also show a typical pattern of peaks in a distance of 111 u (data not shown). It can be observed that in positive-ionisation Kollidon products strongly tend to form sodium adducts ($[M + Na]^+$), even under acidic conditions. Unlike many other polymers and macromolecules, povidones are singly charged throughout. No multiply charged species have been found. That gives evidence to the assumption that the molecules bear the charge only at an end group but not at the pyrrolidone rings of the

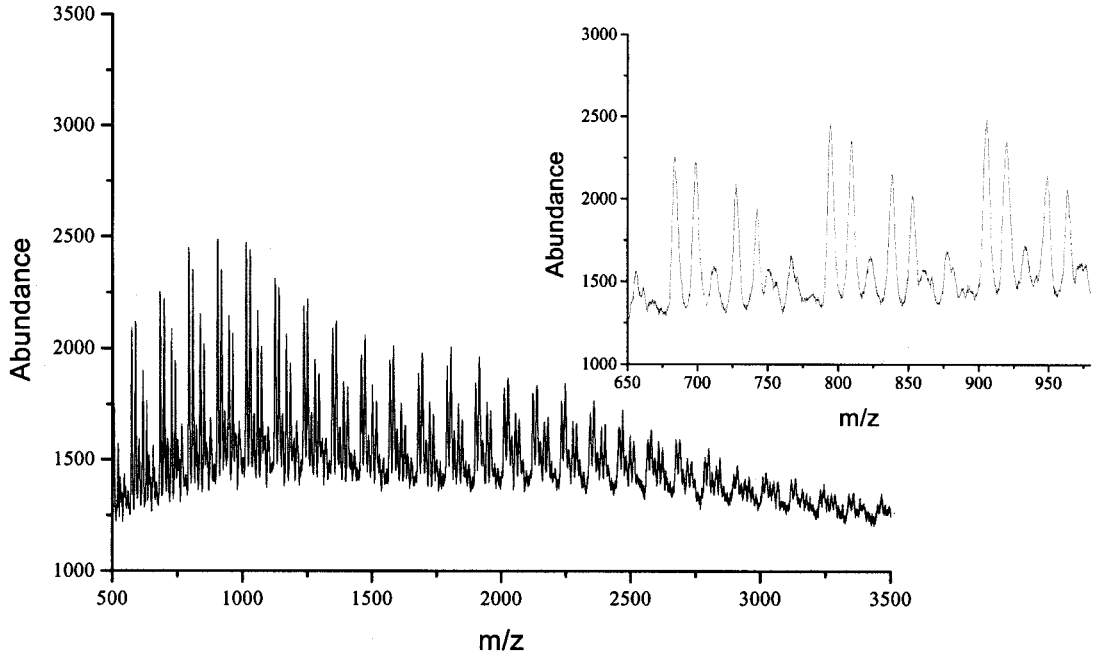

Fig. 3. MALDI-TOF mass spectrum of Plasdone C15. Inset: enlarged section.

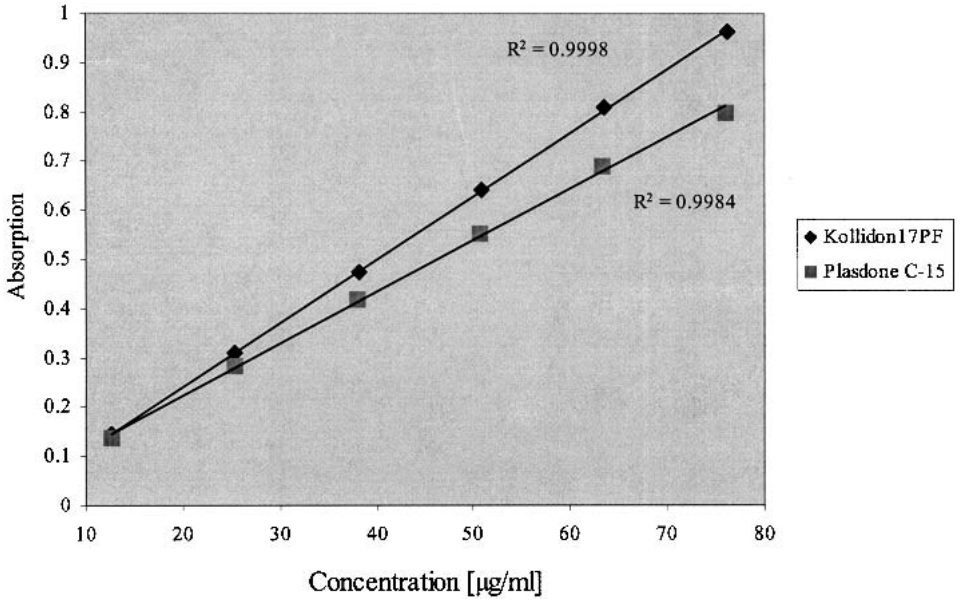

Fig. 4. Iodine binding curves of Kollidon PF17 vs. Plasdone C15 as measured using UV absorbance at $\lambda = 470$ nm.

repeating unit. The observed masses can be calculated according to the formula: $m = (111)_n + 60 + 23$. Although 23 results clearly from common sodium adduct formation as discussed before, 60 is considered to be due to a hydroxyisopropyl endgroup, which is supported by the ^{13}C-NMR results.

Plasdone showed a much weaker ESI response with only pseudomolecular ions ($[M + H]^+$) to be observed (data not shown). The reason for this behavior is presumably the different endgroup structure. The five-peak sets as known from MALDI-TOF spectra cause an additional weakening due to a partition of intensity. Negative-ion electrospray MS is only possible after addition of ammonium formiate to create the formate adducts.

Electrospray tandem mass spectrometry shows in positive-ion mode a water loss (18 u) and a loss of one pyrrolidine-2-one ring, resulting in a loss of 85 u. In MS^3 experiments, water and a ring could be split off again, and a loss of 40 amu occured, which is considered to be due to the formation of an allene leaving group.

Iodine binding experiments with Kollidon PF 17 and Plasdone C15, which both have a *K* value of 17, have been performed to search for differences. The intensity of iodine binding has been monitored by means of UV absorbance spectroscopy at $\lambda = 470$ nm. As pointed out in (2), the response depends on chain length and molecular mass average, respectively. The result of this study was that the responses of both curves are significantly different (Fig. 4).

DISCUSSION

The results presented in this study show that povidone products of different suppliers may have different properties, particularly with respect to end group structures. The solvent used in synthesis is considered to be important. In Kollidon, isopropanol causes a hydroxyisopropyl endgroup. The second series with a 58 u higher mass indicates a second hydroxyisopropyl endgroup instead of a terminal H. In Plasdone, synthesis in water results in hydrogen/hydroxy end groups. The set of five peaks in MALDI-TOF-MS of Plasdone indicates 0–4 additional methylene groups between repeating unit and hydroxy endgroup.

Although the impression is clearly that the average molecular mass obtained from mass spectrometry is below the declared one obtained from viscosity or light scattering, it is difficult to quantify this statement. One has to bear in mind that response in MALDI MS depends on chain length, matrix selection and preparation conditions. Generally, the molecular weight distribution results obtained by mass spectrometry refer to the number-average instead of the weight-average, since signal intensity is proportional to the number of ions detected, independent of mass. In contrast, the weight average detected by means of light scattering as well as the viscosity-average are shifted towards higher masses due to the presence of very few, but large and heavy molecules.

ESI is less suitable for molecular weight distribution surveillance, since the response drops dramatically at higher masses. An intensity maximum can be observed approximately at $m/z = 900$ dependent on instrument parameters (data not shown).

The results from the iodine binding study demonstrate a remarkably different behavior of Kollidon and Plasdone.

Summarizing all results, our study shows that the k-value and pharmacopoeial specifications cannot provide a comprehensive characterization of povidone products. The methods of ^{13}C-NMR spectroscopy, MALDI-TOF, and electrospray mass spectrometry enable deeper insight into molecule structure, particularly concerning end groups of povidone. ESI-MS, MS/MS, and MS^n can easily serve as a fast identity check due to the characteristic spectra.

We suppose that these findings should be considered in terms of the mutual replaceability in pharmaceutical products. Furthermore, it is necessary to correlate these structural findings to physico-chemical properties of the povidone products.

ACKNOWLEDGMENTS

The authors acknowledge financial support from Deutsche Forschungsgemeinschaft (NE 427/10-1). A. Kühn thanks the Landesgraduiertenförderung of Sachsen-Anhalt for her scholarship. We thank BASF and ISP for providing povidone samples as gifts. We appreciate the assistance of Dr. D. Ströhl in performing ^{13}C-NMR.

REFERENCES

1. A. H. Kibbe (ed.), *Handbook of Pharmaceutical Excipients*, APhA, Washington, 2000.
2. V. Bühler. *Kollidon®. Polyvinylpyrrolidone for the pharmaceutical industry*, BASF, Ludwigshafen, 1999.
3. H. Fikentscher and K. Herrle. Polyvinylpyrrolidone. *Modern Plastics* **23**:157–161, 212, 214, 216, 218 (1945).
4. W. Appel and E. Biekert. *Angew. Chemie* **80**:719–725 (1968).
5. V. Bühler and U. Klodwig. *Acta Pharm. Tech.* **30**:317–324 (1984).
6. K. Kreft, B. Kozamernik, and U. Urleb. *Int. J. Pharm.* **177**:1–6 (1999).
7. S. D. Hanton. *Chem. Rev.* **101**:527–569 (2001).
8. U. Bahr, M. Karas, and F. Hillenkamp. *Fresenius J. Anal. Chem.* **348**:783–791 (1994).
9. R. Skelton, F. Dubois, and R. Zenobi. *Anal. Chem.* **72**:1707 (2000).
10. S. Trimpin, A. Rouhanipour, R. Az, H. J. Räder, and K. Müllen. *Rapid Commun. Mass Spectrom.* **15**:1364–1373 (2001).
11. S. Koster, M. C. Duursma, J. J. Boon, and R. M. A. Heeren. *J. Am. Soc. Mass Spectrom.* **11**:536–543 (2000).