The interaction of tetrabutylammonium bromide (TBABr)-based deep eutectic solvents (DESs) with calf thymus DNA (ct-DNA) was investigated by fluorescence and circular dichroism (CD) spectroscopy. The influences of hydrogen bond donors (HBDs), with respect to chain lengths and the presence of OH group were determined through ethidium bromide (EB) fluorescence quenching. Based on the quenching process, it was concluded that hydrophobic interactions were responsible for the DES ability to quench EB from EB-bound DNA. The strong electrostatic attraction between TBA⁺ cation on DES and negative charge phosphate DNA was proved from fluorescence and CD spectroscopy. DNA was able to maintain its double helical structure at 25 °C with a concentration of 25% DES.

**Keyword:** Binding; Deep eutectic solvent; Tetrabutylammonium bromide; Fluorescence quenching; Circular dichroism spectroscopy