Comparison of Analytical Techniques for Thermal Stability Analysis of \(\beta\)-Cyclodextrin for an Ebola Virus Infection Treatment

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ABSTRACT: Each New Drug Application filed with the Food and Drug Administration (FDA) must include the analytical procedures to ensure the identity, strength, quality, purity, and potency of a drug substance and drug product. The BSN389 drug product (being developed to treat Ebola virus infections) includes beta cyclodextrin. Evidence must be provided that the analytical procedures used in testing BSN389 meet proper standards of accuracy, sensitivity, specificity, and reproducibility and are suitable for their intended purpose. The Bootstrap Error-adjusted Single-Sample Technique (BEST) software was used to compare the quantitative and qualitative power of IR and \(^1\)H NMR to differentiate new and partially decomposed samples of beta cyclodextrin, and the best assay will be incorporated into the thermal stability protocol for BCD.

1. INTRODUCTION

\(\beta\)-cyclodextrin, known simply as \(\beta\)-cyclodextrin or \(\beta\)CD or BCD, is a non-reducing cyclic oligosaccharide consisting of seven \(\alpha\)-1,4- linked D-\(\pm\)-glucopyranosyl units (Figure 1). The seven membered ring is produced by enzymatic conversion of starch. This drug has applications not only in pharmaceuticals but also in the food and environmental industry. Toxins can be removed when the ring ensnares specific molecules that are targeted for removal. BCD is also a food additive that acts as a stabilizer for flavors, colors, and some vitamins.\(^2\) BCD’s estimated intake is about 1-1.4 g/day and it is approved by the FDA. Researchers are now taking known information about the cyclodextrin molecule and using it as a carrier for chemotherapeutic cytotoxic anticancer drugs.\(^3\)

The analytical techniques \(^1\)H NMR and infrared (IR) spectroscopy are used to measure the difference between decomposed and stable versions of BCD in this research. The Bootstrap Error-adjusted Single-sample Technique (BEST) software (see Appendix) will then identify the best analytical method to use for the thermal stability regulatory procedure for the drug.

2. METHODS

Preparation of Samples

Approximately two grams of beta cyclodextrin was slightly decomposed thermally by putting the sample on a Pyrex dish and placing it in a conventional oven, heating it slowly to about 232 °C at a linear rate of about 28 °C/5 min until the white powder sample was a slightly yellow color.

Measurements

Six separate samples of the pure and decomposed BCDs were prepared with deuterated water. \(^1\)H NMR spectra were recorded on a 500 MHz JOEL spectrometer, and processed with 16 scans ranging from -2 to 16 ppm. These samples were then also analyzed using a Thermo Scientific Nicolet iS10 infrared spectrometer over a wavenumber range of 4000-500 cm\(^{-1}\).

Analysis

The \(^1\)H NMR data were entered in TopSpin and converted into CVS files. These data along with the IR values were read into MATLAB. Each sample set of data was linked together in a variable with dimensions equal to the number of wavenumbers or chemical shifts. Each set was plotted as described in Appendix 1. The BEST program was used to determine the distance in multidimensional standard deviations (SDs) between the set of samples of pure and decomposed BCD. These values were then compared to the control distances, which were found by finding the distances between the center of the pure BCD validation spectra each pure BCD validation spectrum.

3. RESULTS AND DISCUSSION

Characterization of \(\beta\)CD

As shown in Figure 2 and 3, the structure of BCD was characterized by \(^1\)H-NMR and IR spectroscopy. Figure 2 shows the \(^1\)H-NMR spectrum of decomposed and pure BCD. Results showed only slight left shift of the decomposed sample. Figure 3 shows a comparison of the IR spectra between the pure and decomposed drug. The decomposed sample showed a slight blue shift in the O-H stretch vibrations.
It is worth noting that the Mahalanobis distance between the IR and NMR samples could not be calculated because the number of rows of the data matrix must exceed the number of columns. The BEST software was able to overcome this obstacle and provide a useful result.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

(1) Solutions, N. C. Parchem - fine & specialty chemicals is a Leading Supplier of Enzymes such as Beta Cyclodextrin https://www.parchem.com/news-articles/Parchem-fine-specialty-chemicals-is-a-Leading-Supplier-of-Enzymes-such-as-Beta-Cyclodextrin-N000192.aspx (accessed Dec 12, 2017).


Table 1. BEST results comparing pure and decomposed samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NMR Pure/Decomposed</th>
<th>NMR Pure/Pure</th>
<th>IR Pure/Decomposed</th>
<th>IR Pure/Pure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7103</td>
<td>1.8613</td>
<td>4.3276</td>
<td>1.2500</td>
</tr>
<tr>
<td>2</td>
<td>3.6602</td>
<td>1.8611</td>
<td>4.8218</td>
<td>1.5973</td>
</tr>
<tr>
<td>3</td>
<td>1.6542</td>
<td>1.6542</td>
<td>4.7514</td>
<td>1.7374</td>
</tr>
<tr>
<td>4</td>
<td>11.6069</td>
<td>0.9857</td>
<td>5.0882</td>
<td>1.8101</td>
</tr>
<tr>
<td>5</td>
<td>10.6345</td>
<td>2.1662</td>
<td>5.8849</td>
<td>1.3649</td>
</tr>
<tr>
<td>6</td>
<td>6.5937</td>
<td>2.3055</td>
<td>5.0665</td>
<td>1.4278</td>
</tr>
</tbody>
</table>
APENDIX I

REPLICA PROGRAM

function [BTRAIN,CNTER]=replica(TNSPEC,B)
% TNSPEC=training spectra, B=number of replicates desired.
% REPLICA outputs BTRAIN replicates, and the center of the replicates in CNTER
% "Copyright 2003 Robert A. Lodder & Bin Dai"
[N,D]=size(TNSPEC);
BTRAIN=zeros(B,D);
CNTER=zeros(D,1);
BSAMP=zeros(N,D);
PICKS=rand(B*N,1);
index=find(PICKS==1);
PICKS(index)=0.9999;
PICKS=reshape(PICKS,B,N);
PICKS=fix(N*PICKS+1);
for I=1:B
    BSAMP=TNSPEC(PICKS(I,:),:);
    BTRAIN(I,:)=sum(BSAMP)/N;
end
BTRAIN;
CNTER=sum(BTRAIN)/B;

QB PROGRAM

% Copyright 2003 Robert A. Lodder
function [sds,sdskew] = qb(tnspec,btrain,newspec,cnter,radfrac,sensitiv)
% function definition of qb with parameters
% tnspec= which is training spectra
% btrain =bootstrap replicates calculated using routine replica
% newspec= sample spectrum
% cnter= center of calibration set calculated using routine replica
% radfrac= fraction of points in hypercylinder
% sensitiv= sensitivity
b = size(btrain,1); %finds the number of rows in btrain
qdist = zeros(1,b); %creates a null row matrix
s02 = sqrt(sum((newspec-cnter).^2)); %computing the squareroot of sum of the suares
s0r = sqrt(sum((btrain-repmat(cnter,b,1)).^2,2)); %repmat creates a bX1 tilings of cnter
s2r = sqrt(sum((btrain-repmat(newspec,b,1)).^2,2)); %repmat creates a bX1 tilings of newspe
area = sqrt(sub.*(sub-s02).*(sub-s0r).*(sub-s2r));
radius = (2*area)/s02;
project = sqrt(s0r.^2-radius.^2); %finds the indices where s02*s02 + s0r*s0r
loci = find((s02.^2+s0r.^2) < s2r.^2);
< s2r*s2r( getting the locus)
project(loci) = project(loci)*-1;
qdist = project;
qrr = sort(radius); %sorts the elements in ascending order
radii = qrr(radfrac*b);
qdist(find(radius > radii)) = 0; % setting the elements of qdist to zero where radi-
a > radi
qdist = sort(qdist(find(qdist))); % sorts all non zero elements in qdist
lindex = floor(0.16*length(qdist)); % lower limit found by rouding to nearest inte-
gger
uindex = floor(0.84*length(qdist)); % upper limit found by rounding to nearest inte-
ger
if(length(qdist) < 50)
    '** Need more replicates in hypercylinder **'
\[
\text{sd} = \text{std}(\text{qdist}) \times \sqrt{\text{size}(\text{tnspec},1)}; \quad \% \text{std \ returns \ the \ standard \ deviation}
\]
\[
\text{sds} = \sqrt{\text{sum}((\text{cnter}-\text{newspec}).^2)}/\text{sd}; \quad \% \text{calculation \ of \ the \ standard}
\]
\[
\text{deviation \ distances}
\]
\[
\text{% BIAS \ ADJUSTMENT}
\]
\[
\alpha = \text{normcdf}(-1,0,1); \quad \% \text{computes \ the \ cumulative \ distribution \ function \ with \ mean}
\]
\[
\text{0 \ and \ standard \ deviation} \ 1
\]
\[
\text{za} = \text{norminv}(\alpha,0,1); \quad \% \text{inverse \ of \ the \ cumulative \ ditribution \ function \ with}
\]
\[
\text{mean} \ 0 \text{ and standard \ deviation} \ 1
\]
\[
\text{tcenter} = \text{median}(\text{tnspec}); \quad \% \text{finding \ the}
\]
\[
\text{median \ cs02} = \text{s02};
\]
\[
\text{cs0r} = \sqrt{\text{sum}((\text{tcenter}-\text{cnter}).^2))};
\]
\[
\text{cs2r} = \sqrt{\text{sum}((\text{tcenter}-\text{newspec}).^2))};
\]
\[
\text{cs2r} = (\text{cs02}+\text{cs0r}+\text{cs2r})/2; \quad \text{cs0r} = \sqrt{\text{sum}((\text{tcenter}-\text{cnter}).^2))};
\]
\[
\text{carea} = \sqrt{\text{cs02}*(\text{cs02}-\text{cs0r})*(\text{cs02}-\text{cs2r})}; \quad \text{carea} = \sqrt{\text{cs02}*(\text{cs02}-\text{cs0r})*(\text{cs02}-\text{cs2r})};
\]
\[
\text{cradial} = (2*\text{carea})/\text{cs02}; \quad \text{cradial} = (2*\text{carea})/\text{cs02};
\]
\[
\text{cproject} = \sqrt{\text{cs0r}^2-\text{cradial}^2}; \quad \text{cproject} = \sqrt{\text{cs0r}^2-\text{cradial}^2};
\]
\[
\text{if}((\text{cs02}^2+\text{cs0r}^2) > \text{cs2r}^2)
\]
\[
\text{cproject} = -\text{cproject}; \quad \text{cproject} = -\text{cproject};
\]
\[
\text{n} = \text{length}(\text{qdist}); \quad \% \text{finds \ the \ length \ of \ the \ vector}
\]
\[
\text{if}(\text{floor}(\text{n}/2) == \text{n}/2)
\]
\[
\text{md} = (\text{qdist}(\text{n}/2)+\text{qdist}(\text{n}/2+1))/2;
\]
\[
\text{else}
\]
\[
\text{md} = \text{qdist}(\text{floor}(\text{n}/2+0.5));
\]
\[
\text{end}
\]
\[
\text{cproject} = \text{cproject} * \text{sensitiv} + \text{md}; \quad \text{cproject} = \text{cproject} * \text{sensitiv} + \text{md};
\]
\[
\text{fdist} = \text{qdist}-\text{cproject}; \quad \text{fdist} = \text{qdist}-\text{cproject};
\]
\[
\text{index} = 1: \text{length}(\text{fdist}); \quad \text{index} = 1: \text{length}(\text{fdist});
\]
\[
\text{if}(\text{cproject} > \text{max}(\text{qdist}))
\]
\[
\text{zelement} = \text{length}(\text{qdist})-1; \quad \text{zelement} = \text{length}(\text{qdist})-1;
\]
\[
\text{elseif}(\text{cproject} < \text{min}(\text{qdist}))
\]
\[
\text{zelement} = 1; \quad \text{zelement} = 1;
\]
\[
\text{else}
\]
\[
\text{rootloc} = \text{find}(\text{abs}(\text{fdist}) == \text{min}(\text{abs}(\text{fdist})));
\]
\[
\text{zelement} = \text{rootloc}(1); \quad \text{zelement} = \text{rootloc}(1);
\]
\[
\text{end}
\]
\[
\text{z0} = \text{norminv}(\text{zelement}/\text{length}(\text{qdist}),0,1); \quad \text{z0} = \text{norminv}(\text{zelement}/\text{length}(\text{qdist}),0,1);
\]
\[
\text{if}(\text{abs}(2*z0) > \text{abs}(\text{za}))
\]
\[
\text{error}(' -- \text{Decrease skew sensitivity. --'}); \quad \text{error}(' -- \text{Decrease skew sensitivity. --'});
\]
\[
\text{sensitiv} = \text{abs}(\text{sensitiv}); \quad \text{sensitiv} = \text{abs}(\text{sensitiv});
\]
\[
\text{lowind} = \text{floor}(\text{normcdf}(2*z0+\text{za},0,1)*\text{length}(\text{qdist})); \quad \text{upind} = \text{floor}(\text{normcdf}(2*z0-\text{za},0,1)*\text{length}(\text{qdist}));
\]
\[
\text{if}((\text{lowind} < 2)
\]
\[
\text{*** \ Warning \ ** Too \ few \ replicates'} \quad \text{*** \ Warning \ ** Too \ few \ replicates'}
\]
\[
\text{end}
\]
\[
\text{if}(\text{upind} > \text{length}(\text{qdist})-2)
\]
\[
\text{*** \ Warning \ ** Too \ few \ replicates'} \quad \text{*** \ Warning \ ** Too \ few \ replicates'}
\]
\[
\text{end}
\]
\[
\text{if}(\text{lowind} < 1)
\]
\[
\text{lowind} = 1; \quad \text{lowind} = 1;
\]
\[
\text{end}
\]
\[
\text{if}(\text{upind} > \text{length}(\text{qdist}))
\]
\[
\text{upind} = \text{length}(\text{qdist}); \quad \text{upind} = \text{length}(\text{qdist});
\]
\[
\text{end}
\]
\[
\text{lowlim} = \text{qdist}(\text{lowind}); \quad \text{uplim} = \text{qdist}(\text{upind});
\]
\[
\text{euc} = \sqrt{\text{sum}((\text{cnter}-\text{newspec}).^2))}; \quad \text{fac} = \text{abs}(\text{norminv}(\text{alpha}));
\]
\[
\text{erd} = \sqrt{\text{size}(\text{tnspec},1)};
\]
if(abs(2*z0)>abs(fac))
    '** Warning ** SKEW CORRECTION exceeds replicates'
end

sds = euc/((uplim/fac)*erd);

NMR COMPARISON

>> NMR_stable=zeros(2,2);
NMR_decomposed=zeros(2,2);

>> ppm=zeros(2,2);
>> plot(ppm,NMR_stable);
hold on
plot(ppm,NMR_decomposed);
hold off

[BTRAIN,CNTER]=replica(NMR_stable,1000);

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(1,:),CNTER,0.25,0);
>> sds
sds =
    3.7103

>> [sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(2,:),CNTER,0.25,0);
>> sds
sds =
    3.6602

>> [sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(3,:),CNTER,0.25,0);
>> sds
sds =
    1.6542

>> [sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(4,:),CNTER,0.25,0);
>> sds
sds =
    11.6069

>> [sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(5,:),CNTER,0.25,0);
sds = 10.6345

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_decomposed(6,:),CNTER,0.25,0);  
sds = 6.5937

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(1,:),CNTER,0.25,0);  
sds = 1.8613

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(2,:),CNTER,0.25,0);  
sds = 1.8611

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(3,:),CNTER,0.25,0);  
sds = 1.6542

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(4,:),CNTER,0.25,0);  
sds = 0.9857

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(5,:),CNTER,0.25,0);  
sds = 2.1662

[sds,sdskew] = qb(NMR_stable,BTRAIN,NMR_stable(6,:),CNTER,0.25,0);
>> sds
sds =

2.3055

IR COMPARISON

>> IR_stable=zeros(2,2);
IR_decomposed=zeros(2,2);
wavenumber=zeros(2,2);
>> plot(wavenumber,IR_stable);
hold on
plot(wavenumber,IR_decomposed);
hold off
[BTRAIN,CNTER]=replica(IR_stable,1000);

>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(1,:),CNTER,0.25,0);
>> sds
sds =

4.3276

>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(2,:),CNTER,0.25,0);
>> sds
sds =

4.8218

>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(3,:),CNTER,0.25,0);
>> sds
sds =

4.7514

>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(4,:),CNTER,0.25,0);
>> sds
sds =

5.0882

>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(5,:),CNTER,0.25,0);
>> sds
sds =
   5.8849
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_decomposed(6,:),CNTER,0.25,0);
>> sds
sds =
   5.0665
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(6,:),CNTER,0.25,0);
>> sds
sds =
   1.4278
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(5,:),CNTER,0.25,0);
>> sds
sds =
   1.3649
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(4,:),CNTER,0.25,0);
>> sds
sds =
   1.8101
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(3,:),CNTER,0.25,0);
>> sds
sds =
   1.7374
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(2,:),CNTER,0.25,0);
>> sds
sds =
   1.5973
>> [sds,sdskew] = qb(IR_stable,BTRAIN,IR_stable(1,:),CNTER,0.25,0);
>> mahal(IR_decomposed(1,:),IR_stable)
Error using mahal (line 38)

The number of rows of X must exceed the number of columns.