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A staining technique for evaluating the pore structure variations of microcrystalline cellulose powders

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Abstract

A staining technique was developed for evaluating the pore structure of microcrystalline cellulose powders. This technique used two direct dyes as molecular probes and was based on the differences between the two dyes' molecular size and affinity for microcrystalline cellulose. Direct Blue I has a smaller molecular size and weaker affinity for microcrystalline cellulose compared to Direct Orange 15, which has a larger molecular size and stronger affinity. We used the ratio of adsorbed Direct Orange 15 to adsorbed Direct Blue I after 48 h staining [designated as $(O/B)_{48}$] as the parameter for evaluating the pore structure variations between different microcrystalline cellulose powders. Seven microcrystalline cellulose samples of three different grades—Avicel PH-101, Avicel PH-102 and Avicel PH-103—were analyzed for their $(O/B)_{48}$ ratios, and significant differences were found. These differences are present primarily among different grades and secondly among different batches of the same grade. Among the three grades tested, it was found the their $(O/B)_{48}$ ratios have the following sequence: Avicel PH-103> Avicel PH-101> Avicel PH-102. This suggested that pore structure variations are significant among different microcrystalline celluloses of both different grades and batches, but the degree of variation between different batches is less than that between different grades of microcrystalline cellulose.

Keywords: Pore structure; Microcrystalline cellulose powder; Staining technique

1. Introduction

Microcrystalline cellulose powders are probably the most important pharmaceutical excipients used in tableting, especially for direct compression. Numerous brands of microcrystalline cellulose powders are now available on the market which because of the source of wood and pulp as well as manufacturing process cart have different properties which are not interchangeable in the production of pharmaceutical tablets [1]. Structural variations among these microcrysralline cellulose powders are believed to be responsible for their different functional properties and have been under extensive investigation [1-6]. Chemical composition, such as cellulose, hemicelluloses and lignin content [2]—as well as physical structure such as molecular size [3], crystalline allomorph [2] and crystallinity [3,4], pore structure at dry State [5] and particle size and shape [6] —have been studied by many investigators. Efforts to correlate the structural data to their functional properties have not been successful to date [1], and new structure characterization techniques are being sought to identify the reasons for the differences in functional properties.

Microcrystalline cellulose is made from fibrous cellulose (mainly wood pulp), which consists of an ordered region (microcrystal) and a less-ordered region (amorphous regions). The latter chemically hinges the cellulose miroccrystals together. In the process of acid hydrolysis, the less-ordered region is preferentially hydrolyzed, so the cellulose microcrystals are released. These cellulose microcrystals have a diameter in the range of 10-30 nm [7] and can randomly aggregate together through a hydrogen bond. Between these cellulose microcrystals, pores are formed that generally range in size from several nanometers to 10 nm or more [7]. The size variation was expected to have art influence on the functional properties of microcrystalline cellulose. Here, we report a staining technique used to evaluate this pore structure variation of microcrystalline cellulose powders at wet state.

This technique was developed from Simons' stain [8,9], which is used in the pulp and paper industry to examine the physical structure change of pulp fibers under the microscope. This stain consists of a mixture of two direct dyes: Direct Blue I (Color Index no. 24410) and Direct Orange 15 (Color

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Index no. 40002/3) [10]. Direct Blue I has a smaller molecular size and a weaker affinity for cellulose compared to Direct Orange 15, which has a larger molecular size and a stronger affinity. Therefore, when a mixture of Direct Blue I and Direct Orange 15 is applied to cellulose samples, Direct Orange 15 molecules will preferentially be adsorbed on the cellulose surface where the Direct Orange 15 molecules are accessible. On the surface of small pores, where the Direct Orange 15 molecules are not accessible, the Direct Blue I molecules will be adsorbed. Therefore, the ratio of adsorbed Direct Orange 15 to adsorbed Direct Blue I is anticipated to serve as an indicator of the pore structure-specifically, the pore size population distribution of the cellulose samples.

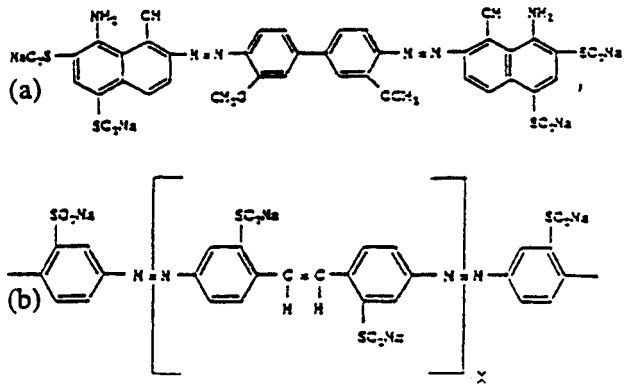

Fig. 1. Chemical structure of Direct Blue I (a) and Direct Orange 15 (b).

2. Separation and purification of the dyes

The Direct Orange 15 was purchased from PYLAM Products under the commercial name Pontamine Fast Orange 6RN. We purified it as follows [9]: 1 g Direct Orange 15 was dissolved in 100 ml distilled water; the solution was then applied to a column packed with Sephadex G-25 and eluted with distilled water. Two orange bands appeared on the column and the front band, which represented the high molecular weight fraction was collected. After evaporating the water, the high molecular weight fraction of Direct Orange 15 was further dried at 80°C under vacuum. The resulting purified Direct Orange 15 was used.

The Direct Blue I was also purchased from PYLAM Products and purified by following the Robinson and Mills procedures [11].

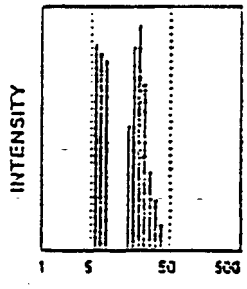

Fig. 2. Light scattering diagram of Dimes Orange 15.

3. Molecular size of the purified Direct Blue I and Direct Orange 15

Direct Blue I has a well defined chemical formula (Fig. 1a) with a molecular weight of 992.82 and a molecular area of 3.6 nm^2 (or a diameter of 1 nm) [12]. Direct Orange 15 is a condensation product of 5-nitro-o-toluenesulfonic acid in aqueous aikali solution. It forms an extended polymer as shown in Fig. 1b, and therefore, its formula and structure are not well defined. Light scattering measurements revealed that the purified Direct Orange 15 has a molecular diameter in the range of 5-36 nm (Fig. 2), which is much larger than the Direct Blue I molecular size. Gel permeation chromatography also demonstrated that purified Direct Orange 15 has a much larger molecular size than Direct Blue I, as shown in Fig. 3.

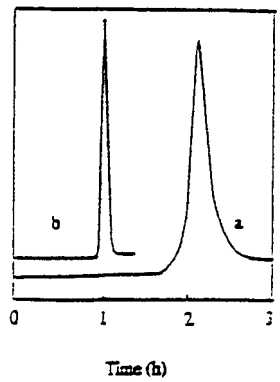

Fig. 3. Gel permeation chromatography of Direct Blue I (a) and Direct Orange 15 (b) on Sephadex G-25 (eluant: water: flow rate: 75 ml/h).

4. Relative affinity of Direct Blue I and Direct Orange 15 for microcrystalline cellulose

The relative affinity of the two dyes was measured by determining the amount of adsorbed dye from a single dye-solution and from a solution containing both dyes. In the mixture solution, the adsorption of the two dyes on the microcrystalline cellulose surface is competitive according to the respective affinity of the dyes. The adsorption of the dye with weaker affinity for the microcrystailine cellulose was significantly less in the mixture solution than was its adsorption with the single-dye solution, whereas the adsorption of the dye with stronger affinity was less affected by the presence of the dye with weaker affinity. The test was carried out on Avicel PH-101 and Avicel PH-103 microcrystalline cellulose. The average results of the three tests and their standard deviations (σ) are shown in Table 1.

The results indicate that the adsorbed Direct Blue I from the solution containing both dyes was about 40% less than that from the single-dye solution, while the presence of Direct

Table 1
Relative affinity and competitive adsorption of Direct Blue 1 and Direct Orange 15

Microcrystalline cellulose	Avicel PH-101	Avicel PH-103
Blue adsorbed in single dye solution (g/100 g) [σ]	1.58 [0.02]	1.51 [0.02]
Orange adsorbed in single dye solution (g/100 g) [σ]	2.86 [0.03]	3.22 [0.04]
Blue adsorbed in mixture solution (g/100 g) [σ]	0.94 [0.02]	0.88 [0.02]
Orange adsorbed in mixture solution (g/100 g) [σ]	2.81 [0.04]	3.05 [0.04]

Blue I had much less effect on the adsorption of Direct Orange 15. This suggested that Direct Orange 15 has a much stronger affinity for microcrystalline cellulose than does Direct Blue I.

5. Determination of the ratio of adsorbed Direct Orange 15 to adsorbed Direct Blue I and its repeatability

The experimental procedures of this analysis are very simple. A 25 mg sample of microcrystalline cellulose powder was placed into a bottle; then sequentially, 10 ml 0.1% Direct Orange 15, 10 ml 0.1 Direct Blue I, 70 ml water and 10 ml 10% NaCl were added. The bottle was sealed and placed in a 75°C water bath for 48 h. The stained microcrystalline cellulose was then filtered out and transferred into another bottle; 20 ml 25% aqueous pyridine was added into the new bottle to strip the adsorbed dyes at 50°C for about 10 h. The strippings were then analyzed with UV/V is for the concentrations of Direct Orange 15 and Direct Blue I (λ_{max} of Direct Blue I and Direct Orange 15 are at 624 nm and 455 nm). The ratio of adsorbed Direct Orange 15 to adsorbed Direct Blue I, which we designated as $(O/B)_{48}$, was then calculated from the absorbencies at 624 and 445 nm. To test the repeatability of this technique. we conducted $(O/B)_{48}$ ratio analysis 10 times on Avicel PH-101 and Avicel PH-103, respectively. The results and error analysis are shown in Table 2.

The results in Table 2 indicate that this technique has a good repeatability with a standard deviation around 1%. Moreover, there is a significant difference between $(O/B)_{48}$ ratios measured for Avicel PH-101 and Avicel PH-103.

6. Pore structure evacuation of different microcrystalline cellulose powders

$(O/B)_{48}$ was the parameter used to evaluate the pore structure of different microcrystalline cellulose. It is clear from previous discussion that large $(O/B)_{48}$ ratios indicate a relatively large population of big pores compared to small pores, while small $(O/B)_{48}$ ratios indicate a large population of small pores compared to large pores. To evaluate the pore structure difference, we tested seven microctystalline cellulose powders for their $(O/B)_{48}$ ratios. Three of the specimens were the same grade of Avicel PH-101 but from different batches. Another three were grade Avicel PH-102, also from different batches, and one was grade Avicel PH-103. The $(O/B)_{48}$ data for these microcrystalline cellulose samples are shown in Table 3. Microcrystalline celluloses of different grades have different functional properties, such as compaction profile and tablet properties, as well as different physical characteristics, such as particle dimensions, crystallinity, molecular weight [1] and especially, specific surface area. which is related to the pore structure and to our staining

Table 2
Repeatability of $(O/B)_{48}$

Replication	Avicel-PH101	Avicel PH-103
1	3.03	3.42
2	2.98	3.41
3	3.02	3.50
4	3.00	3.48
5	3.02	3.42
6	3.03	3.51
7	2.96	3.45
8	2.95	3.46
9	2.99	3.48
10	2.98	3.49
Average $(O/B)_{48}$	3.00	3.46
Standard deviation (σ)	0.03	0.04
Standard deviation in percent [σ/average $(O/B)_{48}$]	0.96%	1.03%
Average standard deviation in percent	1.0%	

Table 3
$(O/B)_{48}$ ratios of different microcrystalline cellulose

MCC	Batch	$(O/B)_{48}$	Surface area (m^2/g)
Avicel PH-101	1	3.00	1.30
	2	3.13	
	3	3.17	
Avicel PH-102	1	2.61	1.26
	2	2.68	
	3	2.75	
Avicel PH-103	1	3.46	1.36

results. It was expected that microcrystalline cellulose with a larger $(O/B)^{48}$ ratio would have a linger surface area, which is accessible to the Direct Orange 15 molecules. Marshall and Sixsmith [13] determined the specific surface of Avicel PH-101, Avicel PH-102 and Avicel PH-103 using mercury intrusion porosimeter with a lower pore size limit up to 5 nm; the data, as comparison, are also shown in Table 3.

The data in Table 3 show that a larger $(O/B)_{48}$ ratio correlates with larger surface area and smaller $(O/B)_{48}$ ratio correlates with smaller surface area. This indicates that $(O/B)_{48}$ reflects the pore structure characteristics of microcrystalline cellulose; although the staining test may not have exactly the same meaning as the mercury intrusion test. Therefore, $(O/B)_{48}$ can be considered as a good indicator of the pore structure.

The results in Table 3 also show that $(O/B)_{48}$ ratios of different microcrystalline celluloses vary significantly. These variations are present not only among different grades, but also different batches of the same grade. The variations among different grades, however, are much larger than those among different batches of the same grade. Among different-grade Avicel microctystilline cellulose. Avicel PH-103 appears to have the largest $(O/B)_{48}$ ratio, followed by Avicel PH-101 and then Avicel PH-102. These results suggest that pore structure variations are present among different microcrystalline celluloses, primarily among different grades and secondarily among different batches of the same grade. Among the three grades of Avicel PH-101 PH-102 and PH-103, we found that Avicel PH-103 has the largest population of large pores, followed by Avicel PH-101, and Avicel PH-102 has the smallest population of large pores.

7. Conclusions

A staining technique has been developed to evaluate the pore structure of microcrystalline cellulose powders. The stain consists of two direct dyes: Direct Orange 15 and Direct Blue I. The ratio of adsorbed Direct Orange 15 to adsorbed Direct Blue I is a good indicator of the pore structure of the test specimen. This technique has a good repeatability; the standard deviation is around 1.0%. We tested seven Avicel microcrystalline cellulose specimens of three grades and found that pore structure variations are remarkable between different-grade microaystalline cellulose. Pore structure variations were also found among different batches of the same grade of microcrystalline cellulose, but the degree of variation was less than that between different grades.

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