J Incl Phenom Macrocycl Chem (2008) 62:143–148
DOI 10.1007/s10847-008-9449-x

ORIGINAL ARTICLE

Physicochemical characterization of sanguinarine-hydroxypropyl-β-cyclodextrin binary and ternary systems

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Received: 22 January 2008 / Accepted: 18 April 2008 / Published online: 2 July 2008

Abstract Complexation of sanguinarine with hydroxypropyl-β-cyclodextrin (HPβCD) in the presence and absence of hydrophilic polymer—polyvinylpyrrolidone K30 was studied. Respective binary and ternary systems were prepared using two techniques, physical mixture and lyophilization, and characterized by FT-infrared spectrometry, differential scanning calorimetry and X-ray diffractometry. The Fourier Transform Infrared spectra of the lyophilized binary and ternary systems showed significant shifts in the regions of 1240–1300 cm^{-1}, 1450–1525 cm^{-1} and 1600–1650 cm^{-1}, where absorptions of –C–O–C– asymmetrical stretching of sanguinarine rings A and F and $\nu_{C=C}$ ring vibrations of sanguinarine benzo[c]phenanthridine system can be observed respectively. Moreover, in the case of ternary products $\nu_{C=O}$ amide band absorption of polyvinylpyrrolidone (1600–1750 cm^{-1}) shifted to the lower wavenumbers in both the physical mixture and the lyophilized product. These changes in the spectra of the studied systems proved the involvement of the respective molecular groups in complexation process. Differential scanning calorimetry and X-ray diffractometry indicated different states of drug amorphization and entrapment in HPβCD in the presence and without polyvinylpyrrolidone. The obtained results let us conclude that obtained binary and ternary systems represent sanguinarine-HPβCD molecular complexes, with different rate of inclusion in the presence and without polyvinylpyrrolidone.

Keywords Differential scanning calorimetry · Drug-cyclodextrin complex · FT-IR spectrometry · Hydroxypropyl-β-cyclodextrin · Polyvinylpyrrolidone · Sanguinarine · X-ray diffractometry

Abbreviations

SANG	Sanguinarine
CD	Cyclodextrin
βCD	β-Cyclodextrin
HPβCD	Hydroxypropyl-β-cyclodextrin
PVP	Polyvynilpyrrolidone
DSC	Differential scanning calorimetry
FT-IR	Fourier transform infrared spectrometry
XRD	X-ray diffractometry
HPLC	High performance liquid chromatography

Introduction

Sanguinarine (SANG), a member of the benzo[c]phenanthridine alkaloids family, is a potent agent with anti-inflammatory, antimicrobial, antifungal and antioxidant properties [1–3]. Moreover, as it has been shown in the recent studies performed by Dr. Nihal Ahmad and his collaborators at the University of Wisconsin-Madison, SANG possesses antiproliferative and pro-apoptotic effects in skin and prostate cancer cell lines [4–6].

SANG exists in solution under physiological pH as either an iminium (Fig. 1, structure I), or a "pseudobase" form (Fig. 1, structure II). The former structure is

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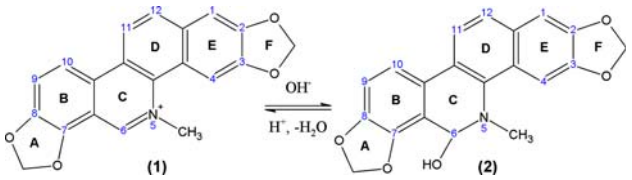

Fig. 1 Sanguinarine iminium (1) and "pseudobase" forms (2)

susceptible to nucleophilic attack at the position 6 and can reversibly react with hydroxyl anion thus forming the latter. The "pseudobase" is an electroneutral substance which is the form expected to penetrate cells where it can act as is or dissociate back into the positively charged iminium form of SANG.

Poor water solubility of SANG is one of the major factors that severely limits its medical applications. Therefore, there is a certain need in developing water soluble forms of SANG as a step to the following new drugs design.

Cyclodextrins (CDs), cyclic oligosaccharides with a hydrophilic outer surface and somewhat hydrophobic central cavity, are known to form stable water-soluble inclusion complexes with a variety of drug molecules [7]. As a consequence of the inclusion process, many physicochemical properties such as solubility, dissolution rate, stability and bioavailability, can be favorably affected. However, the efficiency of complexation is often not very high, and therefore, relatively large amounts of CDs must be used to obtain the desired effect. On the other hand, pharmaceutical dosage forms should contain as little CD as possible because of a variety of reasons: problems of formulation bulk, possible toxicity, reducing of preservative efficacy. So it has been established by several studies that in order to improve both the complexing and the solubilizing efficiencies of CDs, small amounts of water-soluble polymers, such as polyvinylpirrolidone, should be added to a drug-CD system [8–10].

The objective of this study was to investigate the interactions of SANG with hydroxypropyl-β-cyclodextrin (HPβCD) in association with or without water soluble polymer polyvynilpyrrolidone (PVP), as the respective complexing and co-complexing agents for SANG. HPβCD is a partially substituted poly(hydroxpropyl) ether of beta-cyclodextrin (βCD). It is considered the most likely candidate of all the chemically modified CDs for incorporation into formulations used by humans and animals. HPβCD has the best balance of enhanced aqueous solubility, the wide range of drugs with which it forms stable complexes, and the most extensive collection of safety data with no adverse reactions reported. HPβCD is itself very soluble in water (greater than 500 mg/mL at room temperature compared to 18 mg/mL for βCD). An important fact about HPβCD is that the concentration of the complexed drug increases linearly as the concentration of HPβCD increases. This happens due to the formation of AL type complexes between the CD and the drug molecule [11].

PVP was added with the aim of increasing the complexation efficiency of HPβCD toward SANG. PVP is nontoxic polymer often used in pharmaceutical preparations as auxiliary substance. Besides, it is often used in plasma substitute solutions. No carcinogenicity has been reported for PVP as a consequence of oral administration. The only reported biological effect attributed to oral administration of PVP is stool softening or diarrhea.

There are several different techniques frequently applied for preparation of CD inclusion complexes: tumble mixing—to prepare physical mixture, ball-milling—co-ground mixture, kneading, co-precipitation, co-evaporation and lyophilization. Different studies have suggested the lyophilization to be the most efficient one [12, 13]. Namely this technique was applied to prepare the solid binary and ternary systems of SANG-HPβCD. Physical mixtures were also prepared as the main reference systems. The physicochemical characterization of the obtained binary and secondary systems was performed based on Fourier Transform Infrared Spectrometry (FT-IR) and X-ray diffractometry (XRD).

Experimental

Materials

SANG (Sanguinarine chloride hydrate ≥98% (HPLC), empirical formula: $C_{20}H_{14}ClNO_4$, MW 367.78) was purchased from Sigma. PVP (Povidone K30 meets USP testing specifications, linear formula: $(C_6H_9NO)_n$, Avg. MW 40,000) was purchased from Sigma-Aldrich. HPβCD (2-hydroxypropyl-β-cyclodextrin, molecular formula $(C_6H_9O_5)_7(C_3H_7O)_{4.5}$, Avg. MW 1396 (anhyd.)) was purchased from Sigma.

Preparation of physical binary and ternary mixtures

Equimolar physical mixtures (p.m.) of SANG and HPβCD, with and without adding of 15% (w/w) PVP, were prepared by homogeneous blending in agate mortar of exactly weighed amounts.

Preparation of lyophilized binary and ternary products

Equimolar amounts of SANG and HPβCD, with and without adding of 15% (w/w) PVP, were dissolved in water and mixed for 2 h at 35 °C wrapped in the aluminium foil to protect the solutions from the direct light. After the equilibration period of 24 h the clear solutions were frozen

and subsequently lyophilized in a freeze-dryer of Alpha 1-2 LD type.

FT-IR spectrometry

FT-IR spectra of SANG, HPβCD, PVP, and of binary and ternary systems (PMs and lyophilized products) were obtained using the KBr pellet technique. They were collected with Jasco FTIR 6100 spectrometer in the 4000 to 350 cm^{-1} spectral range with the resolution of 2 cm^{-1}.

X-ray diffractometry

Powder XRD patterns of SANG, HPβCD, PVP, their binary and ternary systems (PMs and lyophilized products) were analyzed at room temperature with an automated D8 Bruker powder diffractometer. The patterns were recorded in the 2θ angle range between 3 and 50° and the process parameters were set at: scan step size of 0.1 (2θ); scan step time of 5 s. The XRD traces of all raw materials, binary and ternary systems were compared with regard to peak position, relative intensity, peak shifting, and the presence, and/or absence, of peaks in the certain regions of 2θ values.

Differential scanning calorimetry (DSC)

DSC analysis was performed with a Schimadzu DSC-60. Weighed samples (2.0–2.2 mg) were scanned in aluminium pans at a scan rate of 10 °C min^{-1} over a temperature range of 30–300 °C.

Results and discussion

As it can be seen from the SANG structure in both the iminium and the "pseudobase" forms (Fig. 1), there are two certain possibilities of its molecule to be accommodated inside the cavity of HPβCD. In order to confirm the formation of SANG-HPβCD complex, the FT-IR spectra of the prepared binary and ternary systems have been analyzed and compared with those of individual substances.

The main indicators that confirm the formation of inclusion complexes are changes in peak positions, shapes and intensities observed in the obtained FT-IR spectra of the analyzed systems in comparison with that of the initial substances. These changes can be explained by the implication of different functional groups and parts of the host and guest molecules in the formation of inclusion complex.

Thus, in the 1000–1100 cm^{-1} region (Fig. 2), absorption peaks corresponding to -C-O-C- symmetrical stretching of HPβCD ether groups in the lyophilized SANG-HPβCD product are shifted as compared to those of the physical mixture SANG-HPβCD and pure HPβCD.

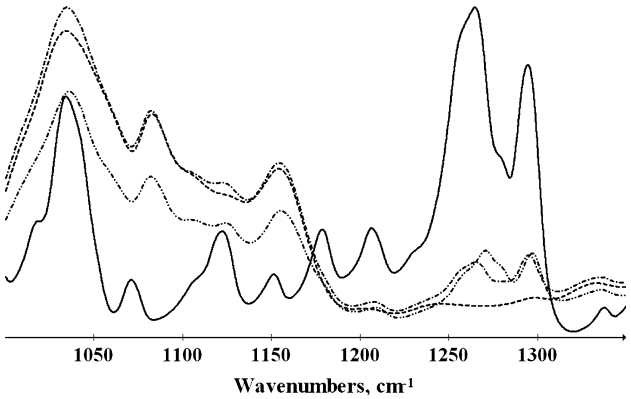

Fig. 2 FT-IR spectra (1000–1350 cm^{-1}) of SANG (——), HPβCD (- - - -), their physical mixture (—·—·—) and lyophilized product (—··—··—)

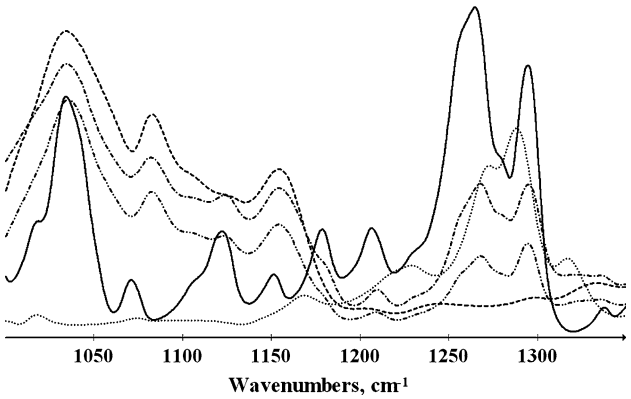

Fig. 3 FT-IR spectra (1000–1350 cm^{-1}) of SANG (——), HPβCD (- - - -), PVP (····), their physical mixture (—·—·—) and lyophilized product (—·—··—)

Even larger shifts to higher wavenumbers can be observed in the 1240–1300 cm^{-1} region, where two peaks of –C–O–C– asymmetrical stretching of SANG rings A and F in the lyophilized SANG-HPβCD product and pure SANG appear. These data already suggest that namely A and F rings are included in the hydrophobic cavity of HPβCD.

In the FT-IR absorption spectra of the ternary systems with PVP (Fig. 3) one can see shifts in the same regions as that of binary systems. However, these shifts are not that drastic or even have the opposite direction. Besides, positions of absorption peaks in the spectra of the physical mixture and the lyophilized product are very close.

In the regions of 1450–1525 cm^{-1} and 1600–1650 cm^{-1} (Figs. 4 and 5) absorptions of $\nu_{C=C}$ ring vibrations of SANG benzo[c]phenanthridine system can be observed. From these, the larger shift is observed in the doublet 1460–

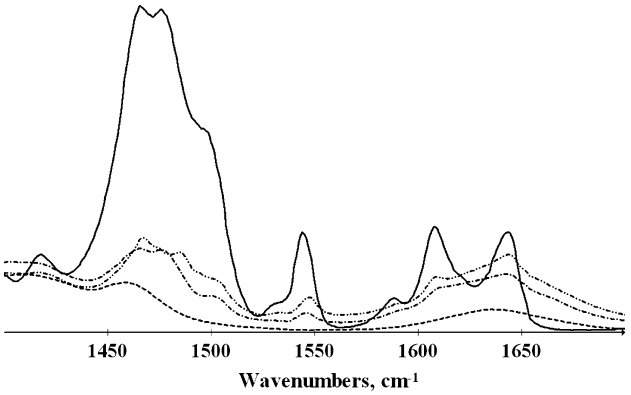

Fig. 4 FT-IR spectra (1400–1700 cm^{-1}) of SANG (——), HPβCD (- - - -), their physical mixture (—·—·—) and lyophilized product (—··—··—)

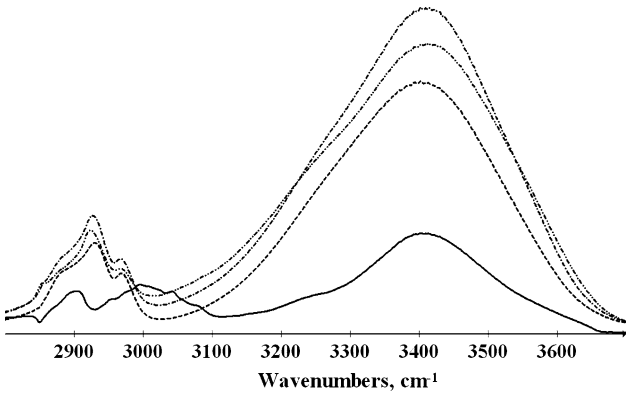

Fig. 6 FT-IR spectra (2800–3800 cm^{-1}) of SANG (——), HPβCD (- - - -), their physical mixture (—·—·—) and lyophilized product (—··—··—)

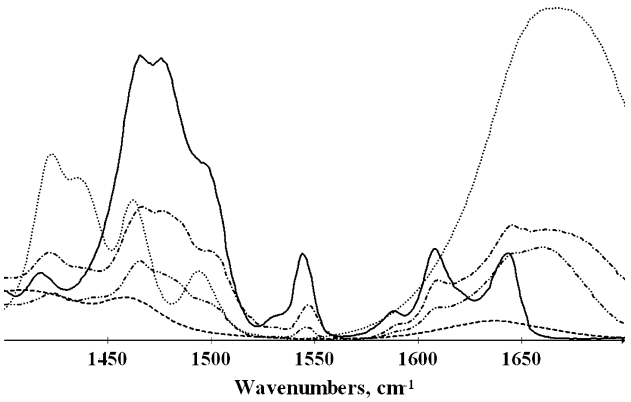

Fig. 5 FT-IR spectra (1400–1700 cm^{-1}) of SANG (——), HPβCD (- - - -), PVP (····), their physical mixture (—·—·—) and lyophilized product (—··—··—)

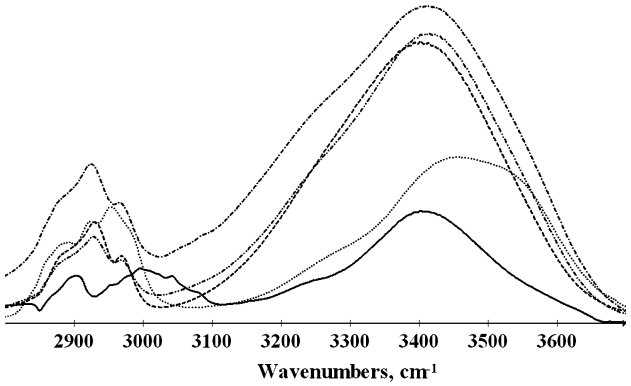

Fig. 7 FT-IR spectra (2800–3800 cm^{-1}) of SANG (——), HPβCD (- - - -), PVP (····), their physical mixture (—·—·—) and lyophilized product (—··—··—)

1475 cm^{-1} of the lyophilized binary product as compared to the respective physical mixture and pure SANG. Less visible changes happened in the region of 1600–1650 cm^{-1} for the same product.

In the case of ternary systems, $\nu_{C=C}$ ring vibrations of SANG enter in superposition with absorptions of δ_{CH_2} (1420–1470 cm^{-1}) and $\nu_{C=O}$ amide band (1600–1750 cm^{-1}) of PVP. Nevertheless, some visible changes, as compared to binary systems, can also be found. Thus, the maximum of $\nu_{C=O}$ amide band absorption shifted to the lower wavenumbers in both the ternary physical mixture and the lyophilized product. Besides, in the case of the lyophilized ternary system, amide band has become much narrower. All of these indicate the possible implication of PVP amide group in the complexation process.

The analysis of FT-IR spectra of the studied binary and ternary systems in the region 2800–3800 cm^{-1} (Figs. 6 and 7) gives us supplementary information. In this spectral region typical absorption bands of the ν_{O-H} stretching vibrational modes that belong to different OH groups of HPβCD can be identified. Besides, in the domain 2800–3000 cm^{-1}, symmetrical and asymmetrical ν_{CH3} and ν_{CH2} stretching modes of aliphatic groups (belonging to SANG, HPβCD and PVP) can be observed. Many of them are represented by overlapped bands.

The maximum of broad ν_{O-H} stretching vibrational band belonging to the lyophilized product of binary system SANG-HPβCD is significantly shifted compared to that of the respective physical mixture and pure HPβCD. In the case of the ternary systems, the frequency of ν_{O-H} stretching of the physical mixture and the lyophilized product are shifted relatively to the maximum of absorption of pure HPβCD.

The thermal profiles of pure components and various binary and ternary products are presented in Figs. 8 and 9. The DSC curve of SANG indicated its crystalline state with melting point at 283 °C. A large endothermal effect, associated with water loss, was recorded for this substance

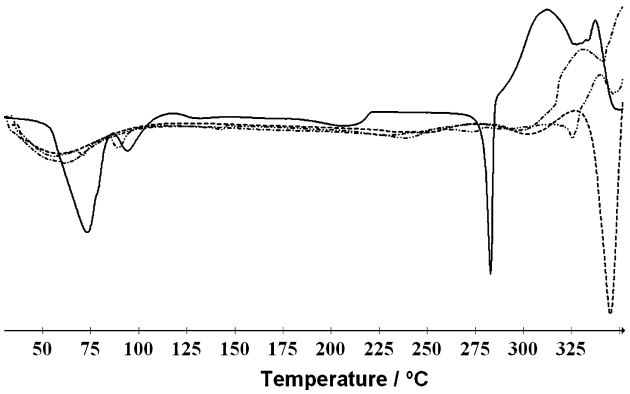

Fig. 8 DSC curves of SANG (——), HPβCD (- - - -), their physical mixture (—·—·—) and lyophilized product (—··—··—)

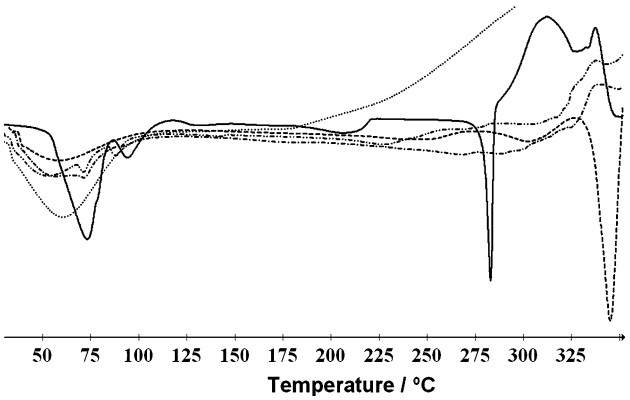

Fig. 9 DSC curves of SANG (——), HPβCD (- - - -), PVP (····), their physical mixture (—·—·—) and lyophilized product (—··—·—)

also. The curve for HPβCD reveal a wide band (range approx. 40–110 °C) that corresponds to loss by evaporation of the water molecules existing as residual humidity ($T < 100$ °C) as well as those included in the cavity ($T > 100$ °C). Besides, at 345.2 °C of the HPβCD DSC curve one can observe another endotherm corresponding to the melting point of oligosaccharide. In the PVP curve, the loss of residual humidity can be observed as a wide band (range approx. 35–100 °C).

In both binary systems, the fusion endotherm of SANG was markedly reduced in intensity or totally absent, as a consequence of interactions between SANG and HPβCD and/or drug amorphization. Such a phenomenon was even more obvious in the ternary systems, in the presence of PVP, where the melting endotherm of SANG was never observed. This indicated the absence of crystalline drug in the obtained systems or its interaction with amorphous carriers during the DSC scan.

The XRD patterns of SANG, HPβCD, PVP, and their lyophilized binary and ternary systems, in the range of 3–50° 2θ, are shown in Fig. 10. The XRD pattern of SANG revealed several high-intensity reflections corresponding to

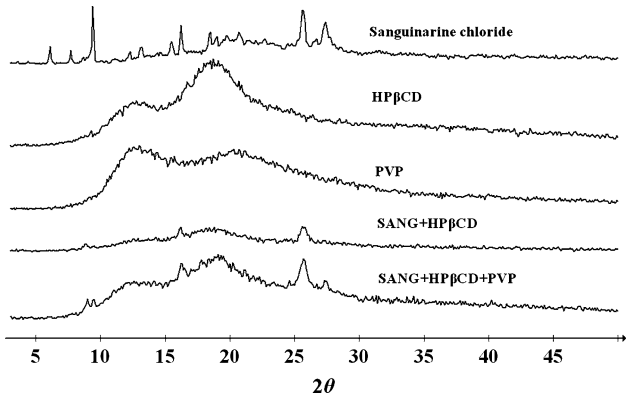

Fig. 10 Powder XRD patterns of SANG, HPβCD, PVP, lyophilized product SANG + HPβCD (1:1), and lyophilized product SANG + HPβCD (1:1), with adding of 15% PVP (w/w)

the diffraction peaks 9.4°, 16.2°, 25.6° and 27,4° (2θ), which were indicative of its crystalline character. For HPβCD and PVP hollow patterns were recorded that demonstrated their amorphous states. The diffractogram of the lyophilized binary SANG-HPβCD product shows a partially amorphous sample by the appearance of two diffraction peaks at 16.2° and a doublet with maximums at 25.6° and 25.8° (2θ), partially overlapped. Both peaks indicate the presence of initial SANG in the mixture representing two of the principal diffraction peaks of SANG. In the XRD pattern of the lyophilized ternary SANG-HPβCD-PVP product a higher degree of SANG in its crystalline form can be observed. At the same time, two new peaks appear in the diffractogram at 9.0° and 16.5° (2θ) indicating the appearance of a new chemical species different from the original substances. All of these indicate at a lower rate of inclusion process than in the case of the binary product. These facts can be explained by a possible higher level of the iminium form of SANG in the inclusion medium as a result of its stabilization at the presence of PVP at the low pH levels. In our recent paper [14] we have shown that only pseudobase form of SANG due to its hydrophobicity can form stable complexes with CDs. This process takes place only after alkalinization of the medium to pH 8.0. Moreover, only at this level of pH presence of PVP can lead to a higher constant of association between SANG and CDs.

Conclusions

All the above suggests that the process of inclusion complex formation between SANG and HPβCD, in association with or without 15% (w/w) PVP, took place. Though, the physicochemical solid-state characterization of SANG-HPβCD binary and ternary systems also indicates the presence of uncomplexed original substance. These facts

indicate that some additional studies on the conditions of the complex formation between SANG and HPβCD need to be performed.

To the best of our knowledge, this is the first study of SANG-HPβCD binary and ternary systems. We believe that it will contribute to the following research attempts in the field of design and development of new SANG based drugs.

Acknowledgements The research described in this publication was made possible in part by Award No. MTFP-012/05 Follow-On Award of the Moldovan Research and Development Association (MRDA) and the U.S. Civilian Research & Development Foundation (CRDF). Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect those of the MRDA or CRDF.

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