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MICROBIOLOGICAL CONSIDERATIONS OF POLYMER SOLUTIONS USED IN AQUEOUS FILM COATING

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ABSTRACT

Environmental and other pressures are causing pharmaceutical manufacturers to convert earlier developed solvent based film coating procedures to aqueous systems. Factors influencing microbiological proliferation in cellulosic polymer solutions are discussed. Commonly employed water soluble cellulosic polymers have been evaluated for their resistance/susceptibility to microbiological growth. The implications of the findings to the use of such aqueous polymer solutions as pharmaceutical film coatings are discussed.

INTRODUCTION

In the early to mid-1950's pharmaceutical film coating was introduced in the drug industry. This technology primarily utilized organic solvent solutions of polymers to form thin coatings or film coatings on pharmaceutical dosages, primarily tablets. Among the initial advantages cited for the film coating process relative to the centuries old sugar coating technology which film coating was replacing, included: the much shorter time required to complete the operation (a few hours versus several days for sugar coating), the fact that the procedure was anhydrous and concern over microbial growth in aquous sugar and other solutions no longer existed, the greater mechanical

and thermal stability properties of the film coatings, the lower weight and smaller size of the film coated product, the fact that the coated tablets were less likely to be mistaken for candy by children, and other advantages. Through the 1960's and into the 1970's many drug companies converted partially or entirely their coated tablet products from sugar coating to film coating. Beginning in the 1970's several economic and regulatory factors began to impinge on solvent based film coating as a pharmaceutical process. The primary economic factor was the rapidly escalating cost of the organic solvents which were always the major cost of materials for the process. However, as solvent cost doubled and redoubled in the 1970's this cost factor became much more appreciable. Of perhaps more concern, however, were the environmental concerns and the federal and state regulations placed on discharge of solvent vapors to the atmosphere (EPA considerations). At the same time, federal and state agencies beginning in the late 1960's and early 1970's began applying protection standards for exposure of workers to all types of noise, chemical and other hazardous exposures. Occupational safety and health administration guidelines set rigid tolerances for worker exposure to solvent vapors in their consideration of hazardous chemical exposure. Many drug companies had employed the use of chlorinated organic solvents in the preparation of their organic solvent coating solutions for two reasons: such solvents reduced the flammability and explosion hazard of the organic solvent solutions employed, and the chlorinated solvent coupled with alcohols and acetone as well as various esters provided a better solvent system for the cellulosic polymers than could otherwise be obtained. Unfortunately the chlorinated solvents were among those of greatest concern to both EPA and OSHA. As a result of these economic and regulatory impacts, in the mid to late 1970's many drug companies began to look for alternative methods of film coating, including the conversion of their solvent based systems to aqueous systems. In converting back to aqueous based coating systems employing polymers some of the advantages of the initially developed film coating process are lost, wholly or in part. Foremost among the advantages which may be lost are the rapidity with which the process may be conducted,

and the amount of thermal energy required to complete the process, together with the resistance to microbiological growth in the coating solution medium. This paper will review some of the latter considerations.

EXPERIMENTAL

Solutions of each polymer studied were prepared in sterile water at room temperature. At selected time points the agar pour-plate method was used to determine the number of organisms in a particular solution based on colony counting. At these selected time points a sterile pipette was used to sample the polymer solution and the sample was diluted 1/1, 1/10, and 1/100. One ml of the each dilution was pipetted into a sterile tube which contained 3 ml of molten agar at 45^{o} and mixed. This mixture was poured into ready made agar plates (about 15 ml), inverted, and allowed to incubate for 5 days at room temperature (25^{o}). Three plates were prepared for each dilution. Microbial counts were made on polymer solutions immediately following the time of dilution preparation (0 time), and at 8 hours, 1 day, 2 days, 4 or 5 days, 9 days, 14 or 15 days. Colony counting was done on plates which had been innoculated to yield between 30 and 300 colonies to insure test precision and accuracy. The counts were recorded as average values at the appropriate dilution, and the standard deviation was also computed. The bacterial colony count data was normalized to 0.1 ml polymer solution initially sampled. The polymers which were utilized in the study are described in Table 1.

In a second part of the experiment the polymers prepared in sterile water were contaminated with selected organisms to evaluate their resistance or susceptability to the proliferation of the microorganisms so added. In this phase of the experiment 100 ml of the polymer solution or of the sterile water control sample was contaminated with either E. coli or A. niger. In the case of E. coli, 100 ml of the polymer solution or the sterile water control was contaminated with the 1 ml of nutrient broth containing 10,000 to 1 million bacteria. Dilutions of these contaminated samples where made in sterile water in ratios of 1:10, 1:100, 1:1000, and 1:10,000. One ml of each diluted

Table 1: Polymers Used in the Microbiological Study.

CMC 7L2 = Sodium carboxymethyl cellulose

Molecular weight (approximate)	90,000	Viscosity (2%)	18 cps

Klucel ^{R} = Hydroxypropyl cellulose (HPC)

Molecular weight (approximate)	HF = 1,000,000	Viscosity (1%)	1500-2500 cps
	MF = 600,000	" (2%)	4000-6500 cps
	GF = 300,000	" (2%)	150-400 cps
	LF = 100,000	" (5%)	75-150 cps
	EF = 60,000	" (2%)	8-10 cps

From: Hercules Incorporated, 910 Market Street, Wilmington, Delaware 19899

Methocel ^{R} = Hydroxypropyl methyl cellulose (HPMC)

Molecular weight	E5 = N.A.	Viscosity (2%)	4-6 cps
	E15 = N.A.	"	13-18 cps
	E50 = N.A.	"	40-60 cps

From: The Dow Chemical Company, Midland, Michigan 48640

contaminated sample was pipetted in 3 ml of molten agar at 45^{o}, mixed, poured into ready made agar plates and incubated as described previously. Three plates were prepared for each diluted sample and the microbial counts were obtained at the time intervals specified in the table in the results section.

In the case of A. niger the procedure was followed as described with the preparation of E. coli contaminated samples except that 0.5 ml of the contaminated sample was placed in the center of an SDA (Sabourand Dextrose Agar Media) plate. The plates were then incubated at room temperature (25^{o}) for five days. The 0.5 ml samples were taken at the time specified in the table in the results section and the diameter of the fungal colonies were measured after the incubation period.

In a third phase of the experiment beakers of each polymer solution were left exposed to the air in the laboratory for several days, were then covered

to prevent further water evaporation, and were then visually evaulated for any evidence of microbial growth.

RESULTS

In the first phase of this study where 1% w/v polymer solution of the polymers listed in Table 1 were prepared in sterile water, the solutions being packaged in sterile glass containers, and aseptically sampled over a 14 day period, no growth was observed in any solution.

Table 2 presents the data of bacterial growth in the E. coli contaminated samples. All of the samples were contaminated to an initial level of about 1300 to 2100 counts per 1 ml of solution. Some interesting trends are seen in the data. In sterile water, where no nutrient is present, the colony count tends to decline over the 14 day test period; an expected result. The hydroxy propyl cellulose (Klucel) solutions had their colony counts decline on aging, with the rate of decline being more rapid as the molecular weight of the polymer decreased. Both carboxymethyl cellulose (CMC) and the lower molecular weight hydroxy propyl methy cellulose (Methocel E5) appear to be good growth media for the test organism. Of the low molecular weight water soluble polymers, Klucel EF and Methocel E5, the former polymer has greatly superior resistance properties to the proliferation of growth of this organism.

To verify the growth of E. coli in aqueous solutions of the commonly used film coating polymer, hydroxy propyl methyl cellulose, recorded in Table 2, new polymer solutions of this polymer were prepared in sterile water, and contaminated with E. coli as previously described. A different lot number of the respective polymers was employed. The results are shown in Table 3. A higher initial contamination level was used (about 10,000 counts) in comparison to the experiment reported in Table 2. Once again the colony counts consistently decline in the sterile water (no nutrients). Also, once again very dramatic bacterial proliferation occurred in the low molecular weight E5 grade. However less difference was seen in the microbial proliferation

TABLE 2. Viable Colony Counts of Bacteria in Cellulosic Polymer Solutions Following the Addition of E. Coli to 1% Polymer Solutions

		Viable Colony Count (Mean ± SD) Normalized to 1 ml Sample						
Polymer		0 hr	8 hr	1 day	2 day	5 day	9 day	14 day
Klucel®	EF	1900±50	2350±100	700±50	43±8	0±0	0±0	0±0
	LF	1883±76	2400±50	1250±50	163±15	0±0	0±0	0±0
	GF	1750±50	2633±76	1583±125	787±35	27±6	13±6	2±1
	MF	1717±76	2100±132	1467±104	693±15	60±10	33±6	6±1
	HF	1583±76	1800±50	900±50	727±50	37±6	37±6	1±1
CMC®	7L2	1333±76	2550±132	2317±104	10,500±500	8333±289	6667±289	24,667±1528
Methocel®	E5	2050±150	2657±76	1267±76	2267±180	66,000±200	130,000±10,000	102,000±2000
	E15	2117±76	2233±76	1333±104	1060±72	83±6	0±0	0±0
	E50	1967±76	2800±50	2083±76	1167±64	90±10	30±10	0±0
Sterile Water Control		2100±50	2033±76	1350±50	713±35	110±10	280±10	113±6

TABLE 3. Viable Colony Counts of E. coli Contaminated 1% Methocel E5, E15 and E 50 Solutions.

Polymer	0 hr	8 hr	1 Day	2 Day	5 Day	9 Day	14 Day
Methocel E5	10,225±810	13,824±1600	14,560±4500	16,574±1580	75,000±3250	142,000±7500	141,250±8200
Methocel E15	9540±350	13,240±240	13,800±850	15,750±1485	45,000±2500	85,000±3450	96,000±5600
Methocel E50	9870±462	14,200±1275	15,900±1390	17,490±1890	65,000±2200	125,000±6500	127,000±7760
Sterile Water Control	9540±320	8575±315	5650±210	3100±250	1890±175	1250±110	789±35

of the three Methocel molecular weight grades than was noted in the earlier study reported in Table 2.

Table 4 reports the changes in colony diameter measurements of polymer solution samples contaminated with A. niger. In the case of this fungal organism the higher molecular weight hydroxy propyl methyl celluloses supported organism growth better than the lower molecular weight material. All three grades support organism growth better than the water control throughout the time periods studied.

In the polymer solutions exposed to the open air and then allowed to stand covered at room temperature, the CMC solutions demonstrated the heaviest visible microbial colony growth, with the shortest time (2 to 3 days). Colony growth was also apparent in the Methocel samples, especially in the solutions of the E5 grade. No visable growth was seen in the Klucel EF solutions.

DISCUSSION

Cellulose is comprised of monomer building blocks of anhydroglucose. As cellulose is broken down, shorter and shorter chains of anhydroglucose are formed, and even glucose may be liberated. Glucose is, of course, an excellent nutrient for microbial growth. It is reported that bacterial degradation of CMC, in which the degree of substitution (D.S.) is less than 1.2, is a common phenomenon. Since the D.S. for the CMC used in this experiment is between 0.4 and 0.7, the finding that the CMC solutions readily supported bacterial growth is in agreement with published microbial degradation information on CMC (1).

E. coli contains a cellulase (β-1,4-Glycosidase) which can convert cellulose derivatives, in which the D.S. is less than about 1, to glucose, which the bacterium uses to maintain its growth (2). This is one reason that this organism was selected for this study. The other is that it is a commonly found pathogen being normal to the human GI tract.

The Klucel solutions supported growth of the bacteria used in this study after innoculation of the solutions with E. coli to the lowest degree.

TABLE 4: Viable Colony Size Measurements of A. niger Contaminated 1% Methacel E5, E15 and E50 Solutions.

Polymer	0 Day	1 Day	2 Day	5 Day	9 Day	15 Day	21 Day	27 Day
Methocel E5	6.58±0.07	6.55±0.06	6.51±0.05	6.50±0.05	6.45±0.05	5.90±0.06	5.75±0.02	5.10±0.05
Methocel E15	6.60±0.06	6.59±0.07	6.59±0.01	6.55±0.06	6.52±0.02	6.10±0.06	5.82±0.01	5.55±0.04
Methocel E50	6.70±0.08	6.70±0.02	6.71±0.04	6.68±0.07	6.62±0.02	6.40±0.04	6.10±0.03	5.80±0.022
Sterile Water Control	6.65±0.03	6.20±0.01	5.89±0.03	5.70±0.06	5.50±0.00	5.32±0.03	4.97±0.04	4.80±0.03

The D.S. of all the Klucel polymer grades used was well above 1 (typically it is about 2.5). Accordingly it is not surprising that Klucel did not support bacterial growth, regardless of the molecular weight of the Klucel used. Another critical factor is the uniformity of substitution along the polymer chain. With even the low molecular weight Klucel the uniformity of substitution appears to be good. Hydroxy propyl methyl cellulose appears to support the growth of E. coli to a much greater extent the hydroxy propyl cellulose. The lowest molecular weight hydroxy propyl methyl cellulose, with the widest use and applicability as a film coating for this polymer, had the greatest susceptibility to proliferation of both test organisms studied.

Aqueous solutions of cellulosic polymers used in film coating can definitely support microbial growth and proliferation. As pharmaceutical manufacturers convert to water based film coatings they should give careful thought to monitoring possible microbiological contamination problems in their coating operations. It is anticipated that microbial contamination of pumps, lines carrying coating solutions, spray equipment, and coating equipment, could become an important source of consistent future product contamination. Holding aqueous cellulosic polymer solutions for prolonged periods, such as over a weekend, even at room temperature, can lead to explosive microbial growth in a contaminated sample. Where solution samples are held for days, or even overnight, refrigeration would probably be a good precaution. Not all water soluble polymers resist (or permit) microbial proliferation to the same extent. A very important consideration in polymer selection for water based film coating should be this factor, resistance of the respective polymer solution to microbial proliferation. This is especially true since product NDAs may not have made provision for incorporation of preservatives in coating solutions or the final product. Furthermore, there are now relatively few effective preservatives available with "gras" status, and preservation of polymer solutions which often have an appreciable ability to complex or bind preservatives, may not be simple or readily accomplished.

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